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Inhibitory effects of additives and heat treatment on the crystallization of freeze-dried sugar

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ABSTRACT

An amorphous matrix of a sugar is frequently used as a bulk-forming and stabilizing agent in the food industry but tends to crystallize as the result of water uptake and increase in temperature. Additives and methods used to inhibit the crystallization of amorphous sugar (sucrose) were screened in this study. Freeze-dried amorphous sucrose containing 0.5–5 wt% of additive, including salts, different types of sugars, and polymers, the crystallization temperature (T_{cry}) and isothermal crystallization characteristics were examined. Certain types of salts markedly increased the T_{cry} and prolonged the induction period for crystal nucleation. The use of 1 wt% $MgCl_2$ was particularly effective in inhibiting sugar crystallization. The heat treatment of crystalline sucrose under appropriate conditions was also found to result in diminished sucrose crystallization. MALDI-TOF mass spectra of the heat-treated sucrose suggested that sucrose derivatives containing multiple pyranose groups were formed, which would closely relate to the crystallization inhibition. Finally, the protein stabilizing effects of the matrices were evaluated. The results indicated that both the addition of additives and the heat treatment resulted in an improvement of the protein stabilizing effect of amorphous sugar matrix, compared to that of sucrose alone.

1. Introduction

An amorphous matrix, comprised of a sugar, is frequently used in the food and drug industries as agents for bulk-forming, encapsulation, and the stabilization of labile ingredients (Manning et al., 1989; Pikal, 1994; Carpenter et al., 1994). Furthermore, a sugar-based solid food, such as candy, generally contains an amorphous phase, which may be closely related to the flavor, taste, and texture of the food. However, the amorphous state is not in thermodynamic equilibrium and is susceptible to being transformed to a crystalline phase. The crystallization of an amorphous sugar matrix dramatically impairs the encapsulation and/or stabilizing effects of the matrix and, what is worse, often accelerates the degradation of unstable substances that are embedded in the matrix (Buera et al., 2005). Hence, sugar crystallization is a subject of interest and has been continuously investigated in the areas of food and pharmaceutical material science (Hartel and Shastry, 1991). To date, many studies dealing with the kinetics of the crystallization from amorphous sugar and the dependency of this on storage conditions. The impacts of storage temperature and water uptake on the crystallization kinetics of amorphous sugar solids have been extensively studied (Saleki-Gerhardt and Zografi, 1994; Kedward et al., 1998, 2000a,b; Levenson and Hartel, 2005; Kawakami et al., 2006; Harnkarnsujarit and Charoenrein, 2011). It also has been reported that the compression of amorphous sugar matrix facilitates crystal nucleation (Imamura et al., 2010a, 2012) as well as the growth rate of sugar crystals (Saleki-Gerhardt and Zografi, 1994; Kawakami et al., 2006; Bhugra et al., 2008).

In the actual utilization of amorphous sugar-based solids, it is also necessary to know how long the amorphous state is maintained under given conditions. Correlations between the melting temperature of a sugar and the induction period before the start of sugar crystallization has been investigated by Godovsky and Slonimsky (1974) and Qiu et al., 2011. The glass transition temperature has been also investigated in terms of its relationship with the induction period for sugar crystallization (Saleki-Gerhardt and Zografi, 1994). The findings reported in our previous study indicated that the induction period was well correlated with the crystallization temperature of an amorphous sugar matrix, which was indicated to hold regardless of the degree of humidification,

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compression and sugar type (sucrose or α-lactose) (Imamura et al., 2012).

Another approach to applications of amorphous sugar-based solids is to develop methodology designed to inhibit or avoid sugar crystallization (Buera et al., 2005). It has been demonstrated that the presence of sugar-compatible substances markedly suppressed the crystallization of a sugar in the amorphous state. The incorporation of a high MW substance such as maltodextrin or polyvinylpyrrolidone (PVP) into an amorphous sugar matrix, to a certain extent (~10%<), was found to have a dramatic effect on inhibiting the crystallization of sugar (Shamblin et al., 1996; Shamblin and Zografi, 1999). However, it has been reported that both the phase stability and the encapsulation/stabilizing effect of an amorphous sugar matrix pose a dilemma: The addition of a sufficient amount of a high MW substance prevents the crystallization of sugar but lowers the ability of amorphous sugar matrix to embed and thus to stabilize labile ingredients (Allison et al., 2000; Imamura et al., 2003). Combinations of salts were also investigated and reported to increase the physical stability of an amorphous sugar matrix (Mazzobra and Buera, 1999; Ohtake et al., 2004). In contrast, the addition of salts above a certain level were found to lower the T_g value (Farahnaky et al., 2009) and impair the protein stabilizing effect of an amorphous sugar matrix (Imamura et al., 2010b).

Based on this background information, the inhibition of sugar crystallization was investigated, focusing on additives that exert high crystallization inhibitory effects when present at low concentrations. On the other hand, the findings indicate that the pre-heat treatment of sucrose crystals also eliminated the occurrence of the crystallization of the heat-treated sucrose. Hence, the appropriate heating conditions for preventing sugar crystallization without any lowering the glass transition temperature was investigated. Finally, the protein stabilizing effects of "hard-to-crystallize" amorphous sugar matrices were compared.

2. Methods

2.1. Materials

Sucrose and all salts (NaCl, KCl, LiCl, $CaCl_2$, NaH_2PO_4 (dihydrate), Na_2HPO_4 (dodecahydrate), and $MgCl_2$ (hexahydrate)) were the products of Wako Pure Chemical Industries, Ltd., (Osaka, Japan). α-D-Glucose, D-fructose, α-maltose (monohydrate), α-lactose (monohydrate) and polyvinylpyrrolidone (abbreviated as PVP) with three different mean MWs (24,500 Da [24 k], 40,000 Da [40 k], and 360,000 Da [360 k]) were also obtained from Wako Pure Chemical Industries. Trehalose (dihydrate) and curdlan (MW ~590,000) were purchased from Hayashibara Biochemical Laboratories, Inc., (Okayama, Japan). Dextrans with mean MWs of 6000 Da and 500,000 Da were products of Fluka Chemie GmbH (Buchs, CH, Switzerland). P_2O_5 and CH_3COOK, used for desiccation and rehumidification, were obtained from Nacalai Tesque (Kyoto, Japan).

All other chemicals were of reagent grade.

2.2. Preparation of samples

A 10 g of powdered sucrose and 0–7.0 g of the powdered additive (a potential crystallization inhibitor) were transferred to a 100 mL measuring flask and dissolved with distilled water to a total volume of 100 mL. Five milliliters of the solution were instantaneously frozen with liquid nitrogen and immediately set to freeze-dryer (FD-5N, EYELA TOKYO RIKAKIKAI Co., Tokyo, Japan) connected to a vacuum pump, followed by lyophilization for 24 h. The freeze-dried samples were thoroughly dehydrated at 37 °C in a vacuum desiccator over P_2O_5 for more than 3 days (Imamura et al., 2008). The residual water contents of the samples were below the detection limit in a Karl–Fischer titration (0.002 g/g-dry matter) (Imamura et al., 2001). Alternatively, the resulting samples were rehumidified at 25 ± 1 °C over saturated LiCl (RH 11%) and CH_3COOK (RH 23%). The rehumidification was preliminarily indicated to be complete within only a single day (Imamura et al., 2011).

2.3. Differential scanning calorimetry and isothermal crystallization of amorphous sugar matrices

Thermal events associated with freeze-dried samples, namely, glass-to-rubber transition and crystallization were analyzed by differential scanning calorimetry (DSC) using a Perkin-Elmer DSC Pyris (Norwalk, CT), as described in our previous study (Imamura et al., 2002; Imamura et al., 2010b). Briefly, a 5–10 mg sample was hermetically sealed in a 20 μl aluminum pan and then scanned at a rate of 10 °C/min from 0 to 200 °C, using an empty aluminum pan as a reference. The glass transition temperature, T_g, and the crystallization temperature, T_{cry}, for the freeze-dried samples were determined as the onset of the endothermic shift in heat flow due to the glass-to-rubber transition and the top of the exothermic peak due to crystallization, respectively, in the obtained DSC curves. The deviations in the T_g and T_{cry} values for three independent experiments were within 3 °C of the average. The exothermic heat due to crystallization, ΔH_{cry}, was also obtained from the DSC thermogram and the sample mass to be analyzed.

The isothermal crystallization processes of the amorphous sugar samples were examined using the Pyris DSC in the same manner as was used in our previous study (Surana et al., 2004; Imamura et al., 2010a; Imamura et al., 2012). The sample, sealed in an aluminum pan, was instantly heated from room temperature to a prescribed temperature (90, 95, 100, 105, 110, 115 and 120 °C) and then maintained at that temperature. During the isothermal maintenance, the rate of heat release due to the crystallization of amorphous sugar sample was monitored. The released heat during the isothermal maintenance was integrated and turned into the degree of crystallinity, by dividing by the sum over the isothermal maintenance.

2.4. Powder X-ray diffractometry

Freeze-dried amorphous sugar samples, containing 10 wt% NaCl, as well as pure sucrose formulation were placed on a sample stage of an X-ray diffractometer Rigaku Rint 2400 system (Rigaku Co., Tokyo, Japan) with Cu-Kα radiation. The X-ray diffraction (XRD) spectra of the samples were then measured at the X-ray tube voltage of 40 kV and the current of 200 mA.

2.5. Heat treatment of sucrose crystals

A 500 mg sample of sucrose crystals was placed in a glass vial that had been heated at a prescribed temperature (150–220 °C) and stored in an oven (NDO-600N, EYELA Co., Tokyo, Japan) at the prescribed temperature. Sucrose crystals that were heated at 205 °C for 1 min (as well as those heated at a rate of 10 °C/min to 200 °C) were dissolved in distilled water to give a final concentration of 100 mg/mL. The resulting solution was freeze-dried and thoroughly dehydrated, followed by an analysis of the crystallization characteristics following the same procedures as described above or evaluated for their protein stabilizing effect, as described later.

2.6. MALDI TOF mass spectroscopy

Heat-treated sucrose crystals as well as untreated samples were dissolved in distilled water to give a final concentration of 100 mg/