Microbiological quality of white-brined cheeses: a review

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INTRODUCTION

White-brined cheeses (also known as white-pickled cheeses) are the most popular varieties of cheeses manufactured in the North-east Mediterranean area and the Balkans. They are manufactured from ovine, buffalo, bovine and/or caprine milk or from mixtures of these milks. Feta cheese (in Greece), domiati (in Egypt), beyaz peynir (in Turkey) and halloumi (in Cyprus) are the best-known cheeses, while other, less well-known varieties include batzos from Greece and brinza from Bulgaria.

It is likely that these cheeses share the same origin, and that they have become differentiated over time according to the particular demands of the people and climate of each country. Most of these cheeses are dry-salted and then matured and stored in brine, and this salting is the main difference from the cheese varieties produced in northern European countries. Traditionally, they were made as artisanal cheeses using raw milk, and the cheesemaker had to rely on the natural microflora of the milk, mainly lactic acid bacteria, to acidify the milk.

Nowadays the demand for white-brined cheeses is so great that tonnage quantities are produced in large dairies. Bovine and/or milk mixtures, usually pasteurized, are used, and the addition of starter cultures is common practice. White-brined cheeses can also be successfully manufactured from ultrafiltered (UF) milk on an industrial scale, and threefold and fivefold concentrations have been used to produce UF-structured and UF-cast cheeses, respectively.

However, as these UF-cheeses have results with respect to acidification of the milk; the latter culture is widely used as it is easy to prepare and preserve. Increasing the level of starter culture results in a cheese with a lower pH and moisture content, together with a lower yield.

The effects of adding various commercial concentrated starters directly to the cheese milk—these do not require bulk culture production—have been studied recently and both mesophilic and thermophilic starters can be used for feta cheese, although use of the latter results in higher levels of proteolysis. Although such starters have not been used commercially for the production of white-brined cheeses, the development of genetically modified starter cultures with enhanced aroma and flavour-producing activity could become important.

The main role of a starter culture is the early acidification of the curd, and yoghurt cultures die...
The species of yeast most frequently found in white-brined cheeses and their brines

<table>
<thead>
<tr>
<th>Species</th>
<th>Type of cheese</th>
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<tbody>
<tr>
<td>Torulaspora delbrueckii</td>
<td>Danish feta (bovine milk)</td>
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<tr>
<td>Debaryomyces Hansenii</td>
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<td>Candida sake</td>
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<td>Yarrowia lipolytica</td>
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<td>Kluyveromyces marxianus</td>
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<td>Candida baiyer</td>
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<td>Saccharomyces cerevisae</td>
<td>Greek feta (ovine milk)</td>
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<td>D. hansenii</td>
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<td>Pichia farinosa</td>
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<td>Candida versatilis</td>
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<td>K. marxianus</td>
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<tr>
<td>Kluyveromyces blattae</td>
<td>French feta (ovine milk)</td>
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<td>Candida spharica</td>
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<tr>
<td>Kluyveromyces thermotolerans</td>
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<td>D. hansenii</td>
<td>Haloumi (ovine milk)</td>
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<td>Candida parapsilosis</td>
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<td>Candida boidini</td>
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<td>C. versatilis</td>
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<tr>
<td>Pichia membranifaciens</td>
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<tr>
<td>Cryptococcus albidos</td>
<td>Halloumi (bovine milk)</td>
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<tr>
<td>P. membranifaciens</td>
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<tr>
<td>S. cerevisae</td>
<td>Brine of Greek feta</td>
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<tr>
<td>Candida famata</td>
<td>(ovine milk)</td>
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<td>P. membranifaciens</td>
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<td>D. hansenii</td>
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<td>Candida baiyer</td>
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<td>T. delbrueckii</td>
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After Refs 15, 17, 19–22

Traditional feta cheese is dry-aged for 5–10 days, depending on the temperature.23,24 During this stage, a slime of surface-growing bacteria and yeasts develops slowly, and this slime makes a major contribution to the maturation of cheese.13 The contribution of this secondary microflora is more important in the middle and late stages of maturation, when high numbers of lactobacilli ($\sim 1.0 \times 10^6$ cfu g$^{-1}$) and yeasts ($2.0 \times 10^5$–$1.0 \times 10^6$ cfu g$^{-1}$) can be detected. Yanai et al.15 reported that as a typical yeast cell has a volume at least 50 times the volume of the largest bacterial cell, the larger biomass of the yeasts might indicate that their role in the maturation of cheese is more important than the counts would suggest.

Enterococci (e.g. Enterococcus faecalis and Enterococcus durans) and pediococci (e.g. Pediococcus pentosaceus and Pediococcus acidilactici) have been found in high numbers in fresh feta cheese;11 however, their numbers declined throughout maturation and both groups were outgrown by the lactobacilli. Enterococcus faecium was the main species recovered from samples of traditional halloumi cheese (1-day-old).15 In another study of microbiological quality feta-type cheeses, the numbers of enterococci were found to be high (up to $3 \times 10^6$ cfu g$^{-1}$) in locally produced cheeses, while imported Danish feta cheeses had between 20 and < 10 cfu g$^{-1}$.26 Litopoulou-Tzanetaki et al.4 reported that the inclusion of Enterococcus durans in a mesophilic LAB starter gave a better flavour, texture and body to feta cheese than the normal yoghurt culture. The differences were attributed to the proteolytic and lipolytic activities of the strain, and an Enterococcus spp. starter has been suggested for the flavour enhancement of domiati cheese.27

In telemche cheese, high numbers of leuconostocs were reported; their numbers increased during the first 30 days but declined thereafter. A salt-resistant species, Leuconostoc paramesenteroides, was found to be the predominant species.28 Rasic29 reported...
that the leucosostos were more frequently found at the beginning of the ripening of white-brined cheese (brinza-type), while the pediococci were more numerous in the later phase of the maturation.

Yanai et al.\textsuperscript{33} reported that coliforms were found in white-brined cheese, even though they were absent from the pasteurized milk and the starter culture, but their number declined in parallel with the reduction in pH. In batzos cheese—a raw ovine milk cheese manufactured in North Greece—\textit{Enterobacteriaceae} and coliforms decreased in number gradually and, by the end of storage, the counts were lower by 2–3 log units over the initial ones.\textsuperscript{36} Because the dominant microbial groups in these secondary microfloras are generally acknowledged as contributing to the maturation process, their addition as ‘adjunct cultures’, namely cultures added for purposes other than acidification of the curd, could accelerate the maturation process. Thus, Vafopoulos-Mastroiannaki et al.\textsuperscript{10} studied the effect of \textit{Pediococcus pentosaceus} on the maturation of feta cheese, and reported that the addition of pediococci reduced the time needed for maturation. As no effect on the extent of lipolysis was observed, the authors speculated that the improvement in flavour was due to the accelerated formation of volatile compounds derived from amino acids. In addition, Vafopoulos et al.\textsuperscript{32} reported that acceleration of the maturation of feta cheese could be achieved by the addition of a heat-shocked yoghurt culture or microbial proteases, together with the addition of small amounts of lamb lipase.

\textcolor{red}{El-Soda et al.\textsuperscript{31}} observed that the ripening of domiati cheese could be accelerated, without impairment of the flavour balance, using crude cell-free extracts from lactobacilli, and more particularly \textit{Lb. plantarum}. The authors speculated that, as the bitter flavours observed in cheddar cheese made with extracts of lactobacilli were absent in domiati, the high salt content of the latter type of cheese either masked the bitter flavours or limited the relevant enzyme activity to an acceptable level.

Feta-type cheese manufactured from bovine milk with the addition of \textit{Lc. lactis} ssp. \textit{lactis} and \textit{Lb. casei} ssp. \textit{casei} as starters and a blend of kid and lamb pregastric esterases developed the flavour and texture of authentic feta.\textsuperscript{34} According to El-Neshawy et al.\textsuperscript{35} low levels of pregastric esterases improved and accelerated the flavour development of domiati cheese, but prolonged ripening gave rise to rancid off-flavours.

**Defects in white-brined cheeses**

Although the secondary microflora might make a beneficial contribution to the development of cheese flavour, components of the same microflora can, on occasions, cause defects.

The most common defect of feta and similar cheeses is ‘early blowing’, a defect that is characterized by the presence of large gas holes in the cheese, whose incidence, in addition, has a spongy texture; this defect is due to coliforms and/or yeasts growing in excessive numbers.\textsuperscript{36} However, the problem is rare in modern dairies, provided that efficient pasteurization and good manufacturing practices are applied. Furthermore, the activity of the starter is crucial in the control of coliforms by decreasing the pH and the amount of lactose in the curd.

The presence of coliforms in cheese, particularly \textit{Aerobacter aerogenes}, has been reported to be responsible for blown tins of domiati cheese;\textsuperscript{37} a salt content in the milk of 90 g L\textsuperscript{−1} can prevent this defect. \textit{Klebsiella aerogenes} was found to be responsible for early blowing and poor cheese quality in other white-brined cheeses.\textsuperscript{38} Excessive yeast growth will cause softening of cheese, a condition that is usually associated with an unpleasant yeasty or ester-like odour\textsuperscript{39} or gas formation;\textsuperscript{30} in the case of white-brined cheeses, swelling of the cans can be caused by yeasts that ferment lactose, e.g. \textit{Kluyveromycetes} spp. Discoloration of the surface of a Portuguese ewes’ milk cheese has been attributed to pigment-producing yeasts.\textsuperscript{40} In addition, yeasts can increase the pH of the cheese surface, thus spurring the growth of \textit{Staphylococcus aureus}\textsuperscript{41} and possibly other pathogenic and/or spoilage bacteria. For feta stored over a year, a definite deterioration of quality was noticed when the pH of the cheese increased to more than 5.0.\textsuperscript{2}

‘Late blowing’ is another defect in cheeses, and this problem is usually attributed to heterofermentative LAB or species of clostridia (e.g. \textit{Clostridium butyricum} and \textit{Clostridium tyrobutyricum}). Although the latter group are sensitive to acid and salt and are more usually associated with problems in Swiss-type and Dutch-type cheeses,\textsuperscript{42,43} some strains of \textit{Cl. tyrobutyricum} are acid-resistant (growing well in a 4.5–7.5 pH zone) and relatively
salt-tolerant (tolerating as much as 55–60 g L\(^{-1}\) NaCl at their optimal pH).\(^{44}\) \textit{Cl. tyrobutyricum} can ferment lactate with the production of butyric acid, which can give an unpleasant aroma at high concentrations, together with carbon dioxide, and it can also generate hydrogen. A similar mixture of gases, released by \textit{Bacillus subtilis}, \textit{Bacillus fastidiosus}, \textit{Bacillus pumilus}, \textit{Bacillus firmus}, \textit{Clostridium paratratrum} and \textit{Clostridium tertium}, was responsible for the swelling of cans of feta cheese.\(^{45}\)

The development of moulds causing visible defects has been reported for various cheeses,\(^{55}\) but because white-brined cheeses are stored in tins filled with brine, the development of moulds is rare, provided that the cheese blocks are completely immersed in the brine.

The growth of psychrotrophs might cause certain defects. More specifically, lipolysis might lead to the excessive formation of free fatty acids (FFAs) and rancid flavours in teleme cheese; this is usually the result of contamination of milk with psychrotrophic bacteria, which produce heat-resistant lipolytic enzymes.\(^{46}\) \textit{Pseudomonas} spp. are the most important group of psychrotrophs associated with cheeses; these produce heat-resistant extracellular proteolytic and lipolytic enzymes, which can cause off-flavours and texture defects.\(^{47}\)

The presence of slime in the brine is a common defect caused by strains of \textit{Lb. plantarum} and/or \textit{Lb. casei} spp. \textit{casei}\(^{48}\) but it can be prevented by ensuring that the pH of the brine is \(-4.0\) and the salt content higher than \(80 \text{ g L}^{-1}\). Chomakov\(^{49}\) reported that strains of \textit{Lactobacillus plantarum} var. \textit{viscosum} were responsible for the formation of ropy substances in the brine of white-brined cheese. The observations highlight the point that the selection of lactobacilli for use as ‘adjunct cultures’ in the manufacture of white-brined cheese must be based on an extensive study of their biochemical activities, as the development of desirable flavours or defective products seems to be strain rather than species dependent.\(^{50}\)

Although most defects are caused by the development of undesirable micro-organisms, some chemically driven defects have been reported. For example, the addition of sorbic acid in feta cheese has been reported to produce 1,3-pentadiene, which gives an unclean odour to the cheese.\(^{51}\)

Fate of pathogens during the manufacture of white-brined cheeses

For many years, dairy products in general and specifically traditionally-manufactured cheeses in particular have been regarded as being nutritious and safe. However, more recently, the dairy industry has had some unfortunate experiences with respect to the presence of pathogenic bacteria in cheese. Pathogens such as \textit{Listeria monocytogenes}, \textit{Yersinia enterocolitica} and \textit{Escherichia coli} \textit{O157:H7} have been involved, along with the more familiar \textit{Staphylococcus aureus}. The dairy industry has responded by further upgrading its already high standards; it has improved processing procedures, sanitation, established Good Manufacturing Practices and implemented Hazard Analysis Critical Control Points (HACCP).\(^{52}\) However, recalls of contaminated batches of cheeses are still being reported.

Traditionally, white-brined cheeses have been manufactured from raw milk, and the flavour of such cheeses is intense and piquant. Raw milk is still used by small dairies, while some larger factories use thermized milk, namely milk heated at \(65^\circ\text{C}\) for \(15–18\) s. Although thermization destroys some pathogenic bacteria, certain pathogens (i.e. \textit{L. monocytogenes} and \textit{E. coli} \textit{O157:H7}) might survive and contaminate the final product. Survival is dependent on several factors, such as the initial level of contamination, the heat, acid and salt tolerance of the species present, the competitive microflora, the composition of the cheese and the conditions of manufacture. For example, white-brined cheeses are often left at high temperatures (i.e. \(16–20^\circ\text{C}\)) to mature (pre-maturation stage) for 2–3 weeks prior to immersion in brine, and, if other parameters are conducive, pathogens can reach high numbers at these temperatures. However, the heat treatment of the cheese blocks is intense and piquant. Raw milk is made from raw milk.\(^{53}\) However, despite their relatively high moisture content, were not involved, probably because of the high salt content and low pH of these cheeses.

Feta cheese might support the growth and survival of \textit{L. monocytogenes},\(^{54}\) as the pathogen survived during 90 days storage at \(4^\circ\text{C}\) when the pH was as low as \(4.3\). In another study of white-brined cheese, the numbers of \textit{L. monocytogenes} increased from \(1.2 \times 10^3\) cfu \(g^{-1}\) at 0 days to \(1.0 \times 10^7\) cfu \(g^{-1}\) after 40 days of storage in brine \((100 \text{ g L}^{-1})\) at \(4^\circ\text{C}\);\(^{55}\) the same micro-organism survived more than 28 days in white-brined cheese made from bovine milk.\(^{56}\) Erkmen\(^{57}\) reported that \textit{L. monocytogenes} could survive in Turkish white cheese during ripening at \(4^\circ\text{C}\) for 90 days, and Gohil et al.\(^{58}\) found that \textit{Listeria} spp. could survive in cans of feta up to the point of retail sale.
Ramsaran et al. found that the counts of *E. coli* O157:H7 had increased almost 100-fold by 10 days after the manufacture of feta cheese, while Hudson et al. reported a 10-fold increase during the first stages of feta cheese manufacture. Aman et al. reported the presence of *E. coli* in domiati cheese and Ahmed et al. found a high incidence of coliforms in domiati cheese; high numbers of enteropathogenic *E. coli* (EEC) were found but *E. coli* O157:H7 was not detected. The authors speculated that the high incidence of EEC was due to a lack of proper sanitation and the use of unpasteurized milk.

*Yersinia enterocolitica* can multiply and survive in feta cheese for 30 days if the pH does not develop normally and remains above 4.5, but Erkmen demonstrated that *Y. enterocolitica* becomes inactive and is finally destroyed in feta cheese when the acidity of the cheese develops due to a lack of proper sanitation and the use of unpasteurized milk. *Salmonella enteritidis* normally. Abu-Donia reported that *Salmonella* typhimurium survived for 34 days in a domiati cheese with 50 g L$^{-1}$ NaCl but for only 16 days when the salt level was increased to 100 g L$^{-1}$. Interestingly, *Salmonella enteritidis* was completely inhibited in feta cheese made from unpasteurized ovine milk, because the strain could not survive the high salt concentrations (70 g L$^{-1}$ NaCl). In a parallel situation, Hargrove et al. concluded that the most important factor controlling *Salmonella* spp. during the manufacture of Cheddar cheese is the acid production by the starter culture; a pH of 5.0 at the time of pressing is sufficient to cause the death of salmonellae. Recently, Melas et al. studied the survival of *Aeromonas hydrophila*—a species potentially pathogenic to humans—in feta cheese, and concluded that initial numbers of $10^7$ cfu g$^{-1}$ were easily inactivated during maturation. The numbers of *S. aureus* in Turkish feta cheese increased during the first day of manufacture, but salting of the cheese, together with the decline in pH, resulted in inhibition of the pathogen. However, it has been reported that increasing the amount of salt in the milk used for the manufacture of domiati cheese enhanced the survival of *S. aureus* in the cheese; this effect was probably due to the inhibition of the LAB by the high salt content.

**Survival of pathogens in brines**

As *Listeria* spp. and other pathogenic bacteria are quite halotolerant, there is an increased concern about the survival and/or growth of such contaminants in the brines used for the storage of white-brined cheeses. Bacteria present in the cheese rapidly contaminate cheese brines, and it has been observed that the pathogen is inactivated more slowly in the brine than in the cheese.

Results in our laboratory showed that the population of *S. aureus* decreased by five log cycles over 10 days storage at 5°C in an acidified model brine (60 g L$^{-1}$ NaCl and pH 4.5), while *L. innocua* survived for 60 days; the latter was eliminated within 20 days when stored at 20°C. The counts of *E. coli* O157:H7 showed a trend similar to *L. innocua*, although the former was slightly less resistant to these specific conditions. The increased survival of these pathogens during cold storage has also been observed by other researchers, and this is of crucial importance in terms of safety, as there is a tendency to rely on low-temperature storage to reduce the risk of pathogens reaching consumers.

Although storage in brine is thought to cause a decrease in the populations of undesirable contaminants, there is great concern that the brine can also serve as a reservoir of certain salt-tolerant pathogens. Larson et al. reported that *L. monocytogenes* survived for 118 days in fresh feta cheese brines (65 g L$^{-1}$ NaCl and pH 6.8) at 4°C and 12°C; moreover, it has been shown that *L. monocytogenes* can grow in salt solutions of up to 60 g L$^{-1}$ NaCl.

As feta and similar white-brined cheeses (i.e. halloumi and domiati) are frequently stored in salted whey containing various concentrations of NaCl, Papageorgiou and Marth studied the fate of *L. monocytogenes* in salted whey. They found that the pathogen was able to grow in salted whey (60 g L$^{-1}$ NaCl, pH 5.65), but was inhibited by a salt concentration of 120 g L$^{-1}$ NaCl in the whey (pH 5.50); large variation in salt tolerance between strains was observed.

Ingham et al. reported that *E. coli* O157:H7 survived for several weeks in model and commercial brines, and that the pH and NaCl concentration of the latter had an effect on the survival rates.

**CONCLUSION**

It is evident that the rate of survival and/or growth of pathogenic bacteria in cheeses depends on the ecological conditions (Aw (water activity), pH, salt content, temperature of maturation) within the cheese and/or brine. The quality of the raw milk, heat treatment of the milk, activity of the starter culture and the salting process—together with the storage in brine—are the most important control points for the prevention of growth/survival of undesirable micro-organisms during the manufacture of white-brined cheeses.

Several regulatory agencies (Codex Alimentarius Commission, Commission of the European Communities, U.S. Food and Drug Administration) have developed specific control requirements for the production of safe foods. These regulations include general requirements (health of personnel, cleanliness and disease control, training and education of personnel) and requirements covering process plant, water supplies, rodent/bird control,
sanitation and handling of equipment and utensils and the establishment of production and process controls. The operation of factories in line with the principles of the HACCP scheme now become mandatory in many countries, and although the cheesemaking process is very complex, this introduction of HACCP can ensure, to a great extent, control of the manufacturing process and thus the production of wholesome, safe and high-quality end-products.

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