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State transitions and freeze concentration in trehalose–protein–cornstarch mixtures

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Abstract

State transitions of solutions of mixtures of trehalose, albumen, gelatin, and cornstarch were studied and state diagrams were established. Maximum freeze concentration was achieved by annealing of solutions at a temperature $T'_m-1\,°C$. The onset temperatures of glass transitions of maximally freeze-concentrated solutes, T'_g, were lower and onset temperatures of ice melting, T'_m, were higher for mixtures of trehalose, proteins and cornstarch when compared with those of pure trehalose. Solute concentration in the maximally freeze-concentrated phase, C'_g, was 79% for trehalose, trehalose/albumin (1:1), and trehalose/cornstarch (1:1). The C'_g of trehalose/gelatin (1:1), and trehalose/cornstarch/gelatin (1:1:1) was 74% and 75%, respectively. The state diagrams established with experimental and predicted T_g values are useful for characterization of thermal phenomena and physical state of carbohydrate–protein–polysaccharide mixtures at various water contents.

Keywords: Trehalose; Annealing; Ice melting; Freeze concentration; State diagrams

1. Introduction

Stability of frozen and freeze-dried systems depends on physical state of their constituents present in an unfrozen phase. The physical state is sensitive to composition, temperature, thermal history, and water content (Le Meste & Huang, 1992; Roos, 1995b; Simatos & Karel, 1988; White & Cakebread, 1966). Freezing often results in component separation into two phases, water in a crystalline ice phase, and an unfrozen phase consisting of solutes with some unfrozen water (Goff & Sahagian, 1996; Karel & Flink, 1973; Le Meste & Huang, 1992; Roos, 1995b; Roos & Karel, 1991b, e, g; Simatos, Faure, Bonjour, & Couach, 1975; Simatos & Karel, 1988). The unfrozen phase is an amorphous, continuous phase with dispersed ice crystals (Levine & Slade, 1988; Roos, 1995a, b). The amorphous phase exists in a thermodynamically nonequilibrium (metastable) state and it is sensitive to variations in temperature. This may affect rates of deteriorative changes (Goff & Sahagian, 1996; Levine & Slade, 1988; Roos, 1995b; Roos & Karel, 1990, 1991a, d, g).

The glass transition temperature (T_g), and temperature of ice melting (T_m), are often related to quality changes and stability of frozen systems. In maximally freeze-concentrated frozen systems, the glass transition temperature is initial concentration independent and referred to as T'_g (Levine & Slade, 1988), while ice melting has its onset at T'_m (Roos & Karel, 1991b). Roos and Karel (1991a, b) found the T'_g as being a small transition preceding T'_m which is more pronounced and was taken as T_g by Levine and Slade (1988). These characteristics of frozen systems are component molecular weight dependent and their temperatures increase

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with increasing molecular weight of the unfrozen phase components (Levine & Slade, 1986).

Both T'_g and T'_m are important transitions controlling the physical state and possibly the shelf-life and quality of frozen systems (Levine & Slade, 1986; Roos, 1987, 1995b; Roos & Karel, 1991b, d, g; Simatos et al., 1975; Slade & Levine, 1991). It has been reported that diffusion-controlled chemical reactions may become kinetically restricted below T'_g due to a high viscosity and low molecular mobility (Roos, 1987, 1995b; Roos & Karel, 1991a). As the temperature increases to above T'_m, ice melting occurs and the viscosity of the unfrozen phase because of dilution decreases rapidly, which results in an increase in molecular mobility and leads to acceleration of physico-chemical changes, such as recrystallization of ice (Roos, 1995a, b; Roos & Karel, 1991a, b, d, g).

Solute concentration and changes in the physical state, which may occur at various temperatures and water contents, can be described using state diagrams (Roos, 1987; Roos & Karel, 1991b, d, g; Simatos et al., 1975; Slade & Levine, 1991). Values for solute concentration in maximally freeze-concentrated sugar and polymer solutions have often been found to be around 80% w/w (Roos, 1995b).

Sugars are often the main components of biological, pharmaceutical and food materials. Trehalose has been used in biological and pharmaceutical industries for improving stability and quality of products during processing and in final products (Richards et al., 2002). Toxicity studies have shown that trehalose is safe in food uses (Richards et al., 2002; Schiraldi, Lernia, & Rosa, 2002). The physio-chemical properties, such as glass forming properties, of trehalose are better than those of many other sugars (Macdonald & Johari, 2000; Mazzobre & Buera, 1999; Mazzobre, Soto, Aguilera, & Buera, 2001; Schebor, Buera, & Chirife, 1996; Schiraldi et al., 2002). It has been found that the reported glass transition temperature of trehalose (Macdonald & Johari, 2000; Roos, 1995a, b; Willart et al., 2001) is higher than those of lactose and sucrose. Several authors (Fuchigami, Ogawa, & Teramoto, 2002; Mazzobre & Buera, 1999; Mazzobre et al., 2001; Nagase, Endo, Ueda, & Nakagaki, 2002; Richards et al., 2002; Schebor et al., 1996; Schiraldi et al., 2002: Sei, Gonda, & Arima, 2002; Xie & Timasheff, 1997; Zhang, Klymachyov, Brown, Ellington, & Grandinetti, 1998) have reported that in the glassy state trehalose is much more effective in stabilizing biological materials (such as microorganisms, proteins and enzymes) than lactose and sucrose.

The thermal behavior of trehalose in complex sugar–protein or sugar–polysaccharide systems is not studied in detail. The purpose of the present study was to investigate frozen state transitions of trehalose/albumin, trehalose/gelatin, trehalose/cornstarch, and trehalose/gelatine/cornstarch systems.

2. Materials and methods

2.1. Materials

Trehalose dihydrate (form *Saccharomyces cerevisiae*), gelatin (Type B: From Bovine Skin), egg albumin (from Chicken Egg, Grade II), and corn starch were products of Sigma-Aldrich Chemie Gmbh, 89552 Steinheim, Germany. These and distilled water were used in experiments.

2.2. Preparation of samples

Two different types of samples, (I) solutions, (II) anhydrous, were made to analyse their thermal behavior using differential scanning calorimetry (DSC).

2.2.1. Preparation of solutions

All model systems were analysed in the solution state at the concentrations of 10%, 20%, and 30% of total solids (w/w). Trehalose, albumin, gelatin, and cornstarch, were weighed with water. Systems with trehalose, proteins and cornstarch had different components in an equal ratio for trehalose/protein (1:1), trehalose/cornstarch (1:1), and trehalose/protein/cornstarch (1:1:1). Trehalose and trehalose/albumin were well stirred to obtain a clear solution at room temperature. Water-bath at 60 °C was used to obtain clear solutions of trehalose/gelatin mixtures. Trehalose/gelatin mixtures were rewarmed using water-bath to facilitate the filling of DSC pans for thermal analysis. For freeze-drying mixtures of trehalose/cornstarch samples were heated in water-bath slightly above 85 °C and held at this temperature for 5 min to complete gelatinization of starch before filling into 20 ml glass vials.

All heated solutions were cooled at room temperature and any water lost as a result of evaporation was added on an analytical balance. DSC samples with cornstarch were gelatinized (discussed later under DSC) in the DSC furnace before measurements.

2.3. Preparation of anhydrous samples

Aliquots of model solutions were filled into Petri dishes up to an approximate height of 0.5–1 cm. Samples in Petri dishes were immediately pre-frozen at −20 °C for 12 h and subsequently transferred to a −80 °C freezer for 24 h. The samples from −80 °C freezer were rapidly transferred to a Finn-Aqua Lyovac GT2 (Germany) freeze-drier (within a time interval of less than 1 min). The samples on the Petri plates were freeze-dried for 72 h at a pressure <0.1 mbar. The freeze-dried samples were stored for 5 days at room temperature in vacuum desiccators over P_2O_5 for further dehydration. After P_2O_5 treatment, samples were crushed to a fine powder with a mortar and pestle. Samples of the powdered