Food Chemistry, Vol. 61, No. 1/2, pp. 139–144, 1998

0308-8146/98 $19.00+0.00

PII: S0308-8146(97)00132-5

Water activity and solubility of trehalose

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(Received 14 January 1997; revised version received 17 May 1997; accepted 17 May 1997)

The water activity, moisture sorption isotherm, and solubility of trehalose were determined. The water activity of a saturated solution of trehalose, as measured by an electric hygrometer, was 0.98 and 0.97 at 20 and 25°C, respectively. The water activity was also measured by a Decagon water activity instrument and found to be 0.96 and 0.95 at 20 and 25°C, respectively. The moisture sorption isotherm of trehalose at 20°C was found to be a Type III isotherm. Trehalose solubility from 10–40°C was determined using a two step method. The solubility of trehalose in water at 10, 20, 30, and 40°C was found to be 42.3, 46.6, 52.3 and 59.7% (g trehalose 100 g^{-1} solution), respectively. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

The nonreducing nature of trehalose (Roser, 1991; Roser and Colaco, 1993; Colaco *et al.*, 1994; O'Brien, 1996;) and its ability to protect cell membranes (Crowe *et al.*, 1984; Carpenter and Crowe, 1989) and volatile flavors and aromas (Roser, 1991; Roser and Colaco, 1993) is well documented in the literature. Anhydrous Tg and Tg′ values of 79°C (Green and Angell, 1989; Slade and Levine, 1991) and −29.5°C (Slade and Levine, 1991), respectively, have been reported. However, little work has been reported regarding the solution properties of trehalose. Portmann and Birch (1995) measured the intrinsic viscosity, apparent specific volume, nuclear magnetic resonance longitudinal and transverse relaxation rates, and sensory characteristics, such as sweetness intensity and persistence, of α,α-trehalose solutions at concentrations of 2.3, 4.6, 6.9, and 9.2% (w/v). Other trehalose papers of interest include Carpenter *et al.* (1987), Carpenter and Crowe (1988), Crowe and Crowe (1991), Levine and Slade (1992), Roberts and Franks (1996).

Other fundamental properties of trehalose, such as the water activity and solubility, are not currently available in the literature. Our goal was to determine the water activity at 20 and 25°C, the moisture sorption isotherm at 20°C, and the saturation solubility of trehalose at 10, 20, 30, and 40°C.

MATERIALS AND METHODS

Materials

The crystalline sucrose and α,α-trehalose used in these experiments were obtained from J.T. Baker Inc. (Philipsburg, NJ) and Ajinomoto Co. (Tokyo, Japan), respectively. The moisture content of sucrose and trehalose (as obtained) measured by Karl Fischer titration was 0.017±0.0001% and 9.66±0.038% wet basis (wb), respectively.

Methods

Water activity and isotherm measurements

A Hygroline Series HTCI-2 hydrometer (Beckman Industrial Corp., Cedar Grove, NJ) and a Decagon Aqualab CX-2 (Pullman, WA) were used to measure the saturated solution water activity of sucrose and trehalose at 20 and 25°C. Sugar solutions were made with distilled, deionized water and either sucrose or trehalose and they were considered saturated when excess sugar crystals remained visible in the solution after mixing with a mechanical stirrer for 24 h. The hygrometer was calibrated with saturated salt solutions (Table 1). All samples were measured in duplicate.

The adsorption isotherm of trehalose was determined by isopiestic equilibration at 20°C, using the proximate equilibration cell (PEC) method developed by Lang *et al.* (1981). PEC's were made with all of the saturated salt solutions listed in Table 1. Equilibrium was obtained

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Table 1. Saturated salt solutions and their respective water activities used for calibration and for trehalose water activity and isotherm determinations at 20 and 25°C obtained from the following references [a]Greenspan (1977), [b]Rockland (1960), and [c]Winston and Bates (1960)

Saturated salt solution	Water activity at 20°C	Water activity at 25°C
Potassium acetate—(CH_3COOK)	0.2311[a]	
Magnesium chloride—($MgCl_2$)	0.3307[a]	0.3278[a]
Potassium carbonate—(K_2CO_3)	0.4316[a]	
Magnesium nitrate—($Mg(NO_3)_2$)	0.5438[a]	0.5289[a]
Potassium iodide—(KI)	0.6990[a]	
Sodium chloride—(NaCl)	0.7547[a]	0.7529[a]
Ammonium sulfate—($(NH_4)_2SO_3$)	0.8134[a]	
Lithium sulfate—(Li_2SO_4)	0.85[b]	
Potassium chromate—(K_2CrO_4)	0.88[b]	
Balium chloride—($BaCl_2$)	0.91[b]	
Potassium nitrate—(KNO_3)	0.9462[a]	0.9358[a]
Potassium sulfate—(K_2SO_4)	0.9759[a]	0.9730[a]
Potassium dichromate—($K_2Cr_2O_7$)	0.98[c]	

when there was no change in the weight of the trehalose after two consecutive measurements. Trehalose reached equilibrium after 3 months. No visible mold growth was observed, even though no antimicrobial agents were used. Triplicate PEC samples were measured.

Solubility measurements

The solubility of sucrose was measured at 10 and 30°C and trehalose was measured at 10, 20, 30, and 40°C. The solubility of the sucrose was done in order to check the reliability of the solubility method used. The solubility method required two steps because the amount of trehalose available from one lot was limited and needed to be conserved. The two step solubility process is described in detail below and given in the flow chart in Fig. 1. Duplicate samples were measured for each sugar.

Step 1: Determination of the refractive index–dry solids (RI–DS) curve. A 5% solution (g sugar $100\,g^{-1}$ solution) of sucrose or trehalose and distilled, deionized water was prepared in a single neck round bottom flask. The initial pH and the initial purity, by HPLC, of the solution were measured. A Buchi Rotovapor RE-120 (Brinkman Instruments, Westbury, KY) was used at 66 ± 0.5°C and 27 ± 1 psi to concentrate the sugar solution. Aliquots of the sugar solution were taken throughout the concentration process for purity analysis, dry solids determination, and refractive index measurements.

The refractive index and dry solids results were used to make a RI–DS curve for each sugar. The purpose of the RI–DS curve was to make a calibration curve whereby the dry solids concentration for a solution of unknown concentration can be obtained by measuring its RI. A second order polynomial equation was used to fit the RI–DS curve using a nonlinear estimation (Statistica; StatSoft, Inc.; Tulsa, OK). The equation was used in Step 2 to calculate the dry solids concentration of the saturated sucrose and trehalose solutions.

Measurement of sugar purity. The purity of the sugar was measured by HPLC to determine if the initial purity of the sugar was maintained throughout the experiment. A Waters 712 WISP autosampler (Waters, Milford, MA) was used to inject $10\,\mu l$ of the sugar solution onto a Bio-Rad HPX-87C carbohydrate analysis column (Bio-Rad, Richmond, CA). The column was kept at a constant temperature of 75 ± 0.10°C using a Waters column heater, and was connected to a Waters 510 Pump and a Waters 410 refractive index detector with an attached Spectra Physics Model 4400 integrator (Mountain View, CA). Degassed Milli-Q water, used as a solvent, was maintained at a temperature of 80 ± 1°C and a flow rate of $0.6\,ml\,min^{-1}$. A run time of 24 min was used to quantify the purity of the injected sample by determining the degree of polymerization. Duplicate samples were measured for each sugar.

RI measurements. An Abbe Refractometer Model 10450 (AO American Optical Scientific Division, Buffalo, NY) was used to measure the refractive index of the sucrose–water and trehalose–water solutions at 45°C. Step One of the solubility method produced viscous syrups and the refractive index was done at 45°C to overcome difficulties associated with measurement of high viscosity syrups at lower temperatures (Corn Refiners Association, 1990). Duplicate samples were measured for each sugar.

Dry solids determination by Karl Fischer titration. An AquaStar V1B Karl Fischer Titrator (EM Science a Division of EM Industries, Cherry Hill, NJ) was used to determine the dry solids content of the solutions at 20 ± 2°C (Corn Refiners Association, 1992). The Karl Fischer solvent system was 20 ml of 98% formamide and 20 ml of Karl Fischer methyl alcohol. The titrant was a single solution, pyridine free Karl Fischer reagent. Duplicate samples were measured for each sugar.

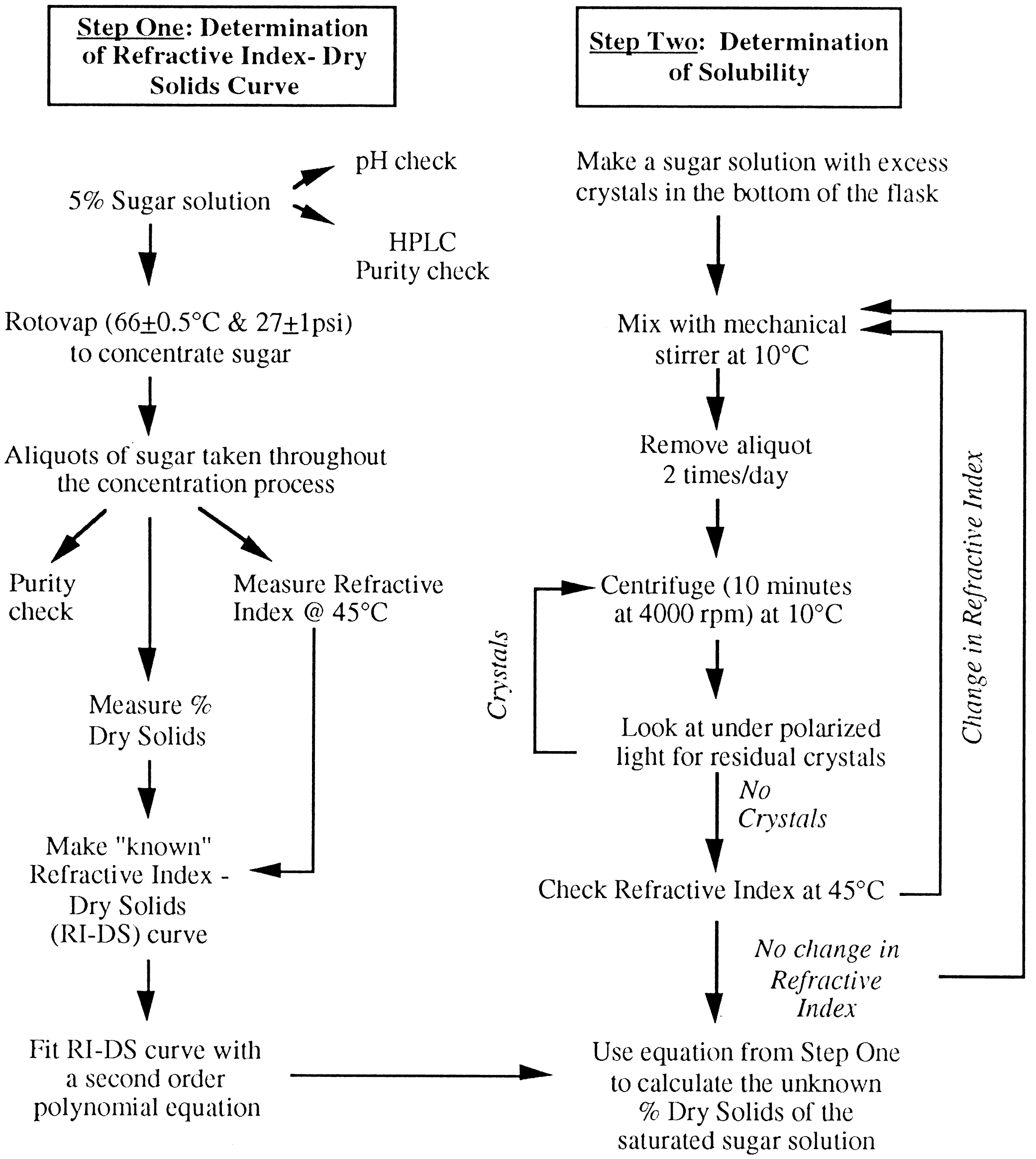

Fig. 1. A flow chart for the two step solubility measurement method used in this experiment.

Step 2: Determination of solubility. A solution of sucrose or trehalose was made in a three neck round bottom flask by adding enough sugar to distilled, deionized water until crystals could be seen by the naked eye (Fig. 1). The round bottom flask was sealed, placed into a MGW Lauda RM 20 water bath (Brinkman Instruments Inc., Des Plaines, IL), and mixed at 10°C with a mechanical stirrer. The saturated solution was monitored to make sure that sugar crystals could be seen by the naked eye throughout the duration of the experiment. If no crystals were present, the solution was undersaturated and more sugar was added. Aliquots of the sugar solution were removed for refractive index measurement twice a day until the refractive index did not change.

The aliquot was centrifuged at 10°C in an IEC Centra-8R Centrifuge (International Equipment Co., a Division of Damon; Needham Heights, MA) for 10 min at 4000 rpm. A drop of the supernatant was placed on a S/P Micro glass slide (Baxter Diagnostics Inc., Deerfield, IL) and examined under an Ortholux polalized light microscope (E. Leitz Inc., Rockleigh, NJ) to determine if any residual sugar crystals were present. If crystals were present, the supematant was centrifuged again until there were no crystals remained, as the crystals may interfere with RI measurements. The RI was measured and used to determine the dry solids content of the supernatant. The sugar–water solution was allowed to mix at 10°C until the RI did not change after two consecutive measurements. At this point, it was assumed that the sugar solution was at its saturation solubility and not supersaturated. The temperature was increased to 20°C followed by 30 and 40°C.

The RI obtained for each sugar, at each temperature, was used in the appropriate second order polynomial equation, (from Step 1) to calculate the amount of dry

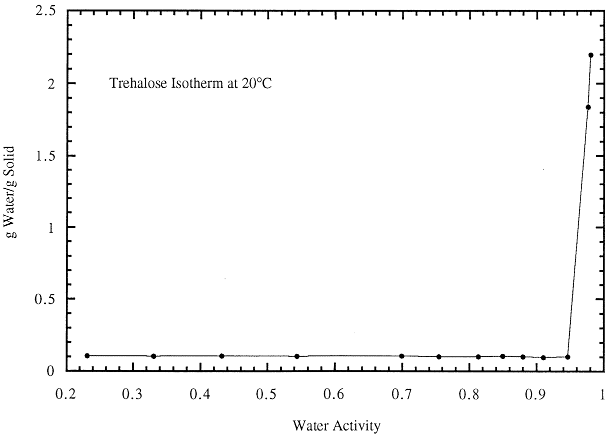

Fig. 2. Trehalose isotherm determined by isopiestic equilibration at 20°C.

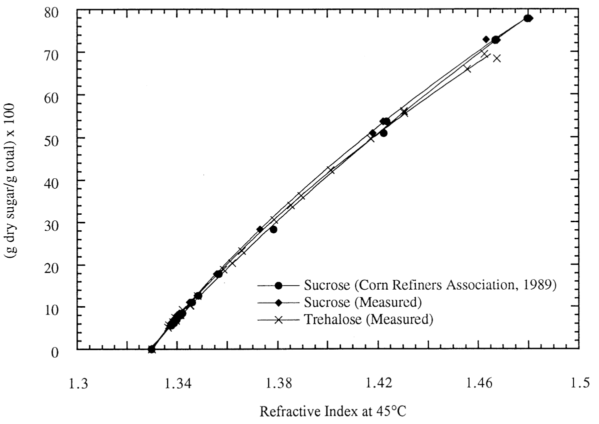

Fig. 3. RI–DS curves for sucrose and trehalose at 45°C.

solids present at saturation. Since trehalose is a dihydrate, the weight of trehalose soluble in 100 g of solution was also calculated in addition to the calculated amount of dry solids saturation solubility.

RESULTS AND DISCUSSION

Water activity and isotherm measurements

The saturated solution water activity and associated standard deviations of sucrose and trehalose measured by the electric hydrometer were 0.85 ± 0.004 and 0.98 ± 0.008 at 20°C and 0.85 ± 0.004 and 0.97 ± 0.004 at 25°C, respectively. The Decagon water activities for sucrose and trehalose were 0.85 ± 0.001 and 0.96 ± 0.001 at 20°C and 0.84 ± 0.001 and 0.95 ± 0.001 at 25°C, respectively. Other water activity values published for sucrose were 0.863 (Lang, 1981) at 20°C and 0.844 (Ruegg and Blanc, 1981), 0.7741 (Shotton and Harb, 1965), and 0.774 (Whittier and Gould, 1930) at 25°C.

The moisture sorption isotherm measured for trehalose can be found in Fig. 2. Our results indicate that the trehalose isotherm is similar to the Type III isotherm described by Brunauer *et al.* (1940) who classified five different types of van der Waals adsorption isotherms.

Solubility

The RI–DS curves for sucrose and trehalose are given in Fig. 3. Experimental RI–DS values for sucrose were similar to previously measured sucrose values (Corn Refiners Association, 1989). Therefore, the method used for determining the RI–DS curve for sucrose was acceptable and we assumed the method would be sufficient for determining a RI–DS curve for trehalose. The measured RI–DS curve for trehalose was similar in shape to the sucrose RI–DS curve. A RI–DS curve for trehalose could not be found in the literature for comparison. An empirical equation (eqn (1)) was fit to the experimental sucrose and trehalose RI–DS data ($r = 0.99987$ for both).

$$y = B_0 + B_1 x + B_2 x^2 \tag{1}$$

where y is dry solids (g solid g^{-1} sample), x is the refractive index, and the regression coefficients with their standard error are: B_0 is -2807.38 ± 101.08, B_1 is 3551.30 ± 144.22, and B_2 is -1082.66 ± 51.37 for sucrose and B_0 is -2915.30 ± 110.63, B_1 is 3720.87 ± 158.81, and B_2 is -1149 ± 56.93 for trehalose. The initial pH of the sucrose and trehalose solutions were 7.0 and 6.4, respectively. Sucrose and trehalose maintained a purity of 98.8 and 99.8%, respectively, throughout the concentration process used to develop the RI–DS curve.

Sucrose and trehalose solubility values were calculated from the measured RI in Step 2 of the solubility procedure using eqn (1) and are given in Table 2. Sugar solubility data are typically fit to a second order polynomial equation (Dodson, 1975). The average standard deviations, averaged over the temperatures measured, for the % dry solids (Table 2) for sucrose and trehalose were ±0.043 and ±0.380, respectively. The sucrose

Table 2. Sucrose and trehalose solubility in water expressed % sugar at different temperatures

	Sucrose % dry solids	g Sucrose 100 g^{-1} solution	g Sucrose 100 g^{-1} water	Trehalose % dry solids	g Trehalose 100 g^{-1} solution	g Sucrose 100 g^{-1} water
10°C	65.6	65.6	190.7	38.2	42.3	73.4
20°C				43.0	46.6	87.3
30°C	68.1	68.1	213.8	47.2	52.3	109.6
40°C				53.9	59.7	148.0

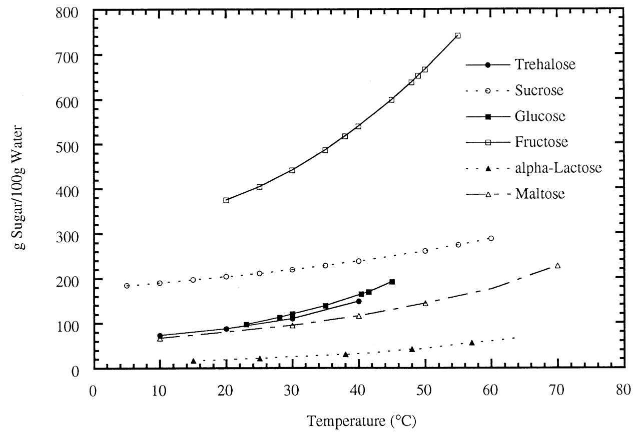

Fig. 4. Comparison of solubilities (g sugar 100 g^{-1} water) of sucrose, glucose, fructose, and α-lactose (Bates, 1942), and maltose (Cakebread, 1971) to trehalose.

Table 3. Regression and correlation coefficients fit to the values in Fig. 4 for the amount of sugar in 100 g water (g sugar 100 g^{-1} water) using eqn (1)

	B_0	B_1	B_2	r^2	Applicable range
Trehalose	71.008	−0.18195	0.050325	0.999	10–40°C
Sucrose	180.86	0.80879	0.015877	0.999	5–60°C
Glucose	87.034	−1.4009	0.082156	0.999	23–45°C
Fructose	331.12	−0.81836	0.15013	1.000	20–55°C
α-Lactose hydrate	17.517	−0.23438	0.015545	0.998	15–65°C
Maltose	68.493	−0.2299	0.034788	0.99806	10–70°C

solubility values determined in this experiment were close to the values reported by Bates (1942) who reported sucrose solubility at 10 and 30°C as 67.09 and 68.70 g sucrose 100 g^{-1} solution, respectively. For comparison purposes, Fig. 4 summarizes solubilities (g trehalose 100 g^{-1} water) of sucrose, glucose, fructose, and lactose (Bates, 1942), and maltose (Cakebread, 1971), and trehalose over a range of temperatures. A second order polynomial equation (same form as eqn (1)) was fit to the solubility data of each of the sugars in Fig. 4, where y is the solubility (g sugar 100 g^{-1} water) and x is temperature (°C). The regression coefficients B_0, B_1, and B_2 are given in Table 3. Trehalose is more soluble than lactose and maltose and less soluble than glucose, sucrose, and fructose in the temperature range of 10–40°C.

ACKNOWLEDGEMENTS

The authors would like to thank the Analytical Department of A.E. Staley Manufacturing Co. (Decatur, IL), Iris McCormick, and Robert Lammert for their contributions to this work and Dr Robert S. Anthony of Kraft Foods (Tarrytown, NY) for donating the trehalose.

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