

A DSC STUDY OF THERMAL TRANSITIONS OF APPLE SYSTEMS AT SEVERAL WATER CONTENTS

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Abstract

The dependence of phase transitions in apple systems at several different water contents has been studied by using differential scanning calorimetry (DSC). The pre-cooled samples with high water fractions show a small but distinct thermal effect at low temperature before the final melting of the ice. The samples with low water content show a second order type transition, characterised by a temperature which increases with decreasing water content. The temperature/composition behaviour is reported in the form of the so-called ‘supplemented’ state diagram, including the solid/liquid coexistence boundaries and the extrapolated glass transition curve. This diagram contributes towards understanding the transformations encountered during the temperature process of partially dried samples of apple. An interpretation is presented about the existence of phase-segregated regions, which could give the observed thermal effects at low temperatures.

Keywords: apple sugars and pectins, frozen water, glass transition, state diagram

Introduction

The understanding of the basic physico-chemical properties of food components and food matrices is a challenging task for the improvement of industrial food processes and for the development of new applications. In recent years, considerable scientific interest has been generated on the basis that these systems can be considered complex, heterogeneous polymeric composites, with additional complications arising from the variability of either the actual concentration or the segregated aqueous phase components [1]. For example, a typical description of a food mixture usually indicates the chemical composition of the dry residual matter. However, such a description ignores all the other features of the structural components in terms of low molecular mass sugars and high molecular mass polysaccharides and therefore completely overlooks the role of the components in terms of the functional properties.

Fruit pulps can be fractionated by mechanical separation into a solid tissue portion and into a pulpy juice portion. Besides the presence of small sugars, the major components of the pectic substances in solid tissues consist of pectins, cellulose and hemicellulose (up to 15% in apples). The pectic substances belong to a group of com-

plex plant polysaccharides in which *D*-galacturonic acid is the principal constituent [2, 3]. They occur in the primary cell walls and in the intercellular substance of land plants. The gel-forming, hot-water soluble polysaccharides are collectively called pectinic acids and are very commonly used as gelling agents and natural additives in foods [4]. In fresh food technology, pectins play an important texture role. Pectins are primarily made of α -1,4-linked galacturonic acid, which is methyl esterified to different extents depending on the material and the maturation of the fruit. It is also relevant, from the polymeric point of view, that recent structural analyses of the pectin structure seem to support the presence of occasional branches, in addition to a non-random distribution of the non galacturonic residues [4]. Reference to the detailed chemical structure may be necessary, not only for the rationalisation of the physico-chemical properties, but also for possible modelling of the system.

While the calorimetric approach to the study of the phase behaviour of foods has been used extensively for the characterisation of starch-based foods, much less attention has been focussed on pectin-based foods. From a more general point of view, the thermodynamic, thermo-mechanical and chemical properties of these systems are of considerable importance in several branches of food industry such as fruit processing and storage. In the food industry, quality, processability and microbial safety can be expressed in terms of thermal and thermo-mechanical parameters of the substrates, with the glass transitions playing an important role. The aim of this work is to study the composition dependence of state transitions, on water-sugars-pectins components, in apple systems at various different water contents by using differential scanning calorimetry. The calorimetric results are discussed and analysed in order to construct the 'supplemented' state diagram for apple dry matter with water.

Experimental

Materials

Stark Delicious apples ('Delizia') were used as they are easily available throughout the year and possess fairly constant characteristics (hardness, turgidity, and maturity level). The composition of Stark Delicious apples is reported in Table 1.

Samples of apple parenchyma tissue were prepared by slicing the apple edible flesh. Tiny slices (with a mass of less than 1 g) were equilibrated for 27 h at 25°C in a vacuum balance. The thickness of the slice decreased from 0.232 to 0.146 cm after the drying. Drying was, however, not complete since the solid sample still contained 8.5% of residual water, as measured by TG analysis. This sample (labelled A1) was subsequently used for the preparation of samples A2, A3, A4 and A5 with water content controlled by exposure to different vapour pressures until equilibration occurred (Table 2). A portion of sample A was further dried by keeping the sample at 50°C for 24 h under vacuum. The TG analysis confirmed the absence of vaporisable water in the fully dried sample (labelled A-dried). Other samples, whose water content was higher than 40%, were obtained by exposing small specimens of fresh apple tissue for different times in a vacuum oven at 30°C. Water content was determined directly by

weighing the samples before and after treatment. Although it was not possible to reproduce a given sample, the reliability of the measurements was based on the consistency of the entire set of experimental data as a function of the appropriate variable (water content).

Table 1 Composition of Stark Delicious apple

Component	g/g (wet basis)	g/g (dry basis)
Water	0.8440	5.410
Dry matter	0.1560	1.000
Soluble sugars	0.1218	0.781
Residual matter	0.0342	0.219

Table 2 Composition of systems at low water content

Sample	g_{H_2O}/g_{tot}	conditions
A-dried	$\approx 0\%$	Vacuum, 50°C, 24 h
A1	0.085	Vacuum, 25°C, 27 h
A2	0.17	$6.3 \cdot 10^{-3}$ atm, $t < t_{eq}$
A3	0.22	$6.3 \cdot 10^{-3}$ atm, $t = t_{eq}$ (ca 2 months)
A4	0.30	$1.9 \cdot 10^{-2}$ atm, $t = t_{eq}$
A5	0.38	$2.2 \cdot 10^{-2}$ atm, $t < t_{eq}$
B	0.85	fresh apple

Methods

Thermal analysis was carried out using a Mettler TA 3000 DSC which had been purged with nitrogen and chilled with liquid nitrogen. Experiments were carried out on samples of about 10 mg at a heating rate of 5°C min^{-1} (unless otherwise specified). The samples were rapidly cooled to the starting temperature and heating scans were terminated well before reaching thermal degradation which occurs at about 200°C , which is lower than that reported for cellulose and starches [5].

The glass transition temperature was assigned to the midpoint of the heat capacity step observed at a heating rate of 5°C min^{-1} . The temperature at which the water melted was reported as the temperature value of the endothermic peak. This choice is mandatory in the presence of possible distributions of crystallite size and stability. Calibrations were similarly carried out by using standard protocols.

Thermogravimetric analysis was carried out with a Netzsch TG 209.

Sorption and equilibration of samples with water vapour was carried out by the microgravimetric method, with custom-made vacuum apparatus and a Rocky Mountain quartz spring balance with an extension of 1.4 cm mg^{-1} . The method has been already described in [6]. For the preparation of the sample A from the apple slice, the

quartz spring was replaced by a steel spring; the calibration of the spring constant gave a value of 4.56 cm g^{-1} .

Results and discussion

Sorption experiments

In order to obtain information on the basic interactions of water with the dry matrix, sorption of water vapour in dried samples was measured at 25°C as a function of water activity $a_w = P/P_0$, where P is the actual pressure to which the sample was exposed and P_0 is the saturation pressure at the temperature of the experiment. The sorption curve of water vapour in the dry matrix as a function of activity a_w is reported in Fig. 1. Like many other literature examples [7, 8], it shows two different trends: in the range up to activity of 0.5, the sorption follows the 'dual sorption mode', whereas at higher activities an almost linear dependence of sorption on activities is evident. In the first range the sorption is typical of systems in which the vapour molecules strongly interact with the substrate, progressively saturating its specific sites. This behaviour is ascribed to the absorption of water molecules firmly interacting with the solid structure. From the data in Fig. 1, the amount of this type of water (bound to specific sites of the matrix) is of the order of 30% (water/d.m) or about $X_w = 0.25$, well in agreement with literature data and predicted behaviour [7].

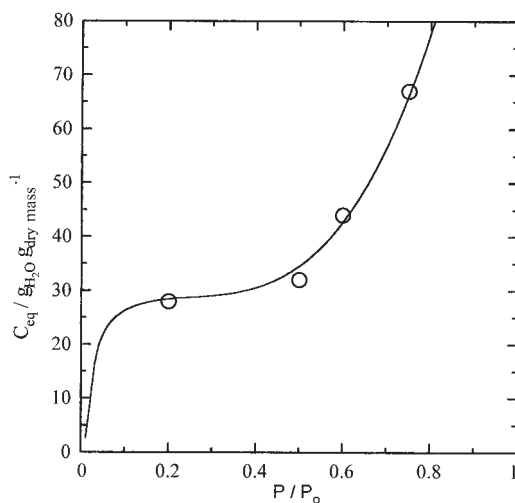


Fig. 1 Sorption curve of water vapour equilibrated with the apple dry matrix at 25°C . The line has been drawn to visually follow the literature trend [7, 8]

Calorimetric analysis

The calorimetric experiments were carried out on samples containing different amount of water between 85 and $\approx 0\%$. Three regions with different thermal behav-

our can be described, depending on their water content: the first one is at high water content (45–85%), the second is a region of transition (30–45%) and the third refers to samples at low water content (less than 30%).

High water content (45–85%)

The thermal behaviour of the samples containing higher proportions of water were studied from -150 to 30°C . The samples were first rapidly cooled in the calorimeter from ambient to the starting temperature (-150°C). The samples were then heated back up to 30°C at a heating rate of $5^{\circ}\text{C min}^{-1}$. Figure 2 shows some of the DSC curves obtained for samples with high water contents. All the curves show a small but distinct thermal effect at low temperature before the final melting of the ice at around 0°C . The water melting occurs over a wide range and covers a ΔT of about 30°C ; the endotherms show an increasing asymmetry upon decreasing the water content. Therefore, the temperature of melting, T_m , is conventionally defined here as the peak temperature; it depends on the concentration of water and changes from 0 to -20°C upon decreasing the water content. As expected for the concentration dependence, the smaller the water content of the sample the lower its melting temperature. In parallel to the melting point depression, the total heat effect (*normalised per gram of sample*) decreases and should reach an extrapolated zero intercept at zero water fraction. However, it is well known that biological macromolecular components easily bind water molecules which become dynamically less mobile (as seen by NMR techniques) and thermodynamically ‘unfrozen’ (as seen by DSC). Although the numerical value of these quantities may differ because of the differences in experimental methodologies (and temperature!), this concept has been widely used and has received a general consensus in the case of phases in thermodynamic equilibrium. It is also necessary to point out that the melting properties of water under confined sur-

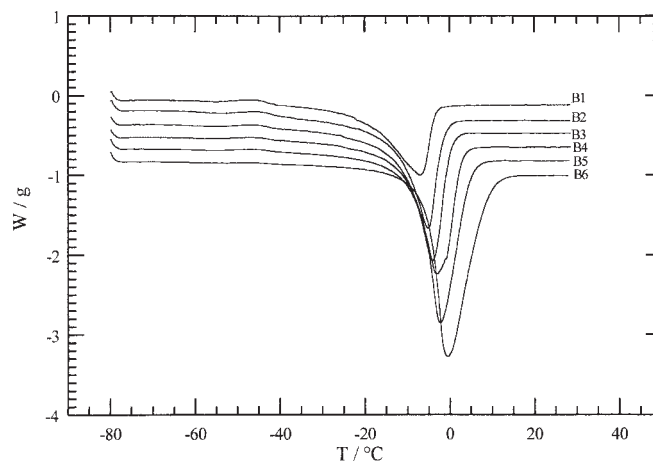


Fig. 2 DSC curves from -80 to 30°C for samples with high water content (B1=0.45, B2=0.51, B3=0.56, B4=0.65, B5=0.72, B6=0.85). For the sake of clarity, the curves have been shifted from each other by a constant value

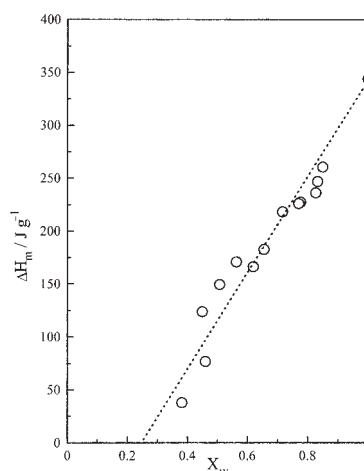


Fig. 3 Plot of the enthalpy of melting (calculated on the basis of the total sample mass) as a function of the fraction of water content in the sample. The value of $X_w=0.25\pm 0.05$ is extrapolated for the amount of 'un-frozen water'. The dotted line indicates the behaviour of a non-interacting mixture

faces or interstitial water have been found to differ significantly from those of bulk water [9]. This fact emphasises that thermodynamic equilibrium is understood to relate to species undergoing phase transitions at equal chemical potential and does not refer to phase transitions between metastable (under-cooled) phases. Notwithstanding the above caveats, all the melting enthalpy data collected in this work and reported in Fig. 3 as a function of water fraction X_w demonstrate that about 25–30% of the water is in the bound state and therefore cannot be crystallised.

A scrutiny of the family of the heating curves discloses some peculiar behaviour since, in all the samples with $X_w \geq 0.45$, an exothermic bump is detected before the melting of water. Figure 4a shows the DSC curve of the sample with $X_w=62\%$ and an enlargement of the region from -80 to -20°C . Such minor but complex thermal effects are very common in all water-carbohydrate systems [10] and have often been thought to be a consequence of a glass transition in a fraction of the mixed aqueous system which then undergoes crystallisation due to its increased mobility. This means that the system is effectively segregated into a crystalline domain of pure water and a glassy/amorphous domain, which contains the solute and some residual water. Reid *et al.* [11] have observed that, on heating, partially lyophilised blackberry samples show two such thermal effects, that are generally ascribed to 'glass transitions'. The question as to whether there are one or two transitions in mixed polymeric systems has been elegantly discussed by Pizzoli and Scandola [12] for synthetic polymers. By analysing these data and other literature results on starch mixtures it has also been argued [13] that, in ternary systems of mutually soluble components, the surface described by the glass transition temperatures is an envelope with a downward curvature. Two distinct transition phenomena are definitely an indication of a phase or domain separation with two different compositions. Therefore, the presence of only one

'bump' should be taken as evidence that the apple system considered here is made up of a single glassy-amorphous phase interspersed with the ice phase.

Moderate water content (30–45%)

In the experiments above reported, our cooling conditions did not enable us to completely vitrify these systems with high water content. During the cooling time, some of the water crystallises and a water-sugars-pectins phase (of constant composition?) separates out. A completely different behaviour is exhibited in Fig. 4b, which shows the curve of the sample containing 38% of water. Similar DSC curves (not shown here) were consistently obtained with 'reconstructed models' of water-sugars-pectins systems with compositions in the same range of water concentration. In this case, given the apparent value of $T_g \approx -60^\circ\text{C}$, the cooling rate allows a so-called 'quasi equilibrium glass' to be formed. On heating, a heat loss, followed by a heat absorption, is

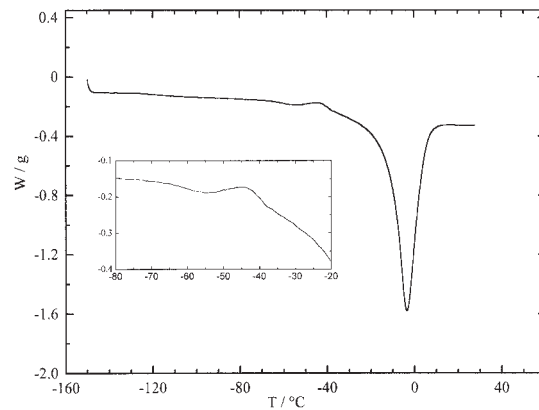


Fig. 4 a) Example of the low temperature behaviour of a mixture with $X_w > 0.45$: the small bump (see the insert) is characteristic of all curves which exhibit a large endothermic peak of water melting around 0°C

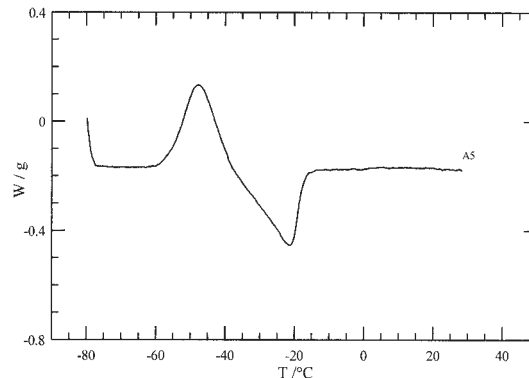


Fig. 4 b) A more evident crystallisation exotherm and the following low melting is observed for the sample with $X_w = 0.38$

observed. As the two heat effects are approximately equal, the two events can be safely ascribed to the crystallisation and to the subsequent following fusion of a small amount of water in the phase-segregated water-sugars-pectins system. No other melting is observed because, apart from the unfrozen water (bound to the solute), all the residual molecules of water are quenched upon cooling. The system must be considered essentially biphasic with a solid glassy matrix made by the mixture of solute and bound water which is able to encapsulate and quench (just below -60°C) the residual 'free' water molecules. Only when heated above -60°C , does the glassy state become a soft amorphous matrix, thus enabling the entrapped water molecules to crystallise and then undergo the melting process.

Low water content (0–30%)

For compositions of, or lower than 30%, there is no melting of water and no transition at -60°C (Fig. 5). For each sample a second order transition can be observed, with a transition temperature that increases with decreasing water content. In particular, for the dry apple sample (A-dried), the mid-point transition temperature is raised to about 30°C . This indicates that the remaining water molecules are very tightly bound to the polysaccharide structure and, for these compositions, only the temperature of the glass transition of the mixture is seen in the thermograms, before reaching the vapourisation of water at around 100°C .

The presence of very small amounts of water in simple carbohydrates, like sucrose, has been shown to have a marked effect on the accuracy of the T_g determination, as well as to mask other controversial phenomena as in the case of polymorphism [14]. From the strong dependence of the T_g on composition, it can be deduced that water acts as a plasticizer by reducing the glass transition temperature of the pectic substances. The data of glass transition temperature as a function of water content have been collected in Fig. 6, which includes all the relevant temperatures for this

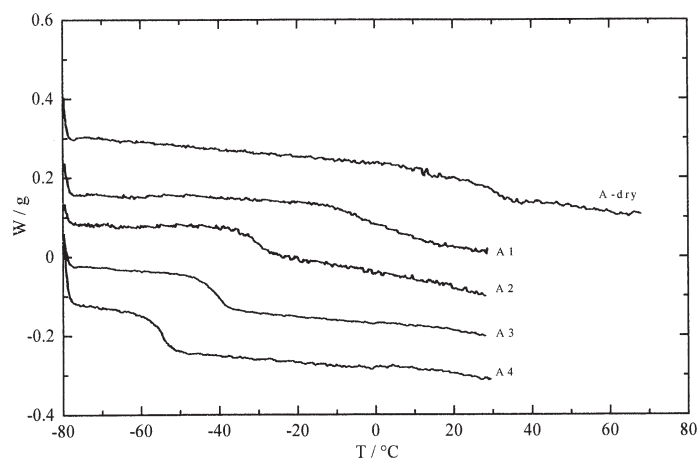


Fig. 5 DSC curves of the samples with low water content ($X_w \leq 0.30$). Only the T_g step is observed

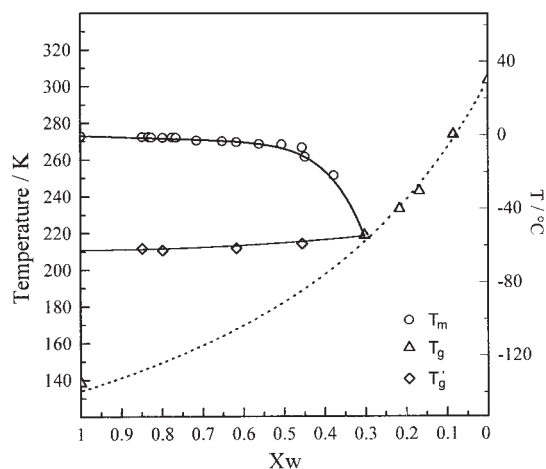


Fig. 6 Supplemented state diagram of the model apple-water system. The lines of the melting temperatures and of glass transition temperatures cross each other at T_g' , the minimum temperature of liquid phase. The line of the glass transition temperatures for the systems with ice-separation ($X_w > 0.30$) is also shown

system. The dependence of glass transition on composition in a miscible polymer-diluent system has been widely described in literature. The simplest is the semi-empirical Gordon–Taylor's equation [15], which gives a continuous curve between the T_g 's of pure components:

$$T_g = \frac{x_1 T_{g1} + k(1-x_1)T_{g2}}{x_1 + k(1-x_1)}$$

where: T_{g1} is the glass transition temperature of water, 138 K [16]; T_{g2} the glass transition temperature of the solid component; and x_1 the water fraction. In this equation k is an empirical constant which, however, in other similar equations, that are formulated on the basis of thermodynamic models, can be related to the changes in the heat capacity at the transition temperature [17]. In our case, since the solute is actually a mixture of several components, it is highly speculative to try to use the few data points of our system as a basis for assuming that the only relevant contribution to ΔC_p is that measured for the pectic substances. In this context it should be mentioned that we have been unable to find any reliable data for chemically characterised pectin samples – the only relevant paper on this system [18] having no T_g values.

It can be noted that for mixtures of simple sugars and water a relation was found between the values of k and the T_g of the sugar [19]. If this correlation is applied to our system, either the value of $k=2.5$ should be too small to fit the data, or the T_g should be as low as -30°C . To reconcile this apparent contradiction, it is enough to recall the well known dependence of the T_g of pure polymers on molecular mass. It should be also mentioned that, in our case, the T_g measured for the 'dry' solid is just a kind of average quantity of the values of the pectic substances and the small sugars (mainly

fructose, glucose and some other monosaccharides). The effect of water is therefore like adding a plasticizer to a polymer, already plasticised by the presence of small monomers [13]. Notwithstanding these uncertainties, an attempt was made to study a 'model' prepared by mixing a sample of purified pectin in the presence of various amounts of small sugars and water. The pectin was purified from a commercial source (Sunskit Growers, California) and characterised in the laboratory of D.A. Brant [20]. The specimens were prepared by adding known amounts of water to a freeze-dried mixture of pectin and sucrose in a ratio of 1:10 by mass. As mentioned above, the curves obtained with samples whose fraction of water exceeded 45, in the range 45–30%, reproduce, in shape and behaviour, those reported in Figs 4a and 4b, respectively. No attempt has been made to prepare mixtures with other sugars (such as fructose), nor dry 'model' samples which could never reproduce the cellular ultra-structure of the pectic substances. The need for accurate nano-structural information on pure pectin polymers with known chemical structures is, however, emphasised and deserves further work.

Supplemented state diagram

The temperature/composition behaviour is reported in Fig. 6 in the form of a so-called 'supplemented' state diagram [21, 22]. It includes the familiar solid/liquid co-existence boundary curve of the equilibrium phase diagram determined by the melting temperatures of the systems with high water content (85–45%). The curve has been drawn to cross the glass transition curve, that was fitted on the experimental glass transition temperatures obtained with the samples at low water content (30–0%). This curve joins the other curve extrapolated from the few data points on the glass transition of the segregated phase in the systems with high water content. A dotted line has also been traced to represent the hypothetical line of the glass transition temperature for a completely miscible system (only in the case of extremely fast cooling when no phase separation would occur).

The three curves reported in Fig. 6 converge well into a single 'triple point', which defines the lowest temperature at which a liquid phase exists in this system. Assuming a thermodynamic equilibrium in the melting/crystallisation region ($T_m = T_c$) the freezing curve and the glassy state curve cross each other at a concentration X^* . In the low concentration domain ($X_w < X^*$), the solvent cannot crystallise since the system passes the glass transition (and becomes viscous) before the temperature of crystallisation is reached.

Conclusions

Besides the heuristic value of the results presented here, a correct interpretation of the DSC signals of a complex mixture, such as those presented here, is still hampered by the presence of small thermal effects which have sometimes been ascribed to glass transitions and enthalpic relaxation occurring in the metastable phase separated system. Arguments in favour of one single convincing interpretation for this behaviour

have not yet been presented in the literature. The phenomena depends on the thermal history (cooling rate, annealing,...), changes of the aqueous phase concentration and changes of the visco-elastic properties of the system (as function of both the temperature and the concentration).

We are not entering into this discussion here, but we wish to present the phase diagram obtained under controlled conditions as a contribution towards understanding the transformations which may be encountered during the thermal processes of apple (dehydrated-rehydrated).

The relevant information emerging from our data is that the 'immobilisation' temperature of water molecules (at least for liquid-like mobility) is far beyond the common freezing temperature of 'super-markets', since it is only below a temperature of -60°C , that either a glassy state or a frozen ice and glassy state are obtained. The temperature T_g' for the present system is much lower than those reported for other carbohydrate-water mixtures, such as those with starch or pullulan, [23]. This implies that dilute juice systems (with $X_w > 0.7$) at low temperature (e.g., -30°C) may actually contain freeze-concentrated soluble bio-catalysts. Therefore, the chemical and enzymatic degradation of biological materials could be even faster than that observed in the samples stored at about 0°C [24].

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