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Interaction between dry starch and plasticisers glycerol or ethylene glycol, measured by differential scanning calorimetry and solid state NMR spectroscopy

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Abstract

The interaction of crystalline amylose and of crystalline and amorphous amylopectin with the plasticisers glycerol or ethylene glycol in the absence of water was studied, by using differential scanning calorimetry (DSC) and solid state nuclear magnetic resonance (NMR) spectroscopy. Upon heating starch freshly mixed with plasticisers, a strong exothermal interaction enthalpy of $\Delta H \sim -35$ J/g was detected by DSC. At room temperature glycerol interacts mainly with the amorphous starch regions, the interaction taking 8 days to reach equilibrium. For ethylene glycol the interaction is faster, taking four days to reach equilibrium, and the rate is not affected by crystallinity. Ethylene glycol interacts in a more ordered manner with amorphous than with crystalline material, resulting in a narrower ethylene glycol cross-polarisation magic angle spinning (CP/MAS) signal when equilibrium is reached at room temperature. Upon heating, more glycerol or ethylene glycol is immobilised, but in a less ordered manner than upon storage at room temperature. This results in a more intense, but broader plasticiser CP/MAS signal upon heating. Interaction in a more ordered manner probably implies interaction with more of the hydroxy groups of the plasticiser. The polysaccharide mobility is increased more when the plasticiser interacts in a more ordered manner, as observed by small starch signals in HP/DEC spectra.

Keywords: Amylopectin; Amylose; Plasticisers; Ageing; Crystallinity; Differential scanning calorimetry; Solid state nuclear magnetic resonance

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1. Introduction

Starch is a cheap biopolymer that is totally biodegradable, ultimately to carbon dioxide and water. Starch is used as a natural food-ingredient and is a main energy provider in the human diet. Thermoplastic starch (TPS) materials are obtained from granular starch mixed with plasticisers to enable melting below the decomposition temperature. TPS is a complex system, since the structure depends mainly on variations in processing conditions (Forssell, Hulleman, Myllärinen, Moates, & Parker, 1999; Forssell, Mikkilä, Moates, & Parker, 1997; van Soest & Borger, 1997; Tharanathan, 1995). The ageing of starch based systems causes the embrittlement of starch plastics and the staling of bakery products (Gudmundsson, 1994; Van Soest, Hulleman, de Wit, & Vliegenthart, 1996b; Thiewes & Steeneken, 1997), leading to deterioration of the product. Plasticisers can be used to influence this ageing induced by retrogradation. For instance, in bread the degree of retrogradation is strongly reduced by the addition of monoglycerides, which interact with the initially amorphous amylopectin (Krog, Oleson, Toernaes, & Joensson, 1989). Van Soest, de Wit, Tournois and Vliegenthart (1994) showed that an increasing glycerol concentration in a waxy maize starch gel reduces the rate of retrogradation. The inhibiting effect of various saccharides on retrogradation has also repeatedly been reported (Bello-Pérez and Parades-López, 1995; Katsuta, Miura, & Nishimura, 1992a; Katsuta, Nishimura, & Miura, 1992b; Kohyama & Nishinari, 1991).

However, specific interactions between plasticiser and starch chains are difficult to elucidate. It is generally accepted that plasticisers lower the number of physical cross-links between starch chains, and consequently retard the rate of retrogradation (Jagannath, Jayaraman, Arya,

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& Somashekar, 1998). Recently, the interaction of glycerol and starch was investigated on a molecular level in the absence of water (Kruiskamp, Smits, van Soest, & Vliegenthart, 2001; Moates, Noel, Parker, & Ring, 2001; Smits, Hulleman, van Soest, Feil, & Vliegenthart, 1999; Smits et al., 2001). By using differential scanning calorimetry (DSC), a strong exothermal transition was found between 50 and 150 °C. Heat treatment gave rise to a strong starch–glycerol interaction. In the present study the interaction of the plasticisers glycerol and ethylene glycol with each of the starch polysaccharides is investigated. Focus is on dry systems in order to exclude influences of water. Solid state nuclear magnetic resonance spectroscopy (NMR) is used to investigate the material before and after heating above the exothermal transition. The time- and temperature-dependence of the interaction was studied by using DSC and solid state NMR spectroscopy.

2. Experimental

2.1. Sample preparation

Amylopectin obtained from granular potato starch, with a remainder of 5% amylose (Amylopectin-UG), and amylose (Amylose-V) were provided by Avebe (Foxhol, Netherlands). Glycerol ($\leq 0.1\%$ H_2O) was obtained from Fluka (Neu-Ulm, Germany) and ethylene glycol ($\leq 0.05\%$ H_2O) from Acros (Geel, Belgium).

The originally (semi-) crystalline amylopectin was fully gelatinised by stirring and heating to 90 °C a 10% dry weight amylopectin dispersion in de-ionised water for 1 h. Even though some crystallinity ($\sim 8\%$) is present, the gelatinised amylopectin will be referred to as amorphous amylopectin. Amylopectin (either crystalline or amorphous) and amylose (crystalline) were dried under reduced pressure in a vacuum-oven at 70 °C. The dried material ($< 3\%$ H_2O) was mixed manually under a nitrogen gas flow with glycerol or ethylene glycol. The plasticiser concentration was 4.3 mmol/g amylopectin for glycerol (corresponding to 29 wt%) and an equal molar amount for ethylene glycol (21 wt%). The samples were stored airtight. Samples exposed to heat treatment for the NMR analyses were heated for 30 min at 165 °C in a small, airtight container.

2.2. Analyses

The crystallinity of the starch samples at atmospheric humidity was examined with wide-angle X-ray scattering, using a Philips PC-APD diffractometer. The crystallinity index X_H is defined as the height of the crystalline diffraction at 17.3° relative to the total height of that peak measured from the baseline (van Soest, Tournois, de Wit, & Vliegenthart, 1995). For comparison, native potato starch ($X_H = 0.60$) has a crystallinity of approximately 25%.

DSC was performed on a Perkin–Elmer DSC7 robotic system. Samples were prepared in stainless steel 80 μl DSC-cups. They were heated from 20 to 180 °C at a rate of 10 °C/min.

Solid state NMR spectra were collected on a Bruker AMX 400 spectrometer operating at 100.63 MHz for ^{13}C. Samples were spun at the magic angle (54.7°) with respect to the static magnetic field. Carbon chemical shifts relative to tetramethylsilane (TMS) were determined from the spectra, using solid glycine at room temperature as an external reference. Samples were packed into 7-mm ceramic rotors and spun at 4 kHz. In ^{13}C cross-polarisation magic angle spinning (CP/MAS) experiments the cross polarisation time was set to 500 μs. In both ^{13}C CP/MAS and ^{13}C high power decoupling (HP/DEC) experiments carbon and proton 90° pulse lengths were 5 μs and the recycle delay was set to 4 s (Gidley, 1992).

3. Results

3.1. Differential scanning calorimetry

When the mixtures of amylose or amylopectin, either crystalline or amorphous, with glycerol or ethylene glycol were heated, a strong exothermal interaction enthalpy ($\Delta H \sim -35$ J/g) could be detected by DSC (Kruiskamp et al., 2001; Smits et al., 1999). The transition enthalpy is proportional to the amounts of glycerol or ethylene glycol added, suggesting that the plasticiser is responsible for the observed exothermic event. The process is irreversible, since reheating of the samples showed no exothermal enthalpy peak. Heat treatment gives rise to a strong starch–plasticiser interaction, most probably caused by H-bond formation. The peak temperature range was 95–110 °C for the amylopectin/glycerol mixtures, 95–105 °C for the amylose/glycerol mixtures, 70–85 °C for the amylopectin/ethylene glycol mixtures, and 65–75 °C for the amylose/ethylene glycol mixtures. In all cases the exothermal transition started well above room temperature. However, when stored for several days at room temperature, the transition enthalpy measured by DSC, decreased (Fig. 1). The decrease in enthalpy for the various mixtures is not accompanied by a significant shift in the interaction temperature.

The mixture of crystalline amylopectin ($X_H = 0.40$) with glycerol proved to be fairly stable in the sense that the interaction enthalpy decreased only gradually during storage at room temperature. After 30 days, a reduction of only 20–25% was observed. For amylose, which is also crystalline ($X_H = 0.35$), results are similar to crystalline amylopectin. After 30 days at room temperature, a reduction in the enthalpy of only 15–20% is found. For amorphous amylopectin ($X_H = 0.20$) the interaction enthalpy decreased to less than 5% of its initial value after 8 days of storage at

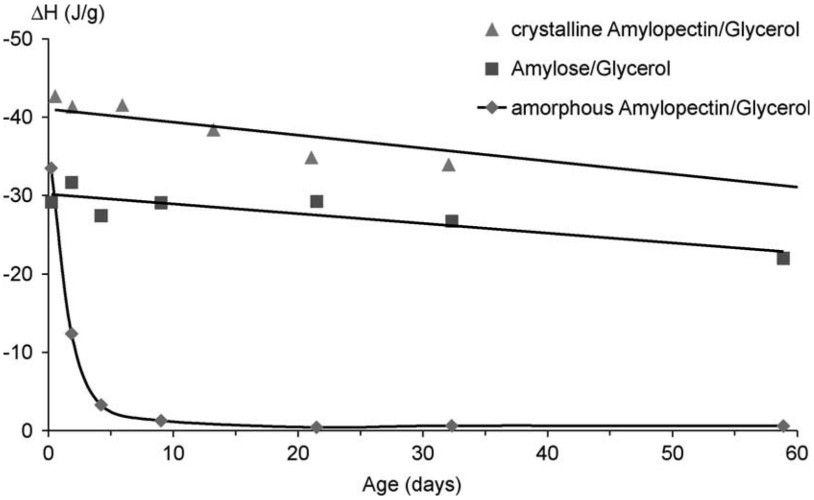

Fig. 1. Enthalpy of the exothermal interaction of dry mixtures of amylopectin (crystalline or amorphous) or crystalline amylose with glycerol, as a function of storage time at room temperature (20 °C).

room temperature. This indicates that the interaction is a kinetically controlled process.

For amylose and amylopectin (amorphous and crystalline) with ethylene glycol, the interaction enthalpy reduced to less than 5% of its initial value already after 4 days of storage at room temperature (Fig. 2). In contrast to glycerol, overall it appeared that the interaction at room temperature between starch and ethylene glycol was independent of the crystallinity of amylopectin. The decrease of interaction enthalpy during storage follows almost identical patterns for all mixtures. When comparing this interaction with that between amorphous amylopectin and glycerol, the interaction enthalpy decreases about twice as fast for ethylene glycol. Because of the smaller size of glycol, it is able to penetrate the starch chains more easily. And the crystallinity does not obstruct this penetration.

Directly after preparation of the mixtures there was a clear difference in behaviour between the amorphous and the crystalline mixtures. Ethylene glycol was readily absorbed, but the rate of this process appeared to be higher for amorphous amylopectin than for crystalline amylopectin or amylose. These different mixing characteristics may explain why the initial exothermal-enthalpy, measured directly after mixing, was higher for crystalline amylopectin than for amorphous amylopectin. The interaction probably started during the mixing period due to mechanical energy input, and the crystalline material is less penetrable. On exploring the influence of mixing on the interaction between crystalline amylopectin and ethylene glycol, it was observed that smaller sample sizes and longer mixing times resulted in a faster interaction. But for the results reported here, similar sample sizes and mixing times were used, in order to largely eliminate this effect.

The kinetic involvement of the starch–ethylene glycol interaction was explored by varying the temperature at which the samples were conditioned. At low temperatures,

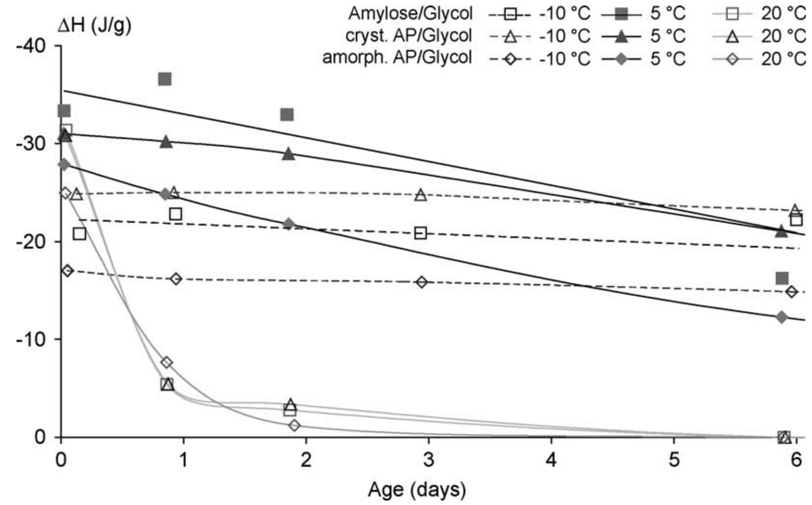

Fig. 2. Enthalpy of interaction between dry amylopectin (crystalline or amorphous) or crystalline amylose and ethylene glycol, as a function of storage time at − 10, 5 °C or room temperature (20 °C).

ethylene glycol molecules become less mobile, and behaviour similar to glycerol at room temperature is expected. In Fig. 2 the course of the interaction enthalpy during storage is shown at conditioning temperatures of − 10, 5 and 20 °C. When conditioned at 20 °C, the interaction takes place very quickly. After 4 days almost no interaction enthalpy was observed by DSC, implying that the interaction has mainly reached equilibrium. At 5 °C the interaction is much slower, taking approximately 17 days to reach equilibrium. The decrease in enthalpy per day of storage (slope in Fig. 2) is similar for the three mixtures. The variation in initial enthalpy value is probably caused by different energy input during sample mixing. When stored at − 10 °C, almost no decrease in interaction enthalpy is observed. It takes approximately 90 days of storage for amylose and amorphous amylopectin/ethylene glycol mixtures before the interaction has reached equilibrium. Because the initial enthalpy value was larger for crystalline amylopectin, the interaction process took longer to reach equilibrium. Also, at low temperatures no differences in the interaction processes between amylose and amylopectin, and between amorphous and crystalline amylopectin can be detected. The crystalline regions do not obstruct the ethylene glycol molecules from penetration.

3.2. Solid state nuclear magnetic resonance spectroscopy

For dry potato amylose or amylopectin mixed with glycerol, sharp peaks appeared in the HP/DEC spectrum, as depicted in Fig. 3a for amorphous amylopectin. Since mobile structures dominate the HP/DEC spectrum it was concluded that the glycerol was highly mobile. After heat treatment of the mixtures, the glycerol peaks in the HP/DEC spectrum became less intense and broader (Fig. 3b), the intensity diminishing to less than 5% of that of the fresh mixtures (Fig. 3a), indicating a decrease in glycerol mobility.

Weak starch signals are detectable in HP/DEC after heat treatment, which may indicate that the starch chains have become somewhat more mobile. In the CP/MAS spectrum, which displays more solid-like material, the glycerol peaks appeared alongside the starch signals after heat treatment (e.g. at $\delta = 63.4$ ppm), indicating the immobilisation of glycerol (Fig. 4) (Kruiskamp et al., 2001; Smits et al., 1999, 2001). A molecular interaction was realised between glycerol and starch.

From the DSC results it was concluded that the interaction also takes place upon storage at room temperature. Crystalline amylopectin and amylose showed similar behaviour, interacting more slowly than amorphous amylopectin. The plasticisers do not seem to discriminate between the two starch polysaccharides. Therefore, the NMR investigation is limited to crystalline and amorphous amylopectin. For crystalline amylopectin with glycerol, no changes in the NMR spectra were observed after several

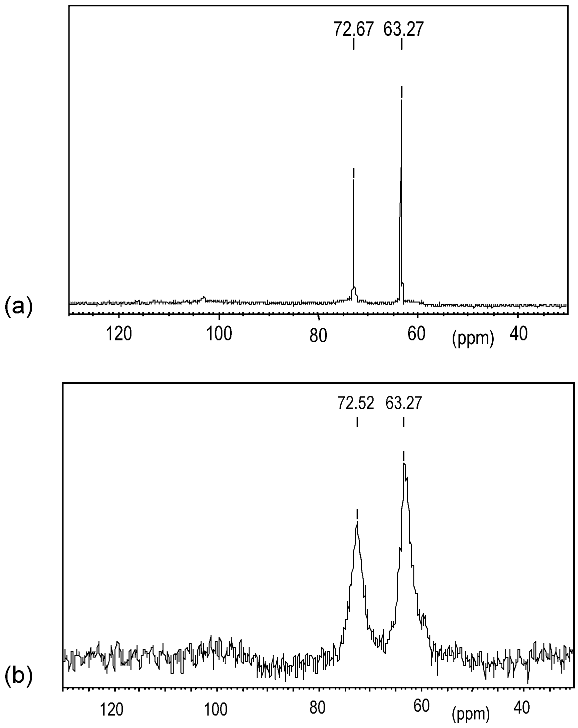

Fig. 3. ^{13}C HP/DEC NMR spectra of dry amorphous potato amylopectin with 29 wt% glycerol, freshly mixed (a), and after heating for 30 min at 165 °C (b).

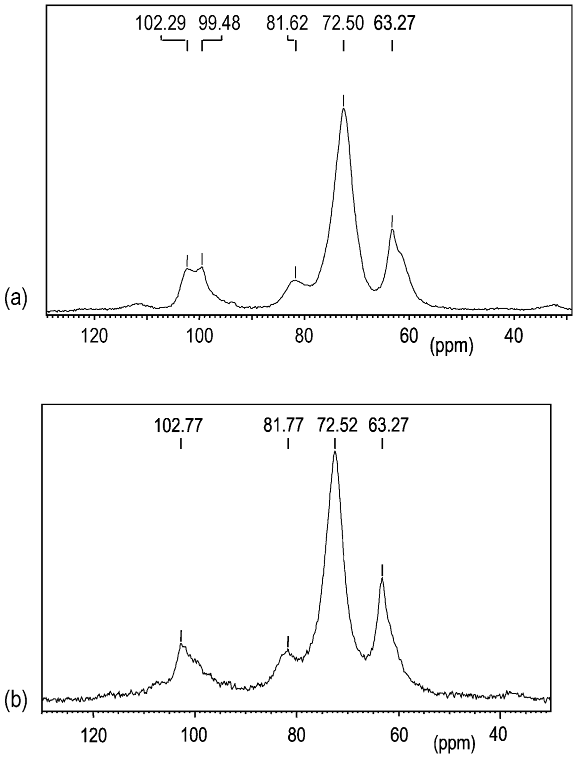

Fig. 4. ^{13}C CP/MAS NMR spectra of dry crystalline (a), and amorphous (b) amylopectin with 29 wt% glycerol, after heating for 30 min at 165 °C. Signals for amylopectin carbon C_1 at 102 ppm, C_4 at 82 ppm, $C_{2/3/5}$ at 72 ppm, and C_6 at 61 ppm. Signals for glycerol central carbon at 72.7 ppm and terminal carbons at 63.3 ppm.

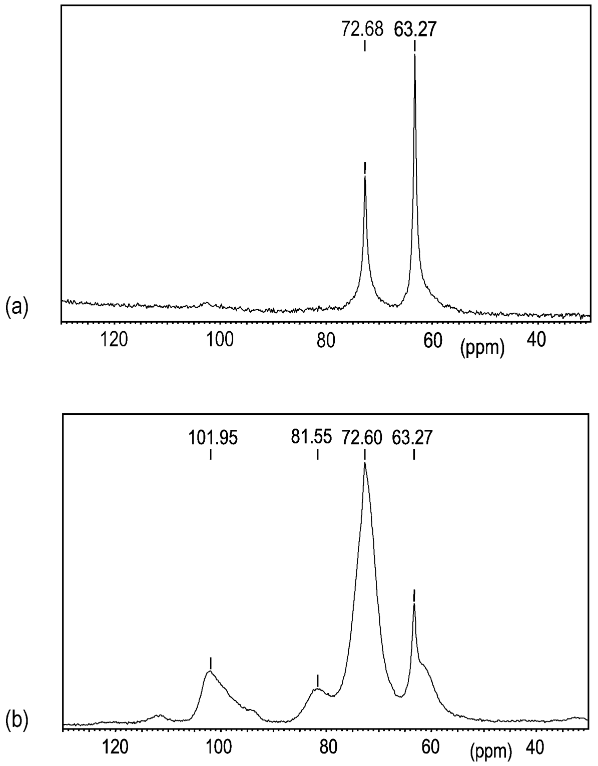

Fig. 5. ^{13}C HP/DEC (a) and CP/MAS (b) NMR spectra of dry amorphous amylopectin with glycerol (29 wt%), after 8 days of storage at room temperature.

days of storage at room temperature. However, as in agreement with the DSC results, for amorphous amylopectin with glycerol, a significant change was found in the NMR spectra after 8 days of storage at room temperature (Fig. 5).

The HP/DEC spectrum showed a considerable broadening and decrease in intensity of the glycerol peaks, as compared with freshly mixed material (Fig. 3a). Also, the glycerol signals appeared in the CP/MAS spectrum. The signal of the terminal glycerol carbons at 63.3 ppm was especially prominent, since the signal of the central glycerol carbon at 72.7 ppm overlapped largely with the amylopectin C2/C3/C5 signals. Comparing the spectra to those after heat treatment (Fig. 3b), the intensity-decrease and broadening of the glycerol signals in the HP/DEC spectrum was relatively small. Moreover, the glycerol signals in the CP/MAS spectrum were less intense but sharper after 8 days of conditioning at room temperature. After 8 days at room temperature, a smaller portion of the glycerol molecules is bound to amorphous amylopectin, explaining the less intense signals. But they are bound in a more ordered fashion than after heat treatment, resulting in a more uniform molecular environment, explaining the sharper signals.

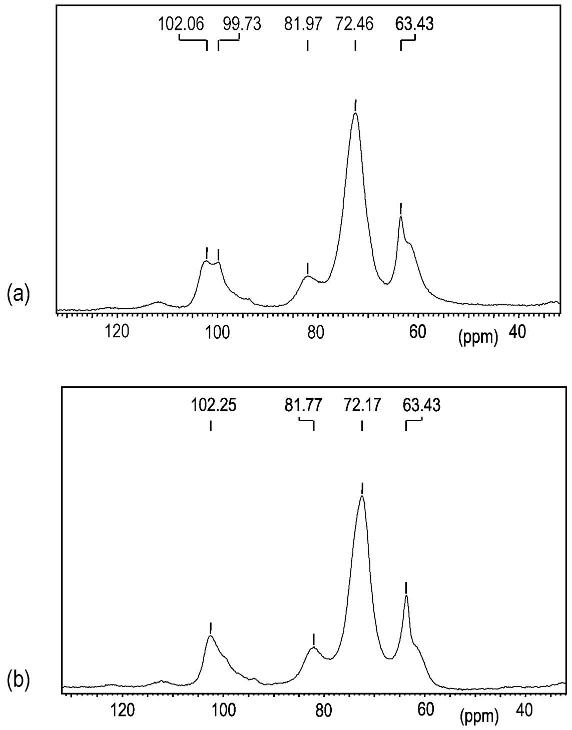

Fig. 6. ^{13}C CP/MAS NMR spectra of dry crystalline (a) and amorphous (b) amylopectin with ethylene glycol (21 wt%), after heating for 30 min at 165 °C. Signal for ethylene glycol carbons at 63.4 ppm.

According to the DSC results, ethylene glycol interacts rapidly with amylose as well as with crystalline or amorphous amylopectin. For mixtures of amylopectin (crystalline or amorphous) with ethylene glycol, in analogy with glycerol, the ethylene glycol signal appeared in the CP/MAS spectra after heat treatment (Fig. 6).

Also, after 5 days of storage at room temperature the CP/MAS spectrum showed an immobilisation of ethylene glycol (Fig. 7). The linewidths of the ethylene glycol signal (63.4 ppm) were about $1\frac{1}{2}$ times larger in the crystalline amylopectin mixture than in the amorphous amylopectin mixture. This may indicate that in crystalline amylopectin ethylene glycol is bound in a less ordered manner than in amorphous amylopectin. The ethylene glycol signal was considerably larger ($\sim 1\frac{1}{2}$ times higher) and about 2 times broader after heat treatment compared to storage at room temperature. The HP/DEC spectra supported this immobilisation of ethylene glycol, both after storage at room temperature and after heat treatment. In the HP/DEC spectra weak amylopectin signals are detected, which are $\sim 10\%$ stronger for amorphous than for crystalline amylopectin. Apparently a more ordered distribution of the plasticiser results in an increased mobility of the starch chains.

In order to probe the flexibility of the polysaccharide and the plasticiser in the mixtures, the CP/MAS contact time was varied. A different response of signal intensity to the contact time within a system, demonstrates that the components do not share a common relaxation pathway (^{1}H $T_{1}\rho$) (Foster, Ablett, McCann, & Gidley, 1996). A series of CP/MAS experiments was performed on dry amorphous amylopectin with glycerol after heat treatment, with a contact time of 100–3500 μs. The glycerol peak intensity,

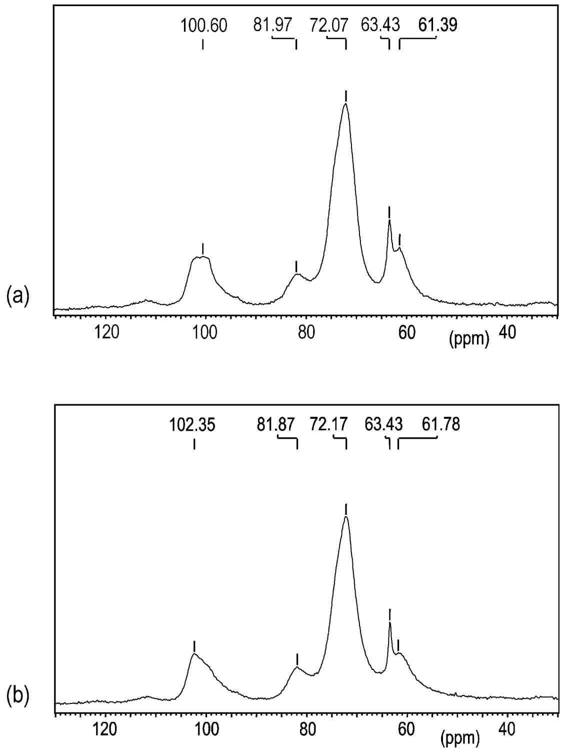

Fig. 7. ^{13}C CP/MAS NMR spectra of dry crystalline (a) and amorphous (b) amylopectin with ethylene glycol (21 wt%), after 5 days of storage at room temperature.

relative to the amylopectin C1 peak, is found to be linearly related to the contact time. It is possible that the intensities are averages of heterogeneous relaxation environments for the two components.

4. Discussion

These results showed that upon heating and upon storage at room temperature for several days, the plasticisers glycerol and ethylene glycol interact through H-bonding with crystalline amylose and crystalline and amorphous amylopectin. At room temperature, ethylene glycol interacts at a similar rate with crystalline amylose and crystalline and amorphous amylopectin. However, glycerol interacts slower with crystalline amylose and amylopectin, than with amorphous amylopectin. The interaction is similar for crystalline amylose and crystalline amylopectin, implying that the plasticiser does not discriminate between the two polysaccharides. This suggests that the interaction with amorphous amylose would be similar to the interaction with amorphous amylopectin. Because of the different rates of interaction of the mixtures at room temperature, this kinetically controlled interaction is mainly effective in the amorphous regions of starch polysaccharides, which allow glycerol to interact with the polymer chains more easily. According to DSC, the interaction between amorphous amylopectin and glycerol or ethylene glycol has reached equilibrium after 8 or 4 days of storage at room temperature, respectively. At room temperature, the crystalline structure largely prevents glycerol from interacting and obstructs ethylene glycol to interact in an ordered manner.

Heat treatment clearly immobilised the plasticisers more than storage at room temperature, although in a less ordered manner. At room temperature the interaction develops more gradually, which explains the difference in order. It may be, that upon storage at room temperature less plasticiser molecules are bound to the polysaccharide, but with more of the hydroxy groups of the plasticiser. Therefore, the plasticiser is bound in a more ordered fashion, but in total the plasticiser is immobilised less because less plasticiser molecules are bound. Weak amylopectin signals are detected in the HP/DEC spectra, that are stronger when the plasticiser is bound in a more ordered fashion. The plasticiser is better capable of mobilising polysaccharide chains when it interacts in a more ordered fashion. When interacting with more hydroxy groups per plasticiser molecule, locally it prevents hydrogen bonding between starch chains in a region of the size of the plasticiser molecule.

Solid state NMR CP/MAS contact time variation experiments showed that the glycerol signal relative to the amylopectin C1 peak is linearly related to the contact time. Highly mobile glycerol would not be detected with CP/MAS. If glycerol were tightly bound to the amylopectin chains, one would expect similar relaxation behaviour for both glycerol and amylopectin. This is not the case, which suggests that the amylopectin/glycerol interaction is relatively weak, or that the average of heterogeneous relaxation environments of glycerol is measured. The latter is the case, since the appearance of the plasticiser in the CP/MAS spectrum suggests that the interaction is strong enough to immobilise plasticiser molecules. The amylopectin/glycerol interaction is involved in some sort of chemical exchange mechanism, in which the glycerol is continuously moving through the mixture and interacting alternately with different sites, as is common for plasticisers.

When processing starch, the ingredients are often premixed. The time dependent interaction with plasticisers affects the molecular mobility and therewith the material properties. In freshly mixed material, starch chains are less flexible and the material may even be glassy or brittle by lack of plasticisation. Due to the interaction with plasticisers starch mobility increases, viscosity reduces, and the material behaves like a rubber. Variation in the time between premixing and processing may influence for example flow properties or resistance to shear, thereby influencing processing conditions.

Recently, Perry and Donald (2000) reported SAXS and WAXS X-ray diffraction results on mixtures of granular potato or waxy maize starch and high concentrations (55–75%) of glycerol, ethylene glycol or 1,4-butanediol. They suggest that the exothermal transition that has been reported previously (Kruiskamp et al., 2001; Smits et al., 1999; van Soest, Bezemer, de Wit, & Vliegenthart, 1996a), results from crystallinity development, comparable with the side by side alignment of helices during annealing (Tester & Debon, 2000). Crystallinity development was also suggested previously, based on dielectric relaxation spectroscopy results. But at the plasticiser concentrations used in this paper (21–29%), which are much lower than those used by Perry and Donald (2000), crystallinity could not be observed with WAXS (Smits et al., 2001). The results in this paper show that the exothermal transition results from interaction development between starch and plasticiser. Because this interaction occurs, the polysaccharide is plasticised and the helices are enabled to align. Crystal lattice perfection thus is a secondary process.

5. Conclusions

It has been previously reported, that upon heating granular or recrystallised starch with glycerol or ethylene glycol in the absence of water, an exothermal transition could be detected by DSC (van Soest et al., 1996a). This was suggested to be caused by an interaction between starch and the plasticisers. The present study shows that upon heating this interaction takes place between glycerol or ethylene glycol with amylose as well as with amylopectin, and that it is independent of the presence of crystallinity of the polysaccharide. The interaction is likely to be caused by H-bond formation.

Furthermore, it was found that a similar interaction takes place during storage at room temperature for several days. Thus, the interaction process is kinetically controlled. This affects the molecular mobility and may influence for example flow properties and resistance to shear when premixing ingredients prior to processing starch. At room temperature, the plasticiser glycerol mainly interacts with the amorphous regions of dry amylopectin or amylose. Ethylene glycol, a smaller molecule, interacts more easily with dry starch polysaccharides than glycerol. This interaction occurs regardless of the presence of crystallinity. Partial immobilisation of the plasticisers is the result of these interactions. NMR results showed that heat treatment of the starch/plasticiser mixtures generally causes further immobilisation of both glycerol and ethylene glycol. Heat treatment also causes broadening of the plasticiser signal, as compared with storage at room temperature. It seems, that upon heating more plasticiser molecules interact, but with less of the hydroxy groups of the plasticiser, than upon storage at room temperature. Starch chains are mobilised more when the plasticiser interacts in a more ordered manner, which occurs when the interaction develops at room temperature. Therefore, when ingredients are premixed, this may facilitate processing. The fact that crystal lattice perfection may result from the mobilisation of starch chains, however, may aggravate processing when the premixed ingredients are stored at room temperature for some days. Premixing is generally accepted as a tool for mixing ingredients without further implications. However, the present study shows that the time between premixing and processing can influence material properties and processing conditions.

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