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Manuscript Title: Influence of Glucose, Sucrose and Trehalose on the Freeze-Thaw Stability of Tapioca Starch Gels

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Abstract: The aim of this study is to study influence of glucose, sucrose and trehalose on the Freeze-Thaw (FT) stability of tapioca starch gels. The effects of glucose, sucrose and trehalose (3%, 6% and 12%, *w*: *w*) on the freeze-thaw stability of Tapioca Starch (TS) gels were investigated at the concentration of starch 6% (*w*: *w*). Syneresis experiment showed the FT stability of TS gels could be improved by sugar addition, according to the sugar type and concentration. And the improvement of syneresis% was in the order: trehalose＞sucrose＞glucose. SEM and DSC experiments were also applied to determine the effects of sugars (6%, *w*: *w*) on the FT stability of TS gels after FT 5 cycles. SEM tests showed that the pore sizes and surrounding matrixes corresponded with the sugars order mentioned above and the pores of gels containing trehalose were smallest and the matrixes surrounding were thickest. DSC experiments showed that retrogradation of starch was retarded in the presence of sugars and trehalose was more effective than sucrose or glucose. Therefore, trehalose is a good candidate for improving the FT stability of TS gels.

Keywords: DSC, freeze-thaw stability, hydration, SEM, syneresis, tapioca starch

INTRODUCTION

As the pace of life continues to increase, all kinds of frozen foods have launched into the world market at high speed. And starch has been used in frozen foods for decades as main raw material or as thickener, stabilizer and food matrix. When starchy foods or starch gels are frozen, ice crystals are formed and a liquid-solid phase separation process happens, which often causes physical stress to food matrix. During thawing, ice crystals are melted and water is easily separated and then spongy networks are formed in starch gels. This process is named as syneresis, which is caused by amylose retrogradation due to molecular association between starch chains, expelling water from the starch gels (Morris, 1990; Karim *et al.*, 2000; Saartratra *et al.*, 2005). Repeated Freeze-Thaw (FT) treatments can facilitate the syneresis process, so the percent of syneresis is a useful index to evaluate the ability of starch to resist the deterioration of the gels during FT treatment (Karim *et al.*, 2000). Meanwhile, SEM and DSC experiments could also be used to assess the FT stability of starch gels. The thawing and dewatering process could soften the food matrix and deteriorate the comprehensive quality (Rahman, 2007), so it was important to improve the FT stability of starchy foods by adjustment of product formulation or improvement of storage condition. Addition of low molecular sugars has been proved to improve the textural properties and storage stability of starch-based products. Baker and Rayas-Duarte (1998) showed adding sugars increased the FT stability of amaranth starch gels when compared with a control. Ahmad and Williams (1999) demonstrated that disaccharides and pentose's improved, while hexoses decreased the FT stability of sago starch gels. Arunyanart and Charoenrein (2008) showed that sucrose addition could effectively enhance the FT stability of rice starch gels subjected to repeated FT cycles. Among sugars studied in above-mentioned literatures, trehalose and protective agent against environment stresses such as desiccation, heat or freezing, was rarely mentioned. As is known to all, tapioca starch is featured by bland flavour, clear paste appearance and high viscosity, so it can be used in food including baby food as a filler material or a thickening and gelling agent (Sanguanpong *et al.*, 2003). And the price of tapioca starch is low in the world market when compared with other starch. So far the use of tapioca starch in food processing has become more and more popular. On the other hand, glucose,

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sucrose and trehalose are universal sweeteners used in food industry. In addition, these sugars originate from nature and are safer than chemical additives. Meanwhile, adding these sugars in frozen foods may ensure the foods of high quality when subjected to repeated FT cycles in supply chains. However, few studies have been reported on the freeze-thawed stability of TS gels containing various sugars especially trehalose, one of the most effective cryoprotectants (Kawai *et al.*, 1992). In particular, the comparative study of different sugar addition on FT stability of starch gels is rarely involved. Thus, the objective of the study was to study in detail the FT stabilities of TS gels in the presence of glucose, sucrose and trehalose by comparing the syneresis% when subjected to repeated FT cycles. DSC and SEM experiments, as auxiliary methods, were applied to evaluate the effects of sugars on the ratio of retrogradation enthalpy to gelatinization enthalpy and the microstructure of freeze-thawed TS gels. All the experiments show that sugars can improve the FT stability of TS gels, with trehalose the best, sucrose the next and glucose the third.

MATERIALS AND METHODS

Materials: Tapioca starch was supplied by Guangxi State Farms Ming yang Biochemical Group, Inc., China. The moisture content and amylase content of tapioca starch was 134.1 and 171.0 g/kg, respectively (American Association of Cereal Chemists, 2000). Trehalose, sucrose and glucose were analytical reagent purchased from Sino harm Group Co., Ltd, China.

Sample preparation: Tapioca starch suspensions (6%, *w*: *w*) containing different levels (0, 3, 6 and 12%, *w*: *w*, based on the mass of starch suspensions) of sugars (sucrose, glucose and trehalose) were added into 250 mL beakers. The beakers were placed in 95°C water bath for 25 min to ensure complete gelatinization. At last, the TS gels were put into sealed tubes for the freezing-thawing experiments. These experiments were conducted in December 2012 in the food property laboratory, Jiangnan University, China.

Freezing and thawing: The TS gels (in triplicate) were placed into a chest freezer and frozen at -18°C for 22 h and then thawed at 25°C water bath for 2 h before the next Freeze-Thaw (FT) cycle. The FT processes were repeated for 1, 2, 3, 4 and 5 cycles and the thawed samples were removed from the syringes for the following tests at the end of FT cycles 1 to 5.

Syneresis measurement: The syneresis measurements were performed in triplicate, according to method 2 of Charoenrein *et al.* (2008) with some modifications. Plastic tube was pre-drilled holes (about 6 mm diameter, 10 holes) at the bottom and a piece of small filter paper (20 mm diameter) was applied to cover the small holes. The plastic tube was covered and placed in a pre-weighted centrifuge tube (W_{t0}), after which the total weight of the tube set was weighted (W_{t1}). The thawed TS gels was added into the inner plastic tube and the tube set with TS gels was weighted again (W_{t2}), then the tube set was centrifuged at 3000×g for 15 min. Liquid was separated from the sample after centrifugation and filtered into the centrifuge tube. The centrifuge tube plus the separated liquid was weighted (W_{t3}) after the plastic tube was pulled out of the centrifuge tube. The syneresis% was calculated according to Eq. (1):

$$Syneresis\% = \frac{W_{t3} - W_{t0}}{W_{t2} - W_{t1}} \times 100 \tag{1}$$

Microstructure of frozen TS gels with SEM: The TS gels in the absence or presence of 6% sugars after FT 5 cycles were cut into sections about 2 mm thickness using a razor blade and immediately freeze-dried in a freeze-dryer (DURA-DRY Corrosion Resistant Freeze-dryer, FTS system, USA) for 3 days. Microstructures of the freeze-dried samples were observed using SEM (Quanta-200 SEM, FEI, Netherlands) at an accelerating voltage of 25 KV.

Differential scanning calorimetric: The thermal behaviours of starch gelatinization and retrogradation were analyzed using a Perkin Elmer Pyris 1 DSC (Perkin Elmer, CT, USA), when compared to an inert reference (empty pan). The instrument was calibrated with metal indium (99.999%) prior to use. Tapioca starch, distilled water or sugar solution were added into aluminum pans to obtain the samples (TS: water = 1:3, *w*: *w*) without (the control) or with sugars (TS: sugars = 1:1, *w*: *w*) and the pans were sealed immediately. The samples were equilibrated at room temperature for 24 h and heated from 30 to 100°C at a heat rate of 10 °C/min. The gelatinization onset, peak and conclusion temperatures (T_o, T_p and T_c, respectively) and gelatinization enthalpy ΔH_1 were determined.

The pans after gelatinization study were freeze-thawed 5 cycles with the methods mentioned above and rescanned from 30 to 100°C at a rate of 10°C/min to determine the degree of starch retrogradation. The melting parameters of the starch recrystallization were analyzed. The Degree of Retro gradation (DR) was calculated as the ratio of retrogradation enthalpy to gelatinization enthalpy (Temsiripong *et al.*, 2005). Three (3) parallel measurements were performed and the averages were reported. The respective enthalpy (J/g) was calculated on the basis of the dry base starch.

Statistical analysis: The results of the syneresis experiments and DSC tests were reported as mean±SD

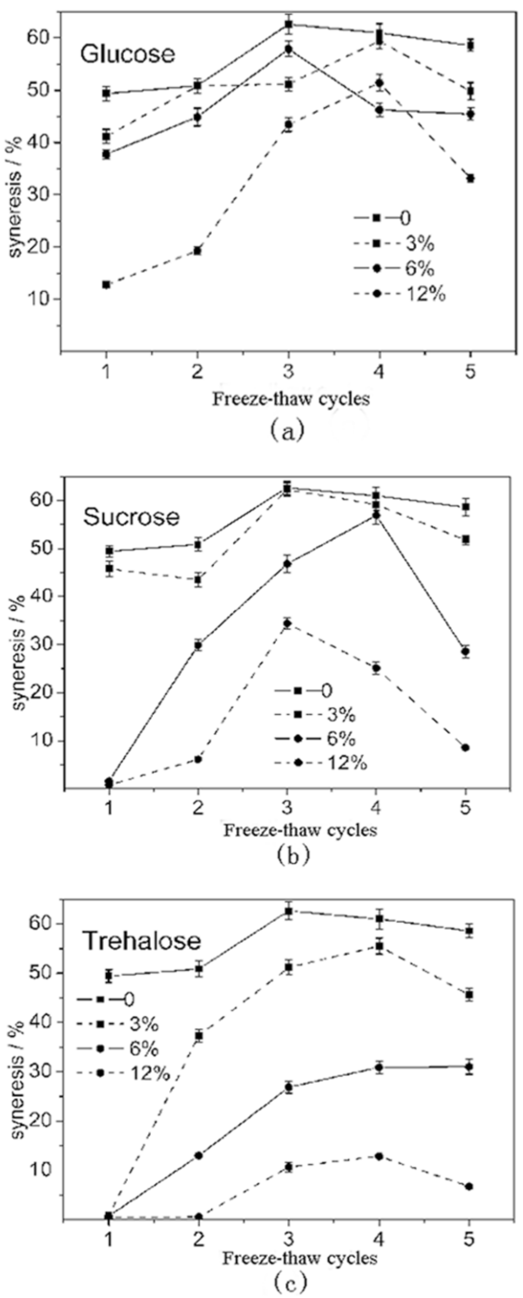

Fig. 1: Effects of sugars (sucrose, glucose and trehalose) on the syneresis% of tapioca starch gels subjected to repeated freeze-thaw cycles: (a) 1 cycle; (b) 2 cycles; (c) 3 cycles; (d) 4 cycles; (e) 5 cycles.

for 3 replications. The difference between means was determined using the Duncan's new multiple range test. All data were statistical analysed using SPSS software (version 12.0 for Windows; SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Percent syneresis of TS gels: To discuss the effects of sugars on the Freeze-Thaw (FT) stabilities of TS gels, syneresis% of TS gels in the absence (the control) or presence of glucose, sucrose and trehalose after freeze-thaw 1-5 cycles were determined and presented in Fig. 1. TS gel containing no sugar showed a high syneresis% (63%) after FT 3 cycles and remained almost unchanged from FT 3-5 cycles. When glucose, sucrose or trehalose was added at the levels of 3, 6 and 12%, syneresis% of TS gels was reduced obviously. Taking glucose addition as an example (Fig. 1a), syneresis% of TS gels increased from FT 1-4 cycles at glucose concentrations of 3 and 12% (FT 3 cycles for 6% glucose addition) and decreased through succeeding cycles. The addition of sucrose (Fig. 1b) and trehalose (Fig. 1c) showed a similarity in the variation trend of syneresis% with glucose addition. The reduction of syneresis% after FT 4 cycles might be attributed to the increases in rigidity and elasticity of the gels due to the formation of spongy gel networks (Yuan and Thompson, 1998). Our findings agreed with Yuan and Thompson (1998) who found that syneresis reached a maximum value within one FT cycle and apparently decreased with additional cycles, in a manner that varied with starch type. Baker and Rayas-Duarte (1998) concluded that amaranth starch gels containing 10% and 20% sucrose greatly improved the stability of the starch gels when subjected to 1 to 3 FT cycles. However, no significant differences were found between starch gels containing sugars and the control after 3 cycles. The difference between Baker and Rayas-Duarte (1998) and ours was probably due to starch types and concentration, measurement method used in each experiment.

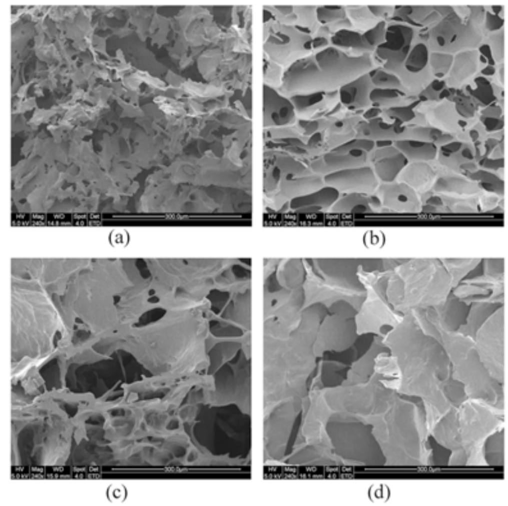

Fig. 2: SEM images of Tapioca Starch (TS) gels (6%, *w*/*w*) without (the control) or with sugars (6%, *w*:*w*) after freeze-thaw 5 cycles (240×, Bar = 300μm): (a) The control, (b) TS gels + 6% glucose, (c) TS gels + 6% sucrose and (d) TS gels + 6% trehalose

Syneresis% of TS gels containing disaccharide (sucrose and trehalose) decreased significantly with the increase of sugar concentrations; the data suggested that the higher the disaccharide concentration, the greater the ability to improve the FT stability of TS gels. The result was consistent with the study of Arunyanart and Charoenrein (2008) who found that 20% sucrose addition in starch gels gave a significantly lower syneresis% after FT 1 (1.25%) or 2 (4.64%) cycles than did 10% sucrose (14.84 and 25.37%, respectively).

However, when glucose (monosaccharide) was added into TS gels, the concentration effect was not obvious after FT 2 cycles and the FT stability was worse than that of starch gels containing disaccharide. Our findings were in accordance with Ahmad and Williams (1999) who demonstrated that the effects of disaccharides on syneresis% of sago starch gels were much better than that of glucose. Last but not least, starch gels containing trehalose had the best FT stabilities than the other 2 sugars for all the FT cycles, especially when subjected to one FT cycle (Fig. 2), which showed low syneresis% (0.46%) even at low trehalose level (3%). Therefore, trehalose is a good candidate for improving the FT stability of starch gels. Thermal fluctuations and phase changes of water were the main causes of the disruption of the gel matrix of starch. Water separated from the gelatinized gels increased with the increase of FT cycles; the structure of retrograded starch was easily destroyed by ice crystal formation which led to higher water separated during thawing (Yuan and Thompson, 1998; Pongsawatmanit *et al*., 2006). Ferrero *et al.* (1994) demonstrated that the disruption was caused by the formation of starch-rich regions and the accompanying partial phase separation. Concretely, the starch-starch interactions were facilitated to form thick fibbers due to the high solid concentration in the regions and water molecules coagulated into ice crystals and formed a separate phase. Upon thawing, ice melted and became water and then released from the polymeric network (syneresis) and leaved the starch gels sponge-like. The difference of syneresis% in the freeze-thawed gels containing different sugars may be due to different hydration ability of sugar addition, the magnitude of which depends on intermolecular hydrogen bonds between sugars and neighbouring water molecules. Equatorial hydroxyl groups of sugars fit well into the hydrogen-bonded water structure and could form stronger hydrogen bonds with water, so the hydration ability of sugar was mainly influenced by the number of equatorial hydroxyl groups ($n_{\text{e-OH}}$) (Kawai *et al*., 1992; Ahmad and Williams, 1999). Trehalose ($n_{\text{e-OH}}$ = 8.0) has the largest number of equatorial OH groups among the disaccharides studied such as sucrose ($n_{\text{e-OH}}$ = 6.3) (Kawai *et al*., 1992), so the former has stronger hydration ability than the latter. And the number of equatorial OH groups in sucrose was larger than that in glucose ($n_{\text{e-OH}}$ = 4.56) (Wu *et al*., 2008). From the analyses mentioned above, the order of hydration ability is trehalose > sucrose > glucose. And thus the “free water” or “available water molecules” of TS gels containing sugars decreased in exactly the same order. Correspondingly, ice crystal formation during freezing and syneresis% during thawing were decreased according to the order of trehalose, sucrose and glucose. Another reason for the lower syneresis with increased sugar concentration was that the proportion of water: solid was lower when more sugar was added to the system without adding more water.

Microstructure of frozen starch gels with SEM: It was worth noting that TS gels containing sugars differed distinctly from the control in their appearance; the formers remained smooth even after FT 2-3 cycles, while the latter became spongy within all FT cycles. The different appearance was another evidence for the effects of sugars on the FT ability of TS gels. To illustrate the relationship between FT gel textures and sugar addition, TS gels (6% *w*/*w*) without or with 6% levels of sugars after FT 5 cycles were selected as representatives to study the effect of sugars on the microstructure of TS gels using SEM (Fig. 2). The figure showed that a spongy structure and simultaneously a fibrillar network of TS gels were formed after FT cycles due to ice crystal formation and amylose retrogradation. The results were consistent with Ferrero *et al*. (1993), who reported the formation of the spongy structure due to the deterioration of the frozen corn starch paste during storage. Obvious difference was observed in the fibrillar network and cavity size for the control and TS gels with sugars. The microstructure of the control was characterized by fragmented structure due to the large pores; the matrix surrounding the pores was very thin (Fig. 2a). The TS gels with glucose formed larger ice crystals (cavities in network) and thinner matrix than gels with sucrose. Comparing the TS gels containing glucose and sucrose, the pores of TS gels containing trehalose were smallest and the matrix surrounding the pores was thickest (Fig. 2d).

The micrographs of the FT gels indicated that addition of sugars could influence or improve the appearance of TS gels subjected to repeated FT cycles. The phenomenon revealed that trehalose addition decreased the ice crystal formation and melting (cavities in network) of TS gels than the other 2 sugars because the amount of available water was decreased by the strong hydration ability of trehalose. Above all, the results of SEM experiments proved the same sugar order in improving the FT stability of TS gels as the syneresis experiment.

Differential scanning calorimetry: Differential Scanning Calorimetry (DSC) has proven to be one of the effective methods to determine the thermal behaviour of starch gelatinization and retrogradation (Karim *et al*., 2000). During the course of

Table 1: DSC parameters of the TS gels (6% *w/w*) in the absence or presence of sugars (6%, *w*: *w*) after freeze-thaw 5 cycles

	Gelatinizatio				Freeze-thaw 5 cycles				
Samples	T_o (ºC)	T_p (ºC)	T_c (ºC)	ΔH_1 (J/g)	T_o (ºC)	T_p (ºC)	T_c (ºC)	ΔH_2 (J/g)	R_1 (ΔH_2 /ΔH_1)
Control	64.1±0.2^d	69.4±0.8^d	73.6±0.5^d	13.3±0.3^b	49.0±0.2^a	58.0±0.2^a	64.0±0.2^a	6.9±0.3^a	0.52±0.03^a
Trehalose	75.8±0.6^b	79.2±1.0^b	83.2±0.6^b	13.9±0.3^a	48.4±0.3^{ab}	57.2±0.2^b	63.3±0.4^a	4.5±0.4^c	0.32±0.02^d
Glucose	73.6±0.5^c	77.1±0.4^c	81.6±0.4^c	14.3±0.4^a	47.8±0.5^b	56.2±0.3^c	63.2±0.3^a	6.4±0.3^a	0.45±0.01^b
Sucrose	77.6±0.6^a	82.9±0.5^a	86.5±0.5^a	14.2±0.2^a	48.6±0.3^a	57.3±0.4^b	63.9±0.2^a	5.4±0.2^b	0.38±0.01^c

Mean ± standard deviation values followed by different superscript within the same column are significantly different ($p \geq 0.05$) by Duncan's multiple range tests.ΔH_2 : melting enthalpy of the freeze-thawed gels; ΔH_1: gelatinization enthalpy of the starch slurries

gelatinization, a certain quantity of heat expressed as gelatinization enthalpy is necessary to breakdown the crystalline areas of starch (Xu *et al*., 2011). As to retrograded starch, the value of melting enthalpy provides a quantitative measure of the energy used to melt the recrystallized starch (Zhou *et al*., 2010). The ratio of melting enthalpy to gelatinization enthalpy (R_1) is considered to be one useful indicator to determine the degree of starch recrystallization. In this study, DSC was used as an auxiliary method to study the effect of sugars on the FT stability of TS gels, where the study systems were selected the same as the SEM experiments.

From Table 1, the gelatinization onset, peak and conclusion temperatures (T_o, T_p and T_c, respectively) of the control were in the range of 64.1-73.6 ºC; when various sugars were added, the gelatinization peaks were significantly increased, for example, 82.9ºC for sucrose addition. The increase of gelatinization temperatures were consistent with the reports of other researchers (Katsuta *et al*., 1992; Kohyama and Nishinari, 1991) and could be interpreted as follows: the available water was reduced by the hydration of sugar and sugar could interact directly with starch by intermolecular hydrogen bonds to stabilize the crystalline regions of TS gels. Another result of the strong sugar-starch interaction was the increased gelatinization enthalpy of TS gels with sugars compared with that of the control (Table 1).

When starch gels were subjected to repeated freeze-thaw cycles, water used in the preparation of starch gels separated due to the tendency of starch molecules to re-associate, forming insoluble aggregates (Baker and Rayas-Duarte, 1998). As shown in Table 1, all DSC parameters of TS gels were lower after freeze-thaw 5 cycles than those of the gelatinization process and the retro gradation enthalpy and peak temperatures changed according to different types of sugar addition, in ranges of 4.5-6.9 J/g and 56.2-58.0^{o}C, respectively. From the values of R_1, the degree of starch retrogradation of TS gels containing sugars increased in the following order: trehalose, sucrose and glucose, which indicated that trehalose was more efficient to retard starch retrogradation than sucrose or glucose. The result was consistent with those of syneresis and SEM experiments as mentioned above.

CONCLUSION

This study shows that sugar addition could improve the FT stability of TS gels; the more amounts of sugars added, the better the FT stability was obtained. Among these tests, the improvement of the FT stability of TS gels was in the order: trehalose > sucrose > glucose. The SEM experiments showed that the changes of pore size and matrix surrounding the pore corresponded with the sugars order mentioned above; and the pores of TS gels containing trehalose were smallest and the matrix was thickest. DSC experiments showed that starch retrogradation caused by FT treatment could be retarded by addition of sugars and trehalose was more efficient to retard starch retrogradation than sucrose and glucose. Therefore, trehalose is a good candidate for improving the FT stability of TS gels.

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