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Abstract

The state diagram is a key parameter when optimizing a freeze-drying cycle, as it allows us to set the optimum processing temperature for a specific product. The purpose of this work is to provide some thermodynamic and physicochemical data for optimizing the freeze-drying cycle of whole milk. Freezing point measurements were determined using differential scanning calorimetry and cryoscopy methods, and glass transitions were evaluated by DSC. The endothermic peak was used as the equilibrium freezing point, a variation from 273.93 K to 264.92 K was detected when the dry matter content varied from 0 to 75%. T_g midpoint temperature was used as glass transition of dry mater. Furthermore, Fox and Gordon-Taylor equations were used to predict the glass transition curve to determine T_g' , this temperature was used as an approximation of the critical temperature T_c. Collapse temperature was determined using the freeze-drying microscopy setup.

Key words: A1. State diagrams, A1 Characterization, A1. Solidification, A1. Optical microscopy, A1. Freeze-drying

1. Introduction

Milk is a complex mixture of ingredients. Liquid water, arguably the most important solvent on Earth, is the main component of whole milk representing more than 86% of the total mass of cow milk. The most important compounds in milk are lactose, fat and protein. Dry food is

thus much more stable than wet food. Water in dairy products contributes to microorganisms growth and acts as a plasticizer for non-fat (carbohydrates and proteins) milk solids [1]. One process that can be used to promote the long-term stability of these products while avoiding thermal degradation of their structure is freeze-drying (also known as lyophilization).

Freeze-drying is a low-temperature, low-pressure dehydration process that is time-consuming and energy-intensive. It consists of three main steps: freezing, primary drying and secondary drying. In the first step, the product can be frozen at an extremely low temperature around -60°C and most of the water solvent is converted to ice. In the second step, the ice is removed from the frozen product by sublimation [2], the primary drying must be performed at a temperature below collapse point T_c (i.e. maximum allowable product temperature during primary drying for an amorphous system). In the final step, the unfrozen water that has not crystallized during freezing and is bound to the product is desorbed at a higher temperature [3]. Primary drying is the most time and energy consuming step in the entire process [4].

Therefore, the optimization of this step is of paramount importance to accelerate the development of freeze-drying process. It is well known that increasing the temperature will significantly increase yield during sublimation [5][6]. Indeed, a temperature increase of only 1°C resulted in a reduction of at least 13% in primary drying time [5][7]. Thus, to optimize the freeze-drying cycles it is necessary to determine the key parameters i.e. T_g , T_g' and T_c . T_g can be defined as the temperature at which an amorphous system changes from glassy to rubbery. It has been used as an indicator of food stability and to predict food behavior, whereas for a concentrated solution at the freezing maximum, the notation is T_g' [8], Glass transition temperature (T_g) of any milk powder, is primarily the effect of amorphous lactose, the amorphous lactose coats milk proteins and fat globules in a glassy powder [9]. The collapse temperature T_c is related to the glass transition temperature T_g' but is not the same the latter being slightly lower even when measured at low rates [5] T_c is usually determined by

freeze-drying microscopy (FDM) while the standard method for T_g' is Differential Scanning Calorimetry (DSC) [10][11]. Small molecules act as plasticizers when mixed with larger molecules by lowering the apparent glass transition temperature of the latter [9]. The most common plasticizer in the food industry is water. As suggested in the literature, the glass transition temperature T_g' can be used as a first approximation of the collapse temperature. The T_g of skimmed milk powder is easier to determine than whole milk powder (WMP). The glass transition temperature of WMP is dependent on the water content, which overlaps more or less with the end point of fat melting in a given water content [9]. To fully understand what occurs in freeze drying, it is also necessary to consider the role of ice formation, both processes are best understood with reference to a state diagram.

State diagram is a tool for optimizing the drying process because it depicts the different states of the food, describes equilibria such as freezing point and glass transition, which helps to understand the complex changes that occur when the water content of a food is modified. It also helps to identify the stability of food during storage and to select the appropriate temperature and moisture content [9]. In order to establish the solid–liquid equilibrium temperature of a mixture, the commonly used methods are cryoscopy and DSC. AOAC (Association of Official Analytical Chemists) usually uses the cryoscopy method to quantify adulteration of milk with water, the solution is cooled until solidification, the temperature-time is observed with a temperature-indicator, and initial or equilibrium freezing point is used as the maximum temperature after ice nucleation (e.i plateau) [12]. While cryoscopy has been shown to be effective, it requires manual observation and often does not provide information on other thermodynamic properties of interest. The amount of solute must be negligible compared to the amount of solvent in a liquid solution [13]. In addition, serious complications can arise from operating cryo-devices at low temperatures. The application of DSC to the study of organic mixtures has shown the development of several methods for the

determination of the liquidus temperature ($T_{liquidus}$) from the thermogram observed during the melting of the solid phase [14]. The freezing point is usually characterized from the endothermic peak during the melting process [15]. The freezing point could be considered as the temperature peak or at the onset point of the endothermic peak [15], although the melting point in mixtures are qualitatively different from those in pure substances. The use of a low heating rate is important to ensure a steady state during heating. It is important to note that the freezing point of water and other liquids can be at the same temperature as the melting point. It won't be higher, but it could easily be lower.

The objective of this study is to realize the state diagram of whole milk by measuring glass transition temperature and freezing curves to find the maximal-freeze-concentration condition, in order to better understand the freeze-drying applied effect. This was possible by using a low-cost and simple cooling curve method (cryoscopy) to measure the freezing point T_f and to compare with DSC results obtained from the melting curve.

2. Experimental Methods.

2.1. Samples preparation.

To plot the state diagram of the water/WMP mixture, samples with different dry matter were prepared using the same brand of milk powder “Commercial name Nido”, with initial moisture content of 3% dry matter (measured using Karl Fisher apparatus). ***Tab.1*** presents the main information about this product. The concentrations of the solutions were calculated using ***Eq1***.

$$W_{p.w} = \frac{m_p}{m_p + m_w} \qquad \textbf{Eq.1}$$

Where $W_{p.w}$ is the weight concentration of the solution, m_p is the mass of Milk powder and m_w is the mass of distilled water.

To complete the discussion about the collapse temperature, some analysis have been done on liquid whole milk, (noted WM) that contains 12.4% of dried matter with (3.6g for 100ml of fat content).

2.2. State diagram of whole milk

2.2.1. Cryoscopy "cooling curve"

Freezing point depression (FPD) is one of the most important thermophysical properties of an aqueous solution for freezing processes [13]. ***Fig.1*** shows the experimental setup used to determine the solid/liquid equilibrium of milk solutions. The cryoscopy apparatus used in this study is from PHYWE, it consists of two cylindrical DURANglass vessels (1-3). One fits into the other and is secured by a screw cap GL 45. The inner cylindrical glass vessel has a capacity of 60 ml with a flat bottom to accommodate a magnetic stirring bar and a side inlet for the introduction of the product. The outer jacket glass is filled with 35-40 ml of ethanol to ensure a uniform heat transport from the inner vessel to the frozen mixture. The set of two cylindrical tubes was immersed in a 1000 ml glass beaker filled with a suitable freezing mixture of crushed ice and sodium chloride to achieve a temperature near to -22 °C. The whole equipment is placed under stirring using magnetic stirrer. The temperatures in the inner cylindrical glass vessel and cooling bath are measured by two Pt100 immersion temperature probes (5) connected to the data acquisition system (6).

The solutions were cooled at a cooling rate of approximately 1.6 °C/min. The experimental study of the solid-liquid equilibrium was carried out for milk solution at concentrations of dry matter ranging from 0 wt % to 50 wt % dry matter. Aqueous solutions were prepared using sucrose as analytical reagent, varied from to 273.15 to 263.39 K when solids content changed from 0 to 49.5%. The FPD of this solution is available in literature, allowing the comparison of our results and the literature data. To further confirm the measurement procedure in the

high concentration range [13]. Equilibrium points are synthesized from a temperature plateau (temperature-time curve) over a long period as shown by ***Fig.2.***

2.2.2. Calorimetric Analysis by Differential Scanning Calorimetry

Freezing point and glass transition of whole milk sample at different dry matter content were measured by DSC. The Differential Scanning Calorimetry instrument used was a TA Instruments DSC Q200 with Universal Analysis Software, to determine the state transition temperatures. Milk solution samples (20-25 mg) from 0% (wt) to 75% (wt) dry matter were prepared, each sample was pipetted into a DSC pan and weighed with a Mettler balance to an accuracy of 0.01 mg. Hermetically sealable aluminum sample pans were used in all measurements with an empty aluminum pan as a reference, equilibrated at 20°C. Afterward, the temperature was decreased to -40°C and then increased to 40°C at a rate of 1°C min^{-1}, two scans were performed for each sample. All measurements were performed under a nitrogen atmosphere with a flow rate of 50 mL min^{-1} used to purge the sample head for all measurements to avoid condensation in the oven. The results reported in this work correspond to data as a function of temperature, with the freezing temperature acquired at the peak of the melting of the heat flow curve at the second melting scan. Fig.3 represents an example of DSC thermogram of whole milk sample containing 20% dry matter.

2.2.3. Freeze-drying microscopy (FDM)

Linkam Scientific FDCS196 freeze drying cryo-stage and optical microscopy LEICA (DM) were used to observe the behavior of the solutions during both cooling and warming periods. A microscope slide made of quartz (16-mm) and oil was used to enhance thermal contact between the sample and the temperature-controlled plate. A drop of 2-3 µl of sample (10wt%, 12.4wt% and 20wt% of WMP and commercial WM) was prepared. A second slide was gently pressed on top to form a seal tight enough to prevent sample water from evaporating, the

149 samples were then transferred into the microscope chamber. A Sony CCD camera with 1600 x
150 1200 pixels was used to record pictures.

151 3. Results and discussion

152 3.1. Experimental Solid−Liquid state Diagram: Freezing point

153 Whole milk state diagram has been studied to identify the phase transitions that can occur at a
154 specific temperature and weight fraction. Experimental state diagram was investigated using
155 two methods: Cryoscopy and DSC analysis to determine freezing point. Whole milk samples
156 were prepared by diluting concentrated milk with distilled water. Cryoscopy method was used
157 for milk solutions with concentrations ranging from 0 to 50% dry mater and the DSC analysis
158 was used for milk solutions with concentrations ranging from 0 to 75% dry matter. According
159 to literature [13], freezing points can be derived from long temperature plateau when
160 cryoscopy method is used, it can be also determined from melting peak at the endothermic
161 curve using DSC [15].
162 ***Fig.4*** represents the melting curves obtained using DSC at a heating rate of 1°C.min^{-1} for
163 different dry matter contents. When the heating rate decreases and approaches zero, the
164 temperature gradient within the sample decreases [16], that is why a low heating rate was
165 applied. Freezing point results are summarized in ***Tab.2***.

166 According to literature work, it is more reasonable to consider the maximum of the
167 endothermic peak as the freezing point to develop the state diagram using DSC, in the case of
168 food products [15][17][18][19].

169 Chen et. al [13] results were used to compare the liquidus temperatures measured by the
170 cryoscopy and DSC as shown in ***Fig.5***. Both methods showed good consistency, increasing
171 amount of milk powder leads to a decreasing of freezing point. However, the equilibrium
172 temperatures given by the synthetic method tends to be lower at certain points than the

temperatures obtained by DSC. For fact that the freezing point of a substance largely depends on supercooling, while supercooling has no effect on the melting point of a substance. Cryoscopy results [13] have been used to determine the freezing point to build the model state diagram of whole milk [9].

However the reliability of cryoscopy results lies in the study of solutions with low solute concentration [13], the cooling curve lost its sensitivity at high dry matter content, due to the low amount of freezable water present in the sample. We find out that using DSC for the construction of equilibrium state diagrams is becoming common; the advantages of DSC lies in its speed of measurement and ease of use. It has been widely used in the study of protein denaturation, carbohydrate stability, and the determination of first and second-order transitions in lipids [20] [21][22]. Based on all of that, the DSC results seem to be more accurate than cryoscopy method in our case.

3.2. Glass transition

The study of the effect of heating rates on glass transition T_g was performed using a commercial whole milk powder containing 3 % water content ***Fig. 6*** shows the graphical results of T_g at different heating rates (1; 5; 10 and 20 °C/min). Heating rate was found to have an effect on the T_g value, with a significant decrease in the glass transition from 65.55 to 54.15 °C when the scanning rate was reduced from 20 to 1°C/min, the use of a low heating rate is important to ensure a steady state during heating. Therefore, the lowest heating rate was selected for the T_g measurements. Glass transition was difficult to detect for WMP between 4 and 8.5 % water content, as reported in the literature [9]. In addition, it was not possible to determine T_g from the DSC curves of fat-containing with a water content range of 23.9 to 44.4 % because the fat-melting endothermic predominates in the same temperature range [23] ***Tab. 3*** shows the T_g results of weight mass fraction (93.3 and 97%) for whole milk

197 powders at a heating rate of 1 °C/min. The glass transitions are reported as the midpoint
198 temperatures: the glass-transition mid-point value was obtained as an average of the onset and
199 end values [24], the glass transition temperature increased as solid content increased.

200 Glass transition of mixtures can be predicted using various empirical equations. The reliability
201 of T_g depends on the accuracy of the determination of total solids in milk. In this paper, the
202 Gordon-Taylor and Fox equations were established to predict the T_g curve of amorphous
203 whole milk, considering that all solid components which contributing to the observed glass
204 transition were miscible and formed a single phase. The Gordon-Taylor equation seems to be
205 most commonly applied to amorphous foods to describe the plasticity of solids by water
206 [9][25].

207 $$T_{g,m} = \frac{W_1\ T_{g,1} + K(1 - W_1)\ T_{g,2}}{W_1 + K(1 - W_1)} \qquad \textbf{Eq.2}$$

208 Where W_1 and W_2 are the weight fractions of solute and water, respectively. The model
209 requires knowledge of three constants: the coefficient K, which represents the non-linearity of
210 plasticizing effect of water. $T_{g,1}$ is the T_g of anhydrous solute and $T_{g,2}$ is the T_g of water.
211 However, it is difficult to dehydrate amorphous food powders by heating without significant
212 chemical changes, recrystallization or structural changes. That is why we used the literature as
213 defining parameters for our datasets: $T_{g,1}$ =373.75 K, K=8.57 [9] ($T_{g,1}$ is close to T_g of pure
214 lactose [23][26][27]), and $T_{g,2}$ =138K (glass transition temperature for pure water) [15][28].
215 The T_g curve with Gordon-Taylor equation for whole milk is presented on ***Fig. 7*** (Orange
216 curve). A predicted glass transition curve is also obtained using the Fox equation (Eq.3) for
217 approximate estimation of T_g of binary system [29].

218 $$\frac{1}{T_{g,m}} = \frac{W_1}{T_{g,1}} + \frac{W_2}{T_{g,2}} \qquad \textbf{Eq.3}$$

Where the definition of W_1 , W_2 , $T_{g,1}$, and $T_{g,2}$ is the same as ***Eq.2***. As mentioned earlier, it is difficult to measure the T_g of anhydrous milk. In this section, we predicted the $T_{g,1}$ using the $T_{g,\mathrm{m}}$ of 97 % dry matter of the commercial powder. The T_g curve of the Fox equation is shown in ***Fig.7*** (blue curve). The most accurate model to build glass transition curve for our state diagram seems to be Gordon-Taylor equation.

T_g' is defined as the intersection of the freezing curve with the glass line. The $T_g{}'$ corresponds to the glass transition of the matrix (mixture of dry matter and unfrozen water) in the frozen sample. It is an important process parameter during freeze-drying. The $T_g{}'$ is approximately read between -48°C and -23 °C, corresponding to a maximally freeze-concentrated solution read between 83% and 88 % of WMP using Gordon-Taylor curve (***Fig.7,)*** result closed to what has already been found by the authors [23]. The $T_g{}'$ of lactose in food are respectively -41°C and -28°C for 0.813 and 0.592g dry solid/g food [30][19]. This prediction is important to determinate the collapse temperature (T_c) currently assimilated to the T_g' as a first approximation.

3.3. Collapse temperature

The collapse temperature (T_c) of whole milk is measured by direct microscopic observation under simulated low-temperature, low-pressure freeze-drying conditions. Samples containing in weight 10%, -12.4%, 20% WMP and a commercial WM with 12.4% were selected for Freeze-drying microscopic observation of the physical state of the WMP/water system. The sample was frozen to a temperature lower than $T_g{}'$ (about -50°C) below the lowest temperature value in the $T_g{}'$ range. Then, the sample was kept at this temperature for 1 min, then the system was placed under vacuum at about 10 Pa (operating limit of our freeze dryer during sublimation). The temperature was increased by 10°C/min until -40°C. At -40°C, we noted that samples were not collapsed (***Fig 8).*** Then the temperature is gradually increased at

0.5°C/min until a collapse was observed up to -36°C, -37°C, -38°C and -35°C respectively for solution 10%, 12.4%, 20% of WMP and WM with 12.4% (***Fig 9),*** the collapse temperature (T_c) is observed by the formation of micro-cracks during the displacement of a front between two zones, a frozen zone and a dried zone as shown in ***Fig. 9.***

We can conclude that the T_c is slightly depending on the WMP amount content of the initial solution of whole milk. This collapse temperature value is in adequation with the T_g', currently T_c is often reported to be higher (1–22°C) than the corresponding T_g'. In last decades, the T_g' has mainly been used as the critical temperature, collapse temperature is now known to better reflect drying conditions [25][31][27] [7].

Conclusion

In this work, DSC data were used to design the solid/liquid state diagram of the water/WMP system with the peak of the endothermic curve as the freezing point and the midpoint as the glass transition temperature. The freezing points of whole milk were varied from to 273.93 to 269.55 K when dry matter content changed from 0 to 75% using DSC, respectively. DSC results were consolidated using cryoscopy experimental data. These data allow us to calculate the glass transition evolution versus mass fraction of WPM using two models, Fox and Gordon-Taylor equations. In addition, the collapse temperature has been evaluated by Freeze-drying microscopy.

These new experimental data concerning whole milk allow us to fix the critical temperatures of freeze-drying cycles of milk formulations in order to optimize the process time and the energy consumption.

Acknowledgment

This study was supported by the Franco-Moroccan Interuniversity Committee, Hubert Curien / Toubkal program, the authors are grateful to the Campus France program for the financial support received during this work "***Number: Toubkal/21/125 - Campus France: 45806RC"***

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Table 1. *Main dry matter components of whole milk powder*

Nutrition	***WMP per 100 g***
Protein	25.7 g
Carbohydrates	36.5 g
Lipids	28.2 g
Salt	0.9 g

Table 2. *Experimental results of cryoscopy and DSC [a] measurement in our laboratory*

$W_{P.M}$ (wt%)	T_f (K)	$W_{P.M}$ (wt%) [a]	T_{onset} (K)	T_{peak} (K)
0	273.15	0	271.92	273.93
2.6	273.05	7	270.88	273.53
2.8	273.03	12	270.35	273.06
3.5	273	20	269.4	272.23
4.0	272.98	25	268.79	271.76
4.9	272.95	30	268.18	271.33
6.5	272.88	35	267.42	270.65
7.2	272.85	40	266.7	270
8.2	272.81	45	266.2	269.76
9.3	272.65	50	266.17	269.55
11.4	272.55	60	265.44	269.05
13.3	272.45	70	262.36	267.2
21.6	272.15	75	258.83	264.92
30	271.45			
34.6	270.83			
39.6	270.21			
42.9	269.82			
44.6	269.47			
49.4	268.63			

[a] Standard uncertainties (u) are ur(wPA) = 0.001, $u(T_{eq}) = u(T_{eut})$ = 1 K, and u(P) = 10 hPa,

Table 3: *Analytical results for whole milk powder*

Mass fraction %	Glass transition K
93.3	304.53
97	327.59

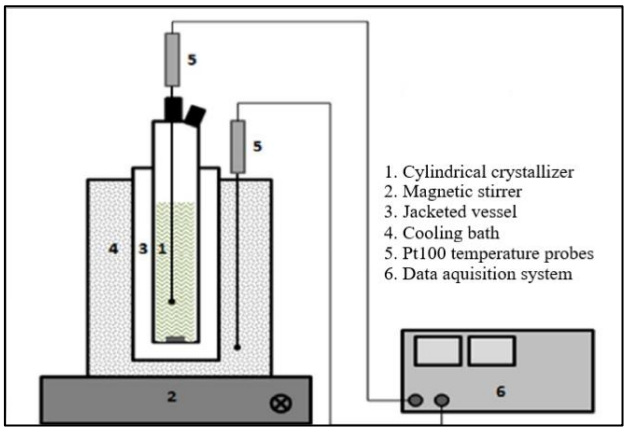
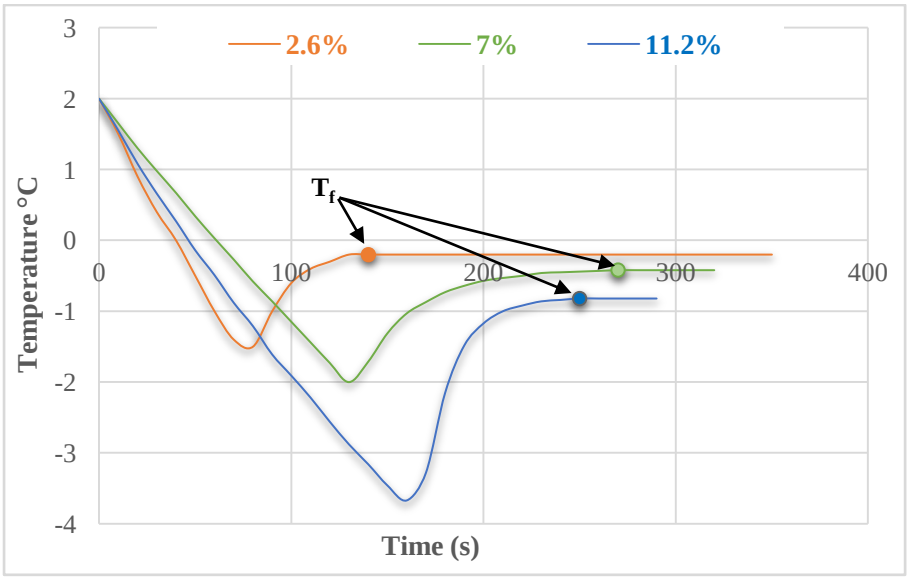


Figure 1. *Experimental setup used for the study of the solid–liquid equilibrium.*



453

454 ***Figure 2**. Freezing temperature for whole milk solutions with 6%; 7% and 11.2% (wt) dry*

455 *matter determined from temperature plateau after nucleation.*

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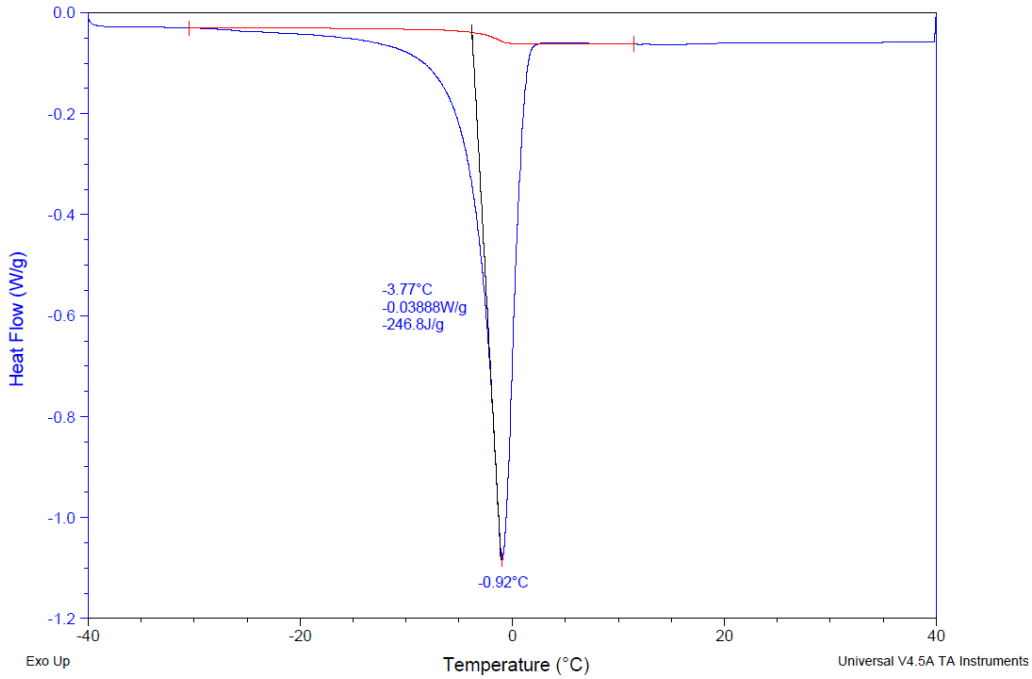


Figure 3. *DSC thermogram of whole milk sample containing 20% dry matter.*

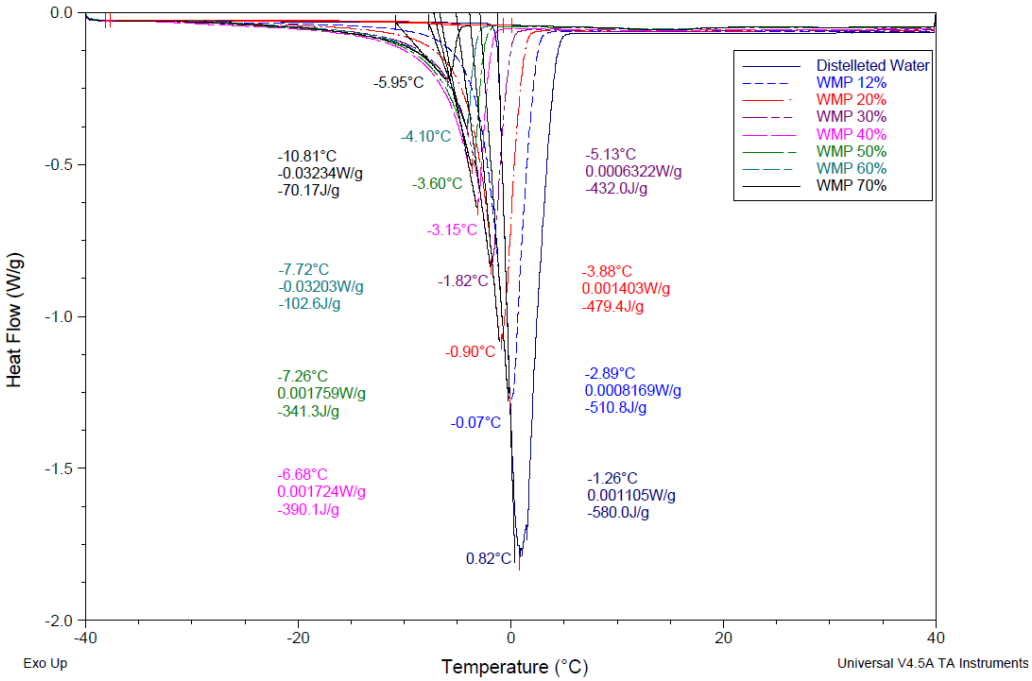
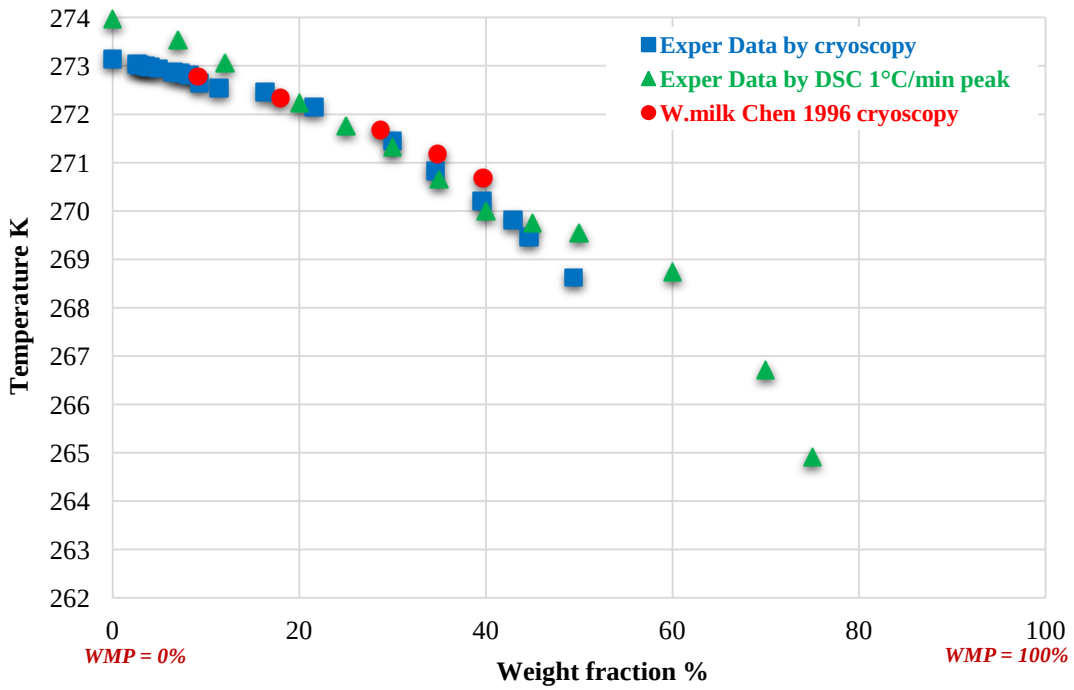


Figure 4. *DSC thermogram of whole milk samples with solids containing 0 to 70% in the 2nd melting cycle showing the onset and peak temperature of each sample.*



489

490 ***Figure 5.*** *Solid-liquid state diagram of whole milk powder (WMP) using DSC results (green*

491 *triangle), cryoscopy data (blue square) and Chen et al results (red circle) [12].*

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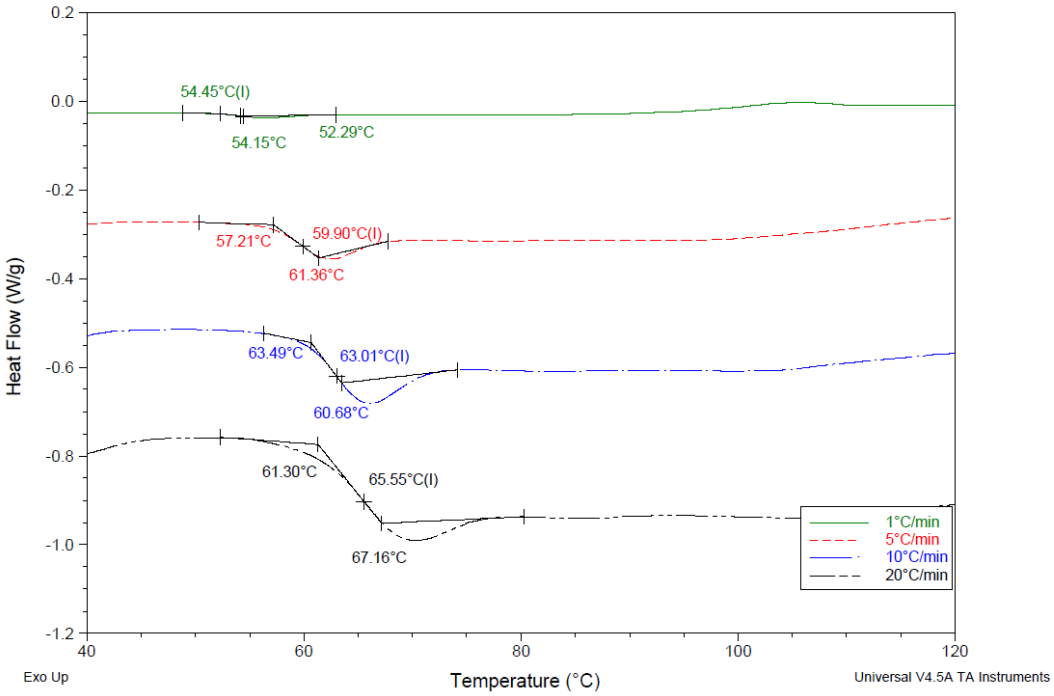

496

497 ***Figure 6.*** *DSC thermographs of whole milk powder (3% water content) at different*

498 *heating rates (1, 5, 10 and 20°C/min respectively in green, red, blue and black*

499 *curves).*

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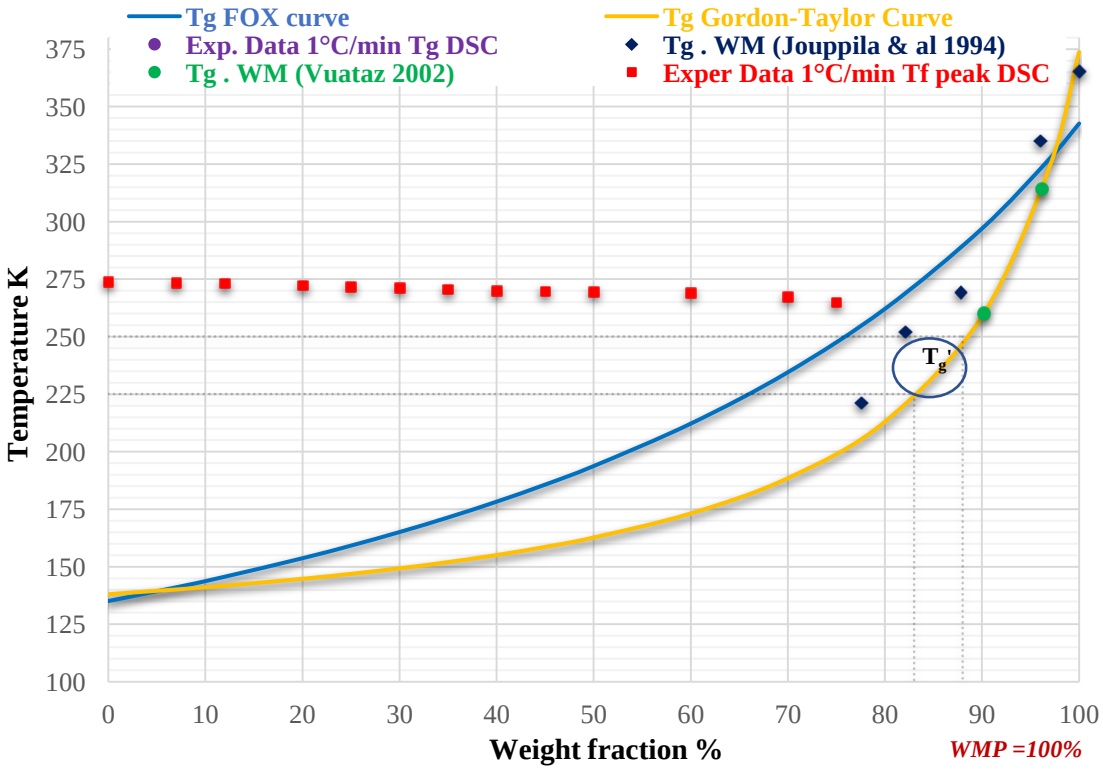

503

504 ***Figure 7.*** *Whole Milk Powder (WMP) state diagram; Freezing temperatures*
505 *measured by DSC (red square); Glass transition temperatures measured by DSC*
506 *(purple circle); Glass transition temperature from bibliography (Vuataz et al. [9]*
507 *(green circle), Jouppila et al. [26] (blue rhombus); Glass transition temperatures*
508 *from Fox equation (blue line) and Gordon–Taylor equation (orange line). The* T_g*'*
509 *range is between 225K and 250K.*

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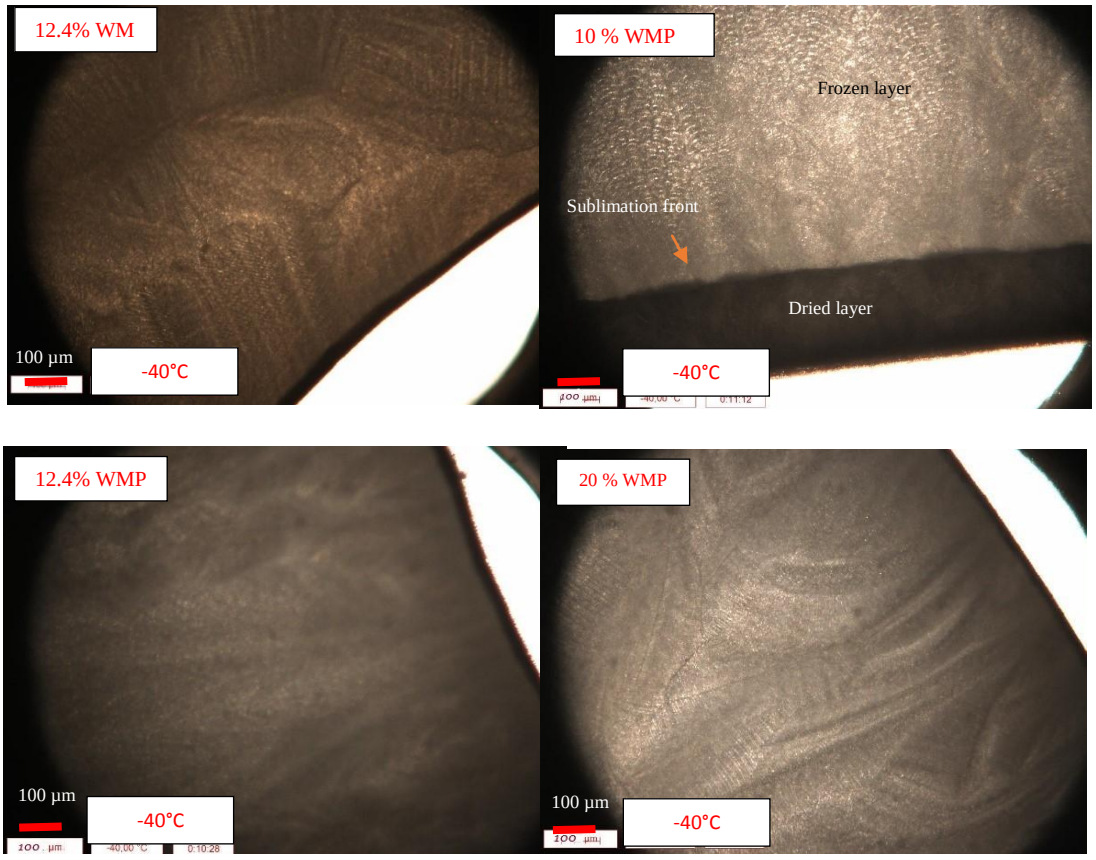
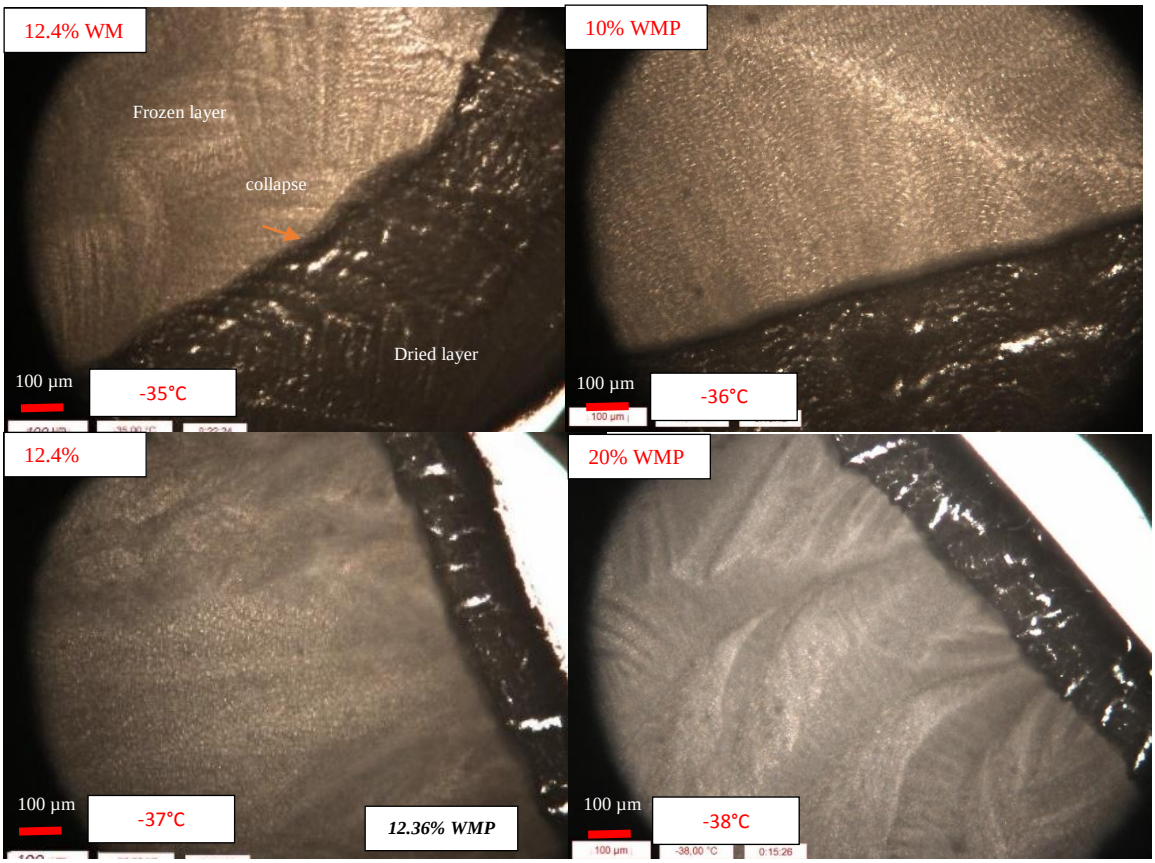


Figure 8 *Microscopic observations of Whole Milk (10%, 12.4%, 20% WMP and 12.4% WM) at -40°C.*



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537

538 ***Figure 9*** *Collapse microscopic observations of Whole Milk (10%, 12.4%, 20% WMP and*
539 *12.4% WM).*

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