From Helical Starch Inclusion Complexes to Supramolecular Starch Assemblies

B. Conde-Petit, J. Nuessli, C. Heinemann and F. Escher

ETH Zurich, Swiss Federal Institute of Technology,
Institute of Food Science and Nutrition,
CH-8092 Zurich, Switzerland, beatrice.conde@ilw.agrl.ethz.ch

ABSTRACT

The present contribution reports on the structural features of aqueous starch dispersions as induced by starch complexation with small ligands. Complexation refers to the physical binding of suitable ligands by starch, where the ligand induces the helication of linear segments of starch. The ligands are non-covalently bound to starch, in particular to the linear starch polymer amylose, by inclusion into the helical cavity or between the helices. Potato starch dispersions were complexed with lactones and terpenes, and the structural properties of complexes were assessed by amperometric iodine titration, differential scanning calorimetry (DSC), wide-angle X-ray diffraction, rheology and microscopy. The strong tendency of starch helices to associate promoted the formation of supramolecular amylose structures. Depending on the complexation kinetics and the type of ligand, starch-ligand complexation led to the formation of a partly crystalline network gel or to spherulitic crystallization.

Keywords: starch, amylose, inclusion complexes, starch network, spherulites

1 INTRODUCTION

Native starch is composed of two immiscible polymers, the essentially linear amylose and the branched amylopectin. In neutral aqueous solution amylose has the characteristics of a random coil, but at the same time it exhibits some helical nature. Amylose readily forms complexes with suitable guest molecules [1-7]. Complexation refers to a non-covalent binding of small molecules to starch where the presence of suitable guest molecules induces the formation of starch helices with an overall diameter of approximately 13.5 Å and an inner channel diameter of around 5.4 Å [8]. The helical channel is hydrophobic and is suited to accommodate lipophilic molecules of appropriate dimensions. Linear aliphatic chains are generally included in the helical cavity, but for some bulky molecules an inclusion between the starch helices is also conceivable as shown schematically in figure 1. The formation of helical inclusion complexes is primarily a property of amylose, while the long external branches of amylopectin are thought to have a limited ability to form complexes. Among the molecules capable of forming inclusion complexes are iodine, which is used for analytical purposes, a number of emulsifiers, fatty acids, alcohols, aldehydes, lactones and terpenes. An important feature of native cereal starches is that a small fraction of amylose is complexed with endogenous lipids [9]. The size of the helical channel can to a certain extent be adapted to the dimensions of the ligand. Linear ligands generally induce a helix with six glucose residues per turn, while for bulky molecules like naphthol helices with eight glucose units per turn were found. The binding affinity of ligands to starch and the binding capacity varies greatly depending on the properties of the guest molecule [3]. Linear ligands exhibit a higher affinity for starch than cyclic molecules like monoterpenes. On the other hand, the binding capacity of cyclic molecules is higher that that of linear ligands. For high amylose starches a maximal guest molecule content of 5 to 10 % has been reported [10]. Positive cooperativity is a further characteristic of amylose inclusion complexation which means that binding of one ligand enhances the binding affinity of the second ligand [3].

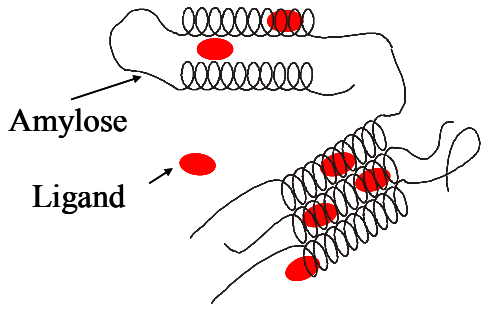

Figure 1: Schematic presentation of amylose-ligand complexes with inclusion of the ligand in the helical cavity or between the helices.

The present contribution reports on the structural properties of starch complexes at different length scales. Native potato starch, which was dispersed in water by hydrothermal treatment, was used for the experiments. This starch is composed of 77 % of amylopectin and 23 % of amylose and is free of endogenous amylose-lipid complexes. Different lactones and terpenes were selected as ligands. These compounds are flavoring agents in food, and from a practical point of view starch-flavor interactions are of interest in connection with flavor retention and release. The flavor compounds can also be viewed as model ligands for studying complexation induced structures that are relevant for all structure related properties.

2 EXPERIMENTAL

2.1 Preparation of complexed starch

Aqueous potato starch dispersions at a concentration of 2 g/100 g db (dry base) were prepared by heating native starch suspensions at 95 °C for 30 min. Thereafter, the complexing agents were mixed with the starch dispersion at 95°C (for all ligands except lactones) or 25 °C (for lactones) for 20 sec and stored at 25 °C until further analysis. The following complexing ligands were added at concentrations between 0 and 200 mmol/mol: γ-nonalactone, γ-decalactone, γ-dodecalactone, δ-decalactone, δ-dodecalactone, decanal, fenchone and geraniol. The molecular structure of selected ligands is presented in figure 2. Samples of potato starch complexed γ-nonalactone were also treated with α-amylase isolated from hog pancreas at an activity of 50U/g dry starch and a temperature of 25 °C.

2.2 Characterization of starch complexes

The extent of starch complexation was assessed by amperometric iodine titration as described in [7]. The voltage of polarization was set to 140 mV. 1 ml of 1mol/L HCl was added to a 30 g sample and titrated with a 0.005 mol/L iodine solution at a rate of 1ml/min. The amount of bound iodine was evaluated graphically and expressed as iodine binding capacity (IBC).

The type of crystalline packing was assessed by wide–angle X-ray diffraction on freeze dried powders. The measurements were carried out in the transmission mode on a powder diffractometer (Siemens Kristalloflex D500, D-Karlsruhe) using a CuK radiation (1.54 Å) with 35 mA and 40 kV.

The melting behavior of complexed starch was measured with differential scanning calorimetry (DSC). The samples were freeze-dried and rehydrated to 30 g dry matter/100 g. Samples of 40 mg were weighed into pressure pans (Perkin Elmer Ltd. Norwalk, CT) and transferred to the DSC instrument (2920 DSC, TA instruments, USA) and measured between 4 and 120°C with a heating rate of 5 °C/min.

Spherulitic crystallization of starch as induced by complexation was followed by light microscopy. A drop of sample was placed on a microscope slide and observed with an Axioplan photomicroscope (Zeiss Ltd, D-Oberkochen) in the polarized light and phase contrast modes.

The viscoelastic properties of complexed starch were followed by small amplitude oscillatoray shear measurements using a stress controlled rheometer (Carri-Med CCSL 100, GB Surrey) with a cone-plate geometry at a frequency of 1 Hz and a temperature of 25 °C.

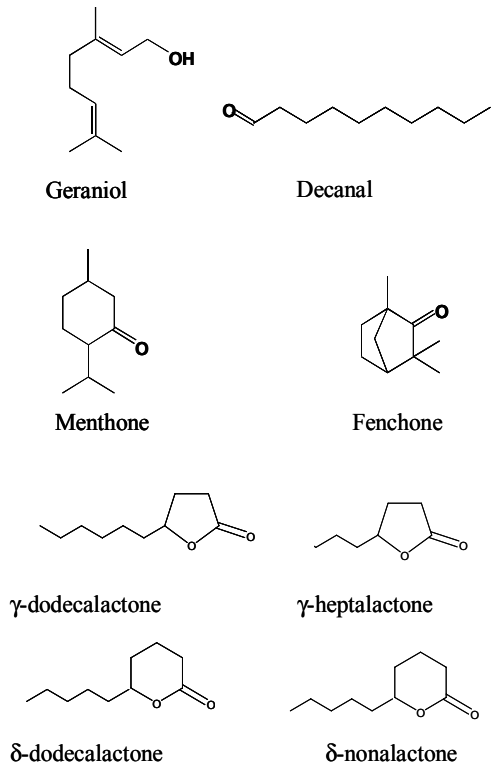

Figure 2: Molecular structure of selected ligands that form complexes with starch.

3 RESULTS AND DISCUSSION

Wide-angle X-ray diffractograms of starch with decanal presented reflections at 7.5, 13 and 20 degrees which are characteristic for Vh type amylose helices with six glucose units per turn (Fig. 3). By analogy to fatty acids it can be assumed that the linear chain of the ligand is entrapped in the helix cavity [5]. The complexation of lactones leads to complexes with the same type of crystalline packing (Vh) as decanal [7].

The diffractograms of starch complexes with geraniol, thymol and fenchone with reflections at 7, 12 and 18.4 ° (Fig. 3) correspond to a six-fold helix, but with a larger space between the helices [11]. The exact location of the ligand for this type of complexes is a matter of debate, and intra- and interhelical starch-ligand associations have been proposed [12]. It is conceivable that in the hydrated form the bulky ligands are included in the helix, and that only upon crystallization of the complex the ligand is excluded form the helix to be accommodated between the helices [13]. Overall, the x-ray diffractograms show rather low intensities and broad reflections indicating low crystallinity and small crystallites of the complexes (Fig. 3).

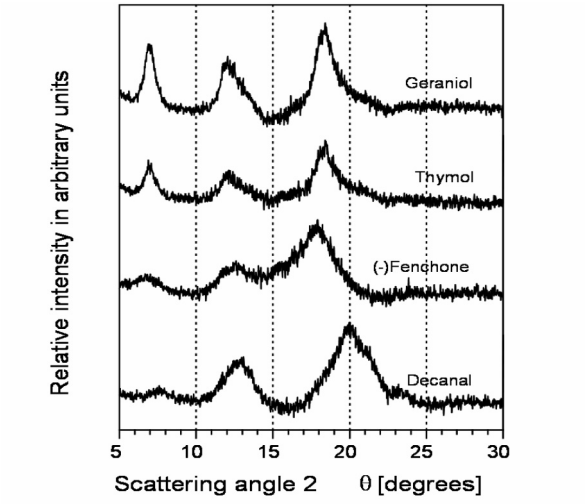

Figure 3: Wide-angle X-ray diffractograms of potato starch complexed with different ligands

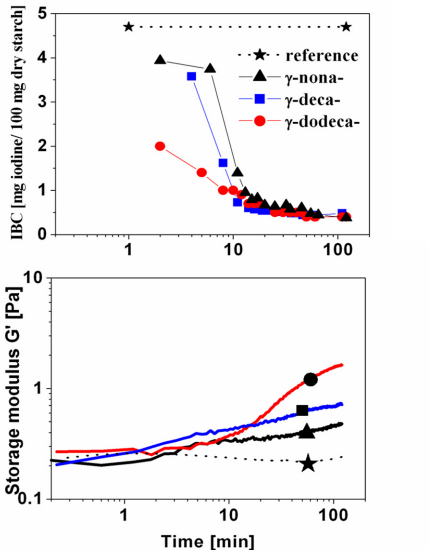

Figure 4: Influence of complexation with different lactones on the iodine binding capacity (IBC) and storage modulus (G') of potato starch dispersions.

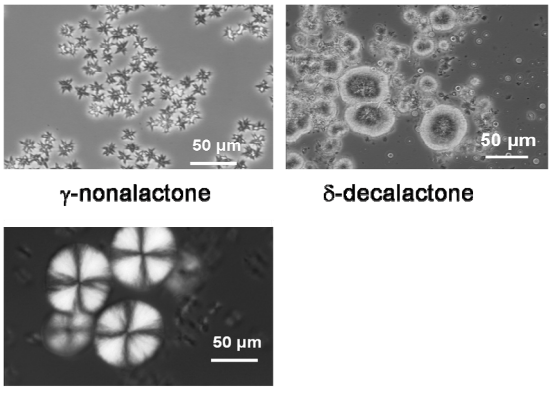

Figure 5: Light micrographs of amylose spherulites formed by complexation of starch with different ligands.

Starch complexation can also be followed by amperometric iodine titration, and a reduced iodine binding capacity is interpreted as complexation (Fig. 4). Experiments with homologous series of lactones showed that the complexation rate increases with the chain length of the ligand. High complexation rates and extensive complexation as found for δ-dodecalactone promoted the gelation of the starch dispersion. This was manifested by an increase of the storage modulus G'. The complexation of starch with lactones with shorter chain length led to a slight increase of G'. The complexation induced gelation of starch was also found with monoglycerides (emulsifiers) [14] and some terpenes like fenchone [15]. The gelation of starch is due to the coil-to-helix transition of amylose which promotes the aggregation of helical amylose segments resulting in a partly crystalline network. In contrast, low complexation rates promote spherulitic crystallization of amylose complexes (Fig. 5) which favor solid-liquid bulk phase separation of starch dispersions.

The different colloidal phenomena, namely the formation of network gels and the self-assembly into well organized supramolecular structures are to a great extent kinetically controlled and can be influenced by the complexation conditions and the type of ligand (Fig. 6). If the spontaneous amylose/amylopectin phase separation progresses faster than amylose aggregation, the formation of a kinetically stabilized amylose network is promoted. On the other hand, spherulitic crystallization is favored if the continuity of the amylose phase falls below a critical level before extensive amylose complexation is reached [16].

Finally, the structural reorganization of supramolecular amylose-γ-nonalactone assemblies upon enzymatic degradation by α-amylase at rather low enzyme activity was investigated. The structural changes were followed by iodine titration, DSC and light microscopy and are summarized in figure 7. The degradation of starch at the molecular level led to a decrease of the iodine binding capacity, and the rate of starch degradation was lower for complexed starch compared to the reference.

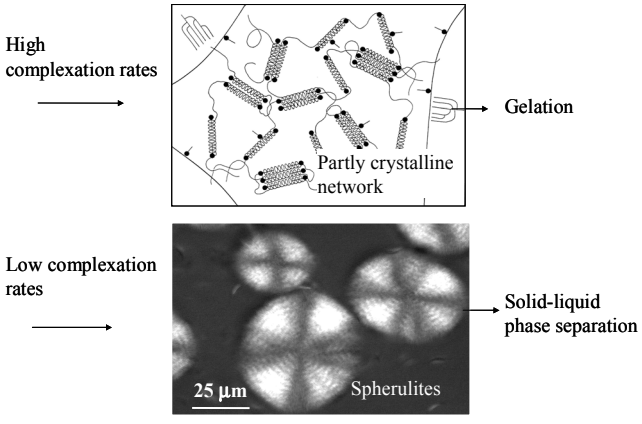

Figure 6: Overview of supramolecular assemblies of amylose as induced by inclusion complexation in aqueous starch dispersion

In the micrometer range, the disintegration of the spherulites could by seen by microscopy, although remnants of the supramolecular assemblies could still be recognized after an incubation time of 24 h. Interestingly, the melting temperature and the melting enthalpy of amylose-γ-nonalactone complexes increased upon enzymatic treatment. These results indicate that at low α-amylase activity the complexes are not completely degraded. By analogy to amylose-lipid complexes it can be assumed that the ordered helical segments are less susceptible toward enzymatic degradation than the amorphous regions [17]. The DSC results suggest that a partial degradation of starch contributes to a higher mobility of the polymer which, in turn, contributes to the formation of more stable crystallites at the expense of less stable ones. This enzymatic annealing of the complexes leads to an increased thermal stability of the remaining complexes and at the same time to a partial disassembly of the supramolecular structure [18].

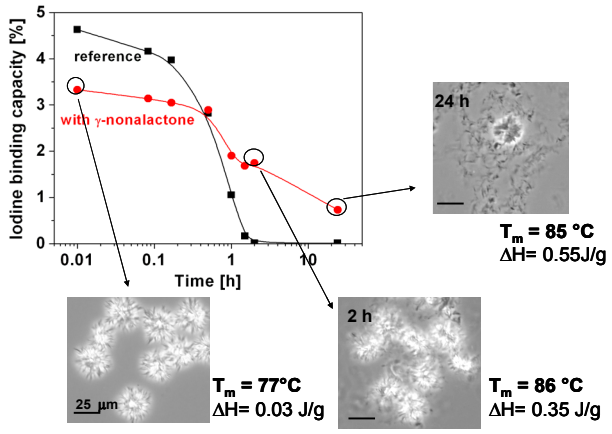

Figure 7: Structural changes of potato starch dispersions complexed with γ-nonalactone (50 mmol/mol starch) upon incubation with hog pancreas α-amylase at 25 °C.

4 CONCLUSIONS

The specific interaction between starch and small molecules capable of inducing the formation of helical amylose complexes in the nanometer range promotes the formation of supramolecular starch structures. The formation of amylose complexes can be viewed as an interaction that drives amylose out of solution since the helical segments have a strong tendency to associate. A variety of supramolecular structures can by generated depending on the ligand and the complexation kinetics. The colloidal phenomena span the range form partly crystalline amylose networks to spherulites of sizes of up to 100 µm. The practical benefit of this molecular encapsulation could be the control of all properties related to structural organization such as rheological behavior, diffusion phenomena or controlled release of the guest molecules.

REFERENCES

[1] T. Kuge and K. Takeo, Agricultural and Biological Chemistry 32, 1231, 1968.

[2] A.C. Eliasson and N. Krog, Journal of Cereal Science 3, 239, 1985.

[3] M. Rutschmann and J. Solms, LWT Food Science and Technology 23, 70, 1990.

[4] C.G. Biliaderis and H.D. Senevirante, Carbohydrate Polymers 13, 185, 1990.

[5] M.C. Godet, V. Tran, M.M. Delage and A. Buléon, International Journal of Biological Macromolecules 15, 11, 1993.

[6] J. Nuessli, B. Conde-Petit and F. Escher, Food Hydrocolloids 11, 27, 1997.

[7] C. Heinemann, B. Conde-Petit, J. Nuessli and F. Escher, Journal of Agricultural and Food Chemistry 49, 1370, 2001.

[8] S. Immel and F.W. Lichtenthaler, Starch/Stärke 52, 1, 2000.

[9] W.R. Morrison, R.V. Law and C.E. Snape, Journal of Cereal Science 18, 107, 1993.

[10] G. Wulff, G. Avgenaki and M.S.P. Guzman, Journal of Cereal Science 41, 239, 2005.

[11] J. Nuessli, J.-L. Puteaux, P. Le Bail and A. Buléon, International Journal of Biological Macromolecules 33, 227, 2003.

[12] C. Rondeau-Mouro, P. Le Bail and A. Buléon, International Journal of Biological Macromolecules 34, 309, 2004.

[13] B. Conde-Petit, F. Escher and J. Nuessli, Trends in Food Science and Technology, 2006 (in press).

[14] B. Conde-Petit and F, Escher, Food Hydrocolloid 6, 223, 1992.

[15] J. Nuessli, B. Conde-Petit, U.R. Trommsdorff and F. Escher, Carbohydrate Polymers 28, 167, 1995.

[16] C. Heinemann, F. Escher and B. Conde-Petit, Carbohydrate Polymers 51, 159, 2003.

[17] H.D. Senevirante and C.G. Biliaderis, Journal of Cereal Science 13, 129, 1991.

[18] C. Heinemann, M. Zinsli, A. Renggli, F. Escher, B. Conde-Petit, LWT Food Science and Technology 38, 885, 2005.