Infant milk formulae processing: Effect of wet-mix total solids and heat treatment temperature on rheological, emulsifying and nutritional properties

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ABSTRACT

This study investigated the effects of total solids (TS) in the wet-mix and pasteurisation temperature on the whey protein (WP) denaturation, rheological behaviour, emulsifying properties and available lysine content of a model infant milk formula (IMF). IMF with 50% or 60% (w/w) TS were produced by wet-mix process and pasteurised at 75 °C or 100 °C for 18 s. Measurements were taken after dispersion, pasteurisation, homogenisation and spray-drying of wet-mix. Pasteurisation at 100 °C caused extensive WP denaturation (above 80%) and increased the viscosity. Available lysine content was 52% higher in powdered IMF obtained from wet-mixes pasteurised at 75 °C compared to 100 °C. A significant reduction in energy consumption could be achieved by producing IMF from 60% TS wet-mixes pasteurised at 75 °C, while obtaining powders with less than 7% WP denaturation, no loss of available lysine during processing and post-reconstitution stability for 2 h.

1. Introduction

Infant milk formula (IMF) composition is based on human milk to deliver the levels of macro and micronutrients required for an adequate development of the infant (Buggy et al., 2017). Powdered IMF is usually produced from cow's milk by the wet-mix process. This process typically involves the rehydration and dispersion of dry ingredients in skim milk or water, followed by pasteurisation, homogenisation to create an emulsion, evaporation to concentrate the mix and spray-drying to produce the powder (Blanchard et al., 2013). The conditions under which the wet-mix is processed have technological, nutritional and functional implications.

Pasteurisation is a critical point of the process, since it is required to reduce the microbial load and ensure the safety of a product to be consumed by newborns. High pasteurisation temperatures, such as 100 °C, are often used in IMF studies (Kelly et al., 2016; Masum et al., 2019; McCarthy et al., 2012; Murphy et al., 2015). Nevertheless, heating at high temperatures may lead to undesirable reactions. As a consequence of heating, Amadori compounds are formed during the early stages of Maillard reactions, rendering lysine biologically unavailable (Krause

et al., 2003). Furthermore, IMF protein fraction is comprised by 60% of whey proteins (WP), which are heat labile and can undergo denaturation and aggregation (Fenelon et al., 2018). The denaturation and aggregation processes of WP can be separated into two stages. First, the native globular structure is reversibly denatured, through dissociation of intramolecular bonds. At temperatures above 70 °C, sulphydryl group interaction and disulphide interchanges together with non-covalent reactions occur, leading to irreversible aggregation (Mulcahy et al., 2017). However, the temperature and extent of these reactions depend on the caracateristics of the mix, such as pH, ionic strength, presence of other components and total solids (TS) level, as well as on the duration and type (direct/indirect) of heat treatment (Akkerman et al., 2016; Anema et al., 2006; Anema and Li, 2003; Guyomarc'h et al., 2009; Marx and Kulozik, 2018; Oldfield et al., 2001). Denaturation and aggregation can cause loss of solubility, with negative nutritional (reduced digestibility) and technological (fouling) implications, and loss of functionality, such as emulsification (Anandharamakrishnan et al., 2008; Carbonaro et al., 1998; Dapueto et al., 2019; Petit et al., 2013). Moreover, these reactions might lead to an increase in the viscosity of the wet-mix, which in turn reduces pump efficiencies and affect the physical properties of the final

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powder (Ho et al., 2019). The size of the droplets created during atomisation, which affects the particle size of the powder, is directly influenced by the viscosity of the liquid in the feed of the spray dryer (Schuck et al., 2005).

During IMF production, a low TS level wet-mix (20–30%) is typically obtained in order to avoid problems related to high viscosities. However, prior to spray-drying, the mix is concentrated until 45–55% TS through evaporation, with the aim of reducing costs associated to drying and to improve the quality of the resulting powder (Blanchard et al., 2013). Increasing TS during the wet-mix preparation reduces energy costs associated to water removal during evaporation and spray-drying, and it could allow skipping the evaporation step prior to spray-drying (Murphy et al., 2013). However, the TS level at the feed of the spray dryer is limited by the viscosity.

Thus, increasing TS of the wet-mix during production of IMF is desirable in order to improve energy efficiency. On the other hand, safety and nutritional aspects are of great importance, and consequently the wet-mix needs to be adequately heat treated and emulsified before feeding the spray dryer.

To the best of the authors' knowledge, the combined impact of heat treatment temperature and increasing total solids above 50% on nutritional and technological properties of IMF has not been previously reported. Thereby, this study aims to evaluate the effect of TS and heat treatment temperature of wet-mixes during processing of model infant formula on their rheological, emulsifying and nutritional properties.

2. Materials and methods

2.1. Materials

Low-heat skim milk powder (SMP) Bützower Dauermilchwerk was obtained from Procudan (Kolding, Denmark); whey protein isolate (WPI, Lacprodan®; 89% protein) and lactose (Variolac 992) were obtained from Arla Food Ingredients (Viby J, Denmark). Fructo-oligosaccharides (FOS, Beneo Orafiti P95) with a degree of polymerisation between 2 and 8 was kindly provided by Alsiano (Birkerød, Denmark). Galacto-oligosaccharides (GOS, Promovita®) was kindly provided by Dairy Crest Limited (Edgmond, Newport, United Kingdom). Sunflower oil (SO) was purchased from a local supermarket.

2.2. Experimental overview

Model infant formulae were produced in a pilot plant scale by the wet-mix process under four different processing conditions: TS in the wet-mix = 50 or 60% (w/w) and pasteurisation temperature = 75 or 100 °C. Each formula was produced in duplicates (total number of trials = 8) and each replicate was produced from scratch on a separate day.

2.3. Model infant formulae preparation

Batches (15 Kg) of model bovine-milk-based infant formula (60:40 whey protein-to-casein ratio) were produced using SMP, WPI, lactose, SO, GOS and FOS. The wet-mixes were formulated to give 1:5 and 1:2.5 total protein-to-lactose and total protein-to-oil ratios, respectively. Oligosaccharides (90% GOS, 10% FOS) were added to the mix, to give 0.06 g in 100 mL of reconstituted final formula (based on reconstitution of 12.5 g of powder in 100 mL of water). The target composition of the model IMF was: 11.2% (w/w) protein, 56.0% (w/w) lactose, 28.0% (w/w) fat, 4.32% (w/w) GOS and 0.48% (w/w) FOS, and was selected to fulfil the European compositional requirements (European Commission, 2016). Deionised water was preheated at 65 °C in a Scanima type SRB-20 mixer (Aalborg, Denmark). The ingredients (Table 1) were added in the following order under vacuum suction: lactose, approximately 10% of the total oil (to reduce foam production), WPI, SMP, GOS, FOS, rest of the oil. The pH was adjusted to 6.7–6.8 (T = 65 °C) using KOH 2 M and the mix was stirred at 65 °C for 15 min. TS of the wet-mixes were determined by oven drying (IDF, 1987) in triplicates (Table 1).

Mixtures were pasteurised at 75 °C or 100 °C for 18 s in a Micro-Thermics Lab tubular heat exchanger (Raleigh, NC, USA) at a 1 L min^{-1} flow rate, immediately after dispersion. The flow was estimated to be in laminar regime for all conditions based on an estimated Reynolds number of 50–362 and average velocity 0.26 m s^{-1}. Following pasteurisation, the wet-mixes were homogenised (approx. 60 °C) using a two-stage APV type B5-14-38 homogeniser (Søborg, Denmark) at a first and second stage pressure of 13 and 3 MPa, respectively, with a flow rate of 2 L min^{-1}. The homogenised wet-mixes were spray-dried using a pilot-scale spray dryer GEA Mobile Minor I (Copenhagen, Denmark) equipped with a two-fluid nozzle atomisation system. The feed was kept at 65 °C, the inlet air temperature at 180 °C and the outlet air temperature at 85 °C. The powders were vacuum packed in aluminium bags and stored at 5 °C for further analysis.

2.4. Quantification of native whey proteins

The reduction in the level of native whey protein (WP) after each processing step (dispersion, pasteurisation, homogenisation, and spray-drying) was determined by reverse-phase HPLC, using the method described by Anandharamakrishnan et al. (2008) with modifications. In this method, the amount of native β-lactoglobulin and α-lactalbumin in the supernatant are determined after precipitation at pH 4.6 of denatured WP (Marx and Kulozik, 2018). Samples were accurately weighed in a 50 mL beaker, to obtain a constant total protein concentration of 6.25 g L^{-1} in the final dilution. Approximately 15 mL of Milli-Q water was added and the mix was vigorously stirred for 5 min. The dispersion was adjusted to pH 4.6 by adding HCl 0.1 N and gently stirred for 30 min. The pH was maintained at 4.6 throughout that period. The dispersion was then transferred to a 50-mL volumetric flask, filled to the mark with Milli-Q water and homogenised by shaking, followed by centrifugation at 9834×g and 4 °C for 30 min. The supernatant was filtered through qualitative filter paper and further filtered through a 0.45-μm Ministart syringe filter. The HPLC system used was an Agilent Technologies 1200 series (Santa Clara, United States). Twenty μL of sample were injected into a Zorbax 300SB-C18, 4.6 × 150-mm, 3.5 μm column (Agilent Technologies, Santa Clara, United States). Elution was carried out using a gradient of solvent A (aqueous phase): 0.1% trifluoroacetic acid in Milli Q water and solvent B (organic phase): 0.1% trifluoroacetic acid and 90% acetonitrile in Milli Q water. The elution gradient was set as follows: 0–10 min 10% B; 10–32 min 10–55% B; 32–33 min 55–100% B; 33–43 min 60-36% B; and 15 min for column equilibration. The flow rate was 1 mL min^{-1} and absorbance was measured at 210 and 280 nm. The experiments were carried out in duplicates for each sample. For the determination of the loss of native WP after each processing step, for each sample the sum of the areas corresponding to α-lactalbumin and β-lactoglobulin was determined. The total area obtained after dispersion was considered 100% native

Table 1
Ingredient quantities used in the formulation of the IMF wet-mixes for a 15 Kg batch.

TS (%)	Water (kg)	Lactose (kg)	SMP (kg)	WPI (kg)	SO (kg)	GOS (kg)	FOS (kg)	TS content obtained after dispersion (%, w/w)[a]
50	7.50	3.42	1.14	0.45	2.01	0.44	0.034	48.9 ± 0.7
60	6.00	4.10	1.37	0.54	2.41	0.53	0.041	58.3 ± 1.1

[a] Mean values ± standard deviation obtained for the 50 and 60% TS batches (n = 4).

WP. The level of native WP in each processing step was calculated as a percentage of the area obtained after dispersion.

2.5. Rheological properties

The rheological properties of each wet-mix were determined after dispersion, heat treatment and homogenisation, at 65 °C, using an ARES-G2 rheometer (TA instruments, New Castle, USA), equipped with a concentric cylinder geometry. The shear rate was increased from 0 to $300 \, s^{-1}$ over 5 min, held at $300 \, s^{-1}$ for 2 min and decreased to $0 \, s^{-1}$ over 5 min, as described by Drapala et al. (2017). The average apparent viscosity was determined at $300 \, s^{-1}$. Flow curves (shear stress versus shear rate) were obtained and fitted to the Power law model (Eq. (1)).

$$\tau = K \times \gamma^n \tag{1}$$

where τ is the shear stress (Pa), γ is the shear rate (s^{-1}), K is the consistency coefficient ($Pa.s^n$) and n is the flow behaviour index. The flow behaviour index indicates Newtonian, shear-thinning and shear-thickening flow behaviour when $n = 1$, $n < 1$ and $n > 1$, respectively.

2.6. Oil droplet size distribution

The effect of each processing step in the particle size distribution (PSD) of the wet-mixes was determined by laser light diffraction using a Mastersizer 3000 (Malvern Instrument Ltd, Worcestershire, UK). Oil droplet size was determined by measuring PSD in the wet-mixes after dispersion, heat treatment and homogenisation, as well as in the reconstituted powdered formulae (12.5% w/w in deionised water at 40 °C) to evaluate the effect of spray-drying. For each sample, the PSD was measured twice (each with three replicates). The optical parameters used were 1.46 and 1.33 as particle and dispersant refractive indexes, respectively, and 0.001 as particle absorbance index (Murphy et al., 2015).

2.7. Emulsion stability after reconstitution

Each spray-dried sample was reconstituted in duplicates (12.5% w/w in deionised water at 40 °C) and stirred at 1500 rpm for 15 s. Five mL of reconstituted sample was added into a cylindrical glass tube immediately after stirring and stored at 20.0 ± 1.0 °C. The physical stability was determined by backscattering (BS) measurement at times 0:00, 0:40, 1:20, 2:00, 3:00, 5:00 and 24:00 h, using a Turbiscan MA 2000 (Formulaction, Toulouse, France). The emulsion stability was calculated as a modification of the Turbiscan Stability Index (Eq. (2)).

$$Emulsion \; stability \; index = \frac{\sum_h |BS_i(h) - BS_0(h)|}{H} \tag{2}$$

where: $BS_i(h)$ is the backscattering (%) at time i for position h, $BS_0(h)$ is the backscattering (%) at time 0 for position h and H is the total sample height.

2.8. Available lysine

Available lysine was quantified in the wet-mixes after dispersion, pasteurisation and homogenisation and in the powders (after spray-drying) using the dye-binding method with Acid-orange 12 as described by Aalaei et al. (2016).

Samples after dispersion, pasteurisation and homogenisation were freeze-dried before analysis of available lysine. Briefly, 300 mg of freeze-dried or spray-dried samples and 2 mL of sodium acetate solution (16.4% w/w) were mixed for 20 min on an orbital lab shaker (GFL, Gesellschaft für Labortechnik, Burgwedel, Germany). Then, 0.2 mL propionic anhydride was added to half of the flasks before mixing for another 20 min. Forty mL of the dye solution ($1.36 \, mg \, mL^{-1}$) were then added and mixed for 60 min, followed by centrifugation at 2599×g and

25 °C for 10 min in a 3K15 centrifuge (Sigma, Osterode, Germany). One mL of the supernatant was filtered using 0.45-μm Q-Max syringe filters. Subsequently, 100 μL of the filtered sample was diluted with the buffer to a final volume of 10 mL and the absorbance was measured at 475 nm using a UV-1280 UV–Vis spectrophotometer (Shimadzu, Kyoto, Japan). The concentration of the available lysine was calculated using a calibration curve of the dye in buffer (y = 54.06 x – 0.01; $R^2 = 0.9972$). The results were expressed as % of available lysine in dry matter basis.

2.9. Estimation of energy consumption

In order to estimate the differences in energy consumption for the processing conditions tested (TS = 50 or 60% and heat treatment temperature: 75 or 100 °C), energy and mass balances were carried out for the pasteurisation and spray-drying step, given that those were the processes that differed between the conditions studied herein.

Thus, the energy required to heat (ΔH_{heat}, kJ/s) and cool (ΔH_{heat}, kJ/s) during pasteurisation were estimated according to the energy balances represented in Eqs. (3) and (4).

$$Q_{heat} = \dot{m} \times (Cp_{heat} \times T_{heat} - Cp_{in} \times T_{in}) \tag{3}$$

$$Q_{cool} = \dot{m} \times (Cp_{heat} \times T_{heat} - Cp_{cool} \times T_{cool}) \tag{4}$$

where: $\dot{m}$ is the massic flow of the wet-mix throughout the pasteuriser = 0.02 kg/s; T_{in} is the temperature of the wet-mix at the entrance of the pasteuriser = 65 °C; T_{heat} is the temperature of pasteurisation = 75 or 100 °C; T_{cool} is the temperature of the wet-mix at the exit of the pasteuriser = 60 °C; and Cp_{in}, Cp_{heat} and Cp_{cool} are the specific heat of the wet-mixes at T_{in}, T_{heat} and T_{cool}, respectively. The Cp (kJ/kg K) values for each wet-mix were calculated based in the composition and temperature of the wet-mix according to the model proposed by Choi and Okos (1986) as described in Singh and Heldman (2014).

The energy balance, based on the mass balance of the product, presented in Eq. (5) was used to estimate the evaporation heat (Q_{evap}) required to spray-dry each wet-mix.

$$Q_{evap} = \dot{m}_f \times \left(1 - \frac{TS_f}{TS_p}\right) \times \Delta H_{vap} \tag{5}$$

where: $\dot{m}_f$ (kg/s) is the massic flow at the feed (wet-mix) of the spray-dryer (0.0011 kg/s for 50% TS wet-mix and 0.0009 kg/s for 60% TS); TS_f and TS_p (%) are the total solids level of the feed (wet-mix) and powder, respectively; and ΔH_{vap} is the specific heat of vaporisation of water at atmospheric pressure = 2258 kJ/kg.

2.10. Statistical analysis

A factorial analysis of variance (ANOVA) was performed to determine the effect of the TS in the wet-mix, heat treatment temperature, processing step and their interactions. Tukey's multicomparison test or *t*-Student test were employed. The level of significance was determined at P < 0.05. Analyses were carried out using JMP® 12.1.0 software (SAS Institute Inc., North Carolina, USA).

3. Results and discussion

3.1. Native whey proteins

The temperature of the heat treatment, the processing step, and their interaction significantly affected (P < 0.001) the level of native WP. Pasteurisation at 75 °C reduced the native WP by 8.5 and 6.5% in the samples with 50 and 60% TS, respectively (Fig. 1). Applying a heat treatment at 100 °C drastically reduced the native WP to 19.8 ± 0.3% in the 50% TS mix and 16.8 ± 3.5% in the 60% TS mix. The homogenisation and spray-drying steps, at the conditions used in the current study, did not contribute to the WP denaturation in any of the samples.
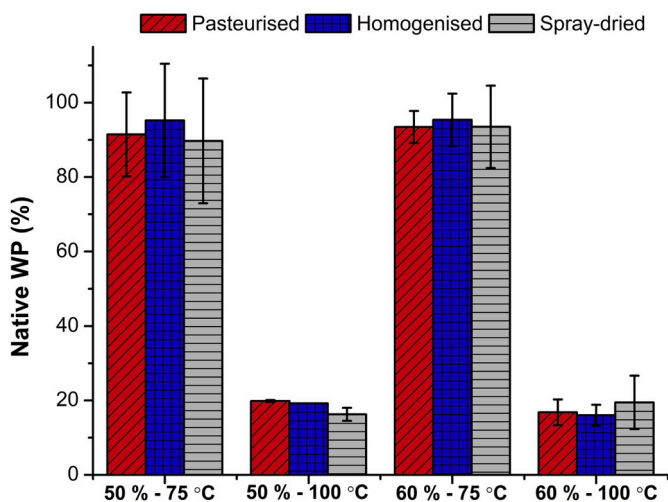
Fig. 1. Level of native whey proteins (WP) obtained in the model infant formulae after pasteurisation, homogenisation and spray-drying, expressed as a percentage of the level after dispersion. Error bars represent standard deviation from production replicates (n = 2).

These results are in agreement with previous studies showing that the main step responsible for the irreversible denaturation of WP during milk powder manufacture is pasteurisation (Guyomarc'h et al., 2000; Murphy et al., 2013; Oldfield et al., 2005). During spray-drying, the temperature of the droplets varies between the wet bulb temperature and the outlet air temperature. The hot air at the inlet evaporates water

from the surface, cooling the droplet. As the droplet falls through the spray dryer the temperature increases, approaching the outlet air temperature but only when most of the water has been evaporated and the proteins cannot undergo conformational changes (Guyomarc'h et al., 2000; Oldfield et al., 2005).

The TS in the wet-mix did not significantly affect the level of native WP (P > 0.05). Increasing TS concentration has been shown to reduce denaturation, due to the increased concentration of lactose that exerts a protective effect over β-lactoglobulin (Anema et al., 2006). On the other hand, increasing the TS level also means increasing the total protein and WP concentration, which has been shown to increase the extent of denaturation (Law and Leaver, 1999).

3.2. Rheological properties of wet-mixes

Wet-mixes produced under different conditions showed different flow behaviours after dispersion, heat treatment and homogenisation (Fig. 2). Those that are reflected in changes in apparent viscosity (η_{300}), consistency coefficient (K) and flow behaviour index (n) were significantly affected (P < 0.05) by TS, heat treatment temperature, processing step and all their interactions (Table 2).

As expected, dispersed wet-mixes with 60% TS had higher apparent viscosity compared to 50% TS (Table 2). Similar results were obtained for K, while n was not significantly affected (P > 0.05) by TS, averaging 0.95 ± 0.04, which indicates Newtonian behaviour (Fig. 2). The effect of the heat treatment on the viscosity also depended on the temperature. Pasteurisation at 100 °C clearly modified the flow curves of wet-mixes (Fig. 2). At 75 °C, viscosity was not significantly affected (P > 0.05) by pasteurisation, while the heat treatment at 100 °C increased (P <
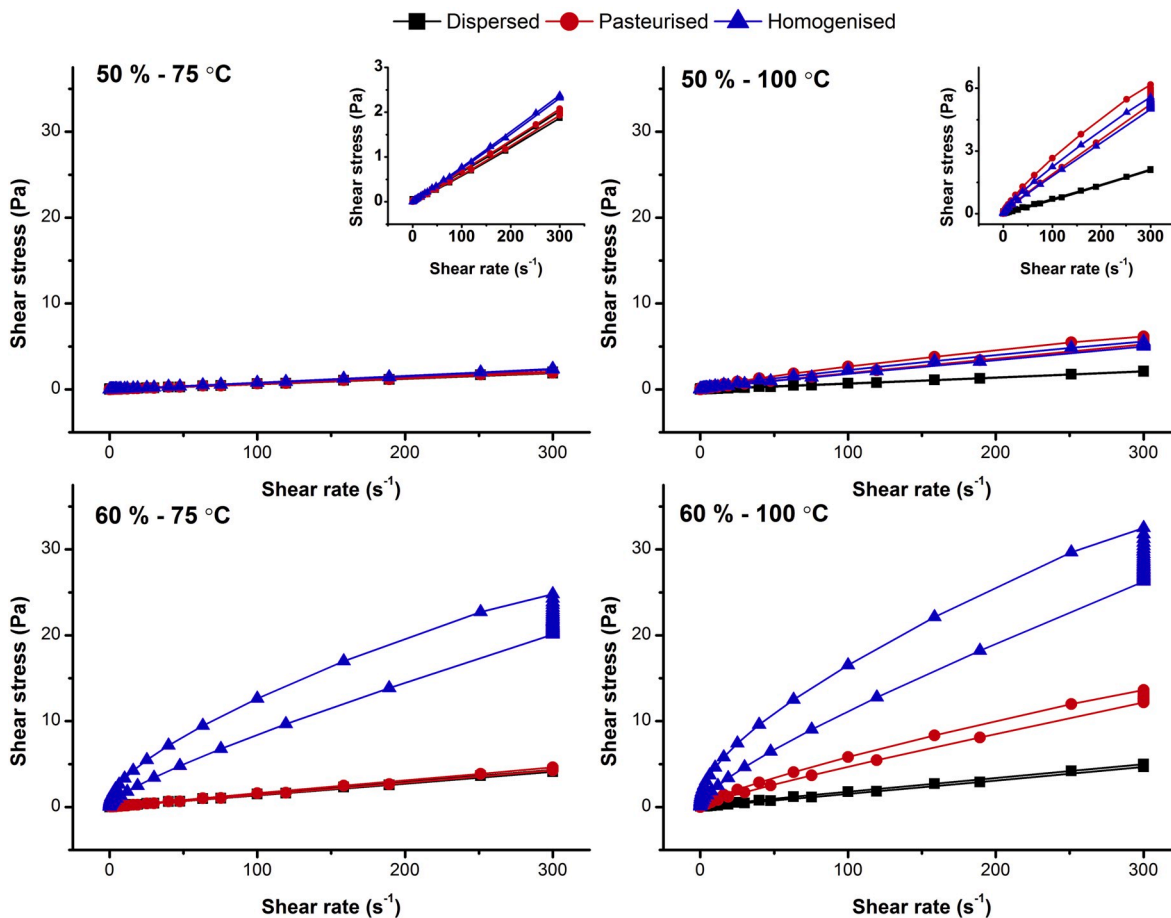

Fig. 2. Flow curves of the model infant formulae wet-mixes produced under different processing conditions (50 or 60% TS, Heat treatment: 75 °C or 100 °C x 18 s), after dispersion, pasteurisation and homogenisation.

Table 2

Rheological properties of the model infant formula wet-mixes following dispersion, heat treatment and homogenisation.

Condition	Dispersed	Pasteurised	Homogenised
	η_{300} (mPa.s)		
50% - 75 °C	6.3 ± 0.6[bA]	6.5 ± 0.2[cA]	7.7 ± 0.2[dA]
50% - 100 °C	7.6 ± 0.8[bB]	18.8 ± 0.4[bA]	17.6 ± 0.5[cA]
60% - 75 °C	15.7 ± 2.1[aB]	15.9 ± 1.5[bB]	49.5 ± 1.9[bA]
60% - 100 °C	16.0 ± 0.2[aC]	46.7 ± 2.9[aB]	95.9 ± 1.0[aA]
	K (Pa.s^n; × 10^2)		
50% - 75 °C	0.7 ± 0.0[bA]	0.7 ± 0.1[aA]	0.6 ± 0.2[bA]
50% - 100 °C	0.7 ± 0.0[bA]	0.8 ± 0.3[aA]	0.7 ± 0.0[bA]
60% - 75 °C	2.3 ± 0.5[aB]	2.2 ± 0.4[aB]	47.8 ± 0.2[bA]
60% - 100 °C	3.0 ± 0.3[aB]	26.7 ± 13.8[aB]	139.8 ± 33.2[aA]
	n		
50% - 75 °C	0.98 ± 0.01[aA]	0.99 ± 0.01[aA]	1.06 ± 0.06[aA]
50% - 100 °C	0.98 ± 0.04[aA]	0.99 ± 0.03[aA]	1.02 ± 0.00[aA]
60% - 75 °C	0.94 ± 0.02[aA]	0.95 ± 0.01[aA]	0.62 ± 0.01[bB]
60% - 100 °C	0.90 ± 0.03[aA]	0.71 ± 0.08[bAB]	0.55 ± 0.04[bB]

η_{300} = apparent viscosity measured at 300 s^{-1} and 65 °C, K = consistency index and n = flow behaviour index.

Mean ± standard deviation from triplicate analysis and duplicate production trials.

For a given parameter (η, K or n), different lowercase letters indicate significant differences (P < 0.05) between conditions and different uppercase letters indicate significant differences (P < 0.05) between processing steps.

0.05) viscosity. These results can be explained by the WP denaturation and aggregation observed in mixes pasteurised at 100 °C (Fig. 1). Heating produces denaturation which increases the WP voluminosity but also the effective casein micelle volume fraction, as a result of interactions between WP and casein micelles and association between casein micelles (Jeurnink and De Kruif, 1993). β-Lactoglobulin, the primary WP, is a compact globular protein with two disulphide bridges and a buried sulfhydryl group per monomer. After denaturation, this free sulfhydryl group becomes exposed and free to participate in intermolecular disulphide-sulfhydryl interchanges, leading to WP-WP aggregation as well as WP-casein (mostly κ-casein) aggregation (Wijayanti et al., 2019).

Heating the 60% TS wet-mix at 100 °C clearly changed its rheological behaviour to shear-thinning (n = 0.71). On the other hand, while after pasteurisation the flow curve of the 50% - 100 °C wet-mix showed a trend to shift from Newtonian towards shear-thinning thixotropic behaviour (Fig. 2), this change was not significant as indicated by n (Table 2).

The effect of the homogenisation was strongly influenced by the TS level. For wet-mixes with 50% TS the homogenisation did not have any significant impact (P > 0.05), while for wet-mixes with 60% TS it affected the flow behaviour, increasing η_{300} and K and reducing n (Fig. 2., Table 2). At the feed of the spray dryer (after homogenisation), mixes with 60% TS showed a reduction in n, as expected due to the increase in viscosity, and a stronger shear-thinning behaviour (n = 0.6). Furthermore, the greatest hystereses were observed for homogenised wet-mixes with 60% TS (Fig. 2). Ramp-up curves were above ramp-down curves, indicating thixotropic behaviour. The presence of thixotropy suggests a structure break down by shearing. Previous studies have shown an increase in the viscosity of the wet-mix after homogenisation (Kelly et al., 2016). The 60% TS wet-mixes have larger amount of particles leading therefore to higher level of interaction. In emulsion systems, shear-thinning can be explained by flocculation of oil droplets, which is denser as TS level of the emulsion increases (Drapala et al., 2017). The viscosity and rheological behaviour of the liquid at the entrance of the spray dryer directly affect the particle size of the resulting powder. Moreover, high viscosities reduce the drying efficiency due to formation of large droplets. Westergaard (2011) suggests that the viscosity of whole milk concentrate at the feed of the spray dryer

should be kept below 60 mPa s, while Blanchard et al. (2013) recommend not exceeding 200 mPa s. In the present study, the maximum apparent viscosity at the feed of the spray dryer was 95.9 mPa s, measured at 300 s^{-1} and 65 °C, for the 60% TS wet-mix heated at 100 °C.

3.3. Oil droplet size

PSD of the oil droplets in model IMF during processing showed significant effects of the pasteurisation and homogenisation steps, depending on the temperature of pasteurisation and TS (Fig. 3). After dispersion, oil droplets size ranged 10–80 μm. A wider peak was observed after pasteurisation, but only at 100 °C (Fig. 3). Homogenised wet-mixes showed bimodal distributions, with the largest population of approximately 0.2–11 μm for the 50% TS wet-mix and 2–60 μm for the 60% TS wet-mix. Therefore, homogenised wet-mixes with the highest viscosities and consistencies and a shear-thinning behaviour (60% TS, Table 2), also presented larger oil droplets (Fig. 3).

Reconstituted spray-dried model formula produced upon the 60% - 100 °C condition showed a shift of the peak towards smaller particles. This result is in line with previous studies reporting a reduction in the particle size after spray-drying, due to a disruption of the oil droplets during atomisation (McCarthy et al., 2012; Ye et al., 2007). On the other hand, a peak ranging from 2 to 30 μm appeared after reconstitution of the 50% - 100 °C spray-dried sample. This result can be explained by the flocculation of small oil droplets during reconstitution.

To better compare the results, the volume mean diameter (D$_{4,3}$) was calculated. Factorial ANOVA showed that oil droplet size was significantly affected by TS in the wet-mix (P = 0.0021), the processing step (P < 0.001) and the interaction between heat treatment temperature and processing step (P = 0.0036). Simple ANOVA were also carried out in order to compare individual conditions and processing steps. Table 3 shows that D$_{4,3}$ was above 1 μm for most of the homogenised mixes, which could have a negative impact on the emulsion stability (Kelly et al., 2016). Although it should be noticed that the model formulae were produced without any non-protein emulsifier, such as lecithin, that are commonly used in the production of commercial formulae (McSweeney, 2008). The homogenised wet-mixes with the lowest and highest D$_{4,3}$ were 50%–75 °C and 60% - 100 °C, respectively (Table 3), which were also the conditions with the lowest and highest viscosities. Previous studies have shown that smaller oil droplet sizes after homogenisation led to lower viscosities (Floury et al., 2000; Kelly et al., 2016; Maher et al., 2014). In the present study, homogenised 60% TS wet-mixes pasteurised at the highest temperature (100 °C) led to emulsions with larger oil droplets. Protein aggregates formed during heat treatment which are not adsorbed in the oil droplet surface, remain in suspension in the continuous phase. At high TS levels, the distance between molecules is reduced and interactions between proteins adsorbed and non-adsorbed in the oil droplet surface are enhanced. These interactions could lead to the formation of larger oil droplets and emulsions with increased viscosity (Buggy et al., 2017).

Even though differences in the PSD of samples before and after reconstitution were evident (Fig. 3), especially for samples preheated at 100 °C, no significant differences (P > 0.05) were obtained in the D$_{4,3}$ values before and after spray-drying (Table 3).

3.4. Emulsion stability of the reconstituted IMF

The stability of the formulae after reconstitution was measured by means of Turbiscan (Fig. 4). An increase of the BS at the top of the samples together with a decrease in the lower part of the samples indicate the appearance of cream at the surface, as a result of flocculation and emulsion instability. This phenomenon was observed for all conditions 24 h after reconstitution. At that time, sample 60% - 75 °C presented the highest instability (Fig. 4), even though D$_{4,3}$ after reconstitution was higher in sample 60% - 100 °C (Table 3). It has been reported that denaturation and aggregation of preheated WP produced
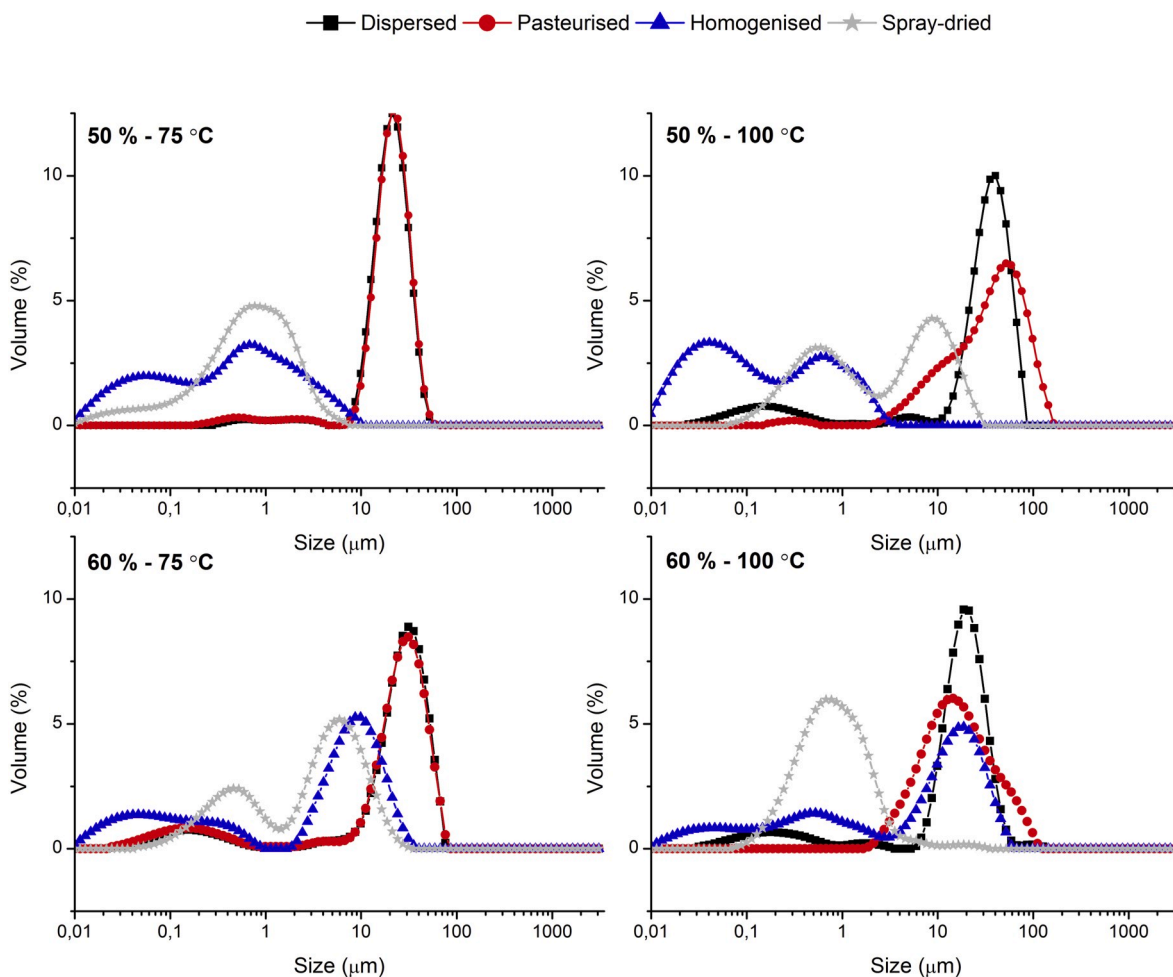
Fig. 3. Particle size distribution of the model infant formulae produced under different wet-mix processing conditions (50 or 60% TS, Heat Treatment: 75 °C x 18 s or 100 °C x 18 s), after dispersion, pasteurisation, homogenisation and spray-drying.

Table 3
Volume mean diameter ($D_{4,3}$; μm) of the model infant formula wet-mixes following dispersion, heat treatment, homogenisation and spray-drying and reconstitution.

Condition	Dispersed	Pasteurised	Homogenised	Spray-dried and reconstituted
50% - 75 °C	26.1 ± 4.8[aA]	28.1 ± 7.5[aA]	0.8 ± 0.1[bB]	1.0 ± 0.2[cB]
50% - 100 °C	22.2 ± 1.2[aA]	24.2 ± 3.0[aA]	5.8 ± 2.0[abB]	6.8 ± 0.1[abB]
60% - 75 °C	30.8 ± 3.6[aA]	31.8 ± 4.2[aA]	6.9 ± 3.0[abB]	4.9 ± 1.0[bcB]
60% - 100 °C	23.0 ± 3.1[aAB]	27.4 ± 2.7[aA]	14.8 ± 4.3[aAB]	11.1 ± 2.0[aB]

Mean ± standard deviation from duplicate analysis and duplicate production trials.
Different lowercase letters indicate significant differences ($P < 0.05$) between conditions (within each column). Different uppercase letters indicate significant differences ($P < 0.05$) between processing steps (within each row).

emulsions with larger oil droplets but with improved stability due to the increase of the viscosity of the continuous phase (Dapueto et al., 2019; Dybowska, 2011). Obtaining a stable emulsion during IMF processing minimises surface free fat in the powders and protects against creaming in reconstituted IMF (Murphy et al., 2015). Samples preheated at 100 °C, which had extensive WP denaturation, were stable for more than 5 h, since the significant increase in the stability index (or reduction in the
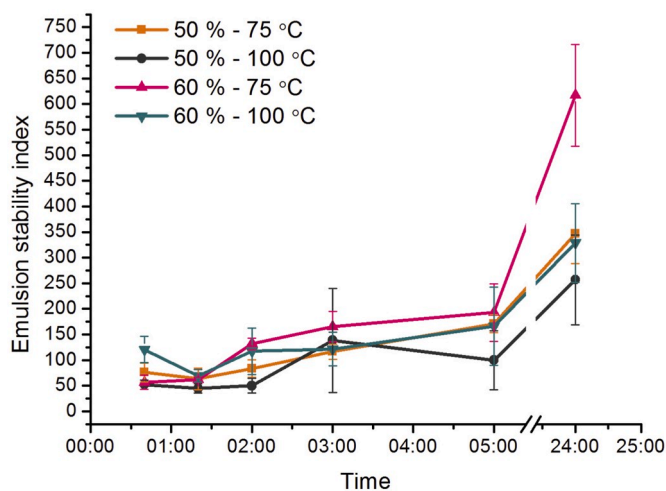

Fig. 4. Emulsion stability index of the model infant formulae produced under different wet-mix processing conditions (50 or 60% TS, Heat Treatment: 75 °C x 18 s or 100 °C x 18 s), during storage at 20 °C. Higher values of the emulsion stability index indicate less stability.

emulsion stability) was observed only at 24 h. On the other hand, samples 50% - 75 °C and 60% - 75 °C showed significant destabilisation ($P < 0.05$) at 5 and 3 h, respectively. Overall, IMF samples produced

under the four conditions were stable for at least 2 h after reconstitution, which can be considered representative of the time within formulae are typically consumed after reconstitution (Murphy et al., 2015).

3.5. Available lysine

Available lysine was significantly affected (P < 0.01) by the heat treatment temperature, the processing step and the interaction between the latter. The concentration of available lysine in the samples heated at 75 °C remained constant (P > 0.05) throughout processing, for both TS levels (Table 4). On the other hand, when the pasteurisation was carried out at 100 °C a significant loss of available lysine was observed after the spray-drying step (32% and 40% reduction for the 50% and 60% TS wet-mixes, respectively). Loss of available lysine occurs during the early stages of Maillard reactions, where the ε-amino group of lysine reacts with the carbonyl groups of reducing carbohydrates, such as lactose (Schmitz-Schug et al., 2013). Heat treatment at 100 °C reduced significantly the level of native WP in comparison to heat treatment at 75 °C (Fig. 1). When WP are denatured, the globular native structure is unfolded and the reactive groups exposed. Hence, this reaction would be expected to increase the accessibility of the groups involved in the Maillard reaction, reducing the concentration of available lysine, while aggregation would generate the opposite effect (O'Mahony et al., 2019). The results obtained in the current work showed that the wet-mixes subjected to higher temperatures, and thus with less percentage of native WP, had a significantly higher (P < 0.05) loss of available lysine. However, the loss was only evident after spray-drying. Optimal conditions for lysine loss include dry state rather that liquid state and low to moderate water activities (Schmitz-Schug et al., 2013). Moreover, spray-drying has been identified as the critical point for lysine loss during IMF wet-mix processing (Ferrer et al., 2000). Therefore, it can be hypothesized that during pasteurisation at 100 °C amino groups present in WP became more accessible, but it was not until spray-drying, that the optimal conditions for the early stages of Maillard reactions were reached (low a_w, higher concentrations of proteins and carbohydrates), leading to the loss of available lysine.

Aalaei et al. (2019) determined the available lysine content by the dye-binding method with Acid-orange 12, in four powdered commercial IMF, and obtained higher concentrations (0.95–1.13% in dry matter) than the ones obtained for the spray-dried samples in the present study (Table 4). This difference can be explained by the intensity of the applied heat treatments and the ingredients (and their thermal history) used for the IMF production.

3.6. Energy consumption

In order to estimate the differences in the energy required to produce IMF powder by the four studied conditions, energy balances were carried out for the pasteurisation and spray drying steps, based on the pilot scale experimental set up used in the current study (Table 5). The pasteurisator and spray dryer have significantly different feed flows and consequently the required energy consumption for the two unit

operations cannot be compared, and the total energy is specific for this study. The results showed that reducing heat treatment temperature from 100 to 75 °C for 50 or 60% TS wet-mixes, resulted in a decrease of energy consumption of approx. 72% at the heating zone and 63% at the cooling zone. Furthermore, it took in average approx. 6% more energy at the heating and cooling zones, to pasteurise the 50% TS wet-mixes than the 60% TS wet-mixes (Table 5). This result can be explained by the higher water content in the 50% TS wet-mixes (approx. 3.1 kJ/kg K) compared to the 60% TS wet-mixes (approx. 2.8 kJ/kg K) (Choi and Okos, 1986). At the spray dryer, the energy required to evaporate water was 32% higher for the 50% TS wet-mixes compared to the 60% TS ones (Table 5). This result was expected as consequence of the difference in the water content of both wet-mixes.

Thus, in our pilot scale study, increasing the TS level from 50 to 60% allows reducing the total energy consumption by 17%, for heat treatments at 75 °C, and by 13%, for heat treatments at 100 °C. Moreover, when the TS level is increased form 50–60% and the heat treatment temperature reduced from 100 to 75 °C, the total energy reduction reaches 59%.

4. Conclusions

Model IMF were produced from high dry matter wet-mixes. TS level in the wet-mix and pasteurisation temperature affected the rheological, emulsifying and nutritional properties of the formulae.

Increasing pasteurisation temperature from 75 to 100 °C drastically increased WP denaturation. After spray-drying, IMF heat treated at 100 °C had reduced the level of native WP by 82%, while samples heated at 75 °C had an 8% loss. IMF powders preheated at 100 °C, which had extensive WP denaturation, showed better emulsion stability (stable for at least 5 h) than powders preheated at 75 °C. The high level of denaturation modified the rheological properties of the wet-mixes, significantly increasing viscosity and consistency index after pasteurisation at 100 °C. Flow behaviour index was dependant on the TS level. While 50% TS wet-mixes had a Newtonian behaviour (n = 1) throughout processing, 60% TS heated at 100 °C shifted to shear-thinning (n < 1) during pasteurisation and 60% TS wet-mixes heated at 75 °C shifted during homogenisation step.

After pasteurisation at 100 °C, oil droplet size distribution showed a wider peak. At the feed of the spray dryer (homogenised wet-mixes) samples with 60% TS had larger oil droplets.

Powdered infant formulae previously pasteurised at 100 °C had an

Table 4
Available lysine (% in dry matter) in the model infant formulae following dispersion, heat treatment, homogenisation and spray-drying.

Condition	Dispersed	Pasteurised	Homogenised	Spray-dried
50% - 75 °C	0.70 ± 0.03[aA]	0.72 ± 0.02[aA]	0.70 ± 0.01[bA]	0.70 ± 0.02[aA]
50% - 100 °C	0.74 ± 0.04[aA]	0.74 ± 0.01[aA]	0.75 ± 0.01[aA]	0.51 ± 0.02[bB]
60% - 75 °C	0.70 ± 0.01[aA]	0.69 ± 0.01[aA]	0.69 ± 0.01[bA]	0.73 ± 0.01[aA]
60% - 100 °C	0.71 ± 0.04[aA]	0.71 ± 0.01[aA]	0.75 ± 0.02[aA]	0.43 ± 0.06[bB]

Mean ± standard deviation from duplicate analysis and duplicate production trials.

Different lowercase letters indicate significant differences (P < 0.05) between conditions (within each column). Different uppercase letters indicate significant differences (P < 0.05) between processing steps (within each row).

Table 5
Estimated energy consumption during pasteurisation and spray-drying in the pilot plant experimental set up used.

Condition	Pasteurisation			Spray-drying		Total Energy
	$\dot{m}$ (kg/s)	Q_{heat} (kJ/s)	Q_{cool} (kJ/s)	$\dot{m}_f$ (kg/s)	Q_{evap} (kJ/s)	Q_{total} (kJ/s)
50% - 75 °C	0.020	0.56 ± 0.01[c]	0.83 ± 0.01[c]	0.0011	1.34 ± 0.01[a]	2.73 ± 0.02[c]
50% - 100 °C	0.020	1.96 ± 0.01[a]	2.23 ± 0.01[a]	0.0011	1.34 ± 0.03[a]	5.53 ± 0.04[d]
60% - 75 °C	0.020	0.52 ± 0.01[d]	0.78 ± 0.01[d]	0.0009	0.96 ± 0.09[b]	2.26 ± 0.10[a]
60% - 100 °C	0.020	1.83 ± 0.01[b]	2.09 ± 0.01[b]	0.0009	0.88 ± 0.04[b]	4.80 ± 0.02[b]

$\dot{m}$ = pasteuriser feed flow, Q_{heat} = energy required at the heating zone of the pasteuriser, Q_{cool} = energy required at the cooling zone of the pasteuriser, $\dot{m}_f$ spray dryer feed flow, Q_{evap} = energy required to evaporate water during spray-drying, Q_{total} = energy required to pasteurise + energy required to evaporate water during spray-drying.

Mean ± standard deviation from production trials.

Different lowercase letters indicate significant differences (P < 0.05) between conditions (within each column).

available lysine content 34% lower than samples pasteurised at 75 °C.

The current study showed that is possible to increase TS in the wet-mix to 60% if pasteurisation is carried out at 75 °C, obtaining an adequate viscosity at the feed of the spray dryer ($<$60 mPa s), low WP denaturation and available lysine loss in the final product and 2-h-stability after reconstitution. Moreover, heating at 75 °C resulted in a reduction in energy consumption during pasteurisation and increasing TS in the wet-mix from 50 to 60% resulted in a significant reduction of the total energy consumption, estimated in 17% at the conditions of our study. These findings show the potential improvement in energy efficiency that could be achieved while maintaining the product quality, by studying the effects of combined processing conditions. Further studies will focus on understanding the effects of varying TS and pasteurisation conditions on the physicochemical and functional characteristics of the resulting powders.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Mariana Rodríguez Arzuaga: Conceptualization, Methodology, Investigation, Formal analysis, Writing - original draft. **Kataneh Aalaei:** Investigation, Writing - review & editing. **Denise Felix da Silva:** Investigation, Writing - review & editing. **Sylvain Barjon:** Methodology, Investigation. **María C. Añón:** Conceptualization, Writing - review & editing, Supervision. **Analía G. Abraham:** Conceptualization, Writing - review & editing, Supervision. **Lilia Ahrné:** Conceptualization, Methodology, Resources, Writing - review & editing, Supervision, Project administration, Funding acquisition.

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