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Microorganisms influence on quality and flavor of cheese

Mikroorganismers betydelse för ostens kvalitet och smak

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Abstract

For this study information about microorganism in cheese and their effect on the final product was gained from science databases, e.g. Science Direct, Web of Science and Prima. The taxonomic structures of microbial communities in cheese can be studied by culture-dependent and culture independent methods. Some problems with production of cheese are early and late gas formation and growth of pathogens. Although these problems can be counteracted by sanitation, adding nitrate, microfiltration, bactofugation, lower water activity and by lowering the pH, they can give the cheese inferior quality. Biological hazards can be avoided by good sanitation. For a good shelf-life cheese, techniques such as fermentation and pasteurization are important. Lactic acid bacteria are very important for flavor development and toxin-producing bacteria can in combination with resistant bacteria be used to control flavor development.

Keywords: cheese, lactic acid bacteria, milk, microorganisms, flavor

Sammanfattning

För denna studie har information om mikroorganismer i ost och deras inverkan på produkten tagits från vetenskapliga databaser, bl.a. Science Direct, Web of Science och Prima. De taxonomiska strukturerna i mikrobiella samhällen i ost kan studeras med hjälp av kulturberoende och kulturoberoende metoder. Några problem i produktionen av ost är tidig och sen gasbildning och tillväxt av patogener. Trots att problemen kan motverkas genom god sanitet, tillförsel av nitrat, baktofugering, tillsats av salt och genom att sänka pH-värdet, kan osten få en sämre kvalitet. Biologiska risker kan undvikas genom god hygien. För en bra hållbarhet i ost är tekniker såsom fermentering och pastörisering viktiga. Mjölksyrebakterier är mycket viktiga för smakutveckling och toxinproducerande bakterier kan användas tillsammans med resistenta bakterier för att styra smakutveckling.

Nyckelord: ost, mjölksyrebakterier, mjölk, mikroorganismer, smaker

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Acronyms and abbreviations

CFU	Colony-forming unit
Cl.	Clostridium
DGGE	Denaturing gradient gel electrophoresis
FHL	Facultatively heterofermentative lactobacilli
FISH	Fluorescence in situ hybridization
LAB	Lactic acid bacteria
Lb.	Lactobacillus
NSLAB	Non-starter Lactic acid bacteria
PAB	Propionic acid bacteria
PCR	Polymerase chain reaction
PFGE	Pulsed-field gel electrophoresis
RAPD	Randomly amplified polymorphic DNA
RFLP	Restriction fragment length polymorphism
SSCP	Single-stranded conformation polymorphism
TTGE	Temporal gradient gel electrophoresis
UHPH	Ultra-high pressure homogenization
UHT	Ultra-high temperature processing

1 Introduction

1.1 Background

Cheese is believed to have been discovered in the Fertile Crescent about 8,000 years ago by a traveller who had some milk with him in a pouch made of a sheep's stomach. Milk, which is the main ingredient in cheese making, is a highly nutritious food containing carbohydrates, fats, proteins, vitamins, minerals and essential amino acids. It has a neutral pH and a high water activity, which makes it an ideal place for both beneficial and non-beneficial microorganisms (Quigley *et al.*, 2013).

1.2 Cheese making

There are different ways to make cheese depending on the type of cheese in question. Cheeses can broadly be categorized into different groups, acid or rennet cheese and natural or processed cheese. In acid cheese, acid is added to the milk to make it to coagulate. Some examples of acid cheeses are cream cheese and queso fresco. In most types of cheese coagulation is achieved by rennet enzyme together with a starter culture; examples are Cheddar and Swiss cheese. The term “natural cheese” is mostly used in the industry referring to cheeses that are directly made from milk. “Processed cheese” is made using natural cheese together with other ingredients to change the texture and/or melting properties and increase shelf life of the cheese (Tamime, 2006).

In a cheese making process the first step is to standardize the milk to optimize the protein to fat ratio to control quality and yield. The milk is then pasteurized to kill spoilage microorganisms and pathogens to get a good environment for the starter culture which is added after the milk has cooled down and reached a suitable temperature for the starter culture. The starter culture produces lactate and gives the cheese its specific characteristics. Non-starter adjunct culture containing mold or bacteria are sometimes added to enhance flavor development in the cheese. For coagulation rennet, containing chymosin, is added, which converts kappa-casein to para-kappa-casein forming the curd whereby glycomacroprotein is lost in the whey. To accelerate the expulsion of whey from the curd, called

syneresis, the cheese is cut into smaller pieces and heated. After the whey has been removed and the cheese has reached the desired pH, the cheese is salted by adding salt to the surface or putting the curd into a salt water solution called brine. The salted cheese is then stored for maturing depending on the kind of cheese that is desired (Tamime, 2006).

1.3 Objective

The objective of this literature study was to find out factors contributing to the variation in quality among cheeses, focusing on the impact of microorganisms. Microorganisms that have been considered in this article include lactic acid bacteria, enterococci, micrococci, staphylococci, coryneforms, propionic acid bacteria, yeasts and molds because of their contribution in cheese manufacturing.

2 Method

The emphasis of data has been gathered from different scientific articles found by using Google search-engine and science databases: Web of Science, Science Direct and Primo. Some search-words used were microorganisms, flavor and cheese. Also the book Dairy Starter Cultures by Cogan, T.M. and J.P. Accolas (1996) was used as a reference.

3 Microorganisms in cheese

3.1 Use of starter culture in cheese making

A starter culture contains lactic acid bacteria (LAB), which have been studied since the sixties and are a relatively heterogeneous group of gram-positive cocci, coccibacilli, and bacilli. LAB have a broad range of ecological niches, low GC content (guacine+cytosine), high acid tolerance, high aerotolerant but not aerobic, unable to synthesize porphyrins and, are strictly fermentative with lactic acid as the major metabolic end product. They are the most dominant microbial population prior to pasteurization and play an important role in making fermented foods (Broadbent & Steele, 2005). Fermentation is an important preservation technique that has widely been practiced since ancient times. It not only increases the shelf life and microbiological safety of the food but also makes the food more digestible (Caplice & Fitzgerald, 1999). Originally the starter culture was a mixture of many undefined microbes but the daily propagation in industries has led to the disappearance of certain strains in the ecosystem and has led to a loss of uniqueness in the cheese (Caplice & Fitzgerald, 1999). Because of this, interest has increased to isolate wild-type strains from traditional cheeses (Beukes *et al.*, 2001; Hébert *et al.*, 2000; Vuyst *et al.*, 2002). Some of the most common LAB used in cheese production are *Lactobacillus delbrueckii* subsp. *L. bulgaricus, delbrueckii* subsp. *lactis* and *L. helveticus*, often used in combination with *Streptococcus thermophilus* for its ability to develop folic and formic acids which are used for purine synthesis (Angelis & Gobbetti, 2011). Today, starter cultures can be bought as freeze-dried preparations, produced on an industrial scale and some can even be directly inoculated into vats (Sandine, 1996).

3.2 Quality defects in cheese caused by microorganisms

Milk in a healthy mammary gland is thought to be sterile but can after secretion be contaminated by microorganisms from teat apex, milking equipment, air, water, feed, grass, soil and other environments (Quigley *et al.*, 2013; Tolle, 1980). Therefore, it is important to remove soil and bacteria on a daily basis from the teats, if not complex bacterial communities can be established that are more difficult to remove from surfaces than free living, vegetative bacterial cells (Boor & Fromm, 2006). After milking the milk temperature needs to be kept below seven degrees Celsius to inhibit growth of mesophilic and thermophilic bacteria. It is also important that the transport trucks have efficient cooling systems (Hantsis-Zacharov & Halpern, 2007). The psychotropic bacteria are still able to grow at low temperature and can cause problems. They have very heat resistant proteases and lipases that can survive Ultra-high temperature processing (UHT) pasteurization and their enzymes can also cause spoilage of stored cheese milk (Sørhaug & Stepaniak, 1997).

Early and late gas development are the most common causes of cheese defects but better sanitation and milk quality control has made it less common today. The

early gas formation can be noticed within a few days as small holes in cheese caused by H_2 produced by coliform bacteria and CO_2 by yeast and is more common in soft cheeses with high water activity (Cogan, 2011). Nitrate is effectively used to control early gas formation. Late gas formation, called 'late blowing', causes off-flavor and gases that produce large holes, called 'eyes' in cheese. This defect happens later in cheese development when butyrate, CO_2 and H_2 is produced by *Clostridium tyrobutyricum* and *butyricum*. To control the late gas production, nitrate, microfiltration, bactofugation, lowering of pH or addition of salt or lysozymes can be used. Lysozymes, destroys the clostridia spores and are often used in Italian cheeses (Cogan, 2011; Sheehan, 2011). Bactofugation is a centrifugation process that removes most of the anaerobic, spore-forming bacteria and spores in cheese milk. Microfiltration removes spores by using a microfiltration membrane (Ávila *et al.*, 2014). Propionic acid bacteria (PAB), can cause large holes in cheese by CO_2, are anaerobic and therefore only grow inside the cheese where they produce propionate, acetate, and CO_2. This is considered to be a defect in Italian cheeses, such as Parmigiano-Reggiano and Grana, but in Emmental and Comté cheeses, the *Propionibacterium freudenreichii* is considered to have an important positive role (Cogan, 2011).

3.3 Microorganisms in flavor development

Most flavor development by microorganisms and their enzymes happen during the cheese ripening process by degradation of carbohydrates, citrate, proteins and lipids. In raw milk cheese, microbial communities are more complex compared to cheese made from pasteurized milk and give the cheese more sensory quality but also brings about safety risks, i.e. development of pathogens (Ayad *et al.*, 1999; Grappin & Beuvier, 1997). LAB produces acetic acid, ethanol, aroma compounds, bacteriocins, exopolysaccharides and enzymes that contribute to the texture and flavor development in cheese (Leroy & De Vuyst, 2004). The diversity of LAB in raw milk have decreased over time due to the use of milk refrigeration and strictly hygienic processing methods in dairying, therefore it has become important to analyze wild strains in good quality raw milk for use as starters to get the unique flavor and characteristics of traditional cheese (Peláez & Requena, 2005). For example, in the absence of facultative heterofermentative lactobacilli (FHL) that convert six-carbon sugars like glucose to lactic acid and five-carbon pentose sugars into a mix of lactic and acetic acid, full flavor of good quality traditional cheese will not be attained (Beuvier *et al.*, 1997; Broadbent *et al.*, 2011; Fernández-García *et al.*, 2002; Swearingen *et al.*, 2001; Wijesundera *et al.*, 1997). FHL in cheese are mainly *L. fermentii* and *L. brevis* (Crow *et al.*, 1995). Selected starter microorganisms can be directly added to cheese making vats but only a limited number of NSLAB strains can be successfully applied as adjunct cultures since many may induce defects or spoilage (Wouters *et al.*, 2002). Adjunct cultures can be defined as selected strains of cheese related microorganisms that are added to the cheese to improve cheese sensory quality and increase ripening (El Soda *et al.*, 2000). Some LAB metabolize citrate giving diacetyl and acetoin, which are important for cheese aroma. In cheddar type cheese these compounds are not

considered important because only homofermentative cultures are used, which do not metabolize citrate.

Enterococci bacteria affect aroma, color, and structure and are found in high numbers in many artisanal cheeses, particularly those produced in southern Europe (Carlos *et al*., 2009; Giraffa, 2003). They are not deliberately added to the milk for cheese making except as probiotic cultures and may originate from the milk used in cheese making. Enterococci are often found in cheeses made of raw milk, but may also be found in cheeses made from pasteurized milk, as many can withstand pasteurization. In recent years, they have been incriminated as the cause of several hospital-acquired infections (Franz *et al.*, 2011). They are promiscuous, easily picking up plasmids encoding resistance to antibiotics (Giraffa, 2003).

Brevibacterium is important in many smear-ripened cheeses, where it grows on the surface and is most known for producing volatile sulphur compounds, e.g. methanethiol, sulfides and thioesters (Arfi *et al.*, 2005; Broome *et al.*, 2011).

Yeasts are considered adventitious contaminants or are deliberately inoculated onto the cheese surface. They are very tolerant to low pH and high salt concentrations and are therefore able to survive on the surface of smear- and mold ripened cheese, for example, Comté, Tilsit, Limburger and Camembert. They produce CO_2 and H_2O by lactate oxidization and NH_3 by deamination of amino acids, causing deacidification and increasing of the pH. This makes the environment suitable for high pH bacteria, like *Debaryomyces hansenii*, *Geotrichum candidum*, *Kluyveromyces lactis*, *Kluyveromyces marxianus*, *Saccharomyces cerevisiae* and *Yarrowia lipolytica* (Cogan, 2011).

3.3.1 Protein degradation and amino acids catabolism

LAB uses amino acids for different purposes, to synthesize proteins, as energy source, to obtain the right internal pH in an acid environment, to generate co-substrates and other complex products. The breakdown of proteins by aminopeptidase enzymes is called peptidolysis and is often specific for a group of amino acids with similar properties and is dependent on environmental conditions such as pH, temperature, water activity and availability of co-substrates. The breakdown of protein is important for cheese taste and also softens the cheese texture by breaking down the casein network (Ardö, 2006; Broadbent & Steele, 2005).

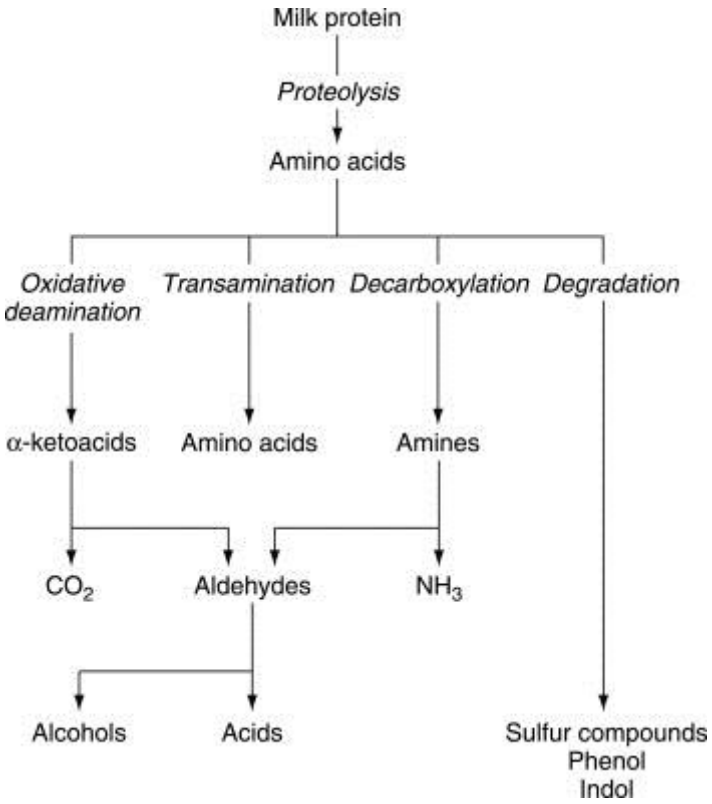

Figure 1. Microbiological catabolism of amino acids during cheese ripening (Molimard & Spinnler, 1996).

The different steps in the amino acid catabolism are decarboxylation, oxidative deamination, transamination and degradation as seen in Figure 1. Volatiles, from proteolysis, can give a malty flavor. Example of such compounds are 3-methylbutanal (isovaleraldehyde), 2-methylbutanal and 2-methylpropanal but these can also give a fruity odour if they are in small quantities (Morgan, 1976; Yvon & Rijnen, 2001). Methional and 3-methylbutanal are among the most potent aroma compounds in Emmental and the typical sweet caramel aroma is from furaneol and homofuraneol (Yvon & Rijnen, 2001). Compounds from methionine give a cabbage and boiled-potato aroma and are important for aroma in Cheddar cheese (Milo & Reineccius, 1997; Yvon & Rijnen, 2001). Dimethyldisulphide and dimethyltrisulphide give a garlic note to cheeses (Yvon & Rijnen, 2001). Indole, p-cresol and skatole are responsible for unclean and rose-like off-flavors (Gummalla & Broadbent, 2001). Leucine is the likely source of a desirable nutty flavor note in cheese (Broadbent & Steele, 2005). Volatile amines are found in cheese, for example, dimethylamine in Camembert and Blue cheese. Many volatile amines are described as fruity, alcoholic, and varnish flavor notes. However amines are usually not the final product, they can undergo oxidative deamination that will result in formation of aldehydes (Le Quéré, 2011). Although many studies have shown positive correlation between aminopeptidase activity and cheese flavor,

there are also studies that do not show this correlation, which would suggests that further catabolic reactions of amino acids may be needed for flavor development (Peláez & Requena, 2005; Weimer *et al*., 1997).

A study by Tammam *et al*. (2000) showed that catabolism of individual amino acids only occurred when α-ketoglutaric acid was present and in another study by Kieronczyk *et al*. (2001), α-ketoglutaric acid was needed to utilize leucine and lysine. Liu *et al*. (2003) showed that serine, asparagine and glutamine were not dependent on α-ketoglutaric acid to be utilized. This would suggest that α-ketoglutarate should be added with the starter to the cheese and that transamination is the primary step in amino acid catabolism of some amino acids but not all by FHL (Yvon *et al.* 1997; Yvon *et al*., 1998). The over-expression of lactococcal aminopeptidase PepN has resulted in enhanced proteolysis and in accelerated flavor development in some occasions but not in others (Christensen *et al*., 1999). Moreover, adding high levels of amino acids to Cheddar cheese did not yield better flavor than adding intermediate levels (Wallace & Fox, 1997).

Sulfur compounds are noticed at very low concentrations and have a whole range of aromatic notes such as for example cheese and garlic. The compound methanethiol are the main contributor to many sulfur flavors in cheese. Bacteria such as lactobacilli, lactococci and *Brevibacterium linens* produce significant amounts of this compound by degradation of methionine. Enzymes involved are methionine-γ-demethiolase, cystathionine-γ-lyase and cystathionine-β-lyase. Further degradation of methanethiol leads to a wide range of sulfur compounds that contribute to the aroma of cheese, for example demethyldisulphide and dimethyltrisulfide, S-methylthioacetate and S-methylthiobutyrate (Le Quéré, 2011).

PAB such as *Propionibacterium freudenreichii* or *Propionibacterium shermanii* are most likely responsible for formation of BCAA (branched chain amino acids) in Swiss-type cheeses (Yvon & Rijnen, 2001).

3.3.2 Fatty acids

Free fatty acids (FAA) come from the hydrolysis of glycerol esters by both milk's indigenous and pregastric and microbial exogenous lipases and gives a somewhat pungent flavor (Hernández *et al*., 2005). The short-chain and medium-chain fatty acids play a bigger part in flavor perception than long-chain fatty acids. The fatty acids, octanic, 4-methyloctanoic, and especially 4-ethyloctanoic acids, have goaty notes. Volatile FFA can contribute to aroma or give a rancid defect to the cheese (Le Quéré, 2011). When developed together with flavor compounds from protein degradation a distinct flavor is obtained. In cheese lacking this sufficient basic flavor from proteolysis, free fatty acids are considered undesirable because they impart a soapy-rancid flavor (Geurts *et al.*, 2006). Adding glutathione to cheese has shown to reduce the concentration of volatiles that produce off-flavors significantly (Castada *et al*., 2015).

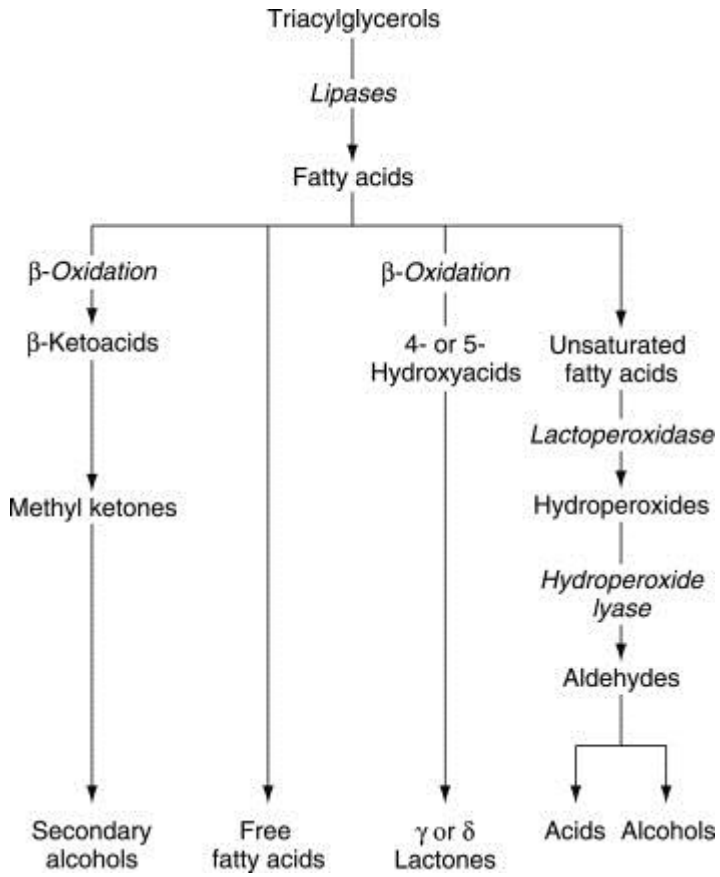

Figure 2. Formation of flavor compounds from lipids (Molimard & Spinnler, 1996).

Fatty acids are aromatic compounds but also precursors of methyl ketones, alcohols, aldehydes and lactones as seen in Figure 2.

Aldehydes are mainly formed from fatty acids. Some examples are hexanal and (*E*)-hex-2-enal that give the note of immature fruit. Octanal, nonanal, decanal and dodecanal are described with an orange resembling note. Benzaldehyde is described as having an aromatic note of bitter almond (Le Quéré, 2011).

Lactones are produced from hydroxylated fatty acids by the influence of pH and sometimes by the help of microorganisms. The ones principally found in cheese are γ-decalactone, δ-decalactone, γ-dodecalactone and δ-dodecalactone and they generally have very fruity notes such as peach, apricot and coconut (Le Quéré, 2011).

The most potent odour in Camembert and Cheddar cheese is butyric acid which gives a sweet flavor and also the ester ethylbutyrate contributing to the Cheddar flavor, although a high amount of esters give a fruitier flavor (Yvon & Rijnen, 2001).

3.3.3 Ketones

Nonan-2-one is the major ketone in surface mold-ripened cheeses and heptan-2-one in blue cheeses. There are both branched chain and unsaturated ketones in cheese. Unsaturated ketones increase during ripening and methyl ketones with even-numbered chains, except for butan-2-one, appear late in ripening. Ketones generally give off floral odor notes, except diacetyl, heptan-2-one, and oct-1-en-3-one which give typical buttery, Blue-cheese, and mushroom odors, respectively (Le Quéré, 2011).

3.3.4 Alcohols

Alcohols are formed in many different ways in cheese. Primary and secondary alcohols and ketones are the most important compounds in aroma of soft and mold-ripened cheeses. The primary alcohols give a floral note. The secondary alcohols 2-heptanol and 2-nonanol constitute 20-30% of all aroma compounds in Camembert-type cheese. Phenylethanol and its esters have a cumulative effect that gives a floral note to Camembert cheeses. Linolenic and linolenic acids are precursors of 8-carbon aroma compounds such as 1-octen-3-ol which has a raw mushroom odour and appear only late in ripening because they are produced by *Penicillium camemberti* (Le Quéré, 2011).

Esterification occurs either directly from alcohols derived from lactose fermentation or amino acid catabolism and short- to medium-chain fatty acids, or by transesterification reaction between ethanol and a partial glyceride. Acetates formed from acetyl-CoA and an alcohol are the most common esters. Enzymes such as carboxylesterases and asylesterases are involved in the esterification reactions. Esters appear in early stages of ripening and are often formed by yeast. Most have fruity odours, except those having a phenyl group, which have floral notes (Le Quéré, 2011).

3.3.5 Future of flavor development

The composition of LAB can be changed by pH, salt, low temperature and nutrition starvation. This can provide knowledge of their biochemistry but also used to optimize pathways for flavor development. One example is restriction of carbohydrates during amino acid metabolism, which has shown to provide branched-chain fatty acids not present in milk fat. *L. casei* output of major and minor compounds will be changed by the amount of lactose they have access to. In a study by Coolbear *et al*. (2011), it was observed that some minor compounds did not change, like propionic acid (pungent, sour milk, nutty), 3-(methylthio) propionic acid (chocolate, roasted) and γ-hexalactone (herbal, coconut, sweet, coumarin). But, other compounds like heptan-2-one (fruity, spicy, sweet, herbal, coconut, woody), nonan-2-one (fresh, sweet, green, weedy, herbal), nonan-2-ol (waxy, green, creamy, citrus) and iso-valeric acid were not found in cultures without lactose limitation, while ethyllactate (sharp, tart, fruity, buttery, butterscotch) was only found under such conditions. Furfuryl alcohol (nutty) was found in lactose-containing cultures.

LAB can be used to make many different new flavors, for example have some fishy, savory and chocolate flavors been reported but there are limitations related to consumer acceptance and limitations using milk as a substrate, application relevance and cost. The drivers of technological success are consistency, predictability, targeting and acceleration (Coolbear *et al.*, 2011). Another use of NSLAB is that some have the ability to produce toxins that kill other microorganisms and may be used in combination with additional resistant bacteria that would become dominant throughout cheese ripening. This approach has been demonstrated in a study by Ryan *et al.* (2001), using lacticin 3147 resistant *L. paracasei subsp. paracasei* together with a bacteriocin-producing strain. This technique can also be used to prevent food spoilage caused by yeasts and molds as demonstrated by Okkers (1999). The use of lysins, phages or bacteriophages for lysis, either by virulent or induction of prophage of the starter cells, will cause the bacteria to release all the intracellular peptidases into the curd. This can be an efficient way to accelerate ripening and control flavor development (Broadbent & Steele, 2005; Peláez & Requena, 2005; Martínez-Cuesta *et al.*, 2001; Morgan *et al.*, 1997). One other ways to accelerate ripening of cheese are by adding cell-free extracted bacteria as a source of tailored enzymes (Calasso *et al.*, 2015).

Bitterness, origination from nitrogen containing compounds, especially small peptides, is one factor that can limit the acceptability of cheese. NaCl is responsible for the salty taste, and other salts often contribute to the bitter taste. It has also been hypothesized that short- and medium-chain fatty acids also contribute to acid taste (Le Quéré, 2011).

4 Methods for identification of microorganisms in cheese

Enzymatic analysis of bacterial strains is today the most widespread tool for predicting the ability of a strain to produce certain flavor compounds. Instrumental analysis of cheese flavor volatile compounds are often done by gas chromatography, whereby elute is sniffed to identify key flavors. Their molecular structure can be further identified by using a coupling mass spectrometry to a gas chromatograph (Geurts *et al*., 2006). Methods for identifying the microbiota of an ecosystem can be either culture-dependent or culture-independent. Culture-dependent methods are appropriate if the amount of nucleic acid is of poor quality, when the milk contain high amounts of PCR inhibitors such as calcium and proteinases and when the sensitivity of culture-independent assays are too low. Culture-dependent methods, however, have some downsides, for example they are time-consuming because of long culturing periods and some species are not able to grow in vitro. Furthermore, the number of bacteria can sometimes not be determined because only a small fraction of the microorganisms will be detected. Also, ecological niches and symbiotic relationships cannot be determined because only predominant species in the habitat will be detected (Fusco & Quero, 2014; Ward *et al*., 1998). Culture-independent methods rely on cloning and sequencing of DNA fragments or on prior amplification of target sequences of the polymerase chain reaction (PCR). Real-time PCR is used to accurately detect individual species or groups as well as total bacterial ecosystems. This has many advantages, for example allowing distinguishing between different species with similar phenotypic characteristics, also within the same species. RNA compared to DNA is better for analyzing viable cells because RNA is more rapidly degraded after cell death (Fusco & Quero, 2014).

5 Discussion and conclusion

Traditionally cheese was made using raw milk but today milk is commonly pasteurized to destroy pathogenic bacteria, also destroying bacteria that add more flavor to cheese. Raw milk cheeses have a richer bacteria flora and can be argued to give a better protection against pathogenic bacteria. This is because a diverse healthy bacteria culture can be protective against pathogens. Good cleaning practices are therefore essential tools for prevention of contaminants in dairy products. Instead of using pasteurization, perhaps bacteria could be added to the milk before cheese production to give protection against pathogens in raw milk. The enterococci bacteria found in raw milk are important for flavor development but in some cases considered emerging pathogens because they have caused several hospital-acquired infections. Therefore it would be a good idea to find good alternative bacteria that could give the same flavor products but without the health risk.

LAB are very important for flavor development in cheese and can be used for development of many different flavors. If the situation will not change, i.e. that cost to produce synthetic flavor compounds would become lower than getting them from other flavor sources, the future of LAB flavor development lies in the hands of dairy industry, already using these technologies.

Acceleration of cheese ripening can be obtained in many ways, for example by adding free extracted bacteria as the source of tailored enzymes. Lysis of LAB, a way to accelerate ripening, can be used to release intracellular peptidases into the curd to control the flavor development. The use of bacteriophages for lysis has been evaluated but shown to have a negative effect on cheese starters and fermentation failures in the milk. Also a-ketoglutaric acid can be added with the starter culture to enhance the proteolysis of proteins. Toxin producing bacteria can be used with or without additional resistant bacteria that would become dominant through the cheese ripening.

Both culture-dependent and culture-independent methods are used to identify microorganisms. Culture-independent methods, based on bulk extraction of DNA and PCR, to amplify a gene which is common in bacteria, are faster and more accurate and can be more adapted to market needs.

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