Journal of Industrial Microbiology & Biotechnology (2001) 26, 90–93
© 2001 Nature Publishing Group 1367-5435/01 $17.00
www.nature.com/jim

The influence of plasticiser on molecular organisation in dry amylopectin measured by differential scanning calorimetry and solid state nuclear magnetic resonance spectroscopy

PH Kruiskamp[1], ALM Smits[1], JJG van Soest[2] and JFG Vliegenthart[1]

[1]*Bijvoet Centre for Biomolecular Research, Utrecht University, P.O. Box 80075, NL-3508 TB Utrecht, Netherlands;*
[2]*Agrotechnological Research Institute ATO, P.O. Box 17, 6700 AA Wageningen, Netherlands*

The interaction of crystalline and amorphous amylopectin with the plasticisers glycerol and ethylene glycol in the absence of water was studied with differential scanning calorimetry (DSC) and solid state NMR. At room temperature glycerol interacts mainly with the amorphous regions, while for ethylene glycol the amylopectin crystallinity does not effect the interaction. After heating the mixtures, an additional immobilisation of the plasticiser occurs. *Journal of Industrial Microbiology & Biotechnology* (2001) **26,** 90–93.

Keywords: amylopectin; plasticisers; ageing; crystallinity; DSC; solid state NMR

Introduction

Starch is a cheap biopolymer that is totally biodegradable forming 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 [3,10,12]. The ageing of starch-based systems causes embrittlement of starch plastics and staling of bakery products [11,15], and thus causes 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 [8]. Van Soest *et al.* [13] showed that an increasing glycerol concentration in a waxy maize starch gel reduces the retrogradation rate. The inhibiting effect of various saccharides on retrogradation has also repeatedly been reported [1][5–7].

However, specific interactions between plasticiser and starch chains are hard to understand. It is generally accepted that plasticisers lower the number of cross-links between starch chains, and consequently retard the rate of retrogradation [4]. Recently, the interaction of glycerol and starch was investigated on a molecular scale in the absence of water [9]. Using differential scanning calorimetry (DSC) a strong exothermal transition is found between 50°C and 150°C. Solid state nuclear magnetic resonance spectroscopy (NMR) was used to investigate the material before and after heating above the exothermal transition. It was concluded that the heat treatment gives rise to a strong starch–glycerol interaction.

In this preliminary report the amylopectin–plasticiser interaction is investigated in more detail, using both glycerol and ethylene glycol as plasticiser. The time- and temperature-dependence of the interaction was studied with DSC and solid state NMR.

Materials and methods

Sample preparation

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

The originally crystalline amylopectin was gelatinised by stirring and heating to 90°C a 10% dry weight amylopectin dispersion in deionised water until the amylopectin was fully gelatinised after 1 h. Amylopectin (gelatinised or crystalline) was 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 29 wt.% for glycerol (corresponding to 4.3 mmol glycerol/g amylopectin) and an equal molar amount for ethylene glycol (21 wt.%). The samples were stored airtight. Samples exposed to heat treatment were heated for 30 min at 165°C in a small, airtight container.

Analyses

The crystallinity of the amylopectin samples were 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 [14].

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

Solid state NMR spectra were collected in 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.

Correspondence: PH Kruiskamp, Research Scientist, Bijvoet Centre for Biomolecular Research, Utrecht University, P.O. Box 80075, NL-3508 TB Utrecht, Netherlands
Received 9 February 2000; accepted 2 May 2000

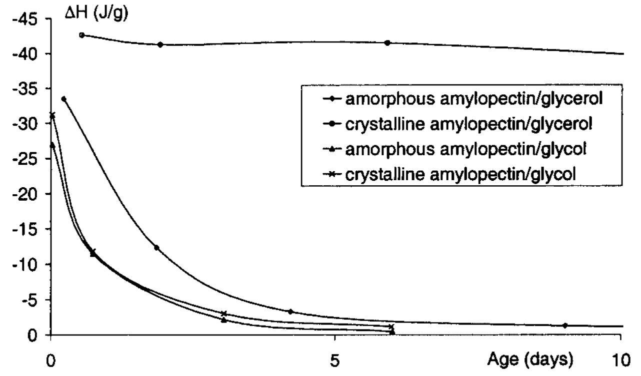

Figure 1 Enthalpy of the exothermal interaction for dry mixtures of amylopectin (crystalline and amorphous) with glycerol and glycol, as a function of storage time at room temperature after mixing.

Carbon chemical shifts relative to tetramethylsilane (TMS) were determined form the spectra, using solid glycine at room temperature as 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 and in both ^{13}C CP/MAS and ^{13}C high-power decoupling (HP/DEC) experiments the recycle delay was set to 4 s [2].

Results and discussion

Differential scanning calorimetry

When the mixtures of amylopectin with glycerol or ethylene glycol were heated with DSC, a strong exothermal interaction enthalpy was detected [9]. This process was irreversible, since reheating of the samples showed no exothermal enthalpy peak. The peak temperature was 100–105°C for the amylopectin/glycerol mixtures, and 75–80°C for the amylopectin/ethylene glycol mixtures. In both cases the exothermal transition started well above room temperature. However, when stored at room temperature, the intensity of the transition enthalpy decreased (Figure 1). The decrease in enthalpy for the various mixtures is not accompanied by a significant shift in the interaction temperature.

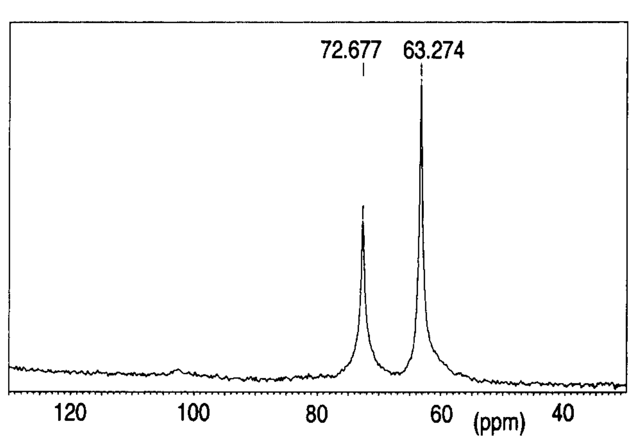

Figure 2 ^{13}C HP/DEC spectrum of physically mixed dry amorphous amylopectin with glycerol (29 wt.%) after 8 days of storage at room temperature.

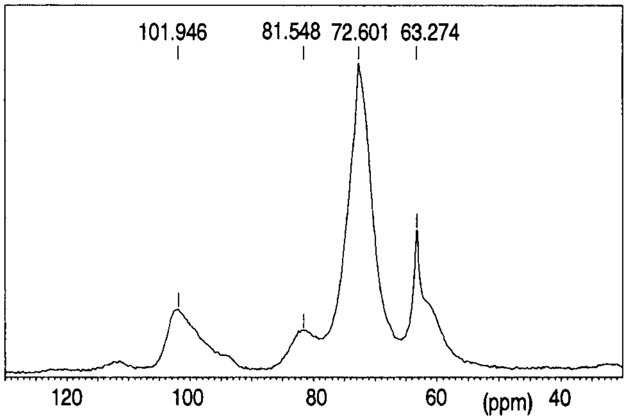

Figure 3 ^{13}C CP/MAS spectrum of physically mixed dry amorphous amylopectin with glycerol (29 wt.%) after 8 days of storage at room temperature.

The mixture of crystalline amylopectin (X_H=0.51) with glycerol proved to be fairly stable in the sense that the interaction enthalpy decreased only gradually with time. After 30 days, a reduction of only 20–25% was observed. For amorphous amylopectin (X_H=0.24) the interaction enthalpy decreased to less than 5% of its initial value after 8 days of storage at room temperature. This indicates that the interaction is a kinetically controlled process. Evidently, because of the different rates of interaction for the two mixtures at room temperature, this kinetically controlled interaction is mainly effective in the amorphous regions of amylopectin, which allowed glycerol to interact with the amylopectin chains more easily.

For amylopectin (amorphous and crystalline) with ethylene glycol, the interaction enthalpy reduced to approximately 5% of its initial value after 5 days of storage at room temperature. Directly after the preparation of the mixture there was a clear difference in behaviour of the two mixtures. In both cases ethylene glycol was readily absorbed by amylopectin, but the rate of this process seemed to be higher for the amorphous amylopectin than for the crystalline amylopectin. These different mixing characteristics might explain why initially the exothermal enthalpy was slightly higher for the crystalline amylopectin than for the amorphous

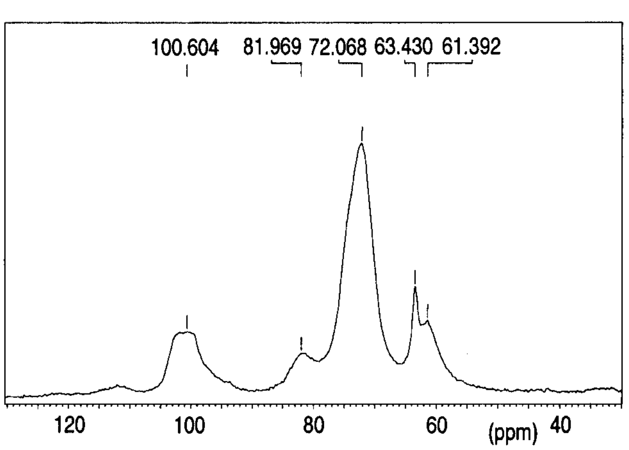

Figure 4 ^{13}C CP/MAS spectrum of physically mixed dry crystalline amylopectin with glycol (21 wt.%) after 5 days of storage at room temperature.

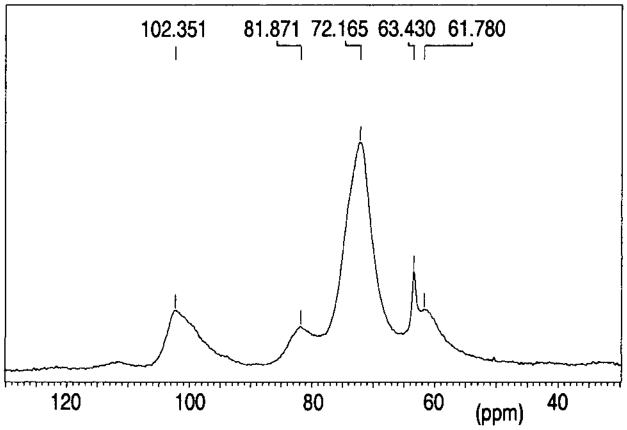

Figure 5 ^{13}C CP/MAS spectrum of physically mixed dry amorphous amylopectin with glycol (21 wt.%) after 5 days of storage at room temperature.

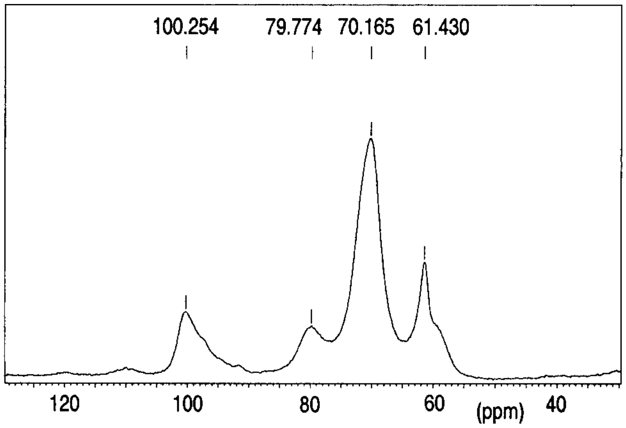

Figure 7 ^{13}C CP/MAS spectrum of physically mixed dry amorphous amylopectin with glycol (21 wt.%) after heating for 30 minutes at 165°C.

amylopectin, since the interaction probably already started during the mixing period. However, overall it appeared that the interaction between amylopectin and glycol at room temperature was independent of the crystallinity of amylopectin. The decrease of interaction enthalpy in time follows an almost identical pattern for both mixtures (Figure 1).

Solid state nuclear magnetic resonance

Smits *et al.* [9] showed that for dry potato starch mixed with glycerol, sharp peaks appeared in the HP/DEC spectrum. Since mobile structures dominate the HP/DEC spectrum it was concluded that glycerol was highly mobile. After heat treatment of the mixtures, the glycerol peaks in the HP/DEC spectrum became less intense and broader, indicating a decrease in glycerol flexibility. In the CP/MAS spectrum, which displays more solid-like material, the glycerol peaks appeared alongside the starch signals after heat treatment, indicating the immobilisation of glycerol [9].

For the amylopectin/glycerol mixtures measured shortly after mixing and after heat treatment, similar results to those of Smits *et al.* [9] were found. For crystalline amylopectin with glycerol, no changes in the NMR spectra were observed after several days of storage at room temperature. For amorphous amylopectin with glycerol, however, a significant change was found in the NMR spectra after 8 days of storage at room temperature. The HP/DEC spectrum (Figure 2) showed a considerable broadening of the glycerol peaks. The glycerol signals also appeared in the CP/MAS spectrum (Figure 3), especially the glycerol signal at 63.3 ppm was quite prominent. The signal at 72.7 ppm overlapped largely with the amylopectin C2/C3/C5 signal. Compared to after heat treatment [9], however, the intensity decrease and broadening of the glycerol signals in the HP/DEC spectrum was relatively small, and the glycerol signals in the CP/MAS spectrum were somewhat less intense and sharper after 8 days of storage at room temperature.

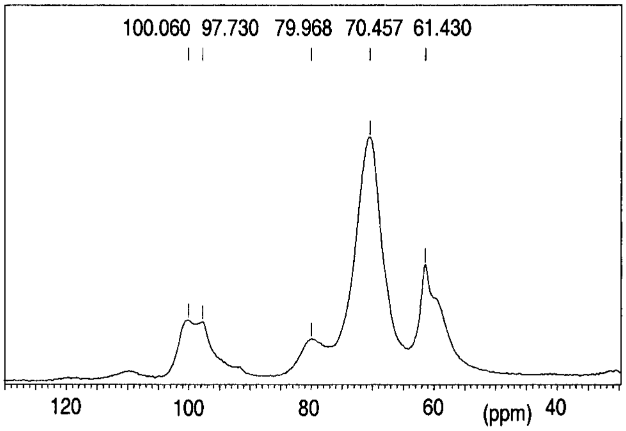

Figure 6 ^{13}C CP/MAS spectrum of physically mixed dry crystalline amylopectin with glycol (21 wt.%) after heating for 30 min at 165°C.

For mixtures of amylopectin (crystalline and amorphous) with ethylene glycol the CP/MAS spectrum showed an immobilisation of ethylene glycol after 5 days of storage at room temperature (Figures 4 and 5). The linewidth of the ethylene glycol signal (61.4 ppm) was slightly larger in the crystalline amylopectin mixture than in the amorphous amylopectin mixture. After heat treatment, ethylene glycol also appeared in the CP/MAS spectra (Figures 6 and 7). The ethylene glycol signal was considerably larger and broader 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 (data not shown).

These results showed that after storage at room temperature, glycerol mainly interacts with amorphous amylopectin, while glycol interacts with both crystalline and amorphous amylopectin. The slightly broader ethylene glycol signal for the crystalline amylopectin mixture compared to the amorphous amylopectin mixture after 5 days of storage indicates that in crystalline amylopectin the ethylene glycol is somewhat less ordered. Apparently the crystalline structure obstructs ethylene glycol to conform to its most favourable conformation. Heat treatment clearly immobilised the plasticisers more than storage at room temperature, although in a less ordered manner. According to DSC, however, the interaction between amorphous amylopectin and glycerol is almost complete after 8 days, and the interaction between amylopectin and ethylene glycol after 5 days of storage at room temperature. Therefore another process has taken place after heating the sample, causing the plasticisers to become immobilised.

Until now it is not clear what immobilises the plasticiser after heat treatment in the mixtures in which the interaction enthalpy as measured by DSC has disappeared after storage at room temperature. It might be that the plasticiser is involved in some kind of exchange mechanism, thereby lowering its overall mobility. Further research will be necessary to understand this mechanism.

Conclusions

Upon storage at room temperature, the plasticiser glycerol interacts mainly with the amorphous regions of dry amylopectin. Ethylene glycol, a smaller molecule, interacts more easily with dry amylopectin, regardless of the presence of crystalline amylopectin. Partial immobilisation of the plasticiser is the result of this interaction. NMR results showed that heat treatment of the amylopectin/plasticiser mixtures generally causes further immobilisation of both glycerol and ethylene glycol. The nature of this extra immobilisation is not yet clear, since no additional interaction enthalpy could be measured with DSC.

Acknowledgements

We thank ATO for the use of the NMR and DSC facilities, and we thank Technology Foundation STW for their financial support (Project UCH.4952).

References

1 Bello-Pérez LA and O Parades-López. 1995. Effects of solutes on retrogradation of stored starches and amylopectins: a calorimetric study. *Starch/Stärke* 47: 83–86.
2 Gidley MJ. 1992. High-resolution solid-state NMR of food materials. *Trends Food Sci Technol* 3: 231–236.
3 Gudmundsson M. 1994. Retrogradation of starch and the role of its components. *Thermochim Acta* 246: 329–341.
4 Jagannath JH, KS Jayaraman, SS Arya and R Somashekar. 1998. Differential scanning calorimetry and wide-angle X-ray scattering studies of bread staling. *J Appl Polym Sci* 67: 1597–1603.
5 Katsuta K, M Miura and A Nishimura. 1992. Kinetic treatment for rheological properties and effects of saccharides on retrogradation of rice starch gels. *Food Hydrocolloids* 6: 187–198.
6 Katsuta K, A Nishimura and M Miura. 1992. Effects of saccharides on stabilities of rice starch gels: 1. Monosaccharides and disaccharides. *Food Hydrocolloids* 6: 387–398.
7 Kohyama K and K Nishinari. 1991. Effect of soluble sugars on gelatinization and retrogradation of sweet potato starch. *J Agric Food Chem* 39: 1406–1410.
8 Krog N, SK Oleson, H Toernaes and T Joensson. 1989. Retrogradation of the starch fraction in wheat bread. *Cereal Foods World* 34: 281–285.
9 Smits ALM, SHD Hulleman, JJG van Soest, H Feil and JFG Vliegenthart. 1999. The influence of polyols on the molecular organization in starch-based plastics. *Polym Adv Technol* 10: 570–573.
10 Tharanathan RN. 1995. Starch – the polysaccharide of high abundance and usefulness. *J Sci Ind Res* 54: 452–458.
11 Thiewes HJ and PAM Steeneken. 1997. The glass transition and the sub Tg endotherm of amorphous and native potato starch at low moisture content. *Carbohydr Polym* 32: 123–130.
12 van Soest JJG and DB Borger. 1997. Structure and properties of compression molded thermoplastic starch materials from normal and high amylose maize starches. *J Appl Polym Sci* 64: 631–644.
13 van Soest JJG, D de Wit, H Tournois and JFG Vliegenthart. 1994. The influence of glycerol on structural changes in waxy maize starch as studied by Fourier transform infra-red spectroscopy. *Polymer* 35: 4722–4727.
14 van Soest JJG, H Tournois, D de Wit and JFG Vliegenthart. 1995. Short-range structure in (partially) crystalline potato starch determined with attenuated total reflectance Fourier-transform IR spectroscopy. *Carbohydr Res* 279: 201–214.
15 van Soest JJG, SHD Hulleman, D de Wit and JFG Vliegenthart. 1996. Changes in the mechanical properties of thermoplastic potato starch in relation with changes in B-type crystallinity. *Carbohydr Polym* 29: 225–232.