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

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Research Article

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Abstract

The experiments reported in this research paper aimed to track the microbiological load of milk throughout a 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.

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.*, 2013*a*; 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

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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 and storage conditions or processing parameters that are favourable 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, addressing the hypothesis that stage of lactation and/or environmental factors related to time of year will influence microbiological quality. 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 and 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 4418 ± 3,066 l and 1786 ± 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 Fig. 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 d 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.

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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 from each other (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. 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 Supplementary Table S2. The mean TBC, PBC, PROT and THERM of the WMS samples were not within the respective confidence intervals.

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. The mean TBC, PBC and PROT of the WMS samples were higher than the CT samples means. 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. 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 Supplementary Table S2. The mean TBC, PBC and PROT of the late-lactation WMS samples were not within the respective confidence intervals.

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 (especially 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

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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[a] ($n = 67$)	CT[a] ($n = 11$)	WMS ($n = 2$)	CS[b] ($n = 2$)	SMS ($n = 2$)	SMP[d] ($n = 9$)
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)[e]	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)[e]	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[a,c] ($n = 150$)**	**CT[a] ($n = 11$)**	**WMS ($n = 2$)**	**CS ($n = 2$)**	**SMS ($n = 2$)**	**SMP[d] ($n = 9$)**
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)

[a]Weighted means calculated considering the volumes and bacterial counts of each farm or CT sample.
[b]Cream samples were not collected during mid-lactation.
[c]Only TBC was measured in the late-lactation farm milk samples.
[d]Bacterial counts in log_{10} cfu/g.
[e]Weighted means calculated not considering the samples in which those bacteria were not detected.
n = number of samples analysed in duplicate
Ranges are given between parentheses.

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; supplementary 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; supplementary 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.*, 2013*b*). 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).

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/or stage of milk production had 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.

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