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Effect of Dehydrated Nystose Addition on the Reduction of Water Activity

Hitoshi MATSUMOTO, Takahisa TOKUNAGA and Masao HIRAYAMA

Bio Science Laboratories, Meiji Seika Kaisha, Ltd., 5-3-1 Chiyoda, Sakado-shi, Saitama, 350-0289, Japan

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Dehydrated nystose was prepared by thermal dehydration of nystose trihydrate, recrystallized from a commercially available syrup of fructooligosaccharides. Dehydrated nystose was found to have the ability to absorb moisture and the ability to maintain water activity (Aw) at a low level during its rehydration. These characteristics remained evident until an 8%(w/w) increase in weight had occurred, corresponding to the restoration of three molecules of crystallization water. As an application of these characteristics, we found that by addition of dehydrated nystose to sucrose powder the Aw is reduced to an appropriate value and the survival of lyophilized *Bifidobacterium* in the mixture is thereby maintained.

Keywords: nystose, dehydration, Aw, probiotics, *Bifidobacterium*

The use of humectants and desiccants to make foods safe and stable has been one of the underlying principles of food preservation. Recently, the health benefits of dietary probiotics have attracted increasing interest (Goldin, 1998) and much attention is being paid to the viability and stability of freeze-dried microbes in food matrices. Aw, the ratio of the water vapor pressure above the surface of a given product to that above pure water, is known to have a major effect on the survival of such microbes; the Aw must be kept at a distinct low level during their processing and storage (Takematsu *et al*., 1982; Troller, 1991). Therefore, a food material highly effective in reducing the Aw in food matrices containing dietary probiotics is needed.

In the present report, we show that dehydrated nystose can be prepared in a practical manner by thermal dehydration of nystose trihydrate which is recrystallized from commercially available fructooligosaccharides (Hirayama & Hidaka, 1993) and that this product has useful functional properties including the ability to absorb moisture and the ability to maintain the Aw at a low level, as compared with other dehydrated carbohydrates. As a typical application, the direct addition of dehydrated nystose to sucrose powder containing freeze-dried *B. longum* was found to reduce the Aw value and to enhance the survival of the bacteria.

Materials and Methods

Materials A commercially available syrup of fructooligosaccharides (Meioligo-P syrup, Meiji Seika Kaisha Ltd., Tokyo) consists of 0.6% monosaccharides, 2.0% sucrose, 31.4% 1-kestose, 34.7% nystose, 6.3% fructofuranosylnystose and 25% water. Dehydrated forms of glucose, maltose, trehalose, raffinose, α-CD, and β-CD were prepared by drying the corresponding hydrates *in vacuo* to a constant weight at 70°C. D(+)-Glucose monohydrate, maltose monohydrate, and D(+)-trehalose dihydrate were purchased from Sigma Chemical Co. (St. Louis, MO). Raffinose pentahydrate, α-CD, and β-CD were from Wako Pure Chemical Industries (Osaka). Other materials were obtained from commercial sources.

Analytical methods Melting points (mp) were uncorrected. Carbohydrate content is expressed as %(w/w), unless otherwise indicated. The composition of carbohydrate mixtures and the content of individual carbohydrates were determined by high-performance liquid chromatography (HPLC) by the method of Hidaka and Hirayama (1988) with some modifications. Water content was determined using a Karl-Fischer Moisture Titrator (MKA-210; Kyoto Electronics Co., Kyoto). ^{1}H and ^{13}C NMR spectra were recorded in D_2O using a JEOL GX-400 spectrometer, and the chemical shifts were referenced indirectly to Me_4Si by setting ^{1}H from HOD at 4.80 ppm and ^{13}C from 1,4-dioxane at 67.4 ppm. Secondary ion mass spectroscopy (SIMS) was performed using a Hitachi M-80A spectrometer. Powder X-ray diffraction patterns were obtained at room temperature using a Rad-2C system (Rigaku Co.) under the following conditions: measurement range, 1–40°, (2θ) range, θ:2θ mode continuous scan; sampling rate 0.01°; scanning speed 3° min-1; using CuKα radiation; generator power 40 kV and 20 mA; slits DS (1.0°), SS (1.0°) and RS (0.15°).

Nystose trihydride A solution of Meioligo-P syrup (1200 g) and water (300 ml) was seeded with fine crystals of nystose (2.4 g) and the mixture was kept at 5°C with slow stirring for 15 days to yield the first crystals (233 g, 88.1% purity with respect to nystose). The crystals were recrystallized at 5°C for 24 h from a supersaturated aqueous solution (72%) to give the second crystals, which were dried *in vacuo* at room temperature (15–20°C) for 24 h to afford colorless prisms [93.2 g, 99.0% purity; mp. 130°C (lit. 129–131°C; Tsuchida *et al*., 1966) from H_2O-MeOH]; the water content was found to be 7.86%, whereas the calculated value for nystose trihydrate is 7.50%]. The ^{1}H and ^{13}C NMR spectra (data not shown) agreed with those reported previously (Timmermans *et al*., 1993). SIMS-MS (*m/z*); 666(M^+ of nystose).

Further recrystallization in a similar manner yielded a single crystal suitable for X-ray analysis. A crystal of dimensions 0.3 × 0.2 × 0.1 mm^3 was mounted on a Rigaku AFC5R diffractometer (Rigaku Co.) and the sample was irradiated with graphite-monochromated CuKα radiation. The cell parameters were deter-

Abbreviations: Aw, water activity; *B. longum, Bifidobacterium longum*; α-CD, α-cyclodextrin; β-CD, β-cyclodextrin.

E-mail: hitoshi_matsumoto@meiji.co.jp

mined from 15 carefully centered reflections ($22<2\theta<35$). The crystal data obtained were as follows: formula, $C_{24}H_{42}O_{21}\cdot 3H_2O$ (FW=720.6); orthorhombic; space group $P2_12_12_1$ (Z=4); a=13.573 (2), b=23.371 (2), and c=10.190 (2) Å; V=3232(1) Å^3; D_c=1.48 gcm^{-3}, μ(CuKα)=11.24 cm^{-1}. In total, 2795 reflections were measured with an ω/2θ scan, and 1270 reflections with $I>2\sigma(I)$ used in subsequent refinement; θmax=120.1 deg. Lorentz and polarization corrections were applied, but no absorption corrections were made. The structure was solved by direct methods using MULTAN88 (Debaerdemaeker *et al*., 1988) and subsequent Fourier techniques (Beurakens *et al.,* 1994). H atoms were put in the calculated positions. The non-H atoms were refined isotopically on F by full-matrix least-squares refinement, whereas H atoms were included but not refined. The R (Rw) values in the final cycle of least-squares refinement were 0.088 (0.101).

Dehydrated nystose The trihydrate (90.0 g) was heated at 80°C *in vacuo* for 24 h yielding a colorless crystalline powder [83.5 g, mp. 128°C, water content 0.80%] of constant weight. The ^{1}H and ^{13}C NMR spectra (data not shown) agreed with those of the trihydrate. After keeping the crystalline powder at 30% humidity for 24 h followed by drying *in vacuo* at room temperature (15–20°C) for 24 h, the increase in weight was found to be 8.11%, which agreed with hydration by three molecules of H_2O.

Measurement of moisture-absorption capacity The moisture-absorption capacity of the dehydrated carbohydrates was measured by a previously reported method (Takematsu *et al*., 1982) at three levels of relative humidity (R.H.) maintained constant by using saturated aqueous solutions of the following salts: calcium chloride (31% R.H.), calcium nitrate (51% R.H.), and ammonium sulfate (81% R.H.). A precisely weighed sample (4.0 g) in a single plastic cylinder was placed at 25°C, and the increase in weight was recorded at one-hour intervals. The moisture-absorption capacity was calculated as the amount of water absorbed in one hour.

Measurement of Aw Aw was measured using a Rotronic Hygroscope DT (Rotronic Instrument Corp., NY). The dehydrated carbohydrate (4.0 g) was allowed to reach equilibrium at 20°C overnight in a single plastic cylinder (diameter, 45 mm; height 16 mm), which contained filter paper wetted with a defined amount of water (0, 0.08, 0.16, 0.24, 0.32, 0.40, or 0.48 g). The Aw and the increase in the weight of the sample were measured.

Effectiveness of dehydrated nystose addition in lowering the Aw of sucrose powder and in enhancing the survival of freeze-dried B. longum Sucrose powder (Aw=0.417; Nippon Beet Sugar Mfg. Co. Ltd., Tokyo) was mixed with five different amounts (2, 5, 10, 15, and 20%) of dehydrated nystose, raffinose, or maltose. Each of the mixtures was placed in a separate plastic cylinder, and the Aw was measured as described above.

Freeze-dried *B. longum* (0.01 g of a preparation containing 1.0×10^{10} cells/g, Meiji Milk Products Co. Ltd., Tokyo) was added to mixtures (10.0 g) of sucrose powder with 10, 7, 5, 2 or 0% dehydrate nystose; the measured Aw values for these mixtures were 0.00, 0.05, 0.10, 0.20 and 0.30, respectively. The initial number of viable bacteria was $3.3–8.7\times10^6$ cells/g. Then 3.0 g of each sample mixture was sealed in an aluminum bag and stored for 8 weeks at 65% relative humidity at 25°C. During the storage period, the Aw was measured and the numbers of surviving bacteria were determined by the method of Mitsuoka *et al*. (1965).

Results

Dehydrated nystose Nystose crystals of high purity (99.0%) could be prepared by two steps of recrystallization from a commercial Meioligo-P syrup, containing nystose (37.4%) as one of the major components. The structure of the carbohydrate moiety was confirmed by NMR and mass spectra analyses. Final confirmation of the structure of the crystals was done by crystallographic X-ray diffraction analysis of 99.8% pure crystals. The crystals were found to be $C_{24}H_{42}O_{21}\cdot 3H_2O$, nystose trihydrate, and the crystal data were similar as those reported previously (Jeffrey & Huang, 1993; Okuyama *et al*., 1993).

Upon heating the trihydrate crystals at 70°C *in vacuo* for sufficient time (48 h) to reach a constant weight, the trihydrate showed a 7.02% decrease in weight and was converted into the dehydrated form, with a mp of 128°C, and a water content of 0.80%. The cumulative thermal loss and the residual water content were both 7.82%, which was close to the calculated value (7.50%) for three molecules of crystallization water. During dehydration, the material showed almost no change in appearance and the dehydrated form showed optical anisotropy as determined by means of a polarization microscope. These observations suggested the retention of the crystal structure. An attempt to obtain the crystallographic X-ray diffraction pattern was unsuccessful because the crystals of the dehydrated form had a rugged surface with cracks. However, the powder X-ray diffraction pattern was found to be almost the same as that of the trihydrate, as shown in Fig. 1. By thermal dehydration in a similar manner, dehydrated forms of six other kinds of carbohydrates were prepared from the corresponding hydrates. The mp, moisture content, and optical anisotropy of each, and a comparison of the

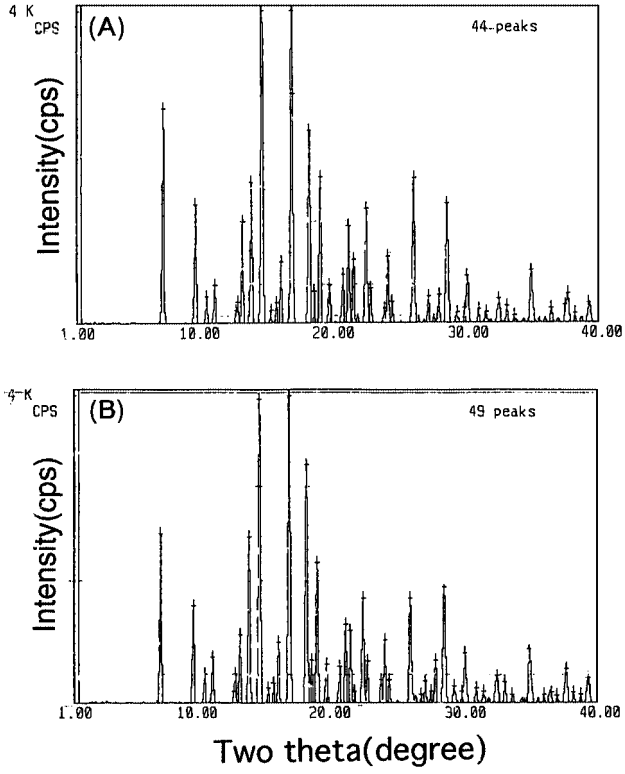

Fig. 1. Powder X-ray diffraction patterns of dehydrated nystose (A) and the trihydrate (B).

powder X-ray patterns of the hydrate and dehydrated forms are summarized in Table 1. In the case all of these carbohydrates, the moisture content decreased to 0.08–0.99% in the dehydrated form, corresponding to 0.01–0.30 molar equivalents of residual water. Differences were observed among them in terms of both the optical anisotropy and the powder X-ray patterns. Loss of optical anisotropy was observed only in the case of dehydrated raffinose. Comparing the powder X-ray patterns before and after dehydration, two carbohydrates (nystose and α-CD) seemed to maintain similar patterns in terms of either the scattering angles or the integrated peak intensities. Figure 1 shows typical patterns observed in the case of nystose before and after dehydration. On the other hand, the patterns changed in the case of four of the carbohydrates examined, showing different peaks after dehydration in the case of β-CD, glucose, and maltose and no detectable peak after dehydration in the case of raffinose.

Table 1. Physical constants and properties of dehydrate carbohydrates.

Dehydrate carbohydrate	Mp. (°C)	H_2O content (%) (mol equiv.)	Optical anisotropy[a)]	Comparison of X-ray pattern[b)]
Nystose	128	0.80 (0.30)	+	similar
α-CD	250<	0.39 (0.18)	+	similar
Raffinose	80	0.24 (0.07)	−	no peak
Trehalose	201–203	0.42 (0.08)	+	differ.
β-CD	250<	0.32 (0.18)	+	differ.
Glucose	150	0.08 (0.01)	+	differ.
Maltose	168	0.99 (0.19)	+	differ.

[a)]Observed by polarization microscope: +, positive; −, negative.
[b)]Comparison of powder X-ray pattern between hydrate and dehydrate forms: similar, similar pattern; no peak, no detectable peak after dehydration; differ., different pattern.

Moisture-absorption capacity The moisture-absorption capacity of dehydrated nystose was monitored upon rehydration at three different levels of relative humidity (Fig. 2A). With increases in humidity, the initial rate of moisture absorption increased and the final amounts of water absorption also increased. That is, 100 g of dehydrated nystose was found to show initial moisture-absorption rates of 2.9, 2.9, and 1.0 g/h and the final amounts of moisture were 11.1, 9.9, and 8.8 g of water at a relative humidity of 81, 51, and 31%, respectively. Figure 2B shows a comparison of the moisture-absorption capacity of dehydrated nystose, dehydrated raffinose, and silica gel at 51% RH. Dehydrated nystose showed a greater initial moisture-absorption rate (2.9 g/100 g, h) than dehydrated raffinose (1.5g/100 g, h) and the rate was almost the same as that in the case of silica gel although the final amount of water absorption (9.1 g/100 g) was smaller than that in the case of silica gel (22.0 g/100 g).

Water activity The Aw of seven dehydrated carbohydrates was measured using a rotronic hygroscope DT meter, and the change in Aw observed is summarized in Fig. 3 in relation to their water content. Only two samples, dehydrated nystose and dehydrated α-CD, showed a characteristic profile wherein the Aw remained at an almost constant low value below a water content of 8% and then increased sharply at a water content above that. On the other hand, four of the carbohydrates examined showed a different pattern in which the Aw increased at rates independent of the increase in water content.

Effectiveness of dehydrated nystose addition in lowering the Aw of sucrose powder and in enhancing the survival of lyophilized B. longum Each of three kinds of dehydrated carbohydrates, nystose, raffinose, and maltose, was mixed with sucrose

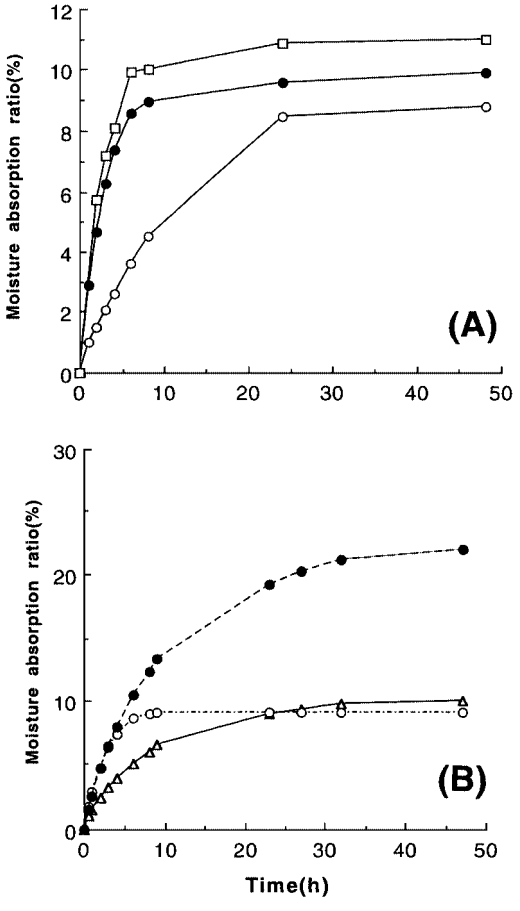

Fig. 2. (A) Effect of relative humidity on the moisture-absorption rate and the amount of moisture absorption upon rehydration of dehydrated nystose. Relative humidity of 31% (○), 51% (●), and 81% (□). (B) Comparison of the moisture-absorption rates and the amounts of moisture absorption upon rehydration of dehydrated nystose (○), raffinose (△), and silica gel (●) at 51% relative humidity.

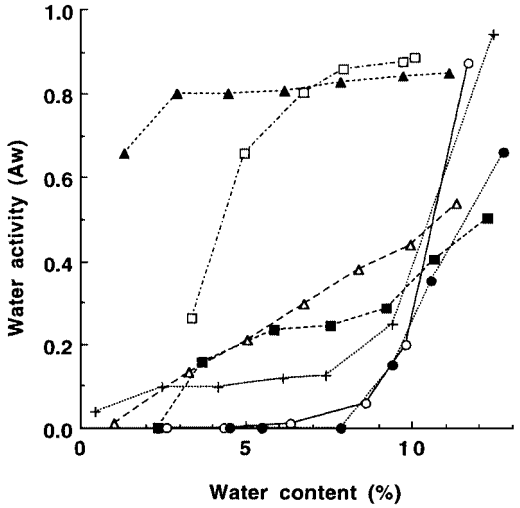

Fig. 3. Relationship between the Aw and the water content of dehydrated carbohydrates. Nystose (○); α-CD (●); raffinose (△); trehalose (+); β-CD (■); maltose (□); and glucose (▲).

powder with appropriate dehydrated carbohydrate content (0.0–20.0%) and each of the resulting mixtures was found to have a lower Aw (0.37–0.02) than that (0.42) of the sucrose powder alone. The effectiveness of each dehydrated carbohydrate in lowering the Aw is summarized in Fig. 4. The results indicated that the Aw of the mixture could be regulated to achieve a desirable level in the range of 0.42–0.02, 0.42–0.12, and 0.42–0.28 by the addition of an appropriate amount of dehydrated nystose, raffinose, and maltose, respectively. Among them, dehydrated nystose was most effective in lowering the Aw to below 0.05.

The effect of the content of dehydrated nystose on the number of surviving cells of lyophilized *B. longum* in sucrose powder was examined in relation to the Aw (0.30–0.00) in the course of storage for 8 weeks. As shown in Fig. 5, as the dehydrated nystose content was increased from 0.0 to 10.0%, the Aw of the mixtures decreased from 0.30 to 0.00. Measurement of the number of surviving cells showed that in the mixtures with a dehydrated nystose content of 0.0% (Aw=0.30) and 2.0% (Aw=0.20) the number of surviving cells decreased almost linearly to 1/30,000 and 1/800 of the initial number, respectively, over the period of 8 weeks. However, in the mixtures with a dehydrated nystose content of 5.0 (Aw=0.10), 7.0 (Aw=0.05), and 10.0% (Aw=0.00), the number of surviving cells decreased to about 1/100 of the initial number in each instance over the 8-week storage period.

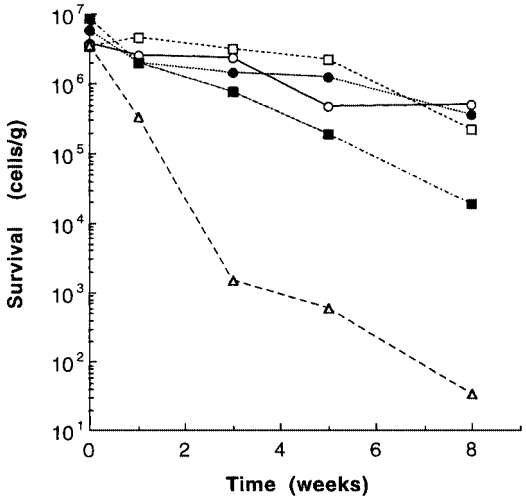

Fig. 5. Effectiveness of dehydrated nystose in enhancing the survival of lyophilized *B. longum* in sucrose powder. Content of dehydrated nystose [Aw]: 0.0% [0.30] (△); 2.0% [0.20] (■); 5.0% [0.10] (□); 7.0% [0.05] (●); and 10.0% [0.00] (○).

Discussion

The dehydrated forms of various carbohydrates have been widely used as desiccants in dietary and pharmaceutical applications, however, in some cases there is a clear limitation in terms of the safety of the final product. Dehydrated maltose is commercially available as a drying agent for food use (Mitsuhashi & Sakai, 1987).

We found that nystose trihydrate, prepared from a commercially available material by two steps of recrystallization, was smoothly converted to the dehydrated form by heating the hydrate at 70 or 80°C *in vacuo*. The crystal structure of nystose trihydrate was determined by X-ray crystallographic analysis. The crystal data were similar to those reported previously (Jeffrey & Huang, 1993; Okuyama *et al*., 1993). An attempt to obtain single crystals of the dehydrated form was unsuccessful because the crystals of suitable size for the analysis were cracked in the process of drying. However, the powder X-ray diffraction analysis yielded a clear pattern, which was almost the same as that of the trihydrate in terms of either the scattering angles or the integrated peak intensities (Fig. 1. A and B). These observations indicated that, for the most part, the dehydrated crystals maintained the matrix structure of the trihydrated form, i.e., the crystal appears to have a zeolite-like structure which would allow absorption and desorption of water molecules through channels reversibly.

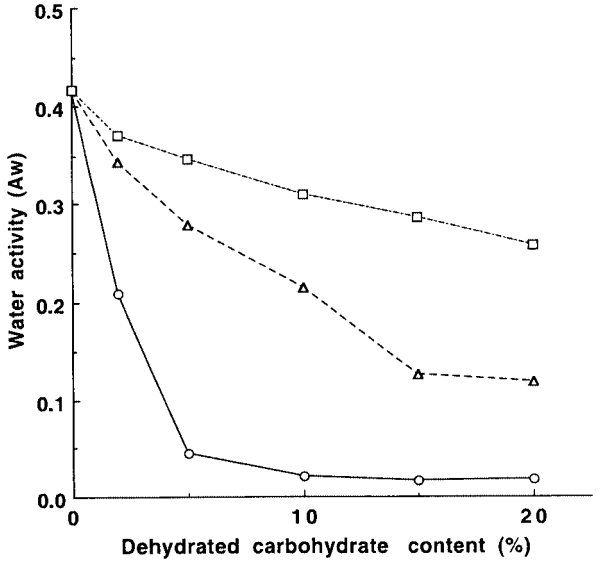

Fig. 4. Effectiveness of dehydrated carbohydrates addition in lowering the Aw of sucrose powder. Relationship between the Aw and the dehydrated carbohydrate content (%). Dehydrated nystose, (○); dehydrated raffinose (△); and dehydrated maltose (□).

Dehydrated nystose was found to be effective in moisture absorption and the Aw decreased during its rehydration. In order to compare its moisture absorption properties with those of other dehydrated carbohydrates, six kinds of hydrates were converted into the dehydrated forms by the same dehydration procedure and tested. The results obtained concerning their moisture absorption properties and the decrease in Aw are shown in Table 1. Compared to the other compounds examined, dehydrated nystose showed a greater moisture absorption capacity at high relative humidity (Fig. 2-A), and the initial absorption rate was greater than that in the case of dehydrated raffinose and almost the same as that of silica gel at 51% relative humidity (Fig. 2-B). As shown in Fig. 3, among the seven dehydrated carbohydrates examined, the Aw was found to be kept at almost the zero level in the case of dehydrated nystose and dehydrated α-CD when the water content was 8% or less, and the Aw was found to increase sharply at a water content greater than 8%. The inflection values were very close to the water content of the rehydration form of nystose trihydrate (7.5%) and that of α-CD hexahydrate (10%) (Manor & Saenger, 1974). In contrast, four other dehydrated carbohydrates showed different Aw profiles, in which the Aw did not remain at a low level but increased with the increase in water content at a rate dependent on the individual compound.

Referring to the H_2O content, optical anisotropy, and powder X-ray diffraction pattern shown in Table 1, all seven hydrates were found to lose their crystallization water to an extent of 0.08–0.99% (0.01–0.30 molar equivalents of residual water), to yield the dehydrated forms. However, comparing the hydrated and dehydrated forms, in some cases there was a difference in optical anisotropy and a difference in powder X-ray diffraction patterns. With respect to the optical anisotropy, dehydration resulted in a change from positive to negative only in the case of raffinose. With respect to the powder X-ray diffraction patterns, the seven dehydrates could be categorized into three groups where: 1) the patterns obtained before and after dehydration were similar, or 2) no peaks were observed after dehydration, or 3) the patterns obtained before and after dehydration were different, as shown in Table 1. The results suggested that only raffinose changed from crystals to an amorphous form whereas in the case of the other six dehydrated compounds the crystal form was maintained through the course of dehydration. Our findings concerning raffinose are consistent with a report by Saleki-Gerhardt *et al*. (1995) indicating that raffinose pentahydrate crystals lost two water molecules with no change in crystal structure but removal of the remaining three water molecules through thermal dehydration caused it to collapse into an amorphous form giving no signal peak upon powder X-ray diffraction analysis. The difference observed in the powder X-ray diffraction patterns obtained before and after dehydration in the case of four of the dehydrated compounds examined could be due to a change in crystal lattice structure as is reported to occur upon dehydration of trehalose dihydrate (Taylor & York, 1998). In the case of nystose and α-CD, it is very interesting that each showed an X-ray pattern after dehydration similar to that of the corresponding hydrate and both were more effective in maintaining the Aw at a low level than the other anhydrous carbohydrates (Fig. 3).

Dehydrated nystose, which was found to be effective in keeping the Aw at a low level, was successfully applied in the regulating Aw of foods by direct addition to them. Figure 4 shows a typical example where addition of dehydrated nystose was effective to lower the Aw of sucrose powder from an initial level of 0.42 to an appropriate level in the range of 0.42–0.02. This served to improve the number of surviving cells of lyophilized *B. longum* in the sucrose powder, as it is known that the Aw must be reduced to below 0.10 to achieve a high survival ratio (Takematsu *et al*., 1982). As shown in Fig. 5, addition of dehydrated nystose was effective to control the Aw of the mixture and the numbers of surviving bacteria remained at about 10^6 cells/g in the mixtures with low Aw (0.00–0.10) after 6 months of storage, whereas the number in the case of sucrose powder alone (Aw=0.30) decreased to 10^2 cells/g.

Many kinds of prebiotics including *Bifidobacterium* and *Lactobacillus* have been used for human and animal consumption and they are now recognized as having beneficial effects in terms of improving health and preventing disease (Goldin, 1998). Lyophilized probiotics for dietary use are prone to display a decrease in cell survival during the storage period. In order to prevent such a decrease in viability, it is important to lower the Aw of the mixture. Dehydrated nystose is considered to be useful for the maintenance of cell viability, because the dehydrated form itself can be added to foods and it is highly effective in maintaining the Aw at a low level.

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References

Beurskens, P. T., Admiraal, G., Beurakens, G., Bosman, W. P., de Gelder, R., Israel, R. and Smits, J.M.M. (1994). The DIRDIF-94 program system of Technical Report of the Crystallography Laboratory; University of Nijmegen: The Netherlands.

Debaerdemaeker, T., Germain, G., Main, P., Refaat, L.S., Tate, C. and Woolfson, M.M. (1988). Computer programs for the automatic solution of crystal structures from X-ray diffraction data. University of York, U.K.

Goldin, B.R. (1998). Health benefits of probiotics. *Br. J. Nutr.,* **80**, Suppl. 2, S203–S207.

Hidaka, H., Hirayama, M. and Sumi, N. (1988). A fructooligosaccharide-producing enzyme from *Aspergillus niger* ATCC 20611. *Agric Biol. Chem*., **52**, 1181–1187.

Hirayama, M. and Hidaka, H. (1993). Production and utilization of microbial fructans. In "Science and Technology of Fructans," ed. by M. Suzuki, N.J. Chatterton. CRC Press, Boca Raton, FL, pp. 273–302.

Jeffrey, G.A. and Huang, D. (1993). The tetrasaccharide nystose trihydrate: Crystal structure analysis and hydrogen bonding. *Carbohydr. Res*., **247**, 37–50.

Manor, P.C. and Saenger, W. (1974). Topography of cyclodextrin inclusion complexes. III. Crystal and molecular structure of cyclohexaamylose hexahydrate, the water dimer inclusion complex. *J. Am. Chem. Soc*., **96**, 3630–3639.

Mitsuhashi, M. and Sakai, S. (1987). New drying agent and its use for dehydration. BE PATENT 905157 (CA, 107, 6041).

Mitsuoka, T., Sega, T. and Yamamoto, S. (1965). Eine verbresserte Methodik der gralitativen und quantitaiven analyse der darmflora von menshen und tieren. *Zentralbl. Bakteriol. I. Abt. Orig*., **A1951**, 455–469.

Okuyama, K., Noguchi, K., Saitoh, M., Ohno, S., Fujii, S., Tsukada, M., Kakeda, H. and Hidano, T. (1993). Crystal structure of nystose trihydrate. *Bull. Chem. Soc. Jpn*., **66**, 374–379.

Saleki-Gerhardt, A., Stowell, J.G., Byrn, S.R. and Zografi, G. (1995). Hydration and dehydration of crystalline and amorphous forms of raffinose. *J. Pharm. Sci*., **84**, 318–323.

Scott, W.J. (1958). The effect of residual water on the survival of dried bacteria during storage. *J. Gen. Microbiol*., **19**, 624–633.

Tatematsu, T., Shimamura, S., Tomita, M. and Okonogi, S. (1982). Effect of water activity on the survival of freeze-dried bifidobacteria and lactic acid bacteria. *Taketsu oyobi Kanso Kenkyukai Kaishi*, **28**, 40–45 (in Japanese).

Taylor L.S. and York, P. (1998). Characterization of the phase transitions of trehalose dihydrate on heating and subsequent dehydration. *J. Pharm. Sci*., **87**, 347–355.

Timmermans, J.W., Waard, P. de, Tournois, H., Leeflang, B.R. and Vliegenthart, J.F.G. (1993). NMR spectroscopy of nystose. *Carbohydr. Res*., **243**, 379–384.

Troller, J.A. (1991). Trends in research related to the influence of "water activity" on microorganisms in food. In "Advances in Experimental Medicine and Biology" Vol. 302; ed. by H. Levine and L. Slade. Plenum Press. New York, NY, pp. 305–313.

Tsuchida, H., Fujii, S. and Komoto, M. (1966). The oligosaccharides produced from sucrose by *Dematium pullulans*. *Agric. Biol. Chem*., **30**, 429–433.