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Structural features of starch–lactone inclusion complexes in aqueous potato starch dispersions: the role of amylose and amylopectin

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Abstract

Starch, in particular the linear amylose, is able to form inclusion complexes with a wide spectrum of ligand molecules, among them flavor compounds such as lactones. The structural properties of potato starch dispersions with lactones were followed with oscillatory measurements, amperometric iodine titration, X-ray diffraction measurements, and light microscopy. The complexation of starch influences the structural properties of starch dispersions at different length scales. At the macroscopic level, the unfavourable interaction of amylose–lactone complexes with water promotes gelation or phase separation, and amylose–lactone complexation leads to spherulitic crystallization. Amylopectin–lactone interactions are thought to contribute to the long-term behavior of starch dispersions. Thereby, the colloidal properties of starch dispersion and spherulite morphology are determined by the type of lactone and the kinetics of starch complexation.

Keywords: Starch; Amylose; Lactone; Spherulite; Amylopectin

1. Introduction

Starch consists of two biopolymers, the linear amylose and the branched amylopectin packed in partially crystalline granules. Most starches contain about 20–35% amylose. Heating of starch in water leads to swelling of the granules, the extent of starch granule solubilization and granule disintegration depending on the processing conditions (Conde-Petit, Nuessli, Handschin, & Escher, 1998). Dispersed or gelatinized starch is a ternary system composed of two immiscible polymers, amylose and amylopectin, and water, the latter being a poor solvent for starch, in particular for the amylose.

The average conformation of amylose in aqueous neutral solution may be viewed as a highly disordered coil involving many discernible sequences of short-range helical structures that are irregular and labile (Braga et al., 1985). The addition of complexing ligands, for instance iodine, fatty acids, emulsifiers or different flavor compounds to aqueous starch dispersions induces the formation of single amylose helices, which display a V type pattern as assessed by X-ray diffraction (Yamashita, 1965; Yamashita & Hirai, 1966; Yamashita & Monobe, 1971). Complexing ligands with a linear structure are thought to be included in the helical cavity of amylose (Godet, Tran, Delage, & Buléon, 1993). For ligands with a cyclic structure such as thymol, a localization of the ligand between the helices in a crystal is also conceivable (Helbert, 1994). Although the complexing ligands interact primarily with the amylose, their interaction with amylopectin is not excluded. Gudmundsson and Eliasson (1990) already found a phase transition for pure amylopectin/emulsifier systems with differential scanning calorimetry (DSC) that indicates interactions of emulsifiers with amylopectin. Nuessli, Handschin, Conde-Petit, and Escher (2000) postulated that interactions between emulsifiers and amylopectin side chains of potato starch contribute to the development of a network in aqueous amylopectin potato starch dispersions.

The complexation of starch with suitable ligands has an influence on the colloidal properties of starch dispersions. The water solubility of amylose is further reduced as a consequence of complexation since the formation of extended helical domains reduces the configurational entropy of the polymer. The result of the interaction between complexed amylose and water is microphase separation as manifested by the appearance of turbidity and changes of the rheological properties that range from bulk phase separation to gelation (Conde-Petit, & Escher, 1992; Heinemann, Conde-Petit, Nuessli, & Escher, 2001;

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Nüssli, 1998). At the microscopic level, the complexation of amylose with suitable ligands can result in the formation of partly crystalline structures, namely spherulites (Davies, Miller, & Procter, 1980; Fanta, Felker, & Shogren, 2002; Schoch, 1942). Such supramolecular structures are polycrystalline and consist of stacks of many lamellar crystals, each crystal being separated by a thin, amorphous interlayer.

The aim of the present study was to investigate the influence of starch–lactone complexation on the supramolecular structure and on the viscoelastic properties of potato starch dispersions. Lactones are important flavor compounds that are found in butter and in fruits like apricot, peach and coconut. Lactones are cyclic esters whose molecular structure is characterized by a five- or six-membered ring and a linear chain. Fig. 1 shows the molecular structure of γ-deca- and δ-decalactone. In a previous publication we showed that lactones are suitable complexing agents for starch (Heinemann et al., 2001). The present study focuses on the influence of time on the extent of starch complexation and related structural changes at the microscopic and macroscopic level. Starch–lactone interactions were followed by rheological measurements, amperometric iodine titration, light microscopy and wide angle X-ray diffraction measurements. To better understand the role of amylose–lactone and amylopectin–lactone interactions in potato starch dispersion, experiments were carried out with conventional potato starch with an amylose content of about 25% and amylopectin potato starch which is almost amylose free.

2. Materials and methods

2.1. Material

Native potato starch was obtained from Blattmann and Co., CH-Wädenswil, and native amylopectin potato starch was obtained from Avebe, NL-Veendam. The ligands γ-nonalactone, γ-decalactone, γ-dodecalactone, δ-decalactone and δ-dodecalactone were supplied by Fluka, CH-Buchs.

2.2. Sample preparation

Starch dispersions at a concentration of 2 g dry starch/100 g dispersion were prepared by heating native starch suspensions in cans in a retort at 1 bar overpressure (121 °C) for 30 min. Thereafter, the dispersions were cooled to 30 °C. The lactones were added to the starch dispersions at concentrations ranging from 0 to 200 mmol/mol glucose. Ligand concentration was based on the molecular weight of anhydroglucose ($C_6H_{10}O_5$) instead of an average molecular weight of starch. The flavor substances were weighed into jars and starch dispersion at 30 °C was added. The jars were closed and the mixture was shaken for about 20 s and the samples were aged at 25 °C.

Lactones also act as fungicide and, therefore, starch dispersions complexed with lactones are not exposed to microbiological spoilage over a period of several weeks. To prevent mould growth in starch dispersion without lactones, the reference was aged in the sealed cans in which they had been autoclaved.

2.3. Iodine binding capacity

The iodine binding capacity (IBC) was determined by amperometric titration using a Polarizer E585, Potentiograph E567 and Dosimat 655 from Methrom (CH-Herisau). The voltage of polarization was set to 140 mV and the attenuation of the polarizer to 5 mA. A 30 g sample containing 100 mg starch (S_{tot}), 1 ml 1 mol/l HCl and deionized water was titrated with 0.005 mol/l iodine solution (Titrisol, Merck) at a titration rate of 1 ml/min. The dispersion was constantly stirred during titration. The amount of bound iodine (I_b) was evaluated graphically as described by Holló and Szejtli (1956). The IBC and the complexation degree C were calculated as follows. A potato starch dispersion without lactone addition (IBC_{ref}) served as reference.

$$\mathrm{IBC} = \frac{I_b}{S_{tot}} \times 100 \qquad (\text{mg iodine}/100\ \text{mg dry starch})$$

$$C = \frac{\mathrm{IBC}_{ref} - \mathrm{IBC}}{\mathrm{IBC}_{ref}} \times 100 \qquad (\%)$$

2.4. Rheological measurements

The viscoelastic properties of starch dispersions were determined by oscillatory measurements using a cone and plate geometry (∅ 6 cm, angle 1°59′, gap 55 μm) using a stress controlled rheometer (Carri-Med CSL 100, GB-Surrey).

To follow the short time aging behavior (0–2 h), the starch dispersion was transferred to the rheometer immediately after lactone addition. The storage (G') and loss modulus (G'') were followed during 2 h at a temperature of 25 °C ± 0.1 °C, a frequency of 1 Hz and a stress of 0.02 Pa which was within the linear viscoelastic range. For the investigation of the long term behavior (1–28 d), the starch–lactone dispersions were aged in jars (reference in cans) and samples were removed at regular intervals. The moduli G' and G'' were determined at 1 Hz and 25 °C in the linear viscoelastic region (between 0.002 and 0.2 Pa). Finally, the frequency dependence of G' and G'' of potato starch dispersions was determined between 0.01 and 1 Hz.

2.5. Light microscopy

A drop of potato starch dispersion was transferred onto a microscope slide. Thereafter, the sample was covered with a

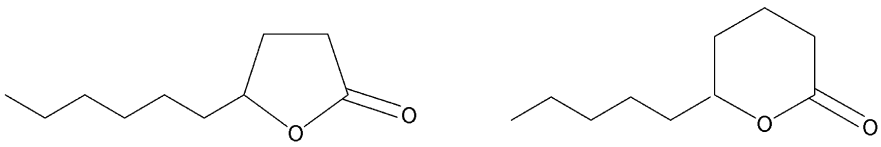

Fig. 1. Molecular structure of γ-decalactone (left) and δ-decalactone (right).

cover glass before examining it under the light microscope (Zeiss Ltd, D-Oberkochen) in the polarized light and phase contrast modes.

2.6. Wide angle X-ray diffraction measurements

For X-ray diffraction measurements the samples were frozen in jars in a freezer at −25 °C and subsequently freeze dried (Sécfroid, CH-Lausanne). The moisture content of the freeze dried samples was adjusted to 10–15 g/100 g moist sample by keeping the freeze dried powder over water for 90 min in a closed vessel. Thereafter, the samples were compressed into tablets of about 1 mm thickness and a diameter of 13 mm. The tablets were fixed on a sample holder. The measurements were carried out in the transmission mode on a powder diffractometer (Siemens Kristalloflex D500, D-Karlsruhe) using Cu Kα radiation (1.54 Å) with 35 mA and 40 kV. A divergence slit of 2 mm and a receiving slit of 1° were chosen. The relative intensity was recorded in a scattering angle range (2θ) of 4–30° with a scintillation counter at a scanning speed of 0.02°/min.

3. Results and discussion

3.1. Rheological properties of aqueous potato starch dispersions complexed with lactones

The influence of starch–lactone complexation on the properties of aqueous potato starch dispersion was followed by amperometric iodine titration and dynamic rheological measurements during an aging period of 2 h. Lactones were added at saturation concentration, the criterion being the complexation of amylose to a degree of ≥90% within 24 h as assessed by amperometric iodine titration (Heinemann et al., 2001). The saturation concentration of lactones for starch complexation depends on the length of the hydrocarbon chain, lactones with shorter linear chain requiring higher lactone concentrations. The results for potato starch dispersions complexed with 200 mmol γ-nonalactone/mol glucose, 50 mmol γ-decalactone/mol glucose, 50 mmol γ-dodecalactone/mol glucose and a lactone-free reference are presented in Fig. 2a–d.

The IBC of the reference potato starch dispersion was 4.7 mg iodine/100 mg dry starch which corresponds to an amylose content of 24 g/100 g dry starch, if an IBC of 19.5 mg iodine/100 mg dry starch is assumed for pure amylose (Banks & Greenwood, 1975). The IBC of the reference did not change during an aging period of 2 h indicating that the amylose did not aggregate. Likewise, the rheological properties of the reference starch dispersion did not show changes during the investigated time period. The addition of lactones induced complexation of amylose as indicated by the decrease of the IBC. In the first minutes the complexation rate was highest for γ-dodecalactone, followed by γ-decalactone and γ-nonalactone. After 10 min all starch dispersions with lactones presented an IBC of <1 mg iodine/100 mg dry starch. While the reference potato starch dispersion was transparent, the addition of lactones induced turbidity, which indicates that starch aggregation occurs.

Starch–lactone interactions led to rheological changes of the starch dispersions. Generally, an increase of the storage modulus G' was found, the crossover of storage and loss modulus marking the transition from a liquid-like to a solid-like behavior (Tung & Dynes, 1982). Although the $G'-G''$ crossover may be a function of frequency, it gives an estimate of the gelation time. As proposed by Curcio, Gabriele, Giordano, Calabrò, de Cindio, and Iorio (2001), the experimental data for all investigated lactones were compared in terms of an estimated gelation time (t_c). In addition, the complex moduli G^* at 1 Hz were evaluated at the $G'-G''$ crossover (G^*_c) and 2 h ($G^*_{2\,h}$) after complexation. G^*_c, and $G^*_{2\,h}$ can be considered as the strength of the critical gel and the firmness of the gel after 2 h, respectively. Furthermore, the IBC at the gelation time was evaluated. The data are summarized in Table 1. Three types of colloidal behavior were found for potato starch dispersions with lactone addition at saturation level, (i) a small increase of the storage modulus G' in the case of γ-nonalactone, (ii) a more pronounced increase of G' that led to the formation of a soft gel for γ-decalactone, γ-dodecalactone and δ-dodecalactone, and finally (iii) bulk phase separation with a solid precipitate and gelation of the supernatant in the case of δ-decalactone.

Gelation of starch as induced by starch–lactone interaction requires complexation of amylose close to the saturation point, which corresponds to IBC values of 0.4–0.8 mg iodine/100 mg dry starch. But on the other hand, full complexation of amylose does not necessarily induce gelation as found for γ-nonalactone. Most likely the length of the linear chain of lactones determines the gelation time and the strength of the resulting gel. The results in Table 1 confirm that increasing length of the hydrocarbon chain of the lactone led to shorter gelation times and firmer gels. The rigidity of the gels increased in the order γ-decalactone < δ-dodecalactone < γ-dodecalactone.

The frequency dependence of G' and G'' of potato starch dispersion complexed with 50 mmol δ-dodecalactone/mol

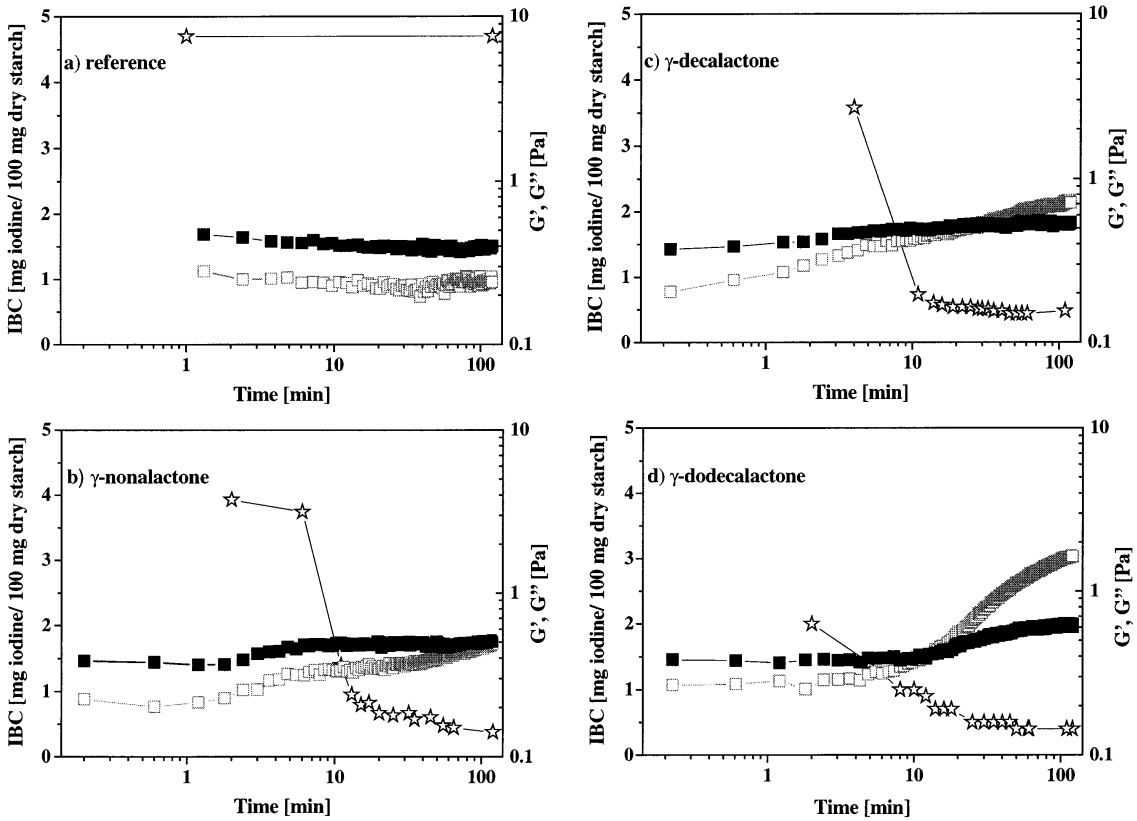

Fig. 2. Influence of the aging time on the viscoelastic behavior G' (open square) and G'' (filled square) and the Iodine Binding Capacity (asterisk) of potato starch dispersion: (a) without addition; (b) complexed with 200 mmol γ-nonalactone per mol glucose; (c) 50 mmol γ-decalactone per mol glucose and (d) 50 mmol γ-dodecalactone per mol glucose within 2 h.

glucose 2 h after complexation is presented in Fig. 3. The sample had an IBC of 0.4 mg iodine/100 mg dry starch indicating full complexation of the amylose. The viscoelastic moduli presented little frequency dependence with G' dominating over G'', which is characteristic for a gel-like behavior.

Although γ-nonalactone and δ-decalactone possess the same carbon chain length and present a similar complexation behavior (Heinemann et al., 2001), the colloidal properties 24 h after complexation were completely different (Table 1). No gelation nor phase separation was observed in case of potato starch dispersion complexed with γ-nonalactone, while δ-decalactone induced a solid–liquid bulk phase separation and a gelation of the supernatant within 24 h. δ-Decalactone has a higher water solubility compared to γ-nonalactone (Burdock, 1995), which may be the reason for the peculiar effect of δ-decalactone in a potato starch dispersion. It should be added that in general lactone concentrations that are below the saturation level of the amylose induce bulk phase separation of potato starch dispersion within 24 h.

The starch δ-decalactone interaction was further investigated by following the rheological changes and the IBC of a potato starch dispersion with a lower concentration of δ-decalactone (50 mmol/mol glucose) over a time period of 28 d. The results are presented in Fig. 4a and b. For the

Table 1
G^* at the G'–G'' crossover (G^*_c) and 2 h ($G^*_{2\,h}$) after complexation and the IBC at the gelation time (t_c) of potato starch dispersions complexed with an excess of lactones and its description 24 h after complexation

Lactone	C-atoms of chain (n)	Concentration (mmol/mol glc)	G^*_c (Pa)	t_c (min)	IBC_c[a]	G^*_{2h} (Pa)	Sample description (24 h after complexation)
None			–	–	–	0.5	Transparent, liquid
γ-Nona	5	200	–	–	–	0.7	Turbid, liquid
γ-Deca	6	50	0.7	29	0.5	0.9	Turbid, very soft gel
γ-Dodeca	8	50	0.6	14	0.8	1.7	Turbid, soft gel
δ-Deca	5	200	–	–	–	2.2[b]	Precipitation, supernatant soft gel
δ-Dodeca	7	50	0.8	25	0.4	1.3	Turbid, soft gel

[a] (mg iodine/100 mg dry starch).
[b] 24 h after complexation, measurement of the supernatant.

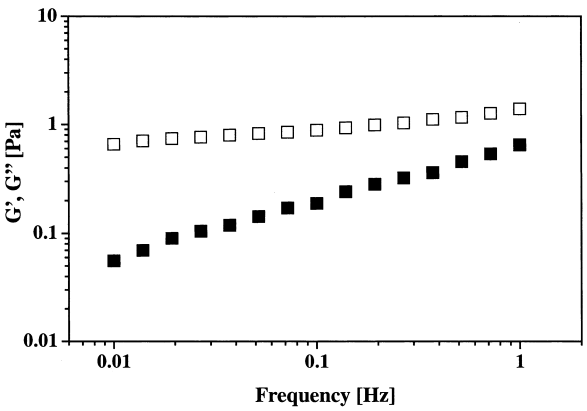

Fig. 3. Frequency dependence of G' (open square) and G'' (filled square) of potato starch dispersion complexed with 50 mmol δ-dodecalactone per mol glucose 2 h after complexation.

determination of the IBC, the sample was shaken to redisperse the solid precipitate and an aliquot was taken. For the rheological measurements, a sample of the supernatant was carefully removed and transferred to the rheometer. The reference potato starch dispersion did not show changes of the IBC nor of the viscoelastic properties during an aging period of 28 d (Fig. 4a and b). The addition

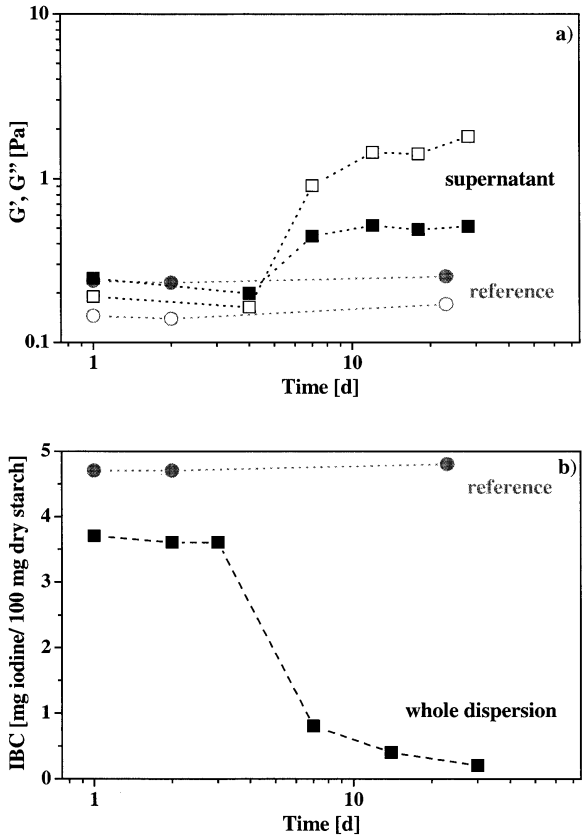

Fig. 4. Influence of the aging time on the viscoelastic properties G' (open symbols) and G'' (filled symbols) of a reference potato starch dispersion without addition and the supernatant of potato starch dispersion complexed with 50 mmol δ-decalactone per mol glucose (a) and on the iodine binding capacity of the reference and a whole, re-mixed potato starch dispersion complexed with 50 mmol δ-decalactone per mol glucose (b) within 28 d.

of δ-decalactone led to solid–liquid bulk phase separation after 24 h although the extent of amylose complexation was rather low. Based on the measurement of the IBC, only 25% of the amylose fraction was complexed within the first three days. Thereafter, a sharp decrease of the IBC was measured and full complexation of amylose was reached after 28 d. The decrease of the IBC of the potato starch δ-decalactone system was accompanied by the gelation of the supernatant. In contrast to the gels formed with other lactones, the gelled supernatant of the system with δ-decalactone was transparent.

The precipitation of amylose upon complexation is a well known phenomenon and is used as a method to separate amylose from amylopectin (Schoch, 1942). Although the supernatant of potato starch δ-decalactone systems became more and more depleted from amylose due to its precipitation, a gelation of this phase was observed. A spontaneous recrystallization (retrogradation) of amylose and amylopectin in the sample with δ-decalactone can be excluded since neither the IBC nor the viscoelastic properties of the reference showed changes during aging. The gelation of the supernatant of the phase separated dispersion may be due to amylopectin-δ-decalactone interactions. It is also conceivable that an intermediate starch fraction between the well-known starch macromolecules amylose and amylopectin contributes to the gelation (Gérard, Barron, Colonna, & Planchot, 2001).

3.2. Amylopectin–lactone interactions

To investigate the interactions of amylopectin with lactones, amylopectin potato starch was included in the investigations that contains about 1 g amylose/100 g starch as assessed by amperometric iodine titration. Thus, in this system the influence of amylose–lactone complexation can be neglected. δ-Lactones were selected since δ-decalactone induced bulk phase separation and a retarded gelation of the supernatant whereas δ-dodecalactone led to gelation of starch dispersion within minutes after complexation. The viscoelastic properties of amylopectin potato starch dispersion without addition and with 200 mmol δ-decalactone/mol glucose and 50 mmol δ-dodecalactone/mol glucose, respectively, were monitored during an aging period of 26 d at 25 °C and the results are presented in Fig. 5.

The reference amylopectin potato starch dispersion showed a storage modulus G' that was higher than the loss modulus G'', both moduli remaining constant during aging. The system with δ-lactones presented lower G' and G'' values than the reference one day after preparation which is mainly due to sample preparation. Since the reference amylopectin potato starch dispersion is prone to microbial degradation, the sample was kept in the sealed can during the aging period and the starch dispersion was not shaken for sample preparation, which resulted in a higher viscoelasticity of the lactone-free system. During aging the sample with δ-dodecalactone was transformed into a soft

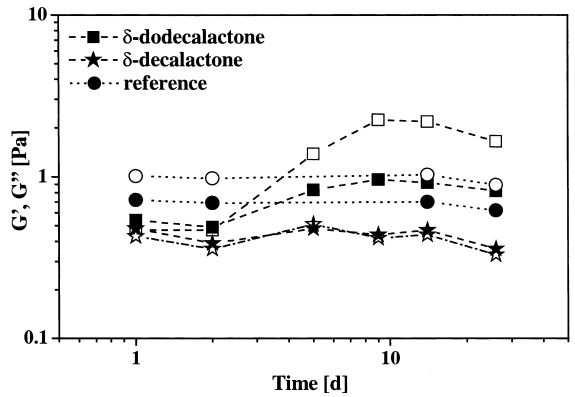

Fig. 5. Influence of aging time on the viscoelastic properties G' (open symbols) and G'' (filled symbols) of amylopectin potato starch dispersion without addition and complexed with 200 mmol δ-decalactone mmol per mol glucose and 50 mmol δ-dodecalactone per mol glucose within 26 d.

gel while δ-decalactone did not induce gelation. The latter result is opposite to the finding that δ-decalactone induces the gelation of the supernatant of phase separated potato starch dispersions.

The amylopectin–lactone interaction was further investigated by X-ray diffraction measurements of freeze-dried systems with moisture contents between 10 and 15 g/100 g wb. The diffraction diagram of freeze-dried amylopectin potato starch dispersion without addition (reference) and complexed with δ-decalactone and δ-dodecalactone stored for 1, 2, 5 and 26 d are presented in Fig. 6. The X-ray diffraction diagram of amylopectin potato starch without addition one and 26 d after processing can be described as an amorphous halo. The X-ray diffraction diagram of amylopectin potato starch dispersion in presence of δ-decalactone and δ-dodecalactone showed peaks at 13.0

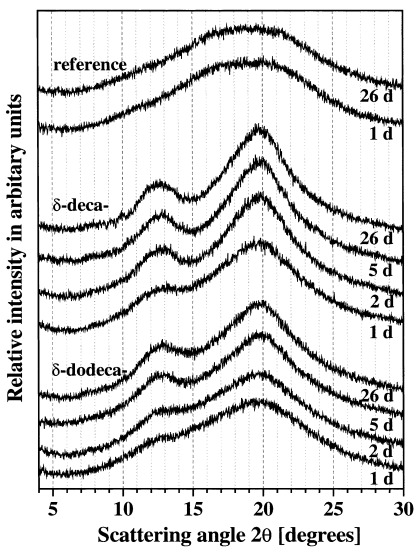

Fig. 6. X-ray diffraction measurements of freeze dried amylopectin potato starch dispersion without addition and complexed with 200 mmol δ-decalactone per mol glucose and 50 mmol δ-dodecalactone per mol glucose aged between 1 and 26 d.

and 20.0° with very low intensities 1 d after complexation, but peak intensities increased during aging. The scattering angles of 13.0 and 20.0° agree well with those described for the amylose–lactone complexes (Heinemann et al., 2001). The X-ray diffraction peaks at 13.0 and 20.0° suggest the formation of a V_h helix although a peak at 7.5° was not readily detectable as it is typical for V_h-type amylose crystals (Buléon, Delage, Brisson, & Chanzy, 1990; Yamashita, 1965). The absence of the low angle peak may be due to the low crystallinity of the system. The results of X-ray diffraction suggest that amylopectin is able to interact with the δ-lactones investigated. It is conceivable that the side chains of amylopectin form helical inclusion complexes with δ-lactones. A further indication that amylopectin-δ-lactone interactions occur is the formation of a gel with δ-dodecalactone. This is in agreement with Nuessli et al. (2000) who found that complexing emulsifiers induce the formation of amylopectin networks. Again, spontaneous recrystallization of amylopectin can be excluded since neither the viscoelastic properties nor the X-ray pattern of the reference changed during aging. The spontaneous recrystallization of amylopectin would lead to a B-type pattern with main peaks at 5.5, 17, 22 and 24°. However, it is unclear why δ-decalactone induced the gelation of the amylopectin rich phase in potato starch but not the gelation of pure amylopectin systems. Differences in the molecular structure of amylopectin from conventional potato starch and amylopectin potato starch may be the reason for contradictory results. Finally, it is not clear if the presence of amylose has an influence on amylopectin–lactone interactions. Small amounts of complexed amylose might act as nuclei for the formation of an amylopectin network.

It should be added that the X-ray pattern of freeze-dried waxy maize and amaranth starch (an amylose free starch) dispersion complexed with δ-lactone also showed peaks at 13.0 and 20.0° some days after sample preparation (results not shown), indicating that the interaction of δ-lactone with amylopectin is independent of the botanical origin of the starch.

Since amylopectin potato starch dispersions in presence of lactones remain transparent, one can assume that helix–helix aggregation due to amylopectin–lactone complexation takes place in the domain of nanometers. In contrast to the fast complexation of the amylose with lactones, amylopectin–lactone complexation is much slower and, thus, requires several days.

3.3. Supramolecular amylose–lactone structure

Light microscopy was applied to investigate the supramolecular structure of amylose–lactone complexes. Since the macroscopic behavior of potato starch dispersion depends on the saturation level of amylose, low and high lactone concentrations were investigated.

A description of the spherocrystalline structures that resulted from the complexation of potato starch dispersion

24 h after complexation is presented in Table 2. Light micrographs in the phase contrast and polarized light mode of potato starch dispersions complexed with low lactone concentrations that is 10 mmol γ-dodecalactone/mol glucose, 50 mmol γ-nonalactone/mol glucose and 10 mmol δ-dodecalactone/mol glucose, respectively are shown in Fig. 7. The complexation of potato starch dispersion with lactones led to the formation of spherocrystalline structures with diameters ranging from 10 to 90 μm. Three different types of structures were observed: small rod-like spherulites with dimensions from 10 to 15 μm, irregular spherulites with diameters from 20 to 50 μm and spherulites ranging from 20 to 90 μm which presented a Maltese cross and a banded extinction pattern when viewed under polarized light.

The complexation of starch dispersion with γ-lactones led to rather small rod-like and irregular spherulites. The complexation of potato starch dispersion with δ-lactones induced the formation of spherulites with Maltese cross independent from the lactone concentration. The complexation of potato starch dispersion with δ-decalactone led to spherulites with a Maltese cross and additionally to irregular spherulites at high and rod-like spherulites at low δ-decalactone concentration.

The partly crystalline spherulites formed in potato starch dispersion with lactones do not represent native granular potato starch structures but new supramolecular amylose–lactone structures since gelatinized potato starch dispersion without addition did not show any structure when viewed with light microscopy. The spherulitic crystallization of starch has been described by several authors. Precipitation of amylose with butanol or ethanol induces the formation of spherulites (Helbert, Chanzy, Planchot, Buléon, & Colonna, 1993; Schoch, 1942). Spherocrystals were also grown from amylose solutions with fatty acids (Godet, Bouchet, Gallant, Colonna, & Buléon, 1996). Davies et al. (1980) showed that spherocrystallites are formed by holding maize starch at high temperatures (60–95 °C). The authors termed this phenomenon high-temperature retrogradation and postulated that it is the result of amylose complexation with internal lipids. Recently, Nordmark and Ziegler (2001) reported the formation of spherulites upon cooling of gelatinized maize starch, the morphology depending on the cooling rate.

Spherulitic crystallization is a self-organization process that is common for small molecules and polymers of natural and synthetic origins (Gedde, 1995; Keller, 1955; Magill, 2001; Wunderlich, 1973). Spherulitic growth can either start at a heterogeneously nucleated complex crystal aggregate or develop from a small initial crystal. Based on computer simulation, Kalinka and Hinrichsen (1997) described the mechanisms that control the growth of spherulites. Linear growth, front branching, side branching and clustering are the different mechanisms of structure formation. The degree of order in the spherulites increases with time. This leads to an increase of crystallinity, which is known as secondary crystallization. Spherulites are commonly characterized optically using polarized light (Magill, 2001) and may present banded extinction patterns as seen in Fig. 7, which results from the twisting of the radiating lamellae.

Growth and morphology of spherulites is kinetically controlled. In the present study the spherulites described in Table 2 and Fig. 7 most likely correspond to different stages of spherulitic growth. The rod-like structures are the result of a few growing cycles, the irregular spherulites represent a more advanced stage of spherulite growth. Late stages of spherulitic growth lead to spherical structures that display a distinct Maltese cross. Similarly, Belamie, Domard, Chanzy, and Giraud-Guille (1999) observed that spherulitic crystallization of chitosan led to different structures which correspond to different steps of spherulite growth. Based on previous studies on starch spherulites it is reasonable to assume that the spherulites grown from starch/lactone systems are composed primarily of amylose. This was confirmed by performing control experiments with amylopectin potato starch. As expected, no supramolecular structures were found in these systems (micrographs not shown). However, it cannot be excluded that amylopectin is entrapped in the interlamellar regions of spherulites.

Amylose aggregates readily in aqueous systems because of the unfavorable interaction between amylose and water. The formation of amylose inclusion complexes further reduces the solubility of amylose and promotes spherulitic crystallization. In the present study this was favored by large undercooling and moderate starch concentrations. When precipitation occurred in starch/lactone systems, the spherulites were generally larger since growth of spherulites is most probably promoted by the absence of an amylose network and by the rather low viscosity of the system. In the case of δ-decalactone at high concentration, the complexation rate was high (results not shown) and spherulite growth rapidly progressed and promoted solid–liquid phase separation before the amylopectin-rich supernatant formed a gel. In systems where starch/lactone complexation induced the gelation within short time, rather small spherulites were found. The size and the morphology of the spherulites did not significantly change over an aging period of several months (results not shown). Thus, it can be assumed that further growth of spherulites was kinetically inhibited in gelled systems due to reduced polymer mobility. Nordmark and Ziegler (2001) also showed that the morphology of spherulites in gelatinized maize starch can be controlled by varying polymer composition and kinetic regimes.

3.4. Structural features of starch/lactone systems at different length scales

Starch–lactone complexation influences the structure of the starch dispersions at different length scales ranging from

Table 2
Types of crystal structures observed with light microscopy 24 h after complexation of potato starch dispersion with lactones

Lactone	Concentration (mmol/mol glc)	Crystalline structure			
		Shape	(μm)	Shape	(μm)
γ-Nona	200	a	10		
	50	a	10	b	20–50
γ-Deca	50	a	10		
	20	a	10		
γ-Dodeca	50	a	10		
	10	a	10		
δ-Deca	200	b	20–50	c	60
	50	a	15	c	20–90
δ-Dodeca	50	c	20		
	10	c	15–60		

a: rod-like spherulites; b: irregular spherulites; c: spherulites with maltese cross.

nano to macroscale. Amylose readily forms inclusion complexes with different lactones, and amperometric iodine titration allows, to follow, the complexation quantitatively. On the other hand, the gelation of amylopectin systems in presence of lactones suggests that the linear side chains of amylopectin also interact with lactones. The short time behavior is dominated by amylose–lactone interactions but for the long time behavior amylopectin–lactone interactions most probably also play a role.

Starch–lactone complexation leads to different colloidal phenomena that span the range from solid–liquid bulk phase separation to gelation. The macroscopic properties are influenced by the type of ligand but also by the complexation rate, which in turn is determined by the ligand concentration and the diffusion of the ligand in the aqueous starch system. This suggests that the different colloidal phenomena are to a great extent kinetically controlled. However, the interpretation of colloidal phenomena is complicated by the occurrence of interrelated and superimposed events that are amylose and amylopectin complexation, amylose/amylopectin phase separation, polymer aggregation and crystallization.

A complexation induced gelation of low concentration potato starch systems requires complexation of amylose close to the saturation point that means an IBC below 1 mg iodine/100 mg dry starch. Amylose inclusion complexes tend to aggregate due to their poor solubility in water. The result is microphase separation and the formation of an interconnected amylose network, which at the macroscopic level is manifested by gelation. Not only a fine stranded network, but also larger aggregates, namely spherulites are formed which act as fillers in the system. It is not clear to which extent the spherulites and the amylose networks are connected. Due to the gelation of the amylose fraction, amylose/amylopectin phase separation is arrested at an early stage, and the system is kinetically stabilized in an out of equilibrium state. The amylopectin-rich phase is trapped in the mixed system, and it is likely that the formation of amylopectin–lactone complexes further increases the rigidity of the system during aging. Further research is

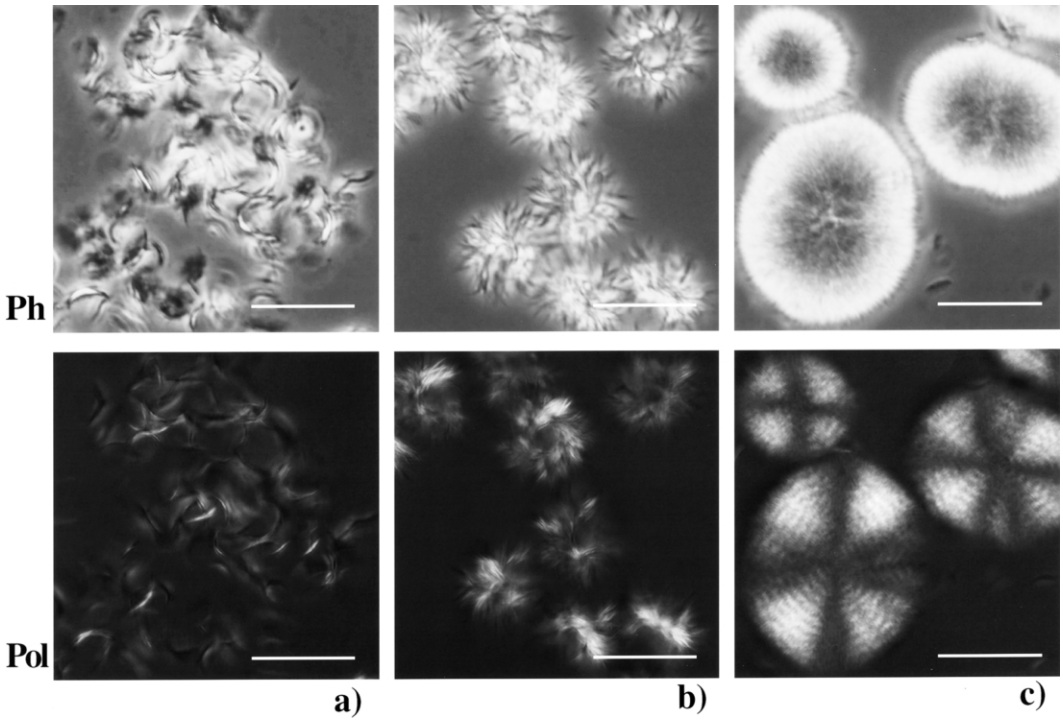

Fig. 7. Polarized (Pol) and phase contrast (Ph) light micrographs of amylose–lactone spherulites, 24 h after complexation (scale bar = 50 μm): (a) rod-like spherulite (10 mmol γ-dodecalactone per mol glucose); (b) irregular spherulite (50 mmol γ-nonalactone per mol glucose); (c) spherulite with Maltese cross (10 mmol δ-dodecalactone per mol glucose).

necessary to establish the kinetics of phase separation in relation to amylose and amylopectin complexation and gelation. On the other hand, low complexation rates promote bulk phase separation. A possible explanation is that amylose/amylopectin phase separation progresses faster than amylose aggregation. If the continuity of the amylose falls below a critical level before amylose saturation is reached, no interconnected network can be formed. Instead, large spherulites are formed which further reduce the phase volume of amylose and finally precipitate. If amylose is not rapidly trapped in a network, the growth of spherulites is favored. An exception is δ-decalactone where high concentrations induce fast saturation of the amylose, but bulk phase separation rather than gelation is found. On the other hand, low δ-decalactone concentration induces the growth of large spherulites within 24 h although the degree of complexation is low (about 20% after 24 h). Regarding the colloidal behavior of starch/lactone systems, δ-decalactone constitutes an exception. Likewise, the X-ray structure of these starch–lactone complexes was not definitively identified as V_h-type amylose helices as all other lactones one day after complexation (Heinemann et al., 2001) but was transformed into V_h-type amylose helices during aging.

4. Conclusions

The thermodynamic conditions of starch dispersions are altered by the presence of a complexing ligand. A variety of structures can be generated by selecting lactones with different chain length and by adjusting the complexation kinetics. Both starch fractions, namely amylose and amylopectin, are able to interact with lactones. The amylose fraction dominates the short time behavior while the amylopectin–lactone interactions develop in the course of several days. The results of this research are relevant to the understanding of the colloidal properties of starch systems. The interaction of suitable ligands with amylose and amylopectin gives the possibility to control the structural properties of starch systems, and ultimately of all properties related to structural organization such as texture and flavor release.

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