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Research Article

Freeze-Concentrated Phase and State Transition Temperatures of Mixtures of Low and High Molecular Weight Cryoprotectants

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Although numerous studies have been conducted on the use of cryoprotectants to prevent the deterioration of food during freezing and frozen storage, scarce reports exist on the thermal transition properties of aqueous cryoprotectant solutions at frozen temperatures. The selection of a suitable cryoprotective medium for the long-term preservation of food requires knowledge of the effects of cryoprotectants and their concentration on the freeze-concentrated unfrozen phase and state transition temperatures known as T_g', T_m', and T_m. Calorimetric measurements were conducted to determine the T_g', T_m', and T_m values of thirty frozen aqueous solutions containing maltodextrin, polydextrose, and glucose, in which a distance-based experimental design was used for mixtures of four components to establish their corresponding mass fractions in the mixtures. Thermograms, measured during heating/rewarming from –70 to 20°C, were used to identify the glass transition and freezing temperatures. Mathematical expressions for T_g', T_m', and T_m as a function of the mass fractions of cryoprotectants and water and their interactions ($p < 0.05$) were developed to aid the formulation of cryoprotective media involving more than two cryoprotectants for adequate frozen conservation of high and intermediate moisture foodstuffs.

1. Introduction

Freezing and frozen storage are widely used for the long-term preservation of food. During freezing, the temperature of the food is lowered to promote the total or partial formation of ice crystals, reducing the availability of water and consequently the growth of microorganisms and enzymatic activity [1–4]. Frozen foods have an extended shelf-life of months compared to days or weeks for chilled or refrigerated foods [5, 6]. Additionally, frozen foods are preferred by consumers because freeze preservation ensures that the food products present better taste, texture, nutritional value, and freshness than those preserved by other methods such as dehydration, concentration, and pasteurization [2].

However, some negative changes in frozen foods caused by physical, chemical, and/or biochemical processes resulting from inadequate freezing rates or storage temperature need to be considered. The major physical change in frozen foods is moisture migration, which causes moisture loss by sublimation, moisture mobility and redistribution in food components, recrystallization of ice, and drip losses in thawed products [7]. It has also been noted that slow-freezing rates during freezing produce extracellular, large, sharp ice crystals, which cause water migration from the cells due to osmotic effects and subsequently lead to cellular dehydration and structural damage [3, 8–11]. On the other hand, among the various chemical changes that take place during freezing and frozen storage are lipid hydrolysis and oxidation, protein denaturation and oxidation, degradation of vitamins, and flavor changes [12].

To alleviate or moderate the deterioration of frozen foods, quick freezing involving the addition of cryoprotectants and phase/state transition concepts have been investigated [13–16]. Quick freezing rates promote the equilibrated formation

of small crystals inside and outside the products. Quick freezing, however, is only suitable for small samples and although fast freezing promotes the formation of small crystals inside the products, during frozen storage at the standard commercial freezing temperature of –18°C, ice crystals undergo metamorphic changes, thus reducing the advantages of fast freezing [1]. This phenomenon occurs because, at –18°C, foods exhibit an unfrozen phase in rubbery state, where mobility of unfrozen water may occur [17]. In addition, the small ice crystals formed under fast freezing conditions are thermodynamically unstable because of their high free energy, so they tend to combine with larger, more stable ice crystals during storage [2, 17]. Therefore, the stability and quality of frozen foods are significantly influenced by the storage temperature and physical state of the unfrozen phase [12, 18, 19].

The temperatures associated with the freeze-concentrated unfrozen phase (T_m' and T_g') are regarded as reference parameters determining the stability of frozen foods. It is assumed that maximum ice formation takes place when food systems are stored between these temperatures [12, 18–20]. It is also known that the unfrozen phase below T_g' leads to a glassy state with substantially increased viscosity (around 10^{10}–10^{12} Pa s), in which molecular motion becomes extremely low, and further crystallization of water into ice and chemical reactions associated with the molecular diffusion of water and other reactants are greatly reduced; therefore, long-term stability may be expected [13, 14, 17, 21–25]. However, the foods in this state are defined as nonequilibrium, metastable, amorphous, disordered materials exhibiting higher free volumes and energy levels than those of crystalline states. Therefore, some factors such as water sorption and temperature changes above T_g' will accelerate the molecular mobility related to chemical and physical changes in glassy frozen foods [12, 13, 19].

In the literature, it has been reported that T_m' and T_g' values exhibit slight variations with the water or solid content but, as a general trend, both parameters depend strongly on the type and molecular weight of the food components [20, 26–28]. Typically, it has been found that the T_m' and T_g' of homologous amorphous polymers such as maltodextrins decrease with the decreasing average molecular weight or increasing amount of plasticizer or moisture content [29–31]. For instance, T_g' values ranging from –15 to –43°C and T_m' values from –11 to –28°C have been reported for frozen solutions elaborated with 40% maltodextrin solutions with a dextrose equivalent (DE) ranging from 5 to 36 and respective molecular weights from 3600 to 500 Da [30].

However, fresh foods usually present high water contents and chemical compositions dominated by low molecular weight (LMW) components with T_m' and T_g' values well below the standard freezing temperature of –18°C. Thus, frozen storage between the T_m' and T_g' or below T_g' is sometimes impractical from an economic point of view [20, 32, 33]. In this context, the use of cryoprotectants with high molecular weight (HMW), such as maltodextrin 5 DE, polydextrose, and hydrocolloids, to manipulate the physical state and deliberately elevate the T_m' and T_g' of frozen foods to above the normal storage temperature of commercial freezers may be an attractive alternative [13, 34–36]. Another approach is the use of LMW cryoprotectants, such as dimethyl sulfoxide, glycerol, and glucose, to depress the freezing point (T_m) and thereby reduce the intracellular ice formation [11, 36–38]. It is important to note that a synergistic increase of T_g' was reported by Harnkarnsujarit et al. [39] for mixtures of LMW sugars (glucose and maltose) and phosphate salts such as Na_3PO_4, $Na_4P_2O_7$, $Na_5P_3O_{10}$, K_3PO_4, and $K_4P_2O_7$ as a result of intermolecular interactions between the components. However, literature reports on the resulting T_m' and T_g' values for aqueous frozen solutions containing mixtures of HMW and LMW cryoprotectants involving a large working range of water content for adequate frozen conservation of high and intermediate moisture foodstuffs, such as fruits, ice cream, purees, jams, etc., remain scarce.

On the other hand, the optimization and selection of cryoprotectants in food formulations are a challenging task because the choice of cryoprotectants is based largely on criteria such as low cost, availability, pleasant or acceptable sensory characteristics in foods, and trial-and-error processes and experience of the food manufacturers [15, 16, 40]. For instance, sucrose and sorbitol concentrations of 4–8% [15, 40], polydextrose concentrations of 1–10% [41, 42], glucose concentrations of 5-15% [43], maltodextrin concentrations of 5-35% [36, 44–46], and maltodextrin-sugars concentrations of 20% (glucose, fructose, and sucrose) [47] have been used as cryoprotectant additives. In some cases, it has been reported that low levels of sugars and sorbitol impart a sweet taste to the products [40, 41, 43]. On the other hand, maltodextrin and polydextrose are nonsweetening and have low viscosities at high solid contents with good solubility [34, 35]. It is evident that the selection of cryoprotectants and their concentration range need to be standardized based on the resulting T_m' and T_g' values rather than on other criteria previously mentioned.

Therefore, this study was based on the assumption that the selection of a cryoprotectant medium suitable for the long-term preservation of food requires knowledge of the effect of cryoprotectants and their concentration on the freeze-concentrated phase and state transition temperatures. The evaluation of the T_g', T_m', and T_m values of different frozen aqueous cryoprotectant solutions elaborated with pure or combined cryoprotectants using differential scanning calorimetry (DSC) involving a large range of water contents was the main focus of this study. For this purpose, thirty aqueous solutions containing maltodextrin, polydextrose, glucose, and their mixtures in a concentration range of 40–95% water were studied.

2. Materials and Methods

2.1. Preparation of Cryoprotectant Solutions. Analytical grade maltodextrin 4–7 DE (product no. 419672; molecular weight 3600), crystalline glucose (product no. G5767; molecular weight 180.16), both from Sigma-Aldrich CO. (St. Louis, MO), and commercial food grade noncrystalline polydextrose (molecular weight 342.3) from Henan Tailijie Biotech Co.

(China) were the employed cryoprotectants. Each cryoprotectant was previously equilibrated over Drierite® (anhydrous calcium sulfate, $a_w \approx 0$) in a desiccator at room temperature for several weeks in order to obtain completely dry samples. The moisture content of these equilibrated samples was then determined by the AOAC method [48] and taken into account for the preparation of solutions, as described below.

Cryoprotectant solutions were prepared gravimetrically by weighing the appropriate amount of solid material using a Mettler-Toledo microbalance (model AG245; RS232 interface; readability 0.1 mg/0.01 mg) and adding the appropriate volume of distilled water. A completely randomized distance-based experimental design for mixtures of four components was used to establish the mass fraction of maltodextrin (X_M), glucose (X_G), polydextrose (X_P), and water (X_W) in the cryoprotectant solutions. The Modde 7.0 (Umetric AB) statistical software was used for the experimental design, in which the constraints $\sum_1^n X_i = 1$ and $0.4 \leq X_W \leq 0.95$ were considered. Five replicate points were used and a total of 30 cryoprotectant solutions, including pure components as well as binary and ternary mixtures, were prepared (Table 1).

Solutions containing high proportions of maltodextrin were dissolved in Eppendorf safe-lock tubes placed in a water bath at a controlled temperature (40°C). In all cases, the solutions were well stirred to obtain clear solutions at room temperature and after stored at 4°C for several days to allow for equilibration of the moisture content throughout the sample [27, 33]. The solid content of the equilibrated cryoprotectant solutions was confirmed with a digital refractometer (AR 200, Leica) at room temperature.

2.2. Phase and State Transition Analysis of Frozen Cryoprotectant Solutions. DSC measurements were carried out using a Q2000 differential scanning calorimeter (TA Instruments, Delaware 19720, USA) with a RCS90 cooling system using ultrapure nitrogen as the purging gas at a flow rate of 50 mL/min.

Samples of the cryoprotectant solutions (approximately 5–10 mg) were weighed in aluminum pans, hermetically sealed, and then placed in the DSC instrument at room temperature; an empty pan was used as the reference. The calorimetric methodology proposed by Sablani et al. [20] and Ruiz-Cabrera et al. [33] was used for determination of the T_m, T_m', and T_g' values of the samples. Before starting the experiments, the samples were equilibrated in the DSC apparatus at 20°C for 2 min. The samples were then cooled to −70°C at 20°C/min to quickly reach the amorphous state, maintained at this temperature for 3 min, and then heated back to room temperature at a rate of 10°C/min. It is important to note that, for cryoprotectant solutions containing water in the range of 40% to 60% wet basis ($0.4 \leq X_W \leq 0.6$, Table 1), an annealing procedure at (T_m' − 1)°C for 30 min was implemented to obtain maximally freeze-concentrated cryoprotectant matrices [33, 49]. The apparent value of T_m' in each experiment was determined from the first thermal analysis in nonannealed state. In these cases, each cryoprotectant solution was cooled to −70°C, held for 3 min, heated at 10°C/min to the annealing temperature (T_m' − 1)°C, and then annealed for 30 min. Following annealing, the samples were cooled at 20°C/min to −70°C, held for 3 min, and then reheated at 10°C/min back to room temperature. The midpoint T_g' and T_m' values were determined using the half-height method from the first and second step changes of the heat flow, as observed in the DSC thermograms during the heating process [33, 50, 51]. In this study, the peak temperature corresponding to the melting endotherm was considered to be the freezing point (T_m). In all cases, the Universal Thermal Analysis software (version 4.4A) was used.

2.3. Statistical Analysis of the Data. Analysis of variance (ANOVA) was performed with a confidence level of 95% ($\alpha = 0.05$) using the Modde 7.0 statistical software (Umetrics, Kinnelon, NJ, USA). The Scheffe cubic model (Eq. (1)) with interactions was used to analyze the effect of the chemical composition of the cryoprotectant solutions (X_M, X_G, X_P, and X_W) on the response variables (T_g', T_m', and T_m) as follows:

$$\begin{aligned} Y = {} & a_0 X_M + a_1 X_G + a_2 X_P + a_3 X_W + a_4 X_M X_G + a_5 X_G X_P \\ & + a_6 X_P X_W + a_7 X_M X_W + a_8 X_G X_W + a_9 X_M X_P \\ & + a_{10} X_M X_G X_P + a_{11} X_M X_G X_W + a_{12} X_M X_P X_W \\ & + a_{13} X_G X_P X_W + a_{14} X_M X_G (X_M - X_G) \\ & + a_{15} X_M X_P (X_M - X_P) + a_{16} X_M X_W (X_M - X_W) \\ & + a_{17} X_G X_P (X_G - X_P) + a_{18} X_G X_W (X_G - X_W) \\ & + a_{19} X_P X_W (X_P - X_W) \end{aligned} \quad (1)$$

where a_0 to a_{19} are the regression coefficients of the model. A backward elimination regression with an α value of 0.10 was applied and hierarchical models were obtained from Eq. (1).

3. Results and Discussion

3.1. Determination of T_g', T_m', and T_m in Frozen Cryoprotectant Solutions. The DSC thermograms showing the process of rewarming from −70 to 20°C for annealed solutions of maltodextrin (Experiment 2), polydextrose (Experiment 1), and glucose (Experiment 27), all conditioned at 40% water, are shown in Figures 1(a)–1(c). No evidence of an exothermic peak associated with the recrystallization of unfrozen water is observed in the rewarming thermograms, confirming that maximally freeze-concentrated phases were obtained after the annealing treatment at T_m'-1°C for 30 min. Instead, the DSC thermograms show thermal events in which the endothermic peak of ice melting is the most visible feature, preceded by one or two changes in the baseline with characteristics typical of a glass transition depending on the cryoprotectant. For instance, only one transition is observed for the maltodextrin solution (Figure 1(a)), while two transitions appear for the mixtures of polydextrose and glucose (Figures 1(b)–1(c)). These results are consistent with numerous previous studies, where solutions of LMW carbohydrates, such as glucose, fructose, sucrose, and trehalose, were found to exhibit two glass transition-like thermal events, while HMW polysaccharides, such as maltodextrin and starch, exhibited

Table 1: Distance-based experimental design for mixture of four components and measured response variables.

Exp No.	Run orden	X_M	X_G	X_P	X_W	T_g'	T_m'	T_m
1	1	0	0	0.6	0.4	-34.6	-22.6	-7.3
2	26	0.6	0	0	0.4	-6.3	-6.3	0.5
3	3	0	0.6	0	0.4	-54.2	-39.2	-15.4
4	4	0.017	0.017	0.017	0.95	N.D.	N.D.	1.8
5	29	0.2	0.2	0.2	0.4	-48.5	-36.1	-15.2
6	12	0.325	0	0	0.675	-7.1	-7.1	1.0
7	8	0	0.325	0	0.675	-53.4	-30.1	-3.9
8	27	0	0	0.325	0.675	-32.7	-20.1	-0.3
9	7	0.4	0.2	0	0.4	-38.6	-18.4	-2.1
10	17	0.2	0.4	0	0.4	-49.7	-28.6	-6.2
11	16	0.4	0	0.2	0.4	-29.9	-14.9	-1.0
12	10	0.2	0	0.4	0.4	-30.1	-16.9	-1.4
13	30	0	0.4	0.2	0.4	-47.2	-28.4	-9.5
14	20	0	0.2	0.4	0.4	-44.8	-29.4	-11.3
15	28	0.108	0.108	0.108	0.675	-38.9	-24.3	-0.6
16	22	0.217	0.2	0	0.583	-40.2	-25.0	-2.5
17	14	0.217	0	0.2	0.583	-25.6	-11.5	1.5
18	24	0	0.217	0.2	0.583	-45.7	-28.1	-4.7
19	11	0.354	0.054	0.054	0.538	-23.8	-14.1	-0.4
20	25	0.054	0.354	0.054	0.538	-51.3	-36.1	-5.4
21	6	0.054	0.054	0.354	0.538	-39.2	-20.5	-2.8
22	19	0.079	0.054	0.054	0.813	-37.0	-21.1	1.1
23	21	0.154	0.154	0.154	0.538	-40.4	-27.0	-1.9
24	23	0.217	0.017	0	0.767	-23.1	-11.0	1.1
25	9	0.017	0.217	0.000	0.767	-51.7	-30.3	-1.3
26	15	0	0	0.6	0.4	-36.5	-22.5	-7.1
27	2	0	0.6	0	0.4	-54.3	-39.2	-15.6
28	5	0.6	0	0	0.4	-7.3	-7.3	1.8
29	18	0.2	0	0.4	0.4	-32.2	-14.4	-1.9
30	13	0	0.2	0.4	0.4	-45.8	-30.0	-11.9

N.D.: not detected.

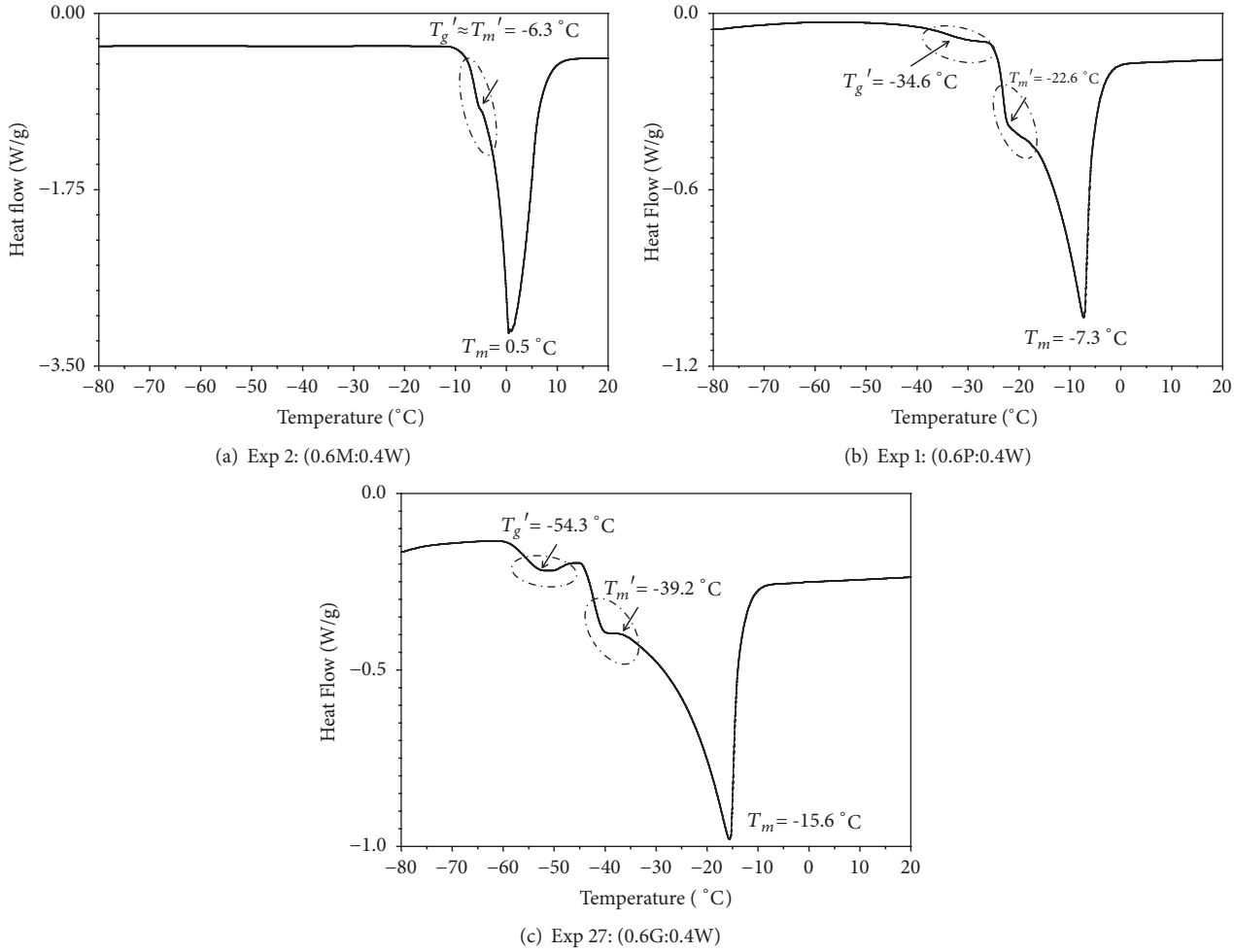

Figure 1: Phase and state transition analysis of frozen cryoprotectant solutions containing pure components prepared at a moisture content of 40% wet basis (X_W = 0.4) and annealed for 30 min at (T_m' − 1)°C: (a) Exp 2, (b) Exp 1, and (c) Exp 27.

a single transition by DSC analysis [20, 30, 32, 43, 51–55]. The origin of these two glass transitions is not completely understood; notions such as incompatibility of different solutes in the mixture, unequilibrated phases trapped around or within nucleated ice crystals, and reduced mobility of some components have been proposed to explain these transition events [51]. For aqueous sugar solutions, it has been suggested that the first transition can be attributed to a glass transition of the maximally freeze-concentrated phase, designated as T_g', while the second transition can be interpreted as the beginning of the melting of ice crystals, often designated as T_m' [30, 32, 33, 50–52]. In the case of protein aqueous solutions, it has been suggested that the first transition corresponds to a glass transition of part of unfrozen water, while the second transition corresponds to a glass transition of the primary chain of the hydrated protein [32]. Thus, a similar approach to those used for sugar solutions was implemented for the determination of the T_g' and T_m' of cryoprotectant solutions.

On the other hand, it has been reported that maltodextrin solutions exhibit a broad glass transition temperature range, attributed to its large molecular weight, and that the difference between T_g' and T_m' is likely to be very small or even null, or the glass transition may overlap with the ice melting [20, 30, 55]. For example, it has been reported by Roos and Karel [30] that the higher the molecular mass of maltodextrin, the lower the difference between the T_g' and T_m' values. However, it is probable that additional factors, such as high viscosity, ability to trap and bind water, maltodextrin-water interactions, or the amount of nonfreezable water, may be involved [56]. Thus, for practical considerations in this study, the T_g' and T_m' values were considered to be identical when solutions of maltodextrin at 40% water were used, as shown in Figure 1(a).

As shown in Figure 1, frozen solutions of pure maltodextrin (Experiment 2, Table 1) exhibited higher values of T_g', T_m', and T_m (_6,–6.3 and 0.5°C) than those obtained for frozen solutions of polydextrose (Experiment 1: –34.6, –22.6, and –7.3°C) and glucose (Experiment 27: –54.3, –39.2, and –15.6°C) at the same water content; this can be attributed to the higher molecular weight of maltodextrin. Roos and Karel [30] also reported that decreasing DE in maltodextrins

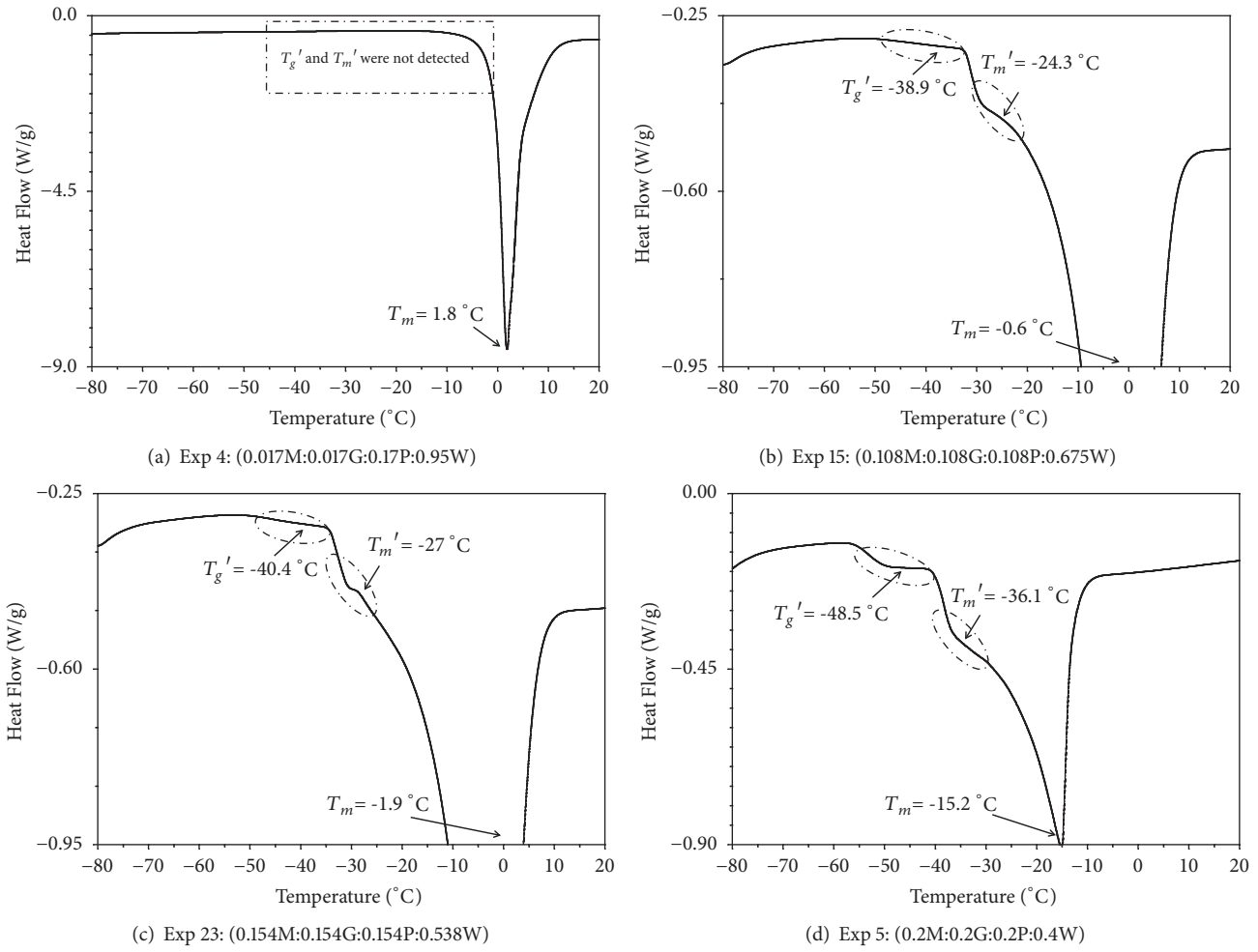

Figure 2: Phase and state transitions analysis of frozen ternary cryoprotectant solutions prepared at moisture contents in the range of 40–95% wet basis ($0.4 < X_W < 0.95$): (a) Exp 4, (b) Exp 15, (c) Exp 23, and (d) Exp 5. Exp 23 and Exp 5 were annealed for 30 min at (T_m' – 1)°C.

increased the molecular weight of the system and shifted the T_g' to a higher temperature. The T_g' and T_m' values determined for the pure maltodextrin solution prepared at 40% water are slightly higher than the values of T_g' (–15°C) and T_m' (–11°C) reported by Roos and Karel [30] when a solution of maltodextrin at 60% water with a similar DE of 5 was prepared. It is likely that the difference in the moisture content in the matrices is the main factor causing these discrepancies [12].

Typically, reports have shown that the higher the molecular weight of the cryoprotectant, the higher the T_g', T_m', and T_m values. The same trend has been reported in other studies when solutions of LMW and HMW carbohydrates were used [36, 43, 55]. In general, T_m' can be increased by addition of high molecular weight compounds as a result of an increase in the viscosity of the unfrozen phase which may delay crystallization of water [30, 57]. With regard to the T_m values, based on the colligative properties of a solution, the freezing point is directly proportional to the molal concentration of the solute [37]. This indicates that the lower the molecular mass of the solute, the higher the freezing point depression, as shown in Figure 1. Information on the freezing point depression is important for chilling and freezing processes, where reduction or inhibition of ice formation is required [3, 4].

For comparison, Figures 2(a)–2(d) show the DSC thermograms obtained for frozen ternary mixtures of cryoprotectants prepared with moisture contents in the range of 40–95% wet basis ($0.4 < X_W < 0.95$). The mixture containing 95% water (Figure 2(a), Experiment 4) showed no apparent glass transition events, because only the ice-melting peak was detected during heating. It is likely that the change in heat capacity (ΔC_p) at the glass transition range decreased with the solid content, resulting in T_g' and T_m' values for this sample outside the limits of detection [32, 54]. However, it was noted that the increase in the concentration of cryoprotectant solutions enhanced the intensity of thermal transitions, whereby the T_g' and T_m' values could be adequately determined in Experiments 15, 23, and 5 (Figures 2(b)–2(d)). It can also be observed in Figures 2(b)–2(d) that, at all moisture contents, the ternary mixtures exhibit well-defined global transitions, indicating good compatibility of the cryoprotectants [33, 56].

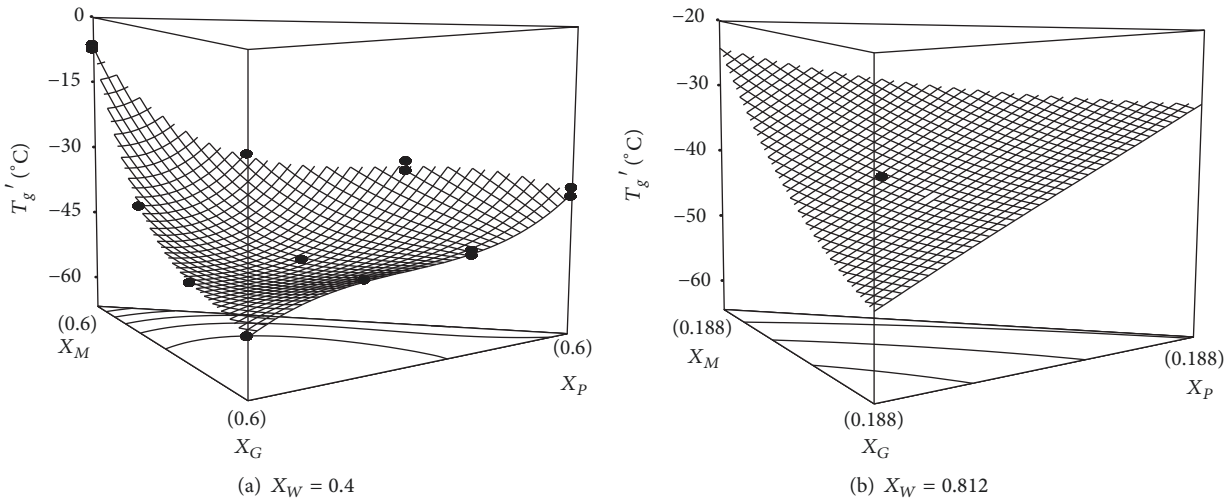

FIGURE 3: Response surface plots showing the variation of the experimental and predicted values of T_g' for different cryoprotectant solutions: (a) $X_W = 0.4$ and (b) $X_W = 0.812$. ● Experimental data.

The same behavior was observed for binary-component cryoprotectant solutions (DSC thermograms not shown).

The values of T_g', T_m', and T_m corresponding to each treatment are presented in Table 1. This table shows that the glass transition temperatures (T_g' and T_m') and freezing point (T_m) values vary from –54.3 to –6.3°C, –39.2 to –6.3°C, and –15.6 to 1.8°C, respectively. In general, these values are of the same order of magnitude as those reported in studies using similar saccharide solutions [30, 43, 55, 58]. According to the data in Table 1, no synergistic increase of T_g' and T_m' was found when mixtures of HMW and LMW cryoprotectants were analyzed. From Table 1, it can be observed that the highest T_g', T_m', and T_m values were found for solutions of maltodextrin at 40% water (Experiments 2 and 28) and the lowest values with solutions of glucose at 40% water (Experiments 3 and 27), with intermediate values for all the other cryoprotectant solutions. Typically, a mixture of compatible biopolymers exhibits intermediate state and phase transition temperatures because the components in the sample act as mutual plasticizers [56, 57]. Harnkarnsujarit et al. [47] had also reported that sugars such as sucrose, glucose and fructose depressed the T_m' and T_g' values of the maltodextrin-sugars systems.

3.2. Regression Analysis and Response Surface Plots. The uncoded regression coefficients, results of variance analysis (ANOVA), coefficients of determination (R^2), coefficients of variation (CV%), and model significance ($p > F$) obtained with (1) for T_g', T_m', and T_m are presented in Table 2. From these results, it can be observed that the response variables show a high level of significance for the regression equation ($p < 0.0001$), being nonsignificant for the lack of fit ($p > 0.05$), indicating the effectiveness of the regression analysis. Additionally, the R^2 values indicated that over 98% of the variability in the responses could be explained by the proposed models, also presented in Table 2.

According to the analysis of variance (Table 2), T_g', T_m', and T_m are all primarily affected by the linear terms of the cubic model, followed by interactions between binary and ternary components. Based on the literature, it is generally accepted that the T_m' and T_g' values are independent of the initial solute concentration or moisture content. The results from some studies have indicated that T_g' and T_m' exhibit slight variations according to the moisture content and, therefore, average values have been reported for several food products [20, 26, 30, 33]. However, the results from the present study show that the moisture content of the cryoprotectant solutions ($p < 0.0001$, Table 2) is also a crucial factor influencing these thermal parameters.

Three-dimensional response surface plots for T_g' (Figure 3), T_m' (Figure 4), and T_m (Figure 5) using two different moisture contents for each response variable were constructed in order to gain better understanding of the interactive effects of the three cryoprotectants (X_M, X_G, X_P) on the corresponding variables. The results presented in Figures 3–5 demonstrate that, in all cases, good agreement exists between the experimental and predicted values ($R^2 > 0.98$). Also, the profiles in Figures 3–5 show that, at all moisture contents, the maximum glass transition and freezing temperatures appear at the points corresponding to pure maltodextrin, while the minimum values were obtained for the samples containing pure glucose. The values in good agreement with the experimental results are presented in Table 1. From the point of view of the glass transition concept, this behavior suggests that maltodextrin exhibits a greater cryostabilizing effect than polydextrose and glucose. For instance, maltodextrin DE 18 showed a higher effectiveness against lipid oxidation that an equiproportional mixture of sucrose and sorbitol in frozen-stored minced muscle of Atlantic mackerel [44]. On the other hand, Rodríguez-Furlán et al. [43] found that inulin exhibits better stabilizing properties than glucose and sucrose in the preservation of

Table 2: Uncoded regression coefficients and variance analysis of the hierarchical mathematical models to evaluate the variation of response variables as a function of chemical composition of the cryoprotectant solutions ($p<0.05$).

Effect	Response variables					
	T_g'		T_m'		T_m^*	
	a values	p>t	*a* values	p>t	*a* values	p>t
X_M	-150.42	< 0.0001	-25.84	< 0.0001	542.57	< 0.0001
X_G	-51.85	< 0.0001	-42.15	< 0.0001	-425.77	0.2184
X_P	109.26	< 0.0001	-154.73	< 0.0001	-364.76	< 0.0001
X_W	-50.10	< 0.0001	-19.77	< 0.0001	490.96	< 0.0001
$X_M X_G$	127.35	< 0.0001	261.83	0.6304	233.10	0.3608
$X_G X_P$	-61.02	0.1873	111.71	0.1788	-45.29	0.8449
$X_P X_W$	-272.34	0.0190	279.03	0.9517	777.85	0.0197
$X_M X_W$	397.90	0.0008	69.14	0.0343	-314.98	0.2683
$X_G X_W$	-13.08	0.7631	-24.86	0.3756	335.40	0.2069
$X_M X_P$	-35.29	< 0.0001	-51.63	0.5986	-3827.75	0.0559
$X_M X_G X_P$	-427.34	0.0296	-1642.90	< 0.0001	-22925.84	< 0.0001
$X_M X_G X_W$	-580.60	0.0160	-672.41	0.0057	-	
$X_M X_P X_W$	-369.15	0.0817	345.36	0.1057	10794.51	0.0052
$X_G X_P X_W$	-281.90	0.3225	-	-	-	
$X_M X_G (X_M - X_G)$	-156.36	0.0171	-	-	-	
$X_M X_P (X_M - X_P)$	-271.59	0.0002	-137.14	0.0201	-2310.27	0.0241
$X_M X_W (X_M - X_W)$	166.11	0.1724	-	-		
$X_G X_P (X_G - X_P)$	130.49	0.0225	183.97	0.0037	2801.85	0.0079
$X_G X_W (X_G - X_W)$	-	-	-	-	-	-
$X_P X_W (X_P - X_W)$	-328.21	0.0311	234.05	0.0942	-	-
Models						
p>F		< 0.0001		< 0.0001		< 0.0001
R^2		0.9971		0.9907		0.9849
Lack of fit		0.2205		0.1066		0.2302
C.V. (%)		3.38		5.90		8.57

∗Note: according to a Box-Cox analysis, a transformation $Tm* = (Tm + 20)^2$ on Tm values was carried out to stabilize the variance.

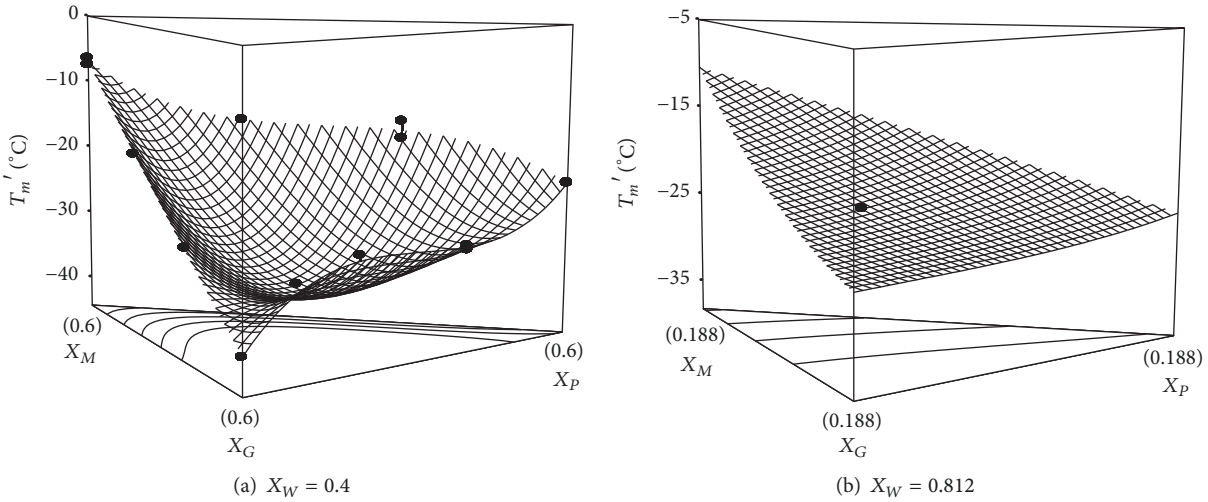

FIGURE 4: Response surface plots showing the variation of the experimental and predicted values of T_m' for different cryoprotectant solutions: (a) X_W = 0.4 and (b) X_W = 0.812. ● Experimental data.

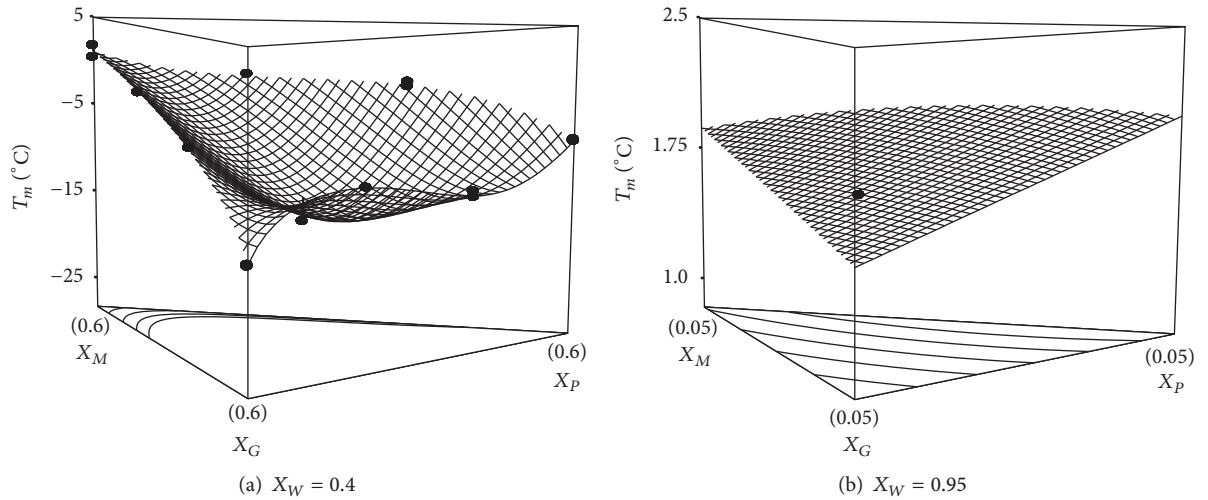

FIGURE 5: Response surface plots showing the variation of the experimental and predicted values of T_m for different cryoprotectant solutions: (a) X_W = 0.4 and (b) X_W = 0.95. ● Experimental data.

frozen bovine plasma protein. However, it is also clearly shown that glucose significantly contributes to the depression of the freezing point in aqueous cryoprotectant solutions and that it can be used as an alternative to reduce or avoid intra- or extra-cellular ice formation during chilling and freezing processes [3, 4]. As previously discussed, intermediate values of T_g, T_m', and T_m were obtained for the other mixtures of cryoprotectants, resulting from the good compatibility and mutual plasticizing action of the different components in the samples [56, 57].

Although numerous studies have been conducted on the use of cryoprotectants to prevent deleterious changes in foods caused during freezing and frozen storage, little research has been carried out on the thermal transition properties of aqueous cryoprotectant solutions at frozen temperatures at a large working range of water contents. In this context, the experimental values of T_g', T_m', and T_m (Table 1) obtained and the mathematical models proposed in this study (Table 2) may be a great aid for the formulation of cryoprotective media containing more than two cryoprotectants to improve the storage stability and quality of frozen food products with high and intermediate moisture content and modifiable formulations, such as ice cream, purees, jams, surimi, sugared yolks, etc. However, further studies are required, which will focus on overcoming the difficulties in the incorporation of cryoprotectants in large samples or foodstuffs, such as whole fruits and meats.

4. Conclusions

The effects of cryoprotectants and their concentration on the glass and phase transition temperatures of frozen aqueous solutions containing maltodextrin, polydextrose, glucose, and their mixtures prepared at moisture contents in the range

of 40–95% water were studied. DSC thermograms revealed the existence of a glass transition for pure maltodextrin solutions and two transitions for all the other solutions. The results indicated that the T_g', T_m', and T_m values increase with the molecular weight of the cryoprotectant. Additionally, statistical analysis of the data ($p < 0.05$) demonstrated that both the solute and water composition should be considered in the formulation of cryoprotective media, as they were found to significantly affect the T_g', T_m', and T_m values ($p < 0.05$). Mathematical expressions for T_g', T_m', and T_m as a function of the mass fractions of the cryoprotectants (X_M, X_G, X_P) and water (X_W) and their interactions were developed to guide the formulation of cryoprotective media involving mixtures of more than two cryoprotectants to improve the storage stability and quality of frozen food products at high and intermediate moisture contents with modifiable formulations, such as ice cream, purees, jams, surimi, sugared yolks, etc.

Data Availability

Data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare no conflicts of interest.

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