

# Microbiological monitoring of raw milk from selected farm in the Trentingrana region

Elena Franciosi<sup>1</sup>, Angelo Pecile<sup>2</sup>, Agostino Cavazza<sup>1</sup>,  
Elisa Poznanski<sup>1</sup>

<sup>1</sup>Fondazione Edmund Mach, Area Qualità Alimentare e Nutrizione,  
Istituto Agrario San Michele all'Adige, Italy

<sup>2</sup>Fondazione Edmund Mach, Area Consulenza e Servizi per le Imprese,  
Istituto Agrario San Michele all'Adige, Italy

*Corresponding author:* Elena Franciosi. Fondazione Edmund Mach, Area Qualità Alimentare e Nutrizione, Istituto Agrario San Michele all'Adige. Via E. Mach 1, 38010 San Michele all'Adige (TN), Italy - Tel. +39 0461 615118 - Email: elena.franciosi@iasma.it

**ABSTRACT** – A study was conducted to monitor raw milk hygiene, and to understand the relationship between different bacterial groups found in raw milk collected from stables in the Trentingrana area. Twenty stables in Trento province were sampled. Each farmer provided one milk sample every 2 months for 8 months. The milk samples were examined for total bacterial count, coliforms, enterococci, psychrotrophic, acidificand and proteolytic microflora. The bacterial counts of four milk samples for each stable, examined over an 8-months period were averaged and expressed as mean bacterial count per milliliter. In most samples, coliforms and enterococci were below 2Log cfu/ml; this suggests that herds and milking hygiene were well controlled by all farmers. The milk refrigeration practice seems to have no influence on the growth of its microflora but to affect that of acidifying and proteolytic bacteria.

*Key words:* Raw cows milk, Trentingrana, Bacteria counts, Refrigerated milk.

**Introduction** – Trentingrana cheese is a hard cooked cheese that undergo a ripening period of almost 2 years, similarly to Grana Padano cheese. It is produced in the Trentino region (Alpine area located in the North part of Italy) from partially skimmed raw cow's milk. Commercial rennet is used, and the whey daily produced at the dairy factory, is added. No starter cultures are added. A milk control program has been implemented to monitor dairy and milk producers. Since the early 1990s, bulk tank milk has been used to diagnose current and potential problems that might exist in a dairy herd related to milk quality (Farnsworth, 1993; Jayarao, *et al.* 2001). Milk refrigeration at stable is actually used in attempts to avoid microbial proliferation during the overnight milk stay between the milking and cheesemaking steps, resulting in better quality cheese-products. Several guidelines have been proposed to interpret milk bacterial counts (Britt *et al.*, 1997; Murphy, 1997). However, many of the guidelines provide little insight into the interrelationship between different groups of bacteria found in milk. This study, conducted in Trentino province from July 2008 through February 2009, was focused on microbial milk analysis to understand the quality of milk to be used in Trentingrana production and the relationship between the presence in milk of different bacterial groups and farming practice at the stable.

**Material and methods** –Twenty stables (Table 1, stables from 1 to 20) holding Italian Brown and Friesian milking cows were selected among those producing milk for Trentingrana. Ten farmers have a “traditional” approach in their stable (Table 1, stables from 1 to 10) they use no refrigeration after milking, feed cow twice a day and collect milk in 50l steel tanks. The others ten (Table 1, stables from 11 to 20) have a “modern” approach: they refrigerate milk at 18°C after milking before delivering it to cheese factories, they feed cows only once a day and collect milk in big steel tanks (each about 500l). In each farm, raw milk was sampled four times throughout a 8 month period (July 2008 – February 2009). Milk samples were removed from the

bulk tank after mixing and stored in liquid nitrogen until arriving in the laboratory, where they were stored at -80°C until microbiological analysis. Milk samples were analyzed in a three-serial of 10-fold dilutions in peptone water, (1 g/l mycological peptone pH 7.0) and each dilution was plated. All counts were the weighted means of results obtained in the four different time sampling. Violet red bile lactose agar (VRBA, Oxoid, UK) was used in coliform counts. Incubation time and temperature were 24 h and 37°C. Enterococci colonies were counted after 24 h at 37°C in Kanamycin Aesculine Agar (Oxoid). Psychrotrophic bacteria were counted on Plate Count Agar (Oxoid) containing 1g/l skim milk powder and incubated at 7°C for 7 days. The acidifying bacteria and the ability to produce extracellular proteolytic enzymes were determined on PCA, supplemented with 1% skim milk powder (Harper *et al.*, 1978) and 0,17 g/l bromcresol purple. The plates were incubated 48h at 30°C: the presence of clear or yellow zones around the colonies were indicative of proteolysis or acidification respectively. Total bacterial counts (TBC) were determined using plate count agar (PCA; Oxoid) with 1g/l skim milk powder and incubated at 30°C for 48 hours. Analyses of variance (ANOVA) were conducted for the viable numbers of the various microbial groups; preliminary logarithmic transformation of the data was required to guarantee that they were independent and normally distributed. The package Statistica 8 was used for statistical analysis.

**Results and conclusions** – The microbial flora counts are given in Table 1.

Table 1. Microflora of raw milk samples. Results of counts (Log cfu/ml) on different media of milk sampled at the stable are reported as mean value (M) ± the standard deviation (SD) of four days of milking over 8 months. Stables are numbered from 1 to 20. In the two lines named TOT are showed M and SD of counts of milk samples at not-refrigerated stables and refrigerated ones.

Stable	Coliforms	Enterococcaceae	TBC	Psychrotrophic	Acidificants	Proteolytics	
	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD	
Not refrigerated milk	1	0.39±0.45	1.13±0.98	3.71±0.35	3.08±0.67	2.50±1.78	2.05±1.50
	2	1.42±1.00	0.19±0.33	3.41±0.27	2.95±0.32	2.13±0.49	1.28±0.85
	3	1.49±0.70	2.54±0.85	5.16±0.19	4.93±0.32	3.69±0.99	3.96±0.50
	4	1.92±0.21	2.72±0.25	4.69±0.30	4.52±0.37	3.88±0.44	2.78±0.33
	5	0.39±0.27	2.06±0.32	3.41±0.26	1.19±1.40	2.22±0.07	1.39±0.96
	6	1.49±0.44	2.38±0.62	3.79±0.83	2.58±1.91	1.77±1.66	2.90±0.74
	7	1.65±0.50	1.46±0.38	5.46±0.64	4.65±0.83	4.71±1.49	3.40±0.35
	8	1.94±1.19	1.73±0.57	4.51±0.91	3.91±0.90	3.42±0.83	2.75±0.86
	9	1.77±0.52	2.87±1.09	4.23±0.45	3.84±0.42	3.61±0.75	2.74±0.23
	10	1.46±0.79	1.33±1.19	3.40±0.52	1.65±1.11	2.21±0.37	0.58±1.15
TOT	1.39±0.56	1.84±0.84	4.18±0.76	3.33±1.27	3.01±0.97	2.38±1.04	
18°C refrigerated milk	11	0.80±0.71	1.62±1.40	3.34±0.27	2.67±0.38	0.57±0.98	2.52±0.15
	12	1.61±0.61	3.14±0.60	3.97±0.14	3.67±0.14	1.69±1.51	2.35±1.61
	13	1.86±0.97	2.23±0.21	3.82±0.21	3.19±0.56	2.62±0.26	2.90±0.28
	14	1.66±0.42	3.15±0.81	4.58±0.81	3.93±0.84	3.23±2.52	3.45±1.33
	15	1.04±0.91	1.67±1.46	3.75±0.33	3.09±0.74	2.18±0.44	1.71±1.71
	16	2.51±0.44	0.73±1.26	4.93±0.48	4.92±0.41	3.73±0.57	3.39±0.87
	17	0.00±0.00	1.13±0.98	3.76±0.45	3.40±0.70	2.03±0.58	2.29±1.65
	18	1.39±0.06	2.19±0.43	4.20±0.76	2.35±1.91	1.49±1.29	2.41±0.37
	19	1.44 ± 0.74	1.86±1.61	3.61±0.21	3.43±0.25	2.42±0.39	2.35±0.55
	20	2.20±0.64	2.12±0.10	4.24±1.05	4.36±1.25	1.67±1.53	3.74±1.04
TOT	1.45±0.72	1.98±0.77	4.02±0.48	3.50±0.76	2.16±0.90	2.71±0,64	

Of the 20 stables examined, 17 had TBC below 4.7 Log cfu/ml. They varied from 3.4 to 5.5 Log cfu/ml and there was no significant correlation between TBC and milk refrigeration. Enterococci average counts were usually higher than coliforms and showed no high variation between samples: enterococci ranged from 0.2 to 3.2 Log cfu/ml and coliform counts from 0 to 2.5 Log cfu/ml. With the exception of stables 16 and 20, all stables produced milk with coliforms below 2 Log cfu/ml. These data indicate that milk produced was of good microbiological quality: in most samples, coliforms and enterococci were low; this is in agreement with previous surveys in other parts of the world (WI. Rea *et al.*, 1992; Desmaures *et al.*, 1997) and suggests that herds and milking hygiene were well controlled in all stables with both modern and traditional approach to cow herding. Psychrotrophic bacteria were present in all samples and were the second most important microbial group of those studied. The average count of psychrotrophic bacteria was 3.4 Log cfu/ml but SD showed very high variation between samples. The counts varied from 1.2 to 4.9 Log cfu/ml and there was no significant correlation between psychrotrophic counts and milk refrigeration, so this means that milk refrigeration is well controlled. Acidifying and proteolytic bacteria were present in all milk samples with highly variable counts. Acidifying bacteria counts ranged from 0.6 to 4.7 Log cfu/ml and proteolytic from 0.6 to 4.0 Log cfu/ml. Both these groups bacteria were more influenced by the refrigeration practice of milk than TBC: the average counts of acidificant and proteolytics in milks collected from refrigerated tanks were 2.2 and 2.7 Log cfu/ml respectively; in non-refrigerated milks their average counts were 3.0 and 2.4 Log cfu/ml; thus the storage temperature seems to have an opposite effect on the two bacterial groups. The microbial composition of raw milk samples studied was highly variable, independently of the season of sampling (summer or winter). Raw milk TBC seems to be correlated with milk counts of microorganisms of faecal origin bacteria like *Enterococcaceae* and coliforms, however their counts were always low (below 3.2 Log cfu/ml). Milk refrigeration seems to have no influence on TBC, coliforms, *Enterococcaceae* and psychrotrophic bacteria. On the contrary, refrigeration seems to have some action on growth of acidifying and proteolytic bacteria.

*The Authors thank the "Consorzio Trentingrana" for technological support; Prof. Mauro Pecorari as consultant and Dott. Massimilano Mazzucchi for milk sampling.*

*Research supported by the Trento Province Government, project "Qualità Filiere Grana Trentino".*

**REFERENCES** – **Britt**, J. S., Hartmann, F., Reinemann, D., 1997. Use of microbiology and strategic site sampling at strategic times to solve high bacteria count problems in bulk tank milk: "An actual case history". pp 80–90 in Proc. 36<sup>th</sup> Natl. Mastitis Council Ann. Mtg., Madison, USA. **Farnsworth**, J. R., 1993. Microbiological examination of bulk tank milk. *Vet. Clin. N. Am. Food Anim. Pract.* 9:469–474. **Harper Jr.** C., Dizikes, J.L., Packard, V.S., 1978. Microbiological methods for butter, margarine and related products. In: Marth, E.H., Steele (Eds.), *Standard Methods for the Examination of Dairy Products*. APHA, Washington, DC, pp. 157–159. **Jayarao**, B. M., Pillai, S. R., Wolfgang, D. R., Griswold, D. R., Hutchinson, L. J., 2001. Herd level information and BTM analysis: Tools for improving milk quality and heard udder health. *Bovine Pract.* 35:23–35. **Murphy**, S. C., 1997. Raw milk bacteria tests: Standard plate count, preliminary incubation count, lab, pasteurization count and coliform count—What do they mean for your farm? pp 34–41 in Natl. Mastitis Council. Reg. Mtg., Syracuse, New York, USA. **Desmaures**, N., Bazin, F., Gueguen, M. 1997. Microbiological composition of raw milk from selected farms in the Camembert region of Normandy. *Journal of Applied Microbiology* 83:53–58. **Rea**, M.C., Cogan, T.M., Tobin, S. 1992. Incidence of pathogenic bacteria in raw milk in Ireland. *J. Appl. Bacteriol.* 73: 331–336.