Microbial stabilization of traditional cheeses made in wooden equipment through selection, characterization and application of autochthonous lactic acid bacteria

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ABSTRACT

In the last years, the demand of typical foods has registered a continuous positive trend and it is still on the increase. With this regards, raw milk cheeses underwent a rediscovery. Following this increasing request, the typical Sicilian cheese PDO (protected denomination of origin) Vastedda della valle del Belìce (VdB) traditionally available only in the summer season is being produced year round. The extended production determined marked differences about cheeses made in different seasons. This depends mainly on the different microbial populations, particularly lactic acid bacteria (LAB), present in the raw milk. This review article reports the efforts made to stabilize the microbial composition and the organoleptic characteristics of VdB cheese preserving its typicality through selection of starter LAB, *in vitro* and *in vivo* application and finally development of a protocol of inoculation respectful of the PDO disciplinary. In particular, it was performed an *ad hoc* biofilm formation with selected *Lactococcus lactis* subsp *cremoris* strains during the microbial activation of the wooden vats used for the traditional VdB cheese making process.

Introduction

Sicily is a southern Italian region characterized by an ancient history of cheese production. The dairy tradition is dated back to the presence of the Phoenician community in the island (OESAAS, 2007). According to one legend, cheese was born in Sicily (in the city of Pergusa within Enna province) thanks to the shepherd Aristeo, the son of Apollo and the nymph Cerere, who taught men how to transform milk into cheese (Betta and Cantarelli, 2000). The first written description of a cheese production in Sicily was reported by Homer in the IX book of Odyssey, when described the activities of the Cyclope Polyphemus in the Etna Volcano (Ballarini, 1999). Recent archaeological discoveries of an oval hut and fragments of sieves, small colanders and perforated vessels within Troina area (Enna

province) support these legends and indicate that the dairy activity in Sicily was conducted daily during the Eneolithic age (end of third – begin of second millennium b.C.) (Ricci, 2017).

In recent years, the food style evolved very fast towards a renewed request of typical products, including cheeses that are produced applying traditional transformation processes. These products, often processed with procedures respectful of the environment, are perceived as "natural" and preferred because do not contain chemical preservatives (Settanni and Moschetti, 2010). These requirements are satisfied by the "recognition of quality" [protected designation of origin (PDO), protected geographical indication (PGI), and traditional specialty guaranteed (TSG),] conferred by the European Community to promote and protect the names of quality of agricultural products and foodstuffs (EU Regulation No 1151/2012).

Several Sicilian cheeses are strongly linked to the territory of origin and are made from the raw milk of indigenous breeds, curdled with animal rennet pastes, processed in traditional wooden equipment without the addition of starter cultures (Scatassa et al., 2015a). Among these cheeses, Pecorino Siciliano, Ragusano, Vastedda della valle Belìce (VdB) and Piacentino Ennese enjoy a PDO status. Due to the production conditions of these non-started cheeses, lactic acid bacteria (LAB) necessary to transform curd in cheese (Settanni and Moschetti, 2010) derive from raw milk (Franciosi et al., 2009; Guarcello et al., 2016), animal rennet (Cruciata et al., 2014), traditional wood equipment (Licitra et al., 2007), and the transformation environment (Scatassa et al., 2015b).

LAB constitute a heterogenous bacterial group characterized mainly by the capacity to produce lactic acid from carbohydrates via homo- or hetero-fermentative metabolisms. Dairy LAB are generally distinguished in starter LAB (SLAB) that are responsible for the rapid acidification of the curd, and non-starter LAB (NSLAB) which drive the ripening process (Settanni and Moschetti, 2010).

Among PDO Sicilian cheeses, VdB undergoes a very short production process that increases the seasonal differences due to the different raw milk characteristics, including the microbial populations. This paper reviews the current knowledge on the ecology of VdB cheese and reports the main findings to minimize the microbial and sensory differences of the final products maintaining the production process traditional.

Production of Vastedda della valle del Belìce cheese

PDO VdB cheese is produced following the general traditional Sicilian cheese making in the western part of the region using exclusively the raw milk of the autochthonous sheep breed Valle del Belìce (Reg. UE n. 971 del 28.10.10; GUUE L 283 del 29.10.10). This cheese is produced applying the stretching ("pasta filata") technology consisting of an acidification followed by the scalding of the acidified curd (Salvadori del Prato, 1998). Briefly, the production process of VdB (Fig. 1) requires the heating of milk at 40°C, its coagulation with lamb rennet paste, obtained from the abomasum of suckling lambs of Valle del Belìce breed, breaking of the curd to rice dimensions, transfer of the mass into rattan baskets to allow whey drain, acidification of the curd and then stretching in hot (80-90°C) water. After production, VdB cheese is sealed under vacuum, refrigerated and sold fresh in local markets (Mucchetti et al. 2008). Up to date, VdB is the only PDO raw ewes' milk cheese that does not undergo to the ripening and, as a matter of fact, is consumed fresh.

The name "Vastedda" originates from its characteristic shape, resembling a local kind of bread loaf, round and flat, called "vastedda". It might also be originated from the dialect word "*vasta*" meaning spoiled. This because it is said that the first production of the cheese was performed with the attempt to recover a spoiled raw ewes' milk cheese; the cheese maker cut the acidified curd, put the small pieces in hot water but when the mass started to stretch it was put in a soup plate acquiring the typical vastedda bread shape.

The production area of VdB includes the whole homonymous Valle del Belìce area that takes its name from the river that flows through it and comprises three provinces (Agrigento, Trapani and Palermo) located in western Sicily. VdB cheese is strongly linked to its production area, since the animals are fed only with fresh forage on grazed grass, hays and straw produced in the same area (Reg. UE n. 971 del 28.10.10; GUUE L 283 del 29.10.10). Furthermore, a specific nutritional model applied to the sheep breed Valle del Belìce, that resulted from the cross between Pinzirita with Comisana and then with Sarda, officially recognized in 1998, enhances the quality of the milk (OESAAS, 2007).

The final characteristics of VdB cheese are strongly influenced by the use of the traditional wooden equipment (Fig. 2) mainly made of chestnut wood. Among these equipment, the wooden vat (tina) used for milk curdling assumes a role of paramount importance to inoculate the desired LAB responsible for the acidification of the curd (Settanni et al., 2012; Scatassa et al., 2015a,b). Due to the contact with milk and whey, the internal surface of the wooden vats are covered by aggregates of microorganisms in which cells that are frequently embedded within a self-produced matrix of extracellular polymeric substance (EPS) adhere to each other and/or to a surface and these microbial structures are known as "biofilms" (Vert et al., 2012). The wooden vat biofilms play beneficial roles during the cheese production processes, especially because they strongly contribute to enrich milk with LAB responsible for curd fermentation and cheese ripening (Cruciata et al., in press; Scatassa et al., 2015b).

In past, VdB cheese was traditionally produced only during the summer season. However, due to an increased demand of this fresh product, mostly appreciated than hard ewes' milk cheeses by several consumers, its production occurs year round. This phenomenon determined a great variability among the production seasons, especially in terms of quality of the final products, as a direct consequence of the different characteristics of the bulk milks (Verzera et al., 2010) and the different dominating LAB populations, mostly

represented by mesophilic species during winter and thermophilic species during warmer seasons (Gaglio et al., 2014a).

The use of wood in traditional Sicilian cheese making

The Commission Regulation (EC) No 2074/2005 allows derogation from Regulation (EC) No 852/2004 for foods with traditional characteristics "as regards the type of materials of which the instruments and the equipment used specifically for the preparation, packaging and wrapping of these products are made" (Commission Regulation, 2005a). Regarding the materials in contact with cheese, the rule CE n. 1935/2004 reports the "principle of no contamination" (Commission Regulation, 2004). However, no limitations to the use of wooden equipment are specifically indicated (Della Ciana, 2015).

The production of artisanal Sicilian cheeses is generally carried out in wooden equipment. Without the addition of starter cultures the biofilms developing onto the surface of the wooden vat used for milk curdling (Fig. 3) are made of autochthonous microorganisms that are adapted to the production area (environment), the local raw materials (substrates) and the traditional protocol (technology) (Settanni and Moschetti, 2014). Once a biofilm is formed the LAB that spontaneously evolved through time to a stable consortium persist and dominate over the lactic acid and non-lactic acid bacterial populations of raw milk (Lortal et al., 2009; Settanni et al., 2012). Vat LAB biofilms influence deeply the characteristics of the final products since they can lead the microbiology of the ripening process and are defining for their typicality (Di Grigoli et al., 2015).

Hystorically, wood was the main material used in cheese making due to its availability, resistance over time and low cost. However, nowadays, the wood used to this purpose is imported from other regions. In general, the tree species employed to produce dairy equipment in Sicily are chestnut and Douglas-fir, genus *Pseudotsuga*. Several cheese producers not involved in PDO productions are introducing the standardization of the

processes through industrial or semi-industrial plants. To this purpose, the wood is replaced by other materials such as plastic polymers or stainless steel that can be easily cleaned. These materials, do not contribute to the syneresis of cheese and affect the flavor and texture of the cheese (Galinari et al., 2014; Scatassa et al., 2015b), firstly because commercial starter cultures have to be added (Settanni et al., 2012). However, when the starter culture is composed of a limited number of strains (commercial preparations produced by a few companies) and is applied to different productions, a flattening of the taste of the final products may occur, with the risk that the final products may no longer be distinguishable by production technology and/or geographical origin (Settanni and Moschetti 2014). Thus, the use of wood is necessary to maintain a product like VdB cheese typical of a given geographical area.

The US Food and Drug Administration declared that "the structure of the wood as porous, would absorb and trap bacteria that may contaminate food products", during the presentation of the advice about Italian and French cheeses ripened on wooden planks (Cutini, 2014). However, several studies (Didienne et al., 2012; Licitra et al., 2007; Lortal et al., 2009; Scatassa et al., 2015b) conducted not only in Sicily, but also in France that is also in derogation from the application of the EC No 852/2004 thanks to the EC 2074/2005, demonstrated that the wooden vats do not host *Salmonella* spp., *Listeria monocytogenes* and other undesired microorganisms and are respectful of the EC No 2073/2005 concerning the microbiological criteria for foodstuffs both for food safety and process hygiene criteria (Commission Regulation, 2005b). Cruciata et al. (in press) better investigate on the observation that pathogens are not generally present in the wooden vat biofilms. These authors carried out VdB cheese productions with milk contaminated by the four main dairy pathogens *Salmonella enteritidis*, *L. monocytogenes*, *Escherichia coli* and *Staphylococcus aureus*. The experimental productions showed that although cheeses were contaminated, the pathogens were not found in vat biofilms. Thus, these results confirmed

the capacity of the LAB biofilm to hamper the adhesion of the undesired bacteria highlighting the positive role of the wooden vat on the microbiological safety of the final products.

In addition to the evaluation of the safety of the wooden vat used during the first stages of chees production, it has been also demonstrated that the aging on wooden planks in several farmhouses reduces the presence of *L. monocytogenes*, showing instead a potential of wood for bioprotection against food pathogens due to resident microbial biofilms (Mariani et al., 2011). Thus, the work of Mariani et al. (2011) implicitly reported the relevance of the wooden ripening shelves for the typicality of the final cheeses. These statement is even more evident considering the data published by Galinari et al. (2014) who reported that the cheese ripening occurred on stainless steel shelves strongly depress the traditional sensory characteristics.

Microbiota of PDO Vastedda della valle del Belìce cheese

Due to the relevance of VdB for the production area, this cheese has been object of several research projects aimed to valorise and ameliorate the production system and the final quality. With this in mind, recent studies analysed the microbial composition of VdB (Reale et al., 2007; Scatassa et al., 2007; Mucchetti et al., 2008; Gaglio et al., 2014a, Todaro et al., 2014, Todaro et al., 2017). LAB resulted always the dominant microbial group, although the growth of this population can be affected in composition and cell density by the geographical location of the dairy factories and the season of production. In general, LAB found in VdB belong to the group of mesophilic cocci (Gaglio et al., 2014a), despite the fact that stretched cheeses are mainly started by streptococci that are thermophilic (Parente et al., 1998). Gaglio et al. (2014b) monitored the behavior of the different groups of LAB in VdB produced under controlled conditions (pasteurized milk in and experimental dairy plant with stainless steel equipment) and registered an increase of

the mesophilic populations, especially lactococci from milk to acidified curd, followed by a decrease during stretching and an increase during refrigerated storage evidencing their potential contribution to maturation even at low temperatures.

The LAB found in VdB showed a certain biodiversity at species level and they were ascribable to the following five main genera: *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc* and *Streptococcus*. The species mostly represented were *Lactococcus lactis* and *Leuconostoc mesenteroides* among mesophilic LAB, while *Streptococcus thermophilus* and *Streptococcus gallolyticus* subsp. *macedonicus* among the thermophilic ones (Gaglio et al., 2014a). Although the last species is not typically reported as dairy starter shows interesting dairy aptitudes during ripening and is being considered as adjunct culture in cheese making (Settanni et al., 2011; Guarcello et al., 2016). The dominating VdB LAB showed inhibitory factors against undesired microorganisms; strains of *L. lactis* were able to produce bacteriocin-like inhibitory substances (BLIS) active against *L. monocytogenes* showing their contribution to the biopreservation of these cheese.

Enterococci are also considered as components of cheese adjunct cultures (Foulquié Moreno et al., 2006), but due to some undesired features, such as virulence factors and antibiotic resistance, their harmlessness has to be proven before deliberate addition during cheese making (Gaglio et al., 2016). Enterococci constitute part of the common LAB community present in raw milk (Franciosi et al., 2009) and they were also found in wooden vat biofilms (Settanni et al., 2012) and animal rennet pastes (Cruciata et al., 2014) and are often found after stretching and during refrigerated storage (Todaro et al., 2017). *Enterococcus* species frequently found in VdB were *Enterococcus durans*, *Enterococcus faecium*, *Enterococcus faecalis* and *Enterococcus gallinarum* (Gaglio et al., 2014a). Harmless enterococci take part to the definition of the sensory profile of the cheeses and contribute to extend their shelf-life through bacteriocins production (Foulquié Moreno et al., 2006). Gaglio et al. (2016) investigated deeply the antimicrobial resistance and

virulence of enterococci collected along the entire production chains of traditional cheeses made in Sicily, including VdB, confirming that dairy enterococci might be a potential source for dissemination of antimicrobial resistances and virulence among bacteria, but also that some harmless strains that did not carry the genes for virulence and antibiotic resistance were found at dominating levels indicating a possible competition with the *Enterococcus* strains expressing the undesired traits.

VdB cheeses have been also extensively investigated for the presence of the four main dairy pathogens *Salmonella* spp., *L. monocytogenes*, β-glucuronidase positive *E. coli* and coagulase positive staphylococci. In general, *Salmonella* spp. and *L. monocytogenes* were never found in any final cheese and any step of production, *E. coli* and *S. aureus* were detected sometimes at low levels in raw milk, but never in cheese (Scatassa et al., 2007, 2009; Todaro et al., 2016). These studies evidenced that the process conditions determine the high microbiological quality of VdB cheese although it is processed from raw milk and does not undergo a ripening stage.

Microbial stabilization

VdB cheese is characterized by marked differences among the production seasons, especially between summer and winter. Furthermore, since the production protocol does not include the addition of starter cultures, the final quality is quite unpredictable. This because LAB populations might be greatly affected by the different temperatures registered in Sicily during the different seasons, going from below 15°C in winter until above 35°C during summer.

The modern systematic approach to minimize the microbial variability among productions and obtain cheeses with stable desired characteristics is basically based on the use of selected starter cultures. In order to develop starters for traditional cheeses, only autochthonous LAB can be considered to maintain their typicality. In particular, for

VdB production, the strains suitable to constitute the starter cultures have to be characterized by heat resistance during stretching and carry out the acidification at high temperatures during summer as well as at low temperatures during winter.

In order to approach this issue, Gaglio et al. (2014a) characterized the indigenous LAB populations isolated from VdB cheeses produced in different seasons. The aim of that work was to test a group of autochthonous strains for VdB able to drive the process year round and maintain the typicality of the cheeses. To this purpose, 74 strains belonging to 16 different species included in the five main dairy LAB genera reported at paragraph 4 were technologically investigated *in vitro* in order to evaluate their acidification, aromatic profiles and antimicrobial properties.

The strains showing the best technological performance were further investigated *in vivo* in order to test their ability to carry out the acidification and dominate the indigenous raw milk LAB populations (Gaglio et al., 2014b). Twelve LAB including three *L. lactis* subsp. *cremoris* and three *L. mesenteroides* among mesophilic strains and three *S. thermophilus* and three *Lactobacillus delbrueckii* among thermophilic strains were tested at a pilot plant level using plastic and stainless steel equipment first in pasteurized milk and then in raw milk in order to evaluate their acidification and dynamics throughout the whole production process and also during the refrigerated storage occurred for 15 d. The strains of LAB were added as starter cultures in multiple combinations following the graphical scheme reported in Fig. (4). The entire experimentation was carried out in February, which is one of the coldest months in Sicily. The temperature was not kept controlled during curd acidification to mimic the conditions of the artisanal cheese factories. In these conditions, the more rapid acidification was observed for all mesophilic LAB in multi-strain combinations per species (only lactococci or leuconostocs) and all together (lactococci and leuconostocs) which determined the drop of pH in the range 5.2 – 5.4, corresponding to the level of acidity necessary for curd

stretching (Niro, 2011) after a few hours from inoculation. The acidification registered with the thermophilic strains was slower and the optimal pH for curdling was reached after 48 h or more. In particular, lactococci showed an acidification kinetics compatible with the 24 h laid down by the PDO disciplinary, while the other mesophilic strains determined a too rapid process. All *Lactococcus* strains were then investigated singly in order to better evaluate their contribution in terms of volatile organic compounds, but the best results at the sensory evaluation were shown by the experimental VdB cheeses produced with the triple *Lactococcus* strain combination which showed an appreciation comparable to that of the traditional commercial VdB cheese.

The results of Gaglio et al. (2014b) indicated that the *L. lactis* subsp. *cremoris* strains selected were able to drive the fermentation process preserving the typicality of the final cheese. However, the entire experimentation was performed in standard conditions, but PDO VdB manufacture is performed with the traditional wooden equipment. Thus, Gaglio et al. (2016) applied a strategy to transfer the selected lactococci in the traditional production system through the development of biofilms onto the surfaces of virgin (new) wooden vats. The system was tested in controlled and uncontrolled conditions. The controlled conditions were represented by cheese productions at pilot plant level with milk from a single farm characterized by low levels of microbial contamination in an experimental dairy plant, while the uncontrolled conditions were those of an industrial plant that transforms bulk milk from several farms. In each condition, two virgin vats were activated, one (control vat) following the traditional protocol applying deproteinized whey from the previous day cheese making and the other one (experimental vat) with a whey fermented by the selected strains. The selected lactococci were able to develop the biofilm necessary for milk inoculation more rapidly than whey of the control vats (Fig 5. biofilm a, b, c). Furthermore, the genotypic monitoring of these strains showed that they dominated cheese LAB populations during storage. The productions performed in winter and in

summer seasons indicated that the biofilm developed *ad hoc* with the autochthonous selected lactococci determined the microbial and sensory stabilization of VdB during the year round production.

Conclusions and future perspectives

The uncontrolled evolution of LAB may determine marked differences among cheese productions and unpredictable final characteristics; on the other hand, the application of commercial starter cultures might determine a flattening of cheese taste with the consequence that cannot be clearly distinguishable by production technology and/or geographical origin. Therefore, the selection and application of autochthonous starter strains is mandatory to drive a fermentation process of typical cheeses. The results presented by this article showed that the addition of selected starters for VdB cheese determined the microbial stabilization of the transformation process and the final product. Furthermore, the implementation of the microbial activation of the virgin wooden vats with selected *L. lactis* subsp *cremoris* strains determined a positive innovation respectful of the traditional process and the PDO disciplinary. Hence, this strategy will be provided to the consortium for the production of PDO VdB cheese in order to stabilize the microbial and sensorial attributes of cheese throughout the year.

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References

Ballarini G (1999). Zootecnodissea. Allevamento degli animali e trasformazione degli alimenti di origineanimale nel mondo omerico, in D. VERA (a cura di), Demografia, sistemi agrari, regimi alimentari nel mondo antico, PRAGMATEIAI, Bari, 1999 (nota 4), pp. 37-51.

Betta P Cantarelli F (2000) Dal mito alla storia il pecorino siciliano. CORERAS Consorzio Regionale per la Ricerca Applicata e la Sperimentazione Palermo

Commission Regulation (2012) Commission regulation no 1151/2012 of 21 November 2012 on quality schemes for agricultural products and foodstuffs. Off J Eur Union 343:1–29. http://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32012R1151

Commission Regulation (2005a) Commission regulation no 2074/2005 of 5 December 2005 laying down implementing measures for certain products under regulation (EC) no 853/2004 of the European parliament and of the Council and for the organisation of official controls under regulation (EC) no 854/2004 of the European parliament and of the Council and regulation (EC) no 882/2004 of the European parliament and of the Council, derogating from regulation (EC) no 852/2004 of the European parliament and of the Council and amending regulations (EC) no 853/2004 and (EC) no 854/2004. Off J Eur Union 338:27–59. http://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX%3A32005R2074

Commission Regulation (2005b) Commission regulation no 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. Off J Eur Union 338:1–26. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2005:338:0001:0026:EN:PDF

Commission Regulation (2004) Commission regulation no 1935/2004 of 27 October 2004 on materials and articles intended to come into contact with food and repealing Directives 80/590/EEC and 89/109/EEC, E P a t council. Off J Eur Union 338:4–17. http://eur-lex.europa.eu/legal-content/IT/ALL/?uri=CELEX%3A32004R1935

Cruciata M, Sannino C, Ercolini D, Scatassa ML, De Filippis F, Mancuso I, La Storia A, Moschetti G, Settanni L (2014) Animal rennets as sources of dairy lactic acid bacteria. Appl Environ Microbiol 80:2050–2061. http://dx.doi.org/10.1128/AEM.03837-13

Cruciata M, Gaglio R, Scatassa ML, Sala G, Cardamone C, Palmeri M, Moschetti G, La Mantia T, Settanni L (*in press*) Formation and characterization of early bacterial biofilms on different wood typologies applied in dairy production. Appl Environ Microbiol http://dx.doi.org/10.1128/AEM.02107-17

Cutini F (2014) Food: Divina (LN), the FDA Declares War on Our Chesees. AgenParl (Agenzia Parlamentare per l'informazione politica ed economica)

Della Ciana M (2015) Il legno nell'industria alimentare. LinkedIn Slide Share 1–11

Didienne R, Defargues C, Callon C, Meylheuc T, Hulin S, Montel MC (2012) Characteristics of microbial biofilm on wooden vats ('gerles') in PDO Salers cheese. Int J Food Microbiol 156:91–101. https://doi.org/10.1016/j.ijfoodmicro.2012.03.007

Di Grigoli A, Francesca N, Gaglio R, Guarrasi V, Moschetti M, Scatassa ML, Settanni L, Bonanno A (2015) The influence of the wooden equipment employed for cheese manufacture on the characteristics of a traditional stretched cheese during ripening. Int J Food Microbiol 46:81–91. https://doi.org/10.1016/j.fm.2014.07.008

Foulquié Moreno MR, Sarantinopoulos P, Tsakalidou E, De Vuyst L (2006) The role and application of enterococci in food and health. Int J Food Microbiol 106:1–24. https://doi.org/10.1016/j.ijfoodmicro.2005.06.026

Franciosi E, Settanni L, Cavazza A, Poznanski E (2009) Biodiversity and technologicsl potential of wild lactic acid bacteria from raw cows' milk. Int Dairy J 19:3–11. https://doi.org/10.1016/j.idairyj.2008.07.008

Gaglio R, Cruciata M, Di Gerlando R, Scatassa ML, Mancuso I, Sardina M T, Moschetti G, Portolano B, Settanni L (2016) Microbial activation of wooden vats used for traditional cheese production and evolution of the neo-formed biofilms. Appl Environ Microbiol 82:585–595. http://dx.doi.org/10.1128/AEM.02868-15

Gaglio R, Francesca N, Di Gerlando R, Cruciata M, Guarcello R, Portolano B, Moschetti G, Settanni L (2014a) Identification, typing, and investigation of the dairy characteristics of lactic acid bacteria isolated from 'Vastedda della valle del Belìce' cheese. Dairy Sci Technol 94:157–180. https://doi.org/10.1007/s13594-013-0150-5

Gaglio R, Scatassa ML, Cruciata M, Miraglia V, Corona O, Di Gerlando R, Portolano B, Moschetti G, Settanni L (2014b) *In vivo* application and dynamics of lactis acid bacteria for the four-season production of Vastedda-like cheese. Int J Food Microbiol 177:37–48. https://doi.org/10.1016/j.ijfoodmicro.2014.02.007

Galinari É, Nóbrega JED, Andrade NJD, Ferreira CLDLF 2014. Microbiological aspects of the biofilm on wooden utensils used to make a Brazilian artisanal cheese. Braz J Microbiol 45:713–20. http://dx.doi.org/10.1590/S1517-83822014000200047

Guarcello R, Carpino S, Gaglio R, Pino A, Rapisarda T, Caggia C, Marino G, Randazzo CL, Settanni L, Todaro M (2016) A large factory-scale application of selected autochthonous lactic acid bacteria for PDO Pecorino Siciliano cheese production. Food Microbiol 59:66–75. https://doi.org/10.1016/j.fm.2016.05.011

Licitra G, Ogier JC, Parayre S, Pediliggieri C, Carnemolla TM, Falentin H, Madec MN, Carpino S, Lortal S (2007) Variability of the bacterial biofilms of the "tina" wood vat used in the Ragusano cheese making process. Appl Environ Microbiol 73:6980–6987. https://doi.org/10.1128/AEM.00835-07

Lortal S, Di Blasi A, Madec MN, Pediliggieri C, Tuminello L, Tanguy G, Fauquant J, Lecuona Y, Campo P, Carpino S, Licitra G (2009) Tina wooden vat biofilm. A safe and highly efficient lactic acid bacteria delivering system in PDO Ragusano cheese making. Int J Food Microbiol 132: 1–8. https://doi.org/10.1016/j.ijfoodmicro.2009.02.026

Mariani C, Oulahal N, Chamba JF, Dubois-Brissonnet F, Notz E, Briandet R (2011) Inhibition of *Listeria monocytogenes* by resident biofilms present on wooden shelves used for cheese ripening. Food Control 22:1357–62. https://doi.org/10.1016/j.foodcont.2011.02.012

Mucchetti G, Bonvini B, Remagni MC, Ghiglietti R, Locci F, Barzaghi S, Francolino S, Perrone A, Rubiloni A, Campo P, Gatti M, Carminati D (2008) Influence of cheese-making technology on composition and microbiological characteristics of Vastedda cheese. Food Control 19:119–125. https://doi.org/10.1016/j.foodcont.2007.02.011

Niro S (2011) Innovazione di processo e di prodotto in formaggi a pasta filata. PhD thesis Università degli Studi del Molise, Italy

Osservatorio sull'Economia del Sistema Agro Alimentare della Sicilia (OEASAAS) (2007) I formaggi storici di nicchia in Sicilia: aspetti produttivi e di mercato ISBN 978-88-95376-17-2

Parente E, Moschetti G, Coppola S (1998) Starter cultures for Mozzarella cheese. Ann Microbiol Enzimol 48:89–109

Reale S, Vitale F, Scatassa ML, Caracappa S, Currò V, Todaro M (2007) Molecular characterization of dominant bacterial population in "Vastedda della Valle del Belice" cheese: preliminary investigation. Ital J Anim Sci 6: 595–597. https://doi.org/10.4081/ijas.2007.1s.595

Ricci M (2017) La scoperta degli archeologi siciliani: a Troina si produceva formaggio oltre 5000 anni fa. http://www.cronachedigusto.it/archiviodal-05042011/325-scenari/21156-la-scoperta-degli-archeologi-siciliani-a-troina-si-produceva-formaggio-oltre-5000-anni-fa.html. Last accessed: 11.27.2017

Salvadori del Prato O (1998) Trattato di Tecnologia Casearia. Edagricole, Bologna, Italy

Scatassa ML, Di Noto AM, Todaro M, Caracappa S (2007) Vastedda della valle del Belicé cheese: Production and microbiological investigation, 379–381 Proceedings of the 5th International Symposium on the challenge to sheep and goat milk sectors, Alghero, Italy

Scatassa ML, Di Noto AM, Cardamone C, Sciortino S, Todaro M, Caracappa S (2009) Vastedda della valle del Belìce: processo produttivo e contaminazione sperimentale con *salmonella spp.*. Sci Tecn Latt-Cas, 60:000–000

Scatassa ML, Gaglio R, Macaluso G, Francesca N, Randazzo W, Cardamone C, Di Grigoli A, Moschetti G (2015a) Transfer, composition and technological characterization of the lactic acid bacterial populations of the wooden vats used to produce traditional stretched cheeses. Food Microbiol 52:31–41. https://doi.org/10.1016/j.fm.2015.06.008

Scatassa ML, Cardamone C, Miraglia V, Lazzara F, Fiorenza G, Macaluso G, Arcuri L, Settanni L, Mancuso I (2015b) Characterisation of the microflora contaminating the wooden vats used for traditional Sicilian cheese procuction. Ital J Food Saf 4:36–9. https://doi.org/10.4081/ijfs.2015.4509

Settanni L, Moschetti G (2014) New trends in technology and identity of traditional dairy and fermented meat production processes: preservation of typicality and hygiene. Trends Food Sci Technol 37:51–58. https://doi.org/10.1016/j.tifs.2014.02.006

Settanni L, Moschetti G (2010) Non-starter lactic acid bacteria used to improve cheese quality and provide health benefits. Food Microbiol 27:691–697. https://doi.org/10.1016/j.fm.2010.05.023

Settanni L, Franciosi E, Cavazza A, Cocconcelli PS, Poznanski E (2011) Extension of Tosèla cheese shelf-life using non-starter lactic acid bacteria. Food Microbiol 28:883–890. https://doi.org/10.1016/j.fm.2010.12.003

Settanni L, Di Grigoli A, Tornambè G, Bellina V, Francesca N, Moschetti G, Bonanno A (2012) Persistence of wild *Streptococcus thermophilus* strains on wooden vat and during the manufacture of a Caciocavallo type cheese. Int J Food Microbiol 155:73–81. https://doi.org/10.1016/j.ijfoodmicro.2012.01.022

Todaro M, Palmeri M, Settanni L, Scatassa ML, Mazza F, Bonanno A, Di Grigoli A (2017) Effect of refrigerated storage on microbiological, chemical and sensory characteristics of a ewes' raw milk stretched cheese. Food Packaging and Shelf Life 11:67–73. https://doi.org/10.1016/j.fpsl.2017.01.005

Todaro M, Bonanno A, Scatassa ML (2014) The quality of Valle del Belice sheep's milk and cheese produced in the hot summer season in Sicily. Dairy Sci Technol 94:225–239. https://doi.org/10.1007/s13594-013-0155-0

Vert M, Doi Y, Hellwich KH, Hess M, Hodge P, Kubisa P, Rinaudo M, Schué F (2012) Terminology for biorelated polymers and applications (IUPAC Recommendations 2012). Pure Appl Chem 84:377–410. http://dx.doi.org/10.1351/PAC-REC-10-12-04

Verzera A, Condurso C, Ziino M, Romeo V, Todaro M, Conte F, Dima G (2010) Free fatty acids and other volatile compounds for the characterisation of “Vastedda della valle del Belìce Cheese Acidos grasos libres y otros constituyentes volátiles para la caracterización de queso “Vastedda della vella del Belìce”", CyTA – Journal of Food, 8:237–243. http://dx.doi.org/10.1080/19476330903450282

Legend to figures.

Fig. 1. Flow diagram of "Vastedda della valle del Belìce" cheese production. (Reference: Gaglio et al., 2014a)

Fig. 2. Main traditional wooden equipment used for PDO Vastedda della valle del Belìce cheese production. **A,** "*tina",* wooden vat used for milk coagulation; **B***, "rotula",* stick for curd breaking; **C**, "*fuscella*", rattan basket for curd acidification; **D**, "*piddiaturi"*, wooden vat used for curd stretching.

Fig. 3. Scanning electron microscopy image of wooden vat microbial biofilm.

Fig. 4. Experimental design of Vastedda-like cheese productions performed at pilot plant scale. **A**, Process 1; **B**, process 2; **C**, process 3. (Reference: Gaglio et al., 2014b).

Fig. 5. Scanning electron microscopy observations of wooden splinters. **A**, Virgin vat after 30 days of hot water treatment; **B**, Vat TZ1 after overnight contact with whey obtained from a traditional Vastedda cheese made with raw milk; **C**, Vat TZ2 after overnight contact with the NWSC developed with the multistrain *Lactococcus* culture. (Reference: Gaglio et al., 2016).

Fig. 1.

raw ewes' milk

↓

heating at 40 °C

↓

addition of lamb rennet

↓

milk curdling (50 min)

↓

hot water (60 °C) addition (10% v/v)

↓

cutting of the curd into rice-size grains

↓

curd extraction

↓

acidification (24-48 h) in cane containers (ambient temperature)

↓

slicing and stretching in hot water (2-3 min at 80-90 °C)

↓

moulding to bun shape

↓

salted in satured brine (30 min)

↓

drying (24 at 7 °C)

↓

"Vastedda della valle del Belìce"

Fig. 2.

Fig. 3.

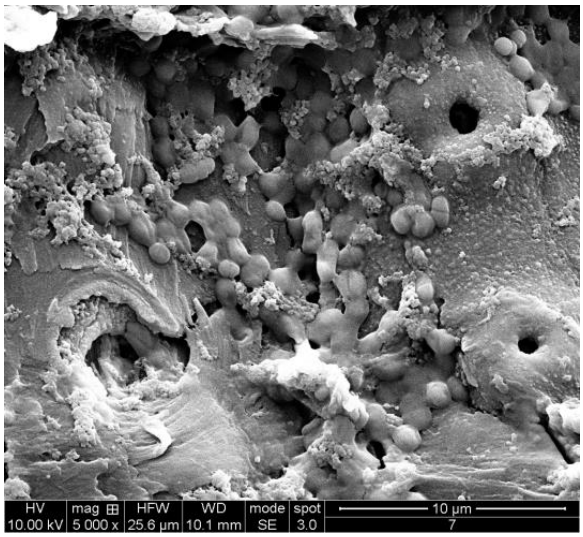

Fig. 4.

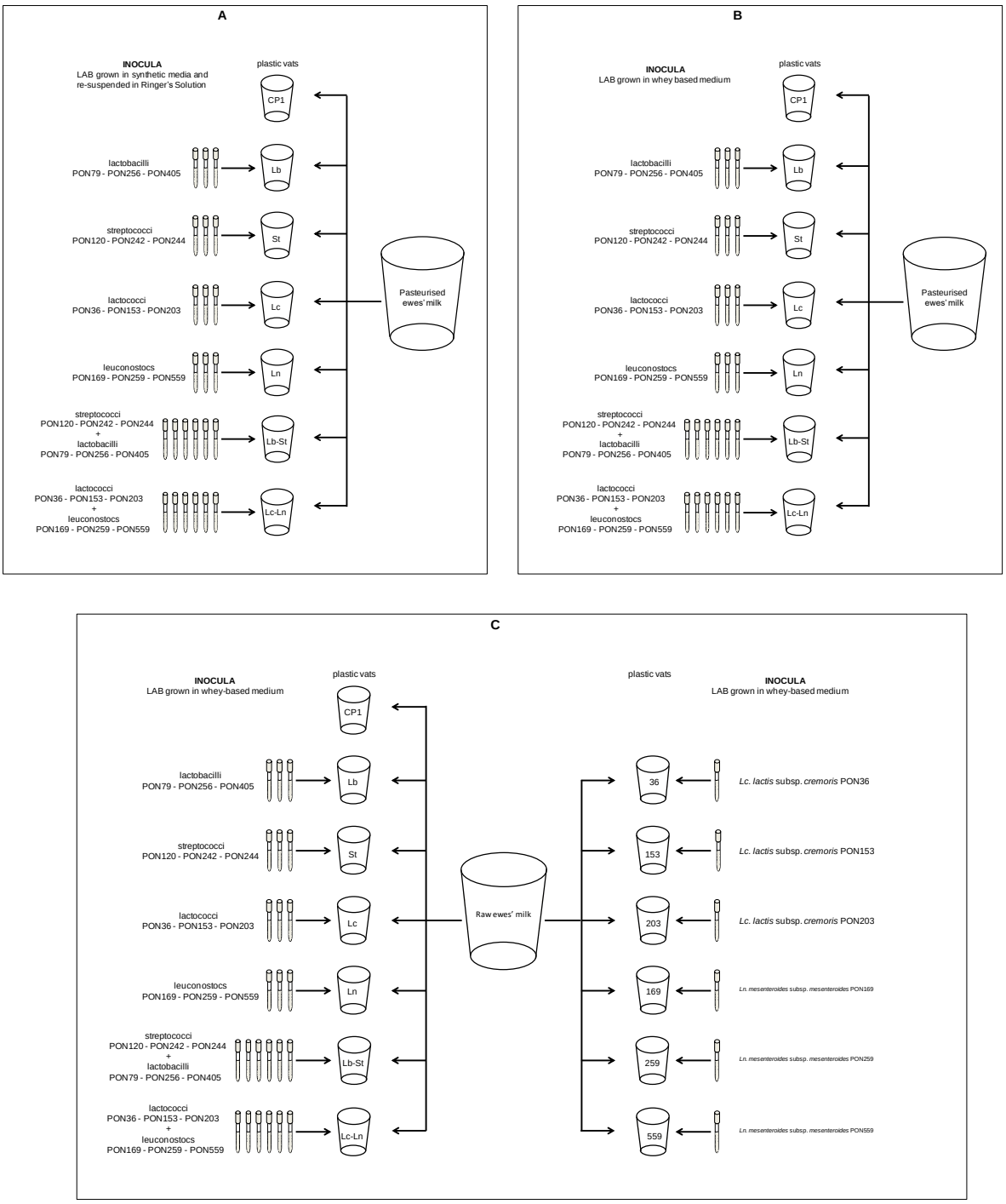
A
INOCULA
LAB grown in synthetic media and re-suspended in Ringer's Solution
plastic vats
CP1
lactobacilli
PON79 - PON256 - PON405
Lb
streptococci
PON120 - PON242 - PON244
St
lactococci
PON36 - PON153 - PON203
Lc
leuconostocs
PON169 - PON259 - PON559
Ln
streptococci
PON120 - PON242 - PON244
+
lactobacilli
PON79 - PON256 - PON405
Lb-St
lactococci
PON36 - PON153 - PON203
+
leuconostocs
PON169 - PON259 - PON559
Lc-Ln
Pasteurised ewes' milk
B
INOCULA
LAB grown in whey based medium
plastic vats
CP1
lactobacilli
PON79 - PON256 - PON405
Lb
streptococci
PON120 - PON242 - PON244
St
lactococci
PON36 - PON153 - PON203
Lc
leuconostocs
PON169 - PON259 - PON559
Ln
streptococci
PON120 - PON242 - PON244
+
lactobacilli
PON79 - PON256 - PON405
Lb-St
lactococci
PON36 - PON153 - PON203
+
leuconostocs
PON169 - PON259 - PON559
Lc-Ln
Pasteurised ewes' milk
C
INOCULA
LAB grown in whey-based medium
plastic vats
CP1
lactobacilli
PON79 - PON256 - PON405
Lb
streptococci
PON120 - PON242 - PON244
St
lactococci
PON36 - PON153 - PON203
Lc
leuconostocs
PON169 - PON259 - PON559
Ln
streptococci
PON120 - PON242 - PON244
+
lactobacilli
PON79 - PON256 - PON405
Lb-St
lactococci
PON36 - PON153 - PON203
+
leuconostocs
PON169 - PON259 - PON559
Lc-Ln
Raw ewes' milk
plastic vats
INOCULA
LAB grown in whey-based medium
36
Lc. lactis subsp. cremoris PON36
153
Lc. lactis subsp. cremoris PON153
203
Lc. lactis subsp. cremoris PON203
169
Ln. mesenteroides subsp. mesenteroides PON169
259
Ln. mesenteroides subsp. mesenteroides PON259
559
Ln. mesenteroides subsp. mesenteroides PON559

Fig. 5.