# Comparison between Emmentaler PDO and generic emmental cheese production in Europe


## The authors

**Walter Bisig, Marie-Therese Fröhlich-Wyder, Ernst Jakob, and Daniel Wechsler**

Agroscope Liebefeld-Posieux Research Station ALP, Berne, Switzerland

*Correspondence to: walter.bisig@alp.admin.ch*



## Abstract

Emmentaler cheese originated 500 years ago in the Swiss region of the river Emme. Nowadays it is locally produced from raw milk in Switzerland whereas other Swiss-type cheeses often are produced in centralised factories out of heat treated milk. Swiss Emmentaler, with a protected designation of origin (PDO), is mostly produced with propionic acid bacteria (PAB) having weak aspartase activity to avoid late fermentation. For the same purpose, facultative heterofermentative lactobacilli (FHL) are added. Emmentaler PDO had higher contents of acetate and propionate and less lactate, butyrate and succinate compared to generic emmental cheese made from thermised milk. It also showed a more pronounced proteolysis. As key odorants, four ketones and aldehydes, three esters, one lactone and two furanones were identified. They were characterised as butter-like (diacetyl), cheese-factory-like, fruity, caramel, spicy, smoky or mushroom-like. Volatile compounds which were significantly higher in Emmentaler PDO were 3-hydroxybutanone, hexanal, butanoic-acid-ethyl-ester and 3-methylheptane. Significantly lower were propan-2-ol and butan-1-ol. A technique to prove the origin of Emmentaler PDO based on the use of specific FHL strains and their identification in matured cheese with the selection of exclusive PCR-primers was tested successfully. Biogenic amines (BA) in mature Emmentaler PDO were lower than in generic emmental. In Emmentaler PDO significant differences in the content of BA between different cheese factories were observed.


*Aust. J. Dairy Technol.* **65**, 206-213

Emmental cheese originated in the region Emmental in canton Bern, Switzerland, and has been produced there since the 12th Century. Emmental is the region of the valleys of the river Emme and its confluent rivers. Its name 'Emmentaler' (coming from the Emmental) was first mentioned in documents in 1542. Originally, it was produced only in summer in huts up in the mountains where the cows were grazing on the alpine pastures. In the 17th and 18th centuries, production was significantly increased and in 1813 the first of many more cheese factories in the lowlands of Bern started to operate. From 1840, production started in other German-speaking cantons of Switzerland and exportation of the cheese began. Since 2002, emmental cheese of Swiss origin has had a protected denomination of origin (PDO) and the Emmental region is still one of its main production areas. Adjacent areas are also included in the PDO production zone.

Emmentaler PDO belongs to the group of Swiss-type cheeses. Emmental cheese in general is probably the best-known Swiss-type cheese and is frequently referred to simply as 'Swiss cheese', mainly outside Europe. There is no recognised definition of Swiss-type cheeses that differentiates them from other varieties. Swiss-type cheeses somewhat resemble Swiss Emmentaler PDO. Many of them have round regular eyes that vary in size from medium to large. For the manufacture of Emmentaler PDO and other Swiss-type cheeses, propionic acid bacteria (PAB) are used to achieve the characteristic eyes and nutty and slightly sweet flavour.

Nowadays, generic emmental is also produced in large quantities in different countries such as France, Germany, Austria, Ireland and Finland. A large variety of other Swiss-type cheeses are also available on the market, including svenbo, jarlsberg, greve, maasdamer, leerdamer, comté and beaufort. Body and texture of these cheeses correspond to those of hard and semi-hard cheeses.

Annual production of Emmentaler PDO in 2009 was 26,853 metric tons (MT) in 180 different village cheese factories, at an average of 150 MT per factory. Seventy-four per cent of this was exported, mainly to European countries and North America. Generic emmental cheese production in Europe is around 500,000 MT with about half of it produced in France.

## Description of Emmental cheese

Emmentaler PDO is a full-fat hard cheese made from 100% raw milk matured for at least four months in the PDO region. The round wheels with a firm, dry, gold-coloured rind have a height of 16 to 27 cm, a diameter of around 85 cm and an average weight of 90 kg. The characteristic eyes are round with a diameter of 2 to 4 cm, evenly distributed in the slightly elastic body with fine to middle fine grains. With maturation, the body develops from being rather elastic to less elastic / slightly crumbly. According to the natural grass and hay-based feeding practices, the colour of the body is ivory in winter and light yellow in summer. The characteristic flavour is given by the PAB fermentation and is slightly acidic, sweet, slightly salty, nut-like, piquant, but not very sharp. The flavour develops from mild and nut-like at four months to more nut-like and more piquant, but not very sharp above 12 months. The fat content is 45% to 55% in dry matter and the maximum water content is 38%. Cave aged Emmentaler PDO may have a brown to black rind with patina (Figure 1).

Generic emmental, according to Codex Standard 269 (Codex 2008), is a ripened hard cheese with cherry to walnut-sized holes and an elastic but not sticky texture. A few openings and splits are acceptable. It can be with or without a hard, dry rind and has a mild, nut-like and sweet flavour.

## Manufacturing technology

For the manufacturing of Emmentaler PDO cheese, 100% raw

milk from silage-free fed cows is used (Table 1) to avoid the presence of *Clostridium butyricum* and *Clostridium tyrobutyricum*. No thermal processing of the milk is allowed before renneting. As processing aids, only natural coagulants, defined lactic acid bacteria cultures originating and produced in the PDO region, PAB, sodium chloride and potable water are used. Cheesemaking occurs in copper vats. Cheese curd is typically scalded at 52 to 54°C for 30 to 60 min, filled into moulds and separated from the whey. During pressing, the temperature remains at around 50°C for many hours. At this temperature syneresis continues and undesirable micro-organisms

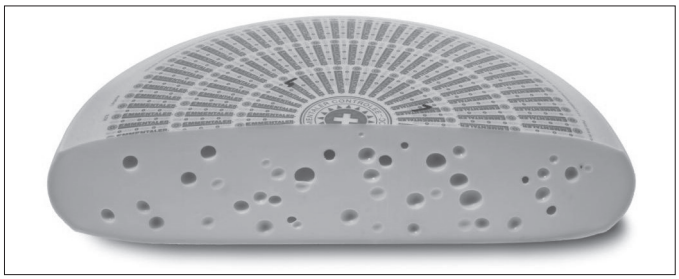

**Figure 1: Emmentaler PDO is a locally produced cheese made from 100% raw milk in village cheese factories. It is produced as a wheel with natural rind, weighing about 90 kg.**

**Table 1: Essential manufacturing characteristics, composition and quality factors: Comparison of Emmtentaler PDO (Switzerland) and Emmental Codex Standard (FOAG 2002, FOAG 2009, Codex 2008, Sieber et al. 1988).**

| | **Emmentaler PDO** | **Emmentaler Codex Standard 269-1967** |
|---|---|---|
| Raw materials | 100% raw milk from the Emmental and adjacent parts of Switzerland, no silage feeding, at least 70% roughage, no GMO feed.<br>Max. 30 km distance to cheese dairy<br>Typically 10 to 30 milk suppliers | Cows' or buffaloes' milk, and products obtained from the milk; longer distances<br><br><br>Large number of milk suppliers |
| Milk storage time | Maximum 18 hours at delivery and 24 hours after milking at cheesemaking | Processing to cheese, often 72 h after milk collection |
| **Permitted ingredients** | | |
| Starter cultures | LAB with defined composition originating in the PDO area<br>No direct vat set cultures<br>PAB cultures with defined composition selected for the production of Emmentaler PDO<br>No GMO cultures | Thermophilic starter cultures of harmless lactic acid producing bacteria and/or flavour producing bacteria<br>Propionic acid producing bacteria, and cultures of other harmless micro-organisms |
| Coagulating enzymes | Natural rennet, no rennet produced by GMO | Rennet or other safe and suitable coagulating enzymes |
| Other | NaCl, potable water | NaCl and KCl, potable water |
| **Essential manufacturing characteristics** | | |
| Place of manufacture | Emmental and adjacent parts of Switzerland (also ripening) | Worldwide |
| Heat treatment of milk | None, never above 40°C | Pasteurisation |
| Other milk treatments | None | Bactofugation or microfiltration |
| Equipment for cheesemaking | Copper vats<br>Max three batches per vat per day | Stainless steel vats<br>Many batches per day |
| Addition of water | 12-18% into milk and curd | Water addition allowed |
| Curd treatment | Heated to 52-55°C for 30-60 min | Heated to a temp. significantly above coagulation temp |
| Food additives | None | Colours; acidity regulators; preservatives (e.g. sorbate, lysozyme, nitrate, nisin, natamycin); ripening enzymes |
| Ripening | Ripening of unpacked wheels with necessary care<br>19-24°C / 70-90% humidity for about 7 weeks<br>11-14°C / 70-89% humidity for further ripening<br>4 months minimum<br>8 months min for reserve<br>12 months min for cave aged | Normally from 2 months at 10-25°C<br>Minimum of 6 weeks for direct consumption<br>Usually ripened within a plastic film |
| Factory size | 1-4 employees<br>Average 150 tonnes per year | Usually large-scale factories >10,000 tonnes per year |
| **Composition** | | |
| Milkfat in dry matter<br>Dry matter<br>Calcium content<br>Salt content | 45-55% (w/w)<br>Min 62%<br>1030 mg /100 g<br>0.4 to 1.0% | Min 45% (w/w), max not restricted, reference 45-55%<br>60% (if 45-50% fat) |
| Labelling | Emmentaler PDO<br>Production site identification<br>Use of raw milk | Country of manufacture shall be declared |

are eliminated by the thermal effect. Brine salting lasts 24 to 72 hours at a brine concentration of 20-22°Bé. To initiate the typical propionic acid fermentation, ripening temperature is increased typically to 21-23°C for about seven weeks. As soon as the eye development is sufficient, the temperature is decreased to between 11°C and 14°C to stop the PAB fermentation and allow further ripening. During ripening, the cheese wheels are regularly washed, turned over and dried on the surface if necessary. The maturation period lasts at least four months, and quite often at least eight months for premium Emmentaler PDO, or at least 12 months partly in a humid climate for cave aged Emmentaler PDO. An important step in the technology of Emmentaler PDO is the addition of water (12-18%) to the milk and into the curd. This leads to a high pH-value after the lactic acid fermentation (5.20-5.30) and consequently accelerates the PAB fermentation, results in a soft and elastic texture and also is the explanation for the high calcium content of the cheese (Table 1, FOAG 2002; FOAG 2009; Bachmann *et al*. 2002).

Nowadays, other Swiss-type cheeses including generic emmental are manufactured in many countries by technologies differing from traditional Swiss procedures. Considering the technological aspects, Swiss-type cheeses are always cooked cheeses. On the other hand, the treatment of milk, the extent of mechanisation, the weight and shape, the average composition (hard or semi-hard varieties both with different fat contents), ripening time and shelf life of foreign Swiss-type and generic emmental cheeses are different from the original. Often the process is specifically designed so that no rind forms on the cheese. Maturation takes place in vacuum-packed plastic wrapping for mass-production purposes (Table 1).

## Revival of the tradition of raw milk cheeses in modern society

The use of raw milk for cheese production was widespread until a few decades ago. Industrialisation of cheese manufacture brought larger factories, longer milk storage and, as a consequence, variable microbiological quality of raw milk and cheese. Since the 1940s heat treatment has been introduced more and more to improve process control in cheesemaking and for food safety reasons. Often no distinction was made for different cheese types as many public health authorities were in favour of pasteurisation. The heat-induced changes in milk and the elimination of the raw milk microflora considerably affects the cheesemaking and ripening process, resulting in cheeses with altered sensory characteristics. It is generally recognised that cheese made from raw milk develops a more distinct flavour than that made from pasteurised milk. A considerable amount of cheese is, therefore, still produced from raw milk. Emmentaler PDO is based on a concept to keep the advantages of raw milk cheese and secure food safety by different hurdles in the process from raw milk quality to the ripening process.

Recently, about 166 cheese varieties, mainly from countries with a long tradition of cheesemaking, such as France, Italy, Spain, Greece and Switzerland, have obtained EU-registration for products with a Protected Designation of Origin (PDO). Emmentaler PDO as well as other PDO cheeses are made from 100% raw milk without any pre-treatments. Locally produced artisanal cheeses made from raw milk are now recognised as speciality products with a unique selling proposition (USP) and find a growing market in the modern globalised world. An increasing number of consumers prefer minimally processed foods, natural products without additives, as well as authentic and artisanal specialities of well-defined origin.

## Particular requirements for the production of raw milk cheeses

Good manufacturing practice for raw milk cheese is challenging. The farmer has to apply good agricultural practices (GAP) on a high level to produce and deliver superior quality milk, transport distances and storage time of the milk have to be limited (Table 1) and food safety has to be assured. The cheesemaker applies rapid tests for raw milk quality to assure the fulfilment of the quality requirements (Table 2), has full traceability to the individual milk suppliers and keeps a personal contact with them. To fulfil these quality and traceability requests, cheese plant size remains limited. For Emmentaler PDO, the biggest cheese plant produced 700 MT in 2009. In addition to the fulfilment of GAP on a high level and the internationally recognised food safety standard ISO 22,000 or an equivalent one, advanced hygienic practices throughout the whole production and processing chain, as well as additional control measures from raw milk production to the point of consumption, are essential to ensure a good product quality and food safety.

**Table 2: Quality requirements for delivered milk for Emmentaler PDO cheese production (Jakob *et al.* 2010).**

| Criteria | Unit | Guideline delivered milk | Vat milk | Remarks |
|---|---|---|---|---|
| Total plate count | cfu/mL | <80,000 | <300,000 | According to Swiss national regulation |
| Aerobic mesophilic germs | cfu/g | <30,000 | | In milk for raw milk cheese production |
| Foreign germs (no acid production) | cfu/g | <20,000 | <20,000 | |
| Staphylococci, coagulase positive | cfu/g | <300 | <100 | Also upper tolerance value for curd before scalding is applied |
| *Enterobacteriaceae* | cfu/g | <300 | <500 | |
| *Escherichia coli* | cfu/g | <50 | <50 | |
| Enterococci | cfu/g | | <100 | |
| Salt-tolerant germs | cfu/g | <5,000 | <5,000 | |
| Propionic acid bacteria | cfu/g | <30 | <10 | |
| Aerobic spore forming germs (MPN) | spores/L | <200 | <140 | |
| *Clostridium tyrobutyricum* (filtration) | spores/L | <25 | <25 | |
| Lipolytic germs | cfu/g | <3,000 | <3,000 | |
| Facultative heterofermentative LAB | cfu/g | <30 | | |
| Psychrotrophic germs | cfu/g | | <5,000 | |

## Food safety of Emmentaler PDO

Phosphatase activity measurement is an accepted method to check whether milk has been subjected to sufficient pasteurisation or not. Its application to raw milk cheese may give confusing results. The typical scalding conditions applied during the manufacture of traditional raw milk cheeses such as Emmentaler PDO result in an almost complete inactivation of the alkaline phosphatase in the curd.

Figure 2 shows the residual activity of alkaline phosphatase found in different zones Z1 to Z14 of Emmentaler PDO cheese; its residual activity may be below the detection limit in the core region. In outside zones, clearly positive test results are obtained due to faster cooling during manufacture.

The food safety of Emmentaler PDO cheese is secured by a combination of technological hurdles. These are strict regulations for milk quality and production hygiene (Table 2), a short storage time for the milk, scalding temperature and sufficient time above 50°C, relatively low water content of a maximum of 38%, a fast and complete lactic acid fermentation, salting and a long ripening period of at least four months. Not to be underestimated are well-trained cheesemakers and their marked sense of duty and great reliability. Bachmann and Spahr (1995) could show that pathogenic germs in hard cheese did not survive after one day (Table 3). In modern chilled prepared food technology, the concept of hurdle technology is now recognised and is increasingly applied to allow high freshness and quality of ready-made food.

## Cultures

Thermophilic homofermentative lactic acid bacteria (LAB) such as *Lactobacillus helveticus, L. delbrueckii* ssp. *lactis* and *Streptococcus salivarius* ssp. *thermophilus* are used for acidification during emmental cheese production. After 24 hours, the lactic acid fermentation is completed. Optimal lactic acid fermentation is important for the control of the raw milk microflora, especially of the undesired species. *L. helveticus* develops a strong proteolytic activity. For generic emmental and other Swiss-type cheeses *L. helveticus* is used for this purpose (Hansen 2009). For Emmentaler PDO it has not been used for some decades as *L. helveticus* favours late fermentation during the longer maturation period. All cultures used for Emmentaler PDO cheese were isolated from Emmentaler PDO cheese factories producing good-quality cheese, starting in 1906, but mainly in the period from 1960 till 1980. They are mainly multi-strain cultures with a specified composition and are developed and produced locally. For generic emmental cheese, starter cultures are mainly from one of the food ingredient companies with worldwide operations.

Propionic acid bacteria (PAB) fermentation is characteristic in all emmental and other Swiss-type cheeses to obtain the typical eyes and the slightly-sweet nutty taste. PAB cultures, usually strains of *Propionibacterium freudenreichii,* are added. Detectable eye formation starts about 30 days after manufacture (Table 1). Reduction of the ripening temperature down to between 11 and 14°C follows to slow down PAB fermentation as much as possible and to further mature the cheese. In cheeses ready for consumption, about $10^8$ to $10^9$ cfu/g of PAB are present. The lactic acid produced by the lactic starters is broken down by the classic metabolic pathway to propionate, acetate and $CO_2$ as follows:

3 lactate $\rightarrow$ 2 propionate + acetate + $CO_2$ + ATP

PAB with strong aspartase activity metabolise lactate and aspartate to higher amounts of acetate, propionate, succinate and $CO_2$ and form larger eyes and a more pronounced flavour. Emmentaler PDO manufacturers seek a controlled PAB fermentation to prevent late fermentation and, therefore, usually avoid PAB with strong aspartase activity (Wyder *et al.* 2001). Lipolysis in Swiss-type cheese is catalysed by bacterial lipases and the indigenous lipoprotein lipase in milk which is, however,

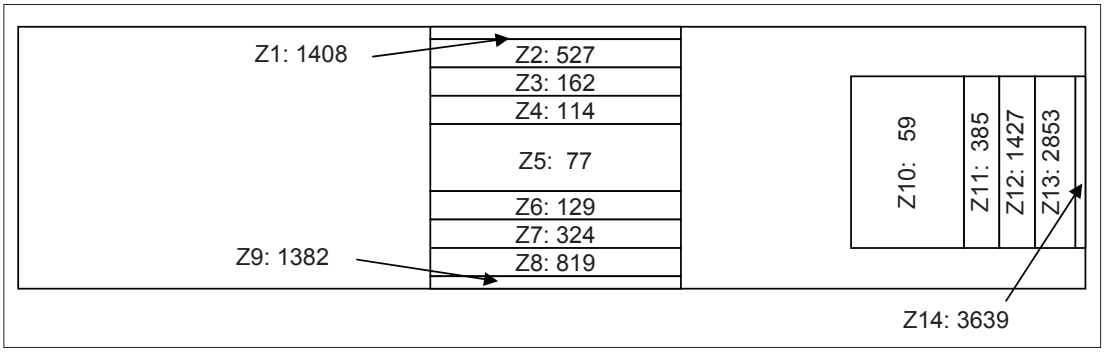


**Figure 2: Residual activity of alkaline phosphatase (IU) measured in different zones of Emmentaler PDO using *p*-nitrophenolphosphate as a substrate.**

**Table 3: Survival of inoculated pathogenic bacteria during manufacture and ripening of Emmentaler PDO cheese manufactured out of raw milk. Detection limit is log cfu/mL = 0 (Bachmann and Spahr 1995).**

| Bacteria | Unit | Milk | Curd after scalding | Cheese 1 day | Cheese 7 days |
|---|---|---|---|---|---|
| *Aeromonas hydrophilia* | log cfu/mL | 5.3 | b.d.l. | b.d.l. | b.d.l. |
| *Campylobacter jejuni* | log cfu/mL | 4.2 | b.d.l. | b.d.l. | b.d.l. |
| *Escherichia coli* | lLog cfu/mL | 6.0 | 2.1 | b.d.l. | b.d.l. |
| *Listeria monocytogenes* | log cfu/mL | 4.6 | 4.5 | b.d.l. | b.d.l. |
| *Pseudomonas aeruginosa* | log cfu/mL | 5.8 | 3.8 | b.d.l. | b.d.l. |
| *Salmonella typhimurium* | log cfu/mL | 5.9 | 2.9 | b.d.l. | b.d.l. |
| *Staphylococcus aureus* | log cfu/mL | 5.8 | 5.0 | b.d.l. | b.d.l. |
| | log cfu/mL | 5.6 | 0.4 | b.d.l. | b.d.l. |

*Notes:*
*b.d.l. = below the detection limit.*

thermo-labile and therefore its activity is reduced by scalding at temperatures above 50°C. Lactic acid bacteria have only limited lipolytic activity, with *Streptococcus thermophilus* having the highest. Propionibacteria show a lipolytic activity 10-100 times stronger than for LAB and which is highly strain dependent. Consequently, lipolysis in Swiss-type cheeses is mainly caused by PAB and is generally recognised as necessary to produce typical Swiss cheese flavour. The amount of free fatty acids present varies from 2 to 7 g/kg (Fröhlich-Wyder and Bachmann 2007).

*L. casei* and *L. rhamnosus* are indigenous to raw milk and belong to the group of facultatively heterofermentative lactobacilli (FHL). They utilise citrate present in young cheese and grow during cheese ripening. For Emmentaler PDO, adjunct cultures of FHL were developed that have been used since 1989 to control PAB fermentation and to minimise late fermentation and therefore improve storage stability. The metabolites acetate and formate of FHL seem to have an inhibitory effect on propionibacteria growth. The metabolism of citrate also leads to a release of complexed copper present in cheese from the copper vat. The concentrations of citrate and copper play an important role in the inhibition. *L. rhamnosus* also produces small but appreciable amounts of diacetyl which has a lethal effect on propionibacteria. As the sensitivity to inhibition by FHL is PAB strain specific, this mechanism can be used to control PAB fermentation very specifically according to the individual needs of a cheese factory (Jimeno *et al*. 1995; Fröhlich *et al*. 2002).

Some Swiss-type cheeses are semi-hard and made from pasteurised milk. Therefore, mesophilic lactic acid bacteria such as *Lactococcus lactis* may also be used. The goals of the cultures are different for Emmentaler PDO and for generic emmental cheese. For Emmentaler PDO, inhibition of late blowing by avoiding excessively active PAB and the selection of LAB with a limited proteolysis are the main goals. Cultures for generic emmental are selected for strong and fast PAB fermentation and strong proteolysis to develop flavour as quickly as possible. The cheeses are consumed at a younger stage and defects of the cheese body and of eye formation are tolerated.

## Ripening – comparison of raw and pasteurised milk cheeses

In addition to eliminating pathogenic bacteria, milk pasteurisation modifies the biochemistry and microbiology of ripening, and directly, through the different ripening pattern, influences cheese flavour and texture. The following effects are known to be associated with milk pasteurisation:

- reduction of vegetative bacteria count including non starter lactic acid bacteria (NSLAB);
- activation of the plasmin / plasminogen complex;
- inactivation of the indigenous lipoprotein lipase, alkaline phosphatase and other indigenous enzymes;
- slight denaturation (7%) of whey proteins;
- impaired rennetability of heated milk mainly due to the formation of complexes of κ-casein with β-lactoglobulin; and
- general increase of the activity of starter LAB and surviving micro-organisms.

Impaired rennetability of heated milk can be compensated by adding $CaCl_2$. Nevertheless, cheeses made from pasteurised milk tend to higher water contents and more elastic textures. The main effect in pasteurised milk cheese is a milder and more uniform flavour and a slower ripening of the cheese. Raw milk micro-organisms are considered to be responsible for the diversity of aromas of raw milk cheese, especially in semi-hard, hard and extra-hard cheese varieties ripened from a few months up to three years. An intense proteolysis, and for some varieties a limited lipolysis, occurs during ripening. Consequently, higher levels of free amino acids (NPN in Table 4), sometimes free fatty acids and some volatile compounds (Table 5) are found in raw milk cheeses (Beuvier and Buchin 2004).

For emmental cheese, the PAB fermentation and proteolysis are the most important factors for ripening and flavour development. In contrast to cheddar or gouda cheese types, for example, rennet is inactivated during the scalding of the emmental curd and does not play a significant role in proteolysis. Indigenous milk proteinases and the proteolytic enzymes of the lactic acid

**Table 4: Volatile organic acids, proteolysis and other metabolites in Emmentaler PDO made out of raw milk compared to generic emmental cheese (Pillonel *et al*. 2005).**

| Compound | Unit | Emmentaler PDO | Emmental France (Brittany) | | Emmental France (East-Central) | | |
|---|---|---|---|---|---|---|---|
| Milk | | raw milk | thermised, bactofuged | | thermised, bactofuged | | |
| No. samples | | 32 | 7 | | 6 | | |
| Ripening time, median | days | 175 | 53 | | 74 | | |
| Formate | mmol/kg | 3.65 | 3.06 | ↓ | 3.22 | | From citrate by FHL |
| Acetate | mmol/kg | 46.22 | 35.78 | ↓ | 36.00 | ↓ | PAB metabolite |
| Propionate | mmol/kg | 58.33 | 33.33 | ↓ | 27.78 | ↓ | PAB metabolite |
| Butyrate | mmol/kg | 0.83 | 3.75 | ↑ | 4.17 | ↑ | Contamination with clostridia |
| Capronate | mmol/kg | 0.31 | 0.21 | | 0.15 | | Indicator for lipolysis |
| TN | g/kg | 44.7 | 44.0 | | 44.95 | | No sig. difference |
| WSN | g/kg | 10.12 | 6.86 | ↓ | 8.22 | ↓ | |
| 12% TCA-SN (NPN) | g/kg | 7.07 | 5.14 | ↓ | 6.00 | | |
| L-Lactate | mmol/kg | 27.43 | 37.14 | ↑ | 60.00 | ↑ | Neg. correlated with actetate and propionate |
| D-Lactate | mmol/kg | 25.00 | 49.17 | ↑ | 54.16 | | same |
| succinate | mmol/kg | 3.57 | 7.86 | ↑ | 6.07 | | Aspartate activity PAB |
| *Lb. helveticus* >3.0 ng | ng/µL DNA | 0% (below 1.2) | 73% | | 93% | | Culture |

*Notes:*
*↓ significantly lower compared to Emmentaler PDO (p≤0.001); ↑ significantly higher compared to Emmentaler PDO (p≤0.001).*

bacteria are mainly responsible for protein breakdown. Table 4 shows metabolites of the primary and secondary fermentation in Emmentaler PDO cheese made from raw milk and in French emmental cheese made from bactofugated thermised milk. Values to characterise proteolysis are shown as well. Formate is produced from citrate by FHL which are added as an adjunct culture to Emmentaler PDO. Emmental cheese from Brittany had a significantly lower content. Acetate and propionate are formed out of lactate by the classic pathway of PAB fermentation and were higher in Emmentaler PDO from raw milk with a ripening period of 175 days compared to 53 or 74 days, respectively, for French emmental. The elevated values of butyrate for French emmental showed that technological measures such as bactofugation or preservatives could not prevent butyric acid fermentation by clostridia completely. The significantly higher contents of water soluble nitrogen (WSN) and 12% TCA-SN in Emmentaler PDO indicated a more pronounced proteolysis. The shorter ripening time of French emmental influenced these values as well. Emmentaler PDO showed a significantly lower value of succinate, indicating and confirming the use of PAB cultures with a low aspartase activity to allow good ripening stability (Fröhlich *et al*. 2002). The low amount of *L. helveticus*-DNA found in Emmentaler PDO and the much higher amounts found in French emmental showed the different starter cultures used for the two emmental types.

Curione and Bosset (2002) identified key odorants in emmental cheese by gas chromatography and olfactometry. Many compounds with a fruity odour like 2-heptaone, ethyl-butyrate, ethyl-hexanoate, δ-decalactone or two different furanones could be identified. Savoury compounds were 3-methylbutanal and 1-octen-3-one. Typical dairy related compounds were the butter-like diacetyl and ethyl-3-methylbutanoate, reminding one of fresh cheese. This explains the often described sweetness of emmental cheese. Also the magnesium- and calcium-propionate contribute to the sweet flavour (Fröhlich-Wyder and Bachmann 2007).

The volatile compounds of Emmentaler PDO from Switzerland and of emmental cheese from thermised and bactofugated milk made in Brittany, France, were analysed by gas chromatography (Table 5, Pillonel *et al*. 2003b). Significant differences for the ketone 3-hydroxybutanone, the aldehyde hexanal, the alcohols propan-2-ol and butan-1-ol, the butanoic-acid-ethyl-ester and the hydrocarbon 3-methylheptane could be found. From the above identified key odorants, for diacetyl a higher level was suggested in French emmental cheese, though the difference was not significant. This is in accordance with present knowledge that less ripened cheese has a higher diacetyl content. All the other mentioned analytes showed at least one significant difference analysing also emmental cheese from Austria, Germany (Bavaria), Finland and Savoie (France). Emmentaler PDO contained much more esters than the French emmental and more volatiles of the group ketones and aldehydes. Hydrocarbons were found in similar amounts and alcohols in PDO were less abundant.

## Proof of origin by naturally occurring genetic markers in lactobacilli

As the market value of Emmentaler PDO cheese made in Switzerland is high, falsified cheese can be found on the market. Besides a system with special cheese labels used for Emmentaler PDO, other ways to prove the origin of Emmentaler PDO were and are being investigated. Naturally occurring genetic markers in lactobacilli are used to verify the authenticity of Emmentaler PDO cheese (Casey *et al*. 2008). From cheese out of 18 generic

**Table 5: Volatile compounds in Emmentaler PDO cheese and French emmental (Brittany). Peak heights are given in arbitrary units (according to Pillonel *et al*. 2003b).**

| Peak No. | Analytes | Retention Index | ANOVA | French Emmental (Brittany) n=3 | | Emmentaler PDO n=6 | |
|---|---|---|---|---|---|---|---|
| | | | | x | $s_x$ | x | $s_x$ |
| | **Ketones and aldehydes** | | | **450** | | **674** | |
| 1 | Propan-2-one | 466 | - | 341 | 230 | 572 | 260 |
| 5 | Butan-2,3-dione | 556 | - | 60 | 29 | 35 | 17 |
| 6 | Butan-2-one (=diacetyl) | 567 | - | 41 | 9 | 21 | 7 |
| 10 | 3-Hydroxybutanone | 683 | *** | 3.4 | 2.6 | 18.7 | 7.9 |
| 18 | Hexanal | 780 | *** | 4.8 | 1.6 | 26.9 | 8.2 |
| | **Alcohols** | | | **589** | | **304** | |
| 2 | Propan-2-ol | 481 | *** | 167 | 80 | 62 | 35 |
| 4 | Propan-1-ol | 535 | - | 148 | 42 | 143 | 94 |
| 7 | Butan-2-ol | 583 | - | 21 | 20 | b.d.l. | – |
| 8 | 2-Methylpropanol | 616 | - | 4.3 | 2.5 | 7.8 | 3.9 |
| 9 | Butan-1-ol | 652 | ** | 143 | 61 | 19 | 21 |
| 11 | Pentan-2-ol | 685 | - | 45 | 38 | 11 | 7 |
| 14 | 2-Methylbutanol | 725 | - | 61 | 9 | 61 | 25 |
| | **Esters** | | | **23** | | **169** | |
| 12 | Butanoic-acid-ethyl-ester | 695 | ** | 23 | 10 | 169 | 66 |
| | **Hydrocarbons** | | | **21** | | **25** | |
| 3 | Pentane | 499 | - | 9.2 | 4.5 | 8.2 | 0.9 |
| 16 | Toluene | 761 | - | 12.0 | 2.8 | 10.6 | 3.7 |
| 17 | 3-Methylheptane | 776 | * | b.d.l. | – | 6.0 | 2.7 |

*Notes:*
*b.d.l. = below the detection limit.*
*** $p \leq 0.001$; ** $p \leq 0.01$; * $p \leq 0.05$

European emmental and from 55 Emmentaler PDO, DNA was extracted. Emmentaler PDO was produced with one of three adjunct cultures of FHL (Table 6). The isolated DNA was amplified using strain-specific primers. Specific PCR-products of specific sizes were obtained. These allowed the identification of Emmentaler PDO. Generic emmental did not contain any of the strain-specific PCR-products as the three adjunct cultures are exclusively used in Switzerland.

## Biogenic amines

The degradation of free amino acids by decarboxylation results in the formation of biogenic amines. Non-starter bacteria such as enterococci, salt-tolerant lactobacilli and enterobacteriaceae may be potent histamine producers (Joosten and Northolt 1987). A specific strain of *L. buchneri*, strain St2A, was identified by Sumner *et al*. (1990) to form histamine during Swiss-type cheese ripening in amounts proportional to the initial number of the strain inoculated in the raw milk. Biogenic amines are important substances in the human metabolism but in higher concentrations they can cause illness, pseudo-allergic reactions or even be toxic (Wechsler *et al*. 2009). An investigation of Emmentaler PDO and generic European emmental by Pillonel *et al*. (2003a) showed that Emmentaler PDO had the lowest content of biogenic amines compared to cheese from Germany (Allgäu), France (Brittany, Savoie), Finland and Austria (Vorarlberg) (Table 7). For the listed biogenic amines cadaverine, histamine, putrescine and tyramine, significant differences between the cheese groups were found. All the detected concentrations did not present any danger for health.

As previously outlined, Emmentaler PDO is manufactured in a way that allows good maturation. It is known that during maturation the occurrence of biogenic amines increases. From nine cheese factories, three wheels each were collected during three consecutive weeks and analysed at 3, 7 and 11 months. From seven out of the nine cheese factories, Emmentaler PDO ripened for a period of 11 months had only moderate contents of biogenic amines with values below 200 mg/kg (Table 8). However, in the two cheese factories L and H, the occurrence of biogenic amines with 807 and 305 mg/kg, respectively, was significantly higher. These data indicate that persistent contaminations may occur in cheese factories or on the farm of individual milk suppliers which lead to an undesirably high formation of biogenic amines. From cheese factory L, further cheeses from another production period were analysed and the high content of histamine and tyramine was confirmed (data not shown). Differences in the ratio between histamine and tyramine in cheeses of the different cheese factories indicate that several micro-organisms are involved in the formation of biogenic amines in Emmentaler PDO. In cheeses of factory L, an increased level of enterococci was detected. Many of them are able to form tyramine. Also *L. buchneri* was found, a compulsory heterofermentative LAB that is a frequent constituent of the flora present in silage. As *L. buchneri* often forms histamine, this probably explains the high contents of histamine in generic emmental (Table 7).

A further study on the biogenic amines in Emmentaler PDO from 10 different factories and matured for 12 months confirmed these findings (Table 9). In the cheeses of two cheese factories, histamine contents of 576 and 911 mg/kg respectively were detected. Average histamine content was much lower (201 mg/kg) and the average total content of biogenic amines with 241 mg/kg can be considered as moderate. Four more cheeses of the factory with the highest detected content of biogenic amines from a production period over six months were analysed and histamine levels of 400, 530, 725 and 1,364 mg/kg, respectively, confirmed the systematically high level over a longer production period in this factory.

In Italian cheese, the formation of cadaverine and putrescine was explained by the presence of Enterobacteriaceae and

**Table 6: PCR products specific for the adjunct cultures FHL (MK 3008, n= 17; MK 3010, n=19; MW 3012, n= 19; Size in base pairs bp; Casey *et al*. 2008).**

| Adjunct culture | Strains | Specific size of PCR product | Found only in |
|---|---|---|---|
| MK 3008 | *L. casei* WS 07.04<br>*L. casei* WS 09.05<br>*L. casei* WS 11.30 | 262 bp | 17 Emmentaler PDO |
| MK 3010 | *L. casei* WS 01.02 | 365 bp | 19 Emmentaler PDO |
| MW 3012 | *L. rhamnosus* WS 10.16<br>*L. rhamnosus* WS 13.25<br>*L. rhamnosus* WS 15.23 | 210 bp | 19 Emmentaler PDO |

**Table 7: Comparison of occurrence of biogenic amines in Emmentaler PDO and generic emmental from European countries and regions (amounts in mg/kg; Pillonel *et al*. 2003a).**

| | ANOVA | Allgäu (n=3) 4 mo. | | Brittany (n=3) 2.5 mo. | | Emmentaler PDO (n=6) 4 mo. | | Finnland (n=2) 3 mo. | | Savoie (n=3) 3 mo. | | Vorarlberg (n=3) 3 mo. | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | x | $s_x$ | x | $s_x$ | x | $s_x$ | x | $s_x$ | x | $s_x$ | x | $s_x$ |
| Cadaverine | ** | 44.0 | 35.0 | 5.6 | 7.0 | 0.2 | 0.3 | 25.0 | 15.0 | 8.6 | 6.7 | 1.1 | 0.8 |
| Histamine | * | 672.0 | 376.0 | 123.0 | 149.0 | 12.3 | 7.4 | 59.0 | 26.0 | 478.0 | 434.0 | 205.0 | 105.0 |
| Putrescine | * | 15.0 | 21.0 | 2.3 | 1.2 | <2.0 | - | <2.0 | - | 24.0 | 19.0 | <2.0 | - |
| Tyramine | * | 178.0 | 121.0 | 8.6 | 8.4 | 54.0 | 123.0 | 220.0 | 45.0 | 403.0 | 269.0 | 117.0 | 91.0 |
| Total | * | 920.0 | 420.0 | 142.0 | 165.0 | 81.0 | 134.0 | 325.0 | 14.0 | 948.0 | 709.0 | 325.0 | 195.0 |

** $p \leq 0.01$; * $p \leq 0.05$

*Pseudomonas* (Innocente and D'Agostin 2002). In Emmentaler PDO, these two biogenic amines only can be found in cheese with microbiological quality defects (Figure 3). The total amount of biogenic amines in that cheese was as high as 1,637 mg/kg. The content of putrescine was unusually high with 481 mg/kg whereas the normal level in Emmentaler PDO is below 3 mg/kg. No secondary propionic acid fermentation was found in the cheese. Excess gas formation was due to the decarboxylation of amino acids to the corresponding biogenic amines. Further work is in progress with the aim to develop suitable methods that allow a rapid identification of the contamination sources in factories producing cheeses with systematically high levels of biogenic amines.

## References


Bachmann, H.P., Bütikofer, U. and Isolini, D. (2002), Cheese: Swiss-Type Cheese. In: *Encyclopedia of Dairy Sciences* (Eds H. Roginski, J.W. Fuquay and P.F. Fox). Elsevier Academic Press, London, pp 363-371.

Bachmann, H.P. and Spahr, U. (1995), The fate of potentially pathogenic bacteria in Swiss hard and semi-hard cheeses made from raw milk. *J. Dairy Sci*. **78**, 476-483.

Beuvier, E. and Buchin, S. (2004), Raw milk cheeses. In *Cheese: Chemistry, Physics and Microbiology*, 3rd edn., vol. 1. (Eds P.F. Fox, P.L.H. McSweeney, T.M. Cogan, and T.P.Guinee). Elsevier Academic Press, London.

Casey, M.G. Isolini, D. Amrein, R. Wechsler, D. and Berthoud, H. (2008), Naturally occurring genetic markers in lactobacilli and their use to verify the authenticity of Swiss Emmental PDO cheese. *Dairy Sci. Technol.* **88**, 457-466.

Codex (2008), Codex Standard for Emmental, Codex Standard 269-1967. Established 1967, Revision 1 2007, Amendment 1 2008. Codex Alimentarius Commission, Joint FAO/WHO food standards programme, Rome, Italy.

Curioni, P.M.G. Bosset, J.O. (2002), Key odorants in various cheese types as determined by gas chromatography-olfactometry. *Int. Dairy J.* **12**, 959-984.

FOAG (2010), Emmentaler PDO. Swiss Federal Office for Agriculture. Accessed at www.blw.admin.ch [April 2010].

FOAG (2002), Zusammenfassung des Eintragungsgesuchs für Emmentaler (GUB) (Summary of the request for the registration of Emmentaler with a PDO). Swiss Federal Office for Agriculture FOAG. Accesed at www.blw.admin.ch [April 2010].

Fröhlich, M.T. Bachmann and H.P. Casey, M. (2002), Interaction between propionibacteria and starter/non-starter lactic acid bacteria in Swiss-type cheeses. *Lait* **82**, 1-15.

Fröhlich-Wyder, M.T. and Bachmann, H.P. (2007), Swiss cheese. In *Cheese problems solved.* (Ed P.L.H. McSweeney). Woodhead Publishing Limited and CRC Press LLC, Cambridge, England, pp 246-267.

Hansen (2009). Product information, Christian Hansen, Denmark.

Innocente, N. and D'Agostin, P. (2002), Formation of biogenic amines in a typical semihard Italian cheese. *J. Food Prot.* **65**, 1498-1501.

Jakob, E. Winkler, H. and Haldimann, J. (2010), Mikrobiologische Kriterien in der Käsefabrikation (Microbiological criteria for cheese making). ALP forum, No. 77, 1-32. Agroscope Liebefeld-Posieux, Bern, Switzerland. Accessed at www.agroscope.ch [May 2010].

Jimeno, J. Lazaro, M.J. and Sollberger, H. (1995), Antagonistic interactions between propionic acid bacteria and non-starter lactic acid bacteria. *Lait* **75**, 401-413.

Joosten, M.H.L.J. and Northolt, M.D. (1987), Conditions allowing the formation of biogenic amines in cheese. 2. Decarboxylative properties of some non-starter bacteria. *Neth. Milk Dairy J.* **41**, 259-280.

Pillonel, L. Albrecht, B. Badertscher, R. Bütikofer, U. Chamba, J.F. Tabacchi, R. and Bosset, J.O. (2003a), Analytical methods for the determination of the geographic origin of Emmental cheese. Parameters of proteolysis and rheology. *Ital. J. Food Sci.* **15**, 49-62.

Pillonel, L. Ampuero, S. Tabachi, R. and Bosset, J.O. (2003b), Analytical methods for the determination of the geographic origin of Emmental cheese: volatile compounds by GC/MS-FID and electronic nose. *Eur. Food Res. Technol.* **216**, 179-183.

Pillonel, L. Badertscher, R. Casey, M. Meyer, J. Rossmann, A. Schlichtherle-Cerny, H., Tabacchi R. and Bosset, J.O. (2005), Geographic origin of European Emmental cheese: Characterisation and descriptive statistics. *Int. Dairy J.* **15**, 547-556.

Sieber, R. Collomb, M. Lavanchy, P. and Steiger, G. (1988), Beitrag zur Kenntnis der Zusammensetzung schweizerischer konsumreifer Emmentaler, Greyerzer, Sbrinz, Appenzeller und Tilsiter. (Composition of mature Swiss Emmentaler, Gruyere, Sbrinz, Appenzell and Tilsit cheeses). *Schweiz. Milchw. Forschung.* **17**, 9-16.

Sumner, S.S. Roche, F. and Taylor, S.L. (1990), Factors controlling histamine production in Swiss cheese inoculated with Lacotbacillus buchneri. *J. Dairy Sci.* **73**, 3050-3058.

Wechsler, D. Walther, B. Jakob, E. and Winkler H. (2009), Bedeutung biogener Amine in der Ernährung und deren Vorkommen in Schweizer Käsesorten (Significance of biogenic amines in nutrition and their occurrence in cheese varieties of Switzerland). ALP forum, No. 73, 1-23. Agroscope Liebefeld-Posieux, Bern, Switzerland. Accessed at www.agroscope.ch [May 2010].

Wyder, M.T. Bosset, J.O. Casey, M.G. Isolini, D. and Sollberger, H. (2001), Influence of two different propionibacterial cultures on the characteristics of Swiss-type cheese with regard to aspartate metabolism. *Milk Sci. Int.* **56**, 78-81.


**Table 8: Total biogenic amines (mg/kg) in Emmentaler PDO from nine cheese factories at the age of 3, 7 and 11 months (n=3 per factory over three consecutive weeks).**

| Factory | L | H | J | I | N | G | B | M | K |
|---|---|---|---|---|---|---|---|---|---|
| 3 months | 125 | 39 | 26 | 30 | 55 | 22 | 12 | 29 | 10 |
| $s_x$ | 38 | 8 | 18 | 21 | 17 | 8 | 4 | 7 | 0 |
| 7 months | 430 | 204 | 77 | 128 | 94 | 131 | 31 | 42 | 25 |
| $s_x$ | 75 | 90 | 35 | 76 | 34 | 70 | 6 | 17 | 5 |
| 11 months | 807 | 305 | 186 | 176 | 172 | 157 | 78 | 74 | 41 |
| $s_x$ | 79 | 31 | 77 | 115 | 27 | 48 | 51 | 40 | 10 |
| Significance 11 months | a | b | c | cd | cd | cd | d | d | d |

*Notes:*
*Different letters in rows mean significant differences ($p<0.05$).*

**Table 9: Biogenic amines in Emmentaler PDO from 10 cheese factories matured for 12 months (n=10).**

| Biogenic amine | Average (mg/kg) | Minimum (mg/kg) | Maximum (mg/kg) | $s_x$ (mg/kg) |
|---|---|---|---|---|
| Cadaverine | <2 | <2 | 3 | <2 |
| Histamine | 201 | <2 | 911 | 303 |
| Isopentylamine | <2 | <2 | 4 | <2 |
| Putrescine | <2 | <2 | 3 | <2 |
| Spermidine | <2 | <2 | < 2 | <2 |
| Spermine | <2 | <2 | < 2 | <2 |
| β-Phenylethylamine | <2 | <2 | < 2 | <2 |
| Tryptamine | <2 | <2 | < 2 | <2 |
| Tyramine | 37 | 28 | 77 | 15 |
| Total biogenic amines | 241 | 34 | 945 | 300 |

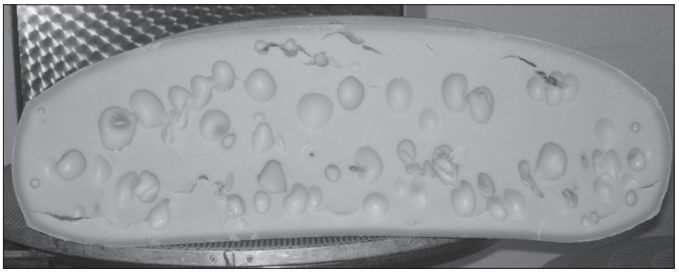

**Figure 3: Emmentaler PDO matured for 5.5 months with serious quality defects such as atypical pungent flavour, symptoms of blowing and high content of biogenic amines (histamine 630 mg/kg, tyramine 505 mg/kg, putrescine 481 mg/kg).**