

Microbiological safety of feta PDO cheese matured in an aged beech wood barrel

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Abstract. The production of traditional feta cheese of protected designation of origin (feta PDO) involves the use of traditional wooden barrels or large tins. Increasingly, studies have highlighted the beneficial role of wooden dairy equipment in enriching milk microbiota and enhancing acidification processes. The present study aimed to assess the hygiene, processing conditions and microbiological quality of feta PDO produced traditionally. Samples were collected at various stages of ripening. These samples included pasteurised milk, biofilm, feta cheese and brine. The present study focused on the hygiene and safety aspects of the production process, including the microbial populations present on the internal surfaces of the wooden barrels used in cheese production. The analysis confirmed that the barrels met safety criteria, as no major pathogenic microorganisms were detected in compliance with legislative standards. The low microbial content of the cheese samples, particularly the absence of *Salmonella* spp., *Listeria monocytogenes* and *Staphylococcus aureus*, reflected excellent hygienic practices throughout the production process. Factors contributing to this outcome included effective heat treatment, proper refrigeration and rigorous hygiene during maturation and storage. On the whole, these findings suggest that feta PDO cheese production follows a well-established,

standardised process that is fully compliant with current national legislation.

Introduction

The Greek feta cheese is a traditional semi-soft white pickled cheese produced from 0-30% goat milk and 70-100% sheep milk or exclusively from sheep milk (1). Since 2002, it has been recognised as a protected designation of origin (PDO) product, in accordance with Commission Regulation (EC) No 1829/2002 (2,3). PDO cheeses ensure the authenticity of the product linked to its geographical indication where it is produced, processed and developed with recognised expertise characterizing traditional and craft production. Greek feta PDO is a regional product of particular areas of Greece (Macedonia, Epirus, Thessaly and mainland Greece) and is matured in tins or wooden barrels for at least 60 days (4). Although there is a variety of >600 different oak species globally (5), only four have been reported to be suitable for feta PDO cheese production (6). These are *Quercus frainetto*, *Quercus pubescens*, *Quercus petraea* and *Quercus robur* and in Greece, these are found in Western Macedonia, Thrace, Eurytania and Pindus National Park (7-10). These species are endemic to specific Greek regions and have traditionally been used in the construction of wooden barrels for the maturation of feta PDO cheese. Alongside oak, beech wood is also considered appropriate.

Wood in contact with raw or pasteurised milk and cheese is rapidly covered by a microbial biofilm (11,12). In previous studies, researchers have shown a high biodiversity of microorganisms that are present in biofilms, not only between the different types of cheeses studied, but also within the same cheese variety, between different wooden vats (tina or gerle) used in different cheese plants (13,14). The biodiversity confirmed and demonstrated the strong characterisation of the territorial origin of the products (15-17). In cheese, the correct maintenance of wooden vats promotes

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the selection of microbial flora, able to play an active role in the achievement of food safety through the biocompetitive activity of lactic acid bacteria (LAB) and their inhibitory activity against pathogenic bacteria, particularly *Listeria monocytogenes* (13,18-20). Greek dairies have traditionally produced feta PDO cheese, using equipment consisting of wooden utensils and wooden barrels of a capacity of up to 60 kg (6) (as illustrated in Fig. 1).

According to the guidelines of the Hellenic Food Safety Authority (EFET) (21), and Hazard Analysis and Critical Control Point (HACCP) principles for the traditional small dairy production units in Greece (22), the wooden barrel can be used among other wooden utensils, to mature traditional feta PDO and white cheese, such as barrel-aged goat cheese. Their porous structure supports beneficial microflora, while their use enhances the texture and flavour through the natural maturation process (23-25). On the other hand, the use of traditional and reusable equipment, particularly in small and medium-sized enterprises producing barrel-aged feta PDO (2), requires special attention concerning the implementation of critical control points (CCPs), that are designed to reduce microbiological (M), chemical (C) and physical (P) risks in line with HACCP guidelines. These CCPs and the related Operational Prerequisite Programs (OPRPs) are outlined throughout the production routine, as illustrated in the flow-chart of the feta PDO cheese production (Fig. 2) (26). Some studies have demonstrated that the wooden vats used for raw milk cheese production allow for the development of a biofilm on the inner surface, which actively participates in the production process by adding unique sensory characteristics to the traditional products, such as aroma, appearance, taste and texture, which is associated with the total viable count of microorganisms, mesophilic LAB, lactic cocci/streptococci and yeast/moulds (11,12,27,28).

The present study aimed to monitor the microbiological parameters during all the stages of the ripening period of the *Fagus sylvatica* (beech) barrel-aged feta PDO cheese (2), to enhance the established control according to the methodology that complies with food safety in the final product.

Materials and methods

Wood type selection for the use of the barrel. A hand-crafted Greek beech barrel constructed by artisans of the Metsovo area was used to produce and mature feta PDO cheese. The evaluation of the suitability of the beech wood for its use in the dairy sector is approved by the State General Chemistry of Greece and the competent Forest Service, which verifies the origin of the timber, lawful harvesting and intended use through official forest marking (29,30).

Beech barrel cleaning. The barrel was inspected for leak tightness and was then vigorously and thoroughly brushed with pressurised water to remove any gross solids, such as salts, brine residues, fat, bacteria and any crumbled feta PDO due to previous usage, which could impede any further natural sanitation treatments reaching into the barrel wood. It was then filled with heated whey of 95°C [from which the majority of the whey proteins had been removed through myzithra cheese production (31)] and left at room temperature for 24 h. The barrel was

then emptied of the whey, washed with high-pressure water, and allowed to dry at ambient temperature for a further 24 h.

Flow diagram of feta PDO cheese production. Following milking (step 1), raw milk is cooled to <4°C and maintained at this temperature during transportation to the dairy facility (steps 2 and 3). Upon arrival, a milk sample is collected and analysed for antibiotic residues, and the pH is measured (step 4) (32,33). The milk is then filtered (step 5) and stored in large silos (step 6).

The milk is processed within 48 h of milking. Pasteurization is performed at 63°C for 30 min (step 7), followed by cooling to 32°C (step 8) with intermittent stirring. At this point, before the stainless steel vat is filled, a starter culture and calcium chloride are added to its base (step 9). Once filled, rennet is added under constant stirring, and coagulation occurs within 45-50 min (step 10).

Following coagulation, the curd is inspected, cut (step 11), and allowed to rest for 10 min until whey appears on the cheese surface (step 12). It is then transferred into perforated moulds (step 13), where it drains and is subsequently reversed (step 14). The mould is removed, the cheese is cut (step 15) and dry salt is applied to the surface (step 16). After two hours, the blocks are flipped and salted again (step 16).

Depending on production needs, the salted cheese blocks are placed into wooden barrels or metal tins on pallets (step 17), where they remain in the facility area for an additional 24 h. The following day, they are transferred to the ripening chamber and held at 18°C for ~15 days, until the pH decreases to <4.6 (step 18). Finally, the barrels or tins are moved to cold storage at 2-4°C for maturation (step 19). The cheese becomes suitable for consumption 2 months following production (step 19) (32). Following that period, feta PDO cheese is being packed into retail packages and is transported at the point of sale (step 20) (Fig. 2).

Cheese sampling. Feta PDO cheese was produced according to the requirements described at EL/PDO/0017/0427 (34). Moreover, the sale of the cheese as feta PDO requires certification by the competent controlling authority ELGO-DEMETER (2,35). In brief, fresh cheese was left to mature in beech-aged wooden barrels. All samples, namely fresh milk, wood, curd and brine, were collected and analysed microbiologically at different time intervals following the maturation schedule of the 1st day of cheese-making, the 3rd and 45th day of cheese-making, and on the 150th day of the ripening period.

Biofilm sampling. The biofilms were collected for the enumeration of total mesophilic count (TMC), and the estimation of the hygiene indicator bacteria from the inside of the barrel. The biofilm was scraped off from the beech wood surface using a sterilised razor blade within a random interior surface of 100 cm² area, delimited with a 10x10 cm sterile plastic mask (80 cm² from the bottom and 20 cm² from the side surface) at a depth of ~1.5 mm within the beech barrel. The detection of *Listeria monocytogenes*, *Staphylococcus aureus* and *Salmonella* spp. was performed on a larger surface, using a 20x20 cm mask and it was then cleaned with a sterile gauze.

Microbiological analysis. All samples were obtained from the dairy company, Pagonis Sisters and Co., and were



Figure 1. Microbiological sampling of the aged beech barrel used for the maturation of feta PDO cheese. The beech barrel is handcrafted in Metsovo, and is used for the ripening of feta PDO made from Greek fresh pasteurised sheep and goat milk. The internal surface of the beech barrel is coated with a biofilm that contributes the transfer of aromatic compounds and defines the organoleptic characteristics of feta PDO cheese (23). The capacity of the reusable barrel is 60 kg. PDO, protected designation of origin.

1. Milking of Goat's and Sheep's milk
2. Milk storage in the farm, at T<4°C raw milk in row milk tanks
3. Collection and transportation
4. Reception of milk and auxiliary materials: rennet, starter culture, salt, CaCl₂, (OPRP)
 5. Filtration (OPRP)
6. Storage in silo tankers, 4°C, (OPRP)
7. Pasteurisation, 63°C*30 min (CCP)
 8. Cooling, 32°C
9. Addition of starter culture, and CaCl₂ (CCP)
 10. Rennet addition
 11. Curd cutting
 12. Curd is left to rest for 10 min
 13. Curd transfer to the perforated moulds
 14. Curd drain-Mould reversal
 15. Mould removal and block cutting
 16. Dry Salting
17. Packaging in wooden barrels or tin cans and storage
 18. Maturation (18°C/15 days) (OPRP)
 19. Storage, 2°C-4°C for two months (OPRP)
20. Packaging in retail packages (CCP) and transportation (OPRP)

Figure 2. General workflow feta PDO production. Beech wooden barrels or stainless-steel tanks are used for the repining and packaging in the dairy plant. CCPs and OPRPs are established to manage microbiological (M), chemical (C) and physical (P) hazards based on HACCP principles. PDO, protected designation of origin; CCPs, Critical Control Points; OPRPs, Operational Prerequisite Programs; T, temperature.

microbiologically analysed. More specifically, samples derived from the pasteurised sheep and goat milk, biofilm samples from the empty barrel, samples of feta cheese during its maturation stages, biofilm samples in the opened barrel and samples of brine were analysed. The enumeration of the TMC was conducted in plate count agar (PCA; Biokar diagnostics) incubated at 30°C for 72 h (ISO 4833-1:2013) (36), Coliforms on Violet Red Bile Agar (VRBA; Oxoid; Thermo Fisher Scientific, Inc.), incubated at 30°C for 24 h (ISO 4832:2006) (37), *Enterobacteria* on Violet Red Bile Glucose Agar (VRBG, Biolife) incubated at 30°C for 24 h (ISO 21528-2:2017) (38), *Escherichia coli* in Tryptone Bile X-Gluc Agar-TBX at 44°C for 24 h (ISO16649-2/2001) (39,40), and in chromIDTM Coli Agar (COLI ID-F) NF VALID (AFNOR BIO 12/19-12/06) for further confirmation.

Listeria monocytogenes, *Staphylococcus aureus* and *Salmonella* spp. were detected in compliance with the microbiological criteria for foodstuffs determined in Commission Regulation (EC) No 2073/2005 (41,42). *Listeria monocytogenes* and *Salmonella* spp. testing was performed using a mini VIDAS Compact automated immunoassay system based on the enzyme linked fluorescent assay (ELFA) principles by Biomerieux using methods according to the protocols, VIDAS *Listeria monocytogenes* II LMO2 BIO-12/11-03/04 and VIDAS Easy *Salmonella* SLM BIO-12/16-09/05, respectively. Both methods were accredited under ISO/IEC 17025:2005. The enumeration of coagulase-positive staphylococci (CPS, *Staphylococcus aureus*) was performed with ISO 6888-2:1999/AMD 1:2003. All methods used for the detection of foodborne pathogens were accredited according to ISO/IEC 17025 by the Hellenic Accreditation System E.SY.D. with certification no. 1111 (43).

pH value measurement. The pH value of cheese was measured using a RL150 pH meter (Russel) throughout the maturation process. The pH was measured by immersing the glass electrode into the cheese sample at independent time points, without interfering with the microbiological assessment.

Results

The data obtained from the microbiological analysis carried out on samples of feta PDO, brine, and biofilm are reported in Tables I-III. The levels of total coliform bacteria, Enterobacteriaceae and *Escherichia coli*, in the pasteurised goat and sheep's milk were zero. Similarly, the 7.6% (w/w) NaCl microbial loads of brine were null. The TMC ranged from 5.67x10⁵ cfu/cm² in the biofilm of the empty barrel to 5.91x10⁵ cfu/cm² in the biofilm of the opened barrel, which according to the literature (12,44), confirms the slight quantitative variation over the ripening period of feta PDO. Coagulase-positive staphylococci, *Listeria monocytogenes* and *Salmonella* spp. were absent in all cheese samples. The absence of pathogens was observed in the biofilm samples taken from the inner surface of the wooden barrel (45). The follow-up of pH measurements in feta PDO revealed a gradual decline from 6.7 to 4.5 within the period of monitoring lasted from the first hours of repining to the fifth month, as shown in Figs. 3 and 4, verifying that acidification proceeded as expected and that pH levels fell within the target range for the ripening of feta PDO cheese.

Discussion

Greek feta PDO cheese is a national treasure, representing 90% of the total cheese production of the country (44). The essential ingredients for its production include milk, salt, rennet and microorganisms (46). The matrix of dairy products facilitates microorganism proliferation, such as *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli*, *Brucella abortus*, *Campylobacter jejuni*, *Salmonella*, *Yersinia enterocolitica*, and several others, compromising the public health status (26,47).

Traceability plays a key role in food chain management, particularly in the case of rapidly deteriorating products, such as milk and cheese (48). In this context, traceability has

Table I. Microbiological analysis of pasteurised milk and feta PDO cheese.

Sample	TMC (log cfu/ml or cfu/g)	Enterobacteriaceae		<i>E. coli</i>		<i>L. Monocytogenes</i>		<i>Salmonella</i> spp.		<i>S. aureus</i> (coagulase ⁺)	
		Coliforms (log cfu/ml or log cfu/g)	log cfu/ml	Limit ^{a,b}	log cfu/ml or log cfu/g	Limit ^a	cfu/25 g	Limit ^{a,b}	cfu/25 g	Limit	cfu/g
D1 milk	3.00	0.00	0.00	n=5, c=2, m<1 cfu/ml, M=5 cfu/ml	0.00						
D1 cheese		3.08			0.00	Absent	Absent	Absent	Absent	<10	<10
D2 cheese		0.00			0.00	Absent	Absent	Absent	Absent	<10	<10
D4 cheese		0.00			0.00	Absent	Absent	Absent	Absent	<10	<10
D15 cheese		0.00			0.00	Absent	Absent	Absent	Absent	<10	<10
D150 cheese		0.00			0.00	<10	n=5, c=2, m=100 cfu/g, M=1,000 cfu/g	n=5, c=0, Absent in 25 g	Absent	n=5, c=0, Absent in 25 g	Absent in 25 g

D1 refers to the first day of fresh cheese production, corresponding to the pasteurisation of milk and before its transfer to the wooden barrel, whereas D150 is the opening day of the wooden barrel, containing the cheese available for sale. The microbial load (log cfu/ml) demonstrated that the concentrations of coagulase-positive Staphylococci, *L. monocytogenes*, *Salmonella* spp., as well as *E. coli* and Coliforms were satisfactory within the legislative limits for final product, with the values for pre-final product samples presented solely for comparative reference as they represent experimental data. ^aSampling parameters defined per Regulation (EC) No. 2073/2005: n, sample units; c, maximum allowable defective units; m, acceptability limit (satisfactory quality); M, rejection limit (unsatisfactory quality); TMC, total mesophilic count. ^bEmpty cells in the 'Limit' columns indicate that no regulatory limits have been established for the corresponding microbiological parameter.

Table II. Evaluation of microbial load (log CFU/ml) of brine following a period of 150 days of feta PDO cheese maturation.

Sample-brine	TMC (log cfu/ml)	Coliforms (log cfu/ml)	Enterobacteria (log cfu/ml)	<i>E. coli</i> (log cfu/ml)	<i>L. monocytogenes</i> (presence/absence)	<i>Salmonella</i> spp. (presence/absence)	<i>S. aureus</i> (coagulase ⁺) (cfu/g)	Regulatory limit for natural brine (reference)
D150	5.77	0.00	0.00	0.00	Absent	Absent	<10	No regulatory limits exist for brine

Coagulase-positive staphylococci, *L. monocytogenes* and *Salmonella* spp. were absent, whereas the concentrations of *E. Coli* and Coliforms were within the limits of the legislation and complied with the microbiological safety criteria for feta PDO cheese. In the absence of regulatory limits specifically for the natural maturation brine of barrel-aged PDO Feta, the interpretation was based on the standards for cheese products made from milk that has undergone heat treatment, as outlined in Commission Regulation (EC) No. 2073/2005, exclusively for research purposes.

Table III. Biofilm microbiological analysis.

Biofilm sample/time point	TMC (log cfu/cm ²)	<i>E. coli</i> (log cfu/cm ²)	<i>L. monocytogenes</i> (cfu/cm ²)	<i>Salmonella</i> spp. (cfu/cm ²)	<i>S. aureus</i> coagulase ⁺ (cfu/cm ²)	Regulatory limit for biofilm (reference)
Biofilm sample of D1 before feta PDO was transferred into the barrel	5.75	0.00	Absent	Absent	<10	No regulatory limits exist for biofilm
Biofilm sample of feta PDO on D150 of ripening	5.77	0.00	Absent	Absent	<10	Same as above

The measurement of the TMC on the biofilm exhibits a slight variation caused by the action of LAB and other microorganisms during feta PDO cheese maturation until the microbial ecosystem reaches an equilibrium. The microbial load (log cfu/ml) related to the concentrations for coagulase-positive Staphylococci, *L. monocytogenes*, *Salmonella* spp., *E. coli* and Coliforms was satisfactory and complied with safety criteria for feta PDO cheese. In the absence of biofilm-specific limits, the interpretation was based on the standards for the final cheese product, as defined by Commission Regulation (EC) No. 2073/2005, and applied exclusively for research purposes. TMC, total mesophilic count; LAB, LAB: lactic acid bacteria; D1, day 1; D150, day 150.

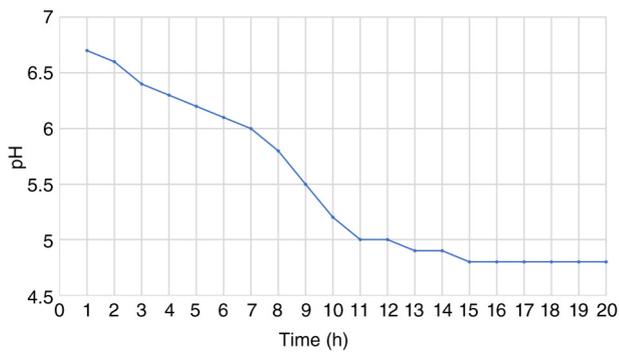


Figure 3. Monitoring of changes in pH during feta PDO production matured in a beech barrel. The pH changes during the cheese curd process of feta PDO production matured in beech barrel exhibit an overall decline from 6.7 to 4.8, reflecting a rapid acidification during the first day. The most notable measurements were recorded at 10-h intervals over a period of 20 h. PDO, protected designation of origin.

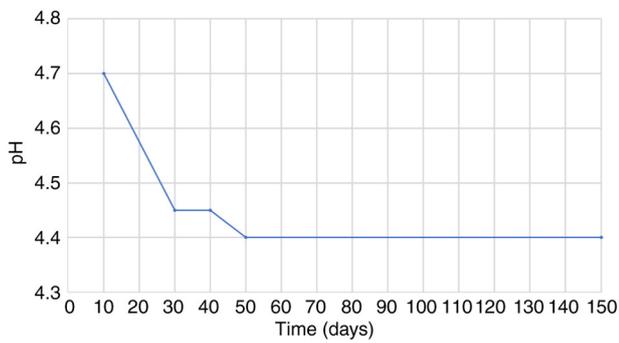


Figure 4. Monitoring of changes in pH values during feta PDO production matured in a beech barrel. The measurements were performed at non-consecutive ripening time points, expressed in days: 10, 30, 40, 50 and 150, independently of the microbiological sampling. The figure illustrates an overall decline from 4.7 to 4.4, being stable after the 50th day. PDO, protected designation of origin.

become a requirement for a better understanding of the life cycle of products and conscientious delight. Thus, the major concern of the dairy companies is the respect of the traditional practices through a certified quality control system according to ISO 22000:2005 and implementing a HACCP daily workflow, illustrated by Casino *et al.* (49), and in the workflow of Fig. 2, to guarantee excellent quality.

Food safety is an increasingly critical public health concern, and the proposed traceability procedure enhances the improved process control for managing any future product recalls and/or product quality. Microbial food contamination in dairy plants is of utmost significance for the monitoring of food quality and safety (6,50). For instance, it is known that *Listeria monocytogenes* has the potential to survive at low temperatures (0°C), low pH values (pH 4.4) and in salt media with 10-20% (w/v) of NaCl (28), conditions that are close to the physicochemical characteristics of feta cheese. For the purpose of the present study, further investigated the food safety of beech barrel-aged Feta PDO, 2.5% (w/w) NaCl, following the monitoring of pH and through the in-depth microbiological analysis across the time-points of the production workflow (Fig. 2).

Previous studies describe that the microbial ecology found in cheese-making establishments exhibits a great differentiation in composition, regarding taxonomy classification, due to the processing procedures (51-53). The conditions that favour microbiome settlement are several and are associated with the age, conservation, quality and nature of the wooden vats. Additionally, the presence of microbial ecosystems and environmental conditioning (54), such as temperature, humidity, or pH, markedly affects the selective survival of the microbiome (55). Humidity, cleaning protocols, nutrient availability and composition also have a regulatory impact on the ability of microbes to develop biofilms. The availability of nutrients in wooden barrels can promote biofilm development. However, this effect is modulated by key environmental factors, such as salinity, moisture and pH, which suppress the proliferation of pathogenic microorganisms even in nutrient-rich conditions. This specific microenvironment favours the development of stable, functional biofilms that enhance the microbial stability and hygiene of the final product (56). In particular, the limited moisture, regulated by the high salinity of the brine, combined with the acidic pH (~4.4), creates a selective ecosystem. Within this environment, the growth of pathogens, such as *Listeria monocytogenes*, *Staphylococcus aureus* and *Salmonella* spp. is inhibited, while microorganisms naturally adapted to high-salt and low-pH conditions are favoured. These salt- and acid-tolerant microbiota include lactic acid bacteria such as *Lactobacillus* spp., as well as probiotic strains such as *Lactobacillus paracasei* and *Bifidobacterium* spp., which form resilient, protective biofilms (17). In the present study, the aggregation of microorganisms referred to as 'biofilm' formed in the wooden barrels used in the production of feta PDO cheese is characterised by minimal variation in TMC, with a quantitative change of <5% following 150 days of ripening. Additionally, the structural and chemical properties of beech wood exert further selective pressure. Cleaning protocols effectively eliminate pathogens while preserving these non-pathogenic, functionally advantageous biofilms. Nutrient limitation during advanced ripening likely restricts microbial overgrowth, supporting a balanced beneficial microbiota and ensuring the microbiological safety and quality of the final product (57-59). However, in the case of feta PDO production, which has always been an artisanal process, the improvements through empirical trials have emerged long before the current scientific knowledge of these fermentations.

The establishment of the LAB protects against spoilage bacteria through differential colonisation capability (60), which creates food bio-protection conditions, providing antifungal and antimicrobial activity. This could be explained through mechanisms of competition for nutrients (61). In the case of the traditional art that characterises feta PDO aged in a wooden barrel, surface spoilage is subject to the irregularity of the surface material, which represents a cardinal issue for compliance with standard hygiene regulations in food processing plants (50). In the present study, it was demonstrated that the traditional practice for the production of feta PDO cheese using wooden-beech barrels for cheese ageing met the standards of Good Manufacturing Practice (GMP) (62), including the appropriate practices of sanitation to ensure food safety.

Wood, due to its organic properties, particularly its porosity and hygroscopic nature, is a renewable natural resource

that allows a progressive inlet of oxygen into the barrel and attributes a sharp flavour to the cheese, but also a slightly harder texture (23,54,60). Previous studies have demonstrated that some commonly used wood species have antimicrobial activities and are suitable for food contact surfaces (63,64). Wooden barrels remain an indispensable requirement characterising the traditional practice of barrel-aged feta PDO. This peculiar maturing process could significantly influence the future and the perspective of this iconic Greek product.

Specifically, the use of beech barrels (*Fagus sylvatica*) allows for controlled micro-oxygenation, as wood enables oxygen to reach the inside of feta cheese as it matures. This oxygen interaction enhances the flavour, contributes to the development of a sharp, piquant and distinct sensory profile, but also defines the texture of the cheese. These organoleptic characteristics are associated to the presence of wood-derived compounds, such as phenolics, for example, syringyl derivatives, and mild, insignificant tree lactones (65-67), as well as tannins, that exert antimicrobial efficacy and structural resilience in brine (68). This maturation technique highlights the artisanal and terroir-specific identity of feta, affirming its value as a PDO product.

Additionally, wooden barrels used in the production of feta PDO support sustainability goals, as they are biodegradable and eco-friendly. Their local sourcing supports circular production and promotes the cultural legacy of cheese, which in turn fosters the rural economy and helps prevent land abandonment. Furthermore, the implementation of forest certification protocols could help prevent and mitigate the decline of tree species commonly used for timber of beech (*Fagus* spp.) populations in Mediterranean regions, while contributing to improved stand structure by protecting mature trees and promoting natural regeneration (69).

Consequently, the long-term commitment to using beech wooden barrels in the production of feta PDO cheese (8,70,71) ensures not only the preservation of the cultural and nutritional heritage, but also highlights the use of sustainable forest management (72,73). This contributes to establishing the continuity and the viability of agricultural livelihoods in Greece.

Despite its advantages, wooden barrel sanitisation poses challenges for product safety, as it carries the risk of microbial contamination due to the porous nature of the wood (74). Nevertheless, forward-looking strategies could further develop Greek feta PDO cheese, ensuring product safety and enhancing its nutritional profile, with the potential to qualify as a functional food through targeted fortification and the integration of adjunct co-culture strains exhibiting probiotic and technological potential (28,75-76). These strategies may not only satisfy consumer expectations for authenticity and safety, but may also boost the market prospects of traditional cheeses, particularly feta PDO cheese.

Although the specifications for Greek feta PDO clearly define the origin of the milk, they do not impose specific requirements on the type of wood used for barrels, since the regulation does not include this parameter (2). For example, chestnut (*Castanea sativa*) has been recommended for fresh cheeses due to its antioxidant properties and its capacity to enhance product stability as well as the shelf life in other agro-industrial by-products (56,77). Likewise, sweet cherry (*Prunus avium* L.)

is known for its high phenolic content, contributing to organoleptic, antioxidant, and anti-hyperglycemic benefits (78,79). At the same time, walnut (*Juglans regia* L.) has been incorporated into functional cheese fortification, as seen in the case of Beyaz cheese (78,79). Despite these favourable attributes, none of these woods aligns with the cultural and historical framework that governs the production of feta PDO.

Although oak is beneficial for cheese ageing, the porosity of the wood, creates an organic wicking effect that favours the conditions of drying and ageing of cheese, because it sustains bacterial growth and the development of biofilms. This is beneficial, as the prevalence of desirable organisms that are immobilised within the thick layer of polysaccharides, promotes the contribution of flavour and positive organoleptic characteristics to the Feta PDO cheese. Specifically, lactococci and lactobacilli are critical as they contribute to the cheese acidification and flavour formation of the feta cheese (23).

To better illustrate how exogenous or endogenous environmental factors impact microbial populations during feta PDO cheese ripening, Table IV summarises the selective pressure acting on several bacterial species, deduced from both our observations and the literature evidence. The perspective is that characterisation and mapping of the microbiome of feta PDO cheese may become a valuable tool for the monitoring of environmental contamination, to improve the quality control in the dairy plants, particularly under routine and experimental conditions such as salinity, moisture, temperature and pH adjustments. Therefore, understanding the interactions between feta cheese and selected bacterial flora could be meaningful for feta PDO cheese manufacturing of a standard quality (50) and variability of organoleptic features. Moreover, the growth of pathogens and the risk of their survival is a fundamental issue and needs to be addressed by validated and frequently monitored good sanitation practices.

In conclusion, the present study provides a comprehensive assessment of the microbiological safety of handcrafted feta PDO cheese aged in wooden barrels, in accordance with current food safety regulations in Greece. The results confirm that the traditional maturation process using beech barrels, when accompanied by proper cleaning protocols inhibit the presence of key foodborne pathogens, including *Listeria monocytogenes*, *Salmonella* spp. and *Staphylococcus aureus*. In particular, to the best of our knowledge, the present study is the first to formally document and evaluate under microbiological criteria the traditional cleaning method of hot whey rinsing during the ripening of Greek artisanal feta PDO cheesemaking in *Fagus sylvatica* barrels, demonstrating that it contributes to the effective hygienic maintenance of wooden barrels used in the maturation process.

The observed TMC in biofilm samples revealed minimal fluctuations ($\leq 5\%$) over a 150-day ripening period, suggesting a stable microbial ecosystem. The dominance of beneficial LAB, fostered by selective environmental pressures such as low pH and high salinity, supports the formation of functional, protective biofilms that contribute to both microbial safety and flavour development. While the complexity and variability of the cheese microbiota pose challenges for standardisation, they also highlight the unique sensory identity of feta PDO. In particular, the presence of beneficial microbial communities within the beech wood-associated biofilm contributes to both

Table IV. Summary of endogenous and exogenous conditions affecting bacterial species during feta PDO cheese production.

Environmental factor	Effect on pathogens (Refs.)	Effect on beneficial bacteria (LAB) (Refs.)	Remarks
pH <4.6-(endogenous)	Inhibits pathogen growth such <i>L. monocytogenes</i> , <i>Salmonella spp.</i> , <i>E. coli</i> (80,81)	Favours <i>Lactobacillus spp.</i> , <i>L. paracasei</i> , <i>Lactococcus spp.</i> (82,83)	Acid-tolerant LAB dominate in low pH environments
NaCl curd 2.5-2.8% (w/w) (endogenous)	Osmotic stress inhibits pathogens, such as <i>S. aureus</i> , <i>Bacillus cereus</i> , <i>Yersinia spp.</i> (84)	Enhances LAB survival; stimulates biofilm formation	LAB possess salt-tolerance mechanisms
Low temperature (4-10°C) (exogenous)	Suppresses mesophilic pathogens (85) i.e., <i>L. monocytogenes</i> , <i>Salmonella spp.</i> , <i>E. coli</i>	<i>Lactobacillus bulgaricus</i> , <i>Lactococcus lactis</i> (85)	Cold ripening favours LAB over pathogens
Environmental humidity (exogenous) and brine (endogenous)	Exogenous factor: Reduces desiccation (86), but limits aerobic pathogen survival (endogenous factors) (87)	Psychrotolerant LAB, such as <i>Lactococcus lactis</i> survive and metabolise slowly (85)	Moist environment promotes biofilm integrity on the wood surface
Wooden barrel (beech or oak)	Surface irregularities complicate pathogen removal (56)	Both exogenous and endogenous factors support LAB surface colonisation and stability	Wood porosity allows oxygen gradients; enhances flavour development
Cleaning (hot whey)	Effectively removes planktonic pathogens	Promotes biofilm of LAB (e.g., <i>Lactobacillus</i> , <i>Bifidobacterium</i>) (56)	Traditional cleaning helps balance hygiene with microbial stability

LAB, lactic acid bacteria; NaCl, sodium chloride; spp., multiple species.

microbial stability and the organoleptic profile of the cheese during ripening. Future studies utilizing advanced molecular approaches, such as next generation sequencing (NGS) and metagenomic analysis, will be essential for elucidating the role of the microbiome in ripening dynamics, biofilm formation, and sensory development.

Overall, the present study reinforces the compatibility of traditional wooden-barrel aging practices with modern food safety requirements and highlights the potential of microbial mapping as a future tool for quality control and process optimisation in artisanal dairy production. Furthermore, the presence of beneficial microbial populations, particularly probiotic strains, provides a promising basis for the development of functional feta cheese with potential health-promoting properties.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

MP, VZ, GTT and ND conceptualised and designed the study. MP, AP, IM and AT were involved in the study methodology. MP, DP and IM provided software (Excel for graph design, GIMP for image editing and LAB Fit for curve fitting). MP and AP were involved in the investigative aspects of the study and in the provision of resources (milk, cheese, brine and biofilm samples). MP and AP confirm the authenticity of all the raw data. AP, IM, DP and NPR were involved in data analysis and curation. MP, VZ and AP were involved in the writing and preparation of the original draft of the manuscript. MP, DP, AP, IM, NPR and GTT were involved in the writing, reviewing and editing of the manuscript. AP, ND and GTT supervised the study. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

MP and AP are co-owners of the company Pagonis Sis & Co. from which the feta was procured. DP, VZ, IM, AT, NPR, GTT and ND declare that they have no competing interests.

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