Supplementary Material

The following sections describes a scale-up pilot plant trial for the production of membrane-filtered infant milk formula (**MEM-IMF**) and high-heat treated infant milk formula (**HT-IMF**) powders.

MATERIALS AND METHODS

Materials

Raw bovine whole milk was collected from the dairy farm at the. Teagasc, Animal and Grassland Research and Innovation Centre (Moorepark, Fermoy, Co., Cork, Ireland). The fat separated centrifugally at 50 °C using facilities at Moorepark Technology Limited (Moorepark, Fermoy, Co. Cork, Ireland). Whey protein isolate powder was obtained from Agropur (Saint-Hubert, Quebec, USA). Lactose was sourced from Glanbia Ingredients Ireland (Ballyragget, Kilkenny, Ireland). The composition of the fresh skim milk and the powder ingredients are provided in Table S1. Rapeseed oil was supplied by Triby Trading Ltd. (Drogheda, Co. Louth, Ireland).

Infant milk formula (IMF) preparation and powder manufacture

The ratio of macronutrients for the first stage (1st stage IMF) and the second stage infant milk formula (2nd stage IMF), protein: lactose: fat, were 1.29: 7: 3.49 and 1.37: 8.3: 3.1, respectively. The protein had a whey protein to casein ratio of 60: 40 for the 1st IMF, while ratio of 50: 50 for the 2nd IMF. Table S1 shows the macro-nutrients composition per 100 g IMF powder. HT-IMF and MEM-IMF powder samples were manufactured independently in a single batch.

Table S1. Macro-nutrient content of ingredients, first stage (1st IMF) and second stage infant milk formula (2nd IMF) are expressed in g per 100g powder.

Product	Fat	Total protein	Carbohydrate
Skim milk	0.1 ± 0.05	3.47 ± 0.08	4.69 ± 0.12[a]
WPI	traces[b]	93 ± 0.31	traces[b]
Lactose	traces[b]	traces[b]	96.67
1st stage IMF	29.1	10.8	58.3
2nd stage IMF	24.2	10.7	65.1

[a] lactose only. [b] traces < 0.1%.

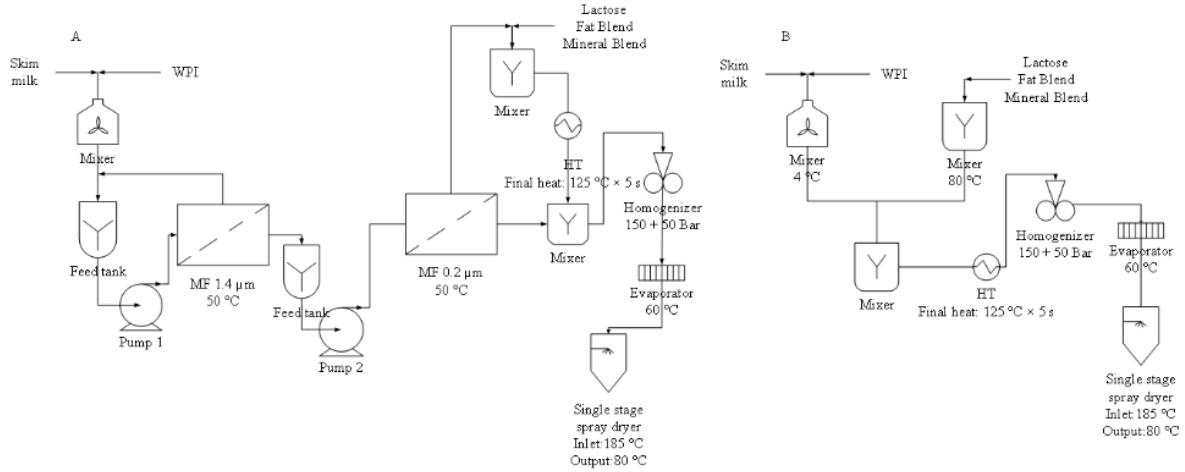

Figure S1. Processing scheme for the production of (A) membrane-filtered infant milk formula (MEM-IMF) and (B) high-heat treated infant milk formula (HT-IMF).

The 1st stage membrane-filtrated infant milk formula (MEM-IMF) processing

Ceramic 1.4 µm membrane microfiltration(MF).

Flowchart for the production of membrane-filtered IMF is described in Figure S1A. Briefly, processes were upgraded from lab-scale to a pilot plant scale.

WPI was rehydrated in skim milk according to the stage of IMFs and stored overnight at 4 °C. The prepared suspension (feed volume shown in Table S2) was pre-heated to 50 °C and was added to a GEA Model F filtration unit (50 L of system volume, GEA Process Engineering A/S, Skanderborg, Denmark) with complete retentate recycling. Three 1.4 µm ceramic membranes with a total surface area of 1.05 m^2 (TAMI Industries, Nyons Cedex, France) were used in parallel. Table S3 shows the processing parameters. The recirculation was stopped when a volumetric concentration factor (VCF) of 11 was reached, while the retentate included in the dead volume within filtration unit was discarded.

PVDF polymeric 0.2 µm membrane microfiltration

The permeate from the 1.4 µm MF was subsequently subjected to two 0.2 µm PVDF spiral-wound polymeric microfiltration membranes, Model 3838 (membrane area 6.7 m2, Synder, CA, USA) arranged in series in the GEA Model F filtration unit. The amount of feed is shown in Table S2. The feed was heated to 50 °C prior to microfiltration. Table S3 shows the processing performance parameters. Processing was completed when a VCF of 9.5 was reached. The retentate was pumped

out of the system using the same amount of RO water as the system volume. Both permeate and retentate streams were collected in separate tanks, and were stored overnight at 4 °C.

Mixing of ingredients, heat treatment, homogenization and spray drying

Fat, lactose and minerals (see Table S2) were weighed and suspended in RO water for 60 min at 80 °C. The mixture was cooled down to 50 °C and mixed with the 0.2 μm MF retentate in a evaporate balance tank. The combined solution was heated at 125 °C × 5 s using an AVP PHE pasteurisers without a homogenization step. This heated stream was mixed with the 0.2 μm MF permeate and homogenized using a Gaulin two stage homogenizer at 15 and 5 MPa for the first and second stages, respectively. The homogenized IMFs were evaporated using a Niro evaporator operated at 60 °C to concentrate solution to 50% total solid. The concentrate was spray-dried using Niro Tall form dryer spray dryer with an inlet temperature of 185 °C and an outlet temperature of 80 °C.

High-heat treated infant milk formula (HT-IMF) powder processing

The pilot-scale process flowchart of high-heat treatment was described in Figure S1B. Facilities applied for the HT-IMF processing are the same as for MEM-IMF processing.

Production of 2nd stage infant milk formula

The process for the production of 2nd stage IMF was the same as for the 1st stage IMF, except the changes in formulation, see Table S1, 2 and 3.

Total protein profile and quantification of native whey proteins in IMF powders

IMFs were measured for their total protein content, protein profiles and native whey protein content using the methods described in the main manuscript (***Protein analysis***). Each individual batch was analysed in triplicates.

Table S2. Weight (in kg) of ingredients and process streams for processing membrane-filtered infant milk formula (MEM-IMF) and high-heat treated infant milk formula (HT-IMF).

Ingredients and streams (kg)	1st stage		2nd stage	
	MEM-IMF Powder	HT-IMF Powder	MEM-IMF Powder	HT-IMF Powder
Skim milk	530	458	629	555
WPI	20	17	14	12.5

1.4 µm MF				
Feed	550		643	
Final VCF	11		12.86	
0.2 µm MF				
Feed	475		577	
Final VCF	9.5		11.54	
Lactose	155	155	167	167
Fat	86	86	70	70

Table S3. Microfiltration (MF) processing performance parameters.

	1st stage		2nd stage	
Parameters	1.4 µm MF	0.2 µm MF	1.4 µm MF	0.2 µm MF
Recirculation flow rate ($kL \cdot h^{-1}$)	14.2	6.8	14.6	6.7
Feed pressure (kPa)	310	310	310	320
Recirculation pressure (kPa)	100	100	120	120
Permeate flow rate ($L \cdot h^{-1}$)	162	583	229	360
Transmembrane pressure (kPa)	210	220	210	220
Trial temperature (°C)	50	50	50	50

Results

As a consequence of scale-up to pilot scale, the VCF of 0.2 µm MF process for the 1st stage MEM-IMF increased to 9.5, thereby more native whey proteins were preserved in the final IMF powder as shown in Table S4. The pilot scale 1st stage MEM-IMF had 59.2 % of the total α-la and 62.5 % of the total β-lg remained in the native form compare to 23 % and 16.7 % at lab scale. Very low levels of

native whey proteins remained in HT-IMFs. The net protein loss in MEM-IMFs were also reduced due to the scale-up leading to a reduction of lost feed in the microfiltration unit at the end of the filtration cycle.

Table S4. Protein profile present in final membrane-filtered infant milk formula (MEM-IMF) and high-heat treated infant milk formula (HT-IMF) powders.

	1st stage		2nd stage	
	MEM-IMF Powder	HT-IMF Powder	MEM-IMF Powder	HT-IMF Powder
Total protein (g 100g $^{-1}$ powder)*	10.9	11.4	10.4	10.5
Protein profile (% of total protein)**				
κ-Casein	6.85	7.31	7.14	7.93
α_s-Casein	17.27	18.60	21.10	22.06
β-Casein	14.24	15.17	18.55	19.69
Total Casein	38.35	41.09	46.79	49.68
α-la	10.23	7.86	8.15	5.80
β-lg B	27.78	30.06	24.37	28.26
β-lg A	23.63	20.98	20.69	16.26
Total whey proteins	61.65	58.91	53.21	50.32
Native whey protein (g 100g $^{-1}$ powder) ***				
α-la	0.66	0.12	0.51	0.12
BSA	0.13	0.02	0.12	0.02
β-lg	3.22	0.17	2.63	0.16
Total native (major) whey proteins	4.02	0.30	3.26	0.30

*Total protein determined by Kjeldahl method; **, ***Protein profile and quantification of native whey proteins determined by RP-HPLC.