Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Faculty of Natural Resources and Agricultural Sciences
Department of Food Science

Cheeses with blowing defect

– Problematics and preventable methods

Feljäsning av ostar

– Problematik och förebyggande metoder

Amanda Alvenäs

Independent Project in Food Science• Bachelor Thesis • 15 hec • Ground G2E
Publikation/Sveriges lantbruksuniversitet, Institutionen för livsmedelsvetenskap, no 412
Uppsala, 2015

Cheeses with blowing defect – problematics and preventable methods
Feljäsning av ostar – problematik och förebyggande metoder

Amanda Alvenäs

Supervisor: Monika Johansson, Swedish University of Agricultural Sciences, Department of Food Science

Examiner: Lena Dimberg, Swedish University of Agricultural Sciences, Department of Food Science

Credits: 15 hec
Level: Ground G2E
Course title: Independent Project in Food Science – Bachelor Thesis
Course code: EX0669
Programme/education: Agronomy in Food Science

Place of publication: Uppsala
Year of publication: 2015
Cover picture: Anders Christiansson, LRF mjölk
Title of series: Publikation/Sveriges lantbruksuniversitet, Institutionen för livsmedelsvetenskap
Serie no: 412
Online publication: http://stud.epsilon.slu.se

Keywords: blowing defect, gas defect, cheese spoilage, cheese problems

Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Faculty of Natural Resources and Agricultural Sciences
Department of Food Science

Abstract

Blowing defect of cheeses refers to undesirable openness of the cheeses interior. It is a result of unwanted microbial metabolism. Production of hard- and semi-hard chesses require methods and/or preservatives to make sure that blowing defect is prevented. Most dairy industries have developed proper methods for this purpose, but it still remain a big problem for many cheese-producers.
In this study problematics behind blowing defect was investigated. Preventing methods was compiled and the magnitude of the problem in the dairy industry was evaluated. Five Swedish large-scale dairy industries as well as two Italian traditional cheese producers have contributed with information about the importance of managing blowing defect at their factories.

Cheeses frequently affected by blowing defect include brine-salted cheeses, cheeses ripened at hot temperatures and hard cheeses that are ripened for extensive periods. Low salt content, high levels of moisture content, water activity and pH as well as high initial amount of spores and/or somatic cell count favours spoilage. Swiss-type-, Dutch-type-, very hard Italian-type-, and Swedish Herrgård- and Grevé-type-cheeses are examples of cheeses susceptible for blowing defect.

Pasteurization of cheese milk is the most common method for the prevention of spoilage bacteria. It inhibits non-sporulating microorganisms causing blowing defect; heterofermentative lactic acid bacteria, propionic acid bacteria, coliform bacteria and yeasts. However, it is not a guarantee for safe dairy products without spoilage organisms since thermoduric microorganisms and enzymes survives the process and sterilized milk is a good substrate for a lot of spoilage microorganisms.

Endospores survive pasteurization and can germinate in the cheese during ripening if environmental conditions are favourable. Endospores are normally reduced by membrane filtration and/or bactofugation of the cheese milk. Sodium nitrate and lysozyme are common additives for the inhibition of spore-forming bacteria as well as other spoilage bacteria. More severe methods for the removal of spores exist, but they normally alter the cheese-making properties too much to be applied in the industry.

Further development of processing techniques of cheese milk and research about combining different methods are needed to be investigated in order to ensure a cheese production without blowing defect, and without altering the cheese-making properties.

Keywords: blowing defect, gas defect, cheese spoilage, cheese problems

Sammanfattning

Feljäsning av ostar orsakas av mikrobiell metabolism och resulterar i oönskad öppen struktur av ostens innanmäte. Produktionen av hårda- och halvhårda ostar kräver metoder och/eller tillsatser för att förhindra problemet. De flesta mejerier har utvecklat lämpliga metoder för detta ändamål men trots detta utgör feljäsning av ostar fortfarande ett problem för många ostproducenter.
I denna studie undersöktes problematiken angående feljäsning av ostar. Förebyggande metoder sammanställdes och omfattningen av problemet i mejeriindustrin utvärderades. Fem svenska storskaliga ostproducenter samt två italienska traditionella ostproducenter har bidragit med information angående omfattningen av feljäsning på deras ystningsverkssamhet.

Osttyper som ofta drabbas av feljäsning inkluderar saltlakesaltade ostar, ostar som mognar vid höga temperaturer och hårdostar som lagras under långa perioder.

Lågt saltinnehåll, hög fuktighet, vattenaktivitet och pH samt höga initiala halter av sporer och/eller somatiska celltal gynnar feljäsning. Schweiziska osttyper-, holländska osttyper-, hårda Italienska osttyper-, samt svensk grevé och herrgårdsost är exempel på ostar som är särskilt mottagliga för feljäsning.

Pastörisering är en metod som förhindrar mikrobiell tillväxt och den tillämpas vanligen på ystmjölk. Pastörisering inhiberar icke-sporulerande mikroorganismer som orsakar feljäsning; heterofermentativa mjölksyrebakterier, propionsyrabakterier, koliforma bakterier och jäst. Metoden kan dock inte garantera helt livsmedelssäkra produkter eftersom termoresistenta mikroorganismer och enzymer överlever processen. Steriliserad mjölk är även ett bra näringssubstrat för flertalet mikroorganismer.

Endosporer överlever pastörisering och om omgivande faktorer är gynnsamma kan sporerna gro i osten under lagringsperioden. Endosporer reduceras vanligen genom membranfiltration och/eller baktofugering av ystmjölken. Natriumnitrat och lysosym är vanliga tillsatser som både inhiberar sporulerande bakterier och andra förstörande mikroorganismer.

Mer omfattande metoder för reduktionen av sporantal finns men de försämrar ofta ystningsegenskaperna vilket gör att de inte används i industrin idag.

Ytterligare forskning angående kombinationen av metoder och utveckling av processandet av ystmjölk behöver utföras för att kunna säkerställa en ostproduktion utan feljäsning samt utan att försämra dess ystningsegenskaper.

Nyckelord: feljäsning, sprängd defekt, ostförstörelse, ostproblem

Table of contents

1 Introduction

Blowing defect of cheeses represent a major risk of spoilage in the dairy industry today. It results in losses in the form of reduced yield, stability and shelf-life of the cheese. Blowing defect is caused by unwanted bacterial gas production which normally appears during the later stages of cheese making. The gasses produce texture defects like holes, slits, eyes and cracks of the cheeses interior. Off-flavours and odours are also usually a consequence of that (Düsterhöft & van den Berg 2007; Hill & Kethireddipalli 2013; Sheelan 2007).

Microbial blowing defect is mainly caused by Clostridia, certain heterofermentative bacteria, coliform bacteria and yeasts. *Clostridium tyrobutyricum* is a well-known cheese spoilage bacteria representing the largest problem due to butyric acid fermentation (Hill & Kethireddipalli 2013; Sheelan 2007)

1.1 Background

Most thermoduric microorganisms and other spoilage organisms are inhibited by normal cheese-making conditions, e.g. by acidity and low water activity. However, if initial counts of spoilage microorganisms are sufficient and environmental conditions allow it, gas defects will occur.

Clostridia are able to produce thermoduric endospores, one of the most resistant survival structures existing. The spores can be transferred from the cattle, end up in the cheese milk, and colonize during ripening of the cheese (Brüggemann & Gottschalk 2009; Giffel & Wells-Bennik 2010; Sheelan 2007; Willey et al. 2012).

1.2 Aim and objectives

The aim of this study was to define and evaluate problematics behind blowing defects of cheeses and to investigate how comprehensive the problem is in the industry today. Causing microorganisms and metabolism are summarised and known preventing methods are compiled and estimated. Scientific literature and interviews with dairies were evaluated.

2 Microbial cheese spoilage

2.1 Factors affecting cheese spoilage

Microbial cheese spoilage organisms lower the quality of cheeses by producing degrading enzymes which alter the texture, flavour and smell of the cheese (Ledenbach & Marshall 2010). Psychrotrophic bacteria, fungi, yeasts, spore-forming bacteria, coliform bacteria and heterofermentative bacteria are the most common ones (Giffel & Wells-Bennik 2010). Factors like water content, temperature, pH, redox potential, nutrient availability, salinity, properties of starter- and adjunct cultures, competitive microflora, oxygen availability, properties of contaminating microorganisms as well as type and quantities of produced enzymes determine the occurrence of spoilage (Johnson 2001; Ledenbach & Marshall 2010).

Soft and fresh cheeses are easily spoilt due to higher pH and moisture content and lower salinity. Hard and ripened cheeses are better preserved due to lower pH, lower water activity (a_w) and salinity (Ledenbach & Marshall 2010). If the curd mass is insufficient pressed and unevenly distributed, it will increase the risk of cheese spoilage (Sheelan 2007).

Most spoilage bacteria need oxygen to grow and are inhibited by the anaerobic ripening environment of the cheeses interior. An exception is Clostridia which are strictly anaerobic bacteria which can germinate and grow in the interior during ripening (Beresford 2007a; Johnson 2001; Zhao et al. 2013).

2.2 Blowing defect

Blowing defects can arise both early and late during cheese maturation. Early gas defect is normally a product by coliforms or yeasts (Hill & Kethireddipalli 2013; Sheelan 2007). Late gas defect is a typical condition of cheeses contaminated with propionic acid bacteria (PAB) or spore-forming Clostridia. Heterofermentative lactic acid bacteria and propionic acid bacteria are able to spoil cheeses by early- as well as late gas defect (Hill & Kethireddipalli 2013). Late blowing defect represent the largest microbial cheese spoilage problem with *Clostridium tyrobutyricum* as the dominant causative agent (Hill & Kethireddipalli 2013).

Clostridial growth produce major texture defects and gas production of the cheeses interior and creates an acidic, fermented flavour as well as a vomit-like odour from butyric acid (Düsterhöft & van den Berg 2007; Zhao et al. 2013).

2.2.1 Cheeses frequently affected

Swiss-type (e.g. Emmentaler, Swiss), and Dutch-type cheeses (e.g. Gouda, Edam) are frequently affected by blowing defect (Ledenbach & Marshall 2010). They are ripened at hot temperatures and are brine-salted. Brine-salting means immersion of formed cheese into a brine. It poses a problem because growth of *C. tyrobutyricum* depend on the rate of diffusion of salt into the interior of the cheese (Düsterhöft & van den Berg 2007; Ledenbach & Marshall 2010; Sheelan 2007). Some brine-salted cheeses, e.g. Cheddar, have rapid S/M (salt to moisture) increase and are not susceptible to spoilage due to brine-salting (Sheelan 2007). All these hard- and semi-hard cheeses have problems with blowing defects as well as cheeses such as Gruyere and Swedish Grevé and Herrgårdsost (Klijn et al. 1995; Thylin 2000). Very hard Italian cheeses like Grana Padano and Parmigiano Reggiano are susceptible for late blowing defect due to extensive ripening periods (Di Cagno & Gobetti 2007; Thylin 2000).

2.3 Factors affecting milk quality

Milk quality has a major impact on final cheese quality and property (Burgess 2010). Somatic cell count (SCC) is often used as a golden standard indicating the milk quality. Cheese milk with high SCC result in lowered yield and affects the final product negatively. Total bacterial count is an important quality marker (Adams & Moss 2008; Hill & Kethireddipalli 2013; Weller 2010). Cheese milk should contain $<10^4$ cfu (colony forming units)/ml and $<10^5$/ml of SCC in order to be classified as high quality, Grade A milk (Johnson 2001; Skeie 2010).

2.3.1 Psychrotrophic organisms

Psychrotrophic microorganisms are microorganisms that can grow at refrigerating temperatures. They have competitive advantage over desired microflora like lactic acid bacteria (LAB) in chilled milk (Hill & Kethireddipalli 2013). Cheese milk with high amount of psychrotrophic bacteria will reduce cheese yield and shelf-life as well as produce off-flavours (Skeie 2010). Psychtrotrophic organisms normally come from Staphylococcus, Pseudomonas, Bacillus, Aerococcus, Lactococcus Micrococcus and coliforms, but some yeasts and molds are also psychrotrophic (Beresford 2007a; Hill & Kethireddipalli 2013; Ledenbach & Marshall 2010). They are killed by pasteurization but some of their hydrolytic enzymes are thermoduric. Psychtrotrophs are most easily inhibited by cooling the milk quickly and using it soon after milking (Ledenbach & Marshall 2010).

2.3.2 Spore-forming bacteria

Clostridia spp. and Bacillus spp. are endospore-forming bacteria. Endospores are dormant structures developed from bacterial cells and are very resistant to environmental stress (Willey et al. 2012). Sporulation protects the bacterial DNA from adverse conditions as extremes in pH and temperatures but also from chemicals, radiation and desiccation. The spores survive pasteurization and pose a big problem in the food industry (Brüggemann & Gottschalk 2009). *Bacillus cereus*, *Bacillus licheniformis* and *Bacillus subtilis* frequently contaminate dairy products, resulting in off-flavours and texture defects (Giffel & Wells-Bennik 2010; Ledenbach & Marshall 2010).

Endospore-forming bacteria and other spoilage organisms can be transferred from the cow to the milk if the udder is contaminated from dirt; either from soil, faeces or the bedding material (Heyndrickx et al. 2010). Straw beds and silage increases the risk of spores, mastitis and average cell counts of the milk (Heyndrickx et al. 2010; Weller 2010). Fast and adequate pH-reduction of the silage by LAB fermentation is necessary regarding the prevention of spoilage microorganisms (Wilkinson 2005). Clostridia are strictly anaerobic which gives them competitive advantage during oxygen free silage and they can grow if the pH-reduction by LAB in the silage is insufficient and a_w is enough (Thylin 2000).

2.4 Pathogens associated with cheese

Major pathogenic bacteria associated with a risk during cheese manufacturing include; *Listeria monocytogenes,* Salmonella spp. and *Escherichi coli* O157:H7. They are common contaminants of raw milk and tolerate the low temperature and pH of cheeses. Some are psychrotrophic and can stay for a long time during the ripening process, e.g. *L. monocytogenes* which is high-risk bacteria in dairy production because it easily colonizes dairy plant environment (Hill & Kethireddipalli 2013; Stessl & Hein 2010).

Other common pathogenic contaminants of milk and dairy products include *Staphylococcus aureus*, Mycobacterium spp., *Yersinia enterocolitica*, *Campylobabter jejuni*, Salmonella spp. and *B. cereus*, but growth and toxin-production is normally inhibited by LAB and low pH (Giffel & Wells-Bennik 2010; Hill & Kethireddipalli 2013).

3 Cheese properties

3.1 Property determinants

Properties of cheeses varies a lot and depend on different choices and conditions during production. Cheese consist of just a few ingredients; milk, culture inoculants, rennet/acid and salt. Even so, properties can change during manufacture since cheeses have a dynamic microflora (McSweeney 2007a). Characteristics and flavor can be altered by choice of culture inoculants, milk type, milk quality and time of renneting and ripening (Adams & Moss 2008).

Texture of cheeses depend on attributes like openness, firmness, elasticity, size of eyes and brittleness (Willey et al. 2012).

3.2 Classification

Cheeses are often classified by hardness and texture, sometimes also size, milk type, ripening process and region of production. Rennet-coagulated cheeses are often classified by hardness, ripening process and manufacturing methods. Examples of classifications include very hard cheese (e.g. Grana padano), hard cheese (e.g. Cheddar), semi-hard cheese (e.g.Gouda), Swiss-type cheese (e.g. Emmental) and Dutch-type cheese (e.g. Edam) (Banks 2007a; Hill & Kethireddipalli 2013; Willey et al. 2012).

Circular holes created from CO_2 and/or H_2 are called eyes. Cheeses with eyes (e.g. Swiss-type) has to be manufactured from an elastic and soft curd, otherwise slits and cracks of the cheese will be produced instead of eyes. Gas development in elastic curds bends the protein network rather than breaks it (Johnson 2001). Eyes will form if gas production is faster than the permeation through the rind of the cheese (Hill & Kethireddipalli 2013). Cheeses with eyes have higher pH (>5.3 within the first days of ripening), than cheeses without eyes (<5.2) (Skeie 2010).

3.3 Lactic acid bacteria

Cheese production depend on microbial culture inoculation for the development of characteristic attributes like flavour and texture, but also in order to prolong shelf-life, prevent quality defects and enhance ripening (Hill & Kethireddipalli 2013).

Lactic acid bacteria (LAB) are used for this purpose. It is inoculated as a starter culture into cheese milk early during cheese-making, often also in the later stages of production as well (Hill & Kethireddipalli 2013). *Lactococcus lactis* is a common starter culture of LAB, both for soft- and hard cheeses (Willey et al. 2012).

LAB are gram-positive non-motile rods or cocci and prefer temperatures over 30 ºC. They produce lactic acid, are mesophilic/termophilic and facultative anaerobes or microaerophilic (Hill & Kethireddipalli 2013). LAB have an advantage in dairy microflora as they produce lactase which makes it possible to utilize the lactose present in milk (Hill & Kethireddipalli 2013). The pH of cheese is lowered by LAB metabolism to about 4.2-5.5. The pH-reduction inhibit many microorganisms, improves the stability of the cheese and enhances texture and flavor (Adams & Moss 2008; Coultate 2009; Hill & Kethireddipalli 2013).

Heterofermentative LAB produce lactic acid, CO_2 and flavour compounds (Hill & Kethireddipalli 2013). In some cheese-types they are inoculated for the characteristic eye development. Some LAB are suitable inoculants for certain cheese types but are highly unwanted in other varieties. Citrate positive LAB (e.g. *Lactococcus lactis* subsp. *Lactis* or *Leuconostoc cremoris*) produce diacetyl, acetate and CO_2 which contributes to characteristic flavor and small eyes for Dutch-type cheeses. However, cheeses like cheddar would get spoiled by the inoculation of the same bacteria (Adams & Moss 2008; Johnson 2001).

3.4 Ripening

Rennet-coagulated cheeses are ripened in order to obtain characteristic flavor, texture, structure and functionality (Hill & Kethireddipalli 2013). Ripening changes chemical and physical properties of the cheese by biochemical processes (Hill & Kethireddipalli 2013). Ripening reduce water, increase hardness and catabolize fatty acids, amino acids and casein micelles (Hill & Kethireddipalli 2013; Willey et al. 2012).

During ripening, lactate is metabolized by yeast, molds and proteolytic bacteria which increases pH gradually with ripening time (Hill & Kethireddipalli 2013). Ripening time depend on desired texture and flavor and is typically 20-24 months for Parmigiano Reggiano and 14-16 months for Grana Padano (Di Cagno 2007; Gobbetti 2007; Hill & Kethireddipalli 2013). Hard cheeses like Swiss and Cheddar require 3 to 12 months of ripening (Willey et al. 2012). Dutch-type cheeses are ripened for at least 4 weeks (Düsterhöft & van den Berg 2007).

Ripening temperature depends on cheese variety (Adams & Moss 2008). Ripening can be accelerated by elevating storage temperatures, high-pressure processing and addition of enzymes (Hill & Kethireddipalli 2013).

4 Blowing defect - causing microorganisms

4.1 Clostridia

Typical Clostridia are endospore-forming, rod-shaped, gram-positive, carbohydrate-fermenting and strictly anaerobic bacteria (Zhao et al. 2013). Normally they have optimal growth at 37 ºC and are inhibited at pH values lower than 4.5 (Thylin 2000; Wilkinson 2005). Some Clostridia produce toxins e.g. *C. botulinum*, *C. perfringens* and *C. butyricum* (Brüggemann & Gottchalk 2009).

C. tyrobutyricum, C. butyricum and *C. sporogenes* cause blowing defect by butyric acid fermentation. *C. tyrobutyricum* is the dominant causative agent and it metabolises lactic acid present in the cheese and produces butyrate, acetate, CO_2 and H_2 as by-products with a high yield (Cocolin et al. 2004; Di Cagno & Gobetti 2007; Klijn et al. 1995; Zhao et al. 2013). From 2 g of lactic acid, 1 g of butyric acid and 1000 mL of gasses are produced (Bachmann & Frölich-Wyder 2007). The gasses create typical blowing defect and large openness of the cheeses interior in hard- and semi-hard cheeses.

C. tyrobutyricum spoil the cheese during the later stages of cheese ripening, often after a few months when pH has increased enough (Di Cagno & Gobetti 2007; Hill & Kethireddipalli 2013; Johnson 2001). Sometimes a gray/green *C. tyrobutyricum*-colony can be observed at the surface of an eye (Di Cagno & Gobetti 2007).

C. butyricum usually spoil the cheese during early ripening because it requires lactose as substrate. *C. sporogenes* spoils the cheese after about one year of ripening time because it use free amino acids as substrate (Di Cagno & Gobetti 2007).

Butyric acid creates an unpleasant fermented, acidic flavour of the cheese and creates a vomit-like smell (Featherstone 2008; Yang et al. 2013). Acetic acid is also produced by the Clostridia, but in smaller amounts; the ratio of butyrate and acetate is depending on pH (Yang et al. 2013; Zhao et al. 2013). Acetic acid causes a sour taste and sharp smell of the cheese (Yang et al. 2013).

Clostridial growth is affected by ripening temperature, initial spore count of the milk, pH, salinity, rate of salt penetration from brines and by types of starter- and adjunct cultures used (Ledenbach & Marshall 2010). Some cheeses require as few as one spore/ml milk to get spoiled by Clostridia (Ledenbach & Marshall 2010).

4.2 Heterofermentative lactic acid bacteria

Heterofermentative lactic acid bacteria can cause blowing defect by the production of CO_2, from the metabolism of residual lactose, galactose, amino acids and/or citrate present in the cheese (Ledenbach & Marschall 2010; Sheelan 2007). They can be hard to detect since LAB are inoculated to the cheese milk as starter cultures, often also as adjunct cultures. Insufficient fermentation by LAB starter culture favours spoilage.

Heterofermentative bacteria, e.g. *Streptococcus thermophilus*- and *Lactobacillus helveticus* strains can cause eyes and cracks in ripened cheeses. If cheese ripening is preceded at 15 ºC rather than 8 ºC, heterofermentative LAB will grow rather than homofermentative starter cultures (Banks 2007a; Johnson 2001; Ledenbach & Marschall 2010).

4.3 Propionic acid bacteria

Propionic acid bacteria (PAB) produce propionic acid, acetic acid, CO_2 and H_2O from the metabolism of lactic acid (Di Cagno & Gobbetti 2007). They can spoil very hard types of cheeses and Swiss-type cheeses by gas-, taste- and flavor defects (Di Cagno & Gobbetti 2007; Hill & Kethireddipalli 2013; Ledenbach & Marschall 2010; Sheelan 2007). Spoilage mainly occurs if the salt diffusion into the cheese and the rate of acidification is too slow (Bachmann & Frölich-Wyder 2007; Di Cagno & Gobbetti 2007; Düsterhöft & van den Berg 2007).

PAB are sometimes used as culture inoculant, e.g. *Propionibacterium freudenreichii* subsp. *shermanii* which is the bacteria behind the characteristic large holes of Swiss-type cheeses. PAB are very sensitive to salinity and such cheeses cannot be salted to a great extent, which pose a problem for the inhibition of spoilage bacteria (Bachmann & Frölich-Wyder 2007; Johnson 2001).

Some strains of PAB have high lactate utilization and aspartase activity (Bachmann & Frölich-Wyder 2007). If they spoil cheeses it might lead to excessive proteolysis and altered texture. During ripening decarboxylation of amino acids is initiated which increases production of CO_2. It can cause enlarged eyes with irregular shape and splits and cracks of the cheese (Bachmann & Frölich-Wyder 2007; Sheelan 2007).

4.4 Coliform bacteria

Coliform bacteria are a group of Gram-negative, lactose fermenting, aerobic- and facultative anaerobic bacteria, e.g. from genera Serratia, Aeromonas, Citrobacter, Escherichia and Enterobacter (Donnelly 2007). Some coliforms can cause off-flavours and early blowing defect by the production of CO_2 and H_2 via lactose metabolism, e.g. *Enterobacter aerogenes* (Alichanidis 2007; Hill & Kethireddipalli 2013).

Coliforms have historically been an important indicator organism for faecal contamination and sanitary of milk and their amount is often analysed in the milk (Hill & Kethireddipalli 2013).

Coliforms are inhibited by salinity and low pH caused by LAB fermentation during cheese making. If the fermentation is insufficient or the acid development by starter cultures is too slow, coliforms might grow rapidly since some of them are psychrotrophs (Hill & Kethireddipalli 2013; Johnson 2001; Ledenbach & Marschall 2010). Pasta-filata cheeses (e.g. mozzarella, provolone) produced from raw milk and washed curd cheeses are the most susceptible cheeses for spoilage by coliform bacteria, since coliforms are easily transferred from water of bad quality (Kindstedt et al. 2004; Ledenbach & Marschall 2010).

4.5 Yeasts

Yeasts are common cheese spoilage organisms e.g. Candida spp., *Kluyveromyces marxianus*, *Debaryomyces hansenii*, *Geotrichum candidum* and Pichia spp. (Johnsson 2001). They tolerate the low pH of cultured dairy products, like cheese, where most other microorganisms are inhibited. Many yeasts can grow at temperatures between 0 ºC to 37 ºC (Ledenbach & Marschall 2010; Thylin 2000).

Yeasts are frequently contaminating brine-salted cheeses since they can live in the brine and are favoured by the high nutrient and water content on the rind of cheeses (Ledenbach & Marschall 2010). Some species can cause early gas defect by the production of CO_2 via lactate/lactose metabolism (Sheelan 2007). Discoloration and fermented/yeasty off-flavours are often also produced (Hill & Kethireddipalli 2013).

Yeast spoilage often favour spoilage by other microorganisms because their degrading enzymes expose different nutrients (Ledenbach & Marschall 2010).

5 Methods for the prevention of blowing defect

5.1 Heat treatment

5.1.1 Pasteurization

Pasteurization is heat treatment applied to cheese milk in order to eliminate microorganisms, inactivate enzymes and partly denaturating whey proteins (Giffel & Wells-Bennik 2010; McSweeney 2007b). HTST (high temperature/short time) is the most common pasteurization method, usually used is 72 ºC for 15s (Heyndrickx et al. 2010; McSweeney 2007b). Pasteurization destroys the most heat-tolerant vegetative milk pathogens; *Coxiella burnettii* and *Mycobacterium tuberculosis*. Mold spores are also destroyed, but not endospores (De Jong 2008; Ledenbach & Marschall 2010).

Traditional cheese-making does not always include pasteurization because raw-milk cheese is considered to develop more intense flavour and sometimes the equipment is not afforded. More focus on hygiene and product safety are required for this kind of cheese-making (West 2008).

UHT (ultra high temperature), ISI (innovative steam injection) and sterilization are other heat treatment methods which inactivates spores, but they are not applied to cheese milk because of its negative effect on cheese making properties e.g. renneting (De Jong 2008; Heyndrickx et al. 2010; McSweeney 2007b; Skeie 2010).

ESL (extended shelf life) pasteurization is heat treatment combined with hygienic processing and microfiltration, and it is expected to be used more extensively in the future (Heyndrickx et al. 2010).

5.1.2 Alternative methods

Alternative methods to heat treatment have during many years been studied but none of these methods have yet had any breakthrough. Examples are high pressure processing, pulsed electric fields (PUF), UV-radiation, IR-processing, ohmic heating, electron beam radiation, microwave heating and high voltage treatment (De Jong 2008; Ledenbach & Marschall 2010). High pressure processing and PUF have the potential of pasteurizing products without lowering the sensory- and nutrional quality (Kelly 2007; Leadley 2008). However, spores require harsher treatment or combinations with other heat treatment to become significantly inactivated and the small quality differences of these methods will probably not overweigh the required equipment costs (De Jong 2008; Leadley 2008; McClements et al. 2001).

Some alternative methods might be used in the dairy industry in the future, but it will probably take some time as new treatments need to be validated, verified and tested to make sure that all processing criteria are achieved (Ledenbach & Marschall 2010).

5.2 Additives

5.2.1 Salt

Besides contributing to flavour, salt addition preserves cheeses from spoilage by lowering the effect of a_w (Guinee 2007). In ripened cheeses, salt to moisture content $\geq$ 3% inhibits Clostridial growth and late blowing defect as well as other unwanted microorganisms (Ledenbach & Marschall 2010). Brine-salting is applied to most cheese varieties e.g. Gouda and Edam (Guinee 2007). It often takes a few weeks before salt has diffused from the rind to the interior which can pose a problem since proper and rapid salting inhibit blowing defects (Di Cagno & Gobbetti 2007; Düsterhöft & van den Berg 2007). Dry-salting is often used additionally at the end of cheese making (Guinee 2007). Early gas defect by coliforms is in a simple and effective way inhibited by pre-salting the curd with 2% salt (Melilli et al. 2004).

5.2.2 Nitrate

Calcium formate and salts of nitric acid e.g. $NaNO_3$,KNO_3 are common additives and are used extensively in many parts of Europe. They are used to prevent both early and late gas defect and they become active when reduced to nitrites (Beresford 2007b; Sheelan 2007; Wilkinson 2005).

Nitrate has been added to cheeses since about year 1830 for the inhibition of butyric acid fermentation (Walstra et al. 1999). Usage is not allowed in some countries, e.g. USA (Johnson 2001).

Nitrates are often added to brine-salted cheeses because of their ability to inhibit Clostridia (Beresford 2007b; Walstra et al. 1999). However, it is not used in Swiss-type cheeses as PAB are also inhibited by nitrites (Beresford 2007b).

Formaldehyde addition inhibit butyric acid fermentation, but it is not permitted in most countries nowadays (Walstra et al. 1999).

5.2.3 Bacteriocins

Bacteriocins are antimicrobial substances produced by bacteria. Some LAB produce bacteriocins and they are classified as lantibiotics. Lantibiotics can be used for the inhibition of non-starter microorganisms. Reuterin produced by *Lactobacillus reuterin* added as culture adjunct was proven by Gómez-Torres et al. (2014) to be effective against late blowing defects. Nisin and lacticin produced by *L. lactis* are other bacteriocins that prevents late blowing defect as well as other spoilage- and pathogenic organisms effectively. New bacteriocin-producing bacteria are an up-to-date investigation area and might be added during cheese production in the future (Gómez-Torres et al. 2014; Hill & Kethireddipalli 2013).

5.2.4 Lysozyme

Lysozyme is often used as a substitute to nitrate- and bacteriocin usage in cheeses. It is an enzyme extracted from egg white, hence needed to be declared on the cheese package because of egg allergens (Schneider & Pischetsrieder 2013).

Lysozyme prevent Gram-positive bacteria like Clostridia. However, lysozyme is less effective compared to nitrate, and higher doses are required (Düsterhöft & van den Berg 2007; Schneider & Pischetsrieder 2013).

5.2.5 Culture adjuncts

LAB can be added to raw milk as a culture adjunct because it inhibit microorganisms effectively, especially important is the inhibition of psychrotrophic bacteria since they easily grow in cold milk (Ledenbach & Marschall 2010). Some strains of LAB are added to silage to prevent yeast deterioration (Thylin 2000).

5.2.6 Carbonation

Addition of CO_2 to milk- and dairy products often inhibit bacterial growth and carbonation has recently been an interesting area of investigation (Heyndrickx et al. 2010). Studies by Loss & Hotchkiss (2002) have proved that thermal survival rates of *B.cereus* spores and heat resistant lipases from *Pseudomonas flourecens* are reduced by carbonation during pasteurization.

Holding raw milk at CO_2-pressure ranging 68-689 kPa and at refrigerating temperatures reduce bacterial count significantly, without the precipitation of proteins (Rajagopal et al. 2005). However, acidic off-flavours of the milk can arise when adding CO_2 and spores are not always significantly affected (Ledenbach & Marschall 2010).

5.3 Membrane filtration and bactofugation

Membrane filtration has become an important method in the food industry and it is commonly applied before cheese-making (Skeie 2010). Membrane filtration is a separation method often based on size and/or charge and shape. The membrane lets through certain components and obstructs others (Grandison & Goulas 2008). "Bactocatching" is collection of the retentate (the filtrated part) which goes through UHT-treatment and is added to the cheese milk again (Skeie 2010).

5.3.1 Microfiltration

Heat treatment combined with microfiltration is the most common way of extending shelf-life of dairy products (De Jong 2008). Microfiltration removes bacteria and spores by membrane filtration ranging 1-10 µm in pore size. Molecular weight over 200 000 Da can be separated (Skeie 2010).

Ultra filtration (UF) is a filtration method which separates molecules with molecular weights ranging 1000 Da to 200000 Da with pore sizes of $10^{-4} – 10^{-3}$ µm (Skeie 2010). UF is most often used for its ability to increase cheese yield, improve gel-formation properties and facilitating uniform milk composition (Banks 2007b).

5.3.2 Bactofugation

Bactofugation extends shelf-life of cheeses by the removal of spores and bacterial cells; around 3% is discarded from the cheese milk by bactofugation at about 9000g (De Jong 2008; Ledenbach & Marschall 2010). Bactofugation is a good method for the removal of spores and at the same time avoiding usage of additives like nitrates (Johnson 2001). Several studies e.g. Daamen et al. (1986) have proven that 98% of spores are removed from the milk by bactofugation.

5.4 Hygiene

Good hygiene and closed production lines can prevent post-pasteurization contamination of cheese milk. Clean equipment is also an important factor since thermoduric bacteria can grow in equipment such as pasteurizers (Hill & Kethireddipalli 2013; Johnson 2001; Sheelan 2007). Spoilage by coliforms and heat resistant enzymes produced by Pseudomonas spp. most often comes from dirty equipment and bad sanitarily (Hill & Kethireddipalli 2013). Surrounding air, water, and personal contact are also factors that could lead to post-pasteurization contamination (Ledenbach & Marschall 2010).

Rapid and adequate cooling of milk is important. Roughly, shelf-life of milk is reduced by 50% for every 2 ºC increase of temperature (Beresford 2007a; Heyndrickx et al. 2010).

5.5 Prevention of late blowing defect

Late blowing by Clostridia and PAB most often occur when salting is insufficient and pH is high (Featherstone 2008). Clostridia can be inhibited by adding preservatives (e.g. nitrate salts, lysozyme, sorbic acid, phenolic antioxidants and polyphosphates) (Featherstone 2008), lowering pH and a_w, heat treatment, bactofugation, membrane filtration as well as addition of lantibiotics (Brüggemann & Gottchalk 2009). Some methods might be combined with other methods like high pressure, voltage discharges, UV light, radiation and laser in the future (Brüggemann & Gottchalk 2009). Hard Italian cheese-types are sometimes pre-ripened in order to prevent Clostridia (Hill & Kethireddipalli 2013). Cold pre-ripening temperatures are preferred for this purpose (Ledenbach & Marschall 2010).

5.5.1 Silage

Silage is the main contamination source of spores in cheese milk and Clostridia are most easily avoided by controlling the spore concentration of the silage (Barbosa-Cánovas & Bermúdez-Aguirre 2010; Heyndrickx et al. 2010; Klijn et al. 1995). Cheeses like Permigiano Reggiano and Emmental are not allowed to be produced from milk harvested from cattle fed with silage and it reduces the risk of spore contamination (Di Cagno & Gobbetti 2007; Heyndrickx et al. 2010).

Additives can be used in order to reduce the risk, e.g. water absorbents, sulphites, sorbic acid, propionic acid and their corresponding salts (Wilkinson 2005). Direct acidification has previously been important for the preservation of silage with formic acid as the most common additive (Wilkinson 2005).

Crops with low nitrogen fertilization and/or low initial level of nitrate are more likely to be contaminated with Clostridia (Wilkinson 2005). In the future, harvested crops might be sterilized before fed to cattle (Wilkinson 2005).

6 Dairy contacts

Several dairy companies were contacted and asked to reply to eleven questions regarding blowing defects at their cheese manufacturing. The purpose was to get an idea about the extent in the dairy industry today and how the problem is handled.

Five Swedish dairies replied, including Arla (Götene mejeri and Boxholms mejeri), Norrmejerier, Skånemejerier and Gäsene mejeri.

Several dairies in other parts of Europe have been contacted as a complement to the Swedish large-scale cheese-making. Two replied, both manufacturing traditional Italian cheeses with extensive ripening.

Answers from the dairy industries are compiled for each question (Appendix).

6.1 Summary of answers

Blowing defect is not an extensive problem at these dairy industries. However, different methods and techniques are required for the prevention. Many dairies experience spoilage by blowing defect occasionally and/or have had more problems previously. If spoilage would occur it would lead to major production losses. (Andersson[1]; reference contact[2]; Palmkvist[3]; Edlund[4]; reference contact[5]; Bertozzo[6]; Mezzadri[7])

Butyric acid fermentation is the main causing agent of blowing defect. In Sweden, blowing defect of Grevé represent a big problem, caused by propionic acid bacteria and interaction with other cultures (Andersson[1], reference contact[2]).

For the removal of spores, Götene mejeri and Boxholms mejeri use bactofugation while other Arla dairies (Kalmar and Östersund) use microfiltration (Andersson[1], Palmkvist[3]). Skånemejerier use filtration as well, also Norrmejerier with a “Bactocatch” (Edlund[4]; Palmkvist[3]).

Sodium nitrate is used instead of filtration at Gäsene mejeri (reference contact[5]). Boxholms mejeri also use sodium nitrate, except for their KRAV-cheese (Palmkvist[3]). KRAV-cheeses are not allowed to use sodium nitrate, hence are more sensitive to spoilage since bactofugation does not always remove enough spores for the prevention of spoilage (Palmkvist[3]; Reference contact[5]).

[1] Fredrik Andersson, Arla-Götene mejeri
[2] Reference contact, Skånemejerier
[3] Tomas Palmkvist, Arla-Boxholms mejeri
[4] Agneta Edlund, Norrmejerier
[5] Reference contact, Gäsene mejeri
[6] Silvia Bertozzo, Latteria sociale mantova
[7] Stefano Mezzadri, Fratelli Mezzadri Zoocasearia

Most dairies does not experience seasonal problematics with spore contamination but winter season may have higher risk of contamination.

Cheeses affected by blowing defect are either discarded, sold as a cheaper varieties or sent to melting plants in Denmark (Andersson[1]; Reference contact[2]; Palmkvist[3]; Edlund[4]; Reference contact[5]; Bertozzo[6]; Mezzadri[7]).

Lysozyme is used as additive in the Grana Padano cheese production. Parmigiano Reggiano is produced without additives and cows are fed non-fermented food, which reduces the risk of contamination of spores (Bertozzo[6]; Mezzadri[7]).

All dairies have different ways of controlling the cheese-making process for spoilage and abnormalities during production. Spores, cell count and amount of coliforms are common control methods, both before and after milk treatment. (Andersson[1]; Bertozzo[6]; Edlund[4]; Palmkvist[3])

7 Discussion

Blowing defect of cheeses does not represent a major problem in the dairy industry today but advanced methods and routines are needed for the prevention and deficiencies during these steps easily arises. Blowing defect can arise if environmental conditions are suitable and initial amount of causing microorganisms are sufficient.

Pasteurization of cheese milk is the most suitable method for the prevention of non-sporulating, vegetative microorganisms. Membrane filtration and bactofugation are good and effective methods for the prevention of endospore-forming bacteria. Additives like sodium nitrate and lysozyme are good substitutes for this purpose.

Other important parameters include sufficient salting and rapid salt diffusion into the cheese, quick and adequate pH-reduction of the curd, suitable temperature- and moisture conditions during ripening, dominating culture inoculants and fast- and adequate temperature reduction of cheese milk.

Hygiene conditions and sanitary handling are important factors to consider, both from personal handling as well as farm environment and dairy equipment. Closed production lines and clean equipment can prevent post-pasteurization contamination. Amount of spores, SCC and coliforms in the cheese milk need to be analysed during production since milk quality has a direct impact on final cheese quality. Proper silage and feeding of the cattle prevent endospores from reaching the milk at the first place.

High pressure processing and pulsed electric fields are examples of methods that could be used instead of HTST, but spores and enzymes are not significantly inactivated. Combinations of new and conventional techniques could possibly be used in the future for the prevention of endospore-forming bacteria (De Jong 2008). Spores are needed to be inactivated by the processing, but cheese making properties are easily altered by these techniques, which poses a problem. Further development of techniques are needed and requires properly testing to solve this problem.

Cheese production without blowing defect remains a challenge in the industry but it can be prevented by using different methods and techniques existing today.

8 Appendix

8.1 Dairy industry contacts

Table 1. Seven dairy industries that have answered to eleven questions regarding the magnitude of blowing defect at their factories, as well as their cheese products.

	Dairy industry	Cheese products
1	**Arla – Götene mejeri** [1]	Wästgöta kloster, Billinge, Riddarost etc. [1]
2	**Skånemejerier** [2]	Hard cheeses e.g. Allerum cheeses, Grevé, Prästost, cheddar etc. [2]
3	**Norrmejerier** [3]	Hard cheeses e.g. Grevé, Herrgård, Prästost [3]
4	**Arla – Boxholms mejeri** [4]	Hard cheeses, e.g. Boxholms gräddost, Boxholms ekologiska [4]
5	**Gäsene mejeri** [5]	Ripened hard cheese e.g. Gäsene special, bondmoran, minimunk, hushållsost etc. [5]
6	**Latteria Sociale Mantova** [6]	Grana Padano [6]
7	**Fratelli Mezzadri Zoocasearia** [7]	Parmigiano Reggiano [7]
	1(Fredrik Andersson, Götene mejeri) 2(Reference contact, Skånemejerier) 3(Agneta Edlund, Norrmejerier) 4(Thomas Palmkvist, Boxholms mejeri) 5(Reference contact, Gäsene mejeri) 6(Silvia Bertozzo, Latteria Sociale Mantova) 7(Stefano Mezzadri, Fratelli Mezzadri Zoocasearia)	1(Arla n.d.) 2(Skånemejerier n.d.) 3(Norrmejerier n.d.) 4(Arla n.d.) 5(Gäsene mejeri) 6(Latteria Sociale Mantova n.d.) 7(Fratelli Mezzadri Zoocasearia n.d.)

8.1.1 Questions

Table 2.

1.	How extensive is the problem with spoilage of cheeses due to bacterial fermentation at your dairy factory?	
1	**Arla- Götene mejeri**	Butyric acid fermentation does not occur. Occasionally spores makes it through the milk treatment. It can appear like a green dot on the cheese and provide with additional gas locally in the cheese
2	**Skånemejerier**	In the current situation, it is not a problem
3	**Norrmejerier**	Not a big problem since the cheese milk is filtrated for the removal of spores
4	**Arla – Boxholms mejeri**	It is not a major problem for us at the moment, but 5-10 years back, we had more problems with it.
5	**Gäsene mejeri**	It depends on the quality of the milk
6	**Latteria Sociale Mantova**	Spoilage of cheese is related to the condition and quality of milk; late blowing is not so extensive in our Grana Padano cheese
7	**Fratelli Mezza-dri Zoocasearia**	Negligible amount, the problem occurs occasionally

Table 3.

2.	How much production losses does it lead to?	
1	**Arla – Götene mejeri**	None. If butyric acid fermentation was to spoil the cheeses it would result in major production losses. We produce about 50 ton cheese/day which would result in an economical loss of approxi-mately 1 250 000 Sek.
2	**Skånemejerier**	None
3	**Norrmejerier**	Negligible amount
4	**Arla – Boxholms mejeri**	When we had problems, it could lead to losses of about 1-2% of the yield
5	**Gäsene mejeri**	2-3 curdlings a year (3000 kg)
6	**Latteria Sociale Mantova**	Between 25-30%
7	**Fratelli Mezza-dri Zoocasearia**	Negligible amount

Table 4.

3. How much hard cheese do you produce in a year (approximately)?		
1	**Arla Götene mejeri**	18 500 ton/year
2	**Skånemejerier**	Around 13.000 ton
3	**Norrmejerier**	Around 8500 ton
4	**Arla – Boxholms mejeri**	Around 1700 ton
5	**Gäsene mejeri**	
6	**Latteria Sociale Mantova**	150.000 wheels
7	**Fratelli Mezza-dri Zoocasearia**	5000 forms of parmesan

Table 5.

4. How do you handle cheeses that has been spoiled due to bacterial fermentation? Do you discard them or sell them as a cheaper variety of a cheese with lower quality?		
1	**Arla – Götene mejeri**	It depends on how big the problem is. If the fermentation is extensive it would not be sold as a cheaper variety, but sold to customers that can handle the cheese
2	**Skånemejerier**	It depends on the degree of spoilage. If the defects are few they can be sold as cheaper class 2 cheese
3	**Norrmejerier**	They are discarded
4	**Arla – Boxholms mejeri**	It depends on the degree of spoilage of the cheese. Usually we sell it in our shop as a second-rate cheese at a lower price (a lot of people like the fermented taste). Really bad cheese is sent to plants in Denmark where it is melted
5	**Gäsene mejeri**	They are sent to Denmark for melting
6	**Latteria Sociale Mantova**	Yes, we sell them as a cheaper variety of a cheese with lower quality
7	**Fratelli Mezza-dri Zoocasearia**	We sell the cheese that has been spoiled due to bacterial fermentation as a cheaper variety with lower quality

Table 6.

5.	**Which are the major bacteria that represents risk of spoiled cheese due to fermentation at your company?**	
1	**Arla – Götene mejeri**	The biggest problem with blowing defect in Sweden right now is spoiled Grevé-cheese by propionic acid bacteria and interaction with other cultures
2	**Skånemejerier**	Butyric acid bacteria
3	**Norrmejerier**	Spores from Clostridia
4	**Arla – Boxholms mejeri**	Butyric acid bacteria
5	**Gäsene mejeri**	Spores
6	**Latteria Sociale Mantova**	Between heterofermentative bacteria, the most common are the propionic acid bacteria
7	**Fratelli Mezzadri Zoocasearia**	The main cause of cheese spoilage is the butyric acid fermentation

Table 7.

6.	**How is the milk controlled before the cheese production begins? For example cell count/different bacteria/ spores.**	
1	**Arla – Götene mejeri**	Spores are analysed in silo and after milk treatment. We follow a control program, the amount of spores use to be around <4/1000 ml after milk treatment. Coliforms are also analysed during the cheese making.
2	**Skånemejerier**	Skim milk is ultrafugated and cream is sterilized in order to minimize the risk of contamination by butyric acid bacteria
3	**Norrmejerier**	Cell counts are analysed at the farm. Total number of bacteria is analysed in the milk from silos. Total number of bacteria and amount of spores are analysed from the filtered milk. Post-pasteurization contamination is controlled by analysing the amount of Enterobacteria.
4	**Arla – Boxholms mejeri**	We analyse total number of Enterobacteriaceae from incoming milk. Organic milk is analysed for the amount of spores
5	**Gäsene mejeri**	Antibiotics, fat content
6	**Latteria Sociale Mantova**	Fat, protein, lactose, RSM, spores inhibitory, somatic cells, bacterial load, cryoscopy, casein, urea, geomaverages, TBC and amount of cells
7	**Fratelli Mezzadri Zoocasearia**	

Table 8.

7. Are specific methods applied in order to remove and/or prevent unwanted bacteria that causes fermented cheese?		
1	**Arla – Götene mejeri**	Yes, we use Götene bactofugation x 3. The plants in Kalmar and Östersund use microfiltration
2	**Skånemejerier**	Filtration
3	**Norrmejerier**	Pasteurization. The milk is filtrated (Bactocatch) before cheese making for the removal of spores
4	**Arla – Boxholms mejeri**	Sodium nitrate is added to the conventional milk in order to prevent butyric acid fermentation. Sodium nitrate is not allowed in our KRAV-cheese making, in this milk bactofugation is used for the removal of spores
5	**Gäsene mejeri**	Sodium nitrate
6	**Latteria Sociale Mantova**	For the Grana padano production we use Lysozyme; other fundamental conditions are hygienic conditions of stables and tanks for milk
7	**Fratelli Mezzadri Zoocasearia**	Additives are not used. Cows are fed non-fermented food

Table 9.

8. Are any cheeses/methods in particular sensitive to fermentation spoilage?		
1	**Arla – Götene mejeri**	Yes, propionic acid cheeses (Grevé types) are sensitive since this type of bacteria thrive in the same environment as Clostridia; slightly higher pH, lower salt content etc.
2	**Skånemejerier**	Grevé and cheeses with low fat content
3	**Norrmejerier**	No
4	**Arla – Boxholms mejeri**	KRAV-cheeses are more sensitive to spoilage since sodium nitrate is not added. Bactofugation does not always remove enough spores
5	**Gäsene mejeri**	Fat cheese
6	**Latteria Sociale Mantova**	
7	**Fratelli Mezzadri Zoocasearia**	Fat content is reduced by natural separation which reduces microbial contamination

Table 10.

9.	Does the problem vary during different times of the year (Winter/summer)?	
1	**Arla – Götene mejeri**	Previously the problem was greater during winter time but nowadays spores occur at summer too. The problem often stems from poorly silage. Cows are normally fed with silage during summer too, also in the cowshed where they are milked
2	**Skånemejerier**	Late winter to early spring is the most susceptible period
3	**Norrmejerier**	Since we filtrate the cheese milk we are free from seasonal variations
4	**Arla – Boxholms mejeri**	A few years ago problems occurred after Christmas and onwards. Nowadays quality is quite good all year around
5	**Gäsene mejeri**	It usually depends on how the harvest was and how the farmer handled the fodder
6	**Latteria Sociale Mantova**	The problem is not seasonal, is all the year
7	**Fratelli Mezzadri Zoocasearia**	The defect is not strictly related to seasonal periods, even though in winter it may be easier to obtain further contamination of spores, because the animals may be dirtier. For this reason poor milking could give rise to a higher contamination

Table 11.

10.	How do you control the process of the cheese in order to prevent fermentation spoilage?	
1	**Arla – Götene mejeri**	Control programs
2	**Skånemejerier**	Control of bacteria in the filtration equipment
3	**Norrmejerier**	Filtration of the cheese milk and analyses of the filtrated milk
4	**Arla – Boxholms mejeri**	By adding sodium nitrate and bactofugation
5	**Gäsene mejeri**	Bactocatch or bactofugation, but this does not provide with complete spore reduction. We don’t use these methods
6	**Latteria Sociale Mantova**	Good raw material is the main thing. Hygienic conditions during cheese production are also important
7	**Fratelli Mezzadri Zoocasearia**	

Table 12.

11. Are any controlling systems applied in order to discover abnormalities of the cheese quality?		
1	**Arla – Götene mejeri**	Yes, we control incoming milk, treated milk, control the cheese after ripening as well as final classification. We have specific customers which have demands regarding analysis of both anaerobic- and aerobic spores. Working with the farms and control programs for the production is important in order to get high quality milk all the time
2	**Skånemejerier**	Control of bacteria in the filtration equipment
3	**Norrmejerier**	Yes, sensory- and visual inspections are preformed to detect abnormalities like swollen cheeses
4	**Arla – Boxholms mejeri**	Each batch is analysed for microorganisms. Sensory and visual controls are also preceded
5	**Gäsene mejeri**	Each curdling tasted before delivery
6	**Latteria Sociale Mantova**	Normally we use X ray to check the cheese after 4-5 months of ripening, also “traditional manner ” with hammer after 6 months
7	**Fratelli Mezzadri Zoocasearia**	The defect shows at the beginning with the beating of the form, increase of the seasoning can be evaluated at the sight

References

Adams, M.R. & Moss, M. O. (2008). *Food microbiology*. 3. ed. Cambridge: Royal Society of Chemistry publishing, pp 123, 332-336

Alichanidis, E. (2007). Cheeses ripened in brine. |: McSweeney, P. L. H. (Ed). *Cheese problems solved*. Cambridge: Woodhead publishing limited, pp 330-342

Bachmann, H.P. & Frölich-Wyder, M.T. (2007). Swiss-cheese.|: McSweeney, P. L. H. (Ed). *Cheese problems solved*. Cambridge: Woodhead publishing limited, pp 246-267

Banks, J.M. (2007a). Analysis of cheese: How reliable is cheese grading? |: McSweeney, P. L. H. (Ed). *Cheese problems solved*. Cambridge: Woodhead publishing limited, pp 174-175

Banks, J.M. (2007b). Ultrafiltration of cheese milk |: McSweeney, P. L. H. (Ed). *Cheese problems solved*. Cambridge: Woodhead publishing limited, pp 30-33

Barbosa-Cánovas, G. & Bermúdez-Aguirre, D. (2010). Other novel milk preservation technologies: ultrasound, irradiation, microwave, radio frequency, ohmic heating, ultraviolet light and bacteriocins. |: Griffiths, M. W. (Ed). *Improving the safety and quality of milk. Vol 1, Milk production and processing.* Cambridge: Woodhead Publishing limited, pp 420-450

Beresford, T. (2007a). Preparation of cheese milk: What problems are caused by psychrotrophs? |: McSweeney, P. L. H. (Ed). *Cheese problems solved*. Cambridge: Woodhead publishing limited, pp 13-15

Beresford, T. (2007b). The microbiology of cheese ripening: What factors affect microbial growth in cheese? |: McSweeney, P. L. H. (Ed). *Cheese problems solved*. Cambridge: Woodhead publishing limited, pp 119-123

Boxholm ost. *Våra ostar.* http://www.boxholmsost.se/vara-ostar/vara-ostar.php?lmId=6 [Accessed 2015-06-06]

Brüggemann, H. & Gottschalk, G. (Eds) (2009). *Clostridia: Molecular Biology in the Post-genomic Era.* Göttingen: Caister Academic Press, pp 1, 29, 216

Burgess, K. (2010). Key requirements for milk quality and safety; a processor's perspective. |: Griffiths, M. W. (Ed). *Improving the safety and quality of milk. Vol 1, Milk production and processing.* Cambridge: Woodhead Publishing limited, pp 65-84

Cocolin, L., Innocente, N., Biasutti, M. & Comi, G. (2004). The late blowing in cheese: a new molecular approach based on PCR and DGGE to study the microbial ecology of the alteration process. *International Journal of Food Microbiology*, 90(1), pp 83–91

Coultate, T. P. (2009). *Food : the chemistry of its components*. 5. Ed. Cambridge: Royal Society of Chemistry Publishing, p 183

Daamen, C. B. G., Berg, G. van den & Stadhouders, J. (1986). Test of the bactofugation efficiency of a self-cleaning hermetic bactofuge, Type BMRPX 618 HGV, manufactured by Alfa-Laval A/B, Lund (Sweden). *NIZO-Rapporten*, (R124), p 19.

De Jong, P. (2008). Thermal processing of milk. |: Britz, T. J. & Robinson, R. K. (Eds). *Advanced dairy science and technology*. Oxford: Blackwell Publishing, pp 1-31

Di Cagno, R. & Gobbetti, M. (2007). Grana-type cheeses and parmesan: What common problems are associated with Grana-type cheeses? |: McSweeney, P. L. H. (Ed). *Cheese problems solved*. Cambridge: Woodhead publishing limited, pp 210-211

Donnelly, C.W. (2007). Pathogens and food poisoning bacteria: What factors should be considered to reduce coliform counts?|: McSweeney, P. L. H. (Ed). *Cheese problems solved*. Cambridge: Woodhead publishing limited, pp 143-144

Düsterhöft. E.-M. & van den Berg. G. (2007). Dutch-type cheeses.|: McSweeney, P. L. H. (Ed). *Cheese problems solved*. Cambridge: Woodhead publishing limited, pp 230-245

Eskin, N. A. M. & Shahidi, F. (Eds) (2013). *Biochemistry of foods [elektronisk resurs]* [online]. London: Academic Press. Available from: http://www.sciencedirect.com/science/book/9780122423529. [Accessed 2015-05-14]

Featherstone, S. (2008). Control of Biodeterioration in Food. |: Tucker, G. (Ed) *Food biodeterioration and preservation*. Oxford: Blackwell Publishing, pp 1-35

Fratelli Mezzadri Zoocasearia. *Our products.* http://www.zoocaseariamezzadri.it/eng/Prodotti.htm [Accessed 2015-06-06]

Giffel, M.C. & Wells-Bennik, M.H.J. (2010). Good hygienic practice in milk production and processing. |: Griffiths, M. W. (Ed). *Improving the safety and quality of milk. Vol 1, Milk production and processing*. Cambridge: Woodhead Publishing limited, pp 179-193

Gómez-Torres, N., Ávila, M., Gaya, P. & Garde, S. (2014). Prevention of late blowing defect by reuterin produced in cheese by a Lactobacillus reuteri adjunct. *Food Microbiology*, 42, pp 82–88

Grandison, A.S. & Goulas, A. (2008). Applications of membrane separation |: Britz, T. J. & Robinson, R. K. (Eds). *Advanced dairy science and technology*. Oxford: Blackwell Publishing, pp 35-74

Guinee, T.P. (2007). Salt in cheese |: McSweeney, P. L. H. (Ed). *Cheese problems solved*. Cambridge: Woodhead publishing limited, pp 80-99

Gäsene mejeri. *Våra ostar.* http://www.gasenemejeri.se/vara-ostar [Accessed 2015-06-06]

Götene mejeri. http://www.arla.se/om-arla/fakta/mejerier/gotene-mejeri/ [Accessed 2015-06-06]

Heyndrickx, M., Marchand, S., De Jonghe, V., Smet, K., Coudijzer, K. & De Block, J. (2010). Understanding and preventing consumer milk microbial spoilage and chemical deterioration. |: Griffiths, M. W. (Ed). *Improving the safety and quality of milk. Vol 2, Improving quality in milk products.* Cambridge: Woodhead Publishing limited, pp 97-135

Hill. A.R. & Kethireddipalli. P. (2013). Dairy products: cheese and yoghurt. |: Eskin, N. A. M. & Shahidi, F. (Eds). *Biochemistry of foods* [online]. London: Academic Press, pp 319-351). Available from: http://www.sciencedirect.com/science/book/9780122423529. [Accessed 2015-06-04]

Johnson, M.E. (2001). Cheese Products. |: Marth, E. H. & Steele, J. (Eds). *Applied Dairy Microbiology, 2. ed.* New York: CRC Press, pp 345-385

Kelly, A.L. (2007) New technologies |: McSweeney, P. L. H. (Ed). *Cheese problems solved*. Cambridge: Woodhead publishing limited, pp 115-116

Kindstedt, P., Caric, M. & Milanovic, S. (2004). Pasta-Filata Cheeses. |: Cogan, T. M. Fox, P. F., Guinee, T. P. & McSweeney, P. L. H. (Eds). *Cheese: Chemistry, Physics and Microbiology. Vol 2, Major cheese groups.* 3. Ed [online]. Academic Press, pp 251-263. Available from: Google Books. [Accessed 2015-06-04]

Klijn, N., Nieuwenhof, F., Hollwerf, J., Vanderwaals, C. & Weerkamp, A. (1995). Identification of Clostridium tyrobutyricum as the Causative Agent of Late Blowing in Cheese by Species-Specific PCR-Amplification. *Applied and Environmental Microbiology*, 61(8), pp 2919–2924

Kristianstadmejeriet. http://www.skanemejerier.se/sv/Kontakta-oss/Vara-anlaggningar/Kristianstadmejeriet/ [Accessed 2015-06-06]

Latteria Sociale Mantova. *Prodotti.* http://www.lsm427.it/it/prodotti.php [Accessed 2015-06-06]

Leadley, C. (2008). Novel commercial preservation methods. |: Tucker, G. (Ed) *Food biodeterioration and preservation*. Oxford: Blackwell Publishing, pp 212-242

Ledenbach, L. H. & Marshall, R. T. (2010). Microbiological Spoilage of Dairy Products. |: Sperber, W. H. & Doyle, M. P. (Eds). *Compendium of the Microbiological Spoilage of Foods and Beverages*. [online]. New York: Springer New York, pp 41-67. Available from: http://link.springer.com/10.1007/978-1-4419-0826-1_2. [Accessed 2015-06-04]

Loss, C. R. & Hotchkiss, J. H. (2002). Effect of dissolved carbon dioxide on thermal inactivation of microorganisms in milk. *Journal of Food Protection*, 65(12), pp 1924–1929

McClements, J.M.J., Patterson, M. F. & Linton, M. (2001). The effect of growth stage and growth temperature on high hydrostatic pressure inactivation of some psychrotrophic bacteria in milk. *Journal of Food Protection*, 64(4), pp 514–522

McSweeney, P.L.H. (2007a). Analysis of cheese: What is the correct way to sample cheese for analysis? |: McSweeney, P. L. H. (Ed). *Cheese problems solved*. Cambridge: Woodhead publishing limited, pp 167-169

McSweeney, P.L.H. (2007b). Preparation of cheese milk: What effects does pasteurisation have on cheesemilk? |: McSweeney, P. L. H. (Ed). *Cheese problems solved*. Cambridge: Woodhead publishing limited, pp 22-23

Melilli, C., Caccamo, D. M., Calvo, M., Schembari, M. A., Barbano, G. & Licitra, G. (2004). Influence of brine concentration, brine temperature, and pre-salting on early gas defects in raw milk pasta-filata cheese. *Journal of Dairy Science*, 87(11), pp 3648–3657

Norrmejerier. *Här finns vi.* http://www.norrmejerier.se/produktkatalog/norrmejerier-ost [Accessed 2015-06-06]
Våra goda produkter. http://www.norrmejerier.se/produktkatalog [Accessed 2015-06-06]

Rajagopal, M., Werner, B. G. & Hotchkiss, J. H. (2005). Low pressure CO_2 storage of raw milk: Microbiological effects. *Journal of Dairy Science*, 88(9), pp 3130–3138

Schneider, N. & Pischetsrieder, M. (2013). Lysozyme allergen in cheese and potential impact on health. |: Preedy, V. R., Watson, R.R. & Patel, V.B. &. (Eds) (2013). *Handbook of cheese in health: production, nutrition and medical sciences*. 2013. ed Wageningen: Wageningen Academic Publishers, pp 767-781

Sheelan. J. J. (2007). The microbiology of cheese ripening: What causes the development of gas during ripening? |: McSweeney, P. L. H. (Ed). *Cheese problems solved*. Cambridge: Woodhead publishing limited, pp 131-132

Skeie, S. (2010). Milk quality requirements for cheesemaking. |: Griffiths, M. W. (Ed). *Improving the safety and quality of milk. Vol 2, Improving quality in milk products.* Cambridge: Woodhead Publishing limited, pp 433-453

Stessl, B. & Hein, I. (2010). Identifying pathogens in milk.|: Griffiths, M. W. (Ed). *Improving the safety and quality of milk. Vol 1, Milk production and processing.* Cambridge: Woodhead Publishing limited, pp 87-112

Thylin, I. (2000). *Methods of preventing growth of Clostridium tyrobutyricum and yeasts in silage*. Diss. Uppsala: Swedish university of agricultural sciences, pp 15, 17.

Walstra, A., van Boekel, M. A. J. S. J., Noomen, A. & Geurts, T. J. (Eds). (1999). *Dairy Technology : Principles of Milk Properties and Processes*. New York: Marcel Dekker Incorporate, p 643

Weller, R. (2010). Improving organic milk. |: Griffiths, M. W. (Ed). *Improving the safety and quality of milk. Vol 2, Improving quality in milk products.* Cambridge: Woodhead Publishing limited, pp 284-303

West, H. G. (2008). Food fears and raw-milk cheese. *Appetite*, 51(1), pp 25–29.

Wilkinson, J. M. (2005). *Silage*. Lincoln: Chalcombe Publications, pp 18, 30, 85-86, 94-95, 107

Willey, J.M., Sherwood, L.M. & Woolverton, C.J. (2012). *Prescott's principles of microbiology*. International edition. Singapore: McGraw-Hill Higher Education, pp 60, 824-825

Yang, S.-T., Yu, M. Chang, W.-L. & Tang, I.-C (2013). Anaerobic fermentations for the production of acetic and butyric acids. |: Yang, S.-T., El Enshasy, H. & Thongchul, N. (Eds). *Bioprocessing technologies in biorefinery for sustainable production of fuels, chemicals, and polymers*. [online]. Hoboken: John Wiley & Sons Inc., pp 351-374. Available from: Ebrary [Accessed 2015-06-04]

Zhao. J., Lu, C., Chen, C.-C. & Yang, S.-T. (2013). Biological production of butanol and higher alcohols.|: Yang, S.-T., El Enshasy, H. & Thongchul, N. (Eds). *Bioprocessing technologies in biorefinery for sustainable production of fuels, chemicals, and polymers*. [online]. Hoboken: John Wiley & Sons Inc., pp 235-261. Available from: Ebrary [Accessed 2015-06-04]