



Concentration of plasticizers applied during heat–moisture treatment affects properties of the modified canna starch



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ABSTRACT

Effects of the concentration of plasticizers applied during heat–moisture treatment (HMT) on the properties of canna starch were investigated. The modified starches were prepared by soaking starch in 0 (water), 1, 3, 5, 10, 20 and 30% w/w glycerol or sorbitol solution for 24 h and adjusting the moisture content to 25% before HMT (100 °C, 1 h). Changes in the pasting profiles of heat–moisture treated starches were more obvious when glycerol solutions were used instead of water. An increase in the concentration of glycerol solution from 1% to 5% resulted in a progressive decrease in paste viscosity; paste viscosity then increased as the glycerol concentration rose from 10 to 30%. A similar trend was observed when sorbitol was used as a plasticizer, but with a lesser effect. A scheme for arrangements of the molecular structure of starch during the process of HMT was suggested.

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1. Introduction

Heat–moisture treatment (HMT) refers to a physical modification of starch through incubation of starch granules at low moisture levels, usually below 30%, and heating at high temperature, above the glass transition temperature but below the gelatinization temperature, for a certain period of time (Jacobs & Delcour, 1998). Research studies on starch modification by HMT, except for a recent report by Juansang et al. (2015), were solely undertaken using water as a plasticizer. The plasticizing effect of water renders starch polymeric chains more flexible, thus facilitating the rearrangement of amylose/amylopectin unit chains, as evidenced by a greater magnitude of changes in paste viscosity, crystallinity and digestibility of modified starches treated with higher moisture content (Kulp & Lorenz, 1981; Olayinka, Adebowale, & Olu-Owolabi, 2008; Watcharatewinkul, Puttanlek, Rungsardthong, & Uttapap, 2009).

Plasticizers can be defined as low-molecular-weight substances that are incorporated into a polymer matrix to enhance the flexibility and processability by increasing the spacing between molecular

chains and increasing the segmental mobility, commonly through reducing the H-bonding between the polymer chains (Mathew & Dufresne, 2002). Besides water, hydrophilic molecules such as glycerol, sorbitol, xylitol and urea have been widely studied as plasticizers, especially in the production of thermoplastic starch (TPS) and starch film. The plasticizing phenomenon that occurs during HMT of starch differs from that of TPS or starch film, since the starch granules remain in an intact form throughout the treatment process. In this instance, plasticization of starch chains is influenced not only by interactions between the plasticizer and starch chains, but also by the ability of the plasticizer to penetrate into starch granules, as well as its diffusion efficiency through amorphous and semi-crystalline regions inside the granules (Juansang et al., 2015).

In our previous study, heat–moisture treated (HMT) canna starches were prepared by soaking starch samples in various plasticizer solutions (propanol, propylene glycol, glycerol, erythritol, xylitol and sorbitol) at a fixed concentration of 5% and then adjusting the moisture content to 25% before heating at 100 °C for 1 h. Their pasting properties were discussed in relation to the molecular weight and hydroxyl groups of the plasticizers. Penetration of plasticizers into starch granules was found to be the limiting step that determined the magnitude of the plasticizing effect. The

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molecular weight of the plasticizer was a key factor affecting structural changes of HMT starches, i.e. larger-sized plasticizers such as sorbitol and xylitol displayed a lower capability to penetrate starch granules, and thus had less plasticizing effect on starch molecules (Juansang et al., 2015). Besides the molecular weight, the concentration of the plasticizer could also play a role in its penetration of the starch granules, since the concentration affects the viscosity of the soaking solution as well as the driving force (the difference between the concentration of plasticizer in the soaking solution and in the granules) of penetration from soaking solution to the granules.

Therefore, the objective of the present study was to investigate the effect of plasticizer concentration on pasting properties and microstructure of the HMT starches. Glycerol (3C, 3OH) and sorbitol (6C, 6OH) were chosen for the reason that they have a significant difference in molecular size. Concentrations of plasticizer solutions were varied at 0 (water), 1, 3, 5, 10, 20 and 30% w/w.

2. Materials and methods

2.1. Materials

Edible canna was grown on experimental plots at the Rayong Field Crops Research Center, Rayong, Thailand. Eight-month-old rhizomes were harvested, and the starch was isolated according to a procedure described by (Puncha-arnon, Puttanlek, Rungsardthong, Pathipanawat, & Uttapap, 2007). Glycerol (liquid, MW 92.09) was purchased from QR&C (Johor Bahru, Malaysia), and sorbitol (powder, MW 182.17) was obtained from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Heat–moisture treatment of canna starch

HMT canna starches were prepared by soaking 100 g of starch in 100 mL of 0, 1, 3, 5, 10, 20 and 30% w/w glycerol or sorbitol solution for 24 h at 25 °C with constant stirring. Excess plasticizer solution in the equilibrated slurry was drawn through filter paper (pore size 20–25 µm) by vacuum suction. The remaining cake was then washed with distilled water and suctioned to obtain a moisture content of around 40%. The starch cake was then air-dried to allow the moisture content to drop to 25%. The obtained starch sample was placed in a 200 mL screw-capped bottle, heated at 100 °C in a hot-air oven for 1 h, and dried at 40 °C overnight.

2.3. Determination of plasticizer content in soaking solution and starch granules

Canna starch (50 g) was soaked in 50 mL of 0, 1, 3, 5, 10, 20 or 30% (w/w) plasticizer solution with constant stirring at 25 °C for 24 h. A 2 mL sample of each starch slurry was centrifuged at 5000 rpm for 5 min. The supernatant was filtered through 0.45 µm filter paper, and plasticizer remaining in the supernatant was determined by high-performance liquid chromatography (HPLC). The HPLC system consisted of a pump (LC-20AD; Shimadzu, Kyoto, Japan), an injector, a column (VertiSep™ OA, 8 µm, 7.8 × 300 mm; Vertical Chromatography, Nonthaburi, Thailand), a refractive index (RI) detector (RID-10A; Shimadzu), and a computer with data analysis software (CLASS-VP version 6.14 SP2; Shimadzu). Temperature of the column was maintained at 50 °C, and the flow rate of the mobile phase (filtered 0.005 N H₂SO₄) was set at 0.8 mL/min. A 40 µL sample of supernatant was injected into the HPLC system and the RI value was recorded. The concentration of plasticizer in the supernatant was calculated from the peak area of each sample compared with the peak area of the corresponding standard plasticizer solution. The data obtained

were used to calculate the amount of plasticizer existing inside starch granules (mol/100 g starch) for different concentrations of plasticizer.

2.4. Viscosities of plasticizer solutions and starch slurries containing plasticizers

Plasticizer solutions at different concentrations (1, 3, 5, 10, 20 and 30% w/w) and starch slurries containing 50 g canna starch and 50 mL of plasticizer solution at different concentrations were continuously stirred for 24 h at 25 °C. Viscosities of the solutions and slurries were then measured at 25 ± 1 °C by a viscometer (model LVDV-IP; Brookfield Engineering Laboratories, Middleborough, MA, USA) equipped with a spindle (No. 61) rotating at a fixed speed of 60 rpm.

2.5. Granule morphology

Native and HMT starch granules were stained with 0.2% I₂/KI and observed under a light microscope (Eclipse E200; Nikon, Tokyo, Japan).

2.6. Pasting properties

Native and HMT starch slurries were prepared at a concentration of 8% (w/w) using distilled water as a dispersing medium. Pasting profiles of the slurries were determined by a rapid visco analyzer (RVA-3D; Newport Scientific, Warriewood, Australia) with a paddle rotating at a fixed speed of 160 rpm. Each starch slurry was heated from 40 to 92.5 °C at a rate of 3 °C/min, maintained at 92.5 °C for 15 min, and then cooled to 40 °C at the same rate.

2.7. Light microscopy of starch gels

Starch gels obtained from rapid visco analysis (RVA) experiments were stained with 0.2% I₂/KI and observed under a light microscope at 100×.

2.8. X-ray diffraction

X-ray diffractograms of native and HMT starches were obtained with an X-ray diffractometer (D8 Discover; Bruker, Billerica, MA, USA) using copper K α radiation under the following operating conditions: the X-ray generator was run at 40 kV and 40 mA, and the scanning angle 2 θ was set from 4° to 45° at a scanning rate of 0.1°/min. Crystallinity (%) was defined as the percentage ratio of diffraction peak area to total diffraction area.

2.9. Statistical analysis

All analyses were carried out in duplicate. The experimental data were analyzed using analysis of variance (ANOVA) and were expressed as mean values ± standard deviations. Duncan's multiple range test was conducted to examine significant differences among experimental mean values ($p \leq 0.05$).

3. Results and discussion

3.1. Contents of plasticizers and hydroxyl (OH) groups in starch granules before HMT

According to our previous report (Juansang et al., 2015), the amount of plasticizers existing in starch granules before heat treatment proved to be a major factor in determining the magnitude of

change of the HMT starch. Although the findings were not conclusive, the numbers of OH groups were also found to take part in the alteration of starch properties. In the present study, therefore, the amounts of plasticizer inside starch granules after soaking in plasticizer solutions at different concentrations were analyzed and their corresponding OH groups were calculated. As shown in Table 1, the amount of plasticizer inside starch granules was clearly dependent on the concentration of the plasticizer solution. For glycerol, increasing the concentration from 1% to 10% resulted in an increase in the glycerol content of starch granules. The maximum content of glycerol in granules was attained at 1.36 g/100 g starch for the sample soaked in 10% glycerol solution. When the glycerol concentration was increased further to 20 and 30%, however, the amount of glycerol in granules declined. The percentage ratio of glycerol penetration into starch granules decreased significantly, from 56.0% for 1% glycerol solution to 3.8% for 30% solution. A similar trend was also observed when sorbitol was used as a plasticizer, but with a lesser extent of penetration. The maximum amount of sorbitol in the granules was achieved at 0.56 g/100 g starch, after soaking in 10% sorbitol solution. Further increases in the concentration of the soaking solution resulted in a slight decrease of sorbitol content in starch granules. The glycerol content (number-based) in the granules was approximately 4–6 times that of sorbitol, while the OH groups were about 2–3 times greater. The results confirmed that smaller size plasticizers can penetrate more effectively than the larger ones.

It was expected that the level of plasticizers in starch granules would increase with increasing concentrations of plasticizer solutions, or would reach a plateau at a certain equilibrium plasticizer concentration, but the experimental results differed from expectations, i.e. plasticizers in starch granules decreased at high plasticizer concentrations (20–30%). Besides the amount and concentration of plasticizers, the viscosity of the soaking solution is thought to be a factor affecting the penetration of plasticizers into the granules. Therefore, the viscosities of plasticizer solutions and starch slurries containing plasticizers at different concentrations were measured; the data obtained are shown in Table 2. It was found that the viscosities of glycerol and sorbitol solutions, as well as starch slurries containing these plasticizers, increased progressively as the concentrations of plasticizers increased. At the same concentrations, sorbitol solutions exhibited slightly higher viscosities than glycerol solutions ($p \leq 0.05$). It is known that the

Table 1

Plasticizer and hydroxyl (OH) group contents in 100 g of starch granules after soaking in plasticizer solutions at different concentrations for 24 h.

Plasticizer concentration (%)	Mass (g) of plasticizer	Amount (mmol) of plasticizer	Amount (mmol) of OH groups
<i>Glycerol</i>			
1	0.56 ^a (56.0)**	6.06 ^a	18.18 ^a
3	0.71 ^b (23.7)	7.67 ^b	23.02 ^b
5	0.90 ^c (18.0)	9.79 ^c	29.38 ^c
10	1.36 ^c (13.6)	14.78 ^c	44.33 ^c
20	1.16 ^d (5.8)	12.58 ^d	37.73 ^d
30	1.13 ^d (3.8)	12.28 ^d	36.85 ^d
<i>Sorbitol</i>			
1	0.17 ^a (17.0)	0.95 ^a	5.71 ^a
3	0.37 ^b (12.3)	2.05 ^b	12.31 ^b
5	0.47 ^c (9.4)	2.59 ^c	15.55 ^c
10	0.56 ^d (5.6)	3.06 ^d	18.37 ^d
20	0.45 ^c (2.3)	2.45 ^c	14.68 ^c
30	0.36 ^b (1.2)	1.96 ^b	11.78 ^b

Means with different superscript letters (a, b, ...) in the same column for each plasticizer are significantly different ($p \leq 0.05$).

* Calculated from glycerol/sorbitol only.

** The numbers in parentheses represent the percentage ratio of plasticizer penetration into starch granules.

Table 2

Viscosities of plasticizer solutions and starch slurries containing plasticizers at different concentrations.

Concentration of plasticizer (% w/w)	Viscosity (cP)			
	Plasticizer solution		Starch slurry	
	Glycerol	Sorbitol	Glycerol	Sorbitol
0	^z 0.83 ^a	^z 0.83 ^a	¹ 1.37 ^a	¹ 1.37 ^a
1	^y 2.23 ^b	^z 3.67 ^b	¹ 4.33 ^b	² 8.67 ^b
3	^y 3.46 ^c	^z 4.42 ^c	¹ 9.28 ^c	² 13.95 ^c
5	^y 5.61 ^d	^z 6.76 ^d	¹ 14.19 ^d	² 19.60 ^d
10	^y 8.56 ^e	^z 10.71 ^e	¹ 17.83 ^e	² 23.53 ^e
20	^y 13.17 ^f	^z 14.32 ^f	¹ 20.49 ^f	² 26.35 ^f
30	^y 16.47 ^g	^z 18.77 ^g	¹ 25.47 ^g	² 30.38 ^g

Means with different superscript letters (a, b, ...) in the same column are significantly different ($p \leq 0.05$).

Means with different superscript letters (y, z) in the same row are significantly different ($p \leq 0.05$).

Means with different superscript numbers (1, 2) in the same row are significantly different ($p \leq 0.05$).

mass transfer rate is proportional to the area, mass transfer coefficient, and driving force: molar flux = mass transfer coefficient \times driving force (Wankat, 2012). In this study, the driving force is the concentration gradient from the bulk plasticizer solution to the interior of starch granules. The mass transfer coefficients will be higher if diffusivities are higher (Wankat, 2012). The concentration dependence of the diffusion coefficient is attributed to a thermodynamic and hydrodynamic factors. In most cases, these factors act in opposition and partly counterbalance the resultant concentration dependence of the diffusion coefficient. The thermodynamic factor usually contributes to positive dependence; that is, to increase the rate of diffusion with increasing concentration. On the other hand, the hydrodynamic factor tends to slow down the diffusion rate with increasing concentration. This factor is attributed to the concentration dependence of the frictional coefficient of the diffusing particles. The frictional coefficient is proportional to the local viscosity of the medium. Thus, the hydrodynamic factor can be estimated from the local viscosity of the medium in which the particles are diffusing. Nishijima and Oster (1960) studied the diffusion coefficient of glycerol in glycerol/water mixtures ranging from zero to 93% glycerol; they found a negative correlation of the diffusion coefficient with glycerol concentration, which indicated that the hydrodynamic factor (viscosity) is predominant. As mentioned above, the mass transfer rate is the net result of the driving force and the mass transfer coefficient; thus, it is possible that at certain plasticizer concentrations, the penetration of the plasticizer at that concentration can be lower than that at a lower concentration. For this reason, at high plasticizer concentrations (20–30% w/w) the decrease of plasticizer content inside starch granules, in spite of the high driving force (concentration difference between the soaking solution and the granule interior), was most likely due to the increased viscosities.

3.2. Morphologies of HMT starches

Granule morphologies of native canna starch and HMT canna starches treated with different concentrations of glycerol solution are shown in Supplementary Fig. 1. Native canna starch granules were rounded, oval or disk-shaped with smooth surfaces. HMT of canna starch plasticized with water (0% glycerol) did not alter the surface morphology of starch granules. Similarly, no noticeable changes could be observed on the granular surfaces of all starches modified by HMT using different levels of glycerol and sorbitol (illustrations not shown). Similar observations have been reported for canna starch treated by HMT at different levels of moisture content (18–25%) (Watcharatewinkul et al., 2009).

3.3. Crystalline structure of HMT starches

X-ray diffractograms of native and HMT starches plasticized with glycerol and sorbitol are presented in Fig. 1. Native canna starch gave a B-type X-ray diffraction pattern, which is typical of tuber and root starches and is characterized by a small peak at 5.6° , only one peak at 17° , and a doublet at 22° and 24° . No noticeable (or a very slight) change in the pattern was observed when the starch was heat-treated using pure water as a plasticizer. However, replacement of water with glycerol solution resulted in an observable change in the crystalline pattern of some HMT starches, especially HMT starches plasticized with glycerol at 1 and 3% – i.e. the peak at 5.6° became smaller and the doublet at 22° and 24° tended to merge into a single peak, which is a characteristic of A-type starch. Even more pronounced changes of these peaks were observed when sorbitol was used as a plasticizer. Sorbitol solutions at all concentrations affected the crystalline structure of the HMT starches, and a significant change was observed at 1, 3 and 5% sorbitol concentrations. Changes in the pattern, even though not obvious, reflected the rearrangement of double helices in amylopectin molecules. Movement of the helices took place during heat treatment at 100°C in the presence of plasticizers. The experimental results also indicated that: 1) sorbitol, although present to a lesser extent in starch granules, displayed a greater effect on the crystalline pattern as compared with glycerol; and 2) a change in the crystalline pattern was limited to a certain concentration of plasticizer; above this level, changes in the pattern became less pronounced. Thus, the reaction of plasticizers inside starch granules is complicated, and not only plasticization takes place. Little information on the interaction of plasticizers and intact starch granules is available in the literature. Only one report, Perry and Donald (2000), investigated the role of low molecular weight solvents such as glycerol, ethylene glycol, and butane-1,4-diol in starch granule assembly. Small- and wide-angle scattering and calorimetric analyses were used as tools in this study. It has been demonstrated that starch granules can be solvated and effectively plasticized by a variety of nonaqueous solvents. Self-assembly upon solvation transforms the disordered structure of dry starch into an ordered, smecticlike lamellar system with pronounced crystallinity. Increasing the molecular weight or decreasing the hydrogen bonding capability of the solvent leads to an elevation of the time/temperature required for lamellar assembly. It should

be noted that the concentration of plasticizers used in this study was around 80–100%.

3.4. Paste and gel properties of HMT starches

3.4.1. Glycerol as a plasticizer

Pasting behavior is the most important characteristic of starch, in terms of its application. In this study, pasting profiles of native and HMT canna starches were investigated using RVA at 8% starch concentration. Fig. 2 shows the pasting profiles of HMT starches plasticized with glycerol; their corresponding pasting parameters and gel morphologies are shown in Supplementary Table 1 and Supplementary Fig. 2, respectively. Native starch displayed high viscosity, slight breakdown and high setback. The image of native starch gel taken at the end of the RVA experiment (Supplementary Fig. 2) revealed that the native canna starch granules were all completely broken. The blue-stained particles distributed throughout the gel were thought to be retrograded particles resulting mainly from the association of the dispersed amylose molecules. These kinds of particles were observed by Fanta, Felker, and Shogren (2002) in diluted solutions of jet-cooked corn starch; the solutions were allowed to slowly cool in an insulated Dewar flask and were recognized as spherocrystalline particles.

When canna starch was subjected to heat treatment using pure water (0% glycerol) as a plasticizer, the modified starch exhibited lower peak viscosity, breakdown and setback as compared with the native starch. Pasting temperature was also raised to a higher temperature. Higher stability of the HMT starch against heating and shearing is supported by the gel images shown in Supplementary Fig. 2. Although the granules were broken, their remnants were still able to hold together. Changes in the pasting profiles were more obvious when glycerol solutions were used instead of water. Increases in the concentration of glycerol solution from 1% to 3% and 5% resulted in a progressive decrease in paste viscosity. The higher stability of these starches could be attributed to a greater interaction of glycerol with starch polymers, when compared with water. For low glycerol concentrations (1–5%), the decrease in viscosity was in proportion to the increasing amounts of glycerol in starch granules (6.1, 7.7 and 9.8 mmol/100 g starch at 1, 3 and 5% glycerol solutions), indicating that with a higher glycerol content in the granules, more plasticization occurred. On the contrary, however, there was an increase in paste viscosity with

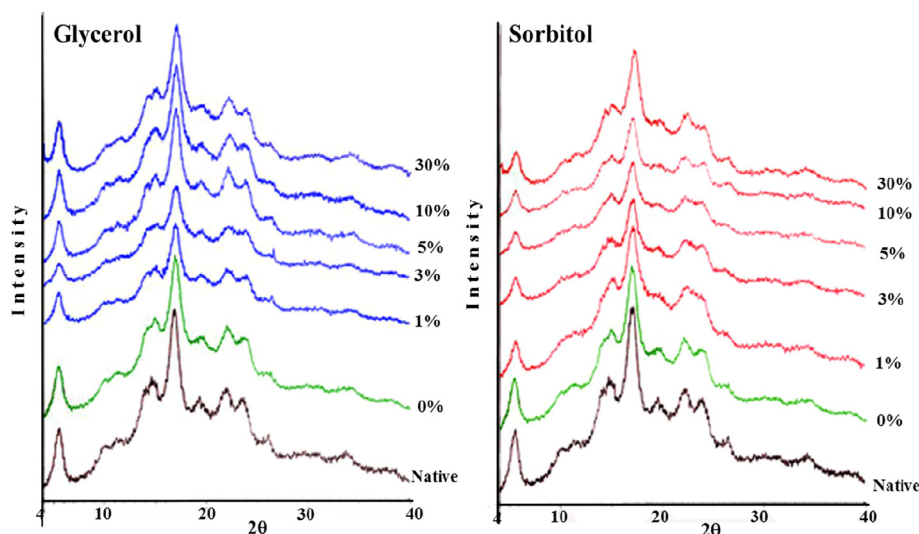


Fig. 1. X-ray diffractograms of native canna starch and HMT canna starches prepared with various concentrations of glycerol and sorbitol.

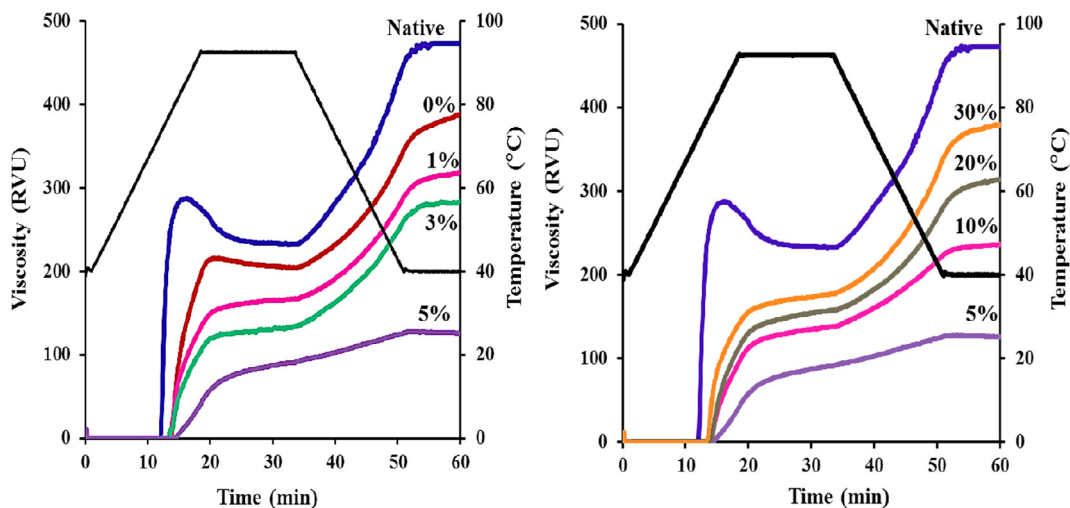


Fig. 2. Pasting profiles of native canna starch and HMT canna starches prepared with various concentrations of glycerol.

a glycerol concentration of 10%, which corresponded to 14.8 mmol glycerol in 100 g canna starch granules. Our expectation was that the effect of glycerol on paste viscosity would reach a maximum at a certain amount of glycerol, and thereafter would level off. However, the experimental results showed that further increases in glycerol concentration, to 20 and 30% (equivalent to 12.6 and 12.3 mmol/100 g starch), had an unanticipated effect on paste viscosity, i.e. viscosity at 30 min increased from 87.8 RVU for 5% glycerol solution to 134.6, 154.6 and 173.5 RVU for 10, 20 and 30% glycerol solutions, respectively. The question then arose as to why the viscosity increased in spite of the increasing amounts of glycerol in the granules. It was therefore postulated that at high glycerol concentrations, not only the interaction of glycerol with starch polymeric chains would occur, but other reactions/interactions would also happen concurrently during heat treatment. One possible interaction that can decrease the interaction of glycerol and starch chains is the self-interaction of glycerol molecules confined in starch granules either via H-bonding or the association of alkyl groups (Dashnau, Nucci, Sharp, & Vanderkooi, 2006), which consequently reduces the amount of glycerol available for interaction with starch chains.

3.4.2. Sorbitol as a plasticizer

A similar trend was observed when sorbitol was used as a plasticizer, although with a lesser effect on paste viscosity (Fig. 3). Viscosity of HMT starch plasticized with sorbitol decreased with increasing sorbitol concentration from 1% to 3% and 5%, respectively. However, further increases of sorbitol concentration to 10%, 20% and 30% resulted in corresponding increases in paste viscosity. The turning point observed beyond 5% sorbitol was the same as that which occurred with glycerol. The pasting profile of HMT starch plasticized with 30% sorbitol was very close to that of HMT starch plasticized with pure water (0% sorbitol), except that the final viscosity was higher (Supplementary Table 1). Morphologies of gels, as shown in Supplementary Fig. 3, were roughly in line with the pasting profiles, i.e. a higher degree of swelling and disruption and higher paste viscosity were observed. However, HMT starch plasticized with 30% sorbitol had a significantly higher setback viscosity compared with HMT starch plasticized with pure water (0% sorbitol); this reflected higher gel network formation, which was not evident from the gel morphologies. The gel morphology of HMT starch plasticized with 30% sorbitol was similar to that of HMT starch plasticized with pure water.

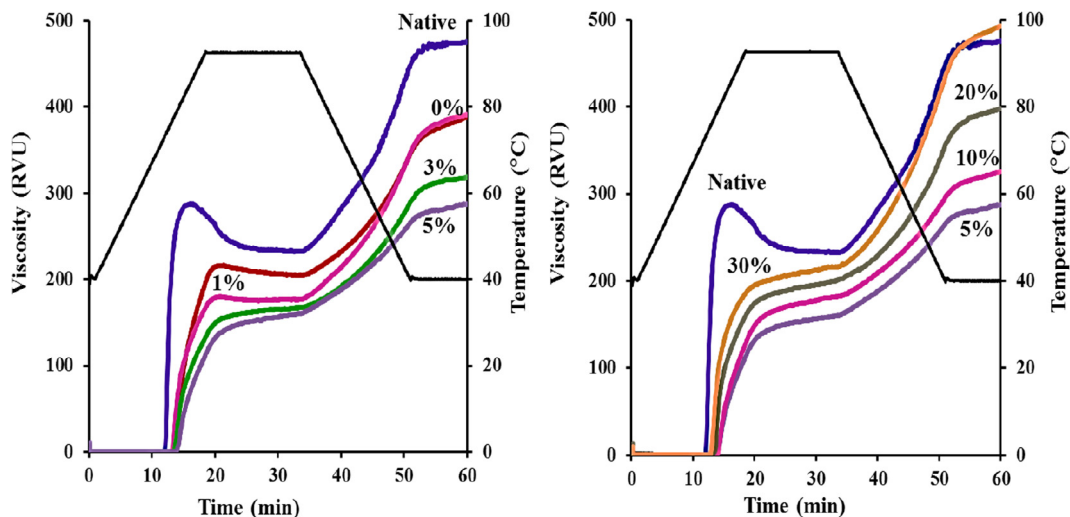


Fig. 3. Pasting profiles of native canna starch and HMT canna starches prepared with various concentrations of sorbitol.

3.4.3. Relationship of pasting properties and the amount of plasticizer in starch granules

The effect of plasticizers on the properties of starch has been extensively studied in starch film formation. The addition of plasticizers, e.g. polyols, affects film properties such as water vapor permeability (WVP), mechanical properties and glass transition temperature. Generally, WVP and elongation increase, while tensile strength and glass transition temperature decrease as the plasticizer content of the film increases (Arvanitoyannis, Psomiadou, & Nakayama, 1996; Gaudin, Lourdin, Le Botlan, Ilari, & Colonna, 1999; Lourdin, Bizot, & Colonna, 1997; Lourdin, Coignard, Bizot, & Colonna, 1997; Mathew & Dufresne, 2002). On the contrary, decreased elongation of polyol-plasticized starch films with increased plasticizer content has been reported for glycerol and sorbitol contents below 12% (Lourdin, Bizot, et al., 1997) and 27% (w/w of solids) (Gaudin et al., 1999), respectively. This unusual property is similar to the antiplasticization effect found in synthetic polymers and might be attributable to the increasing reorganization of starch polymeric chains, and therefore crystallinity, by the plasticizer. It is also possible that strong interaction occurs between the polymer and the plasticizer, producing a cross-linking effect at low concentrations (Lourdin, Bizot, et al., 1997). Zhang and Han (2010) reported that the addition of plasticizers at a low to intermediate concentration (10–20%) facilitated the formation of crystallites in starch films, leading to the antiplasticization phenomenon.

To produce starch film, starch in excess water (usually 2–5% solids) is gelatinized in the presence of plasticizers (around 5–50% w/w of solids). Starch granules are disrupted, and the added plasticizer interacts directly with the well-dispersed starch molecules. On the other hand, HMT starch is produced by heat treatment (at around 90–120 °C) of starch with limited moisture content (less than 30%) in the presence of water or other

plasticizers. Both products are similar in terms of the ingredients – starch polymeric chains, water, and added plasticizer – utilized during treatment. However, the environments for the interactions among the ingredients are substantially different; that is, interactions among the ingredients in starch film occur under conditions of thorough mixing, high dispersion of molecules and plenty of water, while interactions in HMT starch take place under conditions of limited water and much lower plasticizer content (<1.5% w/w starch). In addition, starch polymeric chains (amylose and amylopectin) are still packed in a semi-crystalline structure in intact granules. Plasticization in HMT starch is activated not only by the plasticizer but also by heating at high temperature. Therefore, the explanations of the effect of plasticizers on starch film behavior found in the literature, although they may be helpful, might not be entirely applicable for HMT starch.

A reduction in the paste viscosity of all HMT starches, as well as a decrease in granule disruption compared with native starch (as shown by microscopic images of starch gels), indicated toughening of starch granules as a result of the treatment. This behavior is similar to the antiplasticization effect that occurs in starch films. Róz, Carvalho, Gandini, and Curvelo (2006) suggested that the antiplasticization was caused by an increase in crystallinity. They proposed that plasticizers have two different effects on polymer materials: plasticization and crystallization. At low and intermediate concentration ranges of plasticizers, crystallization prevails over plasticization. The rationale for the crystallization by plasticizers is that they enable starch macromolecules to have increased mobility, which facilitates the packing of starch polymers into crystal lattices (Delville, Joly, Dole, & Bliard, 2003). In the present study, crystallinities of all HMT starches decreased more or less depending on different HMT conditions, as compared to the native starch (Supplementary Fig. 4). Therefore, crystallinity would not account for the toughening of HMT starches. Yet the change in crystallinity

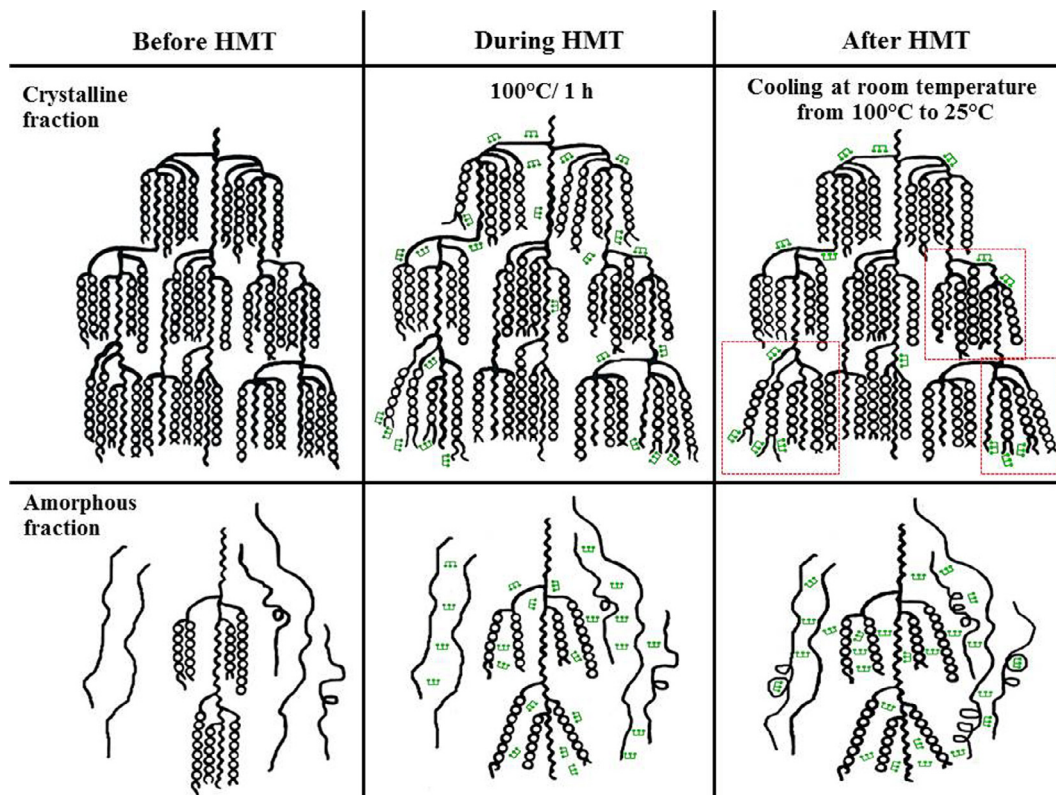


Fig. 4. Suggested schemes for changes in the molecular structure of canna starch during the process of heat–moisture treatment.

(decreased and increased) with treatment conditions implied a difference in interactions between the added plasticizers and starch chains at different plasticizer concentrations. A possible effect of plasticizers on crystalline domains of HMT starches is proposed in the schematic shown in Fig. 4. We supposed that glycerol/sorbitol interacted with chain segments of amylopectin located in amorphous lamellar regions (branching points) via H-bond formation, resulting in a slight movement of some of well-packed double helices and a consequent reduction in crystallinity. The greater influence of sorbitol on crystallinity, in spite of its lower content in starch granules as compared with glycerol, was likely due to the larger molecular size of sorbitol. However, the existing data from our study are not sufficient to explain why the crystallinity increased when the plasticizer concentration increased in the range of 5–30%.

Fig. 4 illustrates our idea on what can happen to starch granules during heat treatment and when they are cooled down to room temperature. Native starch granules consist of alternating layers of amorphous and semi-crystalline growth rings. The crystalline fraction accounts for 15–45% (Zobel, 1988), while the rest is amorphous in nature. The ordered side-chain polymers of amylopectin, which form double helices aligned along the radial axis (crystalline lamellae in a cluster model), are responsible for the crystalline fraction. During heat treatment, plasticization due to the presence of water and glycerol/sorbitol, together with thermal energy input, could increase the segmental mobility of some double helices, mainly as a result of interaction between the plasticizers and the loosely packed fractions (especially the branching segments) via the hydroxyl groups of plasticizers and starch molecules. The terminal segments of double helices might also be unwound into separate strands. When the granules have cooled down, the slightly displaced segments can relocate to their former position; however, some cannot return to their position prior to HMT. Therefore, HMT tends to decrease the crystallinity of starch. The magnitude of the change in crystallinity depends on the extent of the interaction between starch and plasticizer, and also the spacer resulting from these interactions. Thus, a larger-sized plasticizer such as sorbitol, although present in a lesser amount in starch granules, nevertheless had a greater effect on crystallinity reduction.

Crystallinity is believed to be one factor that determines the swellability of starch granules, i.e. a decrease in crystallinity would increase the degree of swelling. In this study, however, when crystallinity decreased swelling decreased as well. Thus, changes in the pasting characteristics of HMT starches (decreased paste viscosity and swelling) would mainly result from alterations of the amorphous fraction in both semi-crystalline and amorphous growth rings. These amorphous fractions include free amylose, amylose (or a segment of amylose) interacting with amylopectin, unordered side chains of amylopectin, and branched segments of amylopectin. The less-ordered structure of these fractions facilitates the access of plasticizers into these areas. Possible interactions of starch segments and plasticizers include: 1) hydrogen bond formation between hydroxyl groups of starch segments and plasticizers; 2) cross-bridging between adjacent starch segments via hydrogen bonding of plasticizers; and 3) inclusion complexing of starch segments and plasticizers. Strengthening of starch granules after HMT was most likely due to these newly generated H-bonds. Accordingly, higher amounts of glycerol, and consequently increased numbers of hydroxyl groups in starch granules, would have a greater effect on the paste viscosity of HMT starches as compared with sorbitol. Zeleznak and Hoseneý (1987) investigated the glass transition temperature (T_g) of wheat starch having different moisture contents, and found that the glass transition occurred below room temperature ($\approx 25^\circ\text{C}$) at moisture contents greater than 22%. Rindlava, Hulleman, and Gatenholma (1997) reported a T_g

of $75\text{--}95^\circ\text{C}$ with 13–15% moisture content for potato starch, and the T_g decreased linearly as the moisture content increased. The T_g of native rice starch with moisture content of 40% was reported to be -6.8°C (Chung, Lee, & Lim, 2002). The HMT in this study was performed on canna starch with a moisture content of 25% at 100°C . Therefore, the temperature during treatment would be much greater than the T_g of starch. At temperatures above the T_g , starch polymeric chains and/or segments of the polymers in the amorphous region have greater mobility and are able to re-orient to achieve their equilibrium conformations. The type and concentration of plasticizers would affect the T_g of the starch, and consequently the ultimate change in properties of the HMT starches. Therefore, measurement of the T_g of the native and modified canna starches in relation to these factors would help in understanding the phenomena occurring in the amorphous regions of starch granules.

4. Conclusion

Besides the type of plasticizer, as shown in our previous work, the present study revealed that the concentration of plasticizer (glycerol or sorbitol) was another important parameter that significantly affected the pasting properties of HMT canna starches. The amounts of glycerol/sorbitol in starch granules seemed to influence the magnitude of change in paste viscosity, but not in a linear proportion. The amount of plasticizer in starch granules was highest when the granules were soaked in 10% plasticizer solution (either glycerol or sorbitol); but the greatest reduction in paste viscosity was found in HMT starch soaked in 5% plasticizer solution. Strengthening of HMT starches could not be explained by the crystalline data; therefore, we suggest that bonding in the amorphous fraction could have a major effect on the pasting properties of HMT starch. The changes occurring in the amorphous fraction can possibly be verified by the comparative determination of iodine binding capacities of the native and modified starches, using wide-angle X-ray diffraction and/or spectrophotometry (K/S spectra). The K/S spectra are reflective of the amount and length of accessible flexible chains that can complex with iodine in the granule (Vamadevan, Hoover, Bertoft, & Seetharaman, 2014). Another approach in verifying the changes in the amorphous region is to determine the glass transition temperature (T_g) of the starches. The T_g reflects the physical state of the amorphous regions in starch granules.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.foodchem.2016.10.134>.

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