Microbiological quality of milk from farms to milk powder manufacture: an industrial case study

Lizandra F. Paludetti[1,2], Alan L. Kelly[2], Bernadette O'Brien[1], Kieran Jordan[3], David Gleeson[1]*

[1]Teagasc Moorepark, Animal & Grassland Research and Innovation Centre, Fermoy, County Cork, Ireland

[2] School of Food and Nutritional Sciences, University College Cork, County Cork, Ireland

[3] Teagasc Moorepark, Food Research Centre, Fermoy, County Cork, Ireland

Short title: **Microbiological quality from farm to milk powder**

Correspondence: David Gleeson,
Teagasc Moorepark
Animal Grassland Research and Innovation Centre
Fermoy
County Cork
Ireland
Tel.: 00353 025 42269
Email address: David.Gleeson@teagasc.ie

Summary

The experiments reported in this research paper aimed to track the microbiological load of milk throughout low-heat skim milk powder (SMP) manufacturing process, from farm bulk tanks to final powder, during mid- and late-lactation (spring and winter, respectively). In the milk powder processing plant studied, low-heat SMP was produced using only the milk supplied by the farms involved in this study. Samples of milk were collected from farm bulk tanks (mid-lactation: 67 farms; late-lactation: 150 farms), collection tankers (CTs), whole milk silo (WMS), skim milk silo (SMS), cream silo (CS) and final SMP. During mid-lactation, the raw milk produced on-farm and transported by the CTs had better microbiological quality than the late-lactation raw milk (e.g., total bacterial count (TBC): 3.60 ± 0.55 and 4.37 ± 0.62 $\log_{10}$ cfu/ mL, respectively). After pasteurisation, reductions in TBC, psychrotrophic (PBC) and proteolytic (PROT) bacterial counts were of lower magnitude in late-lactation than in mid-lactation milk, while thermoduric (LPC – laboratory pasteurisation count) and thermophilic (THERM) bacterial counts were not reduced in both periods. The microbiological quality of the SMP produced was better when using mid-lactation than late-lactation milk (e.g., TBC: 2.36 ± 0.09 and 3.55 ± 0.13 cfu/ g, respectively), as mid-lactation raw milk had better quality than late-lactation milk. The bacterial counts of some CTs and of the WMS samples were higher than the upper confidence limit predicted using the bacterial counts measured in the farm milk samples, indicating that the transport conditions or cleaning protocols could have influenced the microbiological load. Therefore, during the different production seasons, appropriate cow management and hygiene practices (on-farm and within the factory) are necessary to control the numbers of different bacterial groups in milk, as those can influence the effectiveness of thermal treatments and consequently affect final product quality.

Keywords: milk microbiological quality, milk powder quality, lactation period, milk processing.

Bovine milk is used to produce a wide range of dairy products and nutritional ingredients. Each dairy product has to conform with specific quality parameters determined by regulatory authorities and international markets, which could be related to safety, nutritional value, physical and sensory characteristics. Bacterial numbers in milk are one of the main factors that can impact those parameters, and their control throughout processing is essential to achieve dairy products of high quality (Kable *et al*., 2016).

The first stage of the milk supply chain is the farm, where factors such as cow management, stage of lactation and equipment cleaning protocols can affect bacterial numbers in milk (O'Connell *et al*., 2015). A variety of microorganisms could grow in milk, including: mesophilic, psychrotrophic, lipolytic, proteolytic, thermoduric and thermophilic bacteria, as well as pathogenic bacteria. Huck *et al.* (2008) observed that some spore-forming bacteria (*Bacillus*, *Paenibacillus and Sporosarcina*) were identified throughout the processing stages of fluid milk production, from the farm to the packaged product, suggesting that multiple potential entry points for those bacteria into milk are at the farm. Therefore, the production of raw milk under appropriate hygienic conditions is critical to control bacterial numbers, as thermal treatments during dairy processing cannot always completely reduce the bacterial load.

Several studies have focused on quantifying and identifying bacterial types in raw milk on-farm and their effect on dairy products (Barbano *et al*., 2006; Quigley *et al*., 2013a; Murphy *et al*., 2016). However, the combined influence of farm practices, storage conditions, transport and processing conditions on the microbiological quality of final product is not well understood and further investigations are necessary. Kable *et al.* (2016) reported that the microbiota in collection tankers (CTs) can be highly diverse and differ according to season. This diversity may be attributed to contributing on-farm factors, such as cattle skin, bedding, feed, human handling, milking equipment, and on-site bulk tanks used for storage. Thus, each individual supplier could impact differently on the levels of different bacterial groups in the milk within CTs that collect milk from multiple farms.

When milk is collected from farm bulk tanks, it is still prone to further increases in bacterial populations, which can arise due to inappropriate equipment sanitation, favourable storage conditions or processing parameters for rapid bacterial multiplication (Teh *et al.*, 2011; Cherif-Antar *et al.*, 2016). Therefore, dairy processors have to adopt good manufacturing practices and monitor several critical control points throughout the manufacturing processes to guarantee food safety and conformity with legislation or specifications. For example, one of the challenges regarding equipment sanitation concerns heat-resistant spore-forming bacteria. These bacteria can develop cleaning-resistant biofilms on the interior surfaces of pipelines or equipment, enabling cross-contamination of finished products (Jindal *et al*., 2016). Processing parameters could also have an impact on bacterial load, especially thermal treatments. For example, the temperature programme and holding time during pasteurisation should be appropriate to reduce the microbial load and the number of viable pathogens in milk (Tucker, 2015).

The objective of this study was to monitor the microbiological quality of milk throughout the processing of low-heat skim milk powder (SMP), from individual farm bulk tanks to the final powder produced, during mid- and late-lactation periods. This study will aid in determining the association between the quality of milk and subsequent SMP produced, as well as the impact of processing parameters on milk and SMP quality. To our knowledge, this is the first such study that tracked milk quality from individual farms to final product.

Materials & Methods

Milk collection and skim milk powder manufacture

This study was conducted on commercial dairy farms and in a milk powder processing plant, which produced SMP only using the milk supplied by the farms involved in this study. This experiment was carried out during the mid- and late-lactation periods (May 2016 and December 2016, respectively), which corresponded to spring and winter in Ireland. During those periods, cows were grazing outdoors and housed indoors, respectively. The dairy farms involved in this study were located in the Kilkenny and Waterford regions of Ireland. During mid-lactation, 67 Irish dairy farms supplied sufficient milk to the factory to undertake the manufacturing process; during late-lactation, 150 dairy farms were necessary, due to the lower milk yield per cow during that period. During mid- and late-lactation, the average (± SD) milk volume collected from each farm was 4,418 ± 3,066 L and 1,786 ± 1,905 L, respectively. Collection tankers (n = 11) transported a total of 296,003 L and 267,932 L of milk to a commercial SMP factory during mid- and late-lactation, respectively. Those volumes were stored in a whole milk silo (WMS) within the factory. Subsequently, the milk was pasteurised by applying a high temperature/ short time (HTST) treatment (75 ˚C, 25 s). After pasteurisation, the cream was separated and stored in the cream silo (CS), while the skim milk was stored in the skim milk silo (SMS). The skim milk was evaporated in a triple-effect evaporator and afterwards underwent spray-drying process. Approximately 22,000 kg of low-heat SMP were produced during both lactation periods that this study was carried out. Further details regarding the processing parameters are described in the supplementary material.

Sampling procedure

During mid- and late-lactation, samples were collected from the top inlet of the 67 and 150 farm bulk tanks, respectively, using sterilised sample dippers. On arrival at the processing plant, samples were collected from the top inlet of each CT (n = 11) using sterilised dippers.

Samples were also collected from the top and bottom sampling ports of both WMS and SMS using industrial syringes. Additionally, in late-lactation, cream samples were collected from the top and bottom of the CS using industrial syringes, as that cream was produced only using the milk supplied by the 150 farms. All silo samples were collected after the whole milk, skim milk or cream was completely transferred to the respective silos. Additionally, three 25-kg SMP bags were collected within the factory at the start, middle and final stages of the spray-dryer run, giving a total of 9 bags. Powder samples were reconstituted using deionised water (1:10 dilution).

All samples collected in mid-lactation and samples from the factory collected during late-lactation (CT, WMS, CS, SMS and SMP samples) were analysed in the milk quality laboratory in Teagasc Moorepark (Fermoy, Co. Cork, Ireland). Due to the high number of farm milk samples collected in late-lactation, those samples were analysed at the laboratory in the factory. A schematic drawing of the SMP manufacturing process is shown in supplementary Figure S1, as well as the sampling points.

Microbiological analysis

All samples collected during mid-lactation and the CT, WMS, CS, SMS and SMP samples collected during late-lactation were tested in duplicate for a range of bacterial species. All the microbiological analyses were performed according to the *Standard Methods for the Examination of Dairy Products* (Wehr and Frank, 2004). Total (TBC), psychrotrophic (PBC), thermoduric (Laboratory Pasteurisation Count - LPC) and thermophilic (THERM) bacterial counts were measured using Petrifilm aerobic count plates (ready to use media; 1 mL of diluted sample on each plate) (3M, Technopath, Tipperary, Ireland), in accordance with the procedures described by Laird *et al*. (2004). The LPC test consisted of pasteurising the milk samples at 63 ˚C for 35 min, including time to allow samples to reach the required temperature (Frank and Yousef, 2004); afterwards, the samples were cooled to 10 ˚C using iced water before testing. Samples tested for TBC and LPC were incubated for 48 h at 32 ˚C, while samples tested for THERM were incubated for 48 h at 55 ˚C. The Petrifilms corresponding to the PBC test were incubated for 10 days at 7 ± 1 ˚C (Frank and Yousef, 2004). The authors are aware that using Petrifilm at 7 or 55 ˚C is outside the validated temperature range for that media. However, a pre-trial experiment for THERM indicated that, at the same dilution, plate count agar plates were uncountable due to bacterial colonies spreading over the surface of agar plates, whereas Petrifilm plates were countable (data not shown). Regarding PBC, other studies have been using Petrifilm for that test at 7 ˚C

(Ramsahoi *et al*., 2011). A Petrifilm Plate Reader (3M, Technopath, Tipperary, Ireland) was used to assess the number of bacterial colonies.

The proteolytic bacterial count (PROT) test consisted of spread plating the diluted sample (100 µL) on calcium caseinate agar with added skim milk powder (Merck, Darmstadt, Germany). Plates were incubated at 37 ˚C for 48 h. Proteolytic bacterial colonies were identified as colonies surrounded by a clear zone in an opaque medium.

The TBC of the 150 farm milk samples collected during late-lactation were analysed within the factory using a MilkoScan FT2 system (Foss Electric, Hillerød, Denmark).

Statistical analysis

The statistical analyses were performed using the software SAS 9.3 (SAS Institute, 2016). The bacterial counts means (TBC, PBC, PROT, LPC and THERM) of each CT were predicted using the volume and bacterial count measured in the milk of all farms that supplied each CT. The same bacterial counts were predicted for the WMS using the volume and bacterial counts measured in the milk of all CTs that supplied that silo. Those predictions were calculated as volume weighted means with estimated confidence interval. The actual bacterial counts measured in each CT and WMS samples were compared to the respective confidence interval for those predicted means of the bacterial counts. Agreement plots were also used to check for bias in the relationship between actual and predicted bacterial count means. There were insufficient numbers of samples from the factory (WMS, SMS and SMP samples) to determine the statistical differences between the bacterial counts measured in those samples. Therefore, only numerical differences between those samples were reported in this research paper to indicate the possible variations in bacterial load throughout the process. This study was performed once during each mid- and late-lactation periods.

Results

Mid-lactation study

The mean bacterial counts (TBC, PBC, PROT, LPC and THERM) of the samples from the farm bulk tanks, CTs, WMS, SMS and samples of SMP, which were collected during the mid-lactation period, are shown in Table 1. Small increases were observed when comparing all mean bacterial counts of the farm bulk tanks and CTs (Table 1). Pronounced increases in the TBC, PBC and PROT were observed in the WMS samples when compared to the CT samples (Table 1). The mean TBC, PBC and PROT were lower in the SMS samples

compared to the WMS samples; however, the LPC and THERM levels were not different between them (Table 1).

The comparisons between the actual bacterial counts of each CT sample with the respective confidence interval for the predicted means, which were calculated considering the volume and bacterial count of each farm's milk supplied to each CT, are shown in supplementary Table S1. The TBC, PBC, PROT, LPC and THERM of two, three, one, two and four CT samples, respectively, were not within the respective confidence intervals (Table S1).

The comparisons between the actual bacterial counts of the WMS samples and the respective confidence interval for the predicted means, which were calculated considering the volume and bacterial count of each CT milk supplied to the silo, are shown in Table S2. The mean TBC, PBC, PROT and THERM of the WMS samples were not within the respective confidence intervals (Table S2).

Late-lactation study

The mean bacterial counts (TBC, PBC, PROT, LPC and THERM) of the samples from the farm bulk tanks, CTs, WMS, CS, SMS and samples of SMP, that were collected during late-lactation period, are shown in Table 1. The mean TBC of the CT samples was higher than the mean TBC of the farm milk samples (Table 1). The mean TBC, PBC and PROT of the WMS samples were higher than the CT samples means (Table 1). The mean TBC, PBC and PROT of the SMS samples were lower compared to the WMS samples, while their LPC and THERM levels were similar (Table 1).

The comparisons between the actual mean TBC measured in each CT sample with the respective confidence interval for the predicted means, which were calculated considering the volume and TBC of each farm milk supplied to each CT, are shown in the supplementary Table S3. The mean TBC of nine CT samples (1, 3, 5, 6, 7, 8, 9, 10 and 11) were not within the respective confidence intervals (Table S3).

The comparisons between the actual bacterial counts of the WMS samples with the respective confidence interval for the predicted means, which were calculated considering the volume and bacterial count of each CT milk supplied to the silo, are shown in Table S2. The mean TBC, PBC and PROT of the late-lactation WMS samples were not within the respective confidence intervals (Table S2).

Discussion

Production season or storage conditions can affect the bacterial counts of different types of microorganisms in milk, which can impact on the final quality of SMP. In mid-lactation, the mean TBC and PBC of the farm milk samples were below the European limits (EC no 853/2004): 5.00 and 4.22 $\log_{10}$ cfu/ mL, respectively. The TBC was also below the typical limit of 4.70 $\log_{10}$ cfu/ mL applied by some Irish milk processors (Table 1). The mean PROT of the farm samples was below the limit suggested by Vyletelova *et al.* (2000) (4.65 $\log_{10}$ cfu/ mL), at which proteolytic bacteria would produce high levels of heat-resistant proteases. The mean LPC of the mid-lactation farm milk samples was lower than the typical industry specifications, which can range from 2.70 to 3.00 $\log_{10}$ cfu/ mL. Thermoduric and thermophilic bacterial colonies were not detected in 8 and 24 farm milk samples, respectively. In mid-lactation, some individual farm milk samples had TBC, PBC, PROT and LPC higher than the specified limits. However, considering that the milk volumes from all farms would be blended for processing, the comparisons between the weighted mean bacterial counts and the known specifications for raw milk indicated that good quality milk was delivered to the factory for processing in mid-lactation.

The mean TBC of late-lactation farm bulk tank milk samples was also lower than the European and industrial limits; however, 49 farm samples had TBC above those specifications. Statistical comparisons between the mean TBC of the farm samples collected during mid- and late-lactation were not possible, as the group of farms involved in the mid- and late-lactation studies were different and samples from those groups were analysed in different laboratories; however, the figures gave an indication that lower quality milk was produced in late-lactation. The variations in the counts of different bacterial types between lactation periods could be related to seasonal differences in bacterial strains in the environment, cow management, cows' health status (e.g., mastitis), on-farm hygiene practices, or milk storage conditions (Linn, 1988; Lafarge *et al*., 2004).

In mid-lactation, the mean TBC, PBC, PROT and LPC of the CT milk samples were below the limits determined by the European legislation, industry and literature cited; while in late-lactation, the mean TBC and PBC were higher than the European limits (Table 1). The TBC, PBC, PROT, LPC and THERM of the CTs milk were higher in late-lactation compared to mid-lactation, possibly due to the production of milk of inferior quality on-farm during that period. Also, the longer milk collection periods in late-lactation (approximately 8 h) could have contributed to the increased bacterial numbers in the CTs. The CT milk samples that had the bacterial counts higher than the upper confidence limit (mid-lactation: TBC, PBC, PROT,

LPC and THERM; late-lactation: TBC; Tables S1 and S3) indicated that those bacterial numbers could have been influenced by the transport duration, CT cleaning protocol, temperature during transport or by the impact of individual farm suppliers (Kable *et al.*, 2016).

In both lactation periods, some of the bacterial counts measured in the WMS samples were higher than the respective upper confidence limits (mid-lactation: TBC, PBC, PROT and THERM; late-lactation: TBC, PBC and PROT; Table S2). The increase in those bacterial counts could be due to the conditions of the equipment in the milk transfer line (from the CT to the silo) (e.g., pump system and filters), non-effective silo clean-in-place routine, storage time or favourable storage temperature for the growth of some bacterial strains, or could be a result of blending raw milk from different origins and levels of contamination (Pinto *et al*., 2006).

In mid- and late-lactation, the mean TBC of the WMS samples was higher than the limit determined for raw milk prior to processing (5.48 $\log_{10}$ cfu/ mL; EC no 853/2004). However, the temperature-time binomial applied during pasteurisation (75 ˚C, 25 s) reduced the TBC, PBC and PROT, as observed in the SMS samples (Table 1). In both lactation periods, pasteurisation was not efficient in reducing the LPC and THERM, when comparing the figures obtained for the WMS and SMS samples (Table 1), as those bacterial types are capable of surviving the temperatures applied in thermal treatments (Delgado *et al.*, 2013; Quigley *et al.*, 2013b). Thermoduric bacteria are able to survive pasteurisation temperatures (above 63 ˚C), while thermophilic bacteria are able to survive and grow at 55 ˚C or above (Frank and Yousef, 2004). The decreases in TBC and PBC after pasteurisation were of lower magnitude in late-lactation than in mid-lactation (Table 1), indicating that milk may contain higher numbers of heat-resistant bacteria strains during winter. Furthermore, in late-lactation, the THERM levels were higher in the CS samples compared to the WMS and SMS samples (Table 1). Given that cream separation occurred after pasteurisation, the relative abundance of thermophiles in pasteurised whole milk was possibly higher than prior to pasteurisation. Thermophilic bacteria could have migrated with the fat globules due to density (Graham, 2004) or the high levels could be related to the cleaning of the silos, as the persistence of thermophilic bacteria is related to the formation of biofilms (Burgess *et al*., 2010).

Mid-lactation raw milk had better microbiological quality than late-lactation milk; consequently, the SMP produced using mid-lactation milk had lower bacterial counts than that made from late-lactation milk (Table 1). Laboratory-based studies indicated that when TBC in milk is higher than 5.00 $\log_{10}$ cfu/ mL, the solubility index of SMP can increase, as

well as the free fat acid content, while the heat stability decreases (Muir *et al*., 1986; Celestino *et al*., 1997). In relation to thermoduric and thermophilic bacteria, there are no European limits determined for milk powder; however, the SMP produced using mid- and late-lactation milk had THERM levels in accordance to the North American dairy industry requirements (less than 4.00 $\log_{10}$ cfu/ g) (Wehr and Frank, 2004). Furthermore, it is likely that evaporation and spray-drying processes may have contributed to further reductions in TBC, PBC and PROT in the SMP in both periods.

This study highlights the importance of controlling bacterial levels in milk on-farm and during manufacturing, as processing parameters might not be able to reverse the negative effects of high bacterial levels; consequently, compromising the quality of dairy products. For example, when in sufficient numbers, certain bacteria strains can produce lipases and proteases, which could not be eliminated in pasteurisation and could affect essential technological properties of milk for dairy products manufacture (Muir, 1996; Barbano *et al*., 2006). Hygiene practices, cow management and processing parameters can affect the abundance of different bacterial types in milk; and therefore, those should be adequate to guarantee milk powder high quality and safety (Craven *et al*., 2010; Watterson *et al*., 2014).

Conclusion

In conclusion, this was the first study that monitored the quality of milk from farm bulk tank, through processing stages, to skim milk powder. We found evidence that stage of lactation and/or environmental factors related to time of year did influence microbiological quality, but the experimental design did not allow us to statistically validate the hypothesis. The effects of milk quality parameters on the quality of low-heat skim milk powder were observed, as well as how those parameters were affected throughout the manufacturing process. The good microbiological quality of the mid-lactation farm milk resulted in the production of milk powder with lower bacterial counts in contrast to the powder produced during late-lactation with milk of inferior quality. The season and stage of milk production has an influence on the abundance of different bacterial types in milk, which could impact the effectiveness of thermal treatments and consequently affect final product quality. Also, the differences in bacterial counts between production stages are indications of the growth potential of the bacteria in the milk, or even an indication of possible contamination sources in the specific production stage in which changes were observed. The results observed can aid industry in targeting sources of contamination throughout processing stages and practices to control

bacterial numbers, in order to ensure the consistent production of safe high-quality dairy products throughout the year.

Acknowledgements

The authors would like to acknowledge the milk suppliers and processing plant staff involved in this study, Jim Grant for the statistical analysis and Jim Flynn for guidance on the microbiological analysis.

360

361 **References**

362 Barbano DM, Ma Y & Santos MV 2006 Influence of raw milk quality on fluid milk shelf-
363 life. *Journal of Dairy* Science **89** E15-E19

364 Burgess SA, Lindsay D & Flint SH 2010 Thermophilic bacilli and their importance in dairy
365 processing. *International Journal of Food Microbiology* **144** 215 – 225

366 Celestino EL, Iyer M & Roginski H 1997 The effects of refrigerated storage of raw milk on
367 the quality of whole milk powder stored for different periods. *International Dairy*
368 *Journal* **7** 119-127

369 Cherif-Antar A, Moussa-Boudjemaa B, Didouh N, Medjahdi K, Mayo B & Florez AB 2016
370 Diversity and biofilm-forming capability of bacteria recovered from stainless steel
371 pipes of a milk-processing dairy plant. *Dairy Science and Technology* **96** 27-38

372 Craven HM, McAuley CM, Duffy LL & Fegan N 2010 Distribution, prevalence and
373 persistence of *Cronobacter* (*Enterobacter sakazakii*) in the nonprocessing and
374 processing environments of five milk powder factories. *Journal of Applied*
375 *Microbiology* **109** 1044-1052

376 Delgado S, Rachid CTCC, Fernandez E, Rychlik T, Alegria A, Peixoto RS & Mayo B 2013
377 Diversity of thermophilic bacteria in raw, pasteurized and selectively-cultured milk,
378 as assessed by culturing, PCR-DGGE and pyrosequencing. *Food Microbiol*ogy **36**
379 103-111

380 EU Regulation 853/ 2004. Regulation (EC) No 853/2004 of the European Parliament and of
381 the Council of 29 April 2004 laying down specific hygiene rules for food of animal
382 origin. *Official Journal of the European Union* **L226** 22 - 82

383 Frank JF & Yousef AE 2004 Test for groups of microorganisms. Pages 227–248 in Standard
384 Methods for the Examination of Dairy Products, Wehr HM & Frank JF, 17th ed,
385 ed. American Public Health Association Washington DC

386 Graham T 2004 Sampling dairy and related products. Pages 63–91 in Standard Methods for
387 the Examination of Dairy Products, Wehr HM & Frank JF, 17th ed, ed. American
388 Public Health Association Washington DC

389 Huck JR, Sonnen M & Boor KJ 2008 Tracking heat-resistant, cold-thriving fluid milk
390 spoilage bacteria from farm to packaged product. *Journal of Dairy Science* **91** 1218-
391 1228

392 Jindal S, Anand S, Huang K, Goddard J, Metzger L & Amamcharla J 2016 Evaluation of
393 modified stainless steel surfaces targeted to reduce biofilm formation by common
394 milk sporeformers. *Journal of Dairy Science* **99** 9502-9513
395 Kable ME, Srisengfa Y, Laird M, Zaragoza J, McLeod J, Heidenreich J & Marco ML 2016
396 The core and seasonal microbiota of raw bovine milk in tanker trucks and the impact
397 of transfer to a milk processing facility. *MBio* **4** 1-13
398 Lafarge V, Ogier JC, Girard V, Maladen V, Leveau JY, Gruss A & Delacroix-Buchet A 2004
399 Raw cow milk bacterial population shifts attributable to refrigeration. *Applied and*
400 *Environmental Microbiology* **70** 5644-5650
401 Laird DT, Gambrel-Lenarz SA, Scher FM, Graham TE & Reddy R 2004 Microbiological
402 Count Methods. Pages 153–186 in Standard Methods for the Examination of Dairy
403 Products, Wehr HM & Frank JF, 17th ed, ed. American Public Health Association
404 Washington DC
405 Linn JG 1988 Factors affecting the composition of milk from dairy cows. Pages 224-241 in
406 National Research Council (US) Committee on Technological Options to Improve
407 the Nutritional Attributes of Animal Products. Designing Foods: Animal Product
408 Options in the Marketplace. National Academy Press Washington DC
409 Muir DD, Griffiths MW, Phillips JD, Sweetsur AWM & West IG 1986 Effect of the bacterial
410 quality of raw milk on the bacterial quality and some other properties of low-heat
411 and high-heat dried milk. *International Journal of Dairy Technology* **39** 115-118
412 Muir D 1996 The shelf-life of dairy products: 1. Factors influencing raw milk and fresh
413 products. *International Journal of Dairy Technology* **49** 24-32
414 Murphy SC, Martin NH, Barbano DM & Wiedmann M 2016 Influence of raw milk quality
415 on processed dairy products: how do raw milk quality test results relate to product
416 quality and yield? *Journal of Dairy Science* **9** 10128-10149
417 O'Connell A, McParland S, Ruegg PL, O'Brien B & Gleeson D 2015 Seasonal trends in milk
418 quality in Ireland between 2007 and 2011. *Journal of Dairy Science* **98** 3778-3790
419 Pinto CLO, Martins ML & Vanetti MCD 2006 [Microbial quality of raw refrigerated milk
420 and isolation of psychrotrophic proteolytic bacteria]. *Food Science and Technology*
421 **26** 645-651
422 Quigley L, O'Sullivan O, Stanton C, Beresford TP, Ross RP, Fitzgerald GF & Cotter PD
423 2013a The complex microbiota of raw milk. *FEMS Microbiology Reviews* **37** 664-
424 698

425 Quigley L, McCarthy R, O'Sullivan O, Beresford TP, Fitzgerald GF, Ross RP, Stanton C &
426 Cotter PD 2013b The microbial content of raw and pasteurized cow milk as
427 determined by molecular approaches. *Journal of Dairy Science* **96** 4928-4937
428 Ramsahoi L, Gao A, Fabri M & Odumeru JA 2011 Assessment of the application of an
429 automated electronic milk analyser for the enumeration of total bacteria in raw goat
430 milk. *Journal of Dairy Science* **94** 3279-3287
431 SAS 2016 Version 9.3. SAS Institute Inc., Cary NC USA.
432 Teh KH, Flint S, Palmer J, Lindsay D, Andrewes P & Bremer P 2011 Thermo-resistant
433 enzyme-producing bacteria isolated from the internal surfaces of raw milk tankers.
434 *International Dairy Journal* **21** 742-747
435 Tucker G 2015 Pasteurisation: principles and applications. Pages 264-269 in Encyclopedia of
436 Food and Health, Caballero B, Finglas P & Toldra F, 1st ed, Academic Press Oxford
437 UK
438 Watterson MJ, Kent DJ, Boor KJ, Wiedmann M & Martin NH 2014 Evaluation of dairy
439 powder products implicates thermophilic sporeformers as the primary organisms of
440 interest. *Journal of Dairy Science* **97** 2487-2497
441 Wehr HM & Frank JF 2004 Standard Methods for the Examination of Dairy Products. 17th
442 ed, American Public Health Association Washington DC
443 Vyletelova M, Hanus O, Urbanova E & Kopunecz P 2000 The occurrence and identification
444 of psychrotrophic bacteria with proteolytic and lipolytic activity in bulk milk
445 samples at storage in primary production conditions. *Czech Journal of Animal*
446 *Science* **45** 373- 383
447
448
449
450
451
452

Table 1. Mean (± SD) total bacterial count (TBC), psychrotrophic (PBC), proteolytic (PROT), thermoduric (LPC – Laboratory pasteurisation count) and thermophilic (THERM) bacterial counts of the samples collected from the farm bulk tanks, collection tankers (CTs), whole milk silo (WMS), cream silo (CS), skim milk silo (SMS) and samples of skim milk powder (SMP) from the mid- and late-lactation periods.

Mid-Lactation Bacterial counts (log_{10} cfu/ mL)	Farm bulk tanks † (n=67)	CT † (n=11)	WMS (n=2)	CS ‡ (n=2)	SMS (n=2)	SMP ‖ (n=9)
TBC	3.60 ± 0.55 (2.65 to 4.90)	3.90 ± 0.40 (3.22 to 4.62)	5.89 ± 0.02		2.61 ± 0.20	2.36 ± 0.09 (2.26 to 2.50)
PBC	3.54 ± 0.65 (2.70 to 6.00)	3.70 ± 0.53 (2.74 to 5.97)	6.00 ± 0.00		2.00 ± 0.00	1.21 ± 0.15 (1.00 to 1.40)
PROT	3.50 ± 0.56 (3.00 to 5.10)	3.66 ± 0.29 (3.30 to 4.30)	5.72 ± 0.62		2.00 ± 0.00	1.36 ± 0.30 (1.00 to 1.70)
LPC	1.35 ± 0.33 (1.00 to 2.60) ¶	1.44 ± 0.28 (1.00 to 1.98)	1.58 ± 0.17		1.69 ± 0.07	2.45 ± 0.08 (2.30 to 2.51)
THERM	1.43 ± 0.47 (1.00 to 2.52) ¶	1.62 ± 0.35 (1.00 to 2.47)	2.02 ± 0.14		1.85 ± 0.10	3.63 ± 0.11 (3.50 to 3.79)
Late-lactation Bacterial counts (log_{10} cfu/ mL)	**Farm bulk tanks †,§ (n=150)**	**CT † (n=11)**	**WMS (n=2)**	**CS (n=2)**	**SMS (n=2)**	**SMP ‖ (n=9)**
TBC	4.37 ± 0.62 (3.60 to 7.16)	5.12 ± 0.53 (4.32 to 5.96)	5.84 ± 0.09	2.32 ± 0.09	5.00 ± 0.00	3.56 ± 0.08 (3.44 to 3.69)
PBC		5.25 ± 0.58 (4.15 to 5.97)	5.80 ± 0.04	1.15 ± 0.21	5.00 ± 0.00	2.07 ± 0.10 (1.90 to 2.19)
PROT		4.09 ± 0.72 (3.30 to 5.95)	4.68 ± 0.40	4.27 ± 0.27	2.52 ± 0.35	2.18 ± 0.26 (2.00 to 2.54)
LPC		2.60 ± 0.23 (2.35 to 2.99)	2.55 ± 0.03	2.33 ± 0.01	2.61 ± 0.17	3.51 ± 0.09 (3.33 to 3.62)
THERM		2.72 ± 0.19 (2.51 to 2.98)	2.74 ± 0.06	4.54 ± 0.01	2.63 ± 0.04	3.58 ± 0.09 (3.41 to 3.69)

n = number of samples analysed in duplicate

Ranges are given between parentheses.

† Weighted means calculated considering the volumes and bacterial counts of each farm or CT sample.

‡ Cream samples were not collected during mid-lactation.

§ Only TBC was measured in the late-lactation farm milk samples.

‖ Bacterial counts in log_{10} cfu/ g.

¶ Weighted means calculated not considering the samples in which those bacteria were not detected.

Microbiological quality of milk from farms to milk powder manufacture: an industrial case study

Lizandra F. Paludetti[1,2], Alan L. Kelly[2], Bernadette O'Brien[1], Kieran Jordan[3], David Gleeson[1]*

[1]Teagasc Moorepark, Animal & Grassland Research and Innovation Centre, Fermoy, County Cork, Ireland

[2] School of Food and Nutritional Sciences, University College Cork, County Cork, Ireland

[3] Teagasc Moorepark, Food Research Centre, Fermoy, County Cork, Ireland

Short title: **Microbiological quality from farm to milk powder**

Correspondence: David Gleeson,

Teagasc Moorepark

Animal Grassland Research and Innovation Centre

Fermoy

County Cork

Ireland

Tel.: +353 025 42269

Email address: David.Gleeson@teagasc.ie

SUPPLEMENTARY FILE

Materials & Methods

Milk collection and skim milk powder manufacture

The raw milk harvested during mid- and late-lactation were stored within the bulk tanks for an average (± SD) of 44 ± 11 h (range: 2 - 52 h) and 70 ± 19 h (range: 24 – 217 h) prior to tanker collection, at 3.1 ± 0.7 ˚C (range: 0.9 to 4.5 ˚C) and 3.3 ± 1.2 ˚C (range: 0.5 to 9.5 ˚C), respectively. During mid- and late-lactation, the milk volume collected from each farm ranged from 298 to 21,572 L and from 114 to 10,525 L, respectively. Each collection tanker (CT) collected milk from approximately 6 and 14 farms in mid- and late-lactation, respectively; and the temperature in the CTs ranged from 3.7 to 4.2 ˚C. The milk stored in the whole milk silo (WMS) was stored approximately 5.5 h (time between the transference of the first CT milk and the eleventh CT milk to the silo), at an average (± SD) temperature of 4.6 ± 0.2 ˚C, and agitated for 1 min every 29 min. The whole milk was pasteurised by applying a high temperature/ short time (HTST) treatment, during which the milk was heated to 75 ˚C for 25 s. After cream separation, the cream content in the skim milk was 0.075%. In the triple-effect evaporator the skim milk was concentrated from 9% w/w to 52% w/w of total solids content and the final moisture content was 48% w/w. The average moisture content of the skim milk powder (SMP) produced was 3.2 ± 0.2% w/w. The commercial processing plant in which this experiment was carried out detains further details regarding the processing parameters.

Sampling procedure

After agitation, 300-mL milk samples were collected from each farm bulk tanks, CTs, WMS, cream silo (CS) and SMS. All milk samples collected in mid-lactation and samples from the factory collected during late-lactation (CT, WMS, CS and SMS samples) were transported to the milk quality laboratory in Teagasc Moorepark in cooling boxes (<4 ˚C) within 6 h. After delivery, samples were sub-divided into 30-mL sterile bottles for microbiological analysis and analysed within 2 h. The milk samples were manually agitated to avoid unequal fat distribution.

In relation to the low-heat SMP samples, 100 g were taken from the top, middle and bottom of each bag; these were mixed to obtain a representative 300-g sample from each bag. These powder samples were reconstituted using deionised water (1:10 dilutions) and sub-divided into 30-mL sterile bottles for microbiological analysis.

Table S1. Comparison of mean total (TBC), psychrotrophic (PBC), proteolytic (PROT), thermoduric (laboratory pasteurisation count – LPC) and thermophilic (THERM) bacterial counts measured in each collection tanker (CT: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11) during mid-lactation and those predicted (± standard error; S.E.) from the combined farm samples in each CT.

Bacterial counts	CT number	Number of farms	Total volume per tanker (L)	Mean (± SD) volume measured per farm (L)	Mean CT bacterial count (log_{10} cfu/ mL)	Predicted bacterial count (weighted means; S.E.)† (log_{10} cfu/ mL)	95% CI‡		Mean CT bacterial counts covered by predicted C.I.
							LCL	UCL	
TBC									
	1	4	23771	5,943 ± 1,271	3.99	3.93 ± 0.09	3.64	4.23	Yes
	2	5	26503	5,301 ± 2,385	4.38	3.7 ± 0.27	2.95	4.45	Yes
	3	6	29122	4,854 ± 1,763	3.90	3.82 ± 0.32	2.98	4.65	Yes
	4	6	23780	3,963 ± 2,683	4.18	3.64 ± 0.23	3.06	4.22	Yes
	5	8	27585	3,448 ± 2,214	3.88	3.51 ± 0.19	3.05	3.97	Yes
	6	7	28628	4,090 ± 1,208	4.15	3.57 ± 0.2	3.08	4.06	No
	7	7	27188	3,884 ± 2,064	4.62	3.87 ± 0.33	3.06	4.67	Yes
	8	7	28470	4,067 ± 2,437	3.64	3.9 ± 0.08	3.71	4.09	No
	9	2	27147	13,574 ± 11,312	3.22	3.03 ± 0.07	2.2	3.86	Yes
	10	5	25248	5,050 ± 3,877	3.45	3.27 ± 0.13	2.93	3.62	Yes
	11	10	28561	2,856 ± 1,764	3.54	3.35 ± 0.12	3.08	3.62	Yes
PBC									
	1	4	23771	5,943 ± 1,271	3.99	3.61 ± 0.28	2.71	4.51	Yes
	2	5	26503	5,301 ± 2,385	3.52	3.36 ± 0.18	2.86	3.87	Yes
	3	6	29122	4,854 ± 1,763	4.04	3.83 ± 0.33	2.97	4.68	Yes

	4	6	23780	3,963 ± 2,683	3.56	3.51 ± 0.11	3.22	3.8	Yes
	5	8	27585	3,448 ± 2,214	3.74	3.36 ± 0.25	2.76	3.95	Yes
	6	7	28628	4,090 ± 1,208	3.80	3.45 ± 0.1	3.21	3.69	No
	7	7	27188	3,884 ± 2,064	5.97	4.11 ± 0.54	2.78	5.45	No
	8	7	28470	4,067 ± 2,437	3.60	3.97 ± 0.12	3.67	4.28	No
	9	2	27147	13,574 ± 11,312	2.74	3.04 ± 0.04	2.48	3.6	Yes
	10	5	25248	5,050 ± 3,877	3.23	3.35 ± 0.17	2.48	3.6	Yes
	11	10	28561	2,856 ± 1,764	3.51	3.29 ± 0.11	3.04	3.55	Yes
PROT									
	1	4	23771	5,943 ± 1,271	3.70	3.71 ± 0.15	3.24	4.17	Yes
	2	5	26503	5,301 ± 2,385	3.70	3.61 ± 0.41	2.48	4.73	Yes
	3	6	29122	4,854 ± 1,763	3.65	3.68 ± 0.27	2.98	4.38	Yes
	4	6	23780	3,963 ± 2,683	3.98	3.61 ± 0.28	2.9	4.33	Yes
	5	8	27585	3,448 ± 2,214	3.74	3.41 ± 0.15	3.05	3.76	Yes
	6	7	28628	4,090 ± 1,208	3.30	3.67 ± 0.24	3.08	4.26	Yes
	7	7	27188	3,884 ± 2,064	4.30	4.03 ± 0.26	3.39	4.67	Yes
	8	7	28470	4,067 ± 2,437	3.40	3.33 ± 0.09	3.1	3.56	Yes
	9	2	27147	13,574 ± 11,312	3.84	3.06 ± 0.12	1.52	4.61	Yes
	10	5	25248	5,050 ± 3,877	3.30	3.05 ± 0.05	2.9	3.2	No
	11	10	28561	2,856 ± 1,764	3.40	3.37 ± 0.1	3.14	3.6	Yes

LPC								
1	4	23771	5,943 ± 1,271	1.54	1.21 ± 0.06	1.01	1.42	No
2	5	26503	5,301 ± 2,385	1.18	1.35 ± 0.13	0.99	1.71	Yes
3	6	29122	4,854 ± 1,763	1.00	1.07 ± 0.3	0.3	1.84	Yes
4	6	23780	3,963 ± 2,683	1.48	1.34 ± 0.07	1.16	1.52	Yes
5	8	27585	3,448 ± 2,214	1.98	0.79 ± 0.25	0.21	1.38	No
6	7	28628	4,090 ± 1,208	1.30	1.24 ± 0.32	0.45	2.02	Yes
7	7	27188	3,884 ± 2,064	1.60	1.12 ± 0.20	0.62	1.62	Yes
8	7	28470	4,067 ± 2,437	1.18	0.96 ± 0.18	0.51	1.41	Yes
9	2	27147	13,574 ± 11,312	1.70	0.48 ± 0.95	0	12.56	Yes
10	5	25248	5,050 ± 3,877	1.70	1.44 ± 0.1	1.17	1.71	Yes
11	10	28561	2,856 ± 1,764	1.30	1.26 ± 0.08	1.09	1.44	Yes
THERM								
1	4	23771	5,943 ± 1,271	1.30	0.65 ± 0.34	0	1.73	Yes
2	5	26503	5,301 ± 2,385	1.00	1.41 ± 0.19	0.88	1.94	Yes
3	6	29122	4,854 ± 1,763	1.74	0.87 ± 0.32	0.03	1.7	No
4	6	23780	3,963 ± 2,683	1.00	1.08 ± 0.35	0.17	1.99	Yes
5	8	27585	3,448 ± 2,214	1.00	0.19 ± 0.15	0	0.56	No
6	7	28628	4,090 ± 1,208	1.84	1.55 ± 0.33	0.73	2.37	Yes
7	7	27188	3,884 ± 2,064	1.70	0.7 ± 0.3	0	1.44	No
8	7	28470	4,067 ± 2,437	1.40	1.4 ± 0.12	1.12	1.69	Yes

9	2	27147	13,574 ± 11,312	2.47	0.51 ± 1.0	0	13.15	Yes
10	5	25248	5,050 ± 3,877	1.95	0.73 ± 0.25	0.05	1.42	No
11	10	28561	2,856 ± 1,764	1.48	0.92 ± 0.28	0.28	1.55	Yes

†Weighted means were calculated considering the volume of milk supplied by each farm.
‡Confidence interval (CI), lower (LCL) and upper (UCL) confidence limits.

Table S2. Comparison of mean total (TBC), psychrotrophic (PBC), thermoduric (laboratory pasteurisation count – LPC) and thermophilic (THERM) bacterial counts measured in the whole milk silo (WMS) during mid- and late-lactation and those predicted (± standard error; S.E.) from the combined collection tanker (CT) samples.

Stage of lactation	Bacterial count (log_{10} cfu/ mL)	Mean (± SD) bacterial count (WMS)	Predicted bacterial count (weighted means; S.E.)†	95% CI‡		Mean CT bacterial counts covered by predicted C.I.
				LCL	UCL	
Mid-lactation						
	TBC	5.89 ± 0.02	3.9 ± 0.13	3.62	4.18	No
	PBC	6.00 ± 0.00	3.7 ± 0.17	3.33	4.08	No
	PROT	5.72 ± 0.62	3.66 ± 0.09	3.45	3.87	No
	LPC	1.58 ± 0.17	1.46 ± 0.09	1.27	1.65	Yes
	THERM	2.02 ± 0.14	1.64 ± 0.11	1.39	1.88	No
Late-lactation						
	TBC	5.84 ± 0.09	5.1 ± 0.17	4.73	5.47	No
	PBC	5.80 ± 0.04	5.25 ± 0.18	4.84	5.66	No
	PROT	4.68 ± 0.40	4.09 ± 0.23	3.58	4.6	No
	LPC	2.55 ± 0.03	2.61 ± 0.07	2.44	2.77	Yes
	THERM	2.74 ± 0.06	2.73 ± 0.06	2.59	2.86	Yes

Mean (± SD) volume of milk measured per tanker in mid- and late-lactation were 26,909 ± 1,902 L and 24,357 ± 3,768 L, respectively.

†Weighted means were calculated considering the volume of milk supplied by each tanker.

‡Confidence interval (CI), lower (LCL) and upper (UCL) confidence limits.

Table S3. Comparison of mean total bacterial counts (TBC) measured in each collection tanker (CT: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11) during late-lactation and those predicted (± standard error; S.E.) from the combined farm samples in each CT.

CT number	Number of farms	Total volume per tanker (L)	Mean (± SD) volume measured per farm (L)	Mean TBC of each CT (log_{10} cfu/ mL)	Predicted TBC (weighted means; S.E.)† (log_{10} cfu/ mL)	95% CI‡		Mean TBC of each CT covered by predicted C.I.
						LCL	UCL	
1	15	25,743	1,716 ± 2,135	5.64	4.38 ± 0.16	3.95	4.66	No
2	7	19,853	2,836 ± 3,542	5.33	5.12 ± 0.32	4.35	5.89	Yes
3	8	23,460	2,933 ± 2,381	5.96	4.8 ± 0.34	4.0	5.6	No
4	13	24,221	1,863 ± 1,401	4.32	4.14 ± 0.08	3.96	4.33	Yes
5	10	24,274	2,427 ± 2,558	4.64	4.34 ± 0.12	4.06	4.61	No
6	14	24,729	1,766 ± 2,489	5.90	4.24 ± 0.25	3.71	4.77	No
7	19	28,583	1,504 ± 1,168	4.86	4.4 ± 0.08	4.23	4.56	No
8	27	28,322	1,049 ± 881	4.81	4.24 ± 0.08	4.08	4.4	No
9	18	27,606	1,534 ± 1,794	4.84	4.17 ± 0.11	3.93	4.4	No
10	8	15,774	1,972 ± 1,002	5.40	4.27 ± 0.13	3.95	4.59	No
11	13	25,367	2,306 ± 2,221	4.66	4.15 ± 0.06	4.02	4.29	No

†Weighted means were calculated considering the volume of milk supplied by each farm.
‡Confidence interval (CI), lower (LCL) and upper (UCL) confidence limits.

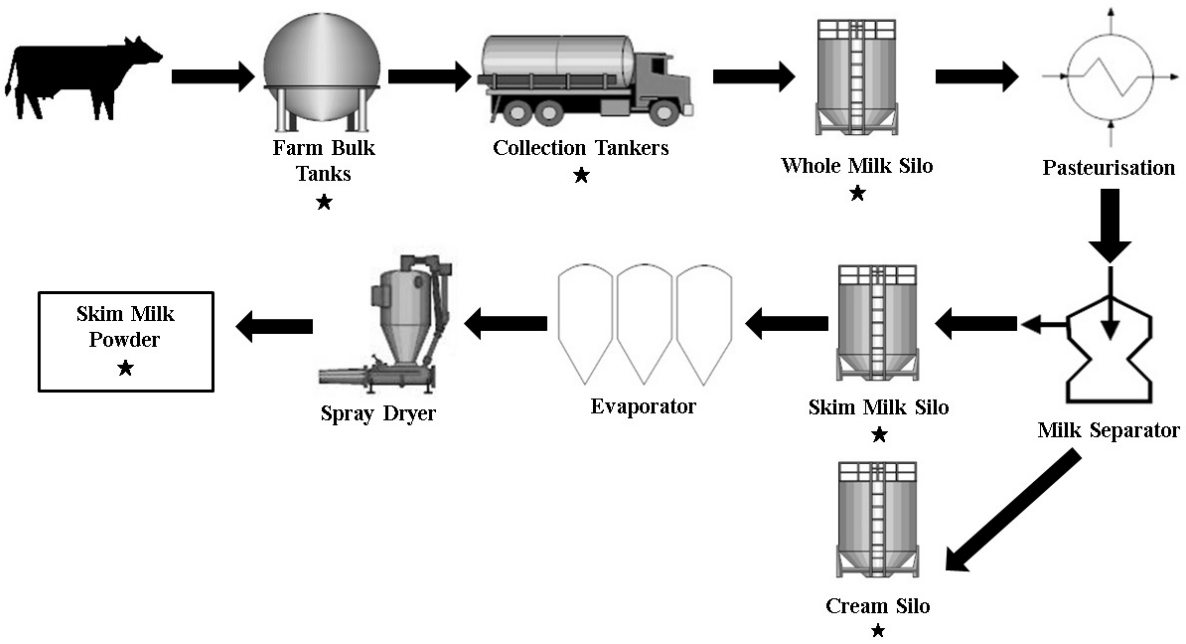

Figure S1. Milk supply chain and manufacturing process for conversion to low-heat skim milk powder, conducted in the mid- and late-lactation periods. The sampling points are indicated with a ★.