Glass Transition and Glycerol Effects on Sucrose Inversion in Pullulan-Sucrose Systems

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ABSTRACT

Glass transition and glycerol effects on sucrose inversion were investigated in pullulan-sucrose systems at various \(a_w\) using DSC, sorption isotherms, and determination of sucrose hydrolysis by spectrophotometry. Water and glycerol plasticized the systems. DSC thermograms indicated phase separation in the systems depending on water content. Glycerol increased sucrose hydrolysis at 0.538 \(a_w\).

INTRODUCTION

Quality and shelf life of low-moisture, amorphous foods are often affected by enzymatic changes during storage. Changes in the physical state and structure often result from glass transition \(^1, 2\). The glass transition occurs over a temperature range and it may be accompanied by an increase in diffusion-controlled physical or chemical processes. In the glassy state, the food systems have a high stability and viscosity, but at temperatures above the glass transition, various time-dependent and diffusion-controlled changes can be observed \(^3\), supporting an assumption of increased rates of chemical and enzymatic reactions\(^2\).

Food materials are significantly plasticized by water. At increasing water contents, the materials also have higher water activities \((a_w)\) and several reactions in low-moisture food systems have been shown to exhibit higher rates above specific water contents and \(a_w\)\(^4\). Other low molecular weight compounds, e.g., polyols\(^5\), are also reported to plasticize food materials. For example, glycerol is a non-toxic and colorless liquid with a sweet taste and syrup-like consistency that can be mixed with water. It is used as a softener or plasticizer to improve flexibility, smoothness and pliability of cellophane film and casings used as sausage skins as well as in starch-based edible films and coatings\(^6\). Our previous studies\(^4, 7\) suggested that invertase activity appear at intermediate water activities, and in lactose-sucrose models the rate of sucrose inversion increases concomitantly with lactose crystallisation.

Pullulan is a water-soluble extracellular polysaccharide. Due to its non-toxicity, non-irritating properties and resistance to oxygen permeability, it is used for producing films, binders, adhesive thickeners, viscosity improvers, and coating agents. Thus, pullulan has a number of potential uses in the pharmaceutical and food industries, and in biotechnology. However, investigations on physical properties of pullulan-based products are still scarce.

The objective of the present study was to investigate the effects of glycerol and water on sucrose inversion in pullulan-sucrose blends in order to elucidate pullulan-sucrose-glycerol interactions and to examine the relationship of the glass transition and invertase activity.
The pullulan-sucrose-invertase-glycerol (PSIG) system was prepared as follows: Glycerol (5g) was mixed to 500ml of distilled water. Sucrose (33.3g) was dissolved into the solution and pullulan (66.6g) was added under a soft heating and stirring until dissolution was completed. The solution was cooled at 5°C. Invertase (58.2mg) dissolved in 5ml of distilled water beforehand was added to the mixture and quickly mixed. Aliquots of the solution (5ml) were prepared in 20ml glass vials and pre-frozen at –20°C for 2h. The samples were then stored in a freezer at –80°C for 24h and freeze-dried for 4 days at a pressure < 0.1 mbar. The freeze-dried samples were stored for 5 days in vacuum desiccators over P2O5 for further dehydration. The same procedure was followed in preparation of pullulan-sucrose (PS) and pullulan–sucrose–invertase (PSI) systems without invertase and glycerol and glycerol addition, respectively. PSI and PSIG were used for water sorption isotherm, differential scanning calorimetry (DSC) and kinetic studies while the PS was used for physical characterization of the system using water sorption isotherm and DSC studies.

RESULTS AND DISCUSSIONS
The GAB and the BET sorption isotherms could be used as models for the experimental data. The GAB model showed a very good fit to experimental water sorption data of PS, PSI, and PSIG over the whole a_w range (Fig.1) while the BET model fitted the experimental data over the whole a_w range for PSIG, and only up to 0.444 a_w, for PS and PSI.

This fitting of the BET model to the experimental data points is possibly due to the transformation of the sorption behaviour of PS and PSI systems attributable to glycerol. The isotherms had the sigmoid shape typical for most food materials. The thermograms obtained from the first scan of samples showed two transitions observable in the 0-0.538 a_w range for PS, PSI and PSIG. Above 0.538 a_w, only one T_g was exhibited. Rescanning of the samples after quenching at room temperature gave only one glass transition for PS samples in the whole a_w range, for the anhydrous PSI sample, and for PSIG samples at all a_w except at 0.239. However PSI and PSIG exhibited two clearly distinct transitions at 0.239, 0.333, and 0.444, and 0.239 a_w, respectively. Fig. 2 shows an example of scan and rescan of PSI sample at 0.239 a_w.

The finding of two T_g values at some a_w suggested phase separation of the systems, on the basis of the well-known single glass transition criterion. Upon the second heating, the endothermic and exothermic events associated with the transition depending on water content, or due possibly
to sub-$T_g$ storage temperature\(^{(10, 11)}\) during drying were removed. The double $T_g$ in PSI and PSIG at some $a_w$ suggested that the systems were not totally miscible in these conditions and increase in humidity led to higher miscibility of the systems. The single $T_g$ in PS in the whole $a_w$ suggested that the systems were not totally miscible in these conditions and increase in humidity leaded to higher miscibility of the system. The single $T_g$ in PS in the whole $a_w$ indicated that miscibility depends on water contents but also on systems composition. However, the double $T_g$ did not seem to depend on gradient of $a_w$ since at $a_w > 0.538$ only one $T_g$ was exhibited by the systems. $T_g$ of anhydrous PSIG was low compared to the two other systems.

This difference could be attributed to glycerol that might act as plasticizer. As $a_w$ increased, the $T_g$ decreased from its value in the anhydrous form. The first responsible of this plasticization is water, but also fructose and glucose resulting from the water-gradient dependence of sucrose hydrolysis by invertase. PSIG was much more plasticized because, in addition to the factors that depressed $T_g$ in PS and PSI, glycerol acted as plasticizer.

In the 0.238-0.444 $a_w$ range, no significant changes was observed in glucose contents with time. Above 0.444 $a_w$ sucrose hydrolysis increased with water activity and time in PSI and PSIG. These features agreed with our previous study\(^{(4, 7)}\). A clear difference in the trend of sucrose hydrolysis was noticeable at 0.538 $a_w$ where glucose content increased more in PSIG than in PSI. Rate constant of sucrose hydrolysis are given in Table 1. At 0.538 $a_w$ the rate constant was $3.84 \times 10^{-4}$ h\(^{-1}\) for PSI and $11.11 \times 10^{-3}$ h\(^{-1}\) for PSIG. Fig.3 illustrates the difference occurring in the systems at this $a_w$. This significant difference in the rate constant is a relevant indication of the impact of glycerol on the activity of the enzyme. It seems like this $a_w$, coupled with glycerol favoured the activity of enzyme by increasing its mobility and probably mobility of the substrate so that hydrolysis was facilitated. Glycerol boosted the activity of the enzyme. At 0.764 $a_w$, the humidity of both systems allowed sufficient and equitable enzyme activity. A plot of rate constants at different $a_w$ versus $T-T_g$ for PSI and PSIG systems is shown in Fig.4. The rate of sucrose hydrolysis was insignificant in the glassy state. An increase in the rate of sucrose hydrolysis occurred in the supercooled liquid state for PSI and PSIG.
T-Tg was higher for PSIG than for PSI. This suggested that although the enzyme activity took place in the rubbery state, there exist some discrepancies concerning the activity of the enzyme in that state. Sucrose hydrolysis at such aw was not significant. It is likely that the activity of the enzyme depended more on the mobility and diffusivity of the system constituents. Water that plays a significant role in mobility and diffusivity of amorphous food systems appears as a key actor in the activity of the enzyme and possibly plasticization of the enzyme itself. This study showed that substance such as glycerol can provide sufficient moisture for sucrose hydrolysis to take place even at very moderate aw, and suggested a particular attention in processing and storage of food and pharmaceutical systems containing glycerol.

REFERENCES


