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Spray and Freeze Drying of Human Milk on the Retention of Immunoglobulins (IgA, IgG, IgM)

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Abstract

Several freeze-drying and spray-drying methods were investigated in relation to the retention of immunoglobulins (Ig) A, IgG and IgM. Spray drying produced human milk powders with 2% humidity and a good retention of IgG (>88%) and IgM (~70%). However, only 38% of IgA remained after spray-dying. For freeze-drying, only the highest heating plate temperature used in this study (40°C) brought IgA content down to 55% in a powder with 1.75% residual humidity, whereas milk samples undergoing lower temperatures had higher preservation rates (75% for IgA and 80% for IgG and IgM) and higher residual moisture contents. From these results, it can be concluded that IgA is the most sensitive Ig lost during drying processing of human milk. The best method to generate human milk powders without a significant loss of Ig was thus freeze-drying at 30°C heating plate temperature, which accelerated the process compared to lower processing temperatures, but still had good overall Ig retention.

KEYWORDS: human milk, freeze-drying, spray-drying, immunological properties

INTRODUCTION

Mother's milk contains compounds that greatly contribute to the development of immune and digestive systems, as to the general growth and immunological support of infants (1). Among a wide array of important compounds such as vitamins and fatty acids, immunoglobulins (Ig) A, IgG, IgM and IgD are all found in human milk. Of these, IgA, which appears to be both synthesised and stored in the breast (2) plays a crucial role. Indeed, IgA sheets the intestinal epithelium, thereby protecting the mucosal surfaces against entry of pathogenic bacteria and enteroviruses. It bestows protection against *E. coli*, *Salmonellae*, *Shigellae*, *Streptococci*, *Staphylococci*, *Pneumococci*, poliovirus and the rotaviruses (3).

When the mother's own milk is unavailable, the American Academics of Pediatrics (4) recommends using donor milk. For this purpose, human milk banks have been created. To reduce the risk of microbial contamination that can occur during collection and handling of human milk, it has to be pasteurized to reduce the number of viable pathogens (5). The guideline for human milk banks in the USA and in Spain is to use low-temperature long-time pasteurization (6), usually 62.5^{o}C for 30 minutes. A study done by Evans et al. (7) on pasteurization of human milk for 30 minutes and temperatures ranging from 60^{o}C to 70^{o}C showed that IgA was preserved with relative little loss until 70^{o}C (33% loss), while IgG and lactoferrin were much more labile, displaying losses of 77.2% and 85%, respectively, at 65^{o}C. Following heating during 30 min at 62.5^{o}C, the initial contents of IgG, lactoferrin and lysozyme were reduced by 34%, 57%, and, 23%, respectively, while IgA content remained stable (7). However, Permanyer et al. (6) found

that pasteurization of human milk, using the same heating conditions, induced a 30% decrease in IgA. Other reports indicate that IgA is stable up to 56^{o}C, but heat-labile at 62.5^{o}C (8) and that temperature but not process time is a critical parameter in determining IgA stability (9). Studies by Friend et al. (10) reported reductions of 47%, 55%, 39% and 73% of lactoperoxidase, lipase, lysozyme and protease, respectively, after pasteurization of human milk at 62.5^{o}C for 30 minutes. Hence, even if human milk is safe for consumption from a microbiological point of view after low temperature-long-time pasteurization, the potential detrimental effect of this traditional type of preservation method on bioactive compounds within human milk should also be taken into account.

Storage of human milk was studied after pasteurization (7; 10), cooling or freezing (7, 11), and freeze-drying (7, 10, 12). Recently, Lozano et al. (12) suggested that the stability of human milk, in terms of vitamins, fatty acids and antioxidant levels, was higher in freeze-dried milk powders than the reported values for frozen or fresh milk after the same length of storage.

Milk can be converted into shelf-stable powders by spray drying or freeze-drying methods. Spray drying is a dehydration method where a liquid/slurry is sprayed in fine droplets in contact with air at elevated temperatures. This method is commonly used to dry milk, whey, yeast, and other high valuable products in industry due to the good final quality of spray-dried powders. Feed flow rate, atomizer rotation speed, and inlet air temperature have been identified as key parameters affecting powder quality during spray drying dairy emulsions such as whole milk (13). Energy consumption is, however, a

restriction in the widespread use of this drying method. In addition, the oxygen present in the large volumes of air mixed with the droplets as well as the high operating temperatures during spray drying could have a negative impact not only in fat-soluble vitamins and in CLA contents in milk due to oxidation but also in other heat-labile compounds such as IgG and IgA. To the best of our knowledge, no studies have been reported on spray-drying of human milk.

Freeze-drying is an alternative dehydration method based on sublimation of the ice contained in a frozen material, and is recognized for producing final products of the highest quality (14). This method is an expensive process due to increased energy consumption during the long processing times under vacuum and thus, its application to the food industry has been limited. Freeze-drying of human milk (previously frozen at -80^{o}C) during 24 hours in a benchtop unit at 10^{-3} mBar pressure and -46^{o}C condenser temperature was proposed as a good alternative to preserve human milk (12). When compared to frozen milk at -20^{o}C, the concentrations of vitamins C and E as well as antioxidant capacity are better retained in freeze-dried human milk. In terms of Ig, a previous study done by Evans et al. (7) suggests that deep freezing at -20^{o}C is a satisfactory procedure compared to the more expensive freeze-drying (no operating conditions specified), which showed no additional benefit in this study.

Most of the previous studies on preservation of human milk have used pasteurization or heat treatments, followed by cool storage or freezing, providing recommendations mainly focused on bacterial content. However, the combination of both processes (heat

processing and storage conditions) can lead to a decrease in bioactive compounds, such as Ig. Other preservation methods like freeze-drying have been studied from the standpoint of feasibility and final quality of the product, but little has been analyzed on the determination of optimal process conditions in order to decrease costs. Also, no previous studies have addressed the effect of human milk dehydration processes on bioactive immunological components. Furthermore, the use of a dehydration method to preserve mother's milk could be more beneficial than pasteurization by producing a powder that can be stored at cold temperatures for long times without loss in bioactive properties. Thus, the aim of this work was to study freeze-drying and spray drying methods with different operating conditions for the conservation of human milk with specific focus on Ig preservation. Determinations of sorption isotherms and glass transition temperature of the final powders complete this work in order to have indicators about their specific storage conditions.

MATERIALS AND METHODS

Ethical Considerations

This study was approved by the Ethical Research Committee of Université Laval (Québec, Canada) in April 2014 (# 2014-034/14-04-2014). Volunteer donors provided a written agreement about the donation of excess human milk for this study.

Human Milk Samples

Surplus human milk was obtained from healthy mothers during lactation between 4 to 8 months after giving birth. Milk was collected in their home using an electric extraction

pump (Medela®), placing the collected milk in 'Pump and Save Bags' (Medela®), stored afterwards in a freezer at -18°C until further processing.

Human Milk Preparation

All human milk samples (for spray or freeze-drying) were thawed in their storage bags in a water bath at 30°C for 20-30 minutes. Several samples (n=10) were randomized and pooled for each repetition. The pooled milk was subjected to one cycle of homogenisation at 5000 psi in an Emulsiflex C50 (Avestin®, Mannheim, Germany).

Freeze-Drying Of Human Milk

Thirty (30) mL of pooled milk were poured in Petri dishes of 9-cm external diameter; the liquid samples having an approximately thickness of 8 mm. The Petri dishes were covered and placed inside a Sanyo medical freezer (MDF 235, Gunma, Japan) at -40°C for a minimum of 9 hours. Then, the samples were placed inside the drying chamber of a Virtis freeze-dryer (Unitop 4001, Gardinier, NY, USA), working under vacuum of less than 30 mTorr, at -85°C condenser temperature (condenser separated from the drying chamber), and at 20, 30, 40 °C heating plate temperatures. In order to establish freeze-drying kinetics, and thus to estimate the final freeze-drying time at each heating plate temperature, drying curves were obtained by periodically weighing the milk samples for up to 10 hours. The temperature at the center of the milk sample was followed throughout the freeze-drying process with a T thermocouple (TMQSS-040G-18, Omega, Stamford, CT, USA), which was inserted in the sample center prior to freezing. Final humidity was determined as described later.

Simplified mathematical models were used to quantify drying kinetics of various food products (15). In this study, experimental data were fitted to the Page's equation (16):

$$\frac{X - X_e}{X_0 - X_e} = \exp\left(-k\,t^n\right) \qquad (1)$$

where X, X_o and X_e are moisture content in dry basis, initial and equilibrium moisture content, respectively, k is the drying constant (h^{-n}), n is the Page's model parameter, and t is the process time (h). Several previous works have suggested that X_e can be neglected in Eqn. (1) since it is significantly lower than moisture content for most of the drying process (17).

Spray-Drying Of Human Milk

Homogenized human milk was kept at 30°C until the spray drying process. Spray-drying was done in a Niro-Atomizer pilot unit with conical base (Model 209/S, Soeborg, Denmark) fed with a Watson Marlow® (Model 503U) peristaltic pump and a nozzle atomizer (3 bar pressure). In order to maximize the yield, preliminary tests based on overall performance and final powder humidity were done, from which the following spray-drying operation variables were selected: 180°C and 160°C inlet air temperature with feeding rates of 5 and 4 mL/min, respectively. Outlet air temperature was recorded and yield could be estimated from gravimetric measurements. Final humidity was determined as described later.

In both spray-drying and freeze-drying processes, the final product was kept in dark conditions, in desiccators at 5°C with the presence of Drierite®, until further analysis.

Humidity And Dry Mass Determination

Total solids and humidity of the dried samples were determined with a halogen balance HR73-P (Mettler Toledo®, Greifensee, Switzerland). Humidity was determined on a wet basis in duplicate measurements.

Immunoglobulin Determination

Human milk powders (1g) were rehydrated to their initial moisture content by dissolution in distilled water (approximately 7.15 mL) at room temperature, followed by agitation homogenization and centrifugation at 1500 rpm for 5 min in order to obtain the Ig in the whey, which was separated and analyzed by ELISA.

ELISA quantification was performed as previously described (18) to determine the concentrations of Ig. Goat antibodies specific to the Fc fragment of human IgG, IgM or IgA were used for capture, and the corresponding HRP-conjugated antibodies, for detection (all purchased from Jackson Immuno Research Laboratories Inc., West Grove, PA). The standard curves were performed using IgA from human colostrum (1 to 10 ng/mL, Sigma-Aldrich Saint Louis, USA), IgG from human serum (2.5 to 50 ng/mL, Sigma-Aldrich Saint Louis, USA) and IgM from human serum (2.5 to 50 ng/mL, Sigma-Aldrich Saint Louis, USA). The results were expressed in micrograms per milliliter (µg/mL) of rehydrated milk.

Sorption Isotherms

Lithium chloride (LiCl), sodium chloride (NaCl), sodium bromide (NaBr), magnesium chloride ($MgCl_2$) and potassium acetate (CH_3COOK) saturated salt solutions were prepared according to the method described by Ratti et al. (19). Relative humidity of the solutions was verified at ambient temperature (20°C) with an AquaLab (Series 3, Decagon Devices Inc, Pullman, Washington, USA): 14.1% (LiCl), 75.6% (NaCl), 57% (NaBr), 33% ($MgCl_2$) and 25.3% (CH_3COOK). Freeze-dried and spray-dried human milk powders (approximately 300 mg) were placed in aluminium cups over the saturated salt solutions in desiccators at constant temperature (20°C) until equilibrium was reached in approximately 7 days.

The dry matter of the solids was determined at 60°C in a vacuum oven using P_2O_5 as desiccant. Sorption experiments were done in duplicate.

Experimental sorption data were fitted to the GAB model (20) using the non-linear regression function of SigmaPlot 11.0 (2008):

$$X_e = \frac{X_m C \cdot K \cdot a_w}{(1 - K \cdot a_w)(1 - K \cdot a_w + C \cdot K \cdot a_w)} \qquad (2)$$

where a_w is the water activity, and X_m, C and K are the GAB model constants.

Glass Transition Temperature (*Tg*)

Glass transition temperature (midpoint) was determined by differential scanning calorimeter using a thermal analysis system DSC Pyris 1 (Perkin Elmer) connected to a PC for simultaneous data treatment (Pyris Software for Windows version 3.52) and to a

refrigeration system with a compressor. The instrument was calibrated for temperature and heat flow with indium (T_m = 156.6°C and ΔH = 28.45 J/g, Perkin Elmer standard) and checked with distilled water for which T_m = 0°C and ΔH = 333 J/g (21). A 30-mg sample of each human milk powder was transferred into a high volume stainless steel pan (Product #03190029, Perkin Elmer), where an O-ring was inserted. The capsule was sealed with a cover and immediately weighed. A similar empty capsule was used as a reference. Capsules were cooled to –20°C. Scanning was performed by heating at 5°C/min from -20°C to 120°C. The glass transition appeared as an endothermic shift in the specific heat capacity. Results were obtained in triplicate.

Statistical Analysis

Due to limited quantity of collected mother's milk available for these studies, only duplicate experiments (n=2) were done for each treatment. Results are reported as the average value with associated standard error. Non-parametric ANOVA was performed on the data (Friedman and Kruskal-Wallis tests) using Dunn's multiple comparison and with initial concentration as control. Differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Spray Drying

Initial solid content of fresh human milk was found to be 11.57 ± 0.58% (w/w). Lawrence (22) reported a solid content of 12.0% in mature human milk and 12.8% in colostrum, whereas Picciano et al. (23) found values of 11.85 ± 1.43%. Table 1 shows the parameters resulting from spray drying of human milk at different operating variables.

For both combinations of inlet temperature and flow rate operating variables, the average of powder humidity obtained after the process was 2.05 ± 0.14%. However, exit air temperature was 10 degrees higher for a 20 degrees increase in inlet air temperature, even if the liquid flow rate was increased from 4 to 5 mL/min.

Freeze-Drying

Figure 1a shows the product temperature (dotted line) during freeze-drying of human milk samples at 20, 30 and 40^{o}C heating plate temperature (solid lines). The temperature curves obtained in this work were similar to most curves available in the literature for product temperature increase during the freeze-drying process (24). The initial temperature of both the heating plate and product after freezing was -30°C. As the temperature of the heating plate was raised, product temperature increased with a delay corresponding to the sublimation time. The duration of the sublimation step had a good correlation with the heating plate temperature (1.3, 3.0 and 4.5 hours for 40, 30 and 20^{o}C, respectively). After all the ice was sublimated, the product temperature increased gradually to reach the heating plate temperature, the times when the product temperature reaches 20, 30 or 40^{o}C being 5.2, 7.7 and 8.5 hours, respectively.

Freeze-drying curves of human milk are presented in Figure 1b. Drying rates were fast during the first phase of drying, and slowed down at the end of the process as expected from the increase of the dry layer resistance to heat. An important effect of heating plate temperature on residual moisture content was observed. This positive effect was amplified as the heating plate temperature increased from 30 to 40^{o}C (see Figure 1b).

Times to complete freeze drying can be extrapolated from kinetic curves as 9, 8 and 6 hours for 20, 30 and 40^{o}C, respectively, which corresponds approximately with the times when the product temperature reached the heating plate temperature.

Experimental data of moisture content decrease as a function of time were fitted to Page's model, Eqn. (1). In Figure 1b, Page's model predictions are shown together with experimental data. As can be observed, this model predicted the freeze-drying kinetics in human milk samples. The Page's model fittings parameters are presented in Table 1. The rate constant (k) in the Page's model involves the moisture diffusion coefficient, which is temperature-dependent. Therefore, as temperature increases, k increases. From Table 1, the rate constant (k) increased slightly between 20 and 30^{o}C, but as observed previously from the kinetic data, the rate constant was markedly increased when using a 40^{o}C heating plate temperature. Table 1 also shows the final moisture content of human milk powders freeze-dried at different heating plate temperatures, which have a mean value of 2.48 ± 0.61%.

Effect Of Dehydration Methods On Iga, Igg And Igm Content

Ig concentrations were measured before and after spray-drying or freeze-drying of human milk and are presented in Table 2. Initial Ig concentrations were 215.80-262.68 □g/mL (IgA), 13.92-19.59 □g/mL (IgG) and 21.95-22.48 □g/mL (IgM). It has to be noted that each experiment for freeze-drying or spray-drying was done with a different pool of homogenized human milk, explaining the different initial Ig content values presented in Table 2. Human milk Ig contents have been reported to vary depending on different

parameters such as the length of breastfeeding, the breastfeeding stage, the time of the day when the milk is extracted, and the geographic origin of the mothers (25, 26). Permanyer et al. (10) reported IgA content in mature human milk from 247 to 488 µg/mL and an average of 13.47 µg/mL for IgG, whereas an IgM concentration of 22.9 µg/mL was observed in the work of Contador et al. (25). Therefore, our results on Ig concentration of human milk are in close agreement with previous studies.

Based on the data presented in Table 2, graphs on Ig retention were constructed (Figure 2a and b for spray-drying and freeze-drying, respectively, at different operation conditions). Spray-drying produced human milk powders having good retention of IgG (higher than 88%) and IgM (higher than 67%) (Figure 2a). However, only 38% of IgA could be preserved after spray-dying. Please note from Table 1, that both spray-dried powders have low residual moisture contents (~2%).

For freeze-drying, only the highest heating plate temperature used in this study (40^{o}C) caused a low retention of immunoglobulin IgA (55%). The other conditions retaining over 76% (IgA), and 80% (IgG and IgM). The reason why different immunoglobulins are retained differently by the same process conditions is unknown to us. It has been previously reported that IgA is stable up to 56^{o}C, but heat-labile at 62.5^{o}C (8) and that temperature rather than process time is a critical parameter in keeping IgA content (9).

The lower retention of IgA at 40^{o}C could be explained by a longer exposure of the milk sample to the heating plate temperature (Figure 1a) as well as its lower residual moisture

content (Table 1). In freeze-drying, the lowest moisture content possible is not necessarily the best optimal condition to preserve proteins (i.e. immunoglobulins) since chemical and physical degradation such as deamination, oxidation and aggregation may occur (27) and accelerate at very low water contents.

Non-parametric ANOVA performed on the Ig concentrations data before and after freeze-drying/spray-drying treatments, failed to reveal evidence significant differences. However, Dunn's multiple comparisons test suggested for spray-drying at 160^{o}C a trend toward a significant reduction of IgA (p = 0.0651) and IgM (p = 0.0911) as well as for IgG at 180^{o}C (p = 0.0651); while for freeze-drying, such a trend was observed for IgA concentrations between Initial *vs* 40^{o}C treatment only (p = 0.0605).

The present results indicated that IgA is the most sensitive Ig during processing human milk by drying. Reduced moisture contents obtained under specific operating conditions as well as higher temperatures could be the causes of lower retention of IgA during freeze-drying and spray-drying. Taking into account that IgA is believed to be the most important Ig in human milk (2), the comparison of both dehydration methods leads to the conclusion that freeze-drying at 30°C is a particularly promising method to preserve human milk. In addition, a heating plate of 30^{o}C throughout the whole sublimation process can accelerate the kinetics (only 8 hours processing time) with good overall immunoglobulin retention (IgA 76.37 µg/mL ± 1.81%, IgG µg/mL 81.65 ± 3.61% and IgM 83.80 µg/mL ± 22.75%).

Sorption Isotherms

The sorption equilibrium isotherms of freeze-dried and spray-dried human milk powders at 20°C are shown in Figure 3. Both curves showed a type-II sigmoid sorption form (28). The sorption curves found in this work showed a progressive increase in water content until a_w = 0.6, then the slope of the curve increases until a_w = 0.9. Soteras et al. (29) found similar behaviour when studying adsorption isotherms at 25°C of whole cow milk samples, dried in an oven. In our study, differences in moisture sorption by spray-dried or freeze-dried human milk samples were not significant.

Human milk freeze-dried at 30°C heating plate temperature for 8 hours presents 3.05% moisture content in wet basis (i.e. 0.0315 kg water/ kg dry solids in dry basis) (Table 1). From Figure 3, it can be observed that at this moisture content, 0.2 is the corresponding approximate water activity at ambient temperature. Thus, storage of this powder at ambient temperature would require a relative humidity lower than 20%, or a moisture barrier packaging, to avoid rehydration of the powder during storage.

The fitted constants of the GAB equation (Eqn. (2)) for human milk are X_m = 0.0596 kg water/ kg dry solids, C = 4.0241 and K = 0.7423. These non-linear regression constants have a standard error estimated to 0.0093 (30). Predictions of GAB equation with fitted parameters are also presented in Figure 3 together with the experimental data. These results showed a good agreement between experimental and predicted data. The X_m parameter is an important sorption value representing the water molecular primary layer. Determining the moisture content for the maximum shelf stability of a dehydrated

product involves the determination of the sorption isotherm and the calculation of the value of X_m in Eqn. (2). The estimated X_m parameter values from our data are in agreement with literature values determined by Garcia-Alvarado et al. (31) and Lim et al. (32), ranging from 0.053 to 0.174 kg water/kg dry solids.

Glass Transition Temperature (T_g)

Figure 4 shows a representative DSC thermograms obtained for human milk powders processed by freeze-drying at different heating plates temperatures (Fig. 6a) and by spray-drying at different air inlet temperatures (Fig. 6b). Since the intensity of the thermograms is solely linked to the mass of the sample and the water content, the comparison of these curves is done by matching the temperatures where peaks and step change in the heat flow appear. The thermograms for freeze-dried and spray-dried human milk powder samples were similar, presenting three main peaks at ~5, 18 and 33^{o}C, which may correspond to the melting of the main fatty acids in human milk. Also, glass transitions are observed in a range of 65 to 75^{o}C, which corresponds to the glass transition of lactose, the main carbohydrate present in human milk, at 2-3% water content (33).

From curves exampled in Figure 4, glass transition temperatures (T_g) for human milk were estimated to 63.90 ± 7.57°C and 69.11 ± 5.55°C for spray-drying at 160 and 180^{o}C inlet air temperature, respectively. For freeze-dried human milk powders, T_g values were 69.07 ± 1.29°C, 72.09 ± 5.29°C and 73.12 ± 0.45°C at 20, 30 and 40^{o}C heating plate temperatures, respectively. Glass transition temperatures of human milk powders were

above 60°C, which indicates a good thermal stability at ambient temperature if the powder is packaged with moisture-barrier materials. Similar T_g results of 61 and 62°C for whole and skim milk, respectively were found for cow milk powders (34, 35, 36).

CONCLUSIONS

The results obtained from this study on dehydration methods and Ig retention in human milk suggested that IgA is particularly sensitive and specifically lost during drying processing. Our data further support the use of freeze-drying at 30°C heating plate temperature to generate human milk powders, which can accelerate the process compared to lower processing temperatures, and minimize the loss of Ig with good retention of IgA (76.37 ± 1.81%), IgG (81.65 ± 3.61%) and IgM (83.80 ± 22.75%). From sorption and glass transition results, storage of this powder at ambient temperature of freeze-dried powders would be possible as long as the milk powder is packaged in moisture-barrier materials. Further research studies based on immunoglobulin structure are recommended in order to explain the differential impact of the same drying conditions on Ig retention values.

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Table 1. Operation variables during spray-drying and freeze-drying of human milk

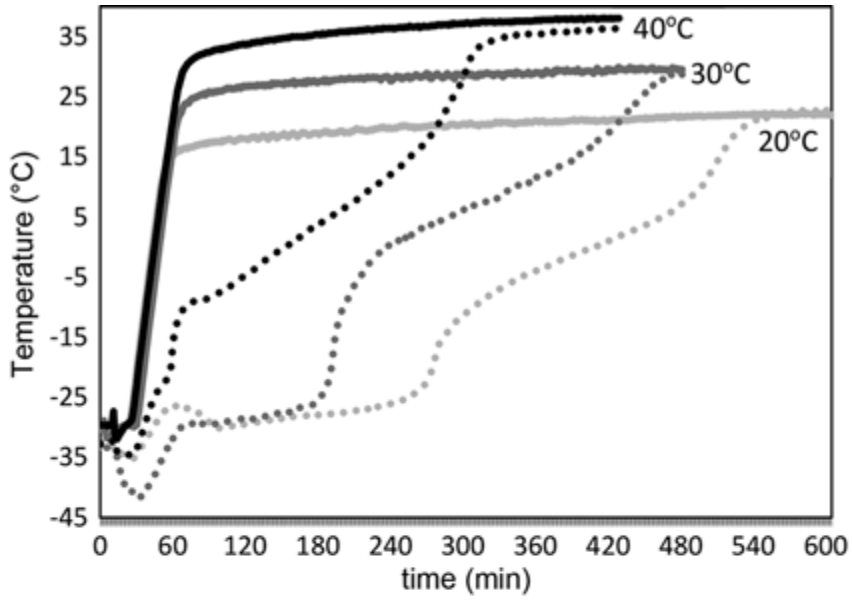
	Initial milk Total solids (%)	T_{inlet} **(°C)**	**Pressure (bar)**	**Flowrate (ml / min)**	T_{exit} **(°C)**	**Final moisture (%)**
Spray-drying	11.57 ± 0.58	160	3	4	77.5 ± 2.5	2.09 ± 0.09
		180	3	5	87.5 ± 2.5	2.00 ± 0.13
	Initial milk Total solids (%)	**Heating plate temperature (°C)**	**Freeze-drying time (h)**	**Final moisture (%)**	***k*** **(h^{-1})**	***n***
Freeze-drying	11.21 ± 0.41	20	9	2.65 ± 005	0.115	1.52
		30	8	3.05 ± 0.15	0.133	1.53
		40	6	1.75 ± 0.075	0.170	1.64

Table 2 IgA, IgG and IgM contents of human milk powder spray-dried or freeze-dried at different operation conditions

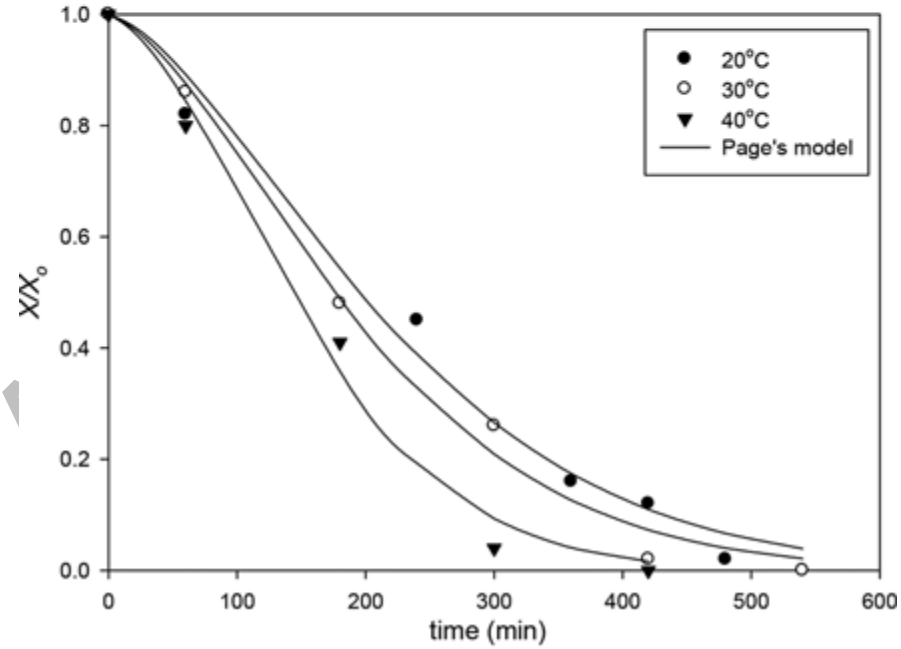
	Temperature (°C)	**IgA** (μg mL^{-1})	**IgG** (μg mL^{-1})	**IgM** (μg mL^{-1})
Spray-drying	Initial	215.80 ± 6.84	13.92 ± 0.80	21.95 ± 5.15
	160 (4 mL/min)	77.76 ± 5.00	13.06 ± 0.17	14.62 ± 2.35
	180 (5 mL/min)	83.20 ± 1.22	12.26 ± 0.55	16.10 ± 3.39
Freeze-drying	Initial	262.68 ± 56.40	19.59 ± 0.17	22.48 ± 5.84
	20	199.48 ± 33.91	15.36 ± 0.33	19.57 ± 6.28
	30	200.62 ± 4.75	16.00 ± 0.70	18.84 ± 5.12
	40	144.72 ± 7.00	15.38 ± 0.20	18.40 ± 8.40

Mean ± standard deviation (n=2)

Figure 1 Freeze-drying of human milk, (a) temperature profile during freeze drying at 20, 30 and 40°C heating plate temperatures (dotted lines represent product temperature while solid lines, heating plate temperature), and (b) freeze-drying kinetic curves of human milk at varying heating plate temperatures (●20, ○ 30 and ▼40°C).

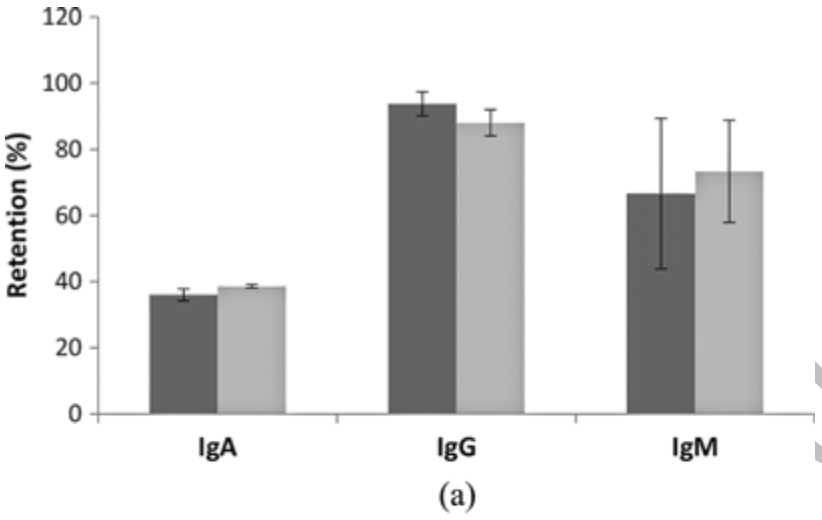


(a)

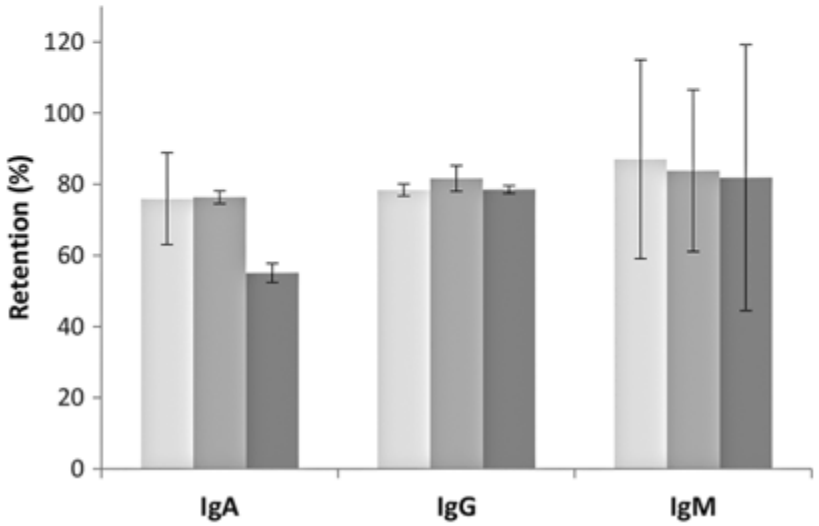


(b)

Figure 2. Human milk IgA, IgG and IgM retention after (a) spray-drying at (▮) 160°C and (▮) 180°C and (b) freeze-drying at heating plate temperatures of (▮) 20°C, (▮) 30°C and (▮) 40°C.



(a)



(b)

Figure 3. Sorption isotherms of human milk freeze-dried or spray-dried powders.

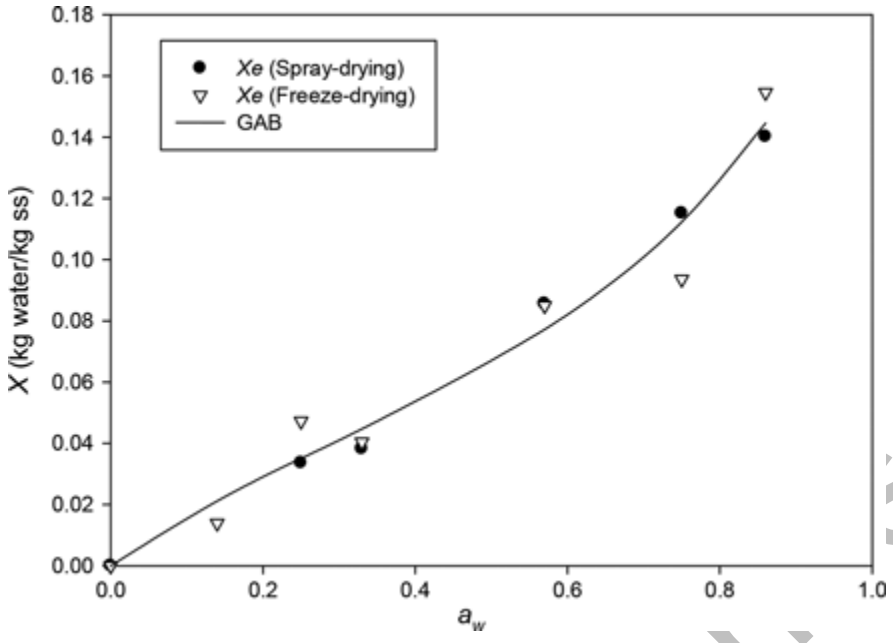
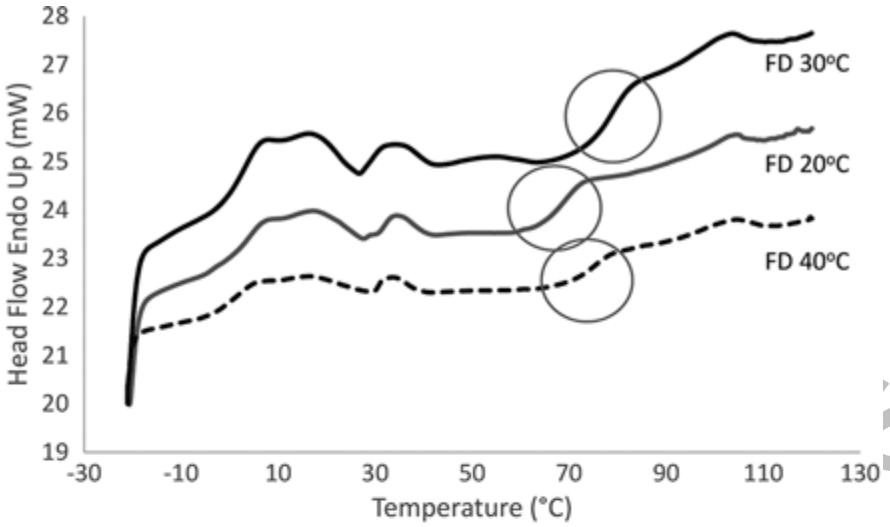
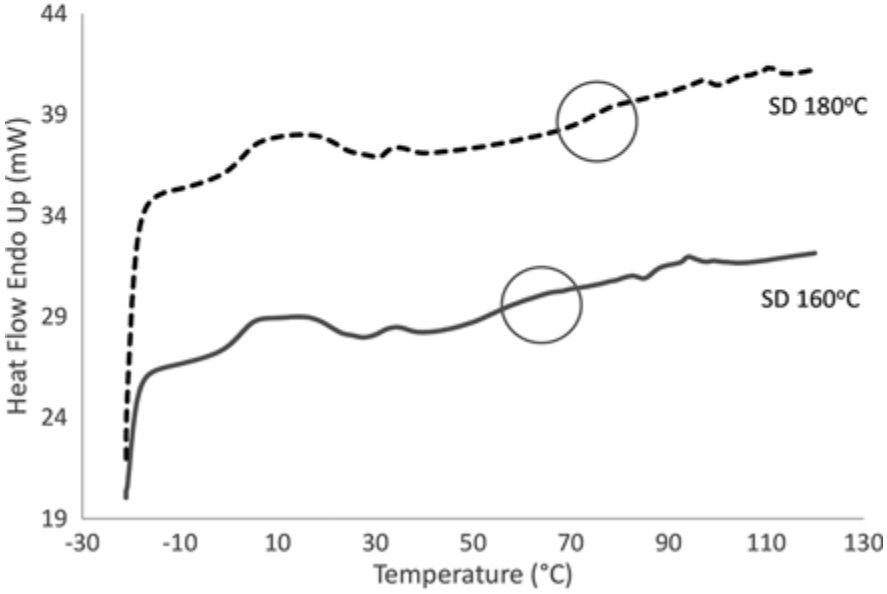


Figure 4. Heat flow curves as a function of scanning temperature. (a: Freeze-drying, b: Spray-drying). The glass transition is marked with light circles.



(a)



(b)