

Evaluating the benefits and risks of organic raw milk cheese. Challenges in the production of organic cheeses made from raw milk.

Master's Thesis 2014



Picture was taken at Caws Teifi Cheese, Wales

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2014.10.10.

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Preface

This master thesis was written as part of the Double Degree MSc programme in Organic Agriculture and Food Systems with the universities of Hohenheim and Aarhus. It is a 30 ECTS literature review Master Thesis with some additional information and remarks from artisan cheese makers. The objective with this project was to improve the decision basis for organic farmers and cheese makers considering producing cheese from organic raw milk. This project collects and summarizes the available literature on the subject of the benefits and risks of raw milk cheeses, especially emphasizing on the differences between organic and conventional cheese products. This master thesis should be seen as a background and guideline summary for farmers and cheese makers who are considering to produce organic raw milk cheese. The project is further aimed at researchers as well as other people interested in the topic of organic raw milk cheese to encourage awareness and further research in this field.

I am very thankful for the advices and guidance of my supervisor Associate Professor Mette Krogh Larsen, Department of Food Science - Differentiated & Biofunctional Foods, University of Aarhus. I would also like to thank John Savage Onstwedder and Tim from Caws Teifi Farmhouse Cheese, and Sam Holding from Hafod organic cheese who helped me in my research and teaching me the craft of cheese making. Also I would like to thank Lutz Mertz from the University of Hohenheim, who helped me on the road to this project and becoming a cheese maker.

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Introduction

Raw milk offered for sale within and into the European Union has to be produced according to the requirements of Commission Directive 89/362/EEC and to meet quality standards described in Council Directive 92/46/EEC. Together the regulations say many things about the quality of milk and the hygienic means of production including guidance and requirements for:

- The means of production, farm specifications etc.
- Processes, milk handling
- Animal health
- Milk composition related to quality - especially antibiotics, cell count and bacterial content (Hillerton & Berry, 2004)

Unpasteurized milk and the associated food products (yogurt, butter, cheese etc.) made from raw milk is safely produced and consumed in many areas around the world. Cheese making is a major industry worldwide, while most of the production comes from large scale industrial producers; a large part is still practiced on a relatively small scale which accounts for the rich diversity of cheeses available. Cheese is made from milk, which contains milk fat and coagulated proteins and preservation is largely achieved by controlling the pH and water activity (Little et al., 2008). In the European Union the production of both fresh and raw milk cheeses is allowed. Between 1995 and 2004 cheese production increased by nearly 15 %, with per capita consumption growing at an average rate of 1.5 % per year. Nearly 40 % of EU milk is consumed as cheese. Four Member States (Germany, France, Italy and the Netherlands) produce more than 75 % of the cheese in the EU. The European dairy industry processes approximately 135 million tons of raw milk into a broad range of products, both for consumption and for use in the production of many food, feed and pharmaceutical products. The raw milk delivered by the EU-25's 1.6 million dairy farmers, processed by the dairy industry, plays a vital role in rural areas (EU, 2006). About 700 000 tons of raw milk cheeses are produced annually in Europe, particularly in France, Italy and Switzerland (Beuviel & Grappin, 1997). The effort to preserve raw milk cheese production in certain countries of the European Union began in the early 1990's. Between 1990 and 1992 the European Union debated the safety of raw milk cheese and was considering the mandatory pasteurization of all dairy products. Some of the northern European countries wanted to forbid the production of raw milk cheeses for sanitary reasons, pointing toward the reduced health risk from pasteurized milk cheeses. They were considering the mandatory pasteurization of all dairy products (Dixon, 2000). Cheese made from raw milk represents an important proportion of the traditional cheeses, particularly in South European countries (Beuviel & Grappin, 1997). In these areas a large variety of traditional cheeses are still produced using raw milk.

The main objective of this thesis was to summarize the available literature concerning the benefits and risks of cheese made from raw organic milk to assist farmers and cheese makers in their decision to produce these products. This was done by carrying out a literature review assessing:

1. EU regulations concerning organic raw milk cheese
2. Comparing the properties of cheese made from raw and pasteurized milk
3. Food safety: pathogens and food poisoning bacteria in raw milk
4. How organic farming and feeding affect milk quality and thereby cheese quality
5. Possible challenges in the production and sale of organic raw milk cheese

Materials and methods

During this Master Thesis project I have collected information and insight, advices from several artisan cheese makers in the South of Wales, who all had experience with raw milk cheeses, and two of them were organic cheese producers. With their help and my supervisor's guidance I could collect and analyse the available literature to highlight some of

the key benefits and risk in cheese produced from raw milk and identifying the benefits and challenges of organic cheese production.

Chapter I. - Regulation of raw and organic milk

In Europe general regulations concerning raw milk and raw milk cheese products began in the 1990s to ensure/help the survival of raw milk cheese products and guaranteeing consumer safety (EEC, 1992). The European Community Directives 92/46 and 92/47 contain regulations for the hygienic production and placing on the market of raw milk, heat-treated milk and milk-based products. These regulations establish hygienic standards for raw-milk collection and transport that focus on issues such as temperature, sanitation and microbiological standards, enabling the production of raw milk of the highest possible quality (Donnelly, 2004).

Scientific documents support the protection and preservation of artisan cheeses. The first European directive concerning the production of raw milk cheeses is from 1992, which laid down the health rules for the production and placing on the market of raw milk, heat-treated milk and milk-based products (EEC, 1992). A number of regulations followed which laid down the framework for raw milk cheese production in the EU. Regulation (EC) No 852/2004 on the hygiene of foodstuffs, (EC) No 853/2004 laid down specific hygiene rules for food of animal origin, (EC) No 2073/2005 on microbiological criteria for foodstuffs and (EC) No 2074/2005 laid down implementing measures for certain products, including raw milk. These regulations describe where it is allowed to produce and market raw milk cheeses as long as certain minimum requirements are met. All the EU member states must follow these minimum regulations, but each country can decide about stricter measures restricting or even banning raw milk cheeses. For example in Denmark raw milk cheeses can only be made if the milk is heated at least to 50°C for 30 minutes, or to 57°C for 15 minutes, or equivalent time/temperature combination, are aged more than 60 days, and the water content is lower than 56%, exceptions are only made with a few artisan cheese products and blue cheeses. France, UK, Ireland, Italy are some of the countries that allow the production and sale of fresh and aged raw milk cheeses (SlowFood, n.d.).

Basic requirements for producing raw milk

Regulation 853/2004 (EC, 2004b) (Annex III, Section IX) states that raw milk must come from animals that do not show symptoms of infectious diseases that can be transmitted to humans through milk. In particular, as regards tuberculosis and brucellosis, this regulation states that raw milk must come from cows (or buffalos) belonging to a herd which is officially free of tuberculosis and brucellosis, that are healthy and that have not been given unauthorized substances or products, and minimum suspension times must have been respected. The bacterial count allowed for raw cow's milk is a maximum of 100,000 bacteria per ml (measured at 30°C) and for other animal milk is a maximum of 500,000 bacteria per ml (measured at 30°C). The somatic cell count is also measured, and the maximum allowed is 400,000 cells per ml (Dixon, 2000; EC, 2004b). If producers cannot meet these criteria, they either cannot continue to sell or process the milk, or they can use it only for specific products (or to make pasteurized cheeses or raw-milk cheeses aged for at least 60 days). If the milk is not processed within two hours of milking, it must immediately be stored in a clean place and chilled to a temperature below 8°C (in case of daily milking) or below 6°C (when milking is not carried out daily) (EC, 2004b). However, food business operators may keep milk at a higher temperature if:

- a) Processing begins immediately after milking, or within four hours of acceptance at the processing establishment
- b) The competent authority authorises a higher temperature for technological reasons concerning the manufacture of certain dairy products

Hygiene requirements (EC, 2004b):

- Milking equipment and premises where milk is stored, handled or cooled must be located and constructed so as to limit the risk of contamination of milk.
- Milking must be carried out hygienically
- Persons performing milking and/or handling raw milk must wear suitable clean clothes and must maintain a high degree of personal cleanliness.

Producing raw milk cheese

The rules for manufacturing raw milk cheeses fall under Article 6 and requirements for the products themselves are contained in Article 7 of regulation 92/46/EEC. In Annex C Chapter I of the 92/46/EEC regulation it is stated that raw milk must be used within 36 hours of acceptance, if the milk is kept at 6 °C or lower, or within 48 hours of acceptance if the milk is kept at 4 °C or lower. Thermized milk must have been obtained from raw milk which, if it is not treated within 36 hours of acceptance, has a standard plate count at 30 °C prior to thermization of 300,000 or less, milk-based products must be prepared in processing establishment that meets the Directive standards and specifications (Dixon, 2000; EEC, 1992).

Processing facilities

The facilities and especially the equipment must be clean, undergo regular maintenance hygiene requirements relating to the premises, equipment and staff are met, which include (Dixon, 2000; EEC, 1992):

- Equipment and instruments used for working on raw materials and products, floors, ceilings or roof linings, walls and partitions, must be kept in a satisfactory state of cleanliness and repair, so that they do not constitute a source of contamination for raw materials or products
- Cross-contamination between operations by equipment, ventilation or staff must be avoided
- Rooms intended for production purposes shall be divided into wet and dry areas, each having its own operating conditions
- The operator of the establishment shall draw up a cleaning program based on risk analysis to ensure that there is no health risk to products as a result of inadequate cleaning methods
- Identification of critical points in the establishment on the basis of the processes used
- Monitoring and checking of such critical points by appropriate methods
- Taking samples for analysis in a laboratory recognized by the competent authority for the purpose of checking cleaning and disinfection methods and for the purpose of checking compliance with the standards

Exception

Member states can grant exceptions regarding processing facilities and materials to businesses that produce traditional cheeses (PDO, IGT, PAT - traditional regional protected food products). If the environment contributes to the development of the cheese's characteristics, the facilities can have walls, ceilings and doors not made from smooth, impermeable, non-absorbent, corrosion-resistant materials and natural geological walls, ceilings and floors. The same applies to the materials used for the tools and equipment for the preparation and packaging of the cheeses. For example in Holland it is still allowed to use open cheese vats covered with wood on the outside, and also to use wooden cheese moulds. Some special cheese varieties also require specific processing methods or ripening facilities, like traditional cave aged cheeses like the Roquefort. (EC, 2004c)

Microbiological characteristics of raw-milk cheese

Moving on from milk to cheese, it is necessary to guarantee the following through regular analyses (EEC, 1992):

- The absence of *Listeria monocytogenes*
- The absence of *Salmonella*
- The absence of staphylococcal enterotoxins
- The control of the presence of bacteria indicating poor hygiene (*Escherichia coli* and coagulase-negative staphylococci)

Labels

The label (packaging, document, label, ring or band) that accompanies products made from raw milk must clearly indicate "made with raw milk" (EEC, 1992).

- 1) The words 'raw milk' for raw milk intended for direct human consumption
- 2) The words 'made with raw milk' for milk-based products manufactured from raw milk whose manufacturing process does not include any heat treatment, including thermization
- 3) For other milk based products the nature of any heat treatment applied at the end of the manufacturing process
- 4) For milk based products in which growth of microorganisms can occur, the use-by or a minimum durability date must be labelled

Regulations on organic milk and cheese production

In Europe regulations concerning organic farming and the production of organic milk began in 1991, when the regulation (EEC) No 2092/91 was published in the official journal of the EU, this year marking the beginnings of official interest in organic agriculture on a European level. Other regulations followed establishing standards for organic production, with rules concerning livestock, feed, housing, labelling (EC, 1999, 2003, 2007, 2008a). Regulations also focus on bacteria, enzymes and GMOs, allowed additives, preservatives and colourings in cheese products (EC, 2008b; EEC, 1993). With these strict standards the authorities ensure that organic milk is of highest possible quality to maintain a high level of consumer confidence.

Organic livestock, dairy farming

Livestock production is fundamental to organic farming because it provides the necessary organic matter and nutrients for the cultivation of the land and accordingly contributes towards soil improvement and the development of sustainable agriculture. In organic farming a wide biological diversity should be encouraged and choosing the suitable breeds able to adapt to local conditions. Dairy farming is very different all over Europe, but all organic dairy farmers have to comply the same rules laid down in the regulations (EEC) No 2092/9, (EC) No 1804/1999 and (EC) 834/2007 concerning feed, breeding, animal welfare, veterinary measures (EC, 1999, 2007; EEC, 1992).

Organic Feed

Organic livestock must be fed on grass, fodder and feeding stuff produced in accordance with the rules of organic farming. Feed materials from organic and in conversion farms cannot enter simultaneously with the same feed materials produced by conventional means into the composition of the product (EC, 1999). The feed has to be 100% organically produced, but because of the difficulty of buying in sufficient amount of organically produced feeding stuff authorization grants provisionally limited number/amount of non-organically produced feeding stuff in restricted quantities. Up to 30% of the feed ratio on average may comprise in-conversion feeding stuffs. When the in-conversion feeding stuffs come from a unit of the own holding, this percentage can be increased to 60%. Also to provide for the basic nutritional requirements of livestock, certain minerals, trace elements and vitamins may need to be used under well-defined conditions (EC, 1999, 2003, 2008a; EEC, 1991). In most cases livestock should have access to free-range exercise areas or grazing if weather

conditions allow it (EC, 1999, 2008a). Grazing systems are to be based on maximum use of pasturage according to the availability of pastures in the different periods of the year. Roughage, fresh or dried fodder, or silage has to be at least 60% of the dry matter in daily rations. The inspection authority or body can permit a reduction to 50% for animals in dairy production for a maximum period of three months in early lactation. (EC, 1999, 2008a; EEC, 1991) Time limited exceptions in feed and feeding are possible under the flexibility rules, in member states where sufficient quantities of organic feed are not yet available (article 43) (EC, 2008a; IFOAM, 2007).

Animal welfare

Housing should satisfy the needs of the animals concerned as regards ventilation, light, space and comfort and sufficient area should accordingly be provided to permit ample freedom of movement for each animal and to develop the animal's natural social behaviour. The livestock must have easy access to feeding and watering. Livestock must have access to a free-range area and the number of animals per unit of area must be limited to ensure integrated management of livestock and crop production on the production unit. It is laid down in Annex VIII. of the Regulation EC 1804/1999 that dairy cows need a minimum indoor area of 6 M²/head and at least an outdoor area of 4,5 M²/head, without pasturage. (EC, 1999) Stocking density is also regulated to maintain animal welfare, prevent over grazing, erosion or pollution (EC, 2007). In smallholdings, as defined by member states, indoor tethering is temporarily permitted according to Article 39 on condition, for example, that there is twice weekly outdoor exercise (EC, 2008a).

Veterinary measures

In organic livestock production the aim is to maintain a high level of animal health mainly through prevention, however, when animals become sick or injured, they should be treated immediately by giving preference to phytotherapeutic or homeopathic medicinal products and by limiting to a strict minimum usage of chemically-synthesized allopathic medicinal products even antibiotics in order to guarantee the integrity of organic production. But the use of these products or antibiotics for preventive treatment is prohibited. According to regulations the maximum usage of antibiotics is 3 treatments within 12 months. In order to protect consumers the withdrawal period after the use of chemically synthesized allopathic medicinal products is doubled (EC, 1999, 2008a; EEC, 1991).

Conversion of livestock and livestock products, certification of farms

If livestock products are to be sold as organic products, the livestock must be reared according to the rules laid down in this Regulation for at least: six months in the case of animals for milk production. Each EU Member State can apply more restrictive rules for the livestock and livestock products produced in their territory (EC, 1999, 2008a; EEC, 1991).

Organic Dairy Products

Organic milk

Organic milk has to come from certified organic farms which produce according to regulations and are being inspected by the authorities.

Organic cheese

Organic cheese is made from organic milk, with restrictions on the usage of additives, preservatives, enzymes and on other substances used during production.

Additives and preservatives

Table 1. The following food additives are allowed in organic cheese production, only the most common are listed here. Source: EU regulations (EC, 2008a; EEC, 1993):

Additive/preservative	Applicability
E 153 Vegetable carbon	Ashy Goat Cheese and Morbier
E 160 Beta-carotene	Colouring the wax in cheese making. The product is regarded as necessary in the making of traditional varieties of cheese such as Edam cheese
E 160b Annatto, Bixin, Norbixin	Red Leicester cheese Double Gloucester cheese Cheddar Mimolette cheese
Lactoflavin (vitamin B2)	Yellow colouring of cheeses
E 500 Sodium carbonates	In 'Dulce de leche' and soured-cream butter and sour milk cheese
E509 Calcium chloride	Improving coagulation during cheese making
Lactic acid	Regulation of the pH of the brine bath in cheese production
Citric acid	Regulation of the pH of the brine bath in cheese production
Hydrochloric acid	Regulation of the pH of the brine bath in the processing of Gouda, Edam and Maasdammer cheeses, Boerenkaas, Friese and Leidse Nagelkaas
E 1105 Lysozyme or egg white	Natural preservative/antimicrobial effect for the production of long ripened cheeses (ICF, 2011)
Wax coating	For ripened cheeses (SAa, n.d.)

Bacteria, Enzymes and GMO-s

When preserving milk, only bacteria cultures and acidification substances of plant origin may be used according to KRAV standards paragraph 5.3.28. In organic production as little chemical preservatives as possible should be used. In biological processes such as fermentation and maturation lactic acid cultures (LAB) and mould cultures (*Brevibacterium linens*, *Penicillium roquefort*, *Penicillium camemberti*) are allowed (Krav, 2006). There is no harmonised European Union (EU) legislation, but there are guidelines for safety assessment of food enzymes (EC, 2008b) which are used for safety evaluations in countries like Denmark and France. According to the EU regulation (EC, 2004a), enzymes produced by fermentation by using a genetically modified microorganism, which is kept under contained conditions, need no GM labelling; this includes Fermentation Produced Chymosin (FPC) (Law & Tamime, 2011). The Codex Alimentarius Commission organic food guidelines allow preparations of micro-organisms and enzymes, specifically, "any preparations of micro-organisms and enzymes normally used in food processing, with the exception of micro-organisms genetically engineered/modified or enzymes derived from genetic engineering" (CODEX, 1999; ICF, 2011). All organic products must not use GM organisms or their derivatives. This includes enzymes which must be from non GM organisms. To ensure that this is the case every cheese manufacturer must submit a GM declaration from their rennet supplier that the material is not derived from a GM organism (SAb, n.d.).

Labelling

Products can be labelled as organic when produced in accordance with organic production standards and certified by a duly constituted certification body or authority. According to regulations organic milk producers and organic cheese makers have to meet strict standards, so that organic milk and cheese products are made without substances used during

production, such as growth hormones, chemical fertilizers, insecticides, herbicides and genetically modified organisms (Narmada, 2007). Genetically modified organism GMOs and products made from or with GMOs are not accepted in organic production and cannot be labelled as organic products (EC, 1999).

Food additives, preservatives and food processing aids such as rennet have to be labelled on the product. An exception under this rule is for example lysozyme which is used to prevent undesired microbial activity. The European Communities Directive no. 95/2/EC authorized the use of Lysozyme in cheese production without needing to declare on the product. Before 2005, lysozyme was declared on the product label as E1105 (EC, 1995).

Chapter II. – Production of raw milk cheese and food safety

For hygienic reasons, most cheeses are produced from pasteurized milk; however, with a production of 700,000 tonnes per year, raw milk cheeses represent a significant proportion of ripened cheeses produced in Europe, particularly in Italy, France and Switzerland. Many artisan cheese makers, customers and researchers claim the benefits/advantages of raw milk cheeses, so it is important to know the consequences and possible implications of pasteurization on the ripening process, and ultimately on the sensory characteristics of cheese (Beuvier & Grappin, 1997).

Raw milk compared with pasteurized milk

The purpose of pasteurization is to increase milk safety for the consumer by destroying disease causing microorganisms (pathogens) that may be present in milk and also reduces the natural occurring fermentation. Pasteurization increases the shelf life and standardizes quality of milk products by destroying spoilage microorganisms and enzymes. The aim is to cause minimum change in composition, flavour acceptability of the milk. While effective heat treatment does not necessarily entail the destruction of all the microorganism originally present, but it must destroy any pathogens, which are mostly sensitive to heat (Kay, C.B.E., & F.R.S., 1962). Milk from healthy cows is usually sterile when it exits the mammary gland. Contamination with pathogens and spoilage bacteria can happen in the following cases (Marler, 2009):

- Mastitis and shedding of the pathogen directly from the cow's udder into the milk
- Entry of bacteria into the milk from the cow's skin, or via manure and dirt in the dairy environment
- Transfer of pathogens by vectors if they come in contact with the raw milk (for example, flies may carry pathogens on their legs and mouthparts)
- Human carriers transferring pathogens from their hands to the milk.

The main goal of milk pasteurization is the elimination of pathogens which may be present in raw milk. Research done to assess the potential risks relating to raw milk - in particular that on staphylococcal enterotoxin published in 2003 by the European Union and the documents on *Listeria monocytogenes* published in 1997 by the Ecole Nationale Vétérinaire de Maisons Alfort fostered the regulations made in order to preserve raw milk cheese making and at the same time maintain public health (Bemrah & Sanaa, 1997; SCVPH, 2003). In order to destroy pathogens and standardise the milk micro flora, pasteurisation of milk has become widespread. Pasteurization makes the milk safer but has an effect on milk quality which can certainly modify the characteristics of the cheese made with it, especially those which are matured for a longer period. Heat treatment reduces the number of mesophilic aerobic organisms, and affects both proteolytic and lipolytic processes in different manners (Hantsis-Zacharov & Halpern, 2007; Hávarria et al., 2000; Mcsweeney, 2007c; P. McSweeney & Weimer, 2007) .

After comparing the same cheeses made from raw and pasteurized milk, Beuvier & Grappin (1997) concluded that, “pasteurization modifies the biochemistry and microbiology of ripening, and the flavour and texture of cheese “ (Beuvier & Grappin, 1997; Dixon, 2000). They also found that besides destruction of pathogenic bacteria, the most significant changes in milk relevant to cheese making, which are induced by pasteurization are:

- Modification of milk rennetability
- Partial elimination of the milk microorganisms which may grow in cheese during ripening,
- Partial or total activation or inhibition of the plasmin/plasminogen complex, cathepsin D, lipoprotein lipase and alkaline phosphatase.
- Whey protein denaturation/denaturation of serum proteins
- Inactivation of acid proteinase

Milk pasteurization – how is milk pasteurized and why

Heat treatment leads to inactivation of many indigenous enzymes. The inactivation of alkaline phosphatase (ALP), is used as an indicator of proper pasteurisation, and inactivation is known to take place at high-temperature, short-time (HTST) treatment of at least 70°C for 16s (Fox, P. & Kelly, A., 2006) Inactivation of ALP indicates that pathogens able to grow in milk have been killed during heating (Hammershøj, Hougaard, Vestergaard, Poulsen, & Ipsen, 2010). Milk is required to be pasteurised commercially by the HTST process, where milk is heated to at least 71.7°C and held at this temperature for 15s (EC, 2005). The heat inactivation of ALP approximates closely to the heat required for destruction of *Mycobacterium tuberculosis* and *Coxiella burnetti* (Dickow, Nielsen, & Hammerhøj, 2012). Raw or unpasteurized milk refers to a dairy product that has not been heat treated to destroy pathogens or spoilage organisms. Pasteurization of milk means the heating of milk or milk products to a certain temperature for a specific period of time. There are different types of pasteurization (Marler, 2009):

- High Temperature Short Time (HTST): uses metal plates and hot water to raise milk temperatures to at least 72°C for not less than 15 seconds following by rapid cooling
- High Heat Short Time (HHST): similar to HTST, but uses slightly different equipment and higher temperatures for a shorter time
- Low Temperature Long Time (LTLT): 63°C for at least 30 minutes
- Ultra Pasteurized (UP/UHT): milk is heated to not less than 135°C for two seconds, which sterilizes the product that can be stored at room temperature for 8 weeks (Cindy, n.d.)

Besides UP pasteurization the milk whether raw or pasteurized eventually spoils, and must be refrigerated to prevent the growth of pathogens. Pasteurization enables the production of milk products almost free of bacteria and improves the consistency of cheese. Heat treatment of milk is essential for the production of cheese of consistent quality in the large, highly mechanized factories that are common today. Although with pasteurization the cheese quality is more consistent than with cheese made from raw milk, but also less highly flavoured. To increase the intensity of the flavour of cheese made from pasteurized milk, it is becoming increasingly common to inoculate pasteurized milk with selected organisms, usually lactobacilli, isolated from good quality raw milk cheese (P. F. Fox, 1993). Also new methods of heat treatments are being developed and used in order to achieve public safety but maintain the qualities of the milk and its micro flora, like Instant infusion pasteurisation and Lenient Steam Injection (Dickow et al., 2012; Hammershøj et al., 2010).

Risk of recontamination

Efficient pasteurization of milk eliminates the risk of pathogenic organism, but does not destroy organisms that grow slowly or produce spores. While pasteurization destroys many microorganisms in milk, improper handling after pasteurization can recontaminate milk (Cindy, n.d.). Both raw and pasteurized milk can be contaminated during bottling, shipment, and storage. Pasteurization only destroys the pathogens in the milk at the time of processing;

if unsanitary conditions allow pathogens to re-enter the milk later, it will be contaminated again. There are two general causes of intentional contamination of pasteurized milk:

- Equipment failure: The pasteurization equipment fails and there is raw milk in the product sold as pasteurized.
- Post-pasteurization contamination: the milk is contaminated after pasteurization, usually through unsanitary handling of the milk (Marler, 2009).

Pathogens in raw and pasteurized milk

According to European legislation, raw milk must be processed within a maximum limit of 48 hours, to reduce the development of pathogens (EEC, 1992). In raw-milk cheese production, checking the acidity (pH and SH of the milk and the curd) along the production chain can help minimize risk. If acidity develops rapidly (in the first 6, 8 or 12 hours since the start of processing), it means that a good part of the microorganisms dangerous to health and product quality have been limited. If the acidity is not optimal, problems will manifest themselves during cheese making and aging. According to Tim, artisan cheese maker at Teifi cheese, when acidity drops too slow or not enough, and the smell is not right, there could be something “fishy going on” with the bacteria; so the milk and cheese has to be checked and carefully monitored.

Independent of post-pasteurization contamination, the growth of milk pathogens in raw milk cheese is highly dependent on the type of cheese and on the technology involved. Cheeses are currently considered to be one of the safest foods consumed; however, pathogenic bacteria can grow and can be transmitted by dairy products. Historically there have been outbreaks of infection associated with the consumption of cheese, and the predominant organisms responsible have included Salmonella, *Listeria monocytogenes*, verocytotoxin producing *Escherichia coli* (VTEC), and *Staphylococcus aureus* (Mcsweeney, 2007b; West, 2008). Detailed investigations have demonstrated that the sources of contamination were raw milk, inadequately pasteurized milk, or post-pasteurization contamination with organisms originally derived from raw milk or from manufacturing environments (Little et al., 2008). It is well documented that pathogens will grow more easily in cheese with high moisture, high pH and a low salt content, than in cooked, long-ripened cheeses (Beuviel & Buchin, 2004; Beuviel & Grappin, 1997). One of the reasons of *Listeria* outbreaks with unripened and smeared cheeses such as: cottage cheese, queso fresco, Hispanic-style cheeses and washed rind cheeses is the recontamination of the cheeses during ripening. It is important to check the surrounding environment during cheese production to check for pathogens like *Listeria*, which can develop in unclean environments and which are tolerant of heat and cold. Roth 2009 found that soft and semi-hard cheeses were more frequently contaminated than hard cheeses, while pasteurized cheeses were more frequently affected than raw milk cheeses (Roth, 2009). According to Rudolf and Scherer (2001) smear cheeses are very sensitive to surface post-contamination by *L. monocytogenes*, as conditions prevailing in the smear may support *Listeria* growth (Rudolf & Scherer, 2001). A study done by Rudolph and Scherer (2001) shows “a higher incidence of *Listeria monocytogenes* in cheese made from pasteurised milk (8 percent) than in cheese made from raw milk (4.8 percent)” (Rudolf & Scherer, 2001). A risk assessment performed by Sanaa et al. (2004) revealed that the predicted probability of contracting severe listeriosis after consumption of both Brie de Meaux cheese and Camembert of Normandy made from raw milk is lower than after consumption of soft cheeses made from pasteurized milk (Sanaa, Coroller, & Cerf, 2004). In 2001, consumption of a homemade Hispanic soft cheese was directly linked to 12 cases of listeriosis in North Carolina (CDC, 2001). Fourteen *Listeria m.* isolates from patients, the implicated cheese, and the dairy supplying the milk all belonged to the same PFGE type (or ‘fingerprint’), which confirmed that the source of the outbreak was homemade raw milk cheese sold illegally by street vendors or by several small Hispanic grocery stores (Knight, Worosz, Todd, Bourquin, & Harris, 2008). Brie de Meaux cheese made from raw cows’ milk was the source of *Listeria monocytogenes* infection among 20 people in France. (Goulet et al., 1995). Desenclos and colleagues (1996) identified an outbreak in 273 people in France

who consumed raw goats' milk cheese where the organism implicated was *Salmonella enterica* serotype paratyphi B infection (Desenclos et al., 1996). Honish et al. (2005) investigated the thirteen cases of *E. coli* hemorrhagic colitis associated with unpasteurized Gouda cheese found in Canada. *E. coli* O157:H7 was isolated from 2 of 26 cheese samples manufactured by the implicated producer. Implicated cheese was found to be contaminated with *E. coli* O157:H7 104 days after production, despite having met regulated microbiological and aging requirements (Honish et al., 2005). Cheese made from unpasteurized cows' milk led to food poisoning also in England and Wales; 42 people who consumed Irish soft cheese were infected with *Salmonella* Dublin. The pathogen was subsequently isolated from cheeses obtained from the manufacturer's premises (Maguire et al., 1992; Taygan, Byron, Rebecca E., Miguel, & Micheál J., n.d.). According to Yousef & Marth (1990) in hard cheese such as Parmesan the environment for the extended survival of *L. monocytogenes* is unfavourable because (Yousef & Marth, 1990):

- Production by the starter culture of bactericidal agents (e.g. bacteriocins)
- Injury of the pathogenic cells during cooking of the cheese curd due to the combined effect of heat and acidity. Conditions in the cheese being unfavourable for the repair of the cells
- Drying the cheese blocks to a low water activity (*a_w*), the relatively high temperature of ripening (12.8°C) and the addition of lipase during the manufacture (with expected release of fatty acids in the cheese) (Lake, Hudson, Cressey, & Gilbert, 2005).

It is accepted that *Listeria* cannot survive in hard cheeses such as: Parmigiano-Reggiano, English Cheddar, Gruyère and Emmenthal, because they're too dry, too low in pH and too high in salt (Brooks et al., 2012).

The quality of the raw milk is especially important for cheese makers, but some members of the natural micro flora of raw milk are undesirable. The composition of psychrotrophic bacterial flora in raw milk has an important role in the determination of milk quality (Hantsis-Zacharov & Halpern, 2007). The primary objective of pasteurization is the killing of pathogens in the milk. Low bacterial counts and low somatic cell counts are the key indicators of milk quality, and as their numbers increase, there is a higher risk for contamination of milk and cheese with pathogens. By looking at the total count of microorganisms in the raw milk above the permitted level of the legislation, we can presume poor hygiene by milking, insufficient cooling, inconvenient storage of milk and possibility of secondary contamination (Kalhotka & Šustová, 2013). When bacteria and somatic cell counts are high, there can be other negative impacts on cheese quality and yield. Minimizing the time from milk collection to the initiation of cheese making reduces the opportunity for the growth of undesirable bacteria in raw milk. On the other hand, long storage time, even when cooled and transported gives the opportunity for pathogen growth, particularly growth of psychrotrophic pathogens, is increased. That is why artisan cheese makers try to make cheese from the freshest milk available, preferably from milk immediately after being milked on the farm, even without cooling (Donnelly, 2004). Also Nisin is used widely in cheese making where regulation allow it, because it shows antimicrobial activity against a broad spectrum of Gram-positive bacteria, such as *Bacillus*, *Clostridium*, *Listeria* and *Staphylococcus* spp. (Berg & Meijer, 2004). To evade sources for zoonotic bacteria in milk good mastitis control, bacterial and cell count limits applying are necessary. Intra mammary infections of the dairy cow by salmonella or listeria species are relatively uncommon so environmental management is most important in limiting their presence in milk from post harvesting contamination. The regulations say that teats must be clean and dry before milking, both are essential to limit coliforms in milk. It is obvious that they are a risk to the milk supply, although very few strains from cattle are pathogenic in man, that they can be controlled and that simple methods exist to measure them in milk. Mycobacterial infections and the potential presence of such zoonoses in milk and raw milk products is of growing importance as the distribution of Johne's disease and the local spread of bovine tuberculosis in many countries becomes more problematic (Hillerton & Berry, 2004).

Spoilage bacteria in raw and pasteurized milk

Raw milk may also contain several spoilage microorganisms, e.g., coliforms (which are unlikely to be a problem today due to improved cooling systems/technology), psychrotrophs (especially if the milk is cold-stored for a long period) and *Clostridium tyrobutyricum*. In case of inadequate cooling spoilage bacteria may still occur, that is why screening for coliforms is still necessary (P. F. Fox, 1993). Jonghe et al. (2010) writes that these spore forming bacteria contaminate the raw milk through both the vegetative form and the heat resistant spore structure which is both problem for raw milk and pasteurized milk cheeses. Contamination sources throughout the dairy chain are very diverse: water, soil, air, feed, especially silages, faeces, udder, milking equipment etc. If the bacteria have contaminated the milk, their spores cannot be destroyed by conventional heating processes, such as pasteurization (e.g. 15 s at 72 °C). The harmful effects concerning food safety and product quality caused by these aerobic spore formers are the following (De Jonghe et al., 2010):

- Production of toxins
- Production of spoilage enzymes and
- Interference with cheese making

Some spore formers are known to cause food poisoning through the production of toxins. According to Jonghe et al. (2010) the production of hydrolytic extracellular enzymes such as proteases, lipases and lecithinases can cause spoilage of pasteurized milk. These spoilage enzymes are responsible for off-flavours and structural defects in pasteurized milk. They are produced as soon as the spores germinate due to heat activation by pasteurization (De Jonghe et al., 2010). Growth of spore forming organism during ripening of most cheese varieties results in a defect known as late gas blowing (LGB) caused by an anaerobic metabolism of lactate to butyrate and H₂. Contamination with *Cl. tyrobutyricum* is minimized by good on-farm hygiene, better quality feed (improved silage quality), contaminants may be removed by bacterofugation or microfiltration, or their growth may be prevented by NaNO₃ or lysozyme (P. F. Fox, 1993). Some of these negative effects only become visibly during the ripening process, such as the blowing of Gouda and Swiss type cheeses, due the anaerobic fermentation bacteria (McSweeney, 2007a). Sodium nitrate is added to milk for Dutch, Swiss, and other cheese varieties to prevent the growth of *Clostridium tyrobutyricum*. In the study of Garde et al. (2012) pasteurized Manchengo cheeses with LGB showed higher counts of clostridial spores and higher pH values than raw milk cheeses with LGB, which were associated with the lowest lactic acid content and the highest concentration of propionic and butyric acids, all attributable to clostridial metabolism. Propionic acid content was significantly lower in raw milk cheeses with LGB, and significantly higher in pasteurized milk cheeses with LGB (Garde, Ávila, Gaya, Arias, & Nuñez, 2012).

Fermentation flora in raw and pasteurized milk – acid bacteria

Although mesophilic lactobacilli are undoubtedly inhabitants of raw milk and the dairy environment, upon acidification of raw milk, they are frequently overgrown by strong acidifiers of the genus *Lactococcus*. However, they do gain access to the cheese making process, because they are often found as secondary flora during the ripening of different cheese varieties. This is especially true for raw-milk cheese, but mesophilic lactobacilli are also common in cheese manufactured with modern technologies, using pasteurisation of the milk, defined-strain starters and hygienic processing (Shakeel-Ur-Rehman, Fox, & McSweeney, 2000; Wouters, Ayad, Hugenoltz, & Smit, 2002). In principle, only thermophilic bacteria survive pasteurization. Usually, the bacterial population in milk, expressed as mesophilic aerobic count or standard plate count, is reduced by at least 90% (or 1 log) by pasteurization. Besides pathogens in their vegetative form, all coliforms and most psychrotrophs are destroyed (Burton, 1986). Thermophilic LAB seem to be more heat resistant out of 60 lactobacilli isolated from Swiss cheeses, only 4 were killed at 71 °C for 18s in milk containing approximately 200,000 microorganisms mL⁻¹ (Bassett & Slatter, 1953). It is clear that low efficiency of pasteurization, as well as post-pasteurization contamination, will allow significant growth of non-starter lactic acid bacteria (NSLAB) in cheese (Beuvier &

Grappin, 1997). The main task of dairy starter cultures is the rapid acidification of the environment, because the resident LAB acidifies the milk slowly. The addition of starter LAB is useful to maintain a certain uniformity of final products, but has the drawback that the raw milk biota may be lost (Wouters et al., 2002). This process may also eliminate bacteria with interesting properties in cheese making (Franciosi, Settanni, Cavazza, & Poznanski, 2009). Beuviel and Grappin (1997) found that the acidification process of Swiss-type cheese by lactic acid bacteria does not seem to be influenced by pasteurization. (Beuviel & Grappin, 1997) If heat treatment was not too severe, it can be partly balanced out by enhanced acidification or with the addition of calcium chloride CaCl.

Enzymes in raw and pasteurized milk

According to Fox (2006), milk contains about 60 indigenous enzymes, of which about 20 have been isolated and characterised in detail. The indigenous enzymes are constituents of the milk as excreted and arise from three principal sources:

- The blood via defective mammary cell membranes;
- Secretory cell cytoplasm, some of which is occasionally entrapped within fat globules by the encircling milk fat globule membrane (MFGM); and
- The MFGM itself (Patrick F. Fox, 2003)

Several enzymes contribute to cheese ripening, from which many have been studied thoroughly. At least four indigenous enzymes contribute to cheese ripening: plasmin, lipoprotein lipase, acid phosphatase, and xanthine oxidase. Although milk contains considerable potential plasmin, relatively little is expressed owing to the presence of inhibitors.

Acid phosphatase, alkaline phosphatase and xanthine oxidase

Acid phosphatase, which has a pH optimum at 4.0 and is very heat stable and may be significant in the dephosphorylation of phosphopeptides in cheese. Acid phosphatase, either indigenous or produced by microorganisms plays an important role in cheese ripening (P.F. Fox & Stepaniak, 1993). LTLT pasteurization causes only 10–20% inactivation and 30 min at 88C is required for full inactivation; when heated in milk at pH 6.7, the enzyme retains significant activity following HTST pasteurization but it does not UHT treatment. In cheese made from pasteurized milk, both indigenous acid phosphatase and bacterial phosphatase are probably responsible for dephosphorylation, but in cheeses made from raw milk like Parmigiano Reggiano or Grana Padano, the alkaline phosphatase seems to be responsible for dephosphorylation (De Noni, Pellegrino, Resmini, & Ferranti, 1997; Pellegrino et al., 1997). According to Beuviel (1997) acid phosphatase and xanthine oxidase, which may be active during ripening, withstand pasteurization, and a slight (7%) denaturation of serum proteins and little or no modification of the cheese making properties (coagulation, acidification by lactic acid bacteria) (Beuviel & Grappin, 1997). Processing conditions that affect the milk fat globule membrane (MFGM) also affect the Xanthine oxidase XO activity in milk, homogenization activates this enzyme at heating temperatures between 60 and 70 C after which pasteurisation reduces XO activity by > 50% (Fox, P. & Kelly, A., 2006; Hammershøj et al., 2010). Xanthine oxidase can catalyse the oxidation of purine bases and reduce nitrate to nitrite in cheese, which may inhibit the germination of spores (P.F. Fox & Stepaniak, 1993).

Lysozyme and lactoperoxidase

More than 75% of the lysozyme activity in bovine milk survives heating at 75°C for 15 min or 80C for 15 sec and is therefore little affected by HTST pasteurization. Lysozyme and lactoperoxidase may have antimicrobial effects in the intestine of the consumer. (Patrick F. Fox, 2003) Most enzymes are inactivated by pasteurization, but some at different temperatures, so they can be useful as time-temperature treatment indicators. Such is lactoperoxidase (LPO), is more heat resistant than AP (alkaline photo) and has also been used for distinguishing between pasteurisations of HTST and HTLT. The LPO in milk is

relatively heat stable, requiring between 5s and 3.5 min at 80°C for complete inactivation depending on the heating method (Hammershøj et al., 2010).

Lipoprotein lipase

Lipoprotein lipase (LPL) is one of the most important indigenous enzyme in milk considering raw milk cheese making. LPL is an enzyme participating in the lipoprotein metabolism where it hydrolyses free fatty acids (FFA) lipoprotein lipase activity is reduced to almost zero by HTST pasteurization, but complete inactivation happens at 80°C for 10 sec. In dairy processing, where indigenous lipase activity is desired, milk is in practice subjected to a so-called thermization treatment at temperatures of 60–67°C, at holding times from 20s to several minutes (Driessen, 1989). The lipolytic action of LPL plays a significant role in flavour development in cheeses made using raw milk, while in cheeses made from pasteurized milk residual LPL activity has a minor role in the lipolytic activity and cheese flavour (Wilkinson, 2007). Other sources of lipolytic activity:

- Rennet paste, used as coagulant in certain Italian cheese varieties, contains a potent lipase, pregastric esterase, which is responsible for lipolysis in cheeses such as Provolone and the Pecorino varieties.
- Lactic acid bacteria are weakly lipolytic, but their enzymes have been shown to contribute to the low level of lipolysis characteristic of Cheddar cheese (Collins, McSweeney, & Wilkinson, 2003a).
- *Pr. freudenreichii* subsp. *shermanii* possesses a lipase which, together with enzymes from the thermophilic starter organisms, contributes to the low level of lipolysis in Swiss cheese.
- *Penicillium roqueforti* produces potent extracellular lipases which are responsible for the extensive lipolysis characteristic of Blue cheese. In the manufacture of e.g. Roquefort and Danish Blue cheese, the activity of indigenous milk LPL is important for the flavour development during maturation of the cheese (Dickow et al., 2012). *P. camemberti* and the complex Gram-positive surface micro flora of smear cheeses also produce extracellular lipases which contribute to lipolysis in surface-bacterial or white mould-ripened varieties (P.L.H. McSweeney, 2004).

Plasmin, plasminogen

Milk contains at least two proteinases, plasmin PL (alkaline milk proteinase) and cathepsin D (acid milk proteinase) and in terms of activity and technological significance, plasmin is the most important of the indigenous proteolytic enzymes. Plasmin is derived by proteolysis of plasminogen (PLG) A complex system of inhibitors, activators and activator inhibitors having different heat sensitivities regulates the activity of PL in milk (Prado, Sombers, Ismail, & Hayes, 2006). Milk also contains an acid proteinase which is now known to be cathepsin D, a lysosomal enzyme. It is relatively heat labile (inactivated by 70°C for 10 min). At least some of the indigenous acid proteinase is incorporated into cheese curd; its specificity on α 1- and β -caseins is quite similar to that of chymosin, but it has very poor milk clotting activity (P L H McSweeney, Fox, & Olson, 1995). It may contribute to proteolysis in cheese, but its activity is probably normally overshadowed by chymosin, which is present at a much higher level in cheese. Heat treatment of milk can affect the total plasmin activity in both negative and positive directions by affecting inhibitors, activators and enzymes to different extents. Plasmin is quite heat stable; it is partially inactivated by heating at 72°C for 15 sec, but its activity in milk increases following HTST pasteurization, probably owing to inactivation of the indigenous inhibitors of plasmin or, more likely, inhibitors of plasminogen activators. It partly survives UHT sterilization and is inactivated by heating at 80°C for 10 min at pH 6.8. There is sufficient plasmin in milk to cause very extensive proteolysis, but this is not realized owing to the presence of inhibitors. Milk contains several broad-specificity plasma-derived proteinase inhibitors. With pasteurization plasmin activity is increased because these proteinase inhibitors are inactivated by heating. Plasmin and plasminogen accompany the casein micelles on the rennet coagulation of milk and are concentrated in cheese in which plasmin contributes to primary proteolysis of the caseins, especially in cheeses that are cooked to a

high temperature, e.g., Swiss and some Italian varieties, in which the coagulant is totally or extensively inactivated (P F Fox & McSweeney, 1996). β -casein is the principal substrate and even in low-cooked cheeses (e.g., Cheddar, Gouda), in which the coagulant is the principal primary proteinase, proteolysis of β -casein is due mainly to plasmin action; some hydrolysis of α s1-casein by plasmin also occurs. The level of plasmin activity in cheese varies substantially with the variety; Emmental, Parmesan, and Dutch-type cheeses contain about three times as much plasmin activity as Cheddar-type cheeses (Nana Y Farkye & Fox, 1990). This is probably due to the greater activation of plasminogen in the former as a result of inactivation of inhibitors of plasminogen activators in high-cooked cheese and their removal from Dutch-type cheese curd on washing (when whey is removed and replaced by water) (Patrick F. Fox, 2003).

According to Ginzinger et al. (1999) after the pasteurization of milk the degradation of α s1-casein proved to be delayed in cheeses made from pasteurized milk due to effects caused by heat treatment and partial elimination of the raw milk flora, an enhanced cleavage of β -casein was found in cheeses from pasteurized milk. These differences were also evident in the water soluble fraction and, partly, in fractions containing smaller breakdown products and were presumably caused by heat-induced changes in the plasmin/plasminogen complex. (Ginzinger, Jaros, Mayer, Rohm, & Tschager-, 1999) They found that in Bergkäse completely different patterns of plasmin activity between cheeses made from raw and pasteurized milk have been observed. During maturation of raw milk cheeses, the plasmin content decreased slowly, on the other hand with pasteurized cheese the plasmin content showed a pronounced increase. These differences are obviously reflected by the degree of degradation of β -casein to γ -casein, which was found to be significantly higher in pasteurized milk cheeses. According to Ginzinger et al. (1999) after the pasteurization of milk the degradation of α s1-casein proved to be delayed in cheeses made from pasteurized milk. In accordance with enhanced primary proteolysis, raw milk treatment affected the distribution of nitrogen (N) compounds and, for example, a higher amount of water-soluble N appeared in Bergkäse made from pasteurized milk (Ginzinger, Jaros, Lavanchy, & Rohm, 1999).

Other enzymes

Other indigenous enzymes and the extracellular proteinases and lipases from psychrotrophic bacteria, which are quite heat stable below 80°C (Driessen, 1983). The influence of psychrotroph proteinases on heat-treated milk is well documented, but their role in cheese ripening is less but their production is associated with the presence of microorganisms, they may have influence during the ripening of the cheese. The role of other enzymes in cheese, such as sulphhydryl oxidase which is partially inactivated (up to 60%) by pasteurization, is not clearly understood. It should also be emphasized that enzymes which are preferentially bound to casein micelles or fat globules will be retained in the cheese curd and therefore may have a significant influence during cheese ripening. However, because moisture represents a large proportion of the cheese, the role of milk serum enzymes should not be overlooked (Beuvier & Grappin, 1997).

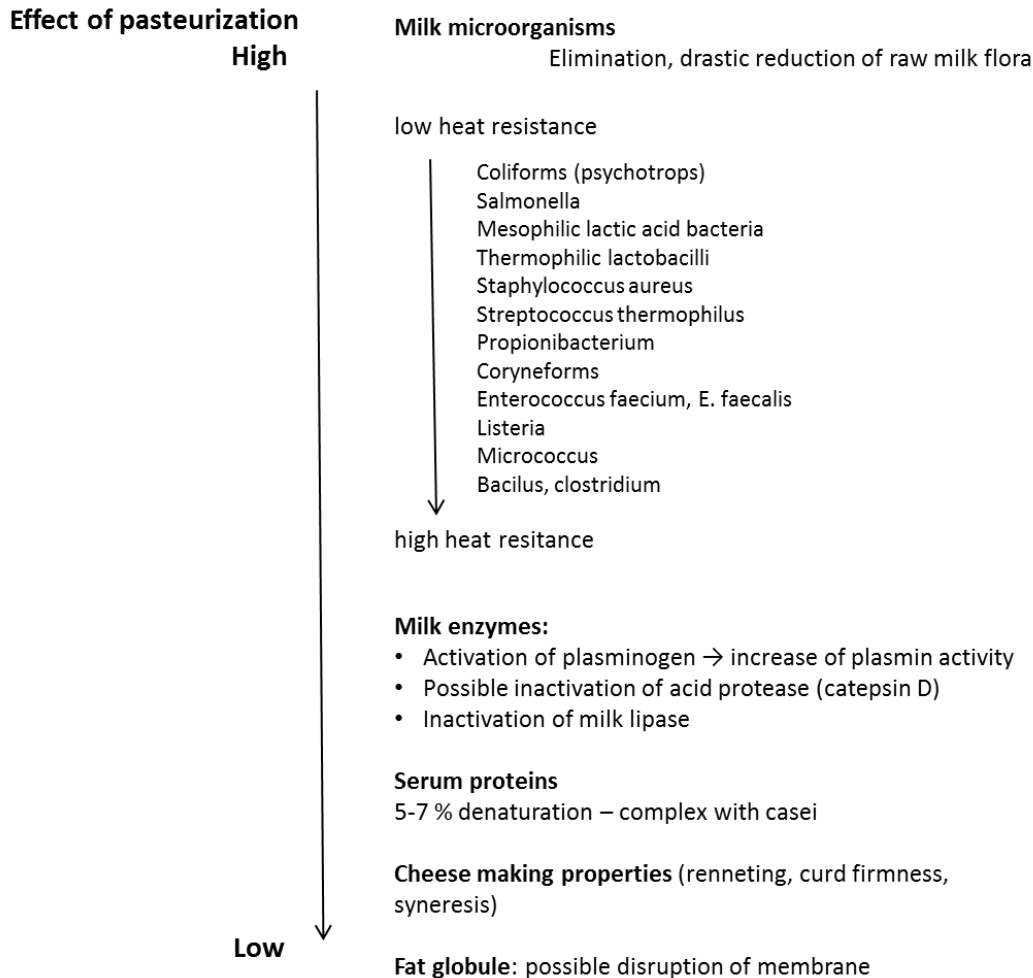


Figure 1. Summary of the consequences of pasteurization on the microbiological, enzymatic and physical characteristics of milk important in cheese making and ripening. Source: Beuviel and Grappin (1997)

Cheese made from raw or pasteurized milk

Cheeses are commonly classified according to their texture (very hard, hard, semi-hard, semi-soft, soft) which is related mainly to the moisture content of the cheese. Cheese is produced sometimes depending on the adventitious micro flora for acidification, but now usually through the growth of cultures of lactic acid bacteria. The properties of rennet-coagulated curds are very different from those produced by isoelectric (acid) precipitation, e.g., they have better syneresis properties, which make it possible to produce low-moisture cheese curd without hardening. Rennet-coagulated curds can, therefore, be converted to a more stable product than acid curds and rennet coagulation has become predominant in cheese manufacture, being exploited for c. 75% of total world production (P. F. Fox, 1993). Almost all acid coagulated and some rennet coagulated cheeses are consumed fresh. The flavour, texture and appearance of the cheese are in their final form at the end of curd production and the curds are not subjected to a period of maturation/ripening. Steps of cheese manufacture:

Step one: produce curds from the protein found in milk

- Milk is pasteurised and a bacteriological starter is added to 'sour' and thicken the milk.

- A renneting agent is added to the milk to form curds. Nowadays, most cheese produced uses a non-animal renneting agent, making the cheese suitable for vegetarians.
- The curd is left to set.

Step two: concentrate the curd

- The curds are cut so that the whey is released (soft cheeses are cut lightly while hard cheeses are cut finely).
- The curds are either 'cooked' or are piled on top of each other (the process known as 'cheddaring'), and further cut to expel whey.
- The curd is milled and salt is added (in some recipes salt is included by immersing the moulded cheese in a bath of brine for several days).
- The curd is pressed into moulds.

Step three: ripen the cheese

- The cheese is placed in storage rooms where temperature and humidity are controlled and varied according to the cheese being produced

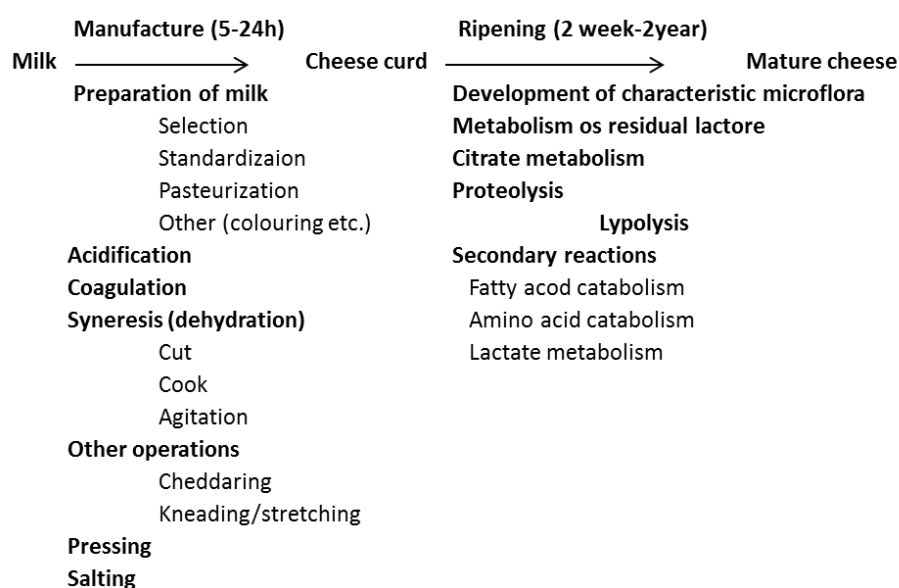


Figure 2. Steps of cheese manufacture. Source: P.F. Fox (1993)

All aspects of cheese curd production (rennet coagulation, gel firmness, syneresis) are affected by the chemical composition of the cheese milk, especially the concentrations of casein, calcium and pH. (P. F. Fox, 1993). The manufacture phase, from milk to a formed cheese can last from a couple of hours up to a day or more, according to the pressing and brining time of the different cheeses, but salting and dehydration, may continue over a longer period. Although the manufacturing protocol for individual varieties differs in detail, the basic steps are common to most varieties; these are: acidification, coagulation of caseins, dehydration (cutting the coagulum, cooking, stirring, separation of whey and curd, pressing, salting and other operations that promote gel syneresis), shaping (moulding and pressing) and salting. During manufacture the fat and casein in milk are concentrated between 6- and 12-fold, depending on the variety through dehydration processes (Patrick F. Fox, 2003). The degree of dehydration is regulated by the extent and combination of the above mentioned operations, in addition to the chemical composition of the milk. In turn, the levels of moisture and salt, the pH and the cheese micro flora regulate and control the biochemical changes that occur during ripening and hence determine the flavour, aroma and texture of the finished product. Thus, the nature and quality of the finished cheese are determined largely by the manufacturing steps. However, it is during the ripening phase that the characteristic flavour

and texture of the individual cheese varieties develop (P. F. Fox, 1993). According to McSweeney (2004) possibly six, sources are involved in the ripening of cheese (Patrick F. Fox, 2003; P.L.H. McSweeney, 2004):

- Enzymes from the coagulant (rennet or rennet substitute)
- Indigenous milk enzymes, especially plasmin
- Starter bacteria and their enzymes, which are released after the cells have died and lysed
- Enzymes from secondary starters (e.g., *Propionibacterium freudenreichii* subsp, *shermanii*, Gram-positive bacteria on the surface of smear-ripened cheese, yeasts and moulds, such as *Penicillium roqueforti* and *P. camemberti*). According to McSweeney (2007) metabolic activity of the secondary microflora often dominates flavour development, and in some cases, e.g., white mould, smear cheeses, have major influence on texture (McSweeney, 2007a)
- Non-starter bacteria, i.e., organisms that either survive pasteurisation of the cheese milk or gain access to the pasteurised milk or curd during manufacture, or during ripening
- Secondary starters & exogenous enzymes added to accelerate cheese ripening

The interaction of these numerous microbiological, biochemical, and chemical events transform the principal constituents of the cheese - the proteins, lipids, and lactose - to primary, and later to secondary, products. Biochemical reactions which occur in cheese during ripening are usually grouped into four major categories (P. F. Fox, Law, McSweeney, & Wallace, 1993):

- Glycolysis of residual lactose and catabolism of lactate,
- Catabolism of citrate, which is very important in certain varieties,
- Lipolysis and the catabolism of free fatty acids and
- Proteolysis and the catabolism of amino acids

The metabolism of lactose to lactate is essentially complete at the end of manufacture or during the early stages of ripening. Most lactose in milk is lost in the whey and that which is retained in the curd is metabolised rapidly after drainage. Lactate contributes to the flavour of cheese, particularly early during maturation, but the major effect of acidification on flavour development is indirect since, together with the buffering capacity of the curd, it influences pH and thus the growth of the secondary flora and the activity of ripening enzymes. The products of citrate metabolism include CO₂, which is responsible for the small eyes often found in Dutch-type cheeses, and important flavour compounds, particularly diacetyl, which contribute to the flavour of these cheeses (P. F. Fox et al., 1993; P.L.H. McSweeney, 2004). Fox (2003) writes that the principal flavour compounds present in most cheese varieties are: peptides, amino acids, amines, acids, thiols, and thioesters (derived from proteins); fatty acids, methyl ketones, lactones, esters, and thioesters (derived from lipids); organic acids (lactic, acetic, and propionic); carbon dioxide; esters; and alcohols (derived from lactose). The different combination and concentration of these compounds give us the diversity of flavours in cheeses all around the world. Proteolysis is essential in all rennet-coagulated cheese varieties, especially internal- and surface-bacterially ripened cheeses in which it is probably the principal biochemical event during ripening. Proteolysis contributes to cheese ripening in at least four ways: (Patrick F. Fox, 2003)

- Makes a direct contribution to flavour, or off-flavour, e.g., bitterness, or indirectly since free amino acids are catabolized to amines, acids, thiols, thioesters, etc.
- Facilitates the release of sapid compounds during mastication
- The production of NH₃ from amino acids released by proteolysis affects flavour and texture
- Changes in texture due to breakdown of the protein network, increase in pH, and greater water binding by the newly formed amino and carboxyl groups

Acidifying bacteria- starter cultures

The activity of the starter is greatly reduced at the end of manufacture or soon thereafter due to the combination of low pH, high NaCl and lack of a fermentable carbohydrate. The inhibition of acid production is particularly abrupt in dry-salted varieties (e.g., Cheddar) where NaCl concentration reaches equilibrium much faster than in brine-salted cheeses. Fresh cheese curd contains a low level of lactose which, in the case of Cheddar cheese, is reduced to trace levels within one month of ripening by the activity of the starter or by the action of the non-starter lactic acid bacteria (NSLAB) (P. F. Fox et al., 1993; P.L.H. McSweeney, 2004).

Starter cultures – LAB

The primary cultures include all the starter lactic acid bacteria and are involved in acid production during cheese manufacture and in cheese ripening (Chamba & Irlinger, 2004). Milk is also a rich source of nutrients for bacteria which contaminate the milk, some species of which utilize milk sugar, lactose, as a source of energy, producing lactic acid. When sufficient acid has been produced, the principal proteins of milk, the caseins, coagulate, i.e., at their isoelectric points (~pH 4.6), to form a gel in which the fat is entrapped (P. F. Fox, 1993). Cheese milk must be free from antibiotics, which totally, or partially, inhibit the starter bacteria; delayed acidification results in an abnormal composition and micro flora and consequently in flavour and textural defects and perhaps very significantly in the growth of harmful, pathogenic or food-poisoning microorganisms. Today frozen starter cultures are wide spread in the dairy industry, because the spontan acidification of milk by the native micro flora acidifies the milk too slow for large scale industrial production. Both industrial and small-scale manufacture of fermented dairy products now almost always relies on industrially prepared starters. These starter cultures are selected and maintained by sub cultivation in milk. The industrial starter culture production has reduced the number of strains present, because mainly a few stable and effective strains are chosen and produced. The use of these starter cultures, which is also necessary when fermenting pasteurised milk, is responsible for a certain uniformity of the milk products and cheeses. The starter culture is responsible for the acidification during the first stages of cheese manufacture and may reach up to 10^9 colony-forming units (cfu) per gram of cheese. During ripening, however, the number of starter cfu generally decreases rather quickly to lower than 10^7 g⁻¹. Non-starter adventitious lactobacilli and lactic acid bacteria, which apparently originate from the milk or the environment, grow out subsequently and may reach numbers higher than those of the starter (Wouters et al., 2002). McSweeney et al. (1993) and Beuvier et al. (1997), working on Cheddar and Swiss-type cheeses, respectively, found that the decline in mesophilic or thermophilic starter bacteria was slower in raw milk cheeses than in pasteurized cheeses. They also found that adventitious bacteria in raw and in pasteurized cheeses generally had similar growth curves but in raw milk cheeses these bacteria reached the maximum faster, because of their high number at the beginning of ripening (Beuvier et al., 1997; P.L.H. McSweeney, Fox, Lucey, Jordan, & Cogan, 1993). Lactic acid bacteria also possess lipolytic enzymes. They hydrolyze tri, di and mono acylglycerols and form FFA. In general, LAB lipolytic activities appear to be quite weak in comparison to other bacteria and moulds. But since it persist in natural cheese their contribution to lipolysis over an extended ripening period may be substantial (Wilkinson, 2007).

Non-starter lactic acid bacteria

The secondary micro flora comprise of non-starter lactic acid bacteria (NSLAB) which grow internally in most cheese varieties and other bacteria, yeasts and/or moulds, which grow internally or externally and are usually unique to specific cheese varieties or closely related types. NSLAB are mesophilic lactobacilli and pediococci, which form a significant portion of the microbial flora of most cheese varieties during ripening. They are not part of the normal starter flora; they generally do not grow well in milk (Cogan et al., 1997), and do not contribute to acid production in the cheese vat. Lactobacilli are traditionally divided into three groups on the basis of being either:

- (I) Obligatory homofermentative,

- (II) Facultatively heterofermentative,
- (III) Obligatory heterofermentative

The NSLAB lactobacilli regularly encountered in cheese are members of the facultatively heterofermentative group (II), and are thus sometimes referred to as facultatively heterofermentative lactobacilli (FHL) (Beresford, Fitzsimons, Brennan, & Cogan, 2001). Non-starter lactic acid bacteria are contaminants that originate from either the raw milk or the factory environment and consist predominantly of *Lactobacillus paracasei* and *Lb. rhamnosus* as well as less common species such as *Pediococcus*, *Lb. brevis*, *Lb. fermentum*, *Lb. plantarum*, *Lb. coryneformis* subsp. *coryneformis*, *Lb. casei* and *Lb. curvatus*. Both the lactic acid starter culture and NSLAB are key agents in the degradation of milk protein during maturation and their proteolytic systems, particularly those of the *Lactococcus* starter cultures, have been extensively investigated (Broome, 2007).

According to several authors the non-starter lactic acid bacteria (NSLAB) in cheese microfloras contain strains, which are essential for producing the characteristic flavours of traditional cheeses (Shakeel-Ur-Rehman et al., 2000; Wouters et al., 2002). Since NSLAB dominate the micro flora of many long ripened cheeses, they are believed to contribute to the maturation of cheese. Non-starter lactic acid bacteria such as mesophilic lactobacilli, grow regularly during ripening, reaching 10^7 - 10^8 cfu g⁻¹ in raw milk cheeses and one or two log cycles lower in pasteurized cheeses at the end of ripening (Beuvier et al., 1997; P.L.H. McSweeney et al., 1993). Since the proteolytic systems of NSLAB are generally similar to those of other LAB, they appear to contribute to proteolysis in a similar way to the starter, but to a lesser extent since maximum NSLAB numbers in cheese (often c. 10^7 - 10^8 cfu g⁻¹) are lower than maximum numbers of starter (c. 10^9 - 10^{10} cfu g⁻¹) (P.L.H. McSweeney, 2004). McSweeney et al. (1993) showed that strains of *Lactobacillus* spp., which dominated the non-starter micro flora in Cheddar cheese, were much more heterogeneous in the raw milk cheese than in the pasteurized cheese. NSLAB contributes to the formation of small peptides and amino acids, which are the precursors for the flavour components (P.F. Fox, 1998; P.L.H. McSweeney et al., 1993). It thus seems likely that the indigenous NSLAB are at least partly responsible for the difference in flavour between pasteurized and raw milk cheese (Wouters et al., 2002). Dasen et al. (2003) found that pasteurization of milk resulted in a significant alteration of the *Lactobacillus* species and strain profile in the cheese (elimination of most of the *Lb. paracasei* strains and all the *Lb. plantarum* strains) (Dasen, Berthier, Grappin, Williams, & Banks, 2003). put diagram here of raw milk NSLAB reach top faster than in pasteurized Beuvier and Grappin (1997) found that facultatively heterofermentative lactobacilli, Micrococcaceae, enterococci, and propionibacteria in Swiss-type cheese, are found at higher levels in raw milk cheese (Beuvier & Grappin, 1997). Among the non-starter flora present in the raw milk cheeses, facultatively heterofermentative lactobacilli FHL played a major role. Besides The FHL, which partly have their origin in recontaminations from the cheese plant environment, did not increase to the same level in the cheeses made from pasteurized milk as in the raw milk cheeses (Eliskases-Lechner & Ginzinger, 1999).

Secondary starters & enzymes

Fox (1989) writes that in some cases additional secondary starters and enzymes are added during cheese manufacture. Enzymes from secondary starters like: propionic acid bacteria, *Brevibacterium linens*, yeasts and moulds, such as *Penicillium roqueforti* and *Penicillium candidum*) are of major importance in some varieties (P.F. Fox, 1989). Also exogenous plasmin can be added to milk, which is incorporated and uniformly distributed during production, in which it accelerates proteolysis and maturation (N. Farkye & Fox, 1992). The casein micelles in bovine milk are capable of binding approximately 10 times as much plasmin as occurs naturally in milk. When other exogenous proteinases are added to cheese milk, much of the added enzyme is lost in the whey, increasing cost and creating potential problems for whey processors. The yield of cheese may also be decreased owing to early hydrolysis of casein in the vat. For Cheddar-type cheese, exogenous proteinases may be added to the milled curds at salting, but the enzyme is concentrated at the surface of the

curd chips. Activation of indigenous plasminogen by added urokinase also accelerates proteolysis (Barrett, Kelly, McSweeney, & Fox, 1999; Bastian & Brown, 1996; Patrick F. Fox, 2003). The addition of selected NSLAB or secondary enzymes to pasteurised milk cheese can improve flavour and accelerate ripening although occasional negative effects do arise (Broome, 2007).

Rennet & coagulation

In most cheese varieties, the initial hydrolysis of caseins is caused by the coagulant and to a lesser extent by plasmin and perhaps somatic cell proteinases (e.g., cathepsin D) which result in the formation of large (water-insoluble) and intermediate sized (water-soluble) peptides which are subsequently hydrolysed by the and enzymes from the starter and non-starter flora of the cheese (Lawrence, Gilles, & Creamer, 2004). The rennet coagulation of milk is a two-stage process. The first (primary) phase involves the enzymatic production of “para-casein” and TCA-soluble peptides (glycomacropeptides), while the secondary phase involves the Ca-induced gelation of para-casein at a temperature in the range of 30–35°C (Patrick F. Fox, 2003). According to Rothe et al. (1977) the production of small peptides and amino acids is caused by the action of microbial proteinases and peptidases, respectively. Preparations of selected aspartyl proteinases are used to coagulate milk. Chymosin is the principal proteinase (88-94%) in traditional calf rennets, the remainder being pepsin (Rothe, Harboe, & Martiny, 1977). Although, the principal role of the coagulant in cheese making is to coagulate milk, some activity is retained in the curd, depending on factors such as coagulant type, cooking temperature and pH at drainage, and contributes to proteolysis in many varieties (P. F. Fox et al., 1993). Cold storage after heat treatment aggravates the adverse effects of heating on the rennet coagulation of milk; this effect is known as rennet hysteresis. Rennets produced from a range of plant species, e.g., fig and thistle, also appear to have been common in ancient times. However, plant rennets are not suitable for the manufacture of long-ripened cheese varieties and gastric proteinases from young animals became the standard rennets until the growth of the dairy industry demanded the introduction of rennet substitutes, and fermentation produced chymosin (P. F. Fox, 1993).

It is well known that heating milk prior to renneting changes the coagulation properties by denaturation of β -lactoglobulin and its subsequent binding to casein, that is why pasteurisation reduces rennetability (clotting time is increased and curd firmness is reduced) and syneresis of milk. Differences in clotting times induced by pasteurization are due to modifications of the mineral equilibrium and formation of complexes between κ -casein and β -lactoglobulin (Ginzinger, Jaros, Mayer, et al., 1999). Many whey proteins are denatured favouring their interaction with κ -casein which, in turn, does not readily interact with chymosin (Lieske, 1997), thus increasing renneting time (Hávarria et al., 2000). According to Hougaard increasing heat treatment also increases the renneting time and curd firming rate in case of instant infusion pasteurization (IIP) at temperatures of 72 °C, 100 °C and 120 °C (Hougaard, Ardö, & Ipsen, 2010).

Fresh cheese

Fresh cheeses are unripened cheeses, which are manufactured by the coagulation of milk, cream or whey using acid, a combination of acid and rennet or a combination of acid and heat. Fresh cheeses are ready for consumption immediately after production. There are various regional types of fresh cheeses, of which some of the more known ones are: Cream cheese, Cottage cheese, Quark or Tvorog, Fromage frais and Ricotta. Fresh cheeses are generally low in dry matter (DM), low in fat and protein and high in lactose/lactate. As most of the calcium is solubilised during the acid coagulation and removed with the whey, fresh cheeses are much lower in calcium than rennet-curd cheeses (Schulz-Collins & Senge, 2004). Fresh cheeses grouped in two categories: acid coagulated cheeses (where rennet might be added) acid-heat coagulated cheeses.

Acid coagulated cheese is for example the cottage cheese, which is produced by acid coagulation of pasteurized skimmilk. The minimum heat treatment given to skimmilk for cottage cheese manufacture is the minimum allowable pasteurization temperature. Excessive heat treatment of milk (i.e., higher pasteurization temperature and/or longer time) results in a soft coagulum from which it is difficult to expel whey. The skimmilk used for cottage cheese manufacture must be of good microbial quality and have a high dry matter (DM) content to ensure good quality and yield of cheese. The mode of setting (incubation) or acidifying milk for cottage cheese manufacture depends on whether cultured or direct-acid cottage cheese is being made. For cultured cottage cheese, acidification is done by harmless mesophilic lactic acid bacteria while for direct-set (or direct acid) cottage cheese, acidification is done using a combination of organic acid, mineral acid or acidogen. The use of food-grade acids or acidogen instead of starter bacteria is an alternate method for manufacturing cottage cheese. A very low level of milk-clotting enzyme (chymosin or chymosin substitute) is required for the manufacture of cottage cheeses (N.Y. Farkye, 2004).

Acid-heat coagulated cheeses are generally fresh, soft, unripened varieties. Several cheeses are manufactured throughout the world by a combination of acid and heat coagulation of milk. Notable are Queso Blanco (Central and South America), Paneer (India) and Ricotta (Italy) (N.Y. Farkye, 2007). Coagulation and curd formation in this group of cheeses is by a combination of acid and heat, usually above 70°C (Phadungath, 2005).

When tasting freshly made curd it is often difficult to differentiate between types of cheeses, because the flavour develops during ripening. Awad et al. (2006) found that Ras cheese made from raw milk at day 1 of manufacture, the moisture content and pH were lower than in pasteurized milk cheeses. In the sensory evaluation conducted by Awad et al. (2006) comparing raw and pasteurized cheeses, the raw milk cheeses received the highest texture and flavour scores by panellists. He concluded that the levels of water-soluble nitrogen, casein breakdown, free amino groups and free fatty acids were higher in cheese made from raw milk than in that made from pasteurized milk. Taste and aroma are very important features of cheese. Consumers make their choice of cheeses primarily on the basis of flavour characteristics. The results of the study done by Awad et al. (2006) confirm the general view that cheese made from raw milk develops the characteristic flavour more rapidly than that made from pasteurized milk. Lactic acid bacteria in raw milk are important not only for the acid development which hastens the milk coagulation and assists in expulsion of whey, but also because of their influence on flavour, body and texture of the final cheese (Awad, 2006).

Small cheeses – surface and inside ripening

Some cheese produced with secondary micro flora such as bacteria, moulds and yeasts. Bockelmann (2004) writes that these secondary cultures contribute significantly to the complexity of cheese manufacture, because these aerobic microorganisms impact on the appearance, flavour and texture development of cheeses substantially, which usually leads to shorter ripening periods of several weeks rather than months. A high level of hygiene must be maintained because the cheese surfaces are exposed to an unsterile environment during ripening and undesirable contaminants will grow immediately if the balance of the cheese surface micro flora is disturbed. Some surface ripened cheeses are ripened with the help of moulds, such as Camembert and Brie. Blue-veined cheeses, such as Roquefort and Gorgonzola, are special surface ripened varieties, where the surface is extended into the core by piercing the freshly made cheeses with needles, allowing the growth of *Penicillium roqueforti* on these inner surfaces. *Penicillium camemberti* and *P. roqueforti* possess several extracellular proteolytic enzymes; these enzymes contribute to cheese-ripening by liberation of amino acids and have a debittering effect in cheeses (Bockelmann, 2007). By bacterial smear ripened cheeses or washed rind cheeses (red smear cheeses) such as Tilsit and Romadour the surface of the cheese is inoculated by washing with a combination of bacteria, yeast and brine solution. Generally, only a few species like *Debaryomyces hansenii* and *Brevibacterium linens* are used as smear adjuncts (Bockelmann, 2002). Deacidification of

smear cheeses by lactate degradation and alkaline metabolites, like ammonia, cause softening of the cheese matrix. The effect of the surface flora on texture is more visible near the surface (0±5mm depth) with a more intense and diverse protein degradation. Volatile aromatic sulphur compounds originating from methionine and cysteine are likely to be key components of smear cheese flavour and contribute to the garlic, cowshed and fecal notes (Bockelmann, 2007; Brennan, Cogan, & Cork, 2004).

Bergkäse represents a surface-ripened cheese variety. Ginzinger and Eliskases-Lechner (1999) compared Bergkäse made from raw milk and pasteurized milk cheeses and observed a trend towards higher contents of lactic and citric acids but lower amounts of succinic, acetic, propionic and formic acids for cheeses made from pasteurized milk. No significant propionic acid fermentation and, consequently, eye formation was observed in any of the cheeses made from pasteurized milk. The levels of the organic acids significantly differed from those of the cheeses made from pasteurized milk presumably due to the growth of the indigenous flora in the raw milk cheeses. The decrease in the content of lactate, which is primarily metabolised by the microorganisms of the smear surface, may be attributed to the migration of lactic acid from the centre to the surface layer. After 3 months of ripening, however a more pronounced decrease in the lactate content for raw milk cheeses was observable in which propionic acid fermentation occurred (Eliskases-Lechner & Ginzinger, 1999). They found that pasteurized mature Bergkäse samples showed reduced intensities (flat sensory character) of the typical Bergkäse flavour as well of smell and aroma, with increased bitterness. Ginzinger et al. (1999) concluded that raw milk and its indigenous micro flora are essential to produce Bergkäse of satisfactory sensory quality (Ginzinger, Jaros, Lavanchy, et al., 1999).

Large cheeses undergoing maturation

Hard cheeses are usually made in bigger size and are ripened after manufacture for periods ranging from a few months to 2 or more years. During this ripening period that the flavour and texture characteristic of the variety develop, which is highly dependent on milk quality, the manufacture methods (cooking temperature, curd size, washing of the curd, size and shape of the cheese, salting, pressing, etc.) and ripening conditions. Cheese ripening usually involves changes to the micro flora of the cheese, often death and lysis of starter cells, the development of an adventitious non-starter micro flora and, in certain cases, the growth of secondary organisms. We differentiate between low temperature hard cheese like Cheddar, high temperature cheeses like Italian and Swiss cheeses and washed semi hard cheeses like Gouda and Edam (Mcsweeney, 2007a).

Remy Grappin and Eric Beuvier (1997) wrote in their report that in raw milk cheeses such as the Swiss, Cheddar, Manchego, Raclette and St. Paulin, the indigenous raw milk micro flora and starter bacteria appeared to be extremely important in the ripening process and subsequent flavour and texture of the cheese. They compared the same cheeses made from raw and pasteurized milk, and concluded that, "pasteurization modifies the biochemistry and microbiology of ripening, and the flavour and texture of cheese" (Beuvier & Grappin, 1997). According to Beuvier & Grappin (1997) raw milk cheeses developed characteristic flavour sooner and the flavour was stronger, richer and more diverse (less uniform) than the same cheeses made from pasteurized milk. They found that facultatively heterofermentative lactobacilli, Micrococcaceae, enterococci, and propionibacteria in Swiss-type cheese, are found at higher levels in raw milk cheese. Milk pasteurization leads to a significant decrease of the amount of small peptides and free amino acids. Also in experimental trials comparing raw and pasteurized cheeses, Beuvier & Grappin (1997) on Swiss-type cheese and Lau et al. (1990) on Cheddar found no significant modification of clotting parameters, but a slightly, not statistically significant, higher level of moisture in cheeses made from pasteurized milk (Beuvier & Grappin, 1997; Lau, Barbano, & Rasmussen, 1990). It has been shown in several studies that hard cheeses made from raw milk show a more pronounced aroma and flavour, which is presumably related to increased proteolysis, and that bitterness defects are

more often in cheeses made from pasteurized milk. During manufacture of Cheddar cheese McSweeney et al. (1994) were successful in improving the cheese flavour by using strains isolated from raw milk cheese. This improvement was believed to be due to increased formation of amino acids (P.L.H. McSweeney et al., 1994; Wouters et al., 2002). In Beuvier and Grappin's (1997) study sensory analysis showed that, in all cases, pasteurized milk cheeses have received lower flavour intensity scores than raw milk cheeses. Beuvier and Grappin (1997) concluded that the indigenous milk micro flora, with its diversity of species and strains, appears to be mainly responsible of the specific sensory properties of raw milk cheeses (Beuvier & Grappin, 1997).

Hávarria et al. (2000) researched the effects of pasteurization on Idiazabal cheeses and found that milk pasteurization significantly affected ($P = 0.01$) decreased the levels of short chain (C4-C10) FFA as well as their total amount. Milk pasteurization reduced the levels of acetic acid by 99% after 90 ripening days at the three times of the year studied (Hávarria et al., 2000). Esters are highly flavoured and are formed when FFAs react with alcohols. In Gouda cheese, the majority of the esters present are ethyl esters of long-chain fatty acids Gouda cheese made from raw milk showed a greater and constant increase in ester formation compared to cheese made from pasteurized milk.(Wilkinson, 2007)

Food safety and cheeses

Eliskases-Lechner and Ginzinger (1999) found that the pasteurisation process eliminated the heat-sensitive raw milk flora such as coliforms, *S. aureus* and, to a lesser extent, lactic acid bacteria. Growth of the microorganisms in the cheese mass was strongly reduced by acidification and curd cooking temperature (Eliskases-Lechner & Ginzinger, 1999). In accordance with Bachmann and Puhan (Bachmann & Puhan, 1994), who studied the behaviour of pathogens in Swiss-type cheeses, they found no incidence of the ability of potential pathogens to survive the production process. *E. coli* and *S. aureus* were $< 0.1 \text{ cfu}^{-9}$ in the cheese after 1 week of ripening (Eliskases-Lechner & Ginzinger, 1999).

HACCP and cheese production

In the European Union in case of raw milk cheeses the safety of consumers is assured with strict sanitation standards and dairy inspections. These are administered by each Member State along the principles of Hazard Analysis and Critical Control Points (HACCP) (Knoll, 2005). For unpasteurized milk cheese, milk production is the first critical control point (CCP) in the cheese maker's Hazard Analysis and Critical Control Point (HACCP) plan (Marler, 2009). The microbiological quality of the milk is out most important, but the quality of cheese is also influenced by equipment and environmental hygiene during manufacture, packaging and handling (Robinson & Tamime, 2002).

PDO cheeses

Animal feeding is especially important for quality labelled products (e.g., Protected Designation of Origin and Protected Geographical Indication), the basis of which is a link with conditions of production related to a "terroir" context (Coppa, Ferlay, et al., 2011). The local forage based diets are part of the basic link between dairy products and their original 'terroir', a notion at the basis of the PDO labelling and image of the product quality from sensory, nutritional, or safety points of view (Engel et al., 2007). The forages are known to confer specific organoleptic and nutritional qualities on the milk products (Martin, Verdier-Metz, Buchin, Hurtaud, & Coulon, 2005) and to provide an added value to the product that could justify its higher price and offer the consumers a healthy image of the environment. Revello et al. (2010) observed significant variability in FA composition and terpene profile in cow milk and "Toma Piemontese" PDO cheese, which was linked to the cows diets. The result confirmed that fresh mountain forage plays a key role to obtain milk with more a favourable FA and terpene profile, than conventionally produced lowland milk. According to Revello et al. (2010) these molecules can be used to trace and/ or confer specific organoleptic and nutritional properties to the dairy products that provide an added value to the product and

justify its higher price, due to elevated production costs (Revello Chion et al., 2010). Preserving certain animal breed specificities is also important for PDO cheese production, like β -casein variant C in the Tarentaise cattle breed for Beaufort cheese production, but also maintaining meadow biodiversity is crucial, so that cheeses best reflect the originality and diversity of the native land where they are produced (Coulon, Delacroix-Buchet, Martin, & Pirisi, 2004). According to Bugaud et al. (2001) the influence of milk production factors on the sensory characteristics of ripened cheeses is of particular importance for cheeses with a “protected denomination of origin”, where the milk used undergoes few or no modifications before cheese making (Bugaud, Buchin, Noël, & Tessier, 2001). This is why many PDO cheese varieties are still produced with raw milk.

Chapter III. – Cheese made from organic milk

Organic cheeses are made with organic milk from farms that follow the organic standards. Cows that produce the milk are fed organic foods with no synthetic chemicals. In addition, farmers maintain their grazing areas without any pesticides or chemical fertilizers, and refrain from using hormones or genetically modified organisms (GMOs). The animal should graze on organic pastures 'wherever conditions allow'. EU waves outdoor access for herbivores in winter under certain conditions. According to regulations the rearing systems for herbivores are to be based on maximum use of pasturage according to the availability of pastures in the different periods of the year (EEC, 1991). Farm management and feeding practices clearly differ between organic and conventional dairy production, organic cows ate less concentrates and forage maize, and more silage of grass clover and hay.

Several studies confirmed the effects of grazing, and hay feeding compared to silage and concentrate feeding on milk and cheese quality (Bugaud et al., 2001; Coulon et al., 2004; Martin et al., 2005; Verdier-Metz, Martin, Pradel, & Albouy, 2005). Organic cheese minimizes exposure to the toxins and pesticides often associated with factory farming practices. For many people this is an important consideration for buying organic milk. However, for others the main reason for buying organic is the idea that organic food is healthier. Tsiplakou et al. (2010) writes that it is complicated discussing the effects of organic farming on food quality (e.g. FA profile of milk fat), because organic production systems are not well-defined, but differ widely between countries, or in some cases even within the same country. In Denmark, for example, the conventional farms compared with the organic ones use different forages, whereas in Sweden the feeding strategies between the two systems are very similar (Toledo, Andrén, & Björck, 2002). There are different feeding strategies and many sources of feeds like pasture, conserved forages (hay, silages, etc.) and concentrates which are used in animal diets and have an effect on the quality of the milk and the cheese (Tsiplakou, Kotrotsios, Hadjigeorgiou, & Zervas, 2010).

Bloksma et al. (2008) found in his study that the organic milk generally scored better than the conventional milk for both the conventional and holistic measures (Bloksma et al., 2008). Butler et al. (2011) also found that organic or low-input management is more likely to result in milk fatty acid profiles that are higher linolenic acid and/or beneficial isomers of conjugated linoleic acid and antioxidants compared with conventional production (Butler et al., 2011). According to Collomb et al. (2008) organic and conventional milks did not significantly differ with respect to saturated fatty acids (SFA) nor trans FA contents, but organic milk had significantly higher contents of polyunsaturated FA (PUFA), conjugated linoleic acid (CLA), n-3 FA and branched FA. Conventional milk had higher contents of monounsaturated FA (MUFA) and n-6 FA. These results can be explained by the significantly higher levels of grasses and lower levels of cereal concentrates in the fodder of organic farming (Collomb et al., 2008). Coulon et al. (2004) writes that another important effect on the sensory characteristics of cheeses is the effect of terpenes. These plant-specific molecules, when

concentrated, have recognised aromatic properties. These molecules very rapidly pass into the milk (Viallonista et al., 2000) and are found in cheese in a much greater quantity when the animals are fed dicotyledon rich natural grass forage than when they are fed concentrate based rations (L Moio, Rillo, Ledda, & Addeo, 1996). Terpenes also seem to have an effect on enzymatic activity in the cheese during ripening (Coulon et al., 2004).

Manufacture of organic cheese

Organic cheese is produced following the same manufacture methods as with non-organic cheese, the only differences are in some substances, which are not allowed in organic cheeses, like GMO produced chymosin and some additives used in conventional production as colorants, flavour enhancers or preservatives.

Organic dairy farming. Effect of different diets on milk and cheese quality

The microbiological and chemical composition of milk and the sensory quality of cheese depends on factors linked to animal management, feeding, genetics and physiology, which all play a crucial role in the milk quality and especially in raw-milk cheese. Partial milk skimming and homogenization to standardise the fat/protein ratio practically obliterates the breed effect on cheese texture. It is therefore likely that certain cheese making technologies are better suited than others to express the effects of some of the above mentioned factors (Coulon et al., 2004). Special characteristics of cheeses are due to the presence of specific molecules or structures in milk directly induced by feeding (carotenes and terpenes) or produced by the animals (plasmin, fatty acids and casein micellar structures) according to their genetic or physiological characteristics or under the effect of specific diets. Modifications of the sensory characteristics of cheeses can depend on different mechanisms (Coulon et al., 2004):

1. Transfer into milk and cheese, without modification, of molecules from feed
2. Protein and fat modifications (content, structure and composition)
3. Transfer in cheese of blood or milk endogenous enzymes which modify proteolysis and/or lipolysis during ripening
4. Microbial ecosystem modifications (composition, dynamics and activity)

In this work we focus on animal feeding, which is important because of its effect on the nutritional properties of milk, in particular the fatty acid (FA) profile. Several studies have been conducted to elucidate the effects of animal feeding on cheese sensory properties (Bonanno et al., 2013; Cifuni et al., 2007; Coulon et al., 2004; Martin et al., 2005) and the differences between pasture and preserved forage derived cheeses are well known. Pasture provides softer, creamier, more elastic, and yellower cheeses, with a more sour and more intense odour, but also a more intense taste (Agabriel et al., 2004; Carpino et al., 2004; Urbach, 1990). According to Coulon et al. (2004) major differences in sensory characteristics were observed between cheeses made with milk produced by cows fed winter diets (based on hay and grass silage) or turned to pasture in the spring. Saint-Nectaire cheeses made with pasture milk were yellower, with a less firm texture, stronger taste and less piquant, less sour and less fruity flavour than those made with winter milk (Verdier, Coulon B., Pradel, & Berdagué L., 1995). In the Comté region, Monnet et al. (2000) found evidence of associations between the floristic typology of pastures and the sensory characteristics of cheeses, they showed that botanical diversity could be associated with more diversified and numerous cheese aromas (Coulon et al., 2004; Monnet, Berodier, & Badot, 2000). Agabriel et al. (2004) found that cheese made from the milk of cows fed more intensively, had stronger flavour and less elastic texture, which was consistent with the trends of their chemical composition: slightly higher pH and proteolytic and lipolytic levels on the average (P. F. Fox et al., 1993). These farms supplemented the feeding throughout the year by a larger proportion of concentrate feeds (Agabriel et al., 2004). Studies show the effect of using maize silage in the diet compared to grass based rations (Martin et al., 2005; Verdier et al., 1995). Cheese made from the milk of cows fed mainly a maize based diet was whiter, slightly firmer and globally rated lower by tasters, by comparison with grass based diets, regardless of its preservation mode. Cheese colour is also dependent on forage composition. Milk

contains variable amounts of pigments. The best known is carotene, which is present in large amounts in green forage and contributes to the yellow coloration of dairy products. Highly sensitive to ultraviolet light, carotene is destroyed during forage drying and preservation, in a manner proportional to light exposure (Park, Anderson, Walters, & Mahoney, 2014). According to Verdier et al. (1995) maize silage contains very little carotene so it produces very white cheeses (Verdier et al., 1995). Coulon et al. (2004) writes that recent results have shown that feeding goat's alfalfa hay led to cheeses with much more intense flavour than maize silage (Coulon et al., 2004). Certain specific defects of flavours could be recorded when silage is poorly preserved (Urbach, 1990). Poor quality silage is especially a problem in cooked cheese (like gouda), where the presence of butyric spores in silage and in milk may lead to major problems in ripening cheeses (late blowing, poor taste and odour) (Demarquilly, 1998). Many of the Cruciferae, when they are fed in large quantities, give off-flavours and a penetrating smell in dairy products due to mustard oil released during digestive fermentation (Vassal, Delacroix-Buchet, & Bouillon, 1994).

Urbach (1990) found that cow diets that are low in lipids, like cows grazing on pastures or hay feeding produces a hard milk fat high in the cheesy flavoured endogenous fatty acids. High lipid diets in the other hand contained less methyl ketones, and coconut peachy-flavored δ -lactones. Lush pastures produce a richly coloured milk fat and introduce phytol, dihydrophytol, phytene, and phytadiene, and probably their lower homologues, into the milk fat. Urbach (1990) also writes that many feeds and weeds cause off-flavours. Many gramineous species are known to have an undesirable effect on the taste and smell of milk, and many legumes can transmit bitter flavours to the milk (Urbach, 1990). Many odour active compounds, such as lactones, ketones in general and aldehydes, are known to derive from the oxidation of milk unsaturated FAs and from lipolysis (Collins, McSweeney, & Wilkinson, 2003b; Luigi Moio, Dekimpe, Etievant, & Addeo, 1993). Differences in milk FA profiles according to pasture botanical composition have also been reported (Collomb, Bütikofer, Sieber, Jeangros, & Bosset, 2002). Coppa et al. (2011) also found that the texture difference between pasture vs. hay derived cheeses, such as the lower firmness and greater creaminess resulted mainly from the difference in feed, which was previously also found by other studies (Agabriel et al., 2004; Carpino et al., 2004; Coppa, Verdier-Metz, et al., 2011). The PUFA richness of pasture diets could induce changes in structure of fat globules, increasing the polarity of their membrane because of high PUFA content (Lopez et al., 2008). According to Coppa et al. (2011) the consequent lower fat cohesion could cause a partial destruction of the fat globules during pressing, which causes oiling-off. They have found that the greater amounts of fat deposited on the rind of the pasture-fed cow cheeses may have partially inhibited the microbial activity responsible for rind appearance (Coppa, Ferlay, et al., 2011). The literature reports various differences in cheese odour, aroma and taste according to botanical composition in on-farm studies. But in the trials of Coppa et al. (2011) the effect of botanical diversity on cheese sensory properties proved surprisingly weak, suggesting that other factors, such as herbage phenology and cow grazing selection (both related to grazing management) may be involved in determining cheese sensory properties. The differences between the grazing systems became perceptible only after long term ripening of the cheeses (Coppa, Verdier-Metz, et al., 2011). On the other hand Bonnano et al. (2013) showed that the physical, chemical, and sensory characteristics of Caciocavallo Palermitano cheese were influenced more by the farming system than by the cheese making technology. Bonnano compared cheese produced through intensive farming and cheese from extensive farming, after which he found that the cheese from extensive farming was richer in polyunsaturated, n-3, and odd- and branched-chain fatty acids, as well as in conjugated linoleic acid (cis-9,trans-11 C18:2) (Bonanno et al., 2013).

Cifuni et al. (2007) found a total of 84 compounds of the following chemical families in mozzarella cheese, comparing hay vs ray-grass silage feeding: hydrocarbons, fatty acids, esters, alcohols, aldehydes, ketones and terpenes. The research data indicated difference between the aromatic profiles of mozzarella cheese as consequence of feeding systems. In

their opinion the differences in mozzarella cheese flavour are primarily caused by concentration differences of a common set of flavour compounds, rather than by the occurrence of compounds uniquely associated with a particular feed (Cifuni et al., 2007; Stefanon & Procida, 2004). Stefano writes that the microbial and chemical fermentations in cheese during ripening affect cheese flavour, rather than a direct transfer of flavour molecules from milk. According to Cifuni et al. (2007) the link between diet and milk composition and cheese quality is even more relevant for cheeses which originate from DOP areas, since very few or no modification of raw milk is allowed (Cifuni et al., 2007). In the research done by Coppa et al. (2011) the cheese making technology seemed to be critical, but also the microbiological and chemical composition of the milk, which depends on animal management, feeding, genetics, and physiology stage played a crucial role. Feeding and genetics are especially important for products in which the raw material is only slightly modified, such as raw-milk cheeses. According to Coppa et al. (2011) grazing management and herbage phenological stage seem to be more relevant for milk and cheese characteristics than pasture botanical composition (Coppa, Ferlay, et al., 2011). Lucas et al. (2001) also found that the botanical composition, maturity stage and preservation mode of forages fed to the ruminants are factors known to influence the milk composition in FA, vitamins and carotenoids (Chilliard, Ferlay, & Doreau, 2001; Lucas et al., 2006). Similarly the quantity and of nature of the, concentrate in the diet the greatly affects the FA profile of the milk (Chilliard, Ferlay, Mansbridge, & Doreau, 2000). Lucas et al. (2006) writes that mineral and vitamin supplementation increases the contents of certain of these micronutrient in the milk. The manufacturing process also plays a crucial role, especially with artisan cheeses, like raw, PDO and regional type traditional cheeses, because cheese making methods affect the composition of the original milk differently. For example, the mineral composition of cheese greatly varies according to the rate of acid production and the pH of whey at draining (Lucey & Fox, 2014), while its content of vitamin B mainly depends on the level of microbial synthesis (Lucas et al., 2006; Reif, Shahani, Vakil, & Crowe, 2014).

Different compounds:

Fatty acids influenced by feed

McSweeney (2004) writes that fatty acids have a direct impact on the flavour of many cheese varieties. In particular, C₄-C₁₀ fatty acids are strongly flavoured. Levels of fatty acids vary considerably between varieties. Free fatty acids (FFAs) are important particularly in Italian-style cheeses. Many internal bacterially ripened varieties (e.g., Edam, Swiss and Cheddar) contain low levels of fatty acids (c. 200-1000 mg kg⁻¹). Very high levels of free fatty acids are found in Blue cheese (c. 30 000 mg kg⁻¹). In addition to their direct role in cheese flavour, fatty acids are important precursors for the production of other volatile flavour compounds during ripening like aldehydes, methyl ketones, esters and lactones. (Qian & Burbank, 2007) Fatty acid esters are produced by reaction of fatty acids with an alcohol; ethyl esters are most common in cheese. (P.L.H. McSweeney, 2004)

According to several studies different feeding systems affect the fatty acid profile of the milk, and consequently the cheese. Collomb et al. (2008) found that organic milk had significantly higher contents of polyunsaturated FA (PUFA) ($\pm 5.5\%$; $P \leq 0.001$), conjugated linolenic acid (CLA) ($\pm 14.9\%$; $P \leq 0.001$), n-3 FA ($\pm 12.3\%$; $P \leq 0.001$) and branched FA ($\pm 4.7\%$; $P \leq 0.001$). Conventional milk had higher contents of monounsaturated FA (MUFA) ($\pm 2.3\%$; $P \leq 0.05$) and n-6 FA ($\pm 4.2\%$; $P \leq 0.01$). These results can be explained by the significantly higher levels of grasses and lower levels of cereal concentrates in the fodder of organic farming. (Collomb et al., 2008) Coppa et al. (2011) writes that cows on pasture produce milk richer in unsaturated FA, especially cis-9-C18:1, trans-11-C18:1, cis-9, trans-11-CLA, and C18:3 n-3, compared with cows fed with preserved forages and concentrates. Pasture derived cheeses often show a variability of sensory characteristics and milk FA composition, which is probably due to the differences in the botanical composition, phenological stage, and grazing management. In Coppa et al. (2011) writes that cows continuously grazing on highly bio diversified pasture,

managed at a low stocking density yielded milk higher in PUFA, which decreased during the season, probably because at the beginning of the season the cows could select high quality vegetative patches. Rotational grazing on less bio diversified grasslands made high quality herbage available throughout the season, resulting in a more stable milk FA profile (Coppa, Ferlay, et al., 2011). Revello et al. (2010) studied the influence of seasonal variation of diets (conserved forage and pasture) on fatty acid (FA) and terpenoid profiles on “Toma piemontese” cheese in a Piedmont mountain farm. They found that the dairy products obtained in summer from pasture-based diets presented a more favourable FA profile. Compared with winter, summer milk had lower contents of saturated FA (SFA) (-15.8%) and higher contents of monounsaturated FA (MUFA) (+33.0%), polyunsaturated FA (PUFA) (+68.2%), conjugated linoleic acid (CLA) (+161%) and vaccenic acid (+148%) (Revello Chion et al., 2010). On the other hand Lejonklev (2013) found that silage from red clover or white clover had similar reduced rate of ruminal hydrogenation of polyunsaturated C18 fatty acid in vitro, compared to perennial ryegrass silages, regardless of whether the plants were cut early or late in the season. The effect may be due to the presence of bioactive plants metabolites in the clover plants, and could result in increased unsaturated C18 fatty acids in milk when used in vitro. Lejonklev (2013) also researched the effects of essential oils like caraway seed and oregano herbs as a bovine in vivo feed additive, but he did not find alterations to milk production levels, or C18 fatty acid contents of milk, even though the oils are known to have antimicrobial properties and hence may affect the microbial metabolism in the rumen (Lejonklev, 2013).

According to Bugaud et al. (2001) the lower melting point of unsaturated fatty acids may produce a more fluid fatty matter, and consequently softer cheeses. The higher fracture strain and, to a lesser extent, the higher fracture stress of cheeses from hay may lead to greater cohesiveness. Bugaud et al. (2001) found that the type of pasture (mountain or valley pastures), affected cheese texture, as did the cheese making process. Cheeses from the mountain were less elastic and less deformable. These rheological characteristics were mainly linked to the proportion of C18 unsaturated fatty acids, which was higher in milk and cheese from mountain pastures (Bugaud et al., 2001). Prandini et al. (2009) found that Grana Padano cheeses made during summer in the mountains had the highest polyunsaturated fatty acids (PUFA) percentage compared to low land cheeses. An increase in PUFA from spring to summer was only observed in mountain cheeses. The saturated fatty acids (SFA) decreased from spring to summer both in lowland and mountain cheeses (Prandini, Sigolo, Cerioli, & Piva, 2009).

Conjugated linoleic acid

Natural products, and specifically dairy fats, reportedly contain over 25 conjugated linoleic acid (CLA) isomers to which studies have attributed a wide range of health effects, e.g. anticarcinogenic, immunomodulating, and antiatherosclerotic effects, immune stimulatory and body fat-reducing properties. Diet is the most significant factor affecting the CLA content in milk fat (Prandini et al., 2009; Revello Chion et al., 2010). Butler et al. (2011) writes that milk produced under organic or low-input management with a high reliance on dietary fresh forage can have elevated concentrations of CLA. The milk was higher in α LA and/or beneficial isomers of CLA and in antioxidants with up to a 2.5- fold increase in some cases compared with milk from conventional production. Pasture grazing in many European regions is not always possible due to extremes in temperature or soil moisture levels, and milk quality may be maintained by the inclusion of oil seed in the dairy diets (Butler et al., 2011). Supplementing dairy cow diets with plant or marine oils resulted in higher CLA values (Lock & Bauman, 2004; Shingfield et al., 2006) also with pasture feeding. Low CLA levels were associated with richer diets in grass and corn silage or mixed grass/corn silage, and concentrate based diets (Chilliard et al., 2001; Dhiman, Anand, Satter, & Pariza, 1999; Prandini et al., 2009). Dhiman et al. (1999) researched the effect of different diets on the CLA content in the milk of cows. In the experiments cows grazing pasture and receiving no supplemental feed had 500% more conjugated linoleic acid in milk fat than cows fed typical

dairy diets. In a third experiment the highest CLA content was measured with a diet supplemented with fish meal and 250g of monensin/cow/day compared to only silage and grain based feeds. Dhiman et al. (1999) also found in the fourth experiment that a grass hay plus grain diet yields a higher CLA content than only alfalfa or grass hay. They proved that the CLA content of cow's milk fat can be increased through different nutritional and management practices. The CLA content of milk can be increased by increasing the proportion of grazed grass from pasture in the diet of dairy cows (Dhiman et al., 1999).

Pasture feeding increases milk CLA, especially with grass at an early growth stage Chilliard et al (2000). writes that the high C18:3 content of young grass and its low fibre content probably interact to increase the production of CLA or its trans C18:1 precursors (Chilliard et al., 2000). Only limited data are available relative to dietary influences on the distribution of individual CLA isomers. The effect of the diet on milk fat CLA is largely accounted for by a change in the concentration of cis-9, trans-11 CLA isomer. Trans-7, cis-9 is typically found as the second largest CLA isomer (3 to 16% of total CLA isomers) in milk fat (Yurawecz et al., 1998). Milk fat was studied from cows fed on pasture and supplemented with dietary oils (peanut, sunflower, linseed and fish oil, which results in the formation of high levels of biohydrogenation intermediates in the rumen. These dietary treatments produced a wide range of cis/trans-CLA isomer concentrations. The following positive associations between dietary treatment and specific cis/trans-CLA isomers in milk fat were observed (Chilliard et al., 2000):

- Pasture feeding and cis/trans 11,13
- Peanut oil and trans-7, cis-9 CLA
- Sunflower oil and trans-10, cis-12
- Linseed oil and cis-12, trans-14
- Fish oil and cis-9, trans-11 CLA

Stanton et al. found that high rapeseed supplementation also increased CLA content in the milk compared with only pasture or low rapeseed supplementation. (Stanton et al., 1997)

Prandini et al. (2009) researched the content of conjugated linoleic acid (CLA) and fatty acid composition of different Grana Padano P.D.O. Italian cheeses made in the mountains or in the low lands. Mountain cheeses had higher levels of CLA, vaccenic (TVA) and α -linolenic (LNA) acid than lowland cheeses. CLA concentrations increased from spring to summer both in mountain and low land cheeses. In the study they found that this rise was greater in mountain cheeses showing an effect of interaction between season and production area ($P < 0.001$). Prandini et al. (2009) observed a more yellow colour by samples of summer Grana Padano with the highest CLA levels, which indicated high β -carotene content. The β -carotene concentration resulted positively correlated with CLA level ($r = 0.92$; $P < 0.01$) in summer cheeses (Prandini et al., 2009). Bergamo et al. (2003) found higher CLA, TVA, LNA, TH (α -tocopherol) and β -carotene contents in their study on fatty acid composition and fat soluble vitamin concentrations in organic than in conventional milk and dairy products. They reported that an organic diet, containing at least 60% dry matter of roughage, fresh or dried fodder (EC Reg. 2092/91 and 1804/99), may well improve microbial biohydrogenation, yielding higher levels of CLA in milk. Further, they suggested that fibre-rich organic diets may improve fat soluble vitamin concentration in milk by decreasing milk yield. (Bergamo, Fedele, Iannibelli, & Marzillo, 2003) In Italy a greater quantity of fresh forage is given to cows during the summer months, this might be the reason that Prandini et al. (2009) found in lowland Grana Padano cheeses higher levels of CLA, oleic acid and MUFA and lower levels of saturated FA compared to spring lowland cheeses. Hays fed during summer could have higher content of CLA precursors than hays fed in winter-early spring, due to the lower oxidation process related to the duration of the storage (Couvreux, Hurtaud, Lopez, Delaby, & Peyraud, 2014; Dewhurst, Shingfield, Lee, & Scollan, 2014). The study of Prandini et al. (2009) proved the role of mountain cheeses obtained from summer milk as a natural source of CLA and beneficial fatty acids for human health (Prandini et al., 2009). Kaiser et al. (2006) writes that feeding legume silages, particularly red clover, may increase the polyunsaturated

fat content of milk, enhancing its health properties for the consumer (Kaiser, Moran, & Piltz, 2006). Dewhurst et al. (2003) confirmed in his research the high intake potential and milk production from legume silages. White clover silage increased milk yield by 6 kg/d increase in comparison with grass silage. They also found that clover silages led to a reduction in the content of palmitic acid and an increase in the content of polyunsaturated fatty acids, particularly the n-3 α -linolenic acid, in milk (Dewhurst, Fisher, Tweed, & Wilkins, 2003).

Table 2. The effect of feeding legume silages on the polyunsaturated fatty acid content (% of total fatty acid) of milk. Source: Dewhurst et al. (2003)

	Silage type and level of concentrate (kg/cow/day)							
	Grass		Red clover		White clover		Lucerne	
	4	8	4	8	4	8	4	8
Experiment 1:								
Milk production (kg/day)	-	24,90	-	28,10	-	31,50	-	27,70
Linoleic acid (C18:2)	-	1,42	-	1,81	-	1,80	-	1,61
Linoleic acid (C18:3)	-	0,43	-	0,81	-	0,91	-	0,54
Experiment 2:								
Milk production (kg/day)	23,50	27,50	25,60	30,20	-	33,20	-	-
Linoleic acid (C18:2)	0,90	1,05	1,47	1,58	-	1,54	-	-
Conjugated linoleic acid (cis-9, trans-1)	0,37	0,36	0,42	0,41	-	0,34	-	-
Linoleic acid (C18:3)	0,48	0,40	1,51	1,28	-	0,96	-	-

Terpenes

Coulon et al. (2004) writes that terpenes have an important effect on the sensory characteristics of cheeses. These plant-specific molecules, when concentrated, have recognised aromatic properties. These molecules very rapidly pass into the milk (Viallonista et al., 2000) and are found in cheese in a much greater quantity when the animals are fed dicotyledon rich natural grass forage than when they are fed concentrate based rations (L Moio et al., 1996), or monospecific forage (S Buchin et al., 2014; Coulon et al., 2004; Viallon et al., 1999). Revello et al. (2010) found that the milk from pasture feed cows presented higher contents of terpenes than did that from winter diets based on hay. Processing of the milk into ripened cheese had no effect on the FA composition and terpene profile of the dairy products (Revello Chion et al., 2010). According to Larsen et al. (2013) the transfer of these molecules is fast and the elevated content of volatile components in milk only persists as long as the volatile components are present in the feed. This composition of volatile compounds in milk does not directly reflect the volatile composition of feed, most likely because some terpenes are metabolized by the cow. The higher terpene content in milk gives a fresher and less stale flavour, but at the applied levels no specific herb flavour. Larsen et al. (2013) concluded that if moderate amounts of herbs are used in feeding the milk flavour may be improved (Larsen et al., 2013).

Phyto estrogens

Using leguminous plants such as clover, lupin, horse beans and peas has increased in organic milk production due to the nitrogen fixation by the these plants fitting well in the crop rotational plan of the farmer and as an important energy and protein source for cows. According to a study done by Andersen et al. (2009) in Denmark looking at the concentration of phyto-oestrogens in herbage for cattle and in milk, the found that clover and other legumes are a potential source of phytoestrogens in cows milk and so in consumers. Feeding clovers, especially red clover increased the concentration of phyto estrogens in the milk. New specially produced organic milk types can be developed with improved taste and potential health benefits, by feeding legumes or herbs containing flavour and special components that are transferred to the milk. Andersen et al. (2009) also found higher concentration of isoflavones in the pastures used in their study than the silages fed in previous studies. They suggest this could be because a higher concentration of phyto-oestrogens in fresh grass than in silage (Andersen et al., 2009). Among the legumes, red clover has the highest total

concentration of phyto-oestrogens varying from 1% to 2.5% of dry matter (DM), while in white clover is 0.02% to 0.06% of DM (Saloniemi, Wähälä, Nykänen-Kurki, Kallela, & Saastamoinen, 1995).

Enzymes and bacteria

Genetic, physiological and dietary factors likely influence the content and activity of milk enzymes. Proteases such as plasmin, which is quite resistant to heat treatment and plays an important role in the ripening of cooked-curd-type cheese is influenced by these factors. Bugaud et al. (2001) writes that the 30 observed differences in cheeses made from late- or middle lactation milk is related to increased proteolysis in late lactation, as shown by the increase in pH and γ -casein content in these cheeses. Plasmin is certainly also involved in the texture differences noted between valley and mountain cheeses in Bugaud's trial. According to Bugaud et al. (2001) the extent of proteolysis was higher in cheeses produced from the milk of mountain pastures. This was contributed to the increased plasmin activity in mountain milks, which can enhance primary proteolysis in the corresponding cheeses (Bugaud et al., 2001). Coulon et al. (2004) writes that the higher concentration of plasmin in milk in certain dietary or physiological situations could be due to increased cellular permeability of mammary tissue following a bacterial infection (mastitis), mammary cells' involution (in late lactation) or the intake of certain plant species (buttercup) present in certain types of meadows only. Differences have also been observed in the degree of milk or cheese lipolysis according to animal casein genotypes. Coulon et al. (2004) writes that in the Tarentaise cattle breed, Beaufort cheeses made with β -casein C milk are more lipolysed and taste more pungent and smell stronger (Coulon et al., 2004). The specific sensory characteristics of cheeses processed with late lactation milk could also be linked to increased lipolysis in late lactation milk. This lipolysis is not due to the increase in lipoprotein lipase concentration, but mainly to the modification of the fat globule structure (Chazal & Chilliard, 1986). Several authors suggest that a part of the differences in micro-organisms diversity in milk from different farms or plants could be due to animal feeding (Demarigny, Beuvier, Dasen, & Duboz, 1996; Desmasure & Gueguen, 1997; Michel, Hauwuy, & Chamba, 2001). According to Coulon et al. (2004) terpenes originating from plant derived feeds, may have an effect on the microbial ecosystem during cheese making and ripening. Negative correlation was observed between terpenes and cheese volatile compounds (such as sulphur compounds) originating from protein breakdown by microbial enzymes or directly by addition of terpenes to cheese milk. (Solange Buchin, Martin, Dupont, Bornard, & Achilleos, 1999; Coulon et al., 2004) Also Verdier-Metz et al. (2011) suggested that some diet effect could be of microbial origin: they found that the marked differences in flavour of Cantal cheese according to diet observed with raw milk disappeared when milk was pasteurised (Coppa, Verdier-Metz, et al., 2011).

Rennet and GMOs

Due to the growth of the dairy industry and the increased demand for rennet, a high share of rennet is made currently with the help of genetically modified organism (GMOs). In the US only 3% of cheese produced is still made with actual calf rennet, according to Mark Johnson, Ph.D., a cheese expert from the Wisconsin Center for Dairy Research. In Europe the share of real calf rennet is higher due to the artisan cheese tradition and PDO cheeses. The other 97% of U.S. cheeses is made with enzymes from microbial sources, one of which is genetically modified and responsible for the vast majority of cheeses at the local grocery store and the other sources are microbial and fungal enzymes (Sarah, 2011). Because of the lower cost and consumer demand for cheeses produced without animal derived products, only five percent of cheeses produced in the United States are created using animal derived rennet (Yacoubou, 2008). The chymosin produced with GM microorganisms consists of 80 to 90 per cent active ingredient and is thereby significantly purer as natural rennet, which contains only between four and eight per cent active chymosin. Since 1997, three chymosin preparations officially have been approved. In Europe generally this chymosin harvested from genetically modified micro-organisms is regarded as a "rennet substitute" and must be

allowed according to the regulations applicable to cheese. These preparations, and the cheese made with their aid, may be marketed without restriction and no particular declaration is required. From personal experience even cheese producers don't always know that the rennet they use is genetically modified. Approval exists in almost all western and eastern European countries (except France and Austria) as well as in the USA. (GMOB, n.d.) The majority of vegetarian rennet contains chymosin produced by this process, so most vegetarian cheese is produced with this rennet.

There are three options for organic cheese makers, either to use animal rennet or vegetarian rennet (derived from plants, not to be mistaken with the fermentation produced rennet, as it is often marketed as such) and microbial rennet. Animal derived rennet could be used to make organic cheese and sour cream; however because the rennet traditionally comes from calves stomachs, vegetarians would likely prefer organic cheese made with microbial or other non-animal sources. Cheeses containing animal derived rennet can be labelled as organic; although the calves would not need to be organic as the enzymes are permitted processing aids so don't have to be organic. Microbial enzymes are often used to make organic cheeses as a form of "vegetarian rennet." Microbial rennet substitutes also are widespread which are derived from (non-GM) microorganisms. These microbial and fungal enzymes are used widely in food processing, and recently some enzymes have been found with similar effect as chymosin, which have not been genetically modified. These enzymes are now produced by a similar fermentation and purification process from these bacteria or fungi and are used to make 'non GM vegetarian rennet'. (SAb, n.d.) True vegetable rennet comes from plants which produce enzymes that have coagulating properties. There is currently no industrial organic plant based rennet production; traditionally it is only common in the Mediterranean. Examples include nettles, cardoon thistle, or fig tree bark. Some disadvantages to using vegetable rennet are that during their use, enzymatic side reactions may occur that lead to undesirable changes in taste, a bitter taste in cheese flavour, especially by aged cheeses and are a little more unpredictable by the coagulation process (ICF, 2011)

Difficulties & challenges in organic cheese production

Feed

Organic cows are fed large amount of silages, especially in winter. Clostridial fermentations in silages can increase the risk of clostridial spores contaminating cheese, adversely affecting the manufacturing process. Good management of silage production to avoid clostridial fermentation will overcome this problem. According to Kaiser et al. (2006) the cheese making properties of milk may be adversely affected when cows are fed aerobically spoiled maize silage (Kaiser et al., 2006). Urbach (1990) writes that poor quality silage and various weeds produce off flavors in the milk due to direct transfer off flavours. This can happen from the breakdown products of weed components, or to the effect of these components on the biochemistry of the cow. Many gramineous species have an undesirable effect on the taste and smell of milk, and many legumes can transmit bitter flavors to the milk. The same negative effects can happen, when these species are fed as hay. Many of the Cruciferae, when they are fed in large quantities, give the milk: a sharp radish-like flavor and a penetrating smell (Urbach, 1990). Weeds can become a serious problem for organic farmers. The control of perennial weeds such as dock and creeping thistle can pose a major challenge to permanent organic grassland management. Weeds are of little agronomic importance in small quantities, but some can spread to infestation levels with consequent reductions in production and quality of forage (Nicholas & Padel, 2004).

Animal welfare

Milk from animals that are sick, abused, suffering from mastitis or treated with antibiotics will not have a balanced composition. This creates problems for the development of lactic bacteria, and as a result allows pathogens to reproduce easily. Good milk must come from

healthy animals. In organic, as well as in non-organic dairy farming the most common animal welfare issues are related to mastitis, lameness and infertility (Marley et al., 2010), with mastitis being the most frequently recorded or quoted disease (von Borell & Sørensen, 2004). Inflammation of the mammary gland, resulting in mastitis, is predominantly caused by bacterial infection and is the most common animal health problem recorded on organic dairy farms (Hardeng & Edge, 2014; Marley et al., 2010; Valle, Lien, Flaten, Koesling, & Ebbesvik, 2007; Weller & Bowling, 2000). Metabolic diseases may be linked to the change in feeding, as organic certification requires that animal diets have a relatively greater level of roughage and lower use of feed supplements and vitamins compared to non-organic enterprises (Marley et al., 2010). According to Sutherland et al. 2013 it is possible that a relatively lower quality and/or restricted diet could place organically managed animals at enhanced risk of metabolic diseases such as milk fever and ketosis (Sutherland, Webster, & Sutherland, 2013). It is essential to have the collaboration of a network of veterinarians who regularly check the livestock's health, the type of water and food they have access to, their stress levels and their living conditions. Additionally the milk must be regularly checked and its bacterial and somatic cell count analysed.

Preservatives

Berg & Meijer (2004) write that grass silage, used as a feed in winter, but sometimes also as a part of the ration in summer, represents the main source of contamination of the milk with spores of spore forming, lactate fermenting butyric acid bacteria (BAB), especially when it is insufficiently preserved. Spores can come from already contaminated silages or grains, and large quantities of spores is excreted with the feces. Spores can get in the raw milk, when cows with contaminated teats or udders are milked. *Clostridium tyrobutyricum* spores survive pasteurization and can cause the late blowing of cheeses due to excessive production of CO₂ and H₂, which causes cracks in the cheese during ripening and also the development of unpleasant flavours and odours (Berg & Meijer, 2004). Late gas blowing is a problem principally in brine-salted cheeses because of the time required for NaCl to diffuse into the cheese and to reach inhibitory concentrations. Strategies to avoid late gas blowing usually involve minimizing spore numbers in milk (e.g. good hygiene, avoidance of silage, inhibition of spore germination and the growth of vegetative cells, for example by the use of lysozyme (P. F. Fox et al., 1993). Addition of Sodium nitrate to prevent LBD is not allowed in organic cheese production (WSDA, 2008). However lysozyme or egg white, which is an enzyme normally used in food processing is allowed according to European regulation (EC) No 889/2008, Article 27, 1(b) (EC, 2008a).

Gouda cheese, as a brined cheese, is especially vulnerable to LBD, especially for large cheeses. Normal eye formation is limited and too large holes are easily detected as a defect. Lysozyme appears to be of value in the control of LBD in organic cheese production. But in case of Gouda, published research indicates that lysozyme used at the current normal addition under normal manufacturing and storage conditions is less effective than the usual amount of nitrate. In this case, lysozyme cannot be considered a suitable alternative to nitrate at present (Patrick F. Fox, 2003). Also Garde et al. (2012) reports that even with the usage of lysozyme LBD was a problem in the production of Manchego cheeses (Garde et al., 2012). Today special starter cultures can be used in organic cheese production to prevent LBD, although more research is needed to verify their efficiency and influence of cheese characteristics (CSK food enrichment, n.d.).

Cheese mites

Cheese makers who produce bandaged or natural rind ripened hard cheeses face the problem of cheese mites, which damage the rind of the cheese, and can cause considerable losses. There are many ways avoiding cheese mites like waxing, clear coating, and vacuum sealing. But in case of organic bandaged and natural rind ripened cheeses, when the infestation already occurred, there are only a few and not completely effective solutions. Mechanical means of limiting mites include brine washing, brushing and vacuuming. Many

cheese makers find the best remedy to be the use of food grade diatomaceous earth (DE). This technique must be employed early in the aging process before the mites have gone below the rind's surface. Ozone machines are being used to limit mite damage in larger aging facilities- and some small ones. Organic cheese makers cannot use the conventional prevention method with methyl bromide, they can use silicium with dill. There are complaints working with this substance that it is very harmful for the lungs so protective wear has to be applied. Organic methods have to be researched and utilized in limiting and eradicating mite infestations for organic cheese makers. (Caldwell & Carroll, 2012; McKnight, 2006; Otherwise, 2014)

Chapter IV. - Discussion

The main objective of this thesis was to improve the decision basis for cheese makers considering switching from nonorganic to organic cheese production and to those considering making raw milk cheeses. This was attempted through a literature review looking at different aspects of the issue. This section discusses the combined results of the review.

In Europe regulations lay down a strict framework for the production of raw milk and organic cheeses, to assure consumer safety, and also achieving high quality products. Cheese making is a complex technology where the quality of the cheese is affected by a large number of factors, from farming methods to the technology of cheese making. In this current research I focused on two main factors. One of them is the effect of pasteurization on the quality of cheeses and the second is the effect of feeding on the quality of cheese from organic or non-organic milk.

During my stay in Wales I have worked with the artisan cheese maker John Savage Onstweder, who is called the Godfather of artisan cheese making in Wales. He has been producing raw milk cheeses for more than 25 years and said to me in a personal conversation: "Why putting all that effort in organically produced milk, if you pasteurize it and because of this loose most of the added value of being organic." Also the artisan cheese maker Sam Holding, who is producing organic cheddars, has explained to me, that for a while he had to pasteurize his milk, and although the cheese was still excellent, the raw milk cheeses had a more complex, deeper and longer lasting taste. With my research I have collected the main points where pasteurization can affect milk and cheese quality and sensory characteristics.

Summarizing the major points researched in this Thesis affecting cheese quality

Pasteurization

Cheese made from raw milk at farm dairies by artisan cheese makers has the reputation of being of the highest quality and is often described as having a more intense flavour than cheese based on pasteurized milk (P.L.H. McSweeney et al., 1993; Muir, Banks, & Hunter, 1997; Roy et al., 1997; Shakeel-Ur-Rehman et al., 2000). The less intense flavour of cheese made from pasteurized milk can be explained by a reduction in the natural bacteria and a partial inactivation of the natural milk enzymes. In my research the following changes have been identified, which are induced by pasteurization (Beuvier & Grappin, 1997; Dixon, 2000):

- Modification of milk rennetability
- Partial elimination of the milk microorganisms which may grow in cheese during ripening,
- Partial or total activation or inhibition many indigenous enzymes: the plasmin/plasminogen complex, cathepsin D, lipoprotein lipase and alkaline phosphatase.

- Whey protein denaturation/denaturation of serum proteins
- Inactivation of acid proteinase

As noted by artisan cheese makers, and reported in many studies all these changes greatly affect the sensory characteristics of cheeses, mainly by long ripened types, resulting in a less intense taste and unique flavoured product (Beuquier & Grappin, 1997; Ginzinger, Jaros, Lavanchy, et al., 1999; P. McSweeney & Weimer, 2007). Although pasteurisation of cheese milk is widespread and has certainly helped to considerably improve average cheese quality, it is often held to be responsible for a certain lack of flavour, especially in well matured varieties. Berg & Meijer (2004) writes this may be due to inactivation of lipoprotein lipase and killing of bacteria that may contribute to raw milk cheese flavour, which usually is more variable than the flavour of cheese made from pasteurised milk. Less severe heat treatment may improve flavour (Berg & Meijer, 2004).

There are no specific studies aimed at the effect of pasteurization on organic milk and cheese, but it can be assumed from the literature in Chapter II that the inactivation of the natural micro flora of the cheese may not fully develop a natural “terroir” flavour and characteristics in the cheese. Starter and secondary cultures and also enzymes can vary according to location, feeding, breed and health of the animal. According to Chapter II and Chapter III it can be assumed that due to the effect of pasteurization, some sensory characteristics (smell, taste, texture) of organically produced cheeses might be changed or decreased. Some of these special flavour characteristics can be attributed to differences in the feeding of organic and non-organic dairy cows.

Feed

The effect of different feed stuffs on the quality of the milk and cheese is well documented and researched. Many studies have proven what artisan cheese makers have been promoting, that the cheese made from the milk of cows grazing on diverse grassland produced more intensive aromas and flavour compounds as well as beneficial fatty acids. Flavour of pasture milk derive from a complex combination of a wide variety of aroma active compounds including: acids, esters, sulfur compounds, indole, skatole, terpenes (Solange Buchin et al., 1999; Bugaud et al., 2001; Coppa, Verdier-Metz, et al., 2011; L Moio et al., 1996; Urbach, 1990). Some evidence suggests that milk from cows grazing on diverse grasslands likely to have higher levels of fat soluble vitamins and other nutrients. Cows fed fresh green forage, especially those grazing grass, have been shown to have higher levels of conjugated linoleic acid (CLA) and essential fatty acids in their milk (Elgersma, Tamminga, & Ellen, 2006). Cows are natural herbivores and are healthiest when they eat grass, rather than the grain they are fed in confinement dairy operations. All findings seems to indicate that organic dairy farming benefits the quality of the cheese more than conventional dairy farming, mainly due to suitable breeds and the difference in feeding. For example very high yielding cows can suffer from fermentation and/or metabolic disturbances, due to low energy intake from herbage available from mainly a pasture based diet Bovolenta et al. (1998) writes that this indicates that high yielding animals may not be suited for pasture based diets like alpine pasture dairy farming (Bovolenta, Malossini, Ventura, & Piasentier, 1998). Milk can gain off-flavours (“feed” flavours) from poor-quality silages. Off-flavours are transmitted rapidly, both through respiratory and digestive routes. Risk factors at farm level:

- Poor silage quality
- Poor air quality in the barn
- Feeding silage just before milking (Kalač, 2011; Mouchili, Wichtel, Dohoo, Keefe, & Halliday, 2004; Urbach, 1990)

Poor quality silages can also lead to LBD problems in cheese making. Conventional diets containing large amounts of concentrates, fats and sugars, which can favour a butyric acid-type fermentation in the rumen, although it increases dry matter intake, and milk fat yield (Ørskov & Oltjen, 1967). Bonanno et al. (2013) found that in an extensive farming system cows fed a pasture based diet, produced milk that was richer in casein and lower in somatic cells compared with the intensive system. The milk from pasture grazed cows produced

cheese in higher yield that had more protein, less fat, a less intense yellow colour, and a better sensory profile, characterized by a smoother paste and sweeter, less bitter and salty, and more acidic taste (Bonanno et al., 2013).

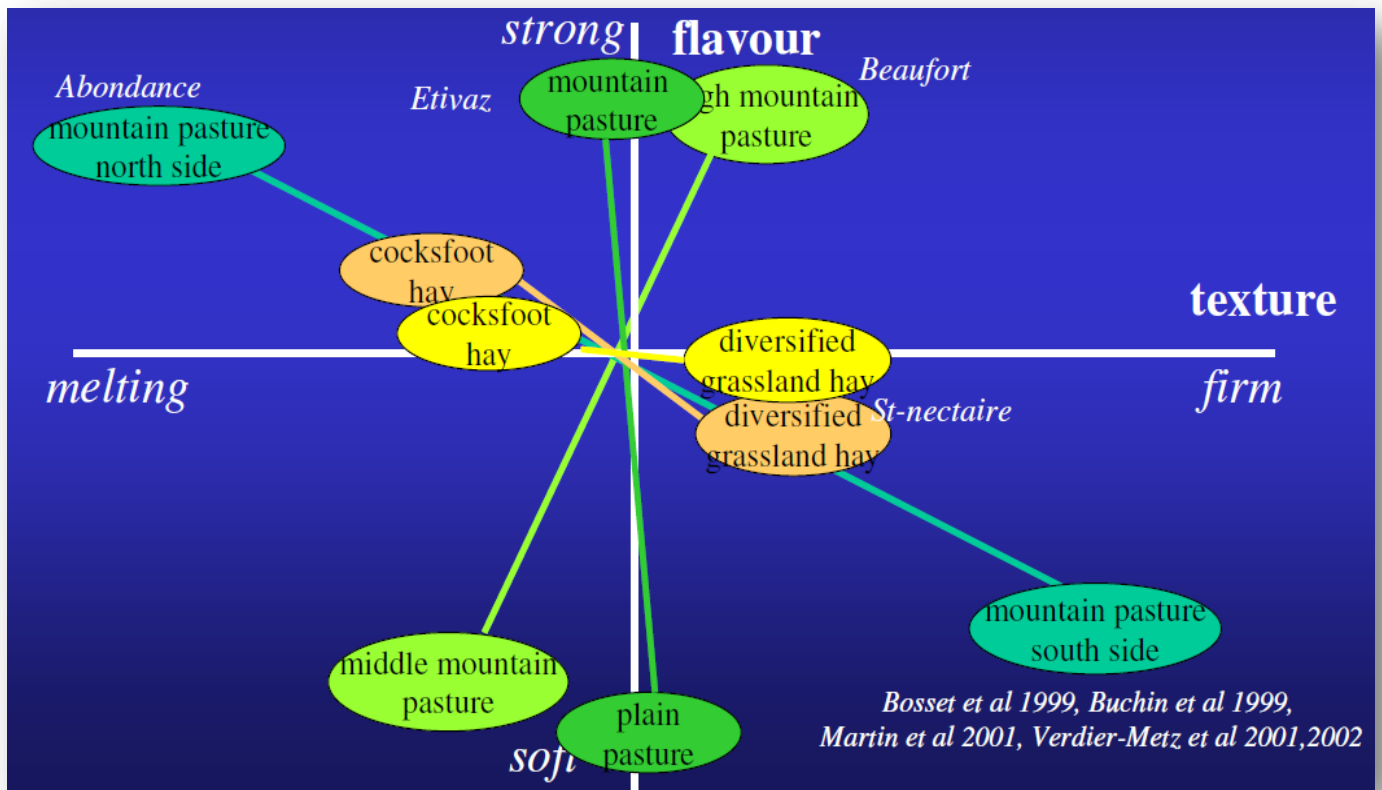


Figure 3. Sensory characteristics and differentiation of cheeses according to grass botanical composition Source: Coulon et al. (2004)

Factors affecting cheese quality and consumer willingness to pay for organic and unpasteurized cheeses.

Organic foods produced according to strictly defined standards are able to satisfy various expectations of today's consumer concerned with various aspect of food quality. Napolitano et al. (2010) writes that information about organic farming, animal welfare and benefits of organic products can increase consumer awareness and willingness to buy organic products. They found in this research that consumer had higher liking expectation for organic cheeses than conventional. Consumers also showed willingness to pay a higher price than for retail price of conventional cheeses. Information and raising awareness among consumers can be a major determinant of cheese liking and to pay extra for organic cheeses. This could be a potential option for product differentiation for small scale and traditional farms. (Napolitano & Braghieri, 2010)

Colonna et al. (2011) found that in his research that more consumers preferred raw milk cheeses than pasteurized milk cheeses. A larger portion of consumers indicated preferences for the raw milk cheese when the cheeses were labelled, which indicates producers of raw milk cheeses would benefit from clearly labelling their cheeses as being made from raw milk, if other concerns such as safety are met. When marketing cheese made from raw milk, producers should inform consumers that raw milk cheese is produced according to the regulation. Consumers of specialty cheeses typically have a broader knowledge of cheese,

spend more money on purchasing cheese, consume a large variety of cheeses, and have little brand loyalty (McCarthy, O'Reilly, & Cronin, 2001). In addition, these consumers tend to be within a wealthier socioeconomic group than the general population, who are willing to spend more for high quality products (Kupiec & Revell, 2001). Specialty cheese consumers purchase cheeses based on many characteristics, such as location of cheese producer, price, size, and quality (Monjardino de Souza Monteiro & Raquel Ventura Lucas, 2001). Another parameter of importance to specialty cheese consumers is whether the cheese is made from pasteurized or raw milk (Murphy, Cowan, Meehan, & O'Reilly, 2004), with different consumer groups preferring either raw or pasteurized milk cheeses or both. The term "local" had a significantly higher average rank than the other characteristics of cheese production. The average rank of terms "organic" and "artisan" were not significantly different from each other but were ranked significantly higher, on average, than either "sustainable" or "farmstead." producers of raw milk cheeses would benefit from clearly labelling their cheeses as being made from raw milk, if other concerns such as safety are met (Colonna, Durham, & Meunier-Goddik, 2011).

Some consumer might have fears concerning the safety of raw milk cheeses and some countries do not allow or restrict the sale of raw milk cheeses. There are plenty of scientific research done to evaluate these risks and benefits. Pasteurization is not a guarantee against bacterial contamination of cheese. There have been outbreaks of pathogens in both raw and pasteurized cheeses (Taygan et al., n.d.). In several countries of Europe where there is a long tradition of raw milk cheese making, producers and raw milk consumers believe that if proper sanitation measures are followed, raw milk cheese is perfectly safe. A healthy population of beneficial bacteria present in the milk is enough to "fight off" any potential bad bacteria. There is currently very little information available about this topic, but artisan cheese makers have been long observing and promoting this idea. Bronwen Percival a raw milk advocate in the UK, who organizes in Somerset County, England a conference called the Science of Artisan Cheese started a Kickstarter project to translate to English a new practical guide/textbook of raw milk microbiology, written by a collective of French scientists and dairy technicians, which was introduced at the last conference by Dr. Christine Montel. This guidebook helps cheese makers to preserve and encourage the natural diversity of their raw milk, which is crucial to the flavour and to the safety of artisan cheeses (Jeanne, 2014; Percival, n.d., 2014). If consumers are informed about the safety measures and the science behind the production methods of raw milk cheeses, they might feel more safe trying out or eating raw milk products.

Conclusion

The reasons why people choose raw milk cheeses or organic cheeses has many reasons, but probably taste is one of the most important ones. Proponents of raw milk cheese argue that it is more delicious than pasteurized cheese, because during pasteurization most of the beneficial micro-organisms, bacteria and enzymes are killed or inactivated. These bacteria and enzymes contribute unique flavours to the cheese that cannot be simply replicated. Cheese makers who choose to use raw milk instead of pasteurized seek the flavour complexities that raw milk lends to the cheeses. It is believed that raw milk cheeses convey the characteristics of their region of origin a concept called "terroir", which means "taste of place." To cheese makers, this means you can taste the grass and herbs that are specific to the region where the milking animals grazed. Raw milk is a live, healthy food, rich in different microorganisms that give each cheese unique characteristics.

In my research I have found that feed type has significant effect on the cheese sensory properties, although a good control of technological factors is necessary to bring out these effects. The effect of different diets can be explained with the presence of molecules in milk and cheeses which are directly transferred from diet or produced by the animals. Further research is needed to understand the link of "terroir" specialty traits, tastes and characteristics in cheeses. Organic and raw milk cheeses should develop appropriate

specifications so that these cheeses reflect best the uniqueness and diversity of the area where they are produced. The demand for milk and cheeses made only with feeding hay is in the increase. Maintaining and enhancing biodiversity on organic grassland may lead to improved cheese sensory properties which can be best utilized by making raw milk cheeses with artisanal techniques.

The demand for organically produced cheese is increasing and if we want this to continue than organic cheeses have to meet the quality that justifies an additional price in relation to the conventionally produced cheese. Careful and minimal processing are two important parameters in organic farming. Cheese making based on raw milk is therefore a natural development within organic production. (Nielsen, Larsen, & Knöchel, 2001)

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