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Effects of emulsifiers on the moisture sorption and crystallization of amorphous sucrose lyophiles

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ABSTRACT

The crystallization of amorphous sucrose can be problematic in food products. This study explored how emulsifiers (a range of sucrose esters, polysorbates, and soy lecithin) impact the moisture sorption and crystallization of amorphous sucrose lyophiles. Solutions containing sucrose with and without emulsifiers were lyophilized, stored in desiccators, and analyzed by X-ray diffraction, infrared spectroscopy, and polarized light microscopy over time. Moisture sorption techniques, Karl Fischer titration, and differential scanning calorimetry were also used. Different emulsifiers had varying impacts on sucrose crystallization tendencies. Polysorbates enhanced sucrose crystallization, decreasing both the RH and time at which sucrose crystallized. These lyophiles did not collapse upon crystallization, unlike all other samples, indicating the likelihood of variations in nucleation sites and crystal growth. All other emulsifiers stabilized amorphous sucrose by up to a factor of 7x, even in the presence of increased water absorbed and independent of glass transition temperatures, indicating emulsifier structure governed sucrose crystallization tendencies.

1. Introduction

In addition to increasing the sweetness of foods, sucrose contributes to the structure, texture, dissolution, and/or taste perception of products ranging from various confectioneries and low moisture baked goods, to powder beverage and seasoning mixtures. The physical state of the sucrose solid affects many characteristics, including stability, dissolution, moisture sorption, and many sensory properties, such as texture and flavor perception (Chirife & Karel, 1974; Mathlouthi, 1995). Amorphous sucrose is often the preferred state for many confectionery products due to the desirable dissolution properties and softer texture. However, amorphous sucrose has a tendency to crystallize to the more thermodynamically stable crystalline form during storage. Crystallization can lead to undesirable texture and flavor changes, impaired solubility, and acceleration of chemical changes such as oxidation and enzymatic activity in other materials in the food matrix (Buera, Schebor, & Elizalde, 2005; Slade, Levine, & Reid, 1991). Therefore, sucrose crystallization is a major area of interest in the food industry, with emphasis placed on the effects of formulations and storage environments on crystallization kinetics (Buera et al., 2005; Kinugawa et al., 2015; Saleki-Gerhardt & Zografi, 1994; Thorat, Forny, Meunier, Taylor, & Mauer, 2017, 2018).

Numerous additives have been shown to disrupt and delay sucrose crystallization by a variety of mechanisms including: decreasing molecular mobility (Saleki-Gerhardt & Zografi, 1994), increasing the glass transition temperature (T_g) and/or viscosity of the co-lyophilized system (Roe & Labuza, 2005; Roos & Karel, 1991), disrupting the crystal lattice due to molecular interactions between sucrose and the additive (Gabarra & Hartel, 1998; Shamblin & Zografi, 1999), and generally inhibiting nucleation and crystal growth (Carstensen & van Scoik, 1990). More recently, a study of the effects of chloride and sulfate salts on amorphous sucrose crystallization found that increasing the cation valency (and corresponding ion hydration shell) delayed or prevented sucrose crystallization even while decreasing T_g, presumably by altering the water dynamics in the matrix (Thorat et al., 2017). A study on the effects of a series of mono-, di-, and tri-saccharides on

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amorphous sucrose stability found that saccharides containing regions of structural similarity as well as structural dissimilarity best inhibited sucrose crystallization, with these structural relationships seemingly having a greater influence on the delay of sucrose crystallization than that of a decrease in T_g due to moisture sorption (Thorat et al., 2018).

While many studies have explored the impact of additives on sucrose crystallization from the solid state, the role of emulsifiers in altering sucrose crystallization has primarily been studied in solutions and is not well-defined. Emulsifiers have been shown to alter the crystallization of compounds by different mechanisms. In solutions, emulsifiers have been shown to both reduce and increase the primary nucleation rate of different compounds (Canselier, 1993; van Hook, 1961). Emulsifiers have also been shown to have conflicting effects on crystal growth rates: the reduction of interfacial tension by the emulsifier can increase the crystal growth rate, but the slowing of mass transfer at the crystal-solution interface due to the presence of the emulsifier can slow the crystal growth rate (Canselier, 1993; van Hook, 1988; Vasanth Kumar & Rocha, 2009). Some emulsifiers have even been shown to both increase and decrease the rate of crystallization, depending on the amount added (Michaels & van Kreveld, 1966). For example, sodium doecyl (tetrapropylene) benzene sulfonate increased the rate of lactose crystallization at low levels of addition but decreased the rate when added in larger amounts (Michaels & van Kreveld, 1966). Regardless, it is agreed upon that the changes emulsifiers cause on the adsorbed crystal surface layer are likely to affect secondary nucleation, and changes in surface energy due to emulsifiers are likely to affect crystal growth (Canselier, 1993; Hartel & Shastry, 1991; Vasanth Kumar & Rocha, 2009); however, these concepts have not been shown to correlate to crystallization from the amorphous state. While understanding formulation effects on crystallization from solutions is certainly important, foods and food ingredients tend to be solids. Therefore, understanding how emulsifiers alter sucrose crystallization from the amorphous state is relevant.

The objective of this study was to determine the effects of different types and concentrations of food-relevant emulsifiers on the crystallization of amorphous sucrose. It was hypothesized that the structure of the emulsifiers would play a significant role in stabilizing amorphous sucrose. Emulsifiers containing a region that is structurally similar to sucrose as well as a structurally dissimilar region were anticipated to provide the greatest inhibition to sucrose crystallization, consistent with the concept shown for the efficacy of how different saccharide structures altered sucrose crystallization (Leinen & Labuza, 2006; Thorat et al., 2018). The structures and properties of the emulsifiers used in this study are shown in Table 1. To test the hypothesis, these emulsifiers were selected to encompass a range of hydrophilic lipophilic balances (HLB, ~2–17), number of monosaccharide units (0–2), number of hydroxyl groups (~0–7), molecular weights, thermal and hygroscopic traits, and structural components.

2. Materials and methods

2.1. Materials

The sucrose used in this study was obtained from Mallinckrodt Chemicals (Philipsburg, NJ), and the emulsifiers were a series of sucrose esters (stearic ester 30% (SP30), stearic ester 50% (SP50), stearic ester 70% (SP70), and palmitic ester 75% (PS750)) varying in the type of fatty acid as well as the percentage of mono-esters (30–75% as shown) relative to di- and tri-esters from Sisterna (Roosendaal, Netherlands); soy lecithin from Modernist Pantry (Eliot, ME); and polysorbate 20 and polysorbate 80 from Florida Laboratories, Inc. (Fort Lauderdale, FL). The emulsifiers were chosen based on common usage in the food industry as well as variable structures of the compounds (as shown in Table 1).

Desiccators were prepared using phosphorus pentoxide (P_2O_5) (Fisher Scientific, Fair Lawn, NJ) to maintain a relative humidity (RH) of ~0% or by using the following saturated salt solutions to control the RH at higher levels: lithium chloride (~11% RH) obtained from Avantor Performance Materials (Center Valley, PA), potassium acetate (~23% RH) obtained from Fisher Scientific, and magnesium chloride (~33% RH) obtained from Fisher Scientific. For use in volumetric one-component Karl Fischer titrations, Karl Fischer reagents including HYDRANAL-Composite 2 (titrant), HYDRANAL-Methanol Rapid (working medium), and HYDRANAL-Water Standard 10 were purchased from Sigma-Aldrich (St. Louis, MO). Water used throughout the study was deionized and purified using a Barnstead E-Pure ultrapure water purification system (ThermoScientific, Waltham, MA) with a resistivity at 25 °C greater than 17.5 MΩ·cm.

2.2. Preparation of amorphous samples

Samples were prepared by freeze drying 10% *w/v* sucrose solutions with and without 1% and 5% (*w/w*) of the co-formulated emulsifier in which both the sucrose and the emulsifier were completely dissolved. There were 7 co-formulated emulsifier additives (Table 1), each added at two concentrations (1% and 5% *w/w* emulsifier/sucrose), giving a total of 14 dispersion preparations in addition to the control sucrose. The solutions were frozen at −20 °C for at least 12 h prior to lyophilization. Lyophilization was completed in a VirTis Genesis 25ES freeze dryer (SP Scientific, Warminster, PA). Samples were initially frozen in the freeze dryer at −40 °C and 300 mTorr (40 Pa) for 6 h. The freeze dryer was then held at −40 °C and 150 mTorr (20 Pa) for 24 h to allow for primary drying to occur. This was followed by an increase in temperature from −40 °C to 20 °C in increments of 10 °C, holding for 9 h at each step to allow for secondary drying. Finally, a heating step was completed at 25 °C and 300 mTorr (40 Pa) for 6 h, after which samples were immediately transferred to desiccators containing P_2O_5 (~0% RH). These samples were stored in the desiccators containing P_2O_5 at ambient temperature (22 ± 2 °C) until further analysis, and all subsequent sample handling was done in a glove box purged with nitrogen (to drop the ambient RH to ~5%).

2.3. Storage treatments

To initiate the RH storage treatments, the lyophiles were transferred from the desiccators containing P_2O_5 into desiccators containing saturated salt solutions of lithium chloride (~11% RH), potassium acetate (~23% RH), or magnesium chloride (~33% RH), which were then stored at 25 °C in a temperature-controlled room. Samples were removed from these desiccators and analyzed periodically over 4 weeks. A single desiccator was used for each timepoint of analysis to avoid exposing the samples to ambient RH until the day of their analysis. Samples were discarded after analysis.

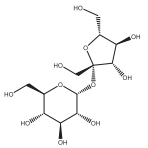
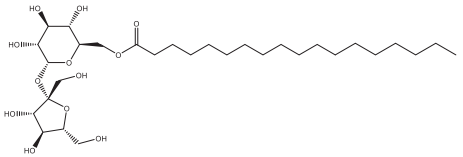
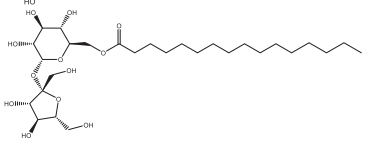
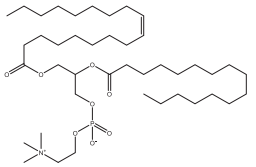
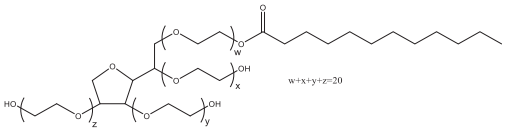
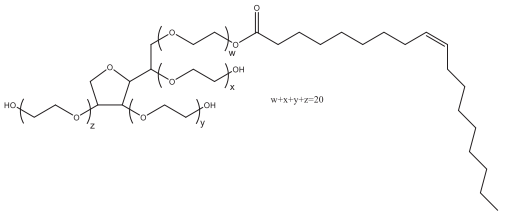
2.4. Determination of crystallinity

A combination of powder X-ray diffraction (PXRD), polarized light microscopy (PLM), and Fourier-transform infrared spectroscopy (FT-IR) was used to monitor the physical state of samples over time and to identify the onset of crystallization of the amorphous lyophiles (Fig. 1). Lyophiles were analyzed on days 0, 7, 14, 21, and 28. Samples that were found to be crystalline on day 7 were further analyzed on days 2 and 4 by preparing fresh samples to narrow down the time at which the onset of crystallization occurred.

2.4.1. Powder X-ray diffraction

PXRD diffractograms were collected using a Rigaku Smartlab diffractometer (Rigaku Corporation, Tokyo, Japan) equipped with a Cu-Kα radiation source set in Bragg-Brentano geometry and operating at 40 kV and 40 mA. Samples were analyzed using a scan range of 10–35° 2θ at a scan speed of 15°/min and a step size of 0.02°. Samples with diffraction patterns consisting of peaks above a signal-to-noise ratio of 3 were

Table 1
Properties of sucrose and emulsifiers used in lyophiles. Uppercase superscript letters denote statistical significance between experimental T_gs, and lowercase superscript letters denote statistical significance between experimental T_ms (T_{m1}s and T_{m2}s).

Component	Average MW (g/mol)	HLB	HBD/HBA	Number of –OH units	Starting Physical State at RT	Structure	Amorphous	Crystalline	
							Onset T_g	Onset T_{m1}	Onset T_{m2}
Sucrose [1]	342.3	–	8/11	8	Crystalline solid		56.5 ± 0.5 °C^{A} (freeze-dried)	187.9 ± 0.7 °C^{a}	–
SP30 [2]	888.55	6	7/12	5.6–6.3	Crystalline solid		–	22 ± 2 °C^{e}	57 ± 1 °C^{b}
SP50 [2]	808.61	11	7/12	6–6.5	Crystalline solid		–	19 ± 2 °C^{e}	46 ± 1 °C^{d}
SP70 [2]	728.67	15	7/12	6.4–6.7	Crystalline solid		–	18.9 ± 0.9 °C^{ef}	52 ± 2 °C^{bc}
PS750 [2]	683.63	16	7/12	6.5–6.75	Crystalline solid		–	14 ± 1 °C^{f}	51 ± 1 °C^{cd}
Soy Lecithin (Phosphatidylcholine) [3]	643.9	2–7	0/8	0	Amorphous solid		41 ± 2 °C^{B}	190.7 ± 0.5 °C^{a}	–
Polysorbate 20 [4,5]	1228	16.7	3/26	3	Liquid		< −50 °C^{C}	−23 ± 2 °C^{g}	–
Polysorbate 80 [4,5]	1310	15	3/26	3	Liquid		< −50 °C^{C}	−19.6 ± 0.8 °C^{g}	–

[1] Slade et al. (1991).
[2] Szűts et al. (2007).
[3] Bueschelberger, Tirok, Stoffels, and Schoeppe (2015).
[4] Cottrell and van Peij (2015).
[5] Amim et al. (2012).

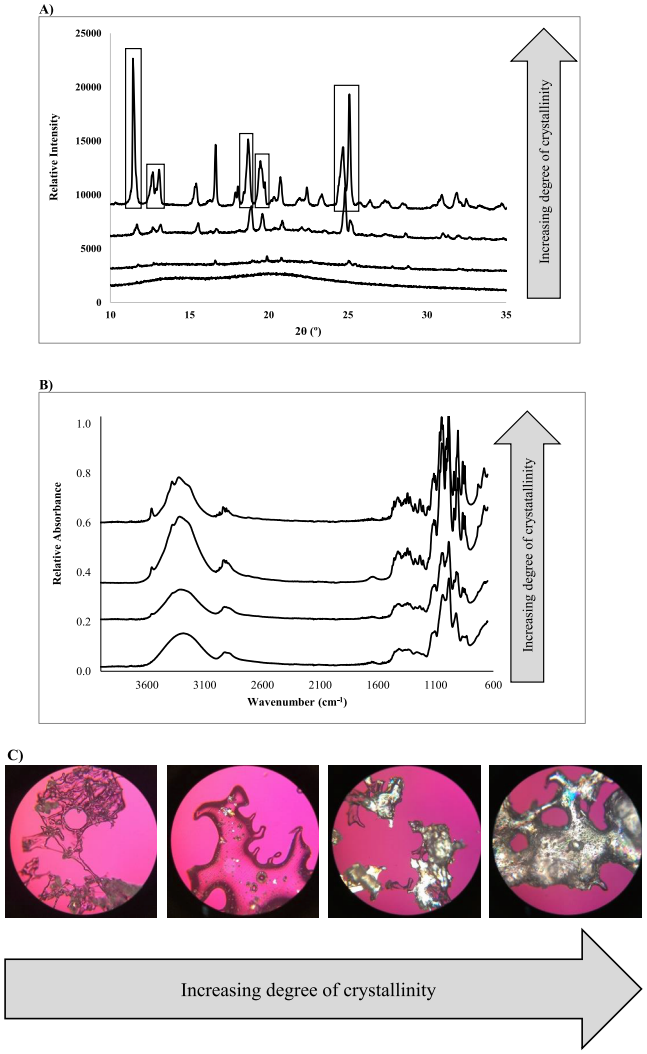


Fig. 1. Analysis of select sucrose lyophiles over time indicating increasing degree of crystallinity, from completely amorphous to completely crystalline, interceded with increasing degrees of crystallinity of A) Powder x-ray diffractograms, where boxed in peaks are the well-defined crystalline sucrose peaks (Leinen & Labuza, 2006), B) FT-IR spectra, where crystallinity was evaluated by the characteristic absorption peaks of crystalline sucrose in the region of 2800–3800 cm^{-1} wavenumbers (Lescure, 1995; Mathlouthi, 1995), and C) PLM images, where birefringence indicates crystallinity.

considered PXRD crystalline. Samples with small peaks above the baseline were labeled partially crystalline, with increasing peak areas/intensities related to increasing crystallinity (Fig. 1A). Samples with no peaks and only an amorphous halo were considered to be PXRD amorphous.

2.4.2. Fourier-Transform Infrared Spectroscopy

FT-IR (TravelIR HCI, SensIR Technologies, LLC, Danbury, CT) with a fixed attenuated total reflectance (ATR) accessory was used to monitor crystallinity of sucrose in the lyophiles using a method described in Lescure (1995) and Mathlouthi (1995). Briefly, crystalline sucrose can be identified by characteristic absorption peaks due to hydrogen bonding in the 2800–3800 cm^{-1} region. The FT-IR was equipped with a TGA detector, resolution was set to 4 cm^{-1}, and samples were scanned 64 times from 650 to 4000 cm^{-1}. Spectra of control crystalline and amorphous sucrose samples were collected and used as comparisons to verify the physical state of the lyophiles. OMNIC Series Software (ThermoScientific) was used to analyze the spectra.

2.4.3. Polarized light microscope

Samples were observed with an Omano polarized light microscope (Omano, China), and crystal identification was done as described by Carlton (2011). Briefly, the appearance of birefringence in the lyophilized samples indicated crystallinity. Photographs to document sample appearance were taken using an iPhone 6 s camera attached to the microscope eyepiece by an iDu LabCam adapter (Detroit, MI). The microscope was also paired with a RH-controlled stage (GenRH, Allentown, PA), and crystallization of a subset of samples was observed over time at 40% RH and ambient temperature (22 ± 2 °C). Timelapse videos of crystallization were taken using the iPhone 6 s camera.

2.5. Dynamic vapor sorption

Three different moisture sorption profiles of all lyophiles were collected at 25 °C using a SPSx-1μ Dynamic Vapor Sorption Analyzer (Projekt Messtechnik, Ulm, Germany). For the first moisture sorption profile, samples (100–200 mg) were placed in a 23-ring sample holder and held at 0% RH for 48 h in the instrument. Samples were then analyzed from 0 to 80% RH in 5% RH increments, with a maximum residence time of 12 h per step and an equilibration end-point criterion of < 0.001% weight change within 30 min. The moisture sorption profile of each lyophile was plotted using the percent change in mass at the end of each RH step as the equilibration moisture gain at that RH. For the second moisture sorption profile, lyophiles (100–200 mg) were placed in a 23-ring sample holder, again held at 0% RH for 48 h, and then the RH was increased to 40% RH, at which samples were held for 96 h or until mass loss indicative of sucrose crystallization had occurred in all samples. The percent change in mass was plotted versus time to generate a moisture sorption/desorption profile with time, and the onset of mass loss was used to identify the onset time of crystallization. For the third moisture sorption profile, lyophiles were prepared and handled the same as was done for the second profiling, but then the samples were held at 33% RH (instead of 40% RH) until mass loss (indicating crystallization) of most samples had occurred. The percent change in mass was plotted versus time, and onset of crystallization data were compared with those from the 40% RH moisture sorption profile as well as crystallization that occurred in the 33% RH desiccators.

2.6. Moisture content

The moisture contents of all initial lyophiles after exposure to 0% RH for 2–4 days, as well as all lyophiles that remained amorphous for the entire 4 weeks of exposure to 11%, 23%, or 33% RH, were determined using a one-component volumetric Karl Fischer titration method (V20S Volumetric KF Titrator, Mettler-Toledo, LLC, Columbus, OH). Approximately 50 mg of each lyophile was added directly to the HYDRANAL-Methanol Rapid working medium to extract water from the sample. The sample was then titrated using the HYDRANAL-Composite 2 titrant, which allowed moisture content to be measured in % moisture (*wb*). Calibration of the Karl Fischer titration system was completed prior to sample analyses using the HYDRANAL-Water Standard 10.0 (10 mg/g = 1% water content).

2.7. Differential scanning calorimetry

All lyophiles and starting materials were analyzed by differential scanning calorimetry (DSC) using a DSC 4000 (PerkinElmer, Waltham, MA). The instrument was calibrated with indium and verified with the melting point of water. Dry nitrogen was used to purge the system at 20 mL/min. Initial lyophiles that had been exposed to 0% RH for 2–4 days (5–10 mg) were weighed into 50 μL aluminum DSC pans (PerkinElmer), hermetically sealed, and punctured with a pinhole to allow water vapor to escape when determining 'dry' T_gs. The onset T_g was determined in a heat-cool-heat protocol from the second scan.

Samples were first scanned by heating the samples in the DSC from 20 °C to 100 °C at a rate of 20 °C/minute. Samples were cooled to 20 °C at a rate of 50 °C/minute and held at 20 °C for 3 min to allow the temperature to equilibrate. A second scan then heated the samples from 20 °C to 100 °C at a rate of 20 °C/minute. All starting ingredients were also analyzed for T_g or melting point (T_m) using the heat-cool-heat protocol described above, only varying the temperature range of the scans based on material. Pyris software (PerkinElmer) was used to calculate the onset T_g or onset T_m, which was defined as the temperature in which the endothermic event characterized by a baseline shift began in the second scan or the temperature in which a sharp endothermic peak began in the second scan, respectively.

2.8. Sample photography

Select samples were analyzed for appearance following crystallization in the second moisture sorption experiment, in which samples were held at 40% RH in the SPS moisture sorption instrument. These samples were photographed in a Deep Professional LED Photography light box and with the polarized light microscope using an iPhone 6 s camera.

2.9. Scanning electron microscopy

Scanning electron microscopy (SEM) was completed using a NOVA nanoSEM Field Emission SEM (FEI Company, Hillsboro, OR) to identify differences in crystal morphology. Lyophiles in which sucrose had crystallized during storage at 33% RH were applied to double-sided carbon tape and coated using a platinum target coating system before analysis.

2.10. Statistical analysis

Samples were analyzed in duplicate for moisture sorption (time of crystallization), moisture content, T_g, and T_m. Single-variable ANOVA using SAS 9.4 (SAS Institute, Cary, NC) was used to determine significant differences in time of crystallization, moisture content, T_g, and T_m. Differences were determined using Tukey's post hoc test for multiple comparisons at a significance level of $\alpha = 0.05$. The HLB value, moisture content (initial, after 4 weeks at 11% RH, and after 4 weeks at 23% RH), T_g, molecular weight, and number of –OH groups were also plotted vs. time to crystallization to determine Pearson's correlation coefficients.

3. Results and discussion

3.1. Stability of amorphous sucrose in RH-controlled desiccators

All of the sucrose lyophiles with and without emulsifiers were initially amorphous, as indicated by PXRD, FT-IR, and PLM, except for the lyophiles containing the higher concentration (5% *w/w*) of polysorbate 80. The effects of storage RH on the time to sucrose crystallization in all lyophiles are summarized in Table 2, wherein it can be seen that different emulsifiers had different effects on the stability of amorphous sucrose. Most of the lyophiles that were initially amorphous remained so for the 4 week duration of storage in desiccators at 11% and 23% RH; however, all lyophiles containing polysorbates at both 1% and 5% (*w/w*) concentrations crystallized at these RHs. Increasing the concentration of either polysorbate and increasing the storage RH both resulted in shorter time to sucrose crystallization.

More varied times to sucrose crystallization (ranging from 2 days to 2 weeks) were found when the RH in the desiccators was increased to 33%. The lyophiles in which sucrose was fastest to crystallize (by day 2) at 33% RH included the control and those containing polysorbates, SP50 1%, and PS750 1%. The lyophiles that were slowest to crystallize at 33% RH (by day 14) were those containing SP70 5% and PS750 5%. Unlike the inverse stability trends seen with increasing polysorbate concentration resulting in decreased amorphous sucrose stability, it appeared that increasing the concentration of the sucrose esters tended to increase amorphous sucrose stability (delay time to crystallization). Sucrose esters containing higher percentages of mono-esters (instead of di- and tri-esters) generally resulted in longer amorphous sucrose stabilization. Based on the desiccator studies, the stabilizing trend of the emulsifiers, as documented by time to crystallization, followed the general trend: polysorbate 80 5% < polysorbate 20 5% < polysorbate 80 1% < polysorbate 20 1% < **sucrose control** ≈ SP50 1% ≈ PS750 1% < SP30 1% ≈ SP30 5% ≈ SP50 5% ≈ SP70 1% < soy lecithin 1% ≈ soy lecithin 5% < SP70 5% ≈ PS750 5%. No evidence of crystallization of the emulsifiers was found in PXRD diffractograms over time. Additional analyses were conducted to better understand the differing effects of the emulsifiers on sucrose crystallization.

3.2. Moisture content of amorphous sucrose lyophiles

The storage RH and sample moisture content are known to affect sucrose crystallization (Mathlouthi, 1995). When exposed to environments with RHs higher than the water activity of the sample, amorphous sucrose will absorb moisture, which results in a decrease in the T_g of the matrix and increase in molecular mobility. If conditions are favorable, molecular rearrangement and crystallization occur, at which point moisture is expelled (Makower & Dye, 1956). To enable comparisons between moisture contents and amorphous sucrose stability, the initial moisture contents of all lyophiles and the moisture contents of lyophiles that remained amorphous after 4 weeks of storage in 11% and 23% RH desiccators were measured (Table 3). All initial moisture contents of the lyophiles except for sucrose:SP30 1% were significantly lower ($p < 0.05$) than the sucrose control. The low initial moisture content found in sucrose:polysorbate 80 5% was likely due to the partially crystalline sucrose in the lyophile even immediately after lyophilization. Increasing the storage RH to 11% or 23% RH significantly increased all sample moisture contents but also resulted in no significant differences in moisture content between any of the lyophiles, including the control, at each RH. Taken together, these findings indicate that the addition of an emulsifier altered the moisture diffusion rates during lyophilization, generally resulting in lower initial moisture contents than the control, and that the addition of 1 and 5% (*w/w*) of the sucrose esters and soy lecithin did not alter the hygroscopicity of the samples at low storage RHs in desiccators compared to the control. Although these samples did not crystallize in these conditions, these data may indicate that matrix effects other than differences in hygroscopicity may contribute to the variations in sucrose crystallization onset times between the formulations.

3.3. Moisture sorption profile and sucrose crystallization

To enable direct comparisons between the samples of moisture sorption leading up to crystallization as well as the RH at which crystallization occurred (indicated by mass loss (Makower & Dye, 1956)), moisture sorption profiles were collected from 0 to 80% RH in a gravimetric moisture sorption instrument (Fig. 2A). A 48 h drying step at 0% RH was done in the instrument prior to this data collection to remove significant differences in the initial moisture contents. While most lyophiles (including the control) crystallized at 40% RH, two lyophiles exhibited delayed sucrose crystallization (sucrose:SP50 1% crystallized between 40 and 45% RH, and sucrose:soy lecithin 5% did not crystallize until 45% RH), and the lyophiles containing polysorbates crystallized at lower RHs (sucrose lyophiles containing polysorbate 20 and 80 at both 1% and 5% crystallized at 30% and 10–15% RH, respectively; however, sucrose:polysorbate 80 5% was partially crystalline initially, so crystallization shown by moisture sorption was affected).

The percent moisture gained before crystallization varied between

Table 2
Physical stability of sucrose lyophiles in controlled RH desiccators measured by a combination of PXRD, FTIR, and PLM as well as time of crystallization of amorphous sucrose lyophiles on exposure to 33% and 40% RH in the SPS instrument and the enhancement compared to the control based on the SPS data. Grayscale shading of desiccator data indicates timeframe of crystallization. Superscript letters denote statistical significance between times of crystallization.

	Co-formulated Additive	Percent Additive	Crystallization in Desiccators at			Crystallization in SPS at 40% RH		Crystallization in SPS at 33% RH	
			11% RH	23% RH	33% RH	Crystallization time (hr)	Enhancement Compared to Control	Crystallization time (hr)	Enhancement Compared to Control
Sucrose	-	-	A	A	Day 2 (PC)	15 ± 2^{BC}	1x	85 ± 3^{E}	1x
Sucrose	SP30	1%	A	A	Day 4	14.6 ± 0.2^{BC}	1x	111 ± 1^{DE}	1.3x
Sucrose	SP30	5%	A	A	Day 4	18.5 ± 0.6^{B}	1.2x	120 ± 20^{CD}	1.4x
Sucrose	SP50	1%	A	A	Day 2 (PC)	17 ± 2^{BC}	1.1x	99 ± 8^{DE}	1.2x
Sucrose	SP50	5%	A	A	Day 4 (PC)	15.55 ± 0.07^{BC}	1x	143 ± 4^{CD}	1.7x
Sucrose	SP70	1%	A	A	Day 4	14.6 ± 0.4^{BC}	1x	120 ± 20^{DE}	1.4x
Sucrose	SP70	5%	A	A	Day 14 (PC)	27.0 ± 0.4^{A}	1.8x	200 ± 10^{B}	2.3x
Sucrose	PS750	1%	A	A	Day 2	18.2 ± 0.6^{BC}	1.2x	80 ± 10^{E}	0.9x
Sucrose	PS750	5%	A	A	Day 14	15 ± 2^{BC}	1x	260 ± 30^{A}	3x
Sucrose	Soy Lecithin	1%	A	A	Day 7	14 ± 1^{C}	1x	90 ± 20^{E}	1x
Sucrose	Soy Lecithin	5%	A	A	Day 7	24.1 ± 0.2^{A}	1.6x	169 ± 7^{BC}	2x
Sucrose	Polysorbate 20	1%	Day 14 (PC)	Day 4	Day 2	3.95 ± 0.07^{D}	0.3x	5.1 ± 0.6^{F}	0.06x
Sucrose	Polysorbate 20	5%	Day 2	Day 2	Day 2	1.8 ± 0.1^{D}	0.1x	0.8 ± 0.4^{F}	0.009x
Sucrose	Polysorbate 80	1%	Day 14 (PC)	Day 2 (PC)	Day 2	3.45 ± 0.07^{D}	0.2x	5.1 ± 0.1^{F}	0.06x
Sucrose	Polysorbate 80	5%	Never fully amorphous			1.5 ± 0.1^{D}	0.1x	0.9 ± 0.2^{F}	0.01x

*Samples that remained amorphous for the entire 4 week desiccator study are marked "A"; length of time prior to evidence of crystallization is indicated otherwise. PC indicates the onset of crystallization before sample was largely crystalline.

samples, with some formulations crystallizing at lower moisture contents than the control, while others did not crystallize until moisture contents were higher. The sucrose control gained 6% weight before crystallizing. The formulations that did not crystallize until moisture contents surpassed 6% were: sucrose:SP70 5% and sucrose:PS750 5%, which gained 8% weight, sucrose:SP50 5% and sucrose:soy lecithin 5%, which gained 7.5% weight, and sucrose:SP70 1%, sucrose:PS750 1%, sucrose:SP30 5%, and sucrose:soy lecithin 1%, which gained 7% weight. Lyophiles that sorbed less water than the control prior to sucrose crystallization were the sucrose:polysorbate 20 and 80 1% and 5% lyophiles, which gained less than 5% and 1% weight before extensively crystallizing, respectively (though sucrose:polysorbate 80 5% was partially crystalline initially). Aside from the polysorbate-containing lyophiles, moisture sorption trends at approximately 11% and 23% RH shown in Fig. 2A indicated no differences between lyophiles, in agreement with the moisture content data (Table 3).

A second set of moisture sorption profiles was collected for samples exposed to a constant 40% RH after drying (Fig. 2B), and the times at which mass loss indicative of crystallization occurred in this treatment are recorded in Table 2. Crystallization differences between lyophiles have been clearly exhibited in a reasonable timeframe between 32% and 43% RH (Saleki-Gerhardt & Zografi, 1994; Shamblin & Zografi, 1999), as was the case in previous studies investigating additive effects on amorphous sucrose stability (Thorat et al., 2017). Here again, differences in the amount of water sorbed prior to sucrose crystallization and the onset time for crystallization were found between the different emulsifier formulations. As in the desiccator studies, the presence of polysorbates resulted in faster sucrose crystallization onset times

Table 3
Percent moisture content (*wb*) of amorphous sucrose lyophiles prior to desiccator storage (Day 0) and samples that remained amorphous after 4 weeks of storage at 11% and 23% RH and onset T_gs of initial (dry) amorphous lyophiles. Uppercase superscript letters on moisture content data denote statistical significance between percent moisture of each lyophile at the specified timepoint, and lowercase superscript letters on moisture content data denote statistical significance between percent moisture of the specified lyophile at each timepoint. Superscript letters on T_g data denote statistical significance between T_gs only. Statistical analysis was run separately for each trial.

	Co-formulated Additive	Percent Additive	Week 0	Week 4 11% RH	Week 4 23% RH	Week 0	
						T_{g1}	T_{g2}*
Sucrose	–	–	$2.2 \pm 0.1\%^{Aa}$	$3.12 \pm 0.07\%^{Ab}$	$5.07 \pm 0.08\%^{Ac}$	$56.5 \pm 0.5\,°C^{BCD}$	
Sucrose	SP30	1%	$1.9 \pm 0.2\%^{ABa}$	$3.08 \pm 0.03\%^{Ab}$	$4.97 \pm 0.03\%^{Ac}$	$61 \pm 2\,°C^{ABCD}$	
Sucrose	SP30	5%	$1.05 \pm 0.04\%^{Ea}$	$2.99 \pm 0.00\%^{Ab}$	$4.7 \pm 0.3\%^{Ac}$	$57 \pm 2\,°C^{BCD}$	$66 \pm 1\,°C$
Sucrose	SP50	1%	$1.29 \pm 0.03\%^{CDEa}$	$3.10 \pm 0.06\%^{Ab}$	$5.005 \pm 0.007\%^{Ac}$	$58.4 \pm 0.4\,°C^{ABCD}$	
Sucrose	SP50	5%	$1.14 \pm 0.02\%^{DEa}$	$3.0 \pm 0.1\%^{Ab}$	$4.9 \pm 0.1\%^{Ac}$	$57 \pm 3\,°C^{BCD}$	
Sucrose	SP70	1%	$1.29 \pm 0.09\%^{CDEa}$	$2.99 \pm 0.02\%^{Ab}$	$4.98 \pm 0.05\%^{Ac}$	$64 \pm 3\,°C^{A}$	
Sucrose	SP70	5%	$1.4 \pm 0.1\%^{CDEa}$	$3.3 \pm 0.1\%^{Ab}$	$4.8 \pm 0.1\%^{Ac}$	$58 \pm 2\,°C^{ABCD}$	
Sucrose	PS750	1%	$1.6 \pm 0.2\%^{BCa}$	$3.2 \pm 0.1\%^{Ab}$	$4.9 \pm 0.2\%^{Ac}$	$58 \pm 2\,°C^{ABCD}$	
Sucrose	PS750	5%	$1.08 \pm 0.06\%^{DEa}$	$3.0 \pm 0.3\%^{Ab}$	$5.1 \pm 0.1\%^{Ac}$	$55.1 \pm 0.5\,°C^{D}$	
Sucrose	Soy Lecithin	1%	$1.17 \pm 0.04\%^{DEa}$	$3.24 \pm 0.08\%^{Ab}$	$5.01 \pm 0.01\%^{Ac}$	$62.3 \pm 0.9\,°C^{AB}$	
Sucrose	Soy Lecithin	5%	$1.285 \pm 0.007\%^{CDEa}$	$3.1 \pm 0.3\%^{Ab}$	$4.9 \pm 0.1\%^{Ac}$	$62.5 \pm 0.6\,°C^{AB}$	
Sucrose	Polysorbate 20	1%	$1.51 \pm 0.09\%^{BCD}$	–	–	$61.3 \pm 0.8\,°C^{ABC}$	
Sucrose	Polysorbate 20	5%	$1.06 \pm 0.08\%^{DE}$	–	–	–	
Sucrose	Polysorbate 80	1%	$1.39 \pm 0.03\%^{CDE}$	–	–	$56.1 \pm 0.8\,°C^{CD}$	
Sucrose	Polysorbate 80	5%	$0.9 \pm 0.2\%^{E}$	–	–	–	

* T_{g2} was found for one sample due to heterogenous nature of the sample at 5% additive.
* No T_g was found for sucrose:polysorbate lyophiles at 5% additive

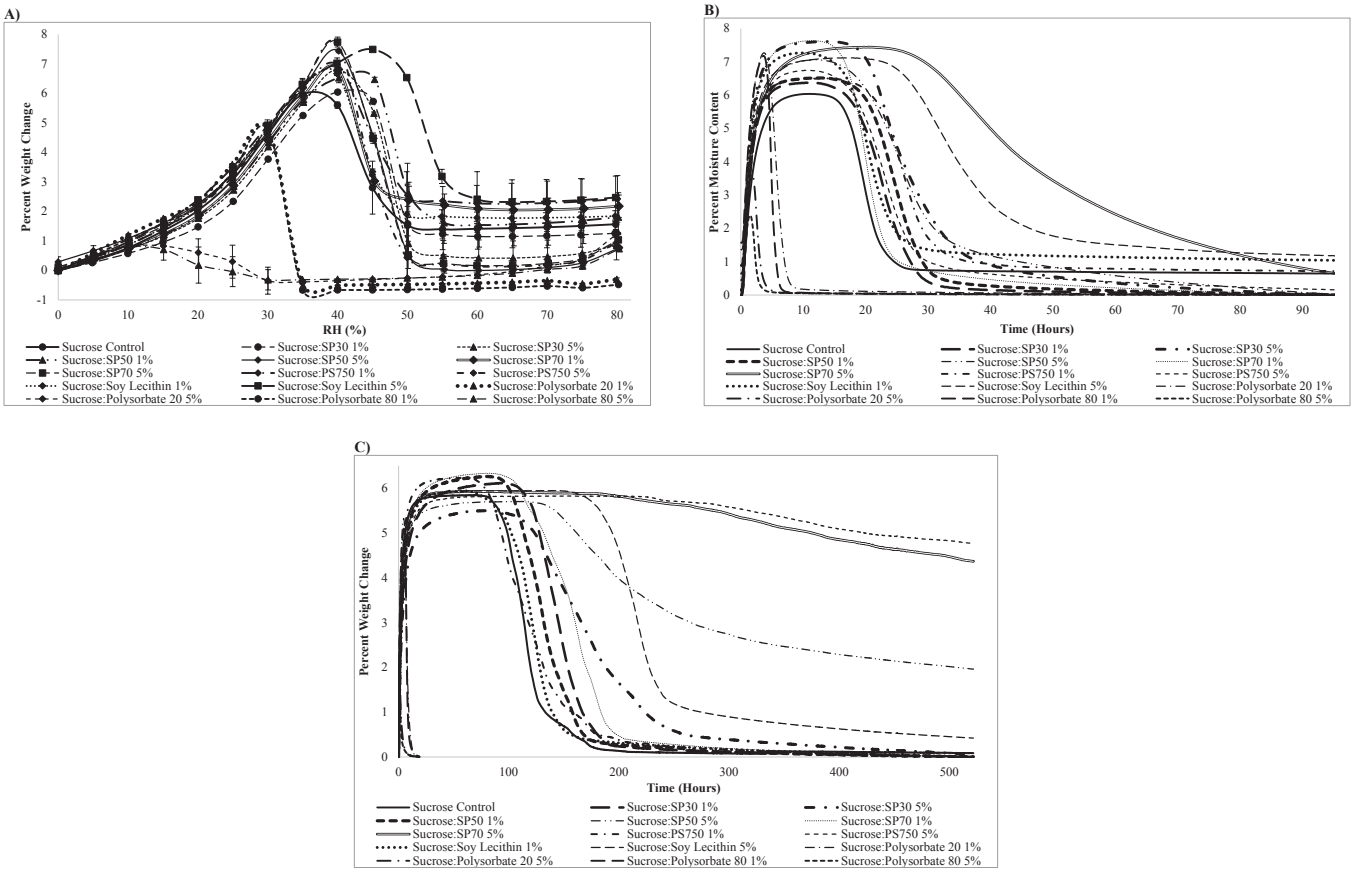


Fig. 2. Moisture sorption profiles of sucrose lyophiles A) from 0 to 80% RH, B) held at 40% RH, and C) held at 33% RH.

(1.5–3.95 h) compared to the control, which did not crystallize until hour 15 (Fig. 2B, Table 2). The emulsifier formulations that most delayed sucrose crystallization were: sucrose:SP70 5%, which crystallized at hour 27, and sucrose:soy lecithin 5%, which crystallized at hour 24. All other lyophiles crystallized between hours 14 and 19, times which were not significantly different than the control sucrose. Unlike trends in the desiccator studies, increasing the degree of mono-esters (as opposed to di- and tri-esters) in the sucrose esters did not correlate to increased time before sucrose crystallization occurred ($R^2 = 0.047$), and increasing the amount of emulsifier (from 1 to 5% *w/w*) did not result in significantly delayed crystallization except for SP70 and soy lecithin.

Based on the 40% RH SPS experiment, the stabilizing trend of the emulsifiers for delaying sucrose crystallization was: polysorbate 80 5% < polysorbate 20 5% < polysorbate 80 1% < polysorbate 20 1% < soy lecithin 1% < SP30 1% ≈ SP70 1% < **sucrose control** ≈ PS750 5% < SP50 5% < SP50 1% < PS750 1% < SP30 5% < soy lecithin 5% < SP70 5% (Fig. 2B, Table 2). This trend differed in several places from that found in the 33% RH desiccator experiments (Table 2), with some formulations providing better stability and others no longer delaying sucrose crystallization compared to the control. Most notably, sucrose:PS750 5% was one of the most stable lyophiles in the desiccator experiment but did not significantly delay sucrose crystallization compared to the control in the 40% RH SPS experiment. Similarly, the sucrose:soy lecithin 1% formulation was the most stable of those containing 1% emulsifier in the desiccators but was not significantly different from the control in the 40% RH experiment. Conversely, sucrose:soy lecithin 5% had similar effects to other emulsifiers in the desiccator experiments but was one of the most stable lyophiles in the 40% RH SPS experiment, remaining amorphous until 24 h into the experiment. Sucrose:PS750 1% was also much more successful at stabilizing sucrose in the 40% RH SPS experiment than in the 33% RH desiccators. Aside from these differences, the polysorbates followed the same trends in both the desiccator and SPS experiments, resulting in more rapid sucrose crystallization than the control, and both experiments found sucrose:SP70 5% to be the most stable lyophile.

Differences in crystallization trends between the SPS experiment at a constant 40% RH and the desiccator studies could have been caused by the drying step done in the SPS experiment, the passive vs. active headspace differences between the treatments, and/or the higher RH of the SPS experiment (40% RH) compared to the 33% RH desiccator. To better determine whether the differences in the experiments were due to the method of storage or specifically as a result of the difference in storage RHs (33% vs. 40%), a third set of sorption profiles was collected in the SPS in which the lyophiles were held at a constant 33% RH (Fig. 2C, Table 2). The stabilizing trend of the emulsifiers in this experiment was: polysorbate 80 5% ≈ polysorbate 20 5% < polysorbate 80 1% ≈ polysorbate 20 1% < PS750 1% < **sucrose control** < soy lecithin 1% < SP50 1% < SP30 1% < SP70 1% < SP30 5% < SP50 5% < soy lecithin 5% < SP70 5% < PS750 5%. Other than the low stability of sucrose:soy lecithin 1% in both the 33% and 40% RH SPS experiments (compared to high stability in the 33% RH desiccator), the overall trend of this 33% RH SPS experiment was more similar to the 33% RH desiccator experiment than the higher 40% RH moisture sorption results, indicating that the difference in RH (33 vs. 40% RH) was likely the main reason for the differing stability trends noted previously. The formulation that exhibited the most RH-dependent properties was sucrose:PS750 5%, which was the most stable amorphous lyophile (along with sucrose:SP70 5%) in both the 33% RH desiccator and SPS experiments, only partially crystallizing in the 3 week SPS experiment, but was not significantly different than the control in the 40% RH SPS experiment. While it is known that RH plays a key role in sucrose crystallization (Mathlouthi, 1995; Shamblin & Zografi, 1999), PS750 was the only emulsifier to show such a dramatic

difference in stabilization of amorphous sucrose between 33% and 40% RH.

3.4. Effect of glass transition temperature on amorphous sucrose stability

The 'dry' T_gs of lyophiles in this experiment are shown in Table 3 and Fig. S1. Although previous studies report an increase in T_g as the underlying reason additives delay crystallization in a variety of food systems, including sucrose matrices (Roos & Karel, 1991; van Hook, 1961), no significant trends were found between T_g and sucrose crystallization time in the current study (when excluding polysorbates, $R^2 = 0.006$). The small amount of emulsifier added relative to sucrose (1% and 5% *w/w*) did not significantly alter the 'dry' T_g compared to the sucrose control (56.5 °C), except for sucrose:SP70 1%, which had a T_g of 64 °C. The other difference noted was that the sucrose:SP30 5% sample had 2 T_gs, presumably due to heterogeneity. Previous studies on sucrose crystallization in the presence of salts and saccharides have also shown that there is not a direct relationship between crystallization onset times and T_g (Thorat et al., 2018). Exposing lyophiles to increasing environmental RHs would be expected to drop the T_gs of all samples in a predictable manner based on moisture content (according to models such as the Gordon-Taylor equation), and therefore crystallization would be expected to correlate with moisture content since no significant differences were found between the majority of the 'dry' T_gs of the lyophiles. However, this was not the case, as shown in Fig. 2. Discrepancies found in Gordon-Taylor modeling of sucrose:saccharide lyophiles with varying moisture contents suggested that factors beyond T_g, specifically structural compatibility of the saccharides with sucrose, contributed to the stabilization of amorphous sucrose (Thorat et al., 2018), and the lack of correlation between T_g and crystallization onset times in sucrose:salt lyophiles was due to ion-water interactions and possible hydration pockets around the ions in the lyophiles affecting T_g and plasticization of amorphous sucrose (Thorat et al., 2017).

Despite the lack of evidence that lyophile T_g correlated to delay in sucrose crystallization, the thermal behaviors of the individual emulsifiers were investigated. Experimental values for T_gs of sucrose and soy lecithin and T_ms of sucrose, sucrose esters, soy lecithin, and polysorbates are provided in Table 1. The T_gs of polysorbates were too low to be measured by this DSC (< −50 °C). Two T_ms were found for the sucrose esters (Fig. S2), which agrees with the report by Szűts, Pallagi, Regdon, Aigner, and Szabó-Révész (2007), although the range of T_ms found differed. It is important to note that the sample storage temperature (25 °C) was above the melting temperature of some of the emulsifiers (Table 1), and T_g is always lower than T_m, often by a factor of $T_g/T_m = 2/3$ (Sakka & Mackenzie, 1971). Polysorbates are known to be plasticizers, having T_gs around −61 °C and T_ms from −15 to 20 °C (Amim, Blachechen, & Petri, 2012; Amim, Kawano, & Petri, 2009). Polysorbates have much lower T_gs and T_ms than sucrose or the other emulsifiers in this study (Table 1). These properties may have led to more localized plasticization of the sucrose matrix when polysorbates were added than in matrices with the other emulsifiers, which may have contributed to the more rapid sucrose crystallization onset times found in these samples. While the T_gs of other emulsifiers were also slightly lower than that of sucrose, indicating that if stability is related to T_g, the samples containing the other emulsifiers should theoretically have been less stable as well, the T_gs of the emulsifiers in this study (except polysorbates) were at most 15 °C less than that of sucrose. This magnitude of difference was small enough that the lowering effect on the T_g of the lyophiles by these emulsifiers was not significant (Table 3). Additionally, the T_gs of these lyophiles remained above room temperature.

3.5. Effect of emulsifier structural properties on amorphous sucrose stability

3.5.1. Role of emulsifier structural similarity to sucrose

It has previously been shown that when T_g is not significantly affected by additives, the structure of the additive plays the major role in influencing comparative stabilization of amorphous sucrose against crystallization (Leinen & Labuza, 2006; Saleki-Gerhardt & Zografi, 1994; Thorat et al., 2018). The stabilizing effect for delaying sucrose crystallization seems to be best when the additive has a region that is structurally similar to sucrose, usually a glucose or fructose unit, that is able to interact with sucrose at the crystal interface and also has a dissimilar structural region that prevents further incorporation of sucrose into the crystal lattice (Thorat et al., 2018). When considering the structures of the emulsifiers used, the sucrose esters had a region that was most structurally similar to sucrose. Theoretically, the glucose and fructose units in the sucrose esters could have interacted with sucrose, and the fatty acid region could have disrupted sucrose crystal growth. This concept is similar to a report on how raffinose disrupts sucrose crystallization: the glucose and fructose units on raffinose attach to the sucrose crystal interface, and the galactose unit disrupts further incorporation into the sucrose crystal lattice, slowing crystal growth (Leinen & Labuza, 2006). However, the success of the sucrose esters at delaying crystallization was minimal in the 40% RH SPS experiment, with only the sucrose:SP70 5% and sucrose:PS750 5% formulations significantly delaying sucrose crystallization time compared to the control (Fig. 2B, Table 2). These emulsifiers (SP70 and PS750) had a higher fraction of mono-esters, and thus less di- and tri-esters, than the other sucrose esters studied. The lower molecular weight of these two sucrose esters led to a greater contribution of molecules since the samples were prepared on a weight basis. Assuming the species adsorb with the head group to sucrose, the presence of the fatty acid tails was what disrupted crystallization. It does not appear that length of the tail, and in effect hydrodynamic radius, played a role in efficacy of delaying crystallization, but rather the prevalence of sucrose head groups determined how effective the sucrose ester was at disrupting crystallization as long as any tail was present.

3.5.2. Role of emulsifier HLB values

While the efficacy of sucrose esters increased with increasing HLB value, when the HLB values of the other emulsifiers studied were considered, there was no correlation between the HLB value of emulsifiers and crystallization time ($R^2 = 0.197$). The ability of the sucrose esters to inhibit sucrose crystallization was more likely due to degree of ester substitution than HLB value since soy lecithin, which has a lower HLB value than the sucrose esters, was more effective at delaying crystallization than many of the sucrose esters, and polysorbates, which have higher HLB values, induced crystallization.

3.5.3. Role of emulsifier structural dissimilarity to sucrose

Although phosphatidylcholine (in soy lecithin) lacks a structurally similar region to sucrose, which may indicate that it would not be successful at delaying sucrose crystallization, soy lecithin contains other phospholipids as well, including phosphatidylethanolamine, phosphatidylinositol (which contains a monosaccharide unit), phosphatidylserine, and phosphatidic acid (Poirier, 2011). The heterogeneity of phospholipids found in soy lecithin may have contributed to the delay of sucrose crystallization seen in this study due to a wider variety of impurities present in the sample despite the absence of many structurally similar regions to sucrose (Gabarra & Hartel, 1998; Smythe, 1967).

The polysorbates also lack a structurally similar region to sucrose and have multiple long hydrophobic chains. However, unlike soy lecithin, both polysorbates at both concentrations induced a much faster rate of crystallization than occurred in the sucrose control (0.1x–0.3x in the 40% RH SPS experiment) (Fig. 2B, Table 2). The lyophile containing 5% polysorbate 80 was never fully amorphous and crystallized faster than lyophiles containing polysorbate 20 in all experiments conducted. The structural differences between these polysorbates (polysorbate 80 contains an oleic acid chain (18:1 *n*-9) and polysorbate 20 contains a lauric acid chain (12:0)) suggested that the longer fatty acid side chain

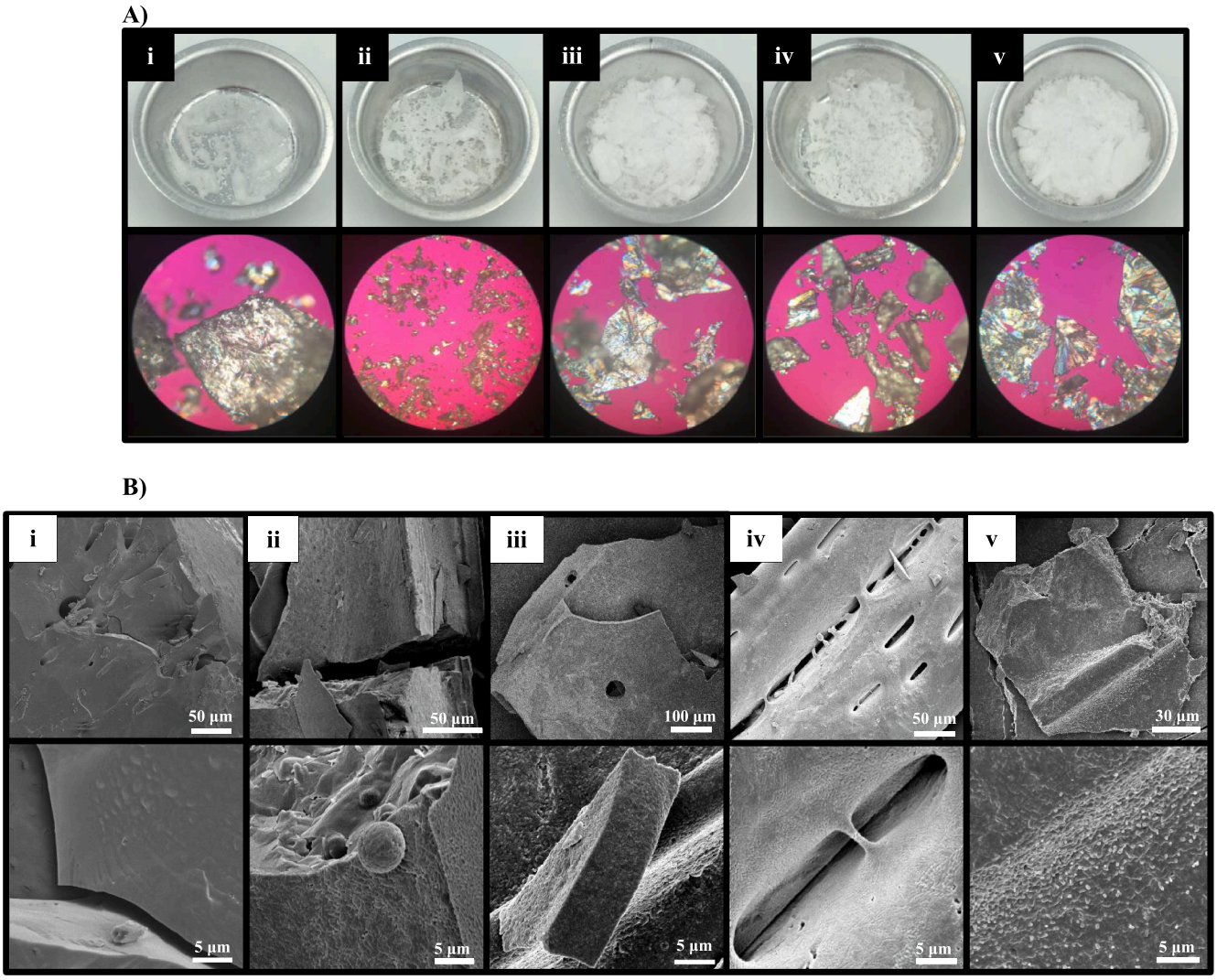


Fig. 3. Images comparing the A) physical and microscopic (PLM) appearance and B) crystal morphology by SEM micrographs after crystallization of the following lyophiles: i) sucrose control, ii) sucrose:polysorbate 20 1%, iii) sucrose:polysorbate 20 5%, iv) sucrose:polysorbate 80 1%, and v) sucrose:polysorbate 80 5%.

may have played a role in inducing crystallization. Increasing the amount of either polysorbate from 1% to 5% resulted in faster sucrose crystallization.

Interestingly, it was observed that while most lyophiles (including the control) collapsed before crystallizing, the sucrose:polysorbate lyophiles did not collapse and did not change much in physical appearance upon crystallization. PLM and light box images of the physical polysorbate lyophiles compared to the control that better illustrate this anomaly are shown in Fig. 3A. The crystallization of these lyophiles at 40% RH was documented using PLM and a RH-controlled microscope stage, with videos of these events provided in the Supplementary material (Figs. S3, S4, and S5). The videos show that while the sucrose control has a changed morphology when exposed to 40% RH as a response to moisture sorption, collapses and becomes rubbery (due to sorbed moisture lowering the T_g), and then crystallizes, the sucrose:-polysorbate lyophiles did not undergo the same extent of physical collapse or plasticization before crystallizing. Collapse precedes crystallization and is caused by decreased viscosity as a response to moisture sorption (Roe & Labuza, 2005). The lack of collapse in the polysorbate-containing lyophiles was presumably because the rate of crystallization was faster than collapse. The videos also suggest that there was a difference in nucleation between the control and the polysorbate-containing lyophiles. The control sucrose had few nucleation sites, which grew larger to eventually completely crystallize the sucrose. Conversely, the sucrose:polysorbate lyophiles had a large number of nucleation sites from which not much crystal growth was seen under the microscope.

The sucrose and sucrose:polysorbate lyophiles were also viewed by SEM after they had crystallized (Fig. 3B). Visual observation of these samples supports the supposition that increased nucleation occurred in the polysorbate-containing lyophiles. While the surface of the control was smooth, the crystals from the polysorbate samples had a bumpy and jagged surface. This rough surface indicated that nucleation was rampant and crystal growth was more limited in the presence of the polysorbates. A smaller crystal size and higher surface area also demonstrated that crystal growth was less extensive (Canselier, 1993). The sucrose crystals formed in the polysorbate lyophiles were porous, as seen in SEM and PLM images, which is consistent with the lack of collapse and the increased nucleation rate causing formation of many small crystallites. The formation of this porous crystalline structure made of very small crystallites generated a large surface area that facilitated rapid release of moisture from the crystallizing amorphous sucrose. Without such fast moisture release from the matrix, the water would have plasticized the remaining amorphous fraction, leading to the collapse that was observed in the other lyophiles in this study. The high surface area of the porous crystals has many additional implications, including altered texture and dissolution. The irregular shape of the crystals may also indicate heterogeneous nucleation and growth of

the sucrose on the surface of the polysorbates (Verma, Zeglinski, Hudson, Davern, & Hodnett, 2018).

3.5.4. Role of emulsifier critical micelle concentration

Another emulsifier property of potential interest is the critical micelle concentration (CMC) since the presence of micelles could contribute to the regions of interaction between the emulsifiers and sucrose in solution prior to lyophilization. While CMCs of the emulsifiers were not measured in this study, the concentrations of polysorbates used (1% and 5%) are greater than reported aqueous CMCs for both polysorbates 20 and 80 (Mahmood & Al-Koofee, 2013; Wan & Lee, 1974), and the concentrations of sucrose esters used in this study were also above the CMC since the CMCs of sucrose esters are generally lower than for polysorbates (Becerra, Toro, Zanocco, Lemp, & Günther, 2008). Because lecithin has such a low HLB value, it does not have a well-reported CMC in water. Since micelles were likely formed in all formulations (micelles were present in solution prior to lyophilization and presumably surfactant was trapped in this form following water removal), except perhaps the sucrose:soy lecithin lyophiles, and polysorbates induced sucrose crystallization while sucrose esters and soy lecithin delayed or had no effect on sucrose crystallization, it was concluded that CMC was not a significant factor in how the emulsifiers altered sucrose crystallization.

3.5.5. Role of emulsifier templating and intermolecular hydrogen bond lifetime with sucrose

Previous studies on the effect of surfactants on crystallization in the pharmaceutical industry have shown that, due to their inherent properties, surfactants with unbranched hydrophobic chains, including polysorbate 80, are more flexible than surfactants with bulky hydrophobic groups and are therefore able to act as a template and align molecules in the optimal configuration to promote nucleation (Berman, June Ahn, & Lio, 1995; Chen, Ormes, Higgins, & Taylor, 2015; Weissbuch, Addadi, Leiserowitz, & Lahav, 1988). Although the studies cited here describe a templating effect by a hydrophobic chain for a hydrophobic crystal, the steric properties indicate that the same effect is worth considering in the case of the numerous nucleation sites and more rapid sucrose crystallization observed in the sucrose:polysorbate lyophiles (Figs. 2, S4, and S5 and Table 2). Generally, a surfactant that is less flexible is unable to have this templating effect and instead inhibits nucleation by mass transfer effects. Assuming the templating theory plays a role in this study, the structural differences between polysorbates and other emulsifiers may have contributed to the absence of this effect in the sucrose ester and soy lecithin lyophiles even though they do not contain exceptionally bulky hydrophobic groups. For example, although sucrose esters also have an unbranched hydrophobic chain, the presence of a sucrose head group caused the sucrose esters to act more like a raffinose additive in which the sucrose group adsorbs to the crystal interface and the hydrophobic chain prevents mass transfer of sucrose into the crystal lattice (Leinen & Labuza, 2006). However, it is interesting to note that when more di- or tri-esters (unbranched hydrophobic chains) were present in the sucrose esters (SP30 and SP50), the sucrose esters were not as successful at stabilizing the amorphous sucrose (Fig. 2, Table 2). This may indicate that there is a contradictory effect between the presence of a sucrose group and the presence of unbranched hydrophobic chains which prevented the sucrose:SP30 and sucrose:SP50 lyophiles from being significantly more stable than the sucrose control. Additionally, soy lecithin also has unbranched hydrophobic chains; however, the presence of multiple types of phospholipids introduces some branched chains and some monosaccharide units, and the higher prevalence of pi bonds decreases the flexibility of the hydrophobic groups. The presence of these bulkier groups in soy lecithin may have prevented the templating effect seen in the sucrose:polysorbate lyophiles.

While the templating effect is a plausible explanation for the increased sucrose nucleation seen in the sucrose:polysorbate lyophiles, an alternative, and possibly more likely, theory is the propensity for hydrogen bonding between sucrose and polysorbates (Cui, Zhang, Yin, & Gong, 2012; Galek, Fábián, Motherwell, Allen, & Feeder, 2007; Verma et al., 2018). It has been reported that when hydrogen bonding is favorable between a compound of interest and a heterosurface (polysorbates in the current study), nucleation is promoted in solution due to the lengthened lifetime of the favorable hydrogen bond (Cui et al., 2012; Verma et al., 2018). Since the polysorbates have only three hydrogen bond donors but have 26 hydrogen bond acceptors, there is a high propensity for hydrogen bonding between the polysorbates and the hydrogen bond donor-rich sucrose molecules. The lengthened lifetimes of these hydrogen bonds promote more sucrose-sucrose interactions and increase the chance that the crystal nucleus survives (Verma et al., 2018). Essentially, the polysorbates create a surface which allows for the clustering and therefore crystallization of the sucrose, which also accounts for the irregular crystal shape shown in Fig. 3B. While phosphatidylcholine found in soy lecithin contains no hydrogen bond donor groups, which could cause it to act like the polysorbates, other phospholipids contained in soy lecithin contain some hydrogen bond donor groups and also some monosaccharides units (Poirier, 2011). The hydrogen bond donor groups on phospholipids may hydrogen bond with other phospholipids rather than with sucrose, lowering the propensity for hydrogen bonding with sucrose. Monosaccharides found in the phospholipids may promote interactions with sucrose as was discussed in crystallization inhibition by raffinose (Leinen & Labuza, 2006), which is why they effectively delay crystallization despite their high density of hydrogen bond acceptor groups. Sucrose esters also contain some hydrogen bond acceptors; however, as previously noted, sucrose esters contain a structurally similar region to sucrose that allowed them to interact with sucrose and prevent further incorporation into the crystal lattice. The amorphous sucrose stabilization induced by the presence of soy lecithin and sucrose esters due to the presence of monosaccharide units despite the presence of hydrogen bond acceptors suggested that when a structurally similar region to sucrose is present in the additive, that emulsifier property outweighed all others when considering the delay of sucrose crystallization.

4. Conclusion

Different emulsifiers had varying effects on the crystallization rates of amorphous sucrose, ranging from accelerating to delaying the onset time of crystallization. Most lyophiles remained amorphous in desiccators at low RHs (11 and 23%RH), except for lyophiles containing polysorbates. Increasing storage RH above 23%RH led to variations in moisture sorption and crystallization tendencies. The structure of the emulsifier was considered to be the major factor contributing to crystallization trends in the sucrose:emulsifier lyophiles, and no correlation was found between moisture sorption, critical micelle concentration, or T_g and crystallization onset time. Sucrose esters contained a structurally similar region to sucrose which was able to interact at the crystal interface, and the ester side chains prevented further incorporation into the crystal lattice, thereby delaying the crystallization of sucrose (by up to 1.8x that of the control at 40%RH and longer at lower RHs). When such a region of structural similarity was not present, intermolecular hydrogen bonding and structural heterogeneity seemed to influence the sucrose crystallization, contributing to the efficacy of soy lecithin at delaying sucrose crystallization (by up to 1.6x at 40%RH). Polysorbates destabilized sucrose crystallization, with crystallization times as low as 0.1x that of the sucrose control at 40%RH, attributed to the long fatty acid and polyoxyethylene side chains that seemed to have a templating effect that increased sucrose nucleation and inhibited structural collapse during crystallization. These findings provide insight into mechanisms by which emulsifiers alter sucrose crystallization and could be useful for designing formulations to alter or control the crystallization of amorphous sucrose in low moisture products.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2019.100050.

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