



Chemical composition, hygiene characteristics, and coagulation aptitude of milk for Parmigiano Reggiano cheese from herds yielding different milk levels

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ABSTRACT - The objective with this research was to compare milk quality parameters among herds characterized by different levels of milk production. The research involved 1080 bulk milk samples collected from 30 dairy herds, which produce milk for Parmigiano Reggiano cheese, during three years. Each milk sample was analysed for fat, crude protein, titratable acidity, total bacterial count, somatic cells, coliform bacteria, and *Clostridia* spores. Least mean values were obtained by ANOVA using milk production intervals from 6000 to 7999 (L-group), from 8000 to 9999 (M-group), and from 10000 to 12000 kg/cow/lactation (H-group) as fixed milk production classes. Increased milk production was associated with a reduction in milk fat content (from 3.54 in the L-group milk to 3.29 g/100 g in the H-group milk). An improvement in the hygienic-sanitary quality of milk was observed with increasing milk production. In fact, a progressive decrease in the total bacterial count (from 95 in the L-group milk to 45 10³ cfu/mL in the H-group milk), number of coliforms (from 2294 in the L-group milk to 1342 cfu/mL in the H-group milk), and somatic cell number (from 382 in the L-group milk to 209 10³ cells/mL in the H-group milk) was observed with the increase in milk production level. Finally, it appears that higher milk yield is connected with significant positive effects on the lactodynamometric properties of milk.

Keywords: dairy cow, dairy product, milk composition, milk fat, milk protein

1. Introduction

Parmigiano Reggiano is a Protected Designation of Origin cheese produced following the method approved by the Safeguarding Consortium. The approved method [Council Regulation (EC) No 510/2006] specifies the raw materials admitted for ration formulation and their *maximum* level in the diet. However, it gives only a few indications about feeding practices and administration systems. The approved method also specifies that the interval between the start of the milking procedure and the milk delivery at the cheese factory must be shorter than 7 h and that during this period, the milk can be cooled only beyond 18 °C. Nevertheless, no recommendation is reported with regard to the milking procedure (i.e. pre- and post-dipping), the milking equipment systems, or the milking cooling system. These general indications leave farmers a broad range of choices with regard to the technology and practices to be implemented on farms.

Various studies demonstrate that the technological investment and management effectiveness of dairy enterprises, such as the procedures adopted in herd care and management, are strongly affected by the productivity and profitability of the herd itself. These variables exert strong effects on milk yield, chemical composition, and hygiene parameters, which have repercussions on milk cheese yield. In particular, Sadeghi-Sefidmazgi and Rayatdoost-Baghal (2014) demonstrated an effect of bedding,

milking hygiene, housing, and cooling systems on the somatic cell count, which in turn affects vat milk quality (Franceschi et al., 2009) and cheese yield (Summer et al., 2015). Simensen et al. (2010) showed that there is an interaction between housing system and herd size and that health and performance are not necessarily better in small free stalls than in tie stalls. However, Summer et al. (2014) showed that milk produced by free-stall herds resulted in lower somatic cell counts and total bacterial counts than milk produced in tie stalls. Furthermore, Franceschi et al. (2012) demonstrated an effect of farm location (e.g. farms in the hills vs farms in the plains) on milk yield and quality.

In the production of raw milk cheeses, the chemical composition of the milk and its microbial characteristics may have significant effects on cheese yield (Le Maréchal et al., 2011; Summer et al., 2015) and cheese quality (Vianna et al., 2008). In the manufacture of Parmigiano Reggiano, cheese yield is directly correlated with milk fat and protein content (Formaggioni et al., 2015) and is also affected by somatic cell content (Le Maréchal et al., 2011; Summer et al., 2015). Moreover, a high total bacterial count and/or the presence of coliforms and *Clostridia* spores may cause structural, colour, and sensory defects in cheese (Beresford et al., 2001).

Our hypothesis was that milk yield levels of the farm can be related to milk compositional and hygienic quality. For this reason, the objective of this study was to compare the chemical composition, physicochemical properties, coliform and *Clostridia* spore counts, and rennet coagulation aptitude of the milk used for manufacturing Parmigiano Reggiano cheese produced on farms with herds characterized by different production milk levels.

2. Material and Methods

The trial involved 1080 bulk milk samples collected during a period of three years from herds located in the Reggio Emilia province (north of Italy) within the Parmigiano Reggiano production area and producing milk for Parmigiano Reggiano cheese. The milk samples were collected directly from the bulk tank at the end of the morning milking. The herds involved in this research were comprised only of Italian Friesian cattle and were all of free-stall type. The management conditions were compatible with the regulation of production for milk intended for Parmigiano Reggiano cheesemaking. Cows were milked twice a day and fed according to the regulations imposed by the Consortium of Parmigiano Reggiano cheese. Herds were divided into three classes (10 herds per class) according to their production level (kg/cow/lactation), from 6000 to 7999 (L), from 8000 to 9999 (M), and from 10000 to 12000 (H), and each herd was sampled monthly. Samples were cooled at 5 °C and immediately transported to the laboratory and subjected to analysis.

The following parameters were determined for each milk sample according to Franceschi et al. (2012): fat and crude protein by infrared analysis, using Milko-Scan (Foss Electric, DK-3400 Hillerød Denmark); titratable acidity titrating 50 mL of milk with 0.25 N sodium hydroxide according to the Soxhlet-Henkel method; total bacterial count by the flow cytometry method with BactoScan FC (Foss Electric, DK-3400 Hillerød Denmark); somatic cell count by the fluoro-opto-electronic method with Fossomatic (Foss Electric, DK-3400 Hillerød Denmark); and coliform bacterial count on Petri dishes with VRBA-agar ground after incubation at 37 °C for 24 h (Standard no. 170; FIL-IDF, 1994). Additionally, in each sample, the number of *Clostridia* spores was determined by MPN (most probable number) with the Weinzirl method modified by Annibaldi (1969). Rennet coagulation properties were determined according to Malacarne et al. (2014), using Formagraph (Foss Electric, DK-3400 Hillerød Denmark). To perform this analysis, 0.2 mL of calf rennet (rennet solution 1:19000; Chr. Hansen, I-20094 Corsico MI, Italy) was added to 10 mL of milk.

Based on the milk clotting time and curd firming time, we then divided the milk samples into 13 lactodynamographic (LDG) types identified with capital letters, 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 Summer et al. (2014): optimal (LDG types A, B, C), suboptimal (EA, EB, EC, D, EF, DD), and poor (E, FE, F, FF). Each class was constituted including milk LDG types with the same technological behaviour during Parmigiano Reggiano cheesemaking.

We grouped the contents of *Clostridia* spores and coliform bacteria into three classes according to Summer et al. (2014): up to 30 spores/L; from 31 to 100 spores/L; more than 100 spores/L and up to 1000 colony forming units (cfu)/mL; from 1001 to 5000 cfu/mL; and over 5000 cfu/mL.

Moreover, rolling geometrical average (RGA) values of somatic cell count and total bacterial count were calculated in compliance with the 853/2004 CEE Regulation. Each monthly value of somatic cell count was averaged (by geometric mean) with those recorded in the previous two months. Concerning the total bacterial count, 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, it was not possible to calculate the RGA values of somatic cells for milk samples collected in January and February of the first year (340 samples) or the total bacterial count for the milk samples collected in January of the first years (350 samples). The RGA values were divided into two classes for both parameters ($\leq 400,000$ or $> 400,000$ cells/mL for somatic cells and $\leq 100,000$ or $> 100,000$ cfu/mL for total bacterial count) in compliance with the legal limits introduced in the 853/2004 CEE regulation.

Furthermore, milk yield per lactation of a single cow was extracted from the dataset of the Italian Breeders Association, and the mean milk production (kg/cow/lactation) for each herd was calculated. Finally, we calculated the logarithm from the somatic cell count, total bacterial count, coliform bacterial count, and *Clostridia* spore number for statistical analysis.

Based on milk yield, as previously mentioned, herds were grouped into three classes according to the milk yield reported by the Italian Breed Association: from 6000 to 7999 (L-group), from 8000 to 9999 (M-group), and from 10000 to 12000 kg/cow/lactation (H-group). We analysed the statistical significance of the difference between mean values of milk parameters by analysis of variance using a general linear model (statistical package IBM SPSS Statics 24, Armonk, New York 10504-1722, USA) after control of variance homogeneity (Levene test). In the model, the effect of the classes of milk produced and the effect of the season were tested as fixed factors, and the effect of the herd was tested as a random effect on the classes of milk produced, according to the following hierarchic model:

$$y_{ijkl} = \mu + P_i + H_{ij} + S_k + \varepsilon_{ijkl}$$

in which y_{ijkl} = dependent variable; μ = overall mean; P_i = classes of milk produced (three levels), $i = 1, \dots, 3$; H_{ij} = herd, $j = 1, \dots, 10$; S_k = season (four levels: winter, from January to March; spring, from April to June; summer, from July to September; autumn, from October to December), $k = 1, \dots, 4$; and ε_{ijkl} = residual error. The significance of the differences was tested with the Bonferroni test. Moreover, the differences between the classes of LDG type, the number of *Clostridia* spores, coliform bacterial content, somatic cells, and total bacterial count were tested by the chi-square method.

3. Results

The cow milk yield (kg of milk per cow per lactation) was on average 7147.90 kg in the L-group, 8901.70 kg in the M-group, and 10798.40 kg in the H-group, and the number of cows raised was on average 40 in the L-group, 50 in the M-group, and 89 in the H-group.

All the parameters reported, except for *Clostridia* spores, showed a significant difference between the L-, M-, and H- groups; fat and protein contents, somatic cells, and total bacterial and coliform bacterial counts with $P \leq 0.001$ and titratable acidity with $P \leq 0.01$. In particular, the milk fat content showed higher values in the L-group milk (3.54 g/100 g) and lower values in the H-group milk (3.29 g/100 g) with intermediate values in the M-group milk (3.37 g/100 g). On the other hand, the milk protein contents showed lower values in the L-group milk (3.26 g/100 g) and higher values in the M and H-group (3.41 and 3.38 g/100 g, respectively).

Total bacterial and coliform bacterial counts were highest in the L-group, lowest in the H-group, and intermediate in the M-group (95, 45 and 53×10^3 cfu/mL and 2294, 1342 and 1664 cfu/mL, respectively) (Table 1). In the L-group milk, the RGA value calculated in two months exceeded the limit of 100,000 cfu/mL more frequently in comparison to the M-group and H-group milks (30.3 vs 6.0 vs

3.4% for the L-, M-, and H-groups, respectively; $P \leq 0.001$). Moreover, the L-group milk showed a higher number of samples with over 5000 coliform bacteria per mL with respect to the M-group and H-group milks (8.1 vs 3.6 vs 2.8% for the L-, M-, and H-groups, respectively; $P \leq 0.01$).

The somatic cell count was higher in the L-group milk (382×10^3 cells/mL) and lower in the H-group milk (209×10^3 cells/mL) with intermediate values in the milk produced in the M-group (253×10^3 cells/mL), which was a similar trend to that shown for the total bacterial count; the L-group milk exceeded the limit of 400,000 cells/mL more frequently than the M- and H-group milks (38.2 vs 10.3 vs 1.5%; $P \leq 0.001$).

In general, the optimal LDG type classes (A, B, C) were the most represented in all the milk yield groups (Table 2). However, the M- and H- groups were characterized by a higher rate of samples with the optimal LDG-type classes (61.9 and 58.9%, respectively) and a lower rate of poor (E, FE, F, FF) LDG-type classes (10.3 and 15.0%, respectively) compared with the L-group milk (showing 52.8 and 20.8% of the optimal and poor LDG types, respectively).

Table 1 - Chemical composition, physico-chemical properties, and microbiological characteristics of the milk produced in herds with different production levels

		L-group ¹ n = 360	M-group ¹ n = 360	H-group ¹ n = 360	SE	P ²
Fat	g/100 g	3.54b	3.37b	3.29a	0.01	***
Protein	g/100 g	3.26a	3.41b	3.38b	0.01	***
Titrate acidity	°SH/50 mL	3.21a	3.26c	3.23b	0.01	**
Somatic cell count	10^3 cells/mL	382c	253b	209a	7	***
Total bacterial count	10^3 cfu/mL	95c	53b	45a	4	***
Coliform bacteria	cfu/mL	2294c	1664b	1342a	118	***
<i>Clostridia</i> spores	spores/L	71	71	63	3	NS

n - number of samples collected; SE - standard error; NS - not significant.

¹ Cow milk production classes: L-group, 6000-7999; M-group, 8000-9999; and H-group 10000-12000 kg/cow/lactation.

² Significance of differences: a, b, and c are different for $P \leq 0.05$; NS - $P > 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

Table 2 - Results of chi-square test for somatic cell (SCC), total bacterial count (TBC), coliforms, *Clostridia* spores, and lactodynamographic (LDG) classes of bulk tank milk samples collected from herds with different production levels

	Class	L-group ¹ n ² = 360		M-group ¹ n ² = 360		H-group ¹ n ² = 360		P ³
		n	%	n	%	n	%	
SCC ⁴	$>400 \times 10^3$ cells/mL	130	38.2c	35	10.3b	5	1.5a	***
TBC ⁵	$>100 \times 10^3$ cfu/mL	106	30.3c	21	6.0b	12	3.4a	***
Coliforms	Up to 1,000 cfu/mL	243	67.5a	287	79.7b	329	91.4c	***
	1,001-5,000 cfu/mL	88	24.4c	60	16.7b	21	5.8a	**
	Over 5,000 cfu/L	29	8.1b	13	3.6a	10	2.8a	**
<i>Clostridia</i> spores	Up to 30 spores/L	59	16.4	51	14.2	57	16.9	NS
	31-100 spores/L	245	68.1	246	68.3	261	72.5	NS
	Over 100 spores/L	56	15.6	63	17.5	38	10.6	NS
LDG	Optimal	190	52.8a	223	61.9b	212	58.9b	**
	Suboptimal	95	26.4	100	27.8	94	26.1	NS
	Poor	75	20.8b	37	10.3a	54	15.0a	**

NS - not significant.

¹ Cow milk production classes: L-group, 6000-7999; M-group, 8000-9999; and H-group 10000-12000 kg/cow/lactation.

² Number of samples collected (for SCC = 340 and for TBC = 350).

³ Significance of differences: a, b, and c are different for $P \leq 0.05$; NS, $P > 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

⁴ SCC = Rolling geometric average values calculated in three months.

⁵ TBC = Rolling geometric average values calculated in two months.

4. Discussion

The average fat content of all milk groups can be considered generally low compared with that reported by Summer et al. (2014), who found an average fat content for milk produced by free-stall herds of 3.82 g/100 g in a study conducted on 504 bulk milk samples collected from 14 free-stall herds producing milk for Parmigiano Reggiano cheese. However, in another study conducted by Franceschi et al. (2012) on 3532 samples, the authors reported an average fat value of 3.47 g/100 g for milk produced in the plain zone of the Parmigiano Reggiano cheese area. This variability in milk fat content in the different studies may be related to differences in feeding strategies. In fact, in most herds with more than 50 cows, especially when housed in free stalls, animals receive a total mixed ration (TMR) diet, while forages and concentrates are distributed separately in smaller herds (traditional feeding). If properly formulated, TMR ensures a better utilization of nutrients, generally allowing for a better exploitation of the genetic potential of dairy cattle for milk quality (Comino et al., 2015). In the present study, the differences in the milk fat content among the groups can be explained by a dilution effect. In particular, according to Righi et al. (2007) and Comino et al. (2015), the low levels of fat observed in the H-group milk could be related to the administration of highly concentrated (i.e. high starch and NSC content), low fibre diets, typical of highly productive, large, and modern farms where the milk fat depression phenomenon is frequently observed.

Differently from the milk fat content, the difference between the average values of milk protein was small and was probably mainly a result of the more intensive genetic selection generally conducted on the larger, modern, and progressive farms.

The titratable acidity did not show a clear trend and was higher in milk produced by the M-group, lower in milk produced in the L-group, and intermediate in milk produced by the H-group. However, it must be noted that although significant, the difference between the average values was very small (0.05 °SH/50 mL), too small to affect the milk transformation process into Parmigiano Reggiano cheese; moreover, all average values were consistent with those reported by Summer et al. (2014) and Franceschi et al. (2012) for milk produced by free-stall herds and in plain herds (3.19 and 3.24 °SH/50 mL, respectively).

The milk produced in the H-group was less contaminated with respect to the milk produced in the other two groups, particularly when compared with the milk produced in the L-group. This finding is probably related to higher hygiene standards in the most productive farms. The average total bacterial count of the M-group and H-group milks was consistent with that reported by Franceschi et al. (2012), for milk produced in the plain zone of Reggio Emilia Province (66833 cfu/mL), and by Summer et al. (2014), for milk produced in the free stall (61135 cfu/mL). On the other hand, the total bacterial count of milk produced by the L-group was considered high, and this observation was confirmed by the number of times the RGA value calculated at two months exceeded the limit of 100,000 cfu/mL for the L-group (30.3%) compared with the other groups. The same observation was shown for the coliform bacterial count, although the average value for the milk from all groups must not be considered high, and the result was in agreement with those verified by other authors, i.e. Summer et al. (2014), who reported an average value of 2041 cfu/mL in milk produced by free-stall herds of Reggio Emilia Province, or by Franceschi et al. (2012), who reported an average value of 1334 cfu/mL in milk produced by herds located in the Reggio Emilia plain zone. The L-group showed a higher percentage of samples with more than 5,000 cfu/mL compared with that shown by Summer et al. (2014), who reported a value of 3.97%.

The somatic cell content of milk produced by the M- and H-groups was lower than that reported by Franceschi et al. (2012), who showed an average value of somatic cells of milk produced in the plain zone of 309660 cells/mL, which was higher than that reported by Summer et al. (2014) for milk produced by free-stall herds (158891 cells/mL). On the other hand, milk produced by the L-group showed high values of somatic cells. This observation was confirmed by the number of times the RGA value calculated at three months exceeded the limit of 400,000 cells/mL for the L-group (38.2%) compared with the other groups.

The trend for a higher somatic cell count in the L-group was in contrast with reports from Sadeghi-Sefidmazgi and Rayatdoost-Baghal (2014), who found a lower average milk somatic cell count in less productive herds (<10000 kg/305 days lactation). This difference is probably related to the different environmental conditions of the two studies and can be explained by a higher standard of milking procedures in dairy enterprises that focused attention on the productivity aspects of the herds. In particular, in Italy, larger farms are generally equipped with modern milking parlours composed of devices that help keep the udders healthy, such as online records of milk yield, milk flow, and milk electric conductivity, as well as automatic cluster removal. Furthermore, milking procedures are usually carried out by a 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 best practices.

The differences among the coagulation properties of milk are difficult to explain since the milk coagulation properties are influenced by many factors, i.e. milk protein and casein contents (Summer et al., 2007), milk mineral content and salt equilibria (Malacarne et al., 2014), or milk hygiene properties (Franceschi et al., 2009).

However, we must observe that the number of samples with optimal coagulation properties is always approximately two times higher in all classes compared with the number of samples with suboptimal properties. This is very important because better coagulation properties correspond to a lower quantity of crude protein and curd that remain in the cooked whey, decreasing cheesemaking losses and, consequently, increasing cheesemaking efficiency, as demonstrated by Franceschi et al. (2019). In this regard, the milk produced by the M- and H- groups is characterized by a higher frequency of samples in the optimal LDG classes and a lower frequency of samples in the poor LDG classes with respect to the milk produced by the L-group. This difference has to be related to the lower somatic cell content of milk from the M- and H- groups. The increase in the number of somatic cells (mainly macrophages, leucocytes, and polymorphic nucleated) in milk is related to the mammary gland inflammation process (Mazal et al., 2007). The inflammatory response is characterized by a transfer of some blood components to the milk (Urech et al., 1999) and by a decrease in secretory activity resulting in reduced milk production (Le Maréchal et al., 2011). Moreover, alterations are observed in the chemical composition of the milk secreted and its physicochemical properties, such as rennet coagulation properties (Franceschi et al., 2009; Summer et al., 2015). In particular, the high frequency of samples with more than 400,000 cells/mL recorded in the L-group milk can influence the high rate samples with poor coagulation properties. This observation is consistent with Malacarne et al. (2014) and Summer et al. (2009); the first research reported a connection between milk mineral content and salt equilibria and milk coagulation properties; and, in a study conducted on single quarter milk collected from homologous quarters with less than 400,000 and with over 400,000 cells/mL, the latter authors showed a worsening of the mineral contents and salt equilibria of milk produced by the quarter with the higher somatic cell content.

Finally, because preservative is strictly forbidden for the production of Parmigiano Reggiano cheese, the contamination of milk by *Clostridia* spores represents a great problem, because it can induce deep defects in cheese wheels that compromise cheese marketability. Because the contamination threshold of *Clostridia* spores for milk destined for Parmigiano Reggiano cheese production is 100 spores/L (Summer et al., 2014), the observed values for all groups can be considered in accordance with those reported in the literature. The values found are in fact in accordance with those reported by Franceschi et al. (2012) and Summer et al. (2014), who reported 94 and 81 spores/L, respectively. This confirms the trend of recent years that show an increase in spore number in milk produced in the Parmigiano Reggiano area (Summer et al., 2014). This observation is also confirmed by the fact that the frequency of samples with more than 100 *Clostridia* spores/L was higher in milk from all groups that ranged from 10.6 to 17.5%. The contamination of milk produced by all groups is consistent with those reported by both Franceschi et al. (2012) and Summer et al. (2014), who reported that the samples with more than 100 spores/L were 19.71 and 22.22%, respectively.

5. Conclusions

The herds characterized by a higher milk yield per cow per lactation show a decrease in milk fat content, which can negatively affect cheese yield. On the other hand, these herds demonstrate more favourable hygiene parameters (lower total bacterial count and lower coliform bacteria count) with a consequent lower risk of blowing defects in cheese when the milk from these herds is employed.

Finally, it appears that higher milk yield is connected to a good hygiene standard with good significant effects on the lactodynamometric properties of milk.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: P. Franceschi, M. Malacarne, F. Righi and A. Summer. Data curation: P. Franceschi, P. Formaggioni and F. Righi. Formal analysis: P. Franceschi and P. Formaggioni. Funding acquisition: M. Malacarne. Investigation: P. Franceschi, M. Malacarne and F. Righi. Methodology: P. Franceschi. Project administration: M. Malacarne. Software: P. Franceschi, M. Malacarne and P. Formaggioni. Supervision: A. Summer. Validation: P. Franceschi, P. Formaggioni and F. Righi. Visualization: P. Franceschi. Writing-original draft: P. Franceschi, M. Malacarne and F. Righi. Writing-review & editing: P. Franceschi, P. Formaggioni, F. Righi and A. Summer.

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