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Dairy Sci. & Technol. (2014) 94:581–590
DOI 10.1007/s13594-014-0181-6

ORIGINAL PAPER

Characteristics of raw milk produced by free-stall or tie-stall cattle herds in the Parmigiano-Reggiano cheese production area

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Received: 10 January 2014 / Revised: 9 July 2014 / Accepted: 10 July 2014 /
Published online: 6 August 2014

Abstract Tie-stalls used to be the common type of housing system for dairy cows in the Parmigiano-Reggiano cheese area; however, the proportion of free-stall herds has greatly increased over the last years. The aim of this study was to compare the milk characteristics between bulk tank milk produced in free-stall and tie-stall herds, in the Parmigiano-Reggiano cheese production area. Fourteen free stalls and 14 tie stalls were involved in the study. Bulk tank milk samples were collected monthly from each herd over a 3-year period. The following parameters were determined for each sample: fat, crude protein, titratable acidity, total bacterial count (TBC), somatic cells (SCC), coliforms, clostridial spores and rennet coagulation properties. Milk produced by free-stall herds (F-milk) showed higher fat, protein and SCC values (3.82 vs 3.61 g.100 g^{-1}; 3.45 vs 3.30 g. 100 g^{-1}; 158,891 vs 426,088 cells.mL^{-1}, respectively) than milk collected from tie-stall herds (T-milk). The percentage of samples with optimal coagulation profiles and exceeding 100 clostridial spores.L^{-1} were higher in F-milk than T-milk. F-milk proved to be more suitable for manufacturing Parmigiano-Reggiano cheese both, in terms of yield and quality, although the presence of clostridial spores should be considered, as these microorganisms are responsible for most Parmigiano-Reggiano cheese defects. Yearly and seasonal variations of milk characteristics were also analysed. During the summer, an increase in the TBC, SCC and coliforms were observed regardless of the housing system. In conclusion, besides the type of housing system, it is advisable to adopt management strategies which will reduce the negative effects on milk quality caused by the adverse climatic conditions which occur in summer in the Po Valley.

Keywords Milk quality · Dairy cattle · Cheesemaking · Housing system · Parmigiano-Reggiano cheese

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1 Introduction

Parmigiano-Reggiano is a very hard protected designated of origin (PDO) cheese produced in a specific area in the north of Italy which can only be made with raw, unheated bovine milk. Cheese milk is obtained by mixing (1:1) partially skimmed evening milk (by means of natural creaming) and full-cream morning milk. Storage of milk is not allowed and both evening and morning milks should be transported to the cheese factory within 6 h from the beginning of the milking procedure (Malacarne et al. 2013). The key features of the cheesemaking procedure are the use of a natural whey starter culture (obtained by the overnight fermentation of the residual previous-day cheesemaking whey), coagulation of milk with calf rennet and a ripening phase which can last from a *minimum* of 12 months and is usually extended to 24 months (Gatti et al. 2014). Moreover, there are strict regulations concerning the diet of the cows producing milk for Parmigiano-Reggiano cheesemaking. Above all, it is essential that 50% of the forage dry matter should be provided by hay and the storage and use of silage is forbidden (www.parmigianoreggiano.com).

Traditionally, tie stalls were commonly used for housing dairy cows in the Parmigiano-Reggiano cheese area. Even if over the last few years, there has been a considerable drop in number of cattle herds, the proportion of cattle housed in free stalls has increased significantly. The two types of housing differ greatly and their influence on animal welfare and their produce have been closely examined over the last 30 years (Norell and Appleman 1981; Smith et al. 1995; Hovinen et al. 2008).

In the production of raw milk cheeses, the chemical composition of the milk and its microbial characteristics may have significant effects on cheese yield (Barbano et al. 1991; Klei et al. 1998; Verdier-Metz et al. 2001) and cheese quality (Coulon et al. 2004; Vianna et al. 2008). In the manufacture of Parmigiano-Reggiano, cheese yield is directly correlated to milk fat and protein contents (Pecorari and Mariani 1990) and is also affected by somatic cell content (Franceschi et al. 2009; Le Maréchal et al. 2011). Moreover, a high total bacterial count and/or the presence of coliforms and clostridial spores may cause structural, colour and sensory defects in cheese (Beresford et al. 2011).

The aim of this study was to compare the chemical composition, physicochemical properties, coliforms and Clostridia counts and rennet coagulation aptitude of the milk used for manufacturing Parmigiano-Reggiano cheese produced by cows housed in two different housing systems (free stall vs tie stall). The cattle herds involved in this research were surveyed on a monthly basis over a 3-year period. Therefore, variations of milk characteristics according to the season and year of sampling were also analysed.

2 Materials and methods

2.1 Experimental design and milk sampling

Twenty-eight Italian Friesian cattle herds (14 tie stalls and 14 free stalls) were surveyed once a month over a 3-year period from January 2006 to December 2008 (36 surveys per herd). The cattle produced milk for Parmigiano-Reggiano cheese and were located in the province of Reggio Emilia (an area in the Po Valley, Northern Italy). During each

survey, a sample of milk was collected at the end of the morning milking, according to International Dairy Federation standards (IDF 2008), and the number of cows milked was recorded. On average, there were 110 cows in lactation at each sampling (minimum 26, maximum 602) and 43 (minimum 18; maximum 111) in free-stall and tie-stall herds, respectively. A total of 1,008 bulk tank milk samples were collected, 504 from free-stall herds (F-milk) and 504 from tie-stall herds (T-milk). An aliquot of milk was collected from each sample in order to carry out the microbial analyses (total bacterial count, coliforms and clostridial spores, see "Milk analyses and classification of samples") before adding sodium merthiolate 0.02% $w.v^{-1}$ ($C_9H_9HgNaO_2S$; Carlo Erba Reagents 20090, Milano, Italy). The samples were transported to the laboratory at 4 °C within 30 min and immediately analysed.

2.2 Milk analyses and classification of samples

The following parameters were determined on the milk samples: fat and crude protein by means of the infrared analysis (Biggs 1978) with Milko-Scan (Foss Electric, DK-3400 Hillerød, Denmark); titratable acidity by titration of 50 mL of milk with 0.25 N sodium hydroxide according to the Soxhlet-Henkel method (Anon. 1963); total bacterial count (TBC) using the flow cytometry method with BactoScan FC (Foss Electric, DK-3400 Hillerød, Denmark) (Grappin et al. 1985); somatic cell count (SCC) using the fluoro-opto-electronic method (Schmidt-Madsen 1975) with Fossomatic (Foss Electric, DK-3400 Hillerød, Denmark); coliform bacterial count on Petri dish with VRBA medium after incubation at 37 °C for 24 h (IDF 1998); number of clostridial spores with most probable number (MPN) using the Weinzirl method modified by Annibaldi (1969); the rennet coagulation properties (RCP) were assessed according to McMahon and Brown (1982) with Formagraph (Foss Electric, DK-3400 Hillerød, Denmark). The milk samples were then divided into 13 lactodynamografic (LDG) types identified with capital letters (Annibaldi et al. 1977), as described in Malacarne et al. (2014): A, B, C, EA, EB, EC, E, D, EF, DD, FE, F and FF. The LDG types were then divided into three classes according to Pecorari (1984): optimal (LDG types A, B, C), suboptimal (EA, EB, EC, D, EF, DD) and poor (E, FE, F, FF).

Rolling geometrical average (RGA) values of SCC and TBC were calculated in compliance with the 853/2004 CEE regulation. In short, each monthly value of SCC was averaged (by geometric mean) with those recorded in the previous 2 months. Concerning TBC, each monthly value was averaged (by geometric mean) with that recorded in the previous month. As the collection of bulk tank milk samples started in January 2006, it was not possible to calculate the RGA values of somatic cells for milk samples collected in January and February 2006 (56 samples) and total bacterial count for those collected in January 2006 (28 samples). The RGA values were divided into two classes for both parameters ($\leq$400,000 or >400,000 cells.mL^{-1} for somatic cells and $\leq$100,000 or >100,000 CFU.mL^{-1} for total bacterial count) in compliance with the legal limits laid down in the 853/2004 CEE regulation.

The milk samples were also grouped in three classes according to the values of clostridial spores (up to 30 spores.L^{-1}, from 31 to 100 spores.L^{-1}, more than 100 spores.L^{-1}) and coliforms (up to 1,000 CFU.mL^{-1}, from 1,001 to 5,000 CFU.mL^{-1}, more than 5,000 CFU.mL^{-1}) (Pecorari 1984).

2.3 Statistical analysis

The statistical significance of the difference was analysed by ANOVA with the statistical package (IBM SPSS Statics 19, Armonk, NY 10504-1722, USA) after control (Levene test) of variance homogeneity, using the following general linear model.

$$y_{ijklm} = \mu + H_i + E_j + S_k + Y_l + (HSY)_{ikl} + \varepsilon_{ijklm}$$

where y_{ijklm}=dependent variable; μ=overall mean; H_i=housing type (i=1, 2; 1 free stall, 2 tie stalls); E_j=herd (j=1,…28; one level for each cattle herd); S_k=season (k=1,…4; 1 winter, from January to March; 2 springs, from April to June; 3 summer, from July to September; 4 autumns, from October to December); Y_l=year (l=1,…3; 1 2006, 2 2007, 3 2008); ε_{ijklm}=residual error. The statistical significance of the differences was tested by means of least significant difference (LSD) control.

The values of SCC, TBC, coliforms and clostridial spores were log transformed before submitting them to ANOVA.

The differences between the distribution of samples in SCC, TBC, LDG, clostridial spores and coliforms classes were tested with the chi-square method.

3 Results and discussion

3.1 Variations of milk characteristics according to the housing system

The values of fat, protein and titratable acidity were higher in F-milk than in T-milk (Table 1). Feeding strategies may have been responsible for the differences of fat and protein percentages observed. In fact, in most free-stall herds, forages and concentrates are given to cows as unifeed while they are distributed separately to tie-stall herds (traditional feeding), allowing the cows to choose among the various feeds. Unifeed ensures a better utilization of nutrients, allowing for a better exploitation of the genetic potential of dairy cattle for milk quality. The high content of protein and fat of F-milk should lead to higher yields of Parmigiano-Reggiano cheese and butter (Aleandri et al. 1989). The lower the value of fat to casein ratio in cheese milk, the stronger the relationships between milk casein and cheese yield. In fact, when making Parmigiano-Reggiano cheese, the fat to casein ratio in cheese milk is lowered to about 1 by means of creaming (overnight gravity separation) of about half of the milk (the evening raw milk) (Malacarne et al. 2008). Furthermore, the surfaced cream is used for making butter. The casein content and titratable acidity values of the milk are both positively associated to its cheesemaking aptitude, in terms of its rennet coagulation ability and the rheological properties of the cheese produced (Pretto et al. 2013). These properties are essential for obtaining a homogeneous and adequate dehydration of the cheese mass during the vat phase (Mariani et al. 2001). Also in this respect, the milk produced in free-stall housing conditions showed better characteristics than milk from tie-stall herds. The difference in titratable acidity mainly depends on the higher casein (presumed by the values of protein) content of F-milk.

Table 1 Effect of the housing type on the chemical composition, physicochemical properties and microbiological characteristics in bulk tank milk samples

		Free stall (F-milk) n=504	Tie stall (T-milk) n=504		
		LS mean	LS mean	SE	P
Fat	g.100 g^{-1}	3.82	3.61	0.03	***
Protein	g.100 g^{-1}	3.45	3.30	0.03	*
Titratable acidity	°SH.50 mL^{-1}	3.27	3.19	0.02	**
Somatic cell count	Cells.mL^{-1}	158,891	426,088	15,568	***
Total bacterial count	CFU.mL^{-1}	61,135	81,997	14,793	ns
Coliform bacteria	CFU.mL^{-1}	2,041	1,875	357	ns
Clostridial spores	Spores.L^{-1}	81	84	12	ns

LS mean least square mean value, *SE* standard error

P=significance of differences: *ns* not significant, $P>0.05$; *$P\leq0.05$; **$P\leq0.01$; ***$P\leq0.001$

The SCC was higher in T-milk than F-milk, while no differences were observed between the two types of milks for the mean values of TBC, coliforms and clostridial spores (Table 1). Besides the mean values, a comparison was carried out between F- and T-milk also concerning the distribution of samples in the classes of SCC, TBC, coliforms, clostridial spores and LDG profiles. In the case of SCC and TBC, we analysed the distribution of the milk samples which showed RGA values above the legal limits (Regulation EC 853 2004). The percentage of milk samples above the legal limits were clearly higher in T-milk than F-milk (Table 2). It is important to note that in the years to which this research refers (2006, 2007 and 2008), Parmigiano-Reggiano cheese milk producers were allowed to process milk samples exceeding the RGA limits for SCC and TBC, due to an exception made by the Italian Ministry of Heath until December 31 2008. The classification used in this study for coliforms, clostridial spores and LDG profiles was based on that employed to reward or penalize producers in the milk quality payment system used in the Parmigiano-Reggiano cheese production area. Compared to T-milk, F-milk showed a higher percentage of samples with coliforms ≤1,000 CFU.mL^{-1} and lower percentages of samples for both 1,001–5,000 and >5000 CFU.mL^{-1} classes (Table 2). A difference between the two types of milk for clostridial spores was only observed in the class >100 spores.L^{-1}, where F-milk showed a percentage of samples higher than T-milk (Table 2). Finally, the percentage of samples in the optimal and poor classes were higher and lower, for F-milk than T-milk, respectively (Table 2).

The relevant differences observed for both mean values of SCC and the percentages of samples with RGA of SCC exceeding the legal threshold are partly influenced by the milking system adopted. In free-stall herds, cows were milked in parlours, whereas in tie-stall herds milk is collected at the stall, using pipeline milking systems. Modern parlours are generally equipped with devices which help to keep the udders healthy, such as online record of milk yield, milk flow and its electric conductivity, as well as automatic cluster removal. Furthermore, milking procedures are usually carried out by specifically trained staff who are able to perform all milking operations (preparation of the udder, pre- and post-dipping, etc.) and are continually updated on new procedures.

Table 2 Results of chi-square test for somatic cell (SCC), total bacterial count (TBC), coliforms, clostridial spores and lactodynamographic (LDG) classes of bulk tank milk samples collected from free-stall herds and collected from tie-stall herds

		Free stall (F-milk)		Tie stall (T-milk)		*P*
	Class	*n*	%	*n*	%	
SCC	>400,000 cells.mL^{-1}	51	10.71	161	33.82	***
TBC	>100,000 CFU.mL^{-1}	40	8.16	93	18.98	***
Coliforms	<1,000 CFU.L^{-1}	424	84.13	358	71.03	***
	1,001–5,000 CFU.L^{-1}	60	11.90	106	21.03	***
	>5,000 CFU.L^{-1}	20	3.97	40	7.94	***
Clostridial spores	<30 spores.L^{-1}	78	15.48	92	18.25	ns
	31–100 spores.L^{-1}	314	62.30	333	66.07	ns
	>100 spores.L^{-1}	112	22.22	79	15.68	**
LDG	Optimal	317	62.90	292	57.94	*
	Suboptimal	121	24.01	127	25.20	ns
	Poor	66	13.09	85	16.86	*

SCC=rolling geometric average values calculated on 3 months, *TBC*=rolling geometric average values calculated on 2 months

P=significance of differences: *ns* not significant, $P>0.05$; *$P\leq0.05$; **$P\leq0.01$; ***$P\leq0.001$

In tie-stall herds, the milking plant is usually of old design and milking operations are carried out by the owner himself, using traditional procedures. The separation of feeding, resting and milking areas in free-stall herds should reduce the contamination of milk with environmental microrganisms during milking operations thus reducing TBC and coliforms. The high content of SCC in T-milk may negatively affect the milk yield and its aptitude to rennet coagulation (Malacarne et al. 2014), as demonstrated by the highest proportion of samples with the worst LDG profiles observed in this research. Furthermore, the increased proteolytic activity towards casein (due to the increased plasmin activity in high SCC milk) may negatively affect the rheological properties of the curd and cheese mass, as well as milk cheese yield (Fleminger et al. 2013; Le Maréchal et al. 2011), as observed in several types of cheese (Politis and Ng-Kwai-Hang 1988; Barbano et al. 1991; Klei et al. 1998).

The large proportion of samples with >100 clostridial spores.L^{-1} in F-milk could depend on the differences in the feeding techniques between free- and tie-stall herds (Colombari et al. 2005). In most free-stall herds, forages and concentrates are given to cows as unifeed. As the use of silage is not allowed, water is added in order to tie the concentrate (as flour) to the hay, in the preparation of unifeed. However, this process also dampens the soil present in the hay which sticks to the hay like flour (Pecorari et al. 2001). Consequently, cows fed with the unifeed method will ingest a higher number of clostridial spores (which are mainly present in soil) than cows fed traditionally, leading to a higher excretion of spores in the faeces. Faeces are the main vehicle of transmission of clostridial spores to milk (Lango and Heinonen-Tanski 1995). Clostridial spores (*Clostridium tyrobutyricu*m, *Clostridium butyricum* and *Clostridium sporogenes*) are

the main cause of early and late blowing defects in Parmigiano-Reggiano cheese (Bassi et al. 2009, 2013).

3.2 Variations of milk characteristics according to year and season

Mean values of milk characteristics according to year and season of sampling are reported in Tables 3 and 4, respectively.

A decrease of fat content and an increase of protein content in milk were observed over the 3-year study period. However, although they were significant, these differences did not appear to affect cheese quality and yield. It is interesting to observe the trend of the fat content, as the official statistics of the Italian Friesian Breeders Association reported an increase of values over the period considered (http://www.anafi.it/english/). The increase of protein is the result of breeding programs aimed at increasing the content of casein in milk (http://www.anafi.it/english/), since about 70% of bovine milk produced in Italy is used for cheese production.

These values were compared with those reported by Tedeschi et al. (2010) and Bertocchi et al. (2014) for bulk tank milk samples collected in the period 2006–2008. In Tedeschi et al. (2010), the milk samples were collected from cattle herds producing milk for Parmigiano-Reggiano cheese. The samples analysed by Bertocchi et al. (2014) were obtained from free-stall cattle herds located in the Po Valley, which is a large area in Northeast Italy, mostly from herds whose milk is not used for making Parmigiano-Reggiano cheese and where the use of silage is not forbidden. This is one of the most important areas in Europe for milk and cheese production. The values of fat were comparable to those observed by Tedeschi et al. (2010), but much lower than those reported by Bertocchi et al. (2014). In general, lower values of milk fat for Parmigiano-Reggiano cheese could depend on the feeding restrictions for cows imposed by the regulation. The protein values were higher than those reported by Tedeschi et al. (2010) and similar to Bertocchi et al. (2014).

Table 3 Effect of the year of sampling (2006, 2007, 2008) on the chemical composition, physicochemical properties and microbiological characteristics of bulk tank milk samples

		2006 n=336 LS mean	2007 n=336 LS mean	2008 n=336 LS mean	SE	P
Fat	g.100 g^{-1}	3.78 c	3.71 b	3.66 a	0.01	***
Protein	g.100 g^{-1}	3.28 a	3.41 b	3.43 b	0.01	***
Titratable acidity	°SH.50 mL^{-1}	3.24	3.23	3.23	0.01	ns
Somatic cell count	Cells.mL^{-1}	245,471 a	257,040 b	281,838 c	6,962	***
Total bacterial count	CFU.mL^{-1}	51,286 b	44,668 a	40,738 a	6,615	***
Coliform bacteria	CFU.mL^{-1}	1,349	1,380	1,380	160	ns
Clostridial spores	Spores.L^{-1}	57	62	56	5	ns

LS mean least square mean value, *SE* standard error

a, b, c=LS mean values not sharing a common letter in a row are different at $P<0.05$

P=significance of differences: *ns* not significant, $P>0.05$; ***$P\leq 0.001$

Table 4 Effect of the season of sampling on the chemical composition, physico-chemical properties and microbiological characteristics of bulk tank milk samples

		Winter n=252	Spring n=252	Summer n=252	Autumn n=252		P
		LS mean	LS mean	LS mean	LS mean	SE	
Fat	g.100 g^{-1}	3.78 b	3.67 a	3.65 a	3.38 b	0.01	***
Protein	g.100 g^{-1}	3.36 b	3.26 a	3.30 ab	3.56 c	0.02	***
Titratable acidity	SH.50mL^{-1}	3.23	3.24	3.22	3.24	0.01	ns
Somatic cell count	Cells.mL^{-1}	239,883 a	251,188 a	301,995 b	251,188 a	8,039	***
Total bacterial count	CFU.mL^{-1}	44,668 a	45,709 ab	52,481 b	38,904 a	7,639	***
Coliform bacteria	CFU.mL^{-1}	1,318 a	1,348 a	1,621 b	1230 a	184	***
Clostridial spores	Spores.L^{-1}	56	56	60	63	6	ns

LS mean least square mean value, *SE* standard error

a, b, c=LS mean values not sharing a common letter in a row are different at $P<0.05$

P=significance of differences: *ns* not significant, $P>0.05$; ***$P\leq0.001$

No significant variations were reported concerning milk titratable acidity. The average yearly values observed were similar to Tedeschi et al. (2010), but clearly lower than those observed in the same area in the past decades (Malacarne et al. 2003; Sandri et al. 2001), confirming an alarming tendency to decrease for this parameter over the years (Formaggioni et al. 2005). As the values of titratable acidity in milk are positively correlated with those of casein and minerals, we can hypothesize that milk minerals may be responsible for the decreasing trend of this parameter.

Although significant from a statistical point of view, the variations of SCC and TBC were small and did not greatly influence the overall processing quality of milk. No significant variations of clostridial spores and coliforms were observed throughout the years. The values of SCC and TBC observed are comparable with those reported by Bertocchi et al. (2014), and slightly lower than those provided by Tedeschi et al. (2010). Furthermore, the values of both parameters are lower than those observed in the same area from 1990 to 2000 (Malacarne et al. 2003; Sandri et al. 2001). The improvement of the hygienic conditions of the cattle herd was actually achieved by means of financial support provided by the 215 "Animal welfare payment" measure (enrd.ec.europa.eu).

Seasonal trends were consistent with those reported by Tedeschi et al. (2010) and Bertocchi et al. (2014). In general, the worst values of all parameters were observed in summer, where adverse climatic conditions had a negative effect on the productive performance of the dairy cows and the hygienic conditions of the herd (Harmon 1994). However, as underlined by Bertocchi et al. (2014) a marked decrease of fat and protein was observed in spring, when climatic conditions should not affect the productive performance of the cows. This variation was probably caused by the increase of the daylight in this period. In fact, the photoperiod positively affects milk production levels, with a subsequent dilution of its constituents (Bertocchi et al. 2014).

4 Conclusions

Milk produced in free-stall herds showed better chemical and physicochemical characteristics, as well as coagulation properties that were more suitable for processing Parmigiano-Reggiano cheese than milk collected in tie-stall herds. Moreover, the high level of SCC in tie-stall herd milk would affect negatively the milk yield, cheese yield and its evaluation in the milk quality payment system, leading to a general economic loss for the producers. On the other hand, the higher risk of blowing defects using free-stall milk may be due to the higher number of clostridial spores observed. Regardless of the housing system, we recommend adopting management strategies which are aimed at reducing the negative influence of the adverse climatic conditions which occur in the Po Valley in summer.

Acknowledgments The authors would like to thank Prof. Primo Mariani for his important contributions in the writing of this paper.

References

Aleandri R, Schneider JC, Buttazzoni L (1989) Evaluation of milk for cheese production based on milk characteristics and Formagraph measures. J Dairy Sci 72:1967–1975

Annibaldi S (1969) Modificazione della prova di Weinzirl per la ricerca dei clostridi nel latte. Sci Tecn Latt-Cas 20:75–79

Annibaldi S, Ferri G, Mora R (1977) New trends for milk technical evaluation: lactodynamographic standardisation. Sci Tecn Latt-Cas 28:15–126

Anonymous (1963) Titratable acidity evaluation with the Soxhlet-Henkel (SH) method. Milchwissenschaft 18: 520

Barbano DM, Rasmussen RR, Lynch JM (1991) Influence of milk somatic cell count and milk age on cheese yield. J Dairy Sci 74:369–388

Bassi D, Cappa F, Cocconcelli PS (2009) A combination of a SEM technique and X-ray microanalysis for studying the spore germination process of *Clostridium tyrobutyricum*. Res Microbiol 160:322–329

Bassi D, Fontana C, Zucchelli S, Gazzola S, Cocconcelli PS (2013) TaqMan real time-quantitative PCR targeting the phosphotransacetylase gene for *Clostridium tyrobutyricum* quantification in animal feed, faeces, milk and cheese. Int Dairy J 33:75–82

Beresford TP, Fitzsimons NA, Brennan NL, Cogan TM (2011) Recent advances in cheese microbiology. Int Dairy J 11:59–274

Bertocchi L, Vitali A, Lacetera N, Varisco G, Bernabucci U (2014) Seasonal variations in the composition of Holstein cow's milk and temperature-humidity index relationship. Animal 8:667–674

Biggs DA (1978) Instrumental infrared estimation of fat, protein and lactose in milk: collaborative study. J Assoc Off Anal Chem 61:1015–1034

Colombari G, Allegretti A, Melani D, Bettoni B, Pecorari M (2005) Growth of clostridial spores in soil, food, faeces, and milk of dairy farm with different levels of technology in production area of Parmigiano-Reggiano. Sci Tecn Latt-Cas 56:309–344

Coulon JB, Delacroix-Buchet A, Martin B, Pirisi A (2004) Relationships between ruminant management and sensory characteristics of cheeses: a review. Lait 84:221–242

Fleminger G, Ragones H, Merin U, Silanikove N, Leitner G (2013) Low molecular mass peptides generated by hydrolysis of casein impair rennet coagulation of milk. Int Dairy J 30:74–78

Formaggioni P, Sandri S, Franceschi P, Malacarne M, Mariani P (2005) Milk acidity, curd firming time, curd firmness and protein and fat losses in the Parmigiano-Reggiano cheesemaking. It J Anim Sci 4(2):239–241

Franceschi P, Summer A, Sandri S, Formaggioni P, Malacarne M, Mariani P (2009) Effects of the full cream milk somatic cell content on the characteristics of vat milk in the manufacture of Parmigiano-Reggiano cheese. Vet Res Commun 33(Suppl 1):281–283

Gatti M, Bottari B, Lazzi C, Neviani E, Mucchetti G (2014) Invited review: microbial evolution in raw-milk, long ripened cheeses produced using undefined natural whey starters. J Dairy Sci 97:573–591
Grappin R, Dasen A, Favenec P (1985) Rapid automatic counting of bacteria in raw milk using the BactoScan. Lait 65:123–147
Harmon RJ (1994) Physiology of mastitis and factors affecting somatic cell counts. J Dairy Sci 77:2103–2112
Hovinen M, Rasmussen MD, Pyörälä S (2008) Udder health of cows changing from tie stalls to free stalls with conventional milking to free stalls either with conventional or automatic milking. J Dairy Sci 92:3696–3703
IDF (1998) Milk and milk products, Enumeration of coliforms. International Dairy Federation Standard 73B. Brussels, Belgium: International Dairy Federation
IDF (2008) Milk and milk products, Guidance on sampling. International Dairy Federation Standard 50/ISO707. Brussels, Belgium: International Dairy Federation.
Klei L, Yun J, Sapru A, Lynch J, Barbano DM, Sears P, Galton D (1998) Effects of milk somatic cell count on cottage cheese yield and quality. J Dairy Sci 81:1205–1213
Langó Z, Heinonen-Tanski H (1995) Occurrence of *Clostridium tyrobutyricum* in cattle slurry and fresh forage grasses. Biores Technol 53:189–191
Le Maréchal C, Thiéry R, Vautor E, Le Loir Y (2011) Mastitis impact on technological properties of milk and quality of milk products—a review. Dairy Sci Technol 91:247–282
Malacarne M, Fossa E, Sandri S, Tosi F, Summer A, Mariani P (2003) Seasonal variation of milk quality in Parmigiano-Reggiano cheese manufacture on a period of 10 years. EAAP Tech Ser 7:100
Malacarne M, Summer A, Formaggioni P, Franceschi P, Sandri S, Pecorari M, Vecchia P, Mariani P (2008) Dairy maturation of milk used in the manufacture of Parmigiano-Reggiano cheese: effects on physico-chemical characteristics, rennet-coagulation aptitude and rheological properties. J Dairy Res 75:218–224
Malacarne M, Summer A, Franceschi P, Formaggioni P, Pecorari M, Panari G, Vecchia P, Sandri S, Fossa E, Scotti C, Mariani P (2013) Effects of storage conditions on physico-chemical characteristics, salt equilibria, processing properties and microbial development of raw milk. Int Dairy J 29:36–41
Malacarne M, Franceschi P, Formaggioni P, Sandri S, Mariani P, Summer A (2014) Influence of micellar calcium and phosphorus on rennet coagulation properties of cows milk. J Dairy Res 81:129–136
Mariani P, Summer A, Formaggioni P, Malacarne M, Battistotti B (2001) Remarks about the main dairy-technological requisites of milk for grana cheese production. Sci Tecn Latt-Cas 52:49–91
McMahon DJ, Brown RJ (1982) Evaluation of Formagraph for comparing rennet solutions. J Dairy Sci 65:1639–1642
Norell RJ, Appleman RD (1981) Change of milk production with housing system and herd expansion. J Dairy Sci 64:1749–1755
Pecorari M (1984) Practical application of payment of milk quality in Parma province. Sci Tecn Latt-Cas 35:231–244
Pecorari M, Mariani P (1990) Casein, milk rennet ability, cheese yield and quality. Sci Tecn Latt-Cas 41:225–244
Pecorari M, Panari G, Guidetti R, Mora R, Summer A (2001) The influence of "unifeed" feeding technique on milk characteristics, cheesemaking technology, quality and typicity of Parmigiano-Reggiano cheese. Sci Tecn Latt-Cas 52:311–323
Politis I, Ng-Kwai-Hang KF (1988) Association between somatic cell count of milk and cheese-yielding capacity. J Dairy Sci 71:1720–1727
Pretto D, De Marchi M, Penasa M, Cassandro M (2013) Effect of milk composition and coagulation traits on Grana Padano cheese yield under field conditions. J Dairy Res 80:1–5
Sandri S, Tosi F, Mariani MS, Vecchia P, Malacarne M, Summer A (2001) Observations on the trends of the main dairy-characteristics of Parmigiano-Reggiano cheese milk during the years 1990-2001. Ann Fac Med Vet Parma 21:235–247
Schmidt-Madsen P (1975) Fluoro-opto-electronic cell-counting on milk. J Dairy Res 42:227–239
Smith, JW, Guthrie LD, Ely LO (1995) Effect of herd size on management variables in Holstein herds. J Dairy Sci 98 (Suppl. 1):318 (Abstr.)
Tedeschi G, Malacarne M, Tosi F, Sandri S (2010) Osservazioni sull'andamento delle principali caratteristiche casearie del latte per Parmigiano-Reggiano dal 2004 al 2008. Sci Tecn Latt-Cas 61:29–45
Verdier-Metz I, Coulon JB, Pradel P (2001) Relationship between milk fat and protein contents and cheese yield. Anim Res 50:365–371
Vianna PCB, Mazal G, Santos MV, Bolini HMA, Gigante ML (2008) Microbial and sensory changes throughout the ripening of Prato cheese made from milk with different levels of somatic cells. J Dairy Sci 91:1743–1750