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

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

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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.

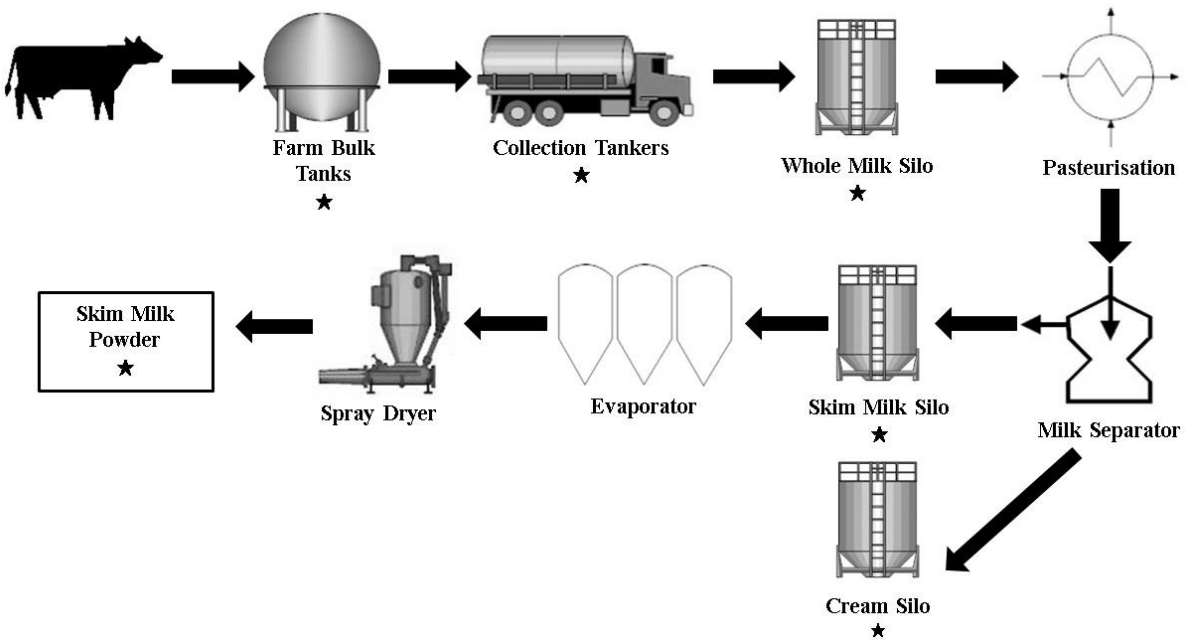

Figure S1. Milk supply chain and manufacturing process for conversion to low-heat skim milk powder, conducted in the mid- and late-lactation periods. The sampling points are indicated with a ★.