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Determination and correlation of the water activity of unsaturated, supersaturated and saturated trehalose solutions

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Abstract

The water activity (a_w) of unsaturated and supersaturated trehalose solutions (and some sucrose solutions) was determined using a dew point hygrometer, and correlated using an equation originally proposed by Norrish [1966. An equation for the activity coefficients and equilibrium relative humidities of water in confectionery syrups. Journal of Food Technology, 1,25] for predicting water activity in binary non-electrolyte solutions. The a_w lowering behaviour of trehalose was found to be almost identical to that of sucrose solutions at same concentrations; however, due to the lower solubility of trehalose the a_w of their saturated solutions is higher than that of sucrose ones. The a_w of saturated trehalose solutions at 20, 25, 30, 35 and 40 °C was also determined and found to be between 0.953 and 0.928. These values agreed well with predictions made using a_w data for trehalose solutions calculated at the solubility concentration at the various temperatures.

Keywords: Trehalose dihydrate; Water activity; Sucrose; Supersaturated; Solubility

1. Introduction

Trehalose (α-D-glucopyranosil-α-D-glucopyranoside) is a naturally occurring non-reducing disaccharide with the same chemical formulae as sucrose but slightly different structure, consisting of two glucose molecules linked by a 1,1 α,α-glycosidic bond. Trehalose is well known for protecting the structure and function of labile sensitive components in foods caused by desiccation (Crowe, Crowe, & Jackson, 1983; Crowe, Mouradian, Crowe, Jackson, & Womrsley, 1984). Apart from this effect many benefits have been reported from adding trehalose to foodstuffs; it is only slightly sweet, does not alter the flavor of foods and it is safe and non-toxic (Portmann & Birch, 1995). Trehalose is a natural sugar which is increasingly available for food application with considerable potential for the food industry which can be used to improve existing products or to create innovative new products. Among its properties, trehalose is 45% as sweet as sucrose, (American Dietetic Association, 2004), it is a non-reducing sugar and therefore does not react with amino acids or proteins according to Maillard browning reaction, and it is also stable under low pH conditions where other disaccharides would hydrolyze into their component monosaccharides (Komes, Lovric, Kovacevic Ganic, & Gracin, 2003; Komes, Lovric, Kovacevic Ganic, Gajdos Kljusuric, & Banovic, 2005). Trehalose has also a low cariogenic potential when compared to sucrose and moderate glycaemic index with low insulinemic response (Cargill, 2004). For these reasons in the past few years trehalose has been used (or proposed to be used) alone or in combination with sucrose in a variety of food products.

The ability of a functional food solute to depress water activity (a_w) is an important property in relation to the microbial stability of foodstuffs (Troller & Christian, 1978). Since trehalose has been proposed to be used to replace or combine with sucrose to optimize sweetness while maintaining product shelf life, knowledge of the water activity lowering properties of trehalose is important.

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In recent years various papers (Miller, de Pablo, & Corti, 1997; Lammert, Schmidt, & Day, 1998) reported data on physical properties of trehalose such as T_g, adsorption isotherm, solubility, viscosity, heat on solution and specific volume. Lammert, Schmidt, and Day (1998) confirmed that trehalose dihydrate does not interact with water until the dissolution of crystals which takes place at very high relative humidity.

It is the purpose of the present study to determine the water activity of trehalose solutions in the whole range of solubility at room temperature; measurements were also extended to supersaturated solutions and to saturated solutions at various temperatures. The main application of these measurements is to evaluate the exact role of trehalose in maintaining reduced water activities compatible with the desired shelf life.

2. Materials and methods

2.1. Sugar solutions

Sucrose was obtained from Laboratorio Anedra, Buenos Aires, Argentina. Crystalline trehalose (dihydrate) was provided by Cargill Inc., Wayzata, MN, USA.

Solutions were prepared by adding distilled water to the sugars; supersaturated trehalose solutions were prepared by heating to about 50 °C (to allow dissolution) and allowed to cool at room temperature. Supersaturated trehalose solutions crystallize with time at room temperature (visible crystals at the bottom of the flask); however, a_w determinations (virtual a_w) were done in the metastable period in which no crystals were formed. This was confirmed because several consecutive water activity readings along a period of time yielded the same value indicating that no crystallization (which would raise water activity) occurred during that time period.

2.2. Determination of water activity

The water activity of unsaturated and supersaturated trehalose solutions and sucrose solutions was determined using a dew point hygrometer, Aqualab Series 3B (Decagon Devices, Pullman, Washington, USA) at 23–24 °C.

Saturated trehalose solutions at different temperatures (20, 25, 30, 35, 40 °C) were prepared and kept overnight in a constant temperature heating plate (Thermal Temperature Plate, Decagon Devices, Pullman, Washington, USA) at those temperatures. The water activity of these saturated trehalose solutions was then determined at 20, 25, 30, 35, 40 °C using an electronic dew-point water activity meter Aqualab Series 3 model TE (Decagon Devices, Pullman, Washington, USA), which allowed temperature control during measurement.

Both a_w measuring devices were calibrated and tested with unsaturated sodium chlorides solutions (Chirife & Resnik, 1984) within the a_w range of interest to this work.

For each a_w determination six replicates were obtained and the average was reported.

2.3. Determination of trehalose solubility

The solubility of trehalose was measured at 30 and 40 °C and compared with literature data reported by Lammert et al. (1998) at 10, 20, 30 and 40 °C.

A trehalose solution was prepared in a test tube by adding enough sugar to distilled water until crystals could be seen by the naked eye. The test tube was sealed and placed in a constant temperature water bath and stirred several times a day for a period of five days. The saturated solution was monitored to make sure that trehalose crystals could be seen during the whole experiment. If no crystals were present the solution was unsaturated and more trehalose was added. After five days in the constant temperature water bath the test tube was given a short centrifugation, placed again in the water bath for several hours and then a sample of the supernatant was used to determine dry solid contents. Dry solid content was determined by drying at 105 °C in a forced convection oven until constant weight; four replicates were used and the average is reported.

3. Results and discussion

Fig. 1 shows the correlation between water activity and concentration of trehalose solutions at 23–24 °C; according to literature data of solubility at this temperature (Lammert et al., 1998) samples with a concentration above about 43% are supersaturated. Measurement of a_w for some sucrose solutions are also included in Fig. 1 for the purpose of comparison. It is to be noted that all the points for the a_w of supersaturated trehalose solutions (virtual a_w) follow a common curve with unsaturated solutions. This behaviour has been previously reported in literature for other supersaturated binary non-electrolyte solutions, such

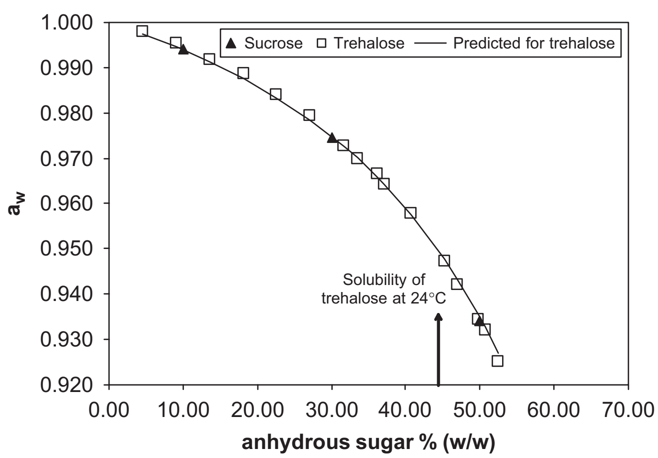

Fig. 1. Measured and predicted water activity values of unsaturated and supersaturated trehalose solutions at 24 °C (data for sucrose are also included).

as sucrose and mannitol (Seow & Teng, 1981). It is to be noted that methods for calculating the water activity of concentrated multicomponent aqueous solutions may require supersaturated binary data, which are not usually available or easily experimentally determined (Seow & Teng, 1981).

The data for trehalose (both unsaturated and supersaturated solutions) were very well correlated using an equation proposed by Norrish (1966) for predicting a_w in binary non-electrolyte solutions,

$$a_w = X_1 \exp(-KX_2^2), \quad (1)$$

where X_1 is molar fraction of water, X_2 is molar fraction of sugar and K a constant to be determined. Chirife, Ferro Fontán, and Benmergui (1980) successfully applied Eq. (1) to several non-electrolyte binary aqueous solutions; these compounds included sugars, polyols and amino acids.

Constant K was estimated by statistical software Infostat using non-linear regression following method proposed by Nelder and Mead (1965) and Press, Flannery, and Vetterling (1986). Constant K was found to be 6.66, and the confidence interval (95%) was 6.39–6.93. Since the K constant for sucrose has been reported in literature (Chirife et al., 1980) to be 6.47 it may be considered that both sugars, trehalose and sucrose, may be described by the same value of K. It is to be noted that a value of $K = 4.54$ has been reported for the disaccharide maltose (Chirife et al., 1980).

Miller, de Pablo, and Corti (1997), reported the solubility of trehalose dihydrate in water at various temperatures, being 35.8%, 40.6%, 46.2% and 52.4% at 10, 20.2, 29.8 and 40.1 °C, respectively. Unfortunately, it is not clear whether these trehalose concentration values were reported on an anhydrous base or for the dihydrate. If the values corresponded to anhydrous trehalose they are not far from the data reported by Lammert et al. (1998), which for similar temperatures found: 38.2, 43.0, 47.2 and 53.9 (% of dry solids), as shown in Fig. 2. For this reason we experimentally determined the solubility at 30 and 40 °C and the results are also included in Fig. 2; literature data for sucrose in the same temperature range, (Bubnik, Kadlec, Urban, & Bruhns, 1995) are also included for the purpose of comparison. Present solubility value of trehalose at 30 °C data is identical to that reported by Lammert et al. (1998) (47.2%, $\sigma = 0.164$) and at 40 °C present value is only slightly lower (52.4%, $\sigma = 0.265$ vs. 53.9%).

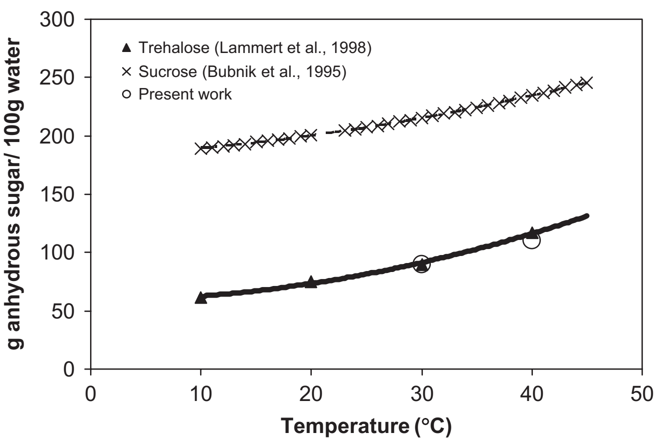

Fig. 2. Solubility of trehalose and sucrose between 10 and 40 °C.

In order to predict the a_w of saturated trehalose solutions at several temperatures, Eq. (1) ($K = 6.66$) was used to predict the a_w at saturation concentration at the various temperatures. It is to be noted that constant K was evaluated from a_w data at 23–24 °C, but used to predict a_w at different temperatures (20–40 °C). However, Ferro Fontán and Chirife (1981) showed that the a_w of sucrose and other non-electrolyte solutions varied very little with temperature (as compared to a reference value at 25 °C) when water activity values considered were above 0.90, as is the case in present work. Table 1 shows predicted and measured water activity of saturated trehalose solutions between 20 and 40 °C; as expected, the saturated a_w decreases with increasing temperature because of solubility increase. The agreement between experimental and predicted water activity for saturated solutions is quite good; at 40 °C the agreement between experimental and predicted value is slightly better when using the solubility measured in present work instead of that reported by Lammert et al. (1998).

Although the a_w lowering behaviour of trehalose was found to be almost identical to that of sucrose solutions at same concentrations, the lowest water activities which may be obtained with trehalose is much higher than that for sucrose and this is due to its lower solubility. This means that trehalose may not be as effective as sucrose to control microbial growth; i.e. the water activity of saturated sucrose solutions is 0.843 (at 25 °C) as compared to 0.953 for trehalose. This has to be taken into account when trehalose is used to substitute sucrose in semimoist foods where reduction of water activity plays a main role in microbial growth control (Troller & Christian, 1978).

It is interesting to note that trehalose is one of the solutes which may be accumulated to a high intracellular concentration in microorganisms capable of growth at low levels of a_w (Nunes, Manaia, Da Costa, & Santos, 1995; Louis & Galinski, 1997; Lamosa, Martins, Da costa, & Santos, 1998). Knowledge of the water activity lowering

Table 1
Predicted and measured a_w of saturated trehalose solutions at selected temperatures

Temperature (°C)	a_w predicted[a]	a_w predicted[b]	a_w experimental
20	0.953	–	0.953
25			0.949
30	0.943	0.943	0.943
35			0.936
40	0.922	0.927	0.928

[a]Using solubility data of Lammert et al. (1998).
[b]Using measured solubility (present work).

behaviour of trehalose may be needed to predict the intracellular water activity of the microorganisms which accumulate trehalose among other solutes (Chirife, Alzamora, & Ferro Fontán, 1983).

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