Impact of high hydrostatic pressure on wheat, tapioca, and potato starches

vorgelegt von
Dipl.-Ing.
Birgit Anika Rumpold

von der Fakultät III – Prozesswissenschaften
der Technischen Universität Berlin
zur Erlangung des akademischen Grades

Doktorin der Ingenieurwissenschaften

- Dr.-Ing. -

genehmigte Dissertation

Berlin 2005
D 83

Danksagung

CONTENT

LIST OF ABBREVIATIONS

ADP-Glc	Adenosine diphosphate glucose
ADP-glucosepyrophosphorylase	Adenosine diphosphate glucosepyrophosphorylase
AOAC	Association of analytical communities
ATP	Adenosine triphosphate
DSC	Differential scanning calorimetry
DTG	Differential thermogravimetry
Frc-6-P	Fructose-6-phosphate
FU	Farinograph Units
GBSS	Granule bound starch synthase
Glc-1-P	Glucose-1-phosphate
Glc-6-P	Glucose-6-phosphate
HMT	Heat-moisture treatment
HP	High pressure
HPDS- High Pressure Cell	Hartmann, Pfeifer, Dornheim, Sommer-High Pressure Cell
HPDTA	High pressure differential thermal analysis
P-glucomutase	Phosphoglucomutase
PTTI	Pressure time temperature integrator
RS	Resistant starch
RT	Room temperature
SEM	Scanning electron microscope
SS	Starch synthase
Susy	Sucrose synthase
T_g	Glass transition temperature
TG	Thermogravimetry
UDP-Glc	Uridine diphosphate glucose
UDP-glucosepyrophosphorylase	Uridine diphosphate glucose glucosepyrophosphorylase

LIST OF FIGURES

LIST OF TABLES

1   INTRODUCTION

Starch is an abundant storage carbohydrate in plants, one of the most important carbohydrate sources for human nutrition and widely used in the food industry. It exhibits unique properties depending on its botanical origin, chemical composition and the chemical, enzymatic, thermal, and/ or mechanical (pre-) treatment applied. The texturising properties of starches are mainly based on a process called gelatinisation defined as a water uptake and swelling of the starch granules accompanied by a viscosity increase of the suspension and melting of starch crystals becoming visible by loss of birefringence. Generally, gelatinisation occurs upon heating but it can also be induced e.g. chemically by addition of alkali. In 1982 Muhr and Blanshard first reported a gelatinisation of wheat starch in excess water at ambient temperature under high hydrostatic pressure (450 MPa).

High hydrostatic pressurisation is a non-thermal physical process where a packed product (e.g. starch suspension) is introduced into a high pressure vessel and pressure is built up either directly or indirectly via a plunger and a pressure transmitting medium (e.g. water). Thus upon pressurisation, reactions involving a reduction in volume are favoured and high hydrostatic pressure can cause denaturation of proteins, inactivation of microorganisms and enzymes at simultaneous preservation of the fresh-like character of a food product at minimum thermal stress.

Up to now, several research groups dealt with the high hydrostatic pressure treatment of starches of different origin. They discovered a swelling of starch granules upon pressurisation associated by a loss of birefringence and gelatinisation. In contrast to heat-gelatinised starches, the granular character was maintained and the textural properties such as water binding, viscosity and gel characteristics differed. Consequently, high hydrostatic pressure treatment of starch suspensions opens up new applications of starches in the food and cosmetics industries with novel properties. Additionally, a relationship between physical starch properties and pressure and time has been observed. Since starch gelatinisation and its physical properties are evidentially temperature-dependent, this raises hope that starch could be applicable as an intrinsic control indicator for pressure, temperature and treatment time applied in a pressurisation process.

However, no thorough, methodical investigation on the impact of high hydrostatic pressure has been performed. The effects of high pressure in combination with temperature and treatment time on degree of gelatinisation and physical properties need to be studied systematically as well as the influence of sugars and salts on pressure-induced starch

gelatinisation. Since differing retrogradation and recrystallisation behaviour and enzymatic degradability were obtained by several research groups comparing heat- and pressure-induced gelatinised starch suspensions and healthy, functional nutrition has gained more and more attention, it would be interesting to investigate the effect of pressure-induced starch gelatinisation in combination with subsequent treatment steps favouring starch recrystallisation on the content of resistant starch. In addition, the impact of pressurised wheat starch replacing part of the flour in dough on the textural properties of wheat bread upon storage appears to be an attractive survey.

2 LITERATURE REVIEW

2.1 Starch Principles

Starch is a major storage product in plants and one of the most important carbohydrate sources for human nutrition. In addition to the high nutritional value, starches play an important role in food manufacturing processes due to their considerable effect on textural properties of food products. They are utilised e.g. as gelling agents for puddings, as thickeners, in sauces and desserts, in baking products, and also in the non-food sector such as in the paper manufacturing industry.

Starch occurs in form of granules varying in size and shape depending on the plant of origin. The granules can be e.g. spherical, oval, polygonal, and lenticular in shape with a diameter of 2 to 175 µm and can be found single, assembled in compounds (Belitz *et al.*, 2001; Tegge, 2004), or ostensibly assembled. For compound starch granules, the single granules do not become visible until after a swelling step. The amount of discrete granules in compound starch granules ranges from a few to up to several thousand granules (Stute, 1985). Wheat, rye and barley even contain two different types of starch granules: a large lenticular type (25-40 µm) and a small spherical type (5-10 µm) (Hoseney, 1986). Depending on their origin, starches have typical properties that are attributed to the size, shape, composition, and crystallinity of the granules (Belitz *et al.*, 2001). In addition to starch, starch granules contain traces of fat, nitrogen, and phosphorous which potentially influence the properties of starches despite negligibly small concentrations (Hoseney, 1986; Belitz *et al.*, 2001). Water is also detectable in starches, e.g. in wheat starch the water content amounts 13-15 %, in potato starch around 20 %. One third thereof exists as free water, the rest is bound by adsorption and capillary forces (Ternes, 1994).

2.1.1 Chemical structure

Starch consists of α-D-glucose molecules and is composed of a mixture of the two glucanes amylose and amylopectin. The ratio of amylose to amylopectin averages 23 ± 3 % amylose to 74-77 % amylopectin (exceptions: waxy starches comprise 100 % amylopectin, amylotype starches contain 70 % amylose.) (Ternes, 1994).

Amylose is predominantly a linear molecule with a partially helical structure consisting of α-1,4-linked D-glucose (Fig. 2.1) with an average molecular weight of 250,000 depending on the type of source plant and its growing stage (Hoseney, 1986; Tegge, 2004). At a degree of polymerisation of about 1000, the amylose molecule exhibits a length of 0.5 µm (Ternes, 1994). The degree of polymerisation of potato amylose can amount up to 4500 corresponding to a molecular mass of 750,000 (Belitz *et al.*, 2001). Amylose has the ability to form complexes with iodine, organic alcohols, and acids, the so-called helical inclusion

Fig. 2.1. Schematic illustration of the amylose molecule.

complexes. It can thus be precipitated from a starch solution by addition of n-butyl-alcohol as a result of forming an insoluble complex with the alcohol (Hoseney, 1986). Additionally, in the presence of salts such as $MgSO_4$ and low molecular fatty acids (caprylic and capric acid), amylose can be separated from starch solutions by means of crystallisation (Belitz *et al.*, 2001). The long, linear structure of amylose is also responsible for its tendency to associate with itself and precipitate from solution. Only at a high pH (e.g. in 1N KOH) amylose stays in solution because small positive charges are induced on the hydroxyl groups that causes adjacent chains to repel each other (Hoseney, 1986).

With a molecular weight of 10^8, amylopectin represents one of the largest molecules found in nature. Just like amylose, amylopectin is composed of α-D-glucose being mostly α-1,4-linked. In contrast to amylose however, it is highly branched and therefore also contains α-1,6-links (4-5 %). The average chain length of the branches amounts up to 20-25 glucose units (Hoseney, 1986). Amylopectin is a two-dimensional, discoidal molecule, which is assumed to have a cluster-like structure (Sivak & Preiss, 1998; Tegge, 2004) and consists of A-, B- and C-chains. A-chains are short, free of side chains and linked to the amylopectin molecule by its reducing end. The longer B-chains bear side chains (A- and B-chains) and can be divided into dense, crystalline sections (cluster) and less dense, amorphous sections without side chains. Parallel A- and B-chains in the same cluster form right-handed double helices originating from branch points with six glucose molecules per turn. The C-chain is characterised by carrying the only reducing end of an amylopectin molecule (Banks & Muir, 1980; Belitz *et al.*, 2001; Tegge, 2004). The molecular structure and an exemplary model of
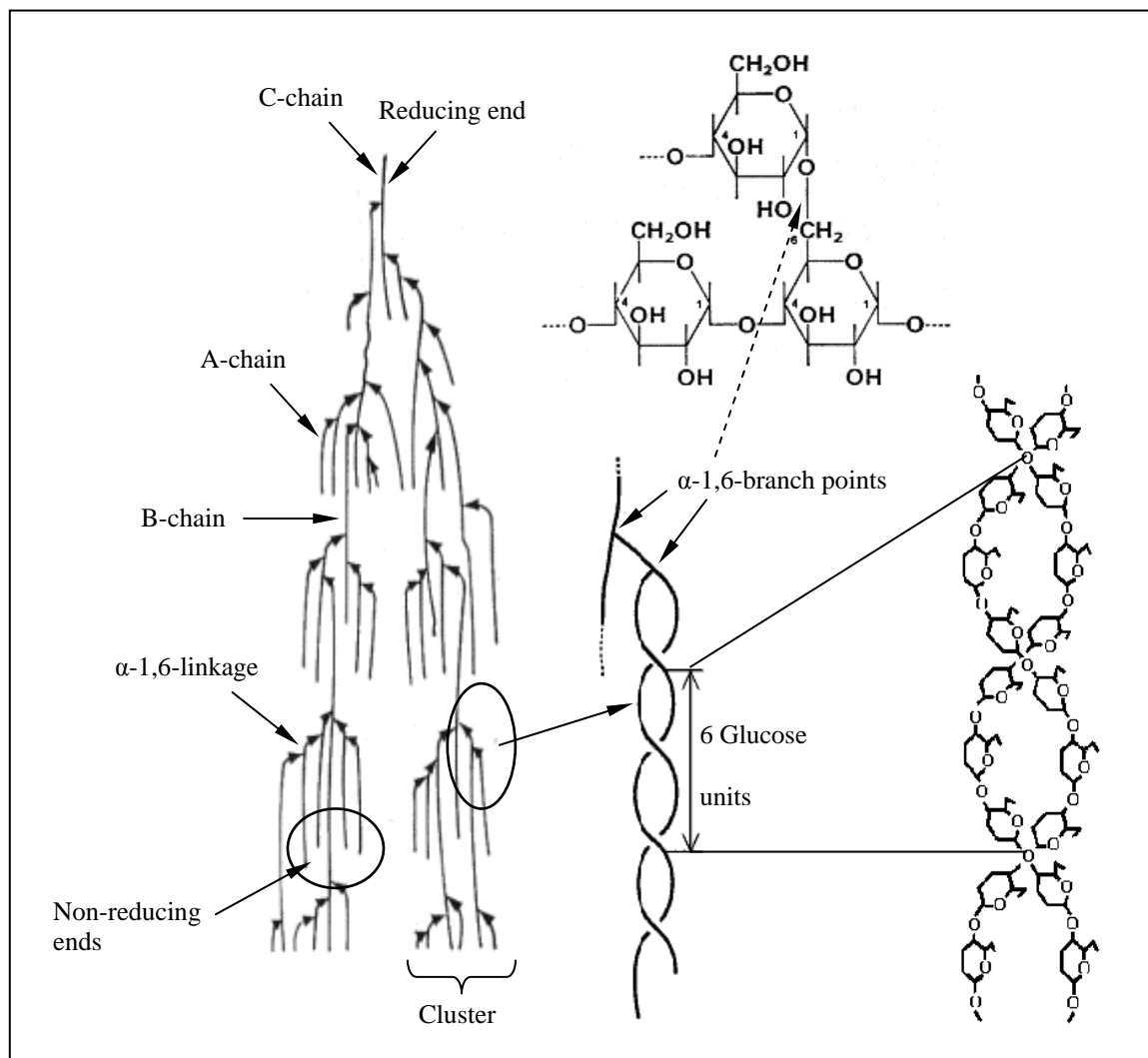
Fig. 2.2. Molecular structure of amylopectin and a model for the helical and cluster-structure of the amylopectin molecule (according to Banks & Muir, 1980; Belitz *et al.*, 2001).

the cluster-structure of amylopectin are depicted in figure 2.2. The amylopectin molecule is radially arranged within the starch granule which leads to a tangential alignment of the crystals. It is oriented with the non-reducing ends of the chains towards the granule surface. The molecule is divided in alternating crystalline and amorphous

Fig. 2.3. Composition of starch blocklets and the assembly of small and large blocklets in soft and hard shells in a starch granule (Gallant *et al.*, 1997).
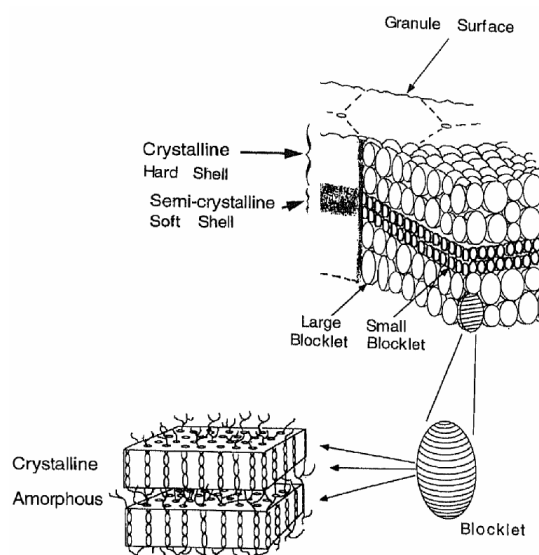
structures of sections (lamellae) with a periodicity of 9-10 nm. In the crystalline lamellae, as mentioned before, the cluster-like structures are located. The amorphous lamellae contain the branching points, the amylose and the amorphous amylopectin. The lamellae form larger, ellipsoid structures, the so-called blocklets, which in turn form crystalline, hard and semicrystalline, soft shells, i.e. growth rings (see fig. 2.6) being several 100 nm thick. The soft shells are made of smaller blocklets (20-50 nm), the harder shells consist of larger blocklets (50-500 nm) as can in detail be seen in figure 2.3 (Gallant, 1997; Tegge, 2004).

2.1.2 Starch crystallinity

Starch is partially crystalline, up to 30 % of starch in a starch granule consist of crystallites (Hoseney, 1986). This crystallinity cannot be attributed to intermolecular interactions between amylose chains analogous to cellulose, but to the branched amylopectin. For amylose being located in the amorphous regions of the starch granules can be leached out of the granule without destruction of the crystalline properties, and amylose-free starches like waxy maize starch also exhibit semicrystallinity (Hoseney, 1986; Sivak & Preiss, 1998; Belitz *et al.*, 2001). An important property of starches is their birefringent character being expressed by the typical dark Maltese crosses in light microscopic examinations (Fig. 2.4). This gives proof of a high degree of order, i.e. molecular orientation within the starch granule which is not to be mistaken with crystallinity. The birefringence is lost upon gelatinisation (Banks & Muir, 1980; Hoseney, 1986).

Fig. 2.4. Birefringent pattern of native potato starch granules in polarised light (x 400).

Investigations of several intact starches by X-ray analysis resulted in the three X-ray diffraction patterns A, B, and C. The pattern for A-type crystals shows three strong peaks at 5.8, 5.2 and 3.8 Å and the B-type pattern exhibits peaks with medium intensity at 15.8-16 Å and 5.9-6.1 Å, a strong peak at 5.2 Å and a doublet with medium intensity at 3.7 and 4.0 Å (Zobel, 1964 & 1988). Most cereal starches as e.g. wheat starches exhibit the A-type crystallinity, potato starch and other tuber starches, amylomaize starch, as well as retrograded starches show B-type crystallinity, and starches of tropical plants and legume starches e.g.

bean starch show C-type X-ray diffraction patterns. The C-type crystallinity is a composite, containing a mixture of A-type and B-type crystallites and exhibits the same X-ray diffraction pattern as A-type crystals with an additional peak at about 16 Å characteristic for B-type crystallinity (Hoseney, 1986; Zobel, 1988; Sivak & Preiss, 1998, Tegge, 2004). In thermally swollen granules a fourth crystallite type called V-type crystallinity has been found (Belitz *et al.*, 2001) with characteristic peaks at 11.3-12 Å, 6.5-6.7 Å and 4.3-4.4 Å depending on the extent of hydration (Zobel, 1964 & 1988). The X-ray diffraction pattern can be changed from the B-type to the A-type pattern by heat-moisture treatment. By swelling and gelatinisation of starches the V-type pattern is formed being characteristic for freshly baked bread. When bread is staling, B-type crystallinity is formed which is the typical crystalline structure for retrograded starches (Sivak & Preiss, 1998; Belitz *et al.*, 2001). According to Stute (1985), starches with B-type crystallinity exhibit higher gelatinisation enthalpies than starches with A-type crystallinity.
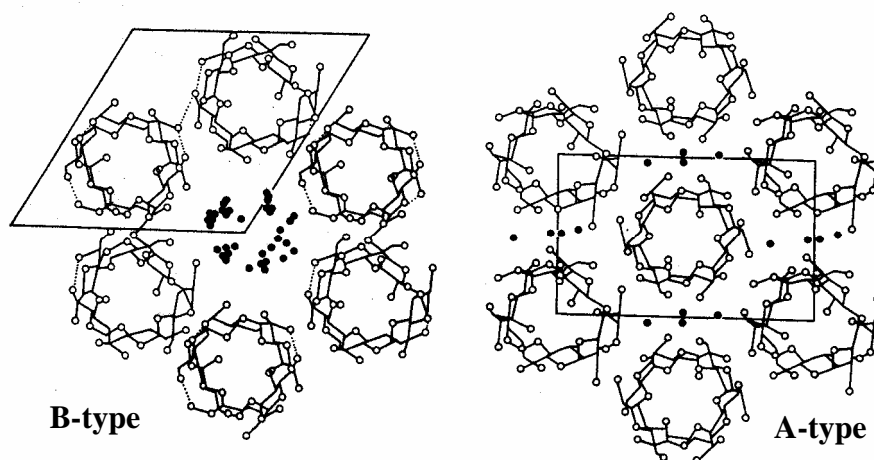
Fig. 2.5. Alignment of double helices in A- and B-type crystals of starches (Blanshard, 1987).

Based on X-ray diffraction patterns of A-type and B-type crystallinity, unit cells made of double helices were estimated (Fig. 2.5). The double helices comprising e.g. amylopectin side chains and representing the structural elements of these unit cells are right-handed, parallelly arranged with 6 glucose units each turn (Sarko & Wu, 1978; Belitz *et al.*, 2001). The assumption of an anti-parallel arrangement of the double helix was not agreeable with the starch biosynthesis according to Buléon *et al.* (1998), Belitz *et al.* (2001) did not exclude this possibility due to existing research results, however. The unit cell of B-type crystalline starch

7

consists of six of these double helices enclosing a cavity filled with 36 water molecules in a hexagonal arrangement. The unit cell of the A-type crystallinity is composed of seven double helices and is therefor more densely packed due to a monoclinic arrangement and contains only eight water molecules per unit cell (Sarko & Wu, 1978; Buléon *et al.* 1998; Belitz *et al.*, 2001).

The conformation of the double helices can be altered by external factors. The presence of e.g. KOH leads to an extension of the helix, KBr causes an even more extended helix with only four glucose molecules per turn. Sufficingly lipophilic molecules like iodine, fatty acids, fatty acid esters of hydroxycarboxylic acids, monoglycerides, and n-butanol are able to form helical inclusion complexes with amylose. By this complexation the before mentioned V-conformation of V-type crystalline starches can be stabilised. A complex of amylose with n-butanol results in a helix with seven glucose molecules per turn, and in the presence of α-naphthol the helix expands to eight sugar molecules per turn (Belitz *et al.*, 2001).

2.1.3 Starch biosynthesis

Starch is synthesised in the plastids, self-replicating organelles surrounded by a double-membrane envelope and divided into amyloplasts and chloroplasts on the basis of their function. In chloroplasts the so-called assimilation starch is synthesised and temporarily stored in case of sugar abundance during photosynthesis, whereas the starch granules generated in the amyloplasts act as permanent storage carbohydrates (Stute, 1985; Sivak & Preiss, 1998). In the amyloplasts the starch granule grows by apposition, i.e. radial deposition of material on the outside, starting from the hilum as growing point (Hoseney, 1986). The hilum is located at the point at which the arms of the Maltese cross being perpendicular to the growth rings meet under polarised light (Banks & Muir, 1980; Sivak & Preiss, 1998). Every new
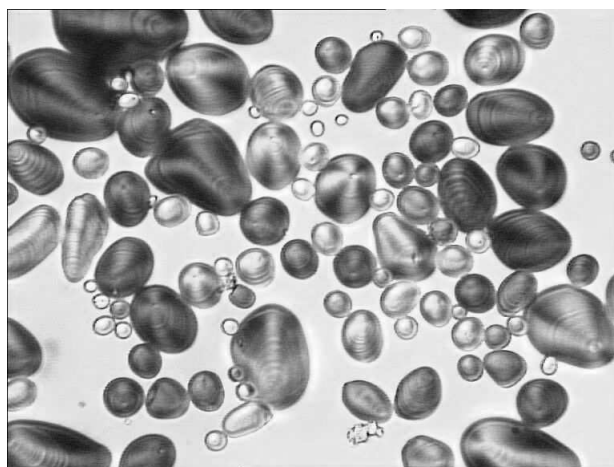
Fig. 2.6. Native potato starch granules stained with Lugols solution under polarised light (x 400). Note the concentric growth rings.

growth ring, representing alternating shells of high and low refractive index, varies in layer thickness, density, and enzyme digestibility depending on the amount of carbohydrates available at that stage of starch synthesis (Hoseney, 1986; Blanshard, 1987). These layers formed during the starch synthesis become visible after a treatment with dilute acid or enzymes of the starch granule (Hoseney, 1986). For potato starch, the growth rings can easily be seen in the intact starch under a light microscope (Fig. 2.6). In wheat starch, the number of growth rings appears to correspond to the days of granule development (Blanshard, 1987).

CYTOPLASM

Amylopectin   **Amylose**

Starch synthase +   Starch synthase
Branching enzymes

Sucrose

Sucrose synthase

α-1,4 Glc$_n$

Fructose   **UDP-Glc**   **ADP-Glc**

fructokinase   UDP-glucosepyro-   ADP-glucosepyrophosphorylase
                      phosphorylase

                                                        PPi

                                                        ATP

Frc-6-P   **Glc-1-P**   **Glc-1-P**

P-hexoseisomerase   Cytosolic   Plastidial Phosphoglucomutase
                      Phosphoglucomutase

                    Glc-6-P   **Glc-6-P**
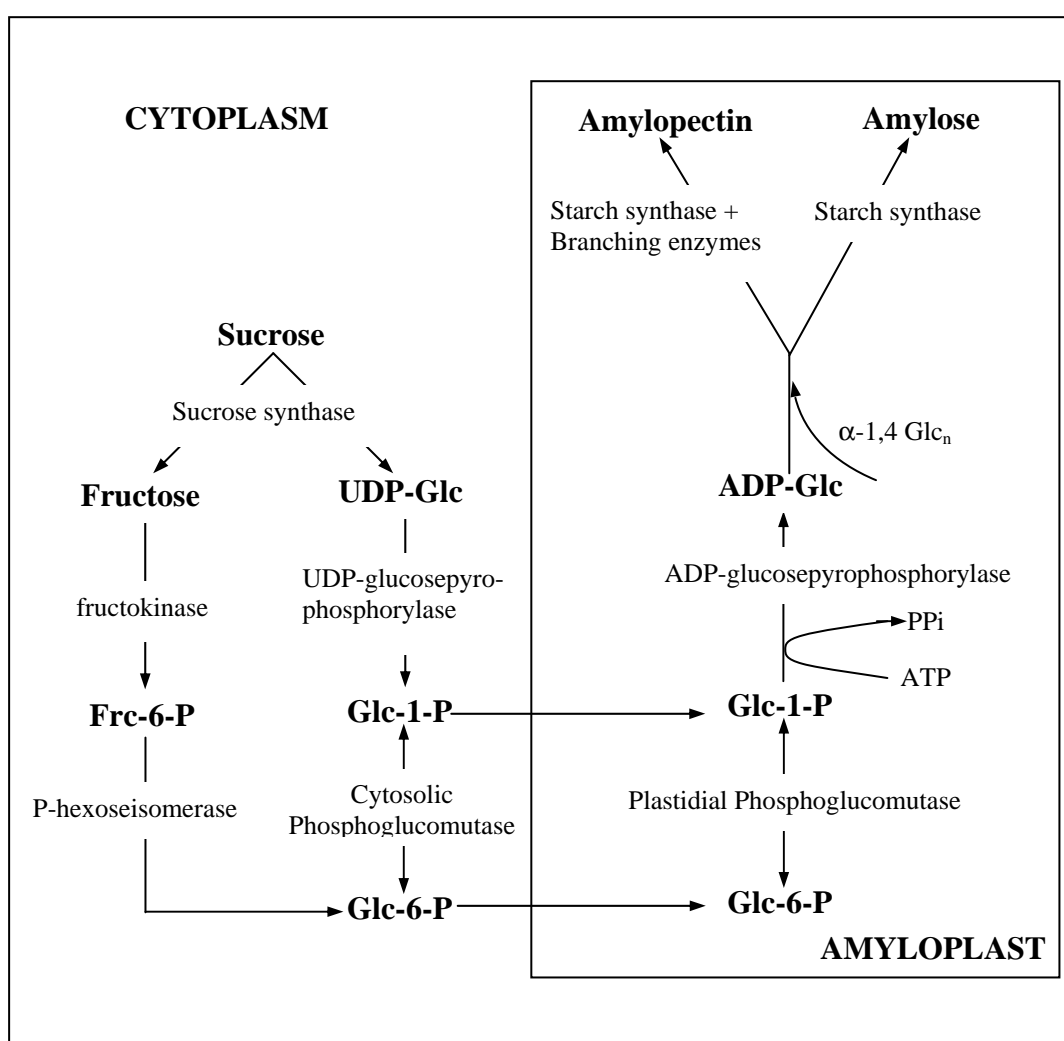
                                        AMYLOPLAST

Fig. 2.7. Schematic overview of starch biosynthesis (Buléon *et al.*, 1998; Sivak & Preiss, 1998; Tegge, 2004).

Since there is no excess of sugars, for starch synthesis the sugar molecules have to be transported to the amyloplasts by a membrane system. Presumably, this transportation is

controlled by the enzymes bound to the double membrane of the plastids (Stute, 1985). The carbon transport form sucrose is metabolised into fructose and UDP-glucose (UDP-Glc) by sucrose synthase (Susy) in the cytoplasm because only hexoses are able to be translocated into the amyloplast. The fructose is transformed into fructose-6-phosphate (Frc-6-P) by fructokinase and further into glucose-6-phosphate (Glc-6-P) by the cytosolic P-hexoseisomerase, while the UDP-glucose is metabolised in glucose-1-phosphate (Glc-1-P) by UDP-glucosepyrophosphorylase and further in glucose-6-phosphate (Glc-6-P) by cytosolic phosphoglucomutase (P-glucomutase). Glc-1-P and Glc-6-P are transferred from the cytoplasm into the amyloplast via translocators of the amyloplast membrane. Within the amyloplast the Glc-6-P is metabolised in Glc-1-P by plastidial P-glucomutase, Glc-1-P is then further converted into ADP-glucose by ADP-glucosepyrophosphorylase. The starch synthase catalyses the transfer of the glucosyl moiety of the ADP-glucosepyrophosphorylase to the non-reducing end of a growing α-1,4-glucan and is involved in amylose as well as amylopectin synthesis. Five kinds of starch synthases (SS) participate in the starch synthesis: The granule bound starch synthase (GBSS), SS I, SS II, SS III, and SS IV, where the GBSS only takes part in the formation of amylose. For the amylopectin synthesis starch branching enzymes are required that participate in the formation of α-1,6-branching points (Buléon *et al.*, 1998; Sivak & Preiss, 1998; Tegge, 2004). The scheme for starch biosynthesis is depicted in figure 2.7.

2.1.4 Raw material for starches

The most significant sources for starches in the industry are maize, potato, tapioca, and wheat. Other raw materials for the starch production include rice, sago, yam, arrowroot, sorghum, lotus root, water chestnut, mung beans, round pea, rye, barley, lentil (Stute, 1985; Belitz *et al.*, 2001), and chestnut (Gassner *et al.*, 1989).

In wheat, the starch granules are situated in the endosperm of the wheat kernel, also called caryopsis, containing about 60 % starch. The starch is produced out of wheat flour by separating it with water from the gluten in a Martin process, a batter process or a slurry process (e.g. Westfalia process). Wheat starch is the only starch being composed of two fractions of starch granules, the larger granules of the A-starch (25-50 µm) and the small granules of the B-starch (2-15 µm) (Hoseney, 1986; Tegge, 2004).

Potato tubers are enlarged underground stems of the plant *Solanum tuberosum* and contain about 19 % starch, which is unevenly distributed throughout the tuber and is mainly

located close to the vascular bundles. Potato starch is produced by wet milling using a process known as rasping. It consists of comparatively large granules (< 100 µm), is quite sensitive to alkali and acid and contains approximately one phosphate ester group per 200 to 400 anhydroglucose units which gives a slight anionic character (FoodStarch.com, 2004; Tegge, 2004).

Tapioca starch, also called manioc or cassava starch, is produced from the tuber-shaped roots of *Manihot utilissima* and *Manihot palmata* containing 22-31 % starch. The tapioca starch production process is similar to the process of potato starch production but tapioca has to be processed within 24 h after harvest due to fast spoilage while potato can be stored for a longer period (Tegge, 2004). The starch production process is not to be further detailed here.

Some properties of wheat, potato, and tapioca starches are listed in table 2.1.

Tab. 2.1. Characteristics of wheat, potato, and tapioca starch granules (Tscheuschner, 1996; Belitz *et al.*, 2001)

Type of starch	Wheat	Potato	Tapioca
Granule size [µm]	< 45	< 100	< 35
Diameter [µm]	2-38	15-100	5-35
Average diameter [µm]	8	27	15
Number of granules per g [$x10^6$]	2 600	60	500
Number of starch molecules per granule[$x10^{12}$]	5	50	4
Specific surface area [m²/ kg]	500	100	200
Amylose content [%]	26-31	21-23	17
Amylopectin content [%]	72	79	83
Gelatinisation temperature [°C]	53-65	58-66	52-64
Water content [%]	13	19	13
Protein [%]	0.30	0.06	0.10
Fat [%]	0.80	0.05	0.10
Ash [%]	0.20	0.40	0.20
Phosphorous [%]	0.06	0.80	0.01

2.2 Thermal starch gelatinisation

When dry, native starch granules are placed in water at 0-4°C, water is absorbed and penetrates the granule. Starch can hold up to 30 % of its dry weight as moisture. The granule

swells slightly (about 5 %) and this change in volume and water uptake are limited and reversible, and heating the system to just below its gelatinisation temperature will not cause any other changes (French, 1984; Hoseney, 1986). However, further heating to temperatures above the gelatinisation temperature results in irreversible changes. Heating of starches containing a limited water amount leads to melting of starch crystals with loss of crystallinity. The melting temperature depends on the water content and lies above 100°C at a low moisture content. In excess water, the melting is accompanied by a hydration and an irreversible swelling of the starches. The viscosity increases with increased heating and the starch granule simultaneously loses its birefringent properties (French, 1984; Hoseney, 1986). The rise in viscosity of starches upon heating in water is the result of starch taking up water and swelling substantially. With continued heating, the starch granules become distorted and soluble starch is released into solution. The soluble starch and the continuous water uptake of insoluble remnants of the starch granules are responsible for the increase in viscosity (Hoseney, 1986). The complete solubilisation of starch granules appears to be dependant only on the treatment temperature, not on an interaction of treatment time and temperature, because holding starch at a constant temperature for a period of time does not increase its solubility. In order to enhance the solubility of starches, the temperature must be raised (Hoseney, 1986). However, Muhrbeck and Svensson (1996) observed that the gelatinisation temperature increased with increasing annealing time (up to 1280 min, 50°C).

Consequently, gelatinisation is regarded as the hydration and irreversible swelling of the granule, the destruction of molecular order within the starch granule, and starch solubilisation and can also be interpreted as the melting of starch crystals (Zobel, 1984; Atwell *et al.*, 1988). This is confirmed by calorimetric examinations showing gelatinisation endotherms (first-order thermal transitions). Although each granule separately gelatinises rather abruptly, a large amount of starch granules of the same species gelatinises over a temperature range of about 8-10°C which is characteristic for each starch species (Snyder, 1984). The gelatinisation process is therefore divided into the onset temperature, the peak temperature and the conclusion or offset temperature. The onset temperature of gelatinisation determined by differential scanning calorimetry (DSC) is in good agreement with values detected by measurements of loss of birefringence. However, DSC measurements demonstrate that yet important structural changes take place after loss of birefringence (Zobel, 1984). Svensson and Eliasson (1995) examining the thermal gelatinisation of potato and wheat starch in limited water found evidence for a two-phase gelatinisation process. First a hydration of amorphous regions of the granule accompanied by a minor reduction in

crystallinity occurred, and secondly melting of starch crystals up to a total loss of crystallinity took place.

2.2.1 Impact of solutes on starch gelatinisation

Water plays an important role in the process of thermal starch gelatinisation as the gelatinisation temperature decreases with increasing water content of starch suspensions. Other liquids than water (e.g. liquid ammoniac, dimethylsulfoxide, formic acid, chloroacetic acid) also influence starch gelatinisation by rupturing hydrogen bonds within the starch granule or by forming soluble complexes with starch. Additionally the presence of alkali, salts, sugars, lipids, alcohol, organic acids and their salts have an impact on the gelatinisation temperature and thus affect the extent of gelatinisation (Zobel, 1984).

The impact of sucrose on the thermal gelatinisation of several starches has been investigated and it was discovered that the gelatinisation temperature increased with increasing sucrose concentration (Evans & Haisman, 1982; Spies & Hoseney, 1982; Chinachoti *et al.*, 1991; Ahmad & Williams, 1999; Jang *et al.*, 2001; Maaurf *et al.*, 2001). Sucrose also caused a rise in pasting temperature (D'Appolonia, 1972) and an increase in starch melting enthalpy (Ahmad & Williams, 1999; Baek *et al.*, 2004). Other low-molecular sugars such as fructose, glucose, maltose etc. exhibited equal effects on thermal starch gelatinisation but influenced the gelatinisation characteristics to different extents (Evans & Haisman, 1982; Beleia *et al.*, 1996; Ahmad & Williams, 1999). This inhibitory effect of sugars on starch gelatinisation can be attributed to the reduction of mobility of the solvent and reduction of the a_W-value thereby impeding the penetration of water into the granule. Spies and Hoseney (1982) suggested that sugar-starch interactions stabilise amorphous regions by sugar molecules forming bridges between starch chains and hence increase the energy requirement for starch gelatinisation. Hansen *et al.* (1989) using carbon-13 nuclear magnetic resonance found evidence for sugar-starch interactions during heating shortly before the onset temperature of starch gelatinisation. Tomasik *et al.* (1995) verified the formation of complexes of low-molecular sugars with starch by polarimetric measurements and concluded that these inclusion complexes develop by penetration of sugar molecules into the starch interior opened by starch swelling.

In the presence of sodium chloride, the gelatinisation enthalpy decreases with increasing salt concentration whereas the gelatinisation temperature rises at NaCl concentrations up to 2M (Sandstedt *et al.*, 1960; Maaurf *et al.*, 2001) and ca. 5 % (Evans & Haisman, 1982) and subsequently decreases sinking below the gelatinisation temperature of pure starch suspensions at a NaCl concentration of 5M (Sandstedt *et al.*, 1960; Maaurf *et al.*, 2001) and approx. 20 % (Evans & Haisman, 1982). Bello-Péres and Paredes-López (1995) examined the influence of sucrose and NaCl on the gelatinisation temperatures of amylopectins of different botanical origin. Their results were analogous to investigations of starches.

The wide variation of effects of salt solutions on the gelatinisation temperature of starches has been shown by several authors (Sandstedt *et al.*, 1960, Gerlsma, 1970; Evans & Haisman, 1982; Jane, 1993). Sandstedt *et al.* (1960) discovered that salt solutions not only shifted the gelatinisation onset temperature but also induced changes in the gelatinisation pattern. According to Gough and Pybus (1973) the capability of salts to influence the gelatinisation temperature depends on their impact on the structure of water. Strongly hydrated ions increase the order of structure of water and simultaneously decrease its capability to gelatinise starch. In contrast, weakly-hydrated ions disrupt the structure of water and facilitate gelatinisation. Consequently, fluorides elevate whereas iodides depress the gelatinisation temperature. Chlorides only have small effects on the structure of water. Thus the increase in gelatinisation temperature at low concentrations is osmotic in origin due to the lowering of the water activity. With increasing chloride concentrations the influence of the partially hydrated ions on the gelatinisation process increases, by playing an increasing role in the attack on the starch granules, and hence the gelatinisation temperature diminishes. At the highest salt concentrations, virtually no penetration of the granule by the solution occurs and erosion proceeds in terms of a pitting corrosion similar to enzyme attack.

Gough and Pybus (1973) discovered three thermal gelatinisation types of wheat starch in the presence of chlorides at high concentrations. In correspondence to results of other research groups mentioned above, they also observed that an increasing chloride concentration first led to a rise, then to a depression and then again to a rise in gelatinisation temperature. Jane (1993) explored the mechanism of starch gelatinisation in neutral salt solutions. The author concluded that the process of gelatinisation of starches in the presence of salts is influenced by two effects: First, in agreement with Gough and Pybus (1973) by water structure-making and structure-breaking effects of ions of high and low charge density,

respectively, and secondly by electrostatic interactions between the salts and hydroxyl groups of the starches (Fig. 2.8).
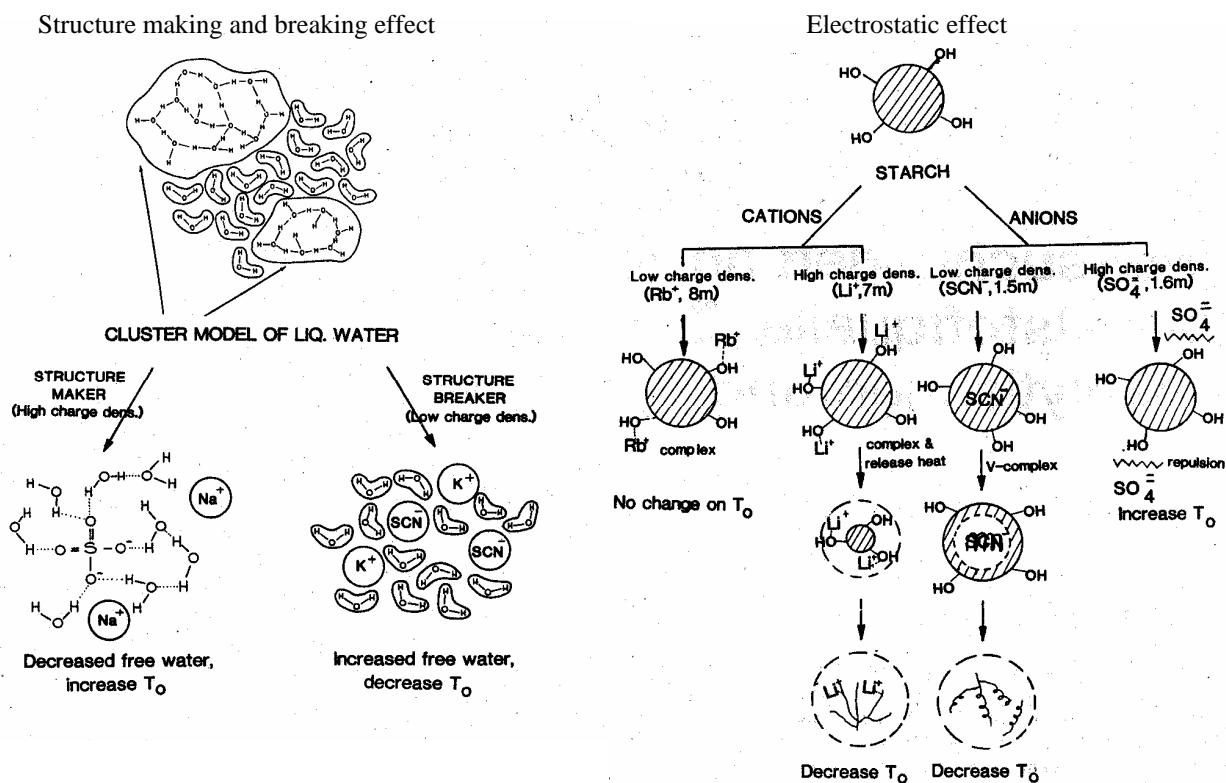
Fig. 2.8. Proposed mechanism of neutral salts affecting starch gelatinisation (Jane, 1993).

Under alkaline conditions starch gelatinises at lower temperatures (Zobel, 1984) and retains its birefringent character despite extensive swelling, deformation and bursting of the granules. This indicates that the mechanisms of merely thermal and alkaline gelatinisation differ (Wootton & Ho, 1989).

The gelatinisation temperature is not only affected by external influence factors but also by the chemical composition of the starch. For example phosphate groups characteristic for potato starch have a retarding effect on gelatinisation properties of potato starch. The higher the degree of phosphorylation, the higher is the gelatinisation temperature (Muhrbeck & Svensson, 1996). Further, Yook *et al.* (1994) discovered that the gelatinisation temperature decreased with increasing degree of substitution of cationised of pea and corn starch.

2.2.2 Monitoring starch gelatinisation

There are numerous methods for the determination of (thermal) starch gelatinisation. The optical analysis by a light microscope is by far the simplest method both in equipment and in application. When starch is heated above its gelatinisation temperature, the granules gelatinise with almost simultaneous loss of the polarisation crosses (Snyder, 1984). This loss in birefringence can easily be observed by light microscopy and simply requires a microscope equipped with polarising attachments and a heating stage (Zobel, 1984). A drop of the starch suspension with a concentration of 0.1-0.2 % is put on a slide and surrounded by oil before the cover glass is applied. This oil barrier is to prevent the development of steam and thus to ensure undisturbed vision. The slide is mounted on the hot stage which is placed on the microscope stage. The temperature of the starch suspension is increased by a constant heating rate of about 2°C per minute starting 20°C below the lowest temperature of the gelatinisation range of the starch. About 100 to 200 starch granules are observed with a magnification sufficient enough to clearly distinguish the polarisation of the smallest granules. Upon heating, the granules are observed and the temperatures corresponding with 2, 10, 25, 50, 75, 90 and 98 % loss of birefringence are recorded (Watson, 1964). For very small granules or granules showing only weak birefringence, granule swelling can also be used as a criterion for gelatinisation where it is a function of temperature and grade of swelling (Zobel, 1984).

Until recently the microscopic analysis in high hydrostatic pressure research was limited to observations before and after the pressure treatment, where the samples were investigated with a microscope before and after the pressurisation step (Begg *et al.*, 1983; Shimada *et al.*, 1993; Sato & Kobori, 1995). Snauwaert and Heremans (1999) and Rubens *et al.* (1999) observed pressure-induced starch gelatinisation in situ in a diamond anvil cell, Douzals *et al.* (1996) in a high pressure microscope. The chair of process engineering of disperse systems at the Technical University München (Freising-Weihenstephan, Germany) in cooperation with the mechanical engineering company Record Maschinenbau GmbH (Königsee, Germany) developed the so called Hartmann, Pfeifer, Dornheim, Sommer - High Pressure Cell (HPDS High Pressure Cell) enabling microscopic in situ analyses of pressure-induced starch gelatinisation under pressures of up to 300 MPa (Hartmann *et al.*, 2003).

It is additionally possible to visually monitor thermal starch gelatinisation by structural changes detected by a scanning electron microscope (SEM). This technique is convenient for granules maintaining integrity throughout the swelling and gelatinisation process (Zobel, 1984). Other complex, physical methods based on optical detection for starch gelatinisation

include light transmission and laser light scattering. The increase in transparency of starch suspensions upon gelatinisation can be determined by light transmission whereas changes in scattering of a polarised laser beam are directly related to changes in the supermolecular organisation of starch macromolecules during gelatinisation (Zobel, 1984). Since high pressure cells with windows exist, this monitoring method appears applicable for pressure induced starch gelatinisation. However, it needs to be determined whether measurable changes in light transmission or light scattering correlating with pressure-induced starch gelatinisation occur during pressurisation of starches.

Differential scanning calorimetry (DSC) is used to evaluate the thermal properties of food samples (Kolbe *et al.*, 1999) where the difference of heat flows between the sample and a reference sample as a function of temperature and time is determined and the heat (reaction enthalpy) absorbed or released by the sample corresponds with the peak area of the DSC curve. The measurement of starch gelatinisation by DSC is based on the detection of endothermic effects such as crystal melting as a result of heating at a constant heating rate (Hemminger & Cammenga, 1989). When starch/ water systems with excess amount of water are heated up to 150°C, two endothermic peaks become visible on the DSC curve, whereas three endothermic peaks are observed for starch/ water mixtures with limited amount of water. The first peak is identified as moisture-mediated disorganisation of starch crystallites, the second peak represents the melting of remaining crystals and is only observed for starch samples with limited amount of water, the third peak is assumed to be attributed to order-disorder-transition of amylose-lipid complexes. Consequently, only the first two peaks are responsible for starch gelatinisation (Fukuoka *et al.*, 2002).
Muhr *et al.* (1982) performed high pressure differential thermal analysis (HPDTA) of starch suspensions and obtained gelatinisation peaks. Consequently, Calorimetric analysis under high pressure is feasible.

Since thermal gelatinisation is accompanied by an increase in viscosity (French, 1984; Hoseney, 1986), the thermal gelatinisation process can also be monitored by viscosimetry using a Brabender Viscoamylograph containing a temperature-controlled rotating bowl and a sensor measuring sample viscosity. The resulting viscogram provides information of the rheological properties of the starch sample as well as beginning of gelatinisation, gelatinisation maximum and temperature (Brabender, 2000). Apparatuses for the online-

measurement of viscosity under pressure are not yet established but the realisation appears feasible.

Wang and Sastry (1997) investigated the effect of starch gelatinisation on electrical conductivity during ohmic heating and detected changes in electrical conductivity synchronously with starch gelatinisation. The electrical conductivity increased with increasing temperature. Comparing the conductivity curves obtained with DSC thermograms, gelatinisation peaks with similar shapes and temperature ranges have been found. This indicated the potential to monitor starch gelatinisation by changes in electrical conductivity. Karapantsios *et al.* (2000) also concluded that conductance measurements had the potential for assessing the extent of starch gelatinisation and the electrical conductance technique of this research group was successfully employed for the on-line monitoring of starch gelatinisation (Sakonidou *et al.*, 2003). Chaiwanichsiri *et al.* (2001) stated as well that electrical conductivity measurements were an effective method to quantify thermal starch gelatinisation, also in on-line applications. The authors explained the rise in electrical conductivity simultaneously with thermal starch gelatinisation by an ion release from starch granules corresponding to the breakdown of crystalline structures. The beginning of the ion release was in accordance with the initiation of starch gelatinisation and the completion of ion release correlated with the disintegration of the starch granules and the total collapse of the crystalline structure. Since the granular structure is maintained after high hydrostatic pressure-induced gelatinisation and no disintegration of the starch granule is observed (see 2.3.3), it was uncertain whether this method could be adapted for the determination of pressure induced starch gelatinisation.

2.2.3 Gelation and Retrogradation

When thermally gelatinised starch pastes with a sufficiently high starch concentration cool down, a gel is formed. A gel is defined as a liquid system with properties of a solid, with a small amount of solid controlling a large amount of water (Hoseney, 1986). Gelation occurs as hydrated and dispersed starch molecules reassociate, i.e. as molecular bonds (e.g. hydrogen bonds) are formed that result in a network. Starches containing amylose normally gel quickly because linear amylose molecules associate more readily than branched amylopectin molecules. Gelation is therefore controlled by the contained amylose, no matter if it dominates or not (Zobel, 1984).

When a gel is ageing or freeze-thawed, starch chains tend to interact strongly and form hydrogen bonds between hydroxyl groups and thus "push" water out of the gel matrix. This forcing of water out of the gel is called syneresis. At longer storage times, the interaction between starch chains increases and eventually crystallisation takes place. This recrystallisation of starch chains within a gel is called retrogradation. With proceeding retrogradation, a gel becomes opaque, rigid and rubbery (Hoseney, 1986; Tegge, 2004). The process of retrogradation, especially amylopectin retrogradation (Fredriksson *et al.*, 2000), is also believed to be involved in the staling of bread and other baked goods (Hoseney, 1986) and the retrogradation behaviour differs depending on the botanical source of the starch (Jacobson *et al.*, 1997; Ottenhof *et al.*, 2005).

Amylose tends to retrograde and this process takes place in three steps: First an extension of amylose helices to linear molecules proceeds, which is followed by an alignment of these stretched amylose chains in groups and the formation of hydrogen bonds between hydroxyl groups of adjacent amylose molecules with a simultaneous release of water (Belitz *et al.*, 2001; Tegge, 2004). This process proceeds analogous for short branches of the amylopectin molecule and the super-helical structure of native starch is thus not regained during retrogradation (Keetels *et al.*, 1996).

In literature it is disputed whether amylose is taking part in starch retrogradation. While Keetels *et al.* (1996), Silverio *et al.* (2000), and Ottenhof *et al.* (2005) defined starch retrogradation as a recrystallisation of amylopectin, Krüsi and Neukom (1984), Jacobson *et al.* (1997), and Garcia-Alonso *et al.* (1999) stated that both starch components, amylose and amylopectin, are involved in the process of starch retrogradation with amylose undergoing retrogradation at a more rapid rate than amylopectin (Jacobson *et al.*, 1997; Fredriksson *et al.*, 2000).

2.2.4 Heat moisture treatment and annealing

The gelatinisation temperature can be altered by thermal treatments and it is differentiated between heat-moisture treatment and annealing.

Heat-moisture treatment (HMT) is characterised by an exposure of starch to higher temperatures above gelatinisation temperature at very limited moisture content of about 18-27 %. The low level of water leads to an elevation of the glass transition temperature (T_g), a physical reorganisation, and an increase in gelatinisation temperature. Although temperatures

above gelatinisation temperature are applied, the starch does not gelatinise during HMT. This is attributed to the restricted amount of water that reduces the molecular mobility and thus prevents gelatinisation (Tester & Debon, 2000). The X-ray diffraction pattern of B-type crystalline starches is changed from the B- to the C-type, i.e. a formation of B-type cells to A-type cells occurs (Banks & Muir, 1980; Stute, 1992). Consequently, starches with B-type crystallinity are much more sensitive to HMT than starches with C-type and A-type crystallinity (Stute, 1992). DSC measurements of heat-moisture treated starches result in a shift to higher gelatinisation temperatures, broader peaks and lower gelatinisation enthalpies. Additionally, sorption isotherms of heat-moisture treated starches exhibit a shift to lower relative humidity (Stute, 1992).

Annealing is defined as a physical reorganisation of starches in excess water at temperatures below gelatinisation temperature but above the glass transition temperature T_g. It is a process that retains granular structure and original order (Jacobs *et al.*, 1998; Tester & Debon, 2000). As upon HMT, a rise in gelatinisation temperature and T_g can be observed upon annealing (Stute, 1992; Tester & Debon, 2000). Additionally, the homogeneity of the starch increases as indicated by narrower DSC peaks. Since X-ray patterns of native and annealed starches are unchanged, the crystal type as well as the crystallinity appears to remain unaltered (Stute, 1992). However, small angle X-ray scattering studies reveal a lower electron density either in the crystalline or amorphous regions of the starch granule as indicated by more pronounced peaks. The repeat distance of the crystalline and amorphous lamellae stays unmodified as implied by unchanged peak positions (Jacobs *et al.*, 1998).

The granule swelling and acid hydrolysis are reduced by annealing. The enzymatic hydrolysis of annealed in respect to native starches is reduced with amyloglucosidase and enhanced with α-amylase. Although amorphous and crystalline lamellae become more ordered, it appears as if the accessibility of the amorphous regions by enzymes is generally facilitated (Tester & Debon, 2000).

2.3 High hydrostatic pressure

At the turn of the last century the capability of high hydrostatic pressure to preserve milk in combination with moderate temperatures (Hite, 1899) and to denature egg albumin (Bridgman, 1914) has been discovered. Since that time, a lot of research has been carried out

in the field of high hydrostatic pressure processing and many useful and pressure specific applications have been found. One of those utilisations of high pressure is the pressure-assisted heating, especially of immiscible foods, where the temperature is increased almost homogeneously throughout the product due to the heat of compression. By this, one of the major drawbacks of conventional heating, where the temperature of the boundary layer of the product exceeds critical temperatures in order to reach the sufficient temperature for microbial inactivation in the centre of the product, can be overcome (Heinz & Knorr, 2002). High hydrostatic pressure is also a potential tool for the improvement as well as the generation of textural properties of food products.

2.3.1 Fundamentals

High hydrostatic pressure treatment is carried out batchwise or semi-continuously. The packed product is inserted into a cylindrical, low alloy steel vessel and pressure is built-up directly or indirectly. For direct, piston-type compression, the pressure medium inside the vessel is pressurised by a piston driven by a low pressure pump at the large diameter end of the piston. The pressure is transferred into the vessel by the small diameter high pressure vessel end of the piston where high pressure is generated by multiplying the low pressure by the ratio of the two piston diameters. For indirect pressurisation, a high pressure intensifier is used to pump pressure medium from a reservoir into the closed high pressure vessel. Thereby the same hydraulic principle of pressure generation is utilised by the intensifier as by the piston for the direct pressurisation (Deplace & Mertens, 1992).

Requirements for the packaging materials for high hydrostatic pressure treatment include flexibility, pressure tightness, heat sealability, by pressure unmodifiable mechanical and gas barrier, and non-toxic, aromatic-proof and sanitary properties (Masuda *et al.*, 1992; Ochiai & Nakagawa, 1992).

Due to a resulting volume decrease upon pressurisation, any phenomena such as chemical reactions, phase transition or molecular configuration changes that are accompanied by a volume reduction are enhanced by high hydrostatic pressure according to Le Chateliers Principle. Consequently, high pressure affects non-covalent bonds (hydrogen, ionic and hydrophobic bonds) and some specific covalent bonds (Tewari *et al.*, 1999).

The compression of the pressure medium and the food product is accompanied by heating, an increase in temperature of about 2-3°C/ 100 MPa occurs at adiabatic conditions. If it is aimed for isothermal conditions, the pressure vessel can be temperature controlled in

order to dissipate heat upon compression and supply heat upon decompression. Otherwise a temperature field is formed because of slow heat conduction in comparison to a fast impulse transport of pressure acting uniformly throughout the vessel (Pfister *et al.*, 2001). It is thus useful to integrate an indicator into the vessel that not only gives information about the pressure applied but also about the temperature conditions during the process.

2.3.2 Pressure time temperature integrator

With the growing demand in nutritionally valuable, fresh-like foods, conventional heating is no longer regarded sufficient to fulfil consumers' demands because heating of potentially sensitive foods might lead to undesired changes in their nutritional, functional and organoleptic quality. Consequently, non-thermal decontamination methods for pasteurisation and sterilisation, which minimise the destructive influence of heat, are developed (Knorr & Heinz, 2001). Altogether, a microbiologically safe pressure treatment is possible (Smelt *et al.*, 2002), for example Yen and Lin (1996) observed that guava puree treated at 600 MPa at 25°C for 15 min retained good quality after storage at 4°C for 40 days and Ogawa *et al.* (1992) found that citrus juice pressurised at 400 MPa at 40°C for 10 min was not spoiled during 2 to 3 months storage. However, control indicators depending on pressure, temperature and treatment time must be integrated into the process to evaluate the high pressure process and thus ensure microbiological safety and stability of the product and obtain regulatory approval.

In thermal processing the impact of the heat treatment is quantified by the application of so-called time temperature integrators (TTIs). These are heat sensitive components extrinsic or intrinsic to the food product that enable measuring directly and quantitatively the impact of the process without knowledge of the actual thermal history (Claeys *et al.*, 2003). Such concepts are also required for pressure processes.

Claeys *et al.* (2003) investigated whether intrinsic components present or formed in milk enabled a direct and quantitative measurement of the impact of the pressure temperature process applied acting as pressure time temperature integrator (PTTI). The authors concluded that the data available regarding high pressure processing of milk was insufficient. Furthermore, Minerich and Labuza (2003) developed a pressure indicator for high pressure processing comprised of a compressed powdered copper tablet decreasing in density with increasing pressure and increasing treatment time. No significant impact of temperature on the copper density was found which limits the application of the copper tablets as PTTIs.

2.3.3 Pressurisation of starch suspensions

Accessorily to the thermal gelatinisation, the crystalline order of starches can also be destroyed by mechanical means such as plastic deformation due to milling e.g. in a swing mill (Meuser *et al.*, 1978) and high hydrostatic pressure treatment (French, 1984). A high pressure treatment up to 150 MPa increases the gelatinisation temperature (Thevelein *et al.*, 1981), above 150 MPa the gelatinisation temperature is decreased (Muhr *et al.*, 1982). Muhr and Blanshard (1982) found a gelatinisation of wheat starch in excess water at ambient temperature and 450 MPa. The pressure range in which gelatinisation occurs is typical for each starch (Stute *et al.*, 1996) and partly depends on their crystalline structure e.g. B-type starches are more resistant to pressure than A- and C-type starches (Muhr & Blanshard, 1982; Ezaki & Hayashi, 1992; Stute *et al.*, 1996; Rubens *et al.*, 1999).

Snauwaert and Heremans (1999) and Rubens *et al.* (1999) observed pressure-induced starch gelatinisation in situ in a diamond anvil cell, Douzals *et al.* (1996) in a high pressure microscope. The authors discovered swelling of starch granules during pressurisation. Douzals *et al.* (1996) also observed further swelling of the granules i.e. an increase in granule volume after pressure release. The authors also detected a decrease in volume of the starch suspension during pressurisation, which partly remained after pressure release, and assumed that starch molecules linked with water occupy less volume than suspended in pure water and therefore the granule hydration would be preferential under pressure according to Le Chateliers Principle. Douzals *et al.* (1996) also viewed pressurisation of iodine stained starch granules and observed a decolouration of the swelling kernels which was regarded as an indication of starch melting.

Rubens *et al.* (1999) proposed a two step-mechanism for pressure-induced gelatinisation similar to the thermal gelatinisation process (Svensson & Eliasson, 1995). In the first step the amorphous regions were hydrated causing a swelling of the granules and a distortion of crystalline regions and in the second step of pressure-induced gelatinisation, the crystalline regions became more accessible to water. Thus under pressure a hydration of starch occurred before changes in crystallinity proceeded during gelatinisation.

As depicted in figure 2.9, the pressure-induced gelatinisation and the thermal gelatinisation differ in other respects e.g. evident by different rheological properties and microstructure of the starches (Stolt *et al.*, 2001). Typical for most pressure-gelatinised starches e.g. as for wheat starch is the limited swelling of the melted granule (up to twice in diameter) and the maintaining of the granular character (Fig. 2.9). However, tapioca starch exhibits an exceptional swelling behaviour expanding six fold in diameter at 600 MPa. (Stute

et al., 1996). Furthermore according to Douzals *et al.* (1998) there is only little and after Stute *et al.* (1996) and Stolt *et al.* (2001) sometimes even no amylose release. Pressurised starch suspensions are more condensed, with a different water binding capacity (Douzals *et al.*, 1998). X-ray diffraction patterns of untreated and pressurised A-type starches in the presence of water show a transformation from the A- to the B-type X-ray diffraction pattern (Hibi *et al.*, 1993; Katopo *et al.*, 2002).
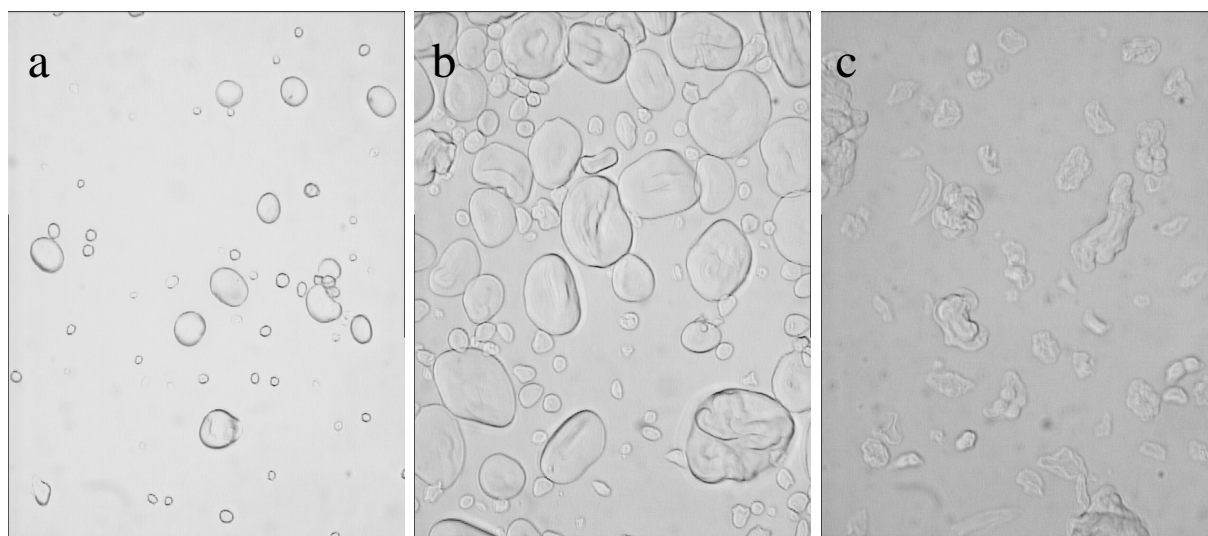
Fig. 2.9. Wheat starch suspensions (5 % w/ w, x 400) native (a), 15 min at 500 MPa, 20°C (b), 15 min at 0.1 MPa, 90°C (c).

Moreover water plays an important role in the high pressure-induced gelatinisation of starches. Suspended in alcohol even at very high pressures (up to 3 GPa) starch granules do not swell (Snauwaert & Heremans, 1999). Katopo *et al.* (2002) suggested that ethanol had a space filling effect stabilising the crystallinity of starches. And according to Stute *et al.* (1996), a high moisture content is required for ultra high pressure gelatinisation. Other process parameters besides water content and type of starch influencing the gelatinisation procedure are pressure, temperature and treatment time.

High hydrostatic pressure also influences the texture of starch suspensions or gels. The storage moduli of 10 % potato starch suspensions and 25 % barley starch suspensions increase with increasing treatment time at constant pressures until a constant G´ value is reached. And the higher the applied constant pressure, the faster the storage modulus increases with increasing treatment time (Stolt *et al.*, 2001; Michel & Autio, 2003). For waxy maize starch suspensions, the storage modulus first increases and then decreases at pressures above 500

MPa with increasing pressurisation time (Stolt *et al.*, 1999). The authors therefore assumed that excessive pressurisation weakens the structure of waxy maize starch gels. Stolt *et al.* (2001), investigating the effect of high pressure on barley starch suspensions with increasing treatment time, determined that consistency index, melting enthalpies and DSC peak temperatures increased with increasing pressure and pressurisation time. Consequently, a relationship between physical starch properties and pressure and time has been observed.

Hayashi and Hayashida (1989) reported an increased amylase digestibility of pressure-treated wheat starch at elevated temperatures relative to alkali treated starch. However, they also discovered the formation of a new, amylase resistant starch structure during long-term pressurisation (17 h). The glucoamylase digestibility rate of pressurised starches was also enhanced at elevated temperatures analysed immediately after pressure treatment and compared to raw starches but did not differ notably relative to thermally treated starches (Takahashi *et al.*, 1994). However, the overall glucose yield by amyloglucosidase hydrolysis of gelatinised wheat starches was considerably improved by pressure-induced gelatinisation compared with thermal gelatinisation (Selmi *et al.*, 2000). Ezaki and Hayashi (1992) observed a slower retrogradation of pressurised starches in comparison to thermally gelatinised starches determined by enzyme digestibility and iodo-starch reaction. These results were in accordance with findings made by Douzals *et al.* (1998). They discovered a quantitatively lower retrogradation of pressure-induced wheat starch gels showing to be less sensitive to ageing and less sensitive to storage conditions known to be favourable for starch retrogradation (4°C). This could open up novel applications of pressure treated starches in bread to reduce bread staling. On the other hand, Stolt et al (2001) observed comparable retrogradation behaviour of heat-induced and pressure-induced starch gels. DSC-investigations of pressurised wheat starch revealed the development of a retrogradation peak showing a faint B-pattern after a pressurisation step leading to the assumption of a rapid retrogradation during or instantaneously after the high pressure treatment (Stute *et al.*, 1996). Katopo *et al.* (2002) presumed that a pressure-induced rearrangement of double helices had occurred in the A-type starch represented by this additional peak. It was therefore interesting whether the starch fraction causing this additional DSC peak observed immediately after pressurisation by Stute *et al.* (1996) and Katopo *et al.* (2002) contributed to the resistant starch (RS) content and thus could prove high pressure to be a feasible alternative for the RS production.

2.4 Resistant Starch

2.4.1 Definition and properties

Several studies have demonstrated that there is a starch fraction resistant to enzymatic digestion in the small intestine (Englyst & Macfarlane, 1986; Englyst & Cummings, 1987; Asp *et al.*, 1992; Tovar *et al.*, 1992; Faisant *et al.*, 1993). The research group of EURESTA (European Resistant Starch research group) defined resistant starch (RS) as the sum of starch and products of starch degradation not absorbed in the small intestine of healthy individuals (Asp, 1992). RS is further partitioned in physically inaccessible starch (RS 1), in resistant starch granules (RS 2), in retrograded starch (RS 3), of which only retrograded amylose is totally

Fig. 2.10. Schematic depiction of RS 1, RS 2 and RS 3 (Cerestar, 2003).

resistant (Fig. 2.10; Englyst & Macfarlane, 1986; Englyst & Cummings, 1987; Englyst *et al.*, 1992; Englyst *et al.*, 1996) and in chemically modified starch (RS 4) (Brown *et al.*, 1995). Analyses indicate that RS consists of crystallised, linear, unbranched, short-chain α-glucans (DP approx. 60-65) (Berry *et al.*, 1988; Siljeström *et al.*, 1989). Since amylose-lipid complexes significantly reduce the availability to α-amylase, an interrelation of complexation with lipids and resistant starch formation is assumed but it is verified that amylose-lipid complexes are not involved in the formation of RS (Sievert & Pomeranz, 1989; Siljeström *et al.*, 1989). However, it was discovered that a removal of lipids from the starch resulted in higher RS yields. This is probably due to an increase of lipid-depleted "free" amylose crystallised (Berry *et al.*, 1988; Cerletti *et al.*, 1993). Derived from heat treated starches, RS displays endothermic transitions between 120 and 177°C in DSC studies that seem to be due to melting of recrystallised amylose (Sievert & Pomeranz, 1990). RS exhibits a B-type X-ray diffraction pattern at low retrogradation temperatures and a mixture of A-type with B-type or V-type pattern if retrograded at high temperatures (95°C) (Siljeström *et al.*, 1989; Sievert *et al.*, 1991; Eerlingen *et al.*, 1993b; Shamai *et al.*, 2003).

Ranhotra *et al.* (1996a) investigated the energy value of RS with rats as test models and concluded that RS provided no energy, whereas Mathers (1992) and Behall and Howe (1996) calculated the mean energy value of RS to be 9-9.5 and 11.7 kJ/ g RS, respectively. Additionally, RS is tasteless, has no satiating effect and acts as a mild laxative (Watzl &

Leitzmann, 1999; Rössler *et al.*, 2002). Nevertheless, RS has several beneficial health effects. Intake of RS improves the intestinal flora by reducing intestinal pathogen levels and promoting colonisation of lactobacilli and bifidobacteria, elevates colonic butyrate levels, stimulates the immune system, and reduces energy intake when substituted for normal starch (Brouns *et al.*, 2002). A RS containing diet may also reduce the risk of colon cancer due to the production of high amounts of butyrate (Watzl & Leitzmann, 1999; Brouns *et al.*, 2002; Puupponen-Pimiä *et al.*, 2002). Schulz *et al.* (1993) stated that RS2 enhanced the magnesium and calcium absorption in rats. Ranhotra *et al.* (1996b) found lowered serum total cholesterol and lowered serum triglyceride levels but also increased liver cholesterol and liver lipid levels in hamsters at a high level of intake of RS.

2.4.2 Resistant starch production

Liljeberg Elmståhl (2002) determined the RS content of starchy foods on the Swedish market and estimated an average daily RS intake of 3.2g in a Swedish diet. Due to its health benefits, it was aimed to develop a RS production process and thus to enhance the RS content in food. Beforehand a standardised determination method had to be invented and validated. Several *in vitro* methods were utilised (Englyst *et al.*, 1982; Berry, 1986; Björck *et al.*, 1987; Englyst *et al.*, 1992; Saura-Calixto *et al.*, 1993; Englyst *et al.*, 1996; Goñi *et al.*, 1996) before Mc Cleary and co-workers developed an official AOAC method for the determination of RS in plant and starch materials (McCleary & Monaghan, 2002; McCleary *et al.*, 2002). This method was applied for our experiments.

The increase of RS content in starches is based on the recrystallisation, i.e. retrogradation of amylose subsequent to thermal gelatinisation. A favoured approach for the enhancement of RS content was an autoclaving step prior to cooling and/ or drying (Berry, 1986; Berry *et al.*, 1988; Siljeström *et al.*, 1989; Eerlingen *et al.*, 1993a; Escarpa *et al.*, 1996; Shamai *et al.*, 2003). Sievert and Pomeranz (1989) further increased the RS yield by up to 20 autoclaving-cooling cycles. Another proceeding for the RS production was an enzymatic debranching of gelatinised starch or starch degradation product followed by a drying step (Chiu *et al.*, 1994; Kettlitz *et al.*, 2000). Factors influencing the yield of RS were the gelatinisation temperature, treatment steps altering the chemical composition of the starches like defatting and debranching by acid hydrolysis or enzyme hydrolysis, the storage temperature, the storage time, and combinations of treatments e.g. freeze-thawing, freeze-

drying, annealing subsequent to acid hydrolysis and autoclaving-storing-cycles (Sievert & Pomeranz, 1989; Eerlingen *et al.*, 1993a; Vasanthan & Bhatty, 1998; Chung *et al.*, 2003).

2.5 Aim

The main intention of this work was to gain knowledge of the impact factors and mechanism of pressure-induced starch gelatinisation of wheat, tapioca, and potato starches, differing in pressure sensitivity. This included fundamental research on the pressure-induced starch gelatinisation, i.e. the influence of temperature, pressure and pressurisation time, and a pressure-temperature phase diagram was to be generated. In this regard it was further aimed to determine the applicability of pressure-induced starch gelatinisation of starch suspensions as a pressure time temperature integrator (PTTI).

With the assistance of the newly developed HPDS High Pressure Cell (Hartmann *et al.*, 2003) in combination with a microscope, it was aimed to validate the efficiency of this high pressure cell for monitoring pressure-induced starch gelatinisation and furthermore to gain visual insight into the process of pressure-induced starch gelatinisation.

Since the determination of degree of gelatinisation by loss of birefringence via a microscope is time consuming and laborious, it was searched for quicker and easier ways to measure starch gelatinisation. Although the use of electrical conductivity to monitor thermal gelatinisation of starches is well established (Wang and Sastry, 1997; Karapantsios *et al.*, 2000; Chaiwanichsiri *et al.*, 2001; Sakonidou *et al.*, 2003), it was unknown if this method could be applied to the measurement of pressure-induced starch gelatinisation due to the retention of the granular character and because the rise in conductivity of a starch suspension with increasing temperature was attributed to an ion release and to amylose leaching out of the granule. In this work it was aimed to find out whether electrical conductivity measurements could be applied to pressure-induced starch gelatinisation of wheat, tapioca, and potato starch suspensions despite the differing gelatinisation process under high hydrostatic pressure as compared to thermal gelatinisation and whether a possible change in electrical conductivity and the gelatinisation process caused by high hydrostatic pressure treatment of starches corresponded.

In addition to process parameters such as temperature, pressure and pressurisation time, the effect of water content and of several salts and sugars on the pressure-induced gelatinisation of wheat starch, tapioca starch and potato starch was to be examined. The

possible change in pH with increasing pressure as well as with increasing salt concentration was neglected. Since the number of equatorial hydroxyl groups of sugars had shown to influence thermotolerance and barotolerance of yeast and proteins (Uedaira & Uedaira, 1980; Tamura, 1999), it was intended to investigate the impact of number of equatorial hydroxyl groups of several sugars on the pressure sensitivity of starches suspended in water. Furthermore the influence of sodium chloride, other chlorides and potassium and sodium salts on pressure induced starch gelatinisation was to be investigated.

It was also aimed to gain insight into the resistant starch (RS) formation of pressure-gelatinised wheat starch and to find treatment processes in combination with high hydrostatic pressure which enhanced the content of wheat starch components resistant to digestion in the small intestine, especially in comparison to merely thermal treatment processes. In this regard, it was searched for a treatment process including pressurisation that produced higher amounts of RS than a comparable RS production process without a high hydrostatic pressure treatment.

The statements in literature regarding the retrogradation behaviour of pressurised starches were contradictory, but a patent specification claiming a decreased firmness of pressurised starch containing bread upon storage (Codovilli, 2004) was found. It was therefore aimed to prove and if possible verify the claims made in this patent specification in order to show potential applications of pressurised starch in foods.

3   MATERIALS AND METHODS

3.1 Starches

The starches utilised included potato starch, wheat starch (both from Overlack, Mönchengladbach, Germany), and tapioca starch (Thai World Import & Export Co., Ltd., Bangkok. Thailand). Distilled water was used for the preparation of starch suspensions.

The commercial starches (Prejel VA70T, Ultra-Tex 2, Instant Clearjel E, C☆Hiform 12742, Paselli P) used to evaluate the generation of sorption isotherms were a kind gift of Dr. R. Stute.

3.2 Chemicals

Glucose was purchased from Sigma (St. Louis, MO, USA), lactose from Oxoid (Basingstoke, U.K.), melibiose from Schuchardt (München, Germany), melezitose from Serva (Heidelberg, Germany), and fructose, sucrose, galactose, mannose, maltose, sodium chloride, calcium chloride, lithium chloride, caesium chloride, potassium chloride, potassium iodide, potassium thiocyanate, potassium bromide, sodium sulphate and sodium hydroxide were purchased from Merck (Darmstadt, Germany). Trehalose was a kind gift from Cargill (Vilvoorde, Belgium).

3.3 Starch analysis

3.3.1 Specific density

The specific density was measured using the pycnometric method by a Multivolume Pycnometer 1305 (micromeritics, Norcross, GA, USA) at the department of food quality and material science, Berlin University of Technology.

3.3.2 Moisture content

The moisture content, and thus the dry matter content, was determined by gravimetric differences before and after 5h at 105°C in oven UT 6060 (Heraeus Instruments, Hanau, Germany).

3.3.3 Ash content

The ash content of the starches was quantified according to EN ISO 3593 (1994) using the oven M104 (Heraeus Instruments, Hanau, Germany).

3.3.4 Fat content

The fat content was quantified according to EN ISO 3947 (1994).

3.3.5 Amylose content

The amylose content of the starches was determined using the amylose/ amylopectin assay kit by Megazyme (Bray, Ireland).

3.3.6 Sorption Isotherms

Sorption isotherms of native, pressure-gelatinised and freeze-dried, and heat-gelatinised and freeze-dried starches were identified by a static gravimetric method. The starch samples were weighed, placed for at least three weeks in air-tight desiccators containing saturated salt solutions providing constant relative humidity environments at room temperature, and again weighed when equilibrium was reached. The moisture contents were calculated from differences in weight based on the dry matter content (Ch. 3.3.2). The relative humidity of each saturated salt solution was established using thermoconstanter novasina (MT-Technologies Meßtechnik GmbH, Gummersbach, Germany) and is shown in table 3.1.

Tab. 3.1. Relative humidity of saturated salt solutions at 25°C

Saturated salt solution	Relative humidity [%]
PO_5	1.9
LiCl	11.4
$MgCl_2$	34.2
$Mg(NO_3)_2$	53.3
NaCl	74.2
KCl	83.8
KSO_4	98.7

Complete pressure-induced gelatinisation of 5 % (w/ w) starch suspensions was achieved by a pressure treatment at room temperature for 15 min at 500 MPa for wheat starch, at 700 MPa for tapioca starch, and at 900 MPa for potato starch, and total thermal

gelatinisation was accomplished by a heat treatment for 15 min at 90°C with continuous manual stirring. The pressure-gelatinised and thermally gelatinised starch suspensions were freeze-dried using the Freeze-Dryer Modulyo (Edwards, Sussex, UK). Potassium sulphate was purchased by Carl Roth (Karlsruhe, Germany) the other salts were obtained by Merck (Darmstadt, Germany).

3.3.7 Water binding capacity

The water-binding capacity of starches and starch suspensions was determined according to Medcalf and Gilles (1965) using a Sorvall RC-5B Refrigerated Superspeed centrifuge (Du Pont Instruments, Bad Homburg, Germany).

3.3.8 Thermoanalytic investigation

The thermal properties of native, dry starches were determined by non-isothermal thermogravimetry (TG) combined with differential thermogravimetry (DTG) and differential scanning calorimetry (DSC) at the department of food quality and material science, Berlin University of Technology. The analysis was performed by a STA409C (Netzsch, Selb, Germany) with a heating rate of 10 K/ min in a temperature range from 0°C to 450°C in a platinum pan under anaerobic nitrogen atmosphere.

3.3.9 X-ray analysis

X-ray analysis of native starches and freeze-dried starch samples was performed at the Rheinische Friedrich Wilhelms-Universität Bonn, Pharmazeutische Technologie with a wavelength of 1.54 Å, a scanning speed of 0.02 °2Θ/ s and a scanning region of 5-45 °2Θ.

3.3.10 Degree of gelatinisation

The pressurised starch suspensions were analysed for their degree of gelatinisation by loss of birefringence via a microscope (Eclipse E400, Nikon, Tokyo, Japan) and by means of their electrical conductivity via a conductivity meter (LF 323, WTW, Weilheim, Germany). All measurements were carried out at least in duplicates and for the determination of degree of gelatinisation by loss of birefringence minimum 200 granules were counted.

3.4 High hydrostatic pressure treatment

Directly before the pressurisation step the samples were thoroughly shaken, quickly inserted into the vessel and pressurised in order to minimise sedimentation and avoid inhomogeneity throughout the sample. It was abandoned to add thickeners such as heat-gelatinised starch in order to increase the viscosity and thus prevent sedimentation, because it was discovered that the reduction of free water depleted the pressure-induced gelatinisation process. Therefore, sedimentation could not be entirely prevented but was neglected due to the good reproducibility of the results.

3.4.1 High pressure multivessel apparatus

Samples of approximately 1.5 ml were filled in Nunc CryoTubes (Nunc A/S, Roskilde, Denmark) and pressurised in a high pressure multivessel apparatus U111 (Unipressequipment, Warsaw, Poland) enabling operating pressures up to 700 MPa and being equipped with a temperature control (Huber CC 245 oil bath, Huber Kältemaschinen GmbH, Offenburg, Germany) generating temperatures between – 45 and 150°C. The pressure build-up was performed in approximately 12 s/ 100 MPa, the pressure release took about 17 s/ 100 MPa, and the pressure transmitting medium was silicon oil (type 6163, Huber, Offenburg, Germany).

The apparatus contains five high pressure vessels submerged in the oil bath and each separately connected with the high pressure pump. This allows pressure treatments at five different pressures for varying treatment times at the same temperature. The high pressure system of the apparatus consists of a high pressure intensifier, eleven manually operated valves (SITEC, Maur/ Zürich, Switzerland), connectors, capillary tubes and a hydraulic power unit (Mannesmann Rexroth Polska Ltd., Warsaw, Poland) composed of a radial piston oil pump, an electric motor controlled by a general-purpose Inverter Freqrol (Mitsubishi, Tokyo, Japan), a block of hydraulic valves and an oil tank with filter.

This apparatus was utilised for the generation of phase diagrams of wheat and tapioca starches, for the examination of pressure-temperature-time relationships on the degree of gelatinisation, and for the influence of sugars and salts on starch gelatinisation.

3.4.2 High volume-pressure unit I (National Forge)

For experiments requiring higher volumes, e.g. water binding capacity, electrical conductivity and production of RS, approx. 150 g of wheat and tapioca starch suspensions

were sealed in polyethylene coated aluminium bags and pressurised in a high pressure vessel (National Forge, St. Niklaas, Belgium) with a maximum operating pressure of 550 MPa, a volume of 800 ml and equipped with double-acting pump P100-05CX-01 (Hydro-Pac Inc., Fairview, PA) and a temperature control (Haake water bath, Karlsruhe, Germany) generating temperatures between 0 and 80°C. The pressure build-up was performed in approximately 7 s/ 100 MPa, the pressure release took about 17 s/ 100 MPa.

3.4.3 High volume-pressure unit II (Unipress)

For measurements requiring higher volumes and pressures above 550 MPa or generally pressures above 700 MPa, pressurisation was conducted by the high pressure single vessel apparatus U400 (Unipressequipment, Warsaw, Poland) with a maximum operating pressure of 1,000 MPa, a volume of approx. 0.75 l and a theoretically operable temperature range of –25 to 100°C. This unit is composed of a biphasic pressure build-up with the initial pump for pressures up to 600 MPa and the intensifier pump for the pressure build-up in the second phase from 600 to 1,000 MPa in combination with a pressure intensifier (transformation ratio 1:16). Up to 600 MPa, pressure build-up took about 5 s/ 100 MPa (30 s from 0.1 to 600 MPa), above 600 MPa it took about 21 s/ 100 MPa (200 s from 0.1 to 950 MPa). Pressure release occurred within about 2 s.

3.4.4 In situ analysis with a high pressure cell

The high pressure cell connected with a microscope is described in detail elsewhere (Hartmann *et al.*, 2003) and consisted of an inverse light microscope (Leica DM IRB HC, Germany), a CCD-camera (Basler, Germany) and the HPDS high pressure cell connected to a screw press. A computer with image analysis software (Aquinto AG, Germany) was coupled with the camera in order to evaluate and archive the pictures recorded.

Unstained and potassium iodide-iodine stained starch suspensions were filled in the HPDS cell onto the microscope stage as described by Hartmann, Pfeifer, Dornheim, and Sommer (2003) and during pressure build-up pictures were taken at 50 MPa intervals. At 300 MPa the pressure was held for one hour, while pictures were taken about every 10 min. In the process of pressure release again pictures were taken at 50 MPa steps. Back at atmospheric pressure the granules were observed another 10 min to detect whether further changes had occurred. The pressure build-up and the decompression required about 7 min, respectively. All experiments were performed at least in duplicates at room temperature. The temperature

increase due to pressure generation is negligible, since the heat is dissipated through the body of the high pressure cell during the slow pressure build-up.

The potassium iodide-iodine solution (Lugols solution) consisted of 0.2 g iodine (Sigma, Deisenhofen, Germany) and 2 g potassium iodide (Merck, Darmstadt, Germany) in 100 ml distilled water.

3.4.5 Statistical evaluation

The statistical evaluation of the impact of pressure and temperature on the degree of gelatinisation of starch suspensions (5 % w/ w) was performed using the software Statgraphics 4.1 at the institute of food quality and material science, Berlin University of Technology. Multifactor analysis of variance was chosen as evaluation tool.

3.5 Resistant starch production

3.5.1 Gelatinisation

For high pressure treatment, approx. 150ml of 5 % (w/ w) wheat starch suspensions sealed in polyethylene-coated aluminium bags were pressurised in a high pressure vessel (see 3.4.2) at 500 MPa for 15 min at room temperature, 53°C, 58°C, and 66°C. Pressure build-up was performed in 35 s, pressure release in 85 s.

Thermal gelatinisation was carried out in a water bath at 90°C for 15 min with continuous manual stirring. Total gelatinisation was achieved after both treatments, respectively, as ascertained by microscopic investigation.

3.5.2 Thermal Treatments

Subsequent to gelatinisation, samples of the starch suspensions were annealed at 40°C for 24 h or 96 h and at 52°C for up to 240 h in a water bath, cooled and stored at room temperature or at 6°C in a refrigerator, or frozen at –20°C. Gelatinised, frozen samples were freeze-dried by Freeze-Dryer Modulyo (Edwards, Sussex, UK) at 0.06 mbar and –50°C.

3.5.3 Hydrolysis

Enzyme hydrolysis of high pressure treated wheat starch was accomplished according to Vasanthan and Bhatty (1998) at 42°C using pullulanase (Megazyme, Bray, Ireland).

High pressure treated wheat starch was also acid hydrolysed at 45°C after Vasanthan and Bhatty (1998). A treatment time of 30 min was chosen since it resulted in the highest yields in RS (Vasanthan & Bhatty, 1998). After hydrolysis the starch suspensions were dried at 30°C over night in an oven.

3.5.4 Pressure-annealing cycles

Up to ten pressure-annealing cycles of untreated, acid hydrolysed, and enzyme hydrolysed starches were performed with a 15 min pressure treatment (500 MPa, room temperature) followed by annealing (23 h 45 min, 52°C).

3.5.5 Resistant starch analysis

The RS content of all samples was determined according to the AOAC 2002.02 method (McCleary & Monaghan, 2002) using α-amylase (Sigma, Mannheim, Germany), amyloglucosidase (3300 U/ ml) and the glucose assay kit K-GLUC (both Megazyme, Bray, Ireland). The method was validated by a sample with a known RS content (C☆ActiStar) which was a kind gift from Cargill/ Cerestar (Vilvoorde, Belgium).

3.6 Bread baking

3.6.1 Recipe and baking procedure

Pouches containing 150 ml of wheat starch suspensions (10 % w/ w) were pressurised for 15 min at 600 MPa. Bread dough without and with pressure-gelatinised wheat starch replacing 5 % of the flour was prepared according to the recipes in table 3.2. The solid components were mixed and simultaneously tempered for 2 min at 30°C in a Farinograph (Brabender, Duisburg, Germany), then the ascorbic acid solution, pressure-treated starch suspension tempered at 30°C contingently on the recipe, and water tempered at 30°C were added until 500 FU were reached. The dough was kneaded for 5 min at 30°C in the farinograph at 63 rpm, afterwards the dough was rested for 15 min at 35°C. After the first rising, each dough was divided into two equal parts (~ 250 g), each portion was machine shaped with 15 turns and put in spray-greased pans, the dough was again allowed to rise for 30 min at 35°C (second rising), and finally baked for 20 min at 210°C including the application of steam.

Tab. 3.2. Recipes for bread dough with and without high pressure-gelatinised wheat starch

	Ref. 1	Ref. 2	Starch 1	Starch 2
Flour type 550 [g]	300	300	285	285
Yeast [g]	15	15	15	15
Sodium chloride [g]	4.5	4.5	4.5	4.5
Sugar [g]	3	3	3	3
Peanut fat [g]	3	3	3	3
Malt flour [g]	1	1	1	1
Ascorbic acid solution (0.2g/ 100 ml) [ml]	3	3	3	3
Water (30°C) added up to 500 FU [ml]	180	189	59	60
Starch suspension (15 min, 600 MPa, 10 %) [g]	0	0	150	150

FU – Farinograph units

3.6.2 Texture analysis

The cooled down loafs of bread were sealed in plastic pouches and stored at room temperature. One and five days after baking, a texture analysis with a Texture Analyser TA-XT2 (Stable Micro Systems, Godalming, UK) was performed two times on two stacked slices out of the centre of the bread loaf (speed 2.0 mm/ s, distance 7.0 mm, one-time repetition after 5 s, trigger force 1.0 g), respectively. One loaf of each set was examined one and five days after baking, respectively, and information about firmness (first peak height) and elasticity (second peak area over first peak area) was obtained.

4   RESULTS AND DISCUSSION

4.1 Starch properties

The wheat, tapioca, and potato starches were analysed for their chemical composition, as well as by DSC, by X-ray diffraction analysis and for their water sorption behaviour in order to become acquainted with the raw material and to understand and infer observations made upon pressurisation from the properties of the starches if possible.

4.1.1 Starch composition

The chemical composition and the specific density of the starches used are given in table 4.1. Potato starch had the highest water as well as ash content while wheat starch exhibited the highest amylose content and contained the highest amount of lipids. This was in accordance with starch compositions as stated in literature (see table 2.1). While the specific density of wheat and tapioca starch was identical, the specific density of potato starch was slightly lower.

Tab. 4.1. Properties and composition of the starches used

Starch type	Wheat	Tapioca	Potato
Specific density [g/ cm³]	1.506	1.506	1.472
Water content [%]	12.690	13.160	19.076
Amylose content [%]	24.246	17.720	18.540
Fat content [%]	0.127	0.014	0.008
Ash content [%]	0.180	0.090	0.290

4.1.2 X-ray analysis

Wheat, tapioca and potato starch were analysed by X-ray diffraction analysis and the resulting spectra are given in figure 4.1 a-c. As anticipated, wheat starch (Fig. 4.1 a) exhibited a diffraction pattern typical for predominant A-type crystallinity, which was characteristic for cereal starches, with strong peaks at 15.19, 17.13, 18.03, and 22.86 °2Θ (5.83, 5.18, 4.92, and 3.89 Å), a medium peak at 20.06 °2Θ (4.43 Å), and weak peaks at 9.51, 11.49, 26.69, and 30.36 °2Θ (9.30, 7.70, 3.34, and 2.94 Å) (Zobel, 1964 and 1988).

The X-ray diffraction pattern of tapioca starch depicted in figure 4.1 b also featured a curve characteristic for A-type crystallinity with strong peaks at 14.85, 17.23, 18.12, and 22.99 °2 Θ

(5.97, 5.15, 4.90, and 3.87 Å) and weak peaks at 9.55, 11.30, 20.08, 26.57, and 30.12 °2Θ (9.26, 7.83, 4.42, 3.36, and 2.97 Å) (Zobel, 1964 and 1988). Except for the lower intensity of the peak at 20.08 °2Θ (4.42 Å), the diffraction pattern of tapioca and wheat starches were virtually congruent. It was expected to obtain a diffraction pattern representing C-type crystallinity for tapioca starch according to Zobel (1964) and Stute *et al.* (1996), but in other papers (Stute, 1985; Moorthy, 2004) it is stated that tapioca starches can exhibit both either A-type or C-type crystallinity.

The X-ray diffraction pattern of potato starch shown in figure 4.1 c exhibited B-type crystallinity, typical for tuber starches in general and potato starches in particular, characterised by a strong peak at 17.10 °2Θ (5.19 Å), medium peaks at 5.76, 14.34, 19,75, 22.30, and 24,09 °2Θ (15.35, 6.18, 4.50, 3.99, and 3.70 Å), and weak peaks at 9.56, 11.67, 26.77, and 34.52 °2Θ (9.25, 7.58, 3.33, and 2.60 Å) (Zobel, 1964 and 1988).
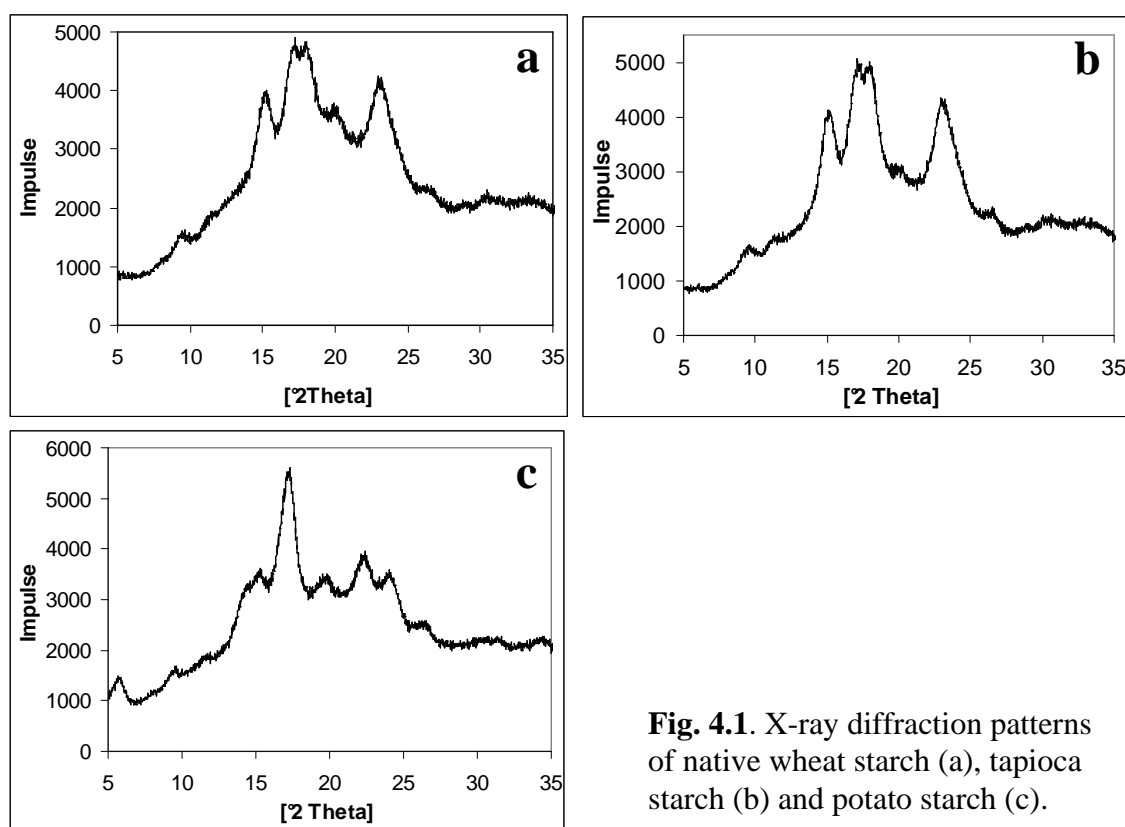
Fig. 4.1. X-ray diffraction patterns of native wheat starch (a), tapioca starch (b) and potato starch (c).

Wheat, tapioca and potato starches were chosen for the investigations of the effect of pressure treatments on starch suspensions due to their assumed crystal structure, because it was discovered that starches with different crystal structure exhibited different pressure

sensitivity and that B-type crystalline starches were more resistant to pressure than starches with A- and C-type crystallinity (Muhr & Blanshard, 1982; Ezaki & Hayashi, 1992; Stute *et al.*, 1996; Rubens *et al.*, 1999). Furthermore, all three raw maerials present significant sources for starch on an industrial level (Tscheuschner, 1996).

As expected, potato starch exhibited B-type crystallinity, while wheat starch showed A-type crystallinity. Tapioca starch also exhibited A-type crystallinity, but due to its by comparison intermediate pressure sensitivity (see chapter 4.4), it proved to be interesting for this study.

4.1.3 Thermal analysis

For native, dry wheat, tapioca, and potato starches TG, DTG, and DSC curves were generated.

The DSC patterns depicted in figure 4.2 emphasise the differences in potato starch and wheat and tapioca starches that have also been observed by X-ray analysis (see chapter 4.1.2). While wheat and tapioca starch exhibit similar DSC curves with four endothermic peaks between 250 and 330°C for thermal degradation of starch, potato starch shows three peaks in this temperature range. This might be due to the differing crystalline structure or the in comparison low fat content of potato starch (see tab. 4.1). At intermediate water content (~ 50 %) potato starch suspensions fail to show the endothermic peak attributed to melting of the amylose-lipid-complex as seen for wheat starch (Svensson & Eliasson, 1995; Jacobs *et al.*, 1998), however this effect might not be transferable to the DSC analysis of dry starches.

Noteworthy is also a considerable DSC peak representing water around 109 to 121°C (see appendix fig. A1) without noticeable change in mass as shown in the TG diagrams. In particular for wheat and tapioca starch a substantial amount of energy is required for the removal of this minor amount of water in comparison to potato starch. This can be explained by the differing crystalline structure. In B crystallites water molecules appear to possess a higher mobility and accessibility due less hydrogen bonds to the starch molecule (Fig. 2.5). The energy required for the removal of water does not correspond with the water content of the starches as shown in table 4.1.

Figure 4.3 shows the TG and DTG curves of dry, native wheat, tapioca and potato starches. The onset, peak, and offset temperatures as well as the points of inflexion are given in the appendix in table A1.
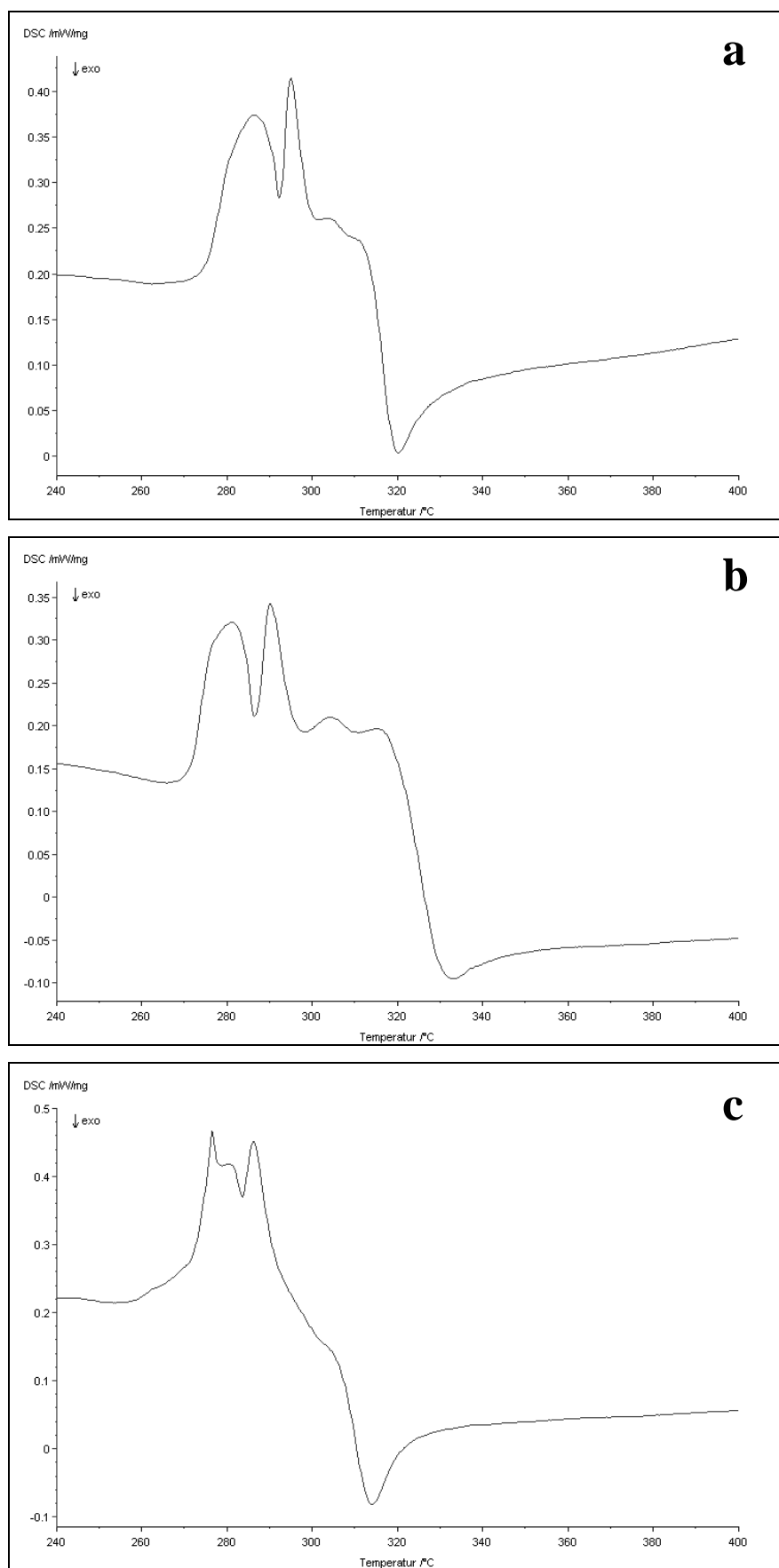
Fig. 4.2. DSC curves of dry, native wheat (a), tapioca (b), and potato (c) starches under anaerobic conditions at a heating rate of 10°K/ min.
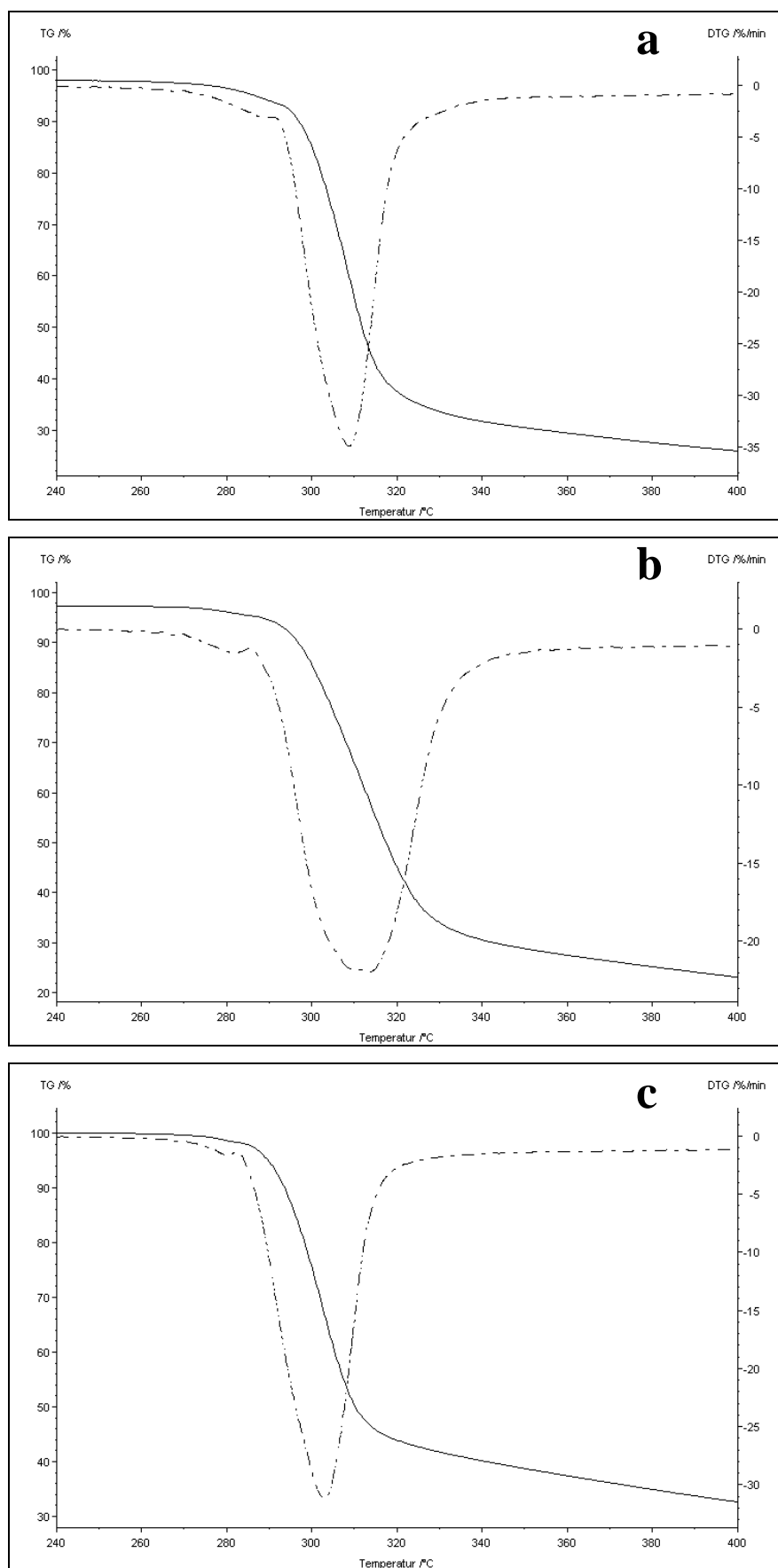
Fig. 4.3. TG (straight line) and DTG (dotted line) curves of dry, native wheat (a), tapioca (b), and potato (c) starches under anaerobic conditions at a heating rate of 10°K/ min.

Comparing the graphs of each starch in figure 4.3, respectively, it can be concluded that the peak temperatures of thermolysis from the DTG signal differ only slightly. Potato starch exhibited the lowest peak temperature, tapioca starch the highest, but the peak temperatures of all three starches examined were located in a temperature range between 303 and 313°C. The same temperature range was obtained for the points of inflexion of the TG signal, accordingly. While the width of the DTG signals of wheat and potato starch amounted about the same, a broader signal was obtained for tapioca starch. Consequently, the thermolysis of tapioca starch occurred over a broader temperature range.

Tapioca starch also exhibited the highest mass loss of the three starches examined with 81.11 % (see appendix tab. A1), whereas wheat starch ranged in between (77.35 %) and potato starch showed the lowest mass loss with 71.68 %. This is in correspondence with the ash content of the starches (see tab. 4.1).

4.1.4 Sorption isotherms

The sorption isotherms of native, heat-gelatinised and freeze-dried, and pressure-gelatinised and freeze-dried wheat, tapioca, and potato starches were generated (Fig. 4.4). Generally, up to an a_W value of 0.84 the water contents of the starches examined were in a close range. The only exception was native potato starch containing a visibly higher amount of water and having thus the best water sorption behaviour at water activities up to 0.84. Between a water activity of 0.84 and 0.99 the water content of the starches increased substantially, at an a_W of 0.99 pressurised wheat starch exhibited the highest water uptake with a water content of 0.49 g water per g oven dry starch.

Generally, no conclusions about the water uptake capability at a water activity of 0.99 could be drawn concerning the type of starch or pre-treatment. While native potato starch exhibited a comparably high water content, native wheat starch and native tapioca starch ranged at the lower end of water absorption of the starches examined. For wheat and tapioca starches drying subsequent to a pre-gelatinisation step resulted in an enhanced water content, but this is not valid for potato starch, where the pre-gelatinisation led to a decrease of water uptake. The water absorption of pressure-gelatinised wheat and potato starches was higher than of heat-gelatinised, whereas thermally gelatinised tapioca starches exhibited the highest water content in comparison to native and pressurised tapioca starches.
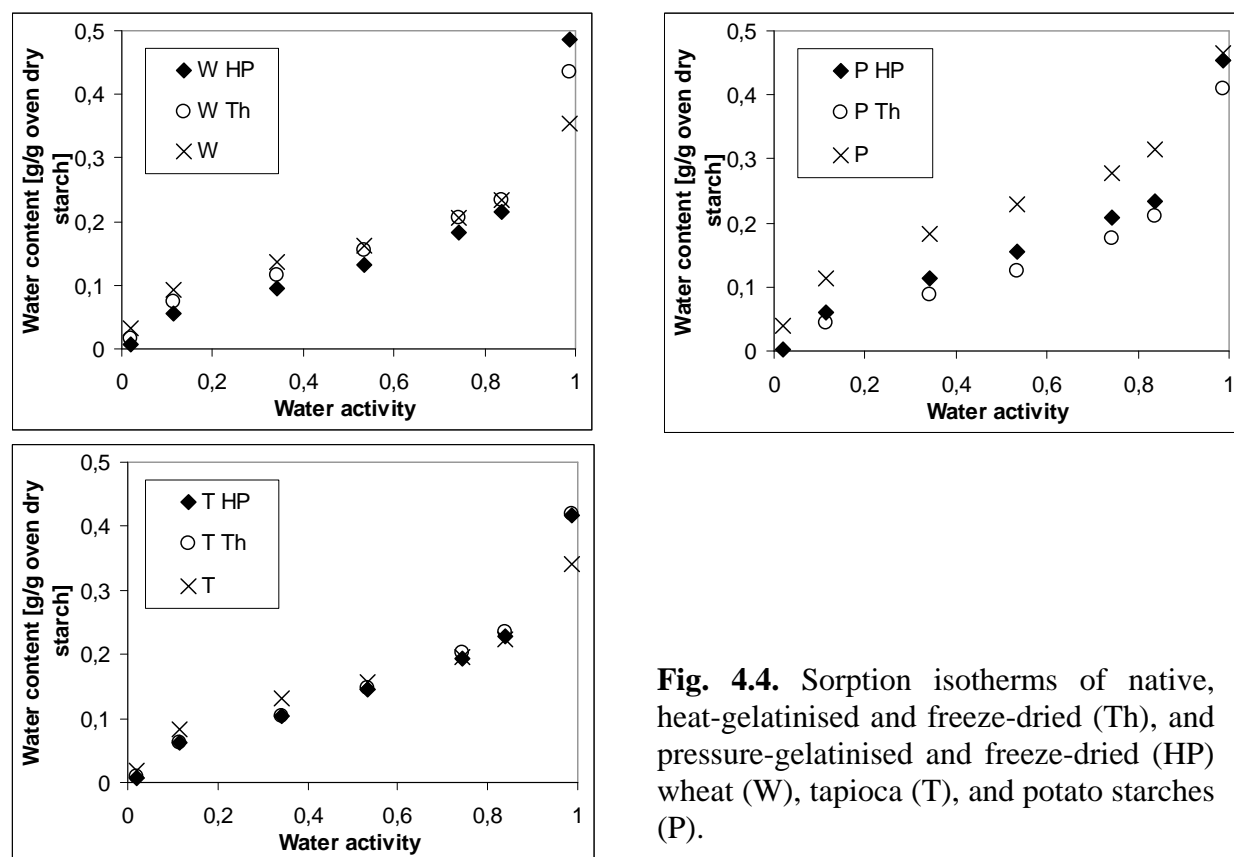
Fig. 4.4. Sorption isotherms of native, heat-gelatinised and freeze-dried (Th), and pressure-gelatinised and freeze-dried (HP) wheat (W), tapioca (T), and potato starches (P).

In order to evaluate the method used and be able to compare the water contents obtained with commercial standards, water contents of the five different commercial starches Prejel VA70T, Ultra-Tex 2, Instant Clearjel E, C☆Hiform 12742, and Paselli P were determined at a water activity of 0.99. They were chemically modified cold water swelling starches derived from waxy maize starch (Ultra-Tex 2, Instant Clearjel E, C☆Hiform 12742), tapioca starch (Prejel VA70T) or potato starch (Paselli P). A comparison of the water content of these five commercial products with native and either pressure-induced or thermally gelatinised wheat, tapioca and potato starches at a water activity of 0.99 is depicted in bar chart of figure 4.5. Overall, all pre-gelatinised starches could match with the commercial starches. It was observed that pressure-gelatinised wheat starch contained the highest amount of water, followed by native potato starch and C☆Hiform 12742. Consequently, it could be concluded that pressure-gelatinised wheat starch exceeded the water absorption capacity of all commercial starches analysed and thus met industrial norms in terms of water uptake. This presents a potential usage of pressure-gelatinised wheat starch as a drying agent, e.g. in rubber gloves, and opens up new possibilities for the application of chemically unmodified food texturisers high good water sorption capacity. The high hydrostatic pressure treatment offers

the opportunity to replace the chemical modification and the heat treatment with concurrent maintenance or even improvement of the water uptake behaviour.
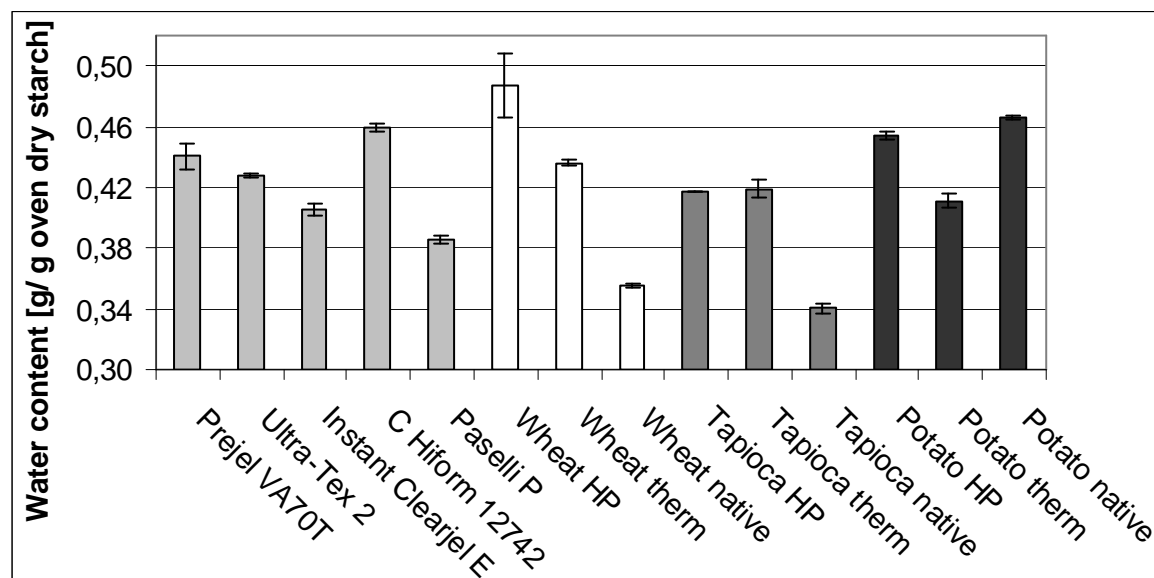
Fig. 4.5. Water content of commercial starches and native, heat-gelatinised and freeze-dried (therm), and pressure-gelatinised and freeze-dried (HP) wheat, tapioca, and potato starches at a water activity of 0.99.

4.2 Optical In situ analysis of starch granules under high pressure

With the development of the HPDS high pressure cell in combination with an inverse microscope (Hartmann *et al.*, 2003), it was possible to obtain in situ images with both high optical resolution and high quality up to pressures of 300 MPa. In cooperation with the chair of process engineering of disperse systems at the TU Munich, the high hydrostatic pressure treatment of native and potassium iodide-iodine stained wheat, tapioca, and potato starch suspensions for 60 min at pressures up to 300 MPa was investigated. The filling of the cell was easy and uncomplicated. Unfortunately, the cell could not be temperature-controlled, and the quality of images with polarised light was too poor for measurements of loss of birefringence.

4.2.1 Visual evaluation of starch pressurisation

Wheat, tapioca and potato starch suspensions were placed in the high pressure cell and observed under pressure through the inverse light microscope. Examining wheat starch

suspensions during the pressure build-up phase, at 200 MPa changes in damaged granules could be observed. Damaged granules seemed to be less resistant to pressure due to cracks in their structure facilitating water absorption. After about 5 min at 300 MPa an obvious swelling of some apparently intact granules had occurred. This was in accordance with observations made by Douzals *et al.* (1996) who detected the beginning of gelatinisation of wheat starch granules below 300 MPa. The number of swollen granules as well as the degree of swelling increased with increasing pressurisation time. In preliminary experiments, a degree of gelatinisation of 26.51 % was obtained after 1 h at 300 MPa in the multivessel apparatus. Correspondingly after one hour at 300 MPa in the high pressure cell, some wheat starch granules were clearly enlarged, but others remained unchanged (Fig. 4.6 b). Douzals *et al.* (1996) also distinguished between these two kinds of wheat starch granules.

During pressure release the size of the swollen granules slightly decreased, but it remained larger than before pressurisation (Fig. 4.6 c). Granules unchanged in size during pressurisation underwent no changes during pressure release. No granules increased in size during pressure release or after 1.5 h at atmospheric pressure. This was contradictory to the results obtained by Douzals *et al.* (1996).
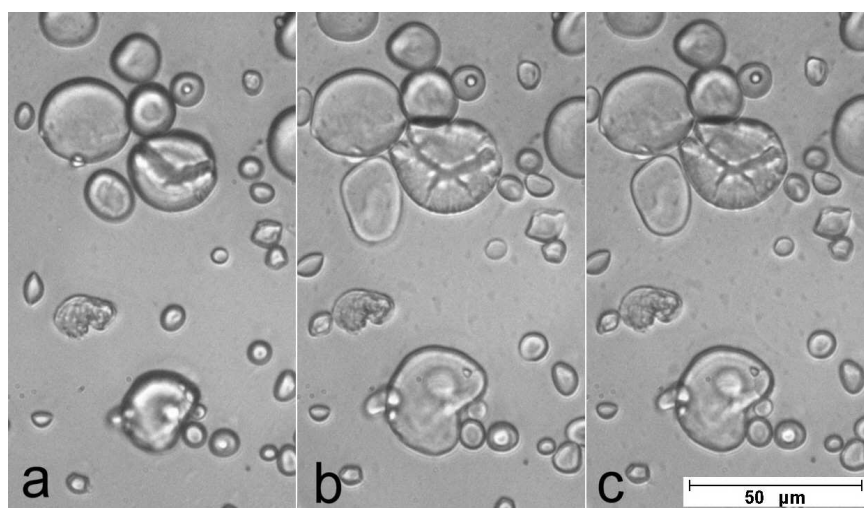
Fig. 4.6. Wheat starch suspension at 0.1 MPa (a), after 1 h at 300 MPa (b) and after pressure release at 0.1 MPa (c).

Investigating potato and tapioca starch suspensions in the high pressure microscope, no detectable changes in granule size became evident during and after pressurisation at 300 MPa for 1 h (data not shown). This was in accordance with preliminary pressure experiments

resulting in a degree of gelatinisation of 0.97 % in the case of 5 % (w/ w) tapioca starch suspensions and 0.00 % for 5 % (w/ w) potato starch suspensions after 1 h at 29°C and 300 MPa in the high pressure multivessel apparatus. Douzals *et al.* (1996) detected an irreversible swelling of 15 % of iodine stained potato starch granules after pressurisation at 300 MPa. Such a slight swelling could not be determined. Nevertheless, it could be concluded that wheat starch was less resistant to pressure than potato starch and tapioca starch.

4.2.2 Starches dyed with Lugol's solution under pressure

The starch suspensions were intensely stained with a potassium iodide-iodine solution (Fig 4.7-4.9) and treated as before. Regarding the granule sizes, the results obtained for undyed starches could be confirmed.

As for wheat starch, first the swelling granules began to lose their coloration (Fig. 4.7 b). As shown in figure 4.7 c, all iodine stained wheat starch granules were totally discoloured after 1 h at 300 MPa, regardless whether they had swollen or not. Additionally the liquid phase became darker and a brownish precipitation appeared. This could be resulting from amylose leaking into the liquid medium. After pressure release the granules did not regain their colouration, and after 10 min at 0.1 MPa a further decolouration was observed (Fig. 4.7 d).
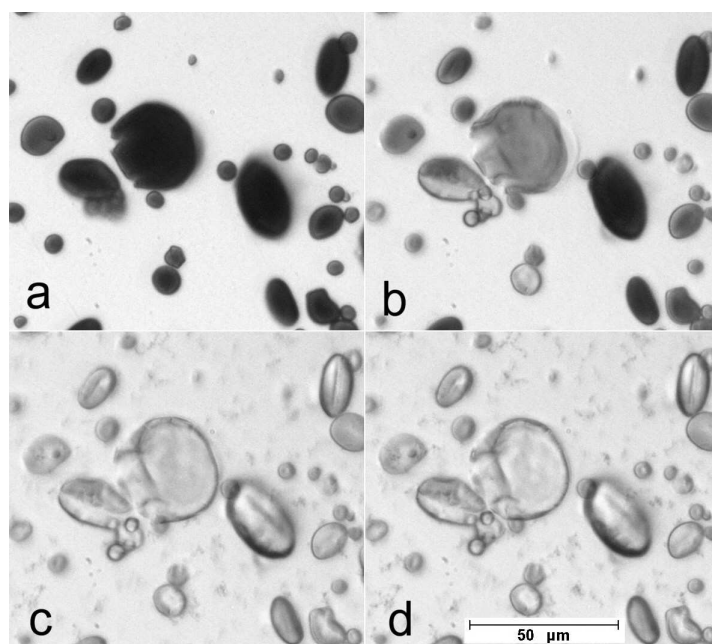
Fig. 4.7. Potassium iodide-iodine stained wheat starch suspension before a pressure treatment (a), after 10 min at 300 MPa (b), after 1 h at 300 MPa (c) and after pressure release at 0.1 MPa (d).

Iodine stained tapioca starch granules were also totally discoloured after 1 h at 300 MPa (Fig. 4.8 b) and a brownish precipitation could be observed in the continuous phase. There was no visible elucidation of the granules after pressure release and 10 min at 0.1 MPa.

The potato starch granules were discoloured only slightly with a minor darkening of the surrounding liquid phase. After pressure release and 10 min at atmospheric pressure, there was a further very slight decolouration of the granules (Fig. 4.9 b).
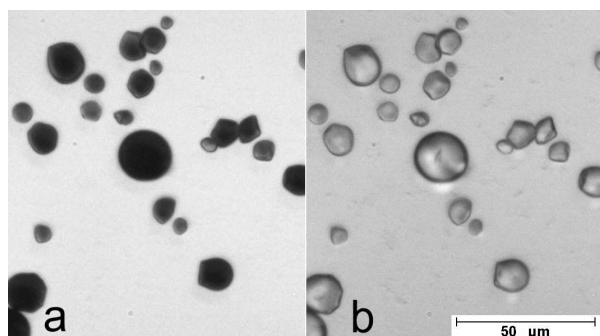
Fig. 4.8. Potassium iodide-iodine stained tapioca starch suspensions before a pressure treatment (a) and after pressure release at 0.1 MPa following a 1 h treatment at 300 MPa (b).
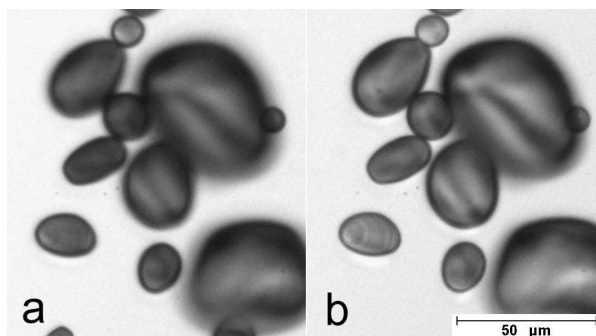
Fig. 4.9. Potassium iodide-iodine stained potato starch suspension before a pressure treatment (a) and after pressure release at 0.1 MPa following a 1 h treatment at 300 MPa (b).

In order to ensure that the decolouration was not attributed to influences other than pressure, e.g. the light or the possible development of heat by the illumination of the microscope, the stained starch suspensions were investigated at atmospheric pressure for 1 h. For the iodine stained tapioca and potato starch suspensions no alterations in colour or size of the granules became visible. However, the continuous phase of the suspensions darkened noticeably. Placing the wheat starch suspension under the light microscope for 1 h at atmospheric pressure led to a very slight elucidation of the granules, which was not comparable to the thorough decolouration at 300 MPa, without a deepening of colour of the surrounding liquid (data not shown). Therefore, this minor elucidation was neglected.

The decolouration observed during and after pressurisation can also not be attributed entirely to an uptake of water and resulting dilution because a pressurisation at 300 MPa for 1 h showed no measurable effects on the granule sizes of tapioca starch as well as potato starch that could be attributed to a water uptake.

According to Sarko and Wu (1978) all starches formed single helical complexes with iodine in their amorphous regions. However, pure, highly oriented crystalline A- and B-amylose molecules were unable to form a complex with iodine due to the double helices (Sarko & Wu, 1978, French, 1984). This can be applied to the crystalline regions of starches consisting of double helices (Sarko & Wu, 1978). Consequently, only the amorphous regions acting more sensitive towards pressure and being the starting point of pressure induced gelatinisation attributed to the colouration with iodine. Therefore the decolouration could not be an indication for starch melting as suggested by Douzals *et al.* (1996) because the crystalline regions are not involved in starch-iodine complexes.

It was striking that the decolouration appeared to be irreversible, since starch could be iodine stained after a pressure treatment and hence had not lost its ability to form complexes with iodine. A degradation of iodine due to pressure could also be excluded. The colour of the iodine solution used remained unchanged in colour intensity and tinge when compared before and after 1 h at 300 MPa (not shown). It is also unlikely that redox reactions could be responsible for the decolouration of the starch granules because the colouring of potato starch remained almost unchanged. If iodine would have been pushed out of the single helices by pressure inducing a disassociation of the amylose-iodine complex, a pressure release should lead to a reformation of the amylose-iodine complexes and a regeneration of the blue colour unless the iodine was to be permanently displaced by water molecules or bound elsewhere. But the permanent displacement of iodine by water molecules is contradictory to the starches ability to form complexes with iodine after a pressurisation step.

It was discovered that the electrical conductivity of pressure-treated starch suspensions increased with increasing degree of gelatinisation (see chapter 4.3). This was regarded as an indication of an ion release during the pressure-induced gelatinisation process. It was possible that leaking of amylose from the starch granule was partly responsible for this rise in electrical conductivity. In literature, the opinions whether amylose release generally occurred during pressurisation were conflicting, according to Douzals *et al.* (1998) there is only little and after Stute *et al.* (1996) and Stolt *et al.* (2001) sometimes even no amylose release.

Kudla and Tomasik (1992) pressurised moistened potato starch with added iodine and concluded that iodine present in starch caused significant deterioration of the starch matrix by gelation and/ or hydrolysis of glycosidic bonds. Accordingly, an amylose release could also originate from the presence of iodine during pressurisation.

The decolouration of the starch granules under high hydrostatic pressure suggested a removal of iodine and the darkening of the surrounding liquid phase led to the assumption

that an accumulation of amylose-iodine complexes in the liquid had occurred. Hence, the decolouration could be caused by an amylose release from the amorphous regions during pressure-induced starch gelatinisation where the released amylose molecules complexed with iodine pushed out of the single helices by pressure and the complexes formed accumulated in the thereby darkening liquid phase. So the removed iodine was again linked to amylose molecules which would explain why the starch granules could still be iodine-stained after a pressure treatment but would not regain their colour after pressure release if stained before the pressure treatment.

Potato starch was apparently not only more resistant to pressure-induced swelling of the granules than wheat starch but also more robust towards amylose release. Although tapioca starch granules showed no visible swelling after 1 h at 300 MPa, the decolouration was clearly evident. This demonstrated that decolouration and swelling were not connected and suggested that the amylose release from the amorphous regions possibly started before the swelling of the granules was initiated.

More data derived from complementary analysis methods is required for explaining the experimental results.

4.3 Physical properties of pressurised starch suspensions

Upon pressurisation at different pressures and pressurisation times, the electrical conductivity and the water binding capacity of wheat, tapioca, and potato starch suspensions were determined and compared with its degree of gelatinisation. It was searched for possible correlations analogous to the thermal gelatinisation and furthermore for a quick method for the determination of the degree of gelatinisation.

4.3.1 Pressure gelatinisation curves and electrical conductivity

Wheat, tapioca, and potato starch suspensions (5 % w/ w) were pressurised at 29°C for 15 min at various pressures and the degree of gelatinisation as well as the electrical conductivity of the starch suspensions were determined. Figure 4.10 shows the degree of gelatinisation and the electrical conductivity of the pressure-treated starch suspensions in relation to the pressure applied. The degree of gelatinisation of all three starches examined increased with increasing pressure and the gelatinisation curves obtained strongly resembled

thermal gelatinisation curves with their typical sigmoid shape. Hence the process of pressure-induced gelatinisation appeared to proceed similarly to the process of thermal gelatinisation.
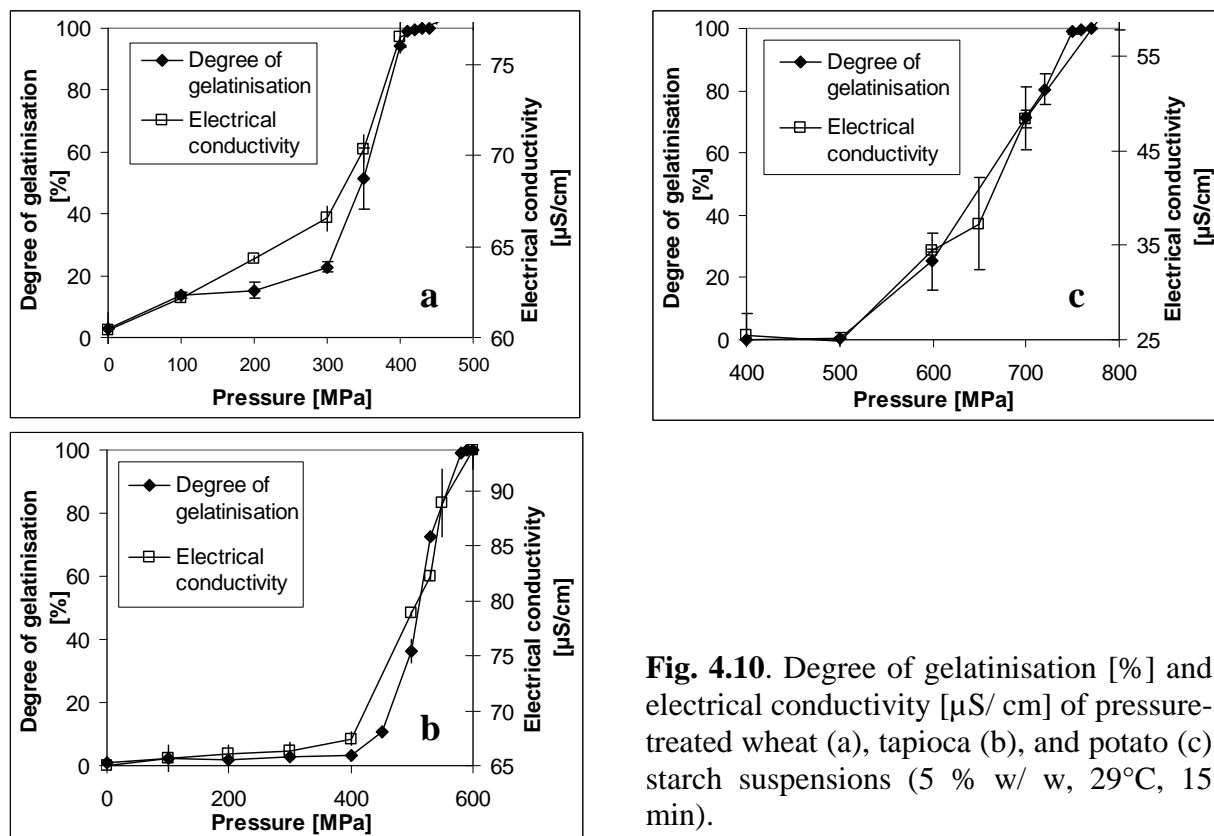
Fig. 4.10. Degree of gelatinisation [%] and electrical conductivity [μS/ cm] of pressure-treated wheat (a), tapioca (b), and potato (c) starch suspensions (5 % w/ w, 29°C, 15 min).

Only at approximately 200 MPa there was a slight minimum in the gelatinisation curve of tapioca starch and a reduced rise in the degree of gelatinisation for wheat starch. Both wheat and tapioca starches seemed to be more stable towards pressure around 200 MPa. This could be due to a change in volume owing to the pressure. For example, the gelatinisation temperature was slightly increased by pressurisation up to 150-250 MPa and subsequently decreased with rising pressure (Thevelein *et al.*, 1981, Muhr & Blanshard, 1982). Muhr *et al.* (1982) investigated starch gelatinisation using a HPDTA. Applying the Clapeyron equation, the authors calculated the volume change (ΔV) to decrease with increasing pressure and to be zero around 150-250 MPa and suggested that the initial increase and subsequent decrease in gelatinisation temperature following a pressure treatment was a consequence of a decrease in ΔV. Potato starch was less sensitive to pressure than tapioca and wheat starch, and since it

exhibited a higher resistance to pressure in comparison to wheat and tapioca starches and did not begin to gelatinise below 500 MPa, this does not apply for potato starch.

Tapioca starch gelatinised comparatively instantaneously within a pressure range of around 200 MPa, complete gelatinisation was achieved at 600 MPa at the conditions applied (5 % w/ w, 29°C, 15 min) while wheat and potato starch gelatinised over a broader pressure range. Tapioca starch ranged intermediate in terms of pressure sensitivity of the three starches investigated. Wheat starch exhibited the lowest resistance towards pressure and completely gelatinised at 440 MPa at the conditions applied, whereas 100 % gelatinisation of potato starch, showing the highest pressure resistance, was achieved at 770 MPa.
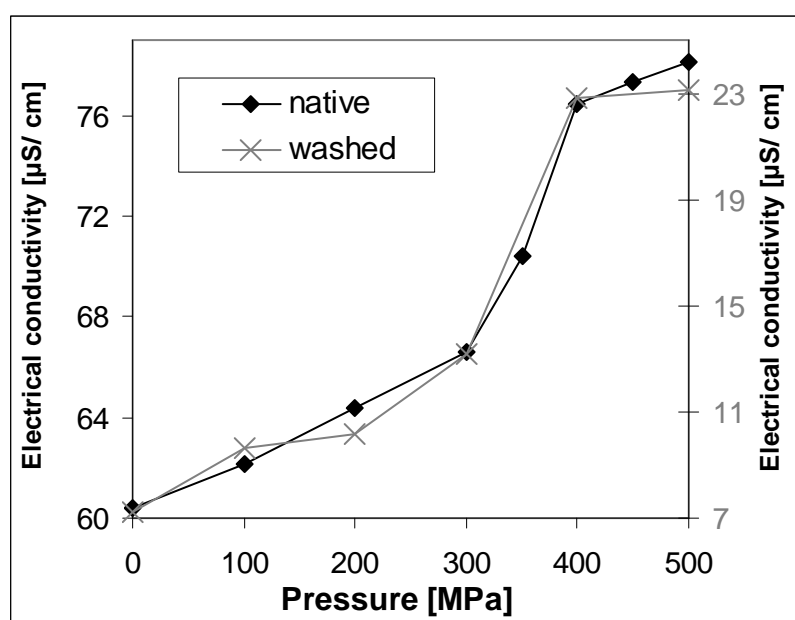
Fig. 4.11. Electrical conductivity [μS/ cm] of pressurised (black) and washed and then pressurised (grey) wheat starch suspensions (5 % w/ w, 29°C, 15 min).

The electrical conductivity of the starch suspensions rose with increasing pressure. Therefore there must have been an ion release during pressure-induced gelatinisation although the starches retained their granular character. In order to prove that this increase in electrical conductivity was based on an ion release from within the starch granule and not from ions attached to the granule's surface, the rise in electrical conductivity with increasing pressure of native and three times washed wheat starch suspensions was compared, as depicted in figure 4.11. Since the absolute value of the increase in electrical conductivity was almost identical for native and washed wheat starch suspensions, it was shown, that the there must be an ion

release from within the granule. In addition it is possible that a concentrating effect due to water uptake of the starch granules contributes to the rise in electrical conductivity.

On the whole, the electrical conductivity of the pressurised samples corresponded well with the gelatinisation curves. As aforementioned, the tapioca starch suspensions gelatinised over a comparatively narrow pressure range starting around 400 MPa and ending at 600 MPa, and there was also a considerable rise in electrical conductance of the tapioca starch suspension within this range (Fig. 4.10 b). Wheat starch being less resistant to pressure and gelatinising over a much wider range (Fig. 4.10 a) practically started to gelatinise at 100 MPa and ended at 440 MPa. There was a rise in the slope of the gelatinisation curve around 300 MPa, which correlated with a rise of the slope of the electrical conductivity. The rise in electrical conductivity of potato starch suspensions due to a pressure treatment also correlated well with its degree of gelatinisation (Fig. 4.10 c).

Generally, the initial electrical conductivity of 5 % starch suspensions of different origins differed, which might be due to the manufacturing process of the starches resulting from the embedding of the starch granules in the plant and to the content of mineral nutrients of the starches. Additionally, the absolute value of increase in electrical conductivity differed dependant on the type of starch, e.g. the electrical conductivity of wheat starch suspension increased by 16.92 µS/ cm, of tapioca starch suspension by 28.68 µS/ cm, and of potato starch suspension by 36.40 µS/ cm upon pressurisation at the gelatinisation pressure, defined as the pressure where all starch granules have lost their birefringent properties. The increase in electrical conductivity might be influenced by the water availability due to differing viscosity increase.

4.3.2 Impact of pressurisation time on electrical conductivity

The influence of the pressure treatment time on the degree of gelatinisation and electrical conductivity of the starch suspensions was also investigated (Fig. 4.12 a-c). Because wheat starch, tapioca starch, and potato starch showed a different stability towards pressure, different pressures were applied for each starch, i.e. 350 MPa for wheat starch, 530 MPa for tapioca starch, and 700 MPa for potato starch suspensions. At constant temperature and pressure, the degree of gelatinisation increased with increasing treatment time for all three starches examined. The impact of treatment time was the most pronounced during the first hour of pressurisation where a steep slope of the gelatinisation curve can be observed. The

electrical conductivity of wheat and tapioca starch suspensions also rose with increasing pressurisation time and the course of the conductivity curve corresponded well with their gelatinisation curve. Tapioca starch was nearly fully gelatinised after a 4 h treatment at 530 MPa (Fig. 4.12 b) whereas wheat starch showed a much slower rise in degree of gelatinisation with increasing pressurisation time at 350 MPa (Fig. 4.12 a).

As depicted in figure 4.12 c, during the first 60 min, the degree of gelatinisation and the electrical conductivity of potato starch suspensions also increased with increasing treatment time. Between a treatment time of 1 h and 4 h the degree of gelatinisation almost stagnated, whereas the electrical conductivity even slightly decreased. It is possible that the gel-like texture of pressure-treated potato starch suspensions inhibits the measurement of its electrical conductivity. Additionally, the determination of the degree of gelatinisation of pressure-treated potato starch suspensions was complicated due to starch sedimentation and consequential inhomogeneity of the sample which is also emphasised by the high standard deviations. This did not apply for the measurement of degree of gelatinisation of wheat and tapioca starch suspensions.

Due to the different pressures applied, the gelatinisation curves of the three starches are not comparable.

Fig. 4.12. Degree of gelatinisation [%] and electrical conductivity [µS/ cm] of wheat starch suspensions (a; 5 % w/ w, 29°C, 350 MPa), tapioca starch suspensions (b; 5 % w/ w, 29°C, 530 MPa), and potato starch suspensions (c; 5 % w/ w, 29°C, 700 MPa) pressurised for 5, 15, 30, 60, and 240 min, respectively.
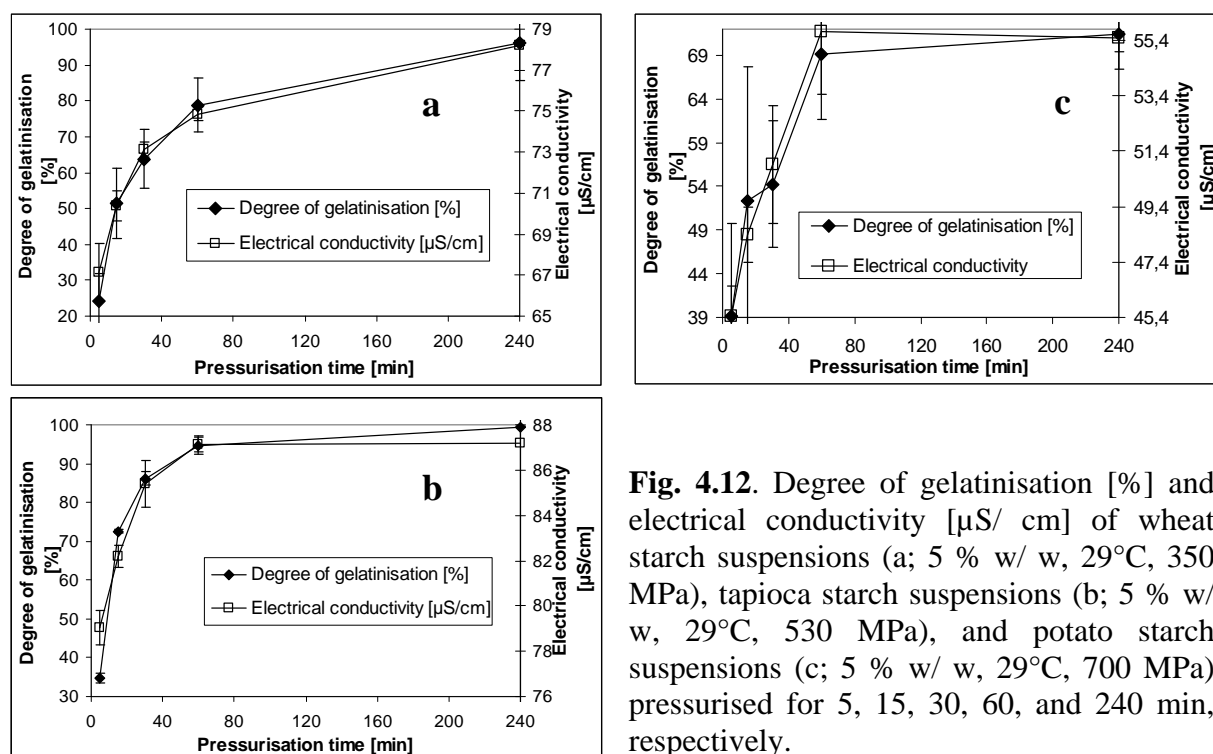
4.3.3 Correlation of degree of gelatinisation and electrical conductivity

The correspondence of the degree of gelatinisation and electrical conductivity of the three starches used is depicted in figure 4.13 a-c, respectively. The data points shown were derived from pressurisation experiments at a constant pressurisation time of 15 min (see chapter 4.3.1) and also from pressure treatments at constant pressures and varying pressurisation periods between 5 min and 4 h (see chapter 4.3.2). The values of the electrical conductivity data were normalised, the lowest data point was defined as 0 % and the highest as 100 %. The resulting adjusted linear curve is also displayed. The coefficients of determination R^2 showed a good linear relationship between the degree of gelatinisation and the electrical conductivity for the starches. Moreover the slope of the adjusted curve was close to one ($y \approx x$) proving that there was a conformity of the degree of gelatinisation and electrical conductivity after pressure treatment.
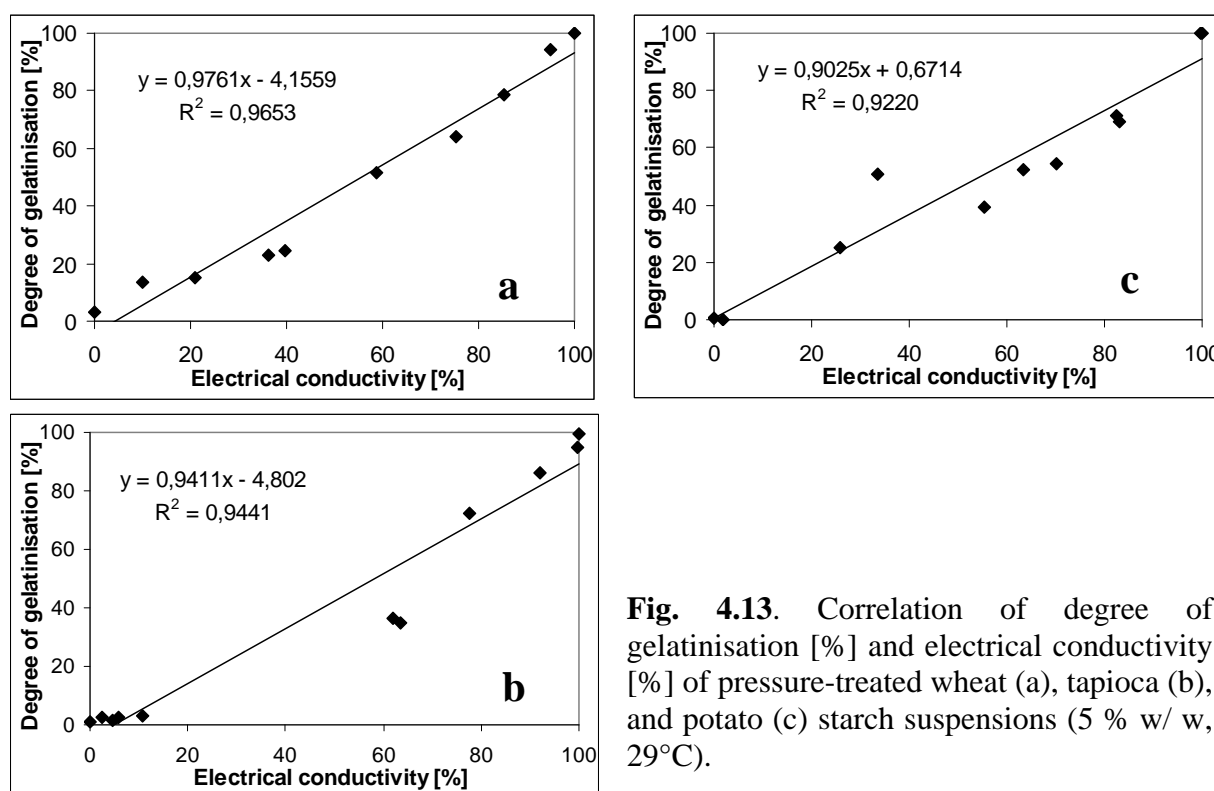
**Fig. 4.13**. Correlation of degree of gelatinisation [%] and electrical conductivity [%] of pressure-treated wheat (a), tapioca (b), and potato (c) starch suspensions (5 % w/ w, 29°C).

It can be seen that the electrical conductivity corresponds with the degree of gelatinisation of the starches investigated after pressure treatment and is an effective tool for the quick determination of pressure-induced starch gelatinisation. The only complication to be

considered is the initial electrical conductivity of the starch suspension to be pressurised. There was always a slight irregularity in the initial conductivity due to the inhomogeneity of the starches or inaccuracy in weighing during the preparation of the sample which made a comparison of the electrical conductivity data obtained difficult. However, this limitation could be overcome by washing the starches beforehand and weighing as precisely as possible since the course of the electrical conductivity curve was not altered by a washing step (Fig. 4.11) and it was experienced that the exact sample preparation conducted was sufficient as long as reproducible results were obtained.

4.3.4 Water binding capacity

Equivalent to the electrical conductivity measurements, the water binding capacity of pressurised starch suspensions was determined. As depicted in figure 4.14, the water binding capacity of all three starch suspensions (5 % w/ w) increased with increasing pressure after 15 min of pressurisation at 29°C and corresponds with its degree of gelatinisation. Therefore, water binding capacity is also a potential indicator for pressure-induced starch gelatinisation.
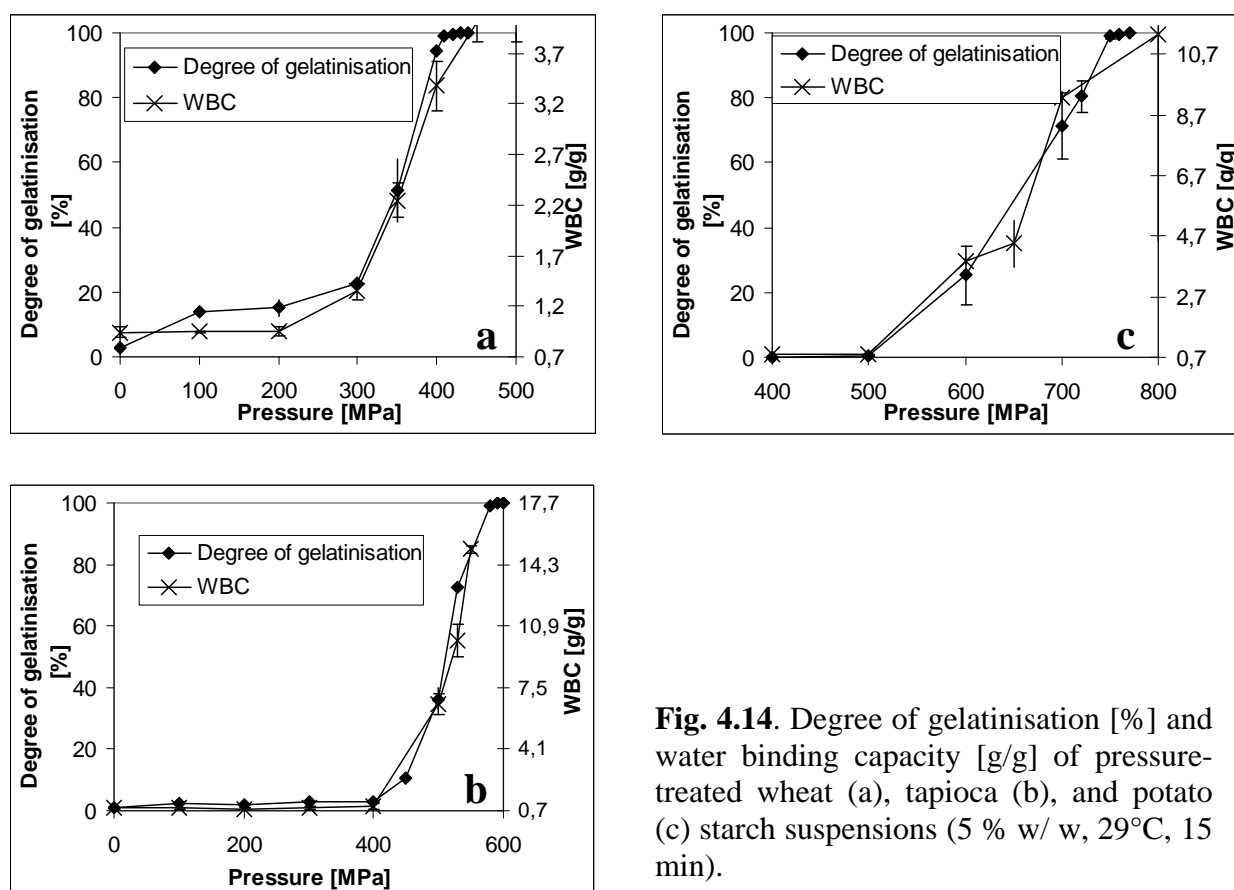
Fig. 4.14. Degree of gelatinisation [%] and water binding capacity [g/g] of pressure-treated wheat (a), tapioca (b), and potato (c) starch suspensions (5 % w/ w, 29°C, 15 min).

Comparing the water binding capacity of the pressurised starch suspensions at a degree of gelatinisation of 100 %, it is lowest for wheat starch, about threefold higher for potato starch, and 4.5 times higher for tapioca starch. Consequently, completely pressure-gelatinised tapioca starch exhibits the highest water binding capacity upon pressurisation of the three starches examined. However at 450 MPa, wheat starch shows the highest water binding capacity, since it is completely gelatinised, while the degree of gelatinisation of tapioca starch is below 20 % and potato starch remains almost unaltered below 500 MPa.

4.4 Impact of pressure, temperature and treatment time on starch gelatinisation

The effect of pressure, temperature, and treatment time on the degree of gelatinisation of wheat, tapioca, and potato starch suspensions was systematically investigated including a statistical evaluation determining whether temperature and pressure have a statistically significant effect on the degree of gelatinisation of wheat, tapioca, and potato starch at the 95.0 % confidence level, respectively. In this context, it was additionally scrutinised and discussed if starch suspensions could be utilised as PTTI (see chapter 2.3.2).

4.4.1 Phase diagrams

The impact of pressure, temperature and treatment time on wheat, tapioca, and potato starch suspensions was systematically determined. Primarily, a pressure-temperature phase diagram of complete gelatinisation of the three starches was generated (Fig. 4.15).

As expected from previous findings (Muhr & Blanshard, 1982; Ezaki & Hayashi, 1992; Stute *et al.*, 1996; Rubens *et al.*, 1999), the wheat starch with an A-type X-ray diffraction pattern proved to be the most sensitive to pressure. The course of the phase transition curve of wheat starch was parabolic at temperatures above 0°C. This was in accordance with results obtained by Douzals *et al.* (1999 and 2001). Below 0°C, the pressure of phase transition increased highly, which was probably due to reduced water availability because of freezing (data not shown).

The phase transition of tapioca starch occurred at higher temperatures and pressures than the phase change of wheat starch but below the phase transformation of potato starch. The course of the transition line of tapioca starch strongly resembled the pressure-induced gelatinisation diagram of rice starch obtained by Rubens and Heremans (2000). Between

10°C and 48°C the phase change appeared to be almost solely dependent on the pressure. A decrease in the gelatinisation pressure with rising temperature of only 10 MPa/ 10°C could be observed. Nearly regardless of the temperature applied, the gelatinisation pressure remained around 600 MPa leading to a plateau-like progression of the phase transition line. Above 48°C the pressure of complete gelatinisation decreased rapidly with increasing temperature. This course of the phase transition line between 10 and 50°C gave the impression that the tapioca starch investigated was not applicable as a pressure time temperature integrator (PTTI; see chapter 2.3.2) because the required temperature dependence was not ensured if complete gelatinisation is the critical criterion chosen.
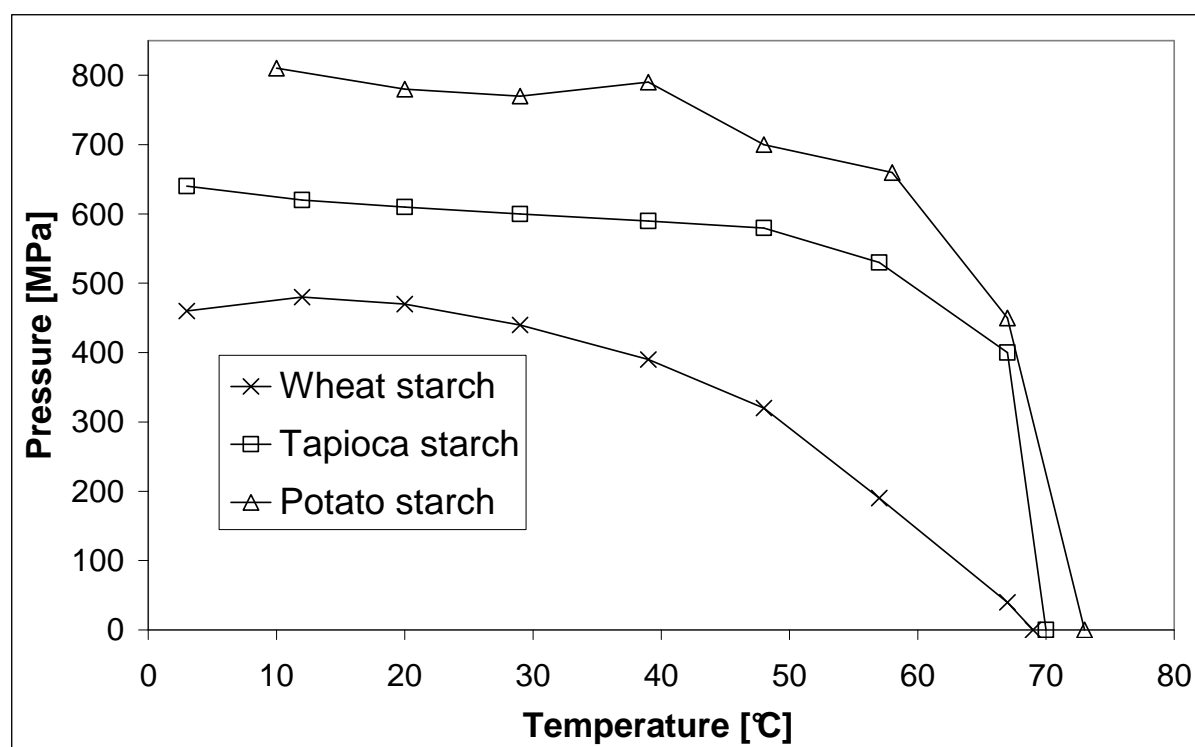
Fig. 4.15. Phase diagram of complete gelatinisation of wheat, tapioca, and potato starch suspensions (5 % w/ w) after 15 min of pressure-temperature treatments.

Potato starch having a B-type crystalline pattern had the highest resistance to pressure. Albeit at higher pressures, the phase transition line of potato starch proceeds similarly to the line of wheat starch between 10 and 48°C except for an outlier at 39°C. The pressure of complete gelatinisation decreases consistently with increasing temperature up to 58°C and then declines rapidly. Assuming that the value for the pressure of complete gelatinisation of potato starch at 39°C was derived from an error in measurement, potato starch suspensions as

well as wheat starch suspensions are applicable as PTTIs if complete gelatinisation is the chosen indicator.

4.4.2 Pressure-induced gelatinisation curves

As depicted in figure 4.16, the pressure dependence of wheat starch, tapioca starch and potato starch suspensions at 29°C was investigated. As already observed before (Chapter 4.2.1; Fig. 4.15), wheat starch was more sensitive to pressure than tapioca and potato starch. The degree of gelatinisation increased with increasing pressure. And for all three starches examined, sigmoid shaped gelatinisation curves similar to thermal gelatinisation curves were derived. As reported before (see chapter 4.3.1) the only discrepancy was a reduced rise of the degree of gelatinisation around 200 MPa in the case of wheat starch and a minor local minimum in the case of tapioca starch. This was probably due to a change in volume (Δv) owing to the pressure (Muhr *et al.*, 1982). Consequently for pressures below 200 MPa at 29°C, tapioca starch was not applicable as a pressure time temperature integrator (PTTI) because at a constant, known temperature the pressure could not unambiguously be derived from the degree of gelatinisation.
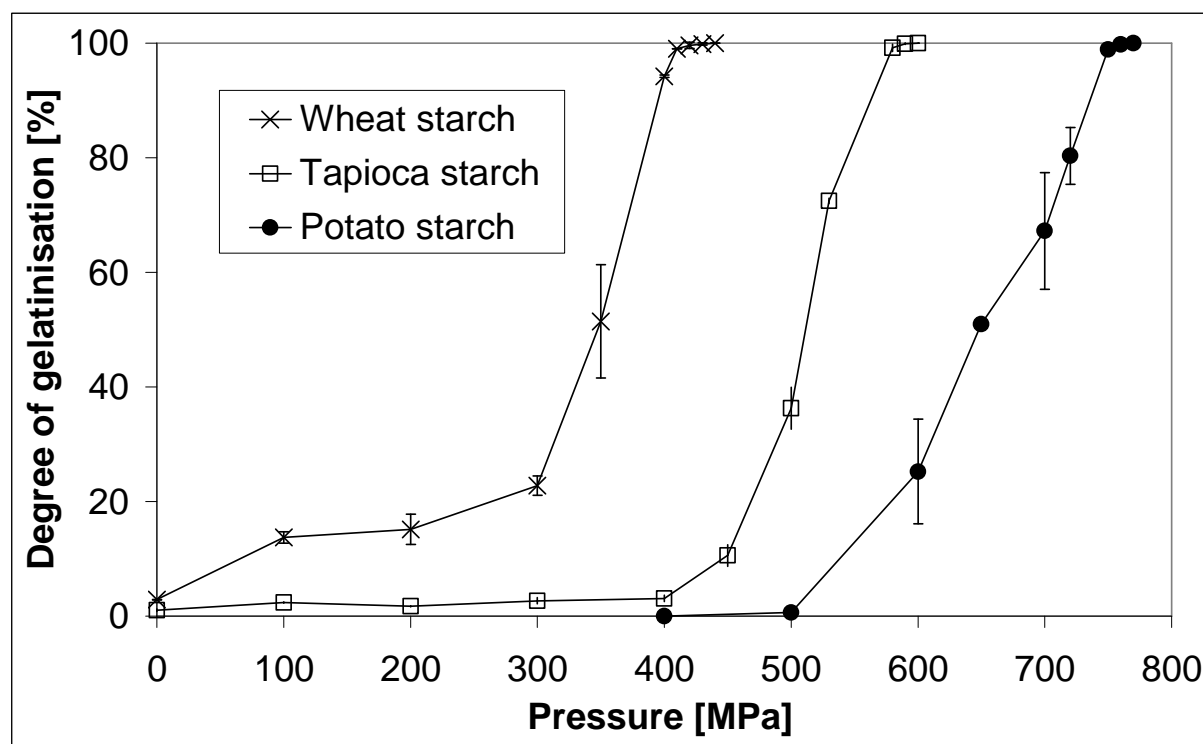
Fig. 4.16. Pressure-induced gelatinisation curves of wheat, tapioca and potato starch suspensions (5 % w/ w) after pressurisation for 15 min at 29°C.

Above 200 MPa, tapioca starch gelatinisation possessed a satisfactory pressure dependency at a constant temperature to function as a PTTI. Since most current high pressure applications are performed above 200 MPa, this does not seem to be a major limitation of the applicability of starches as PTTIs. Regardless of the change in slope of the gelatinisation curve, wheat starch gelatinisation proved to have an adequate pressure dependency to yield distinct information about the pressure applied at given temperatures and treatment times. Potato starch could be applied as a PTTI at higher pressures above 500 MPa. For high pressure processes below 500 MPa, potato starch would not be suitable.
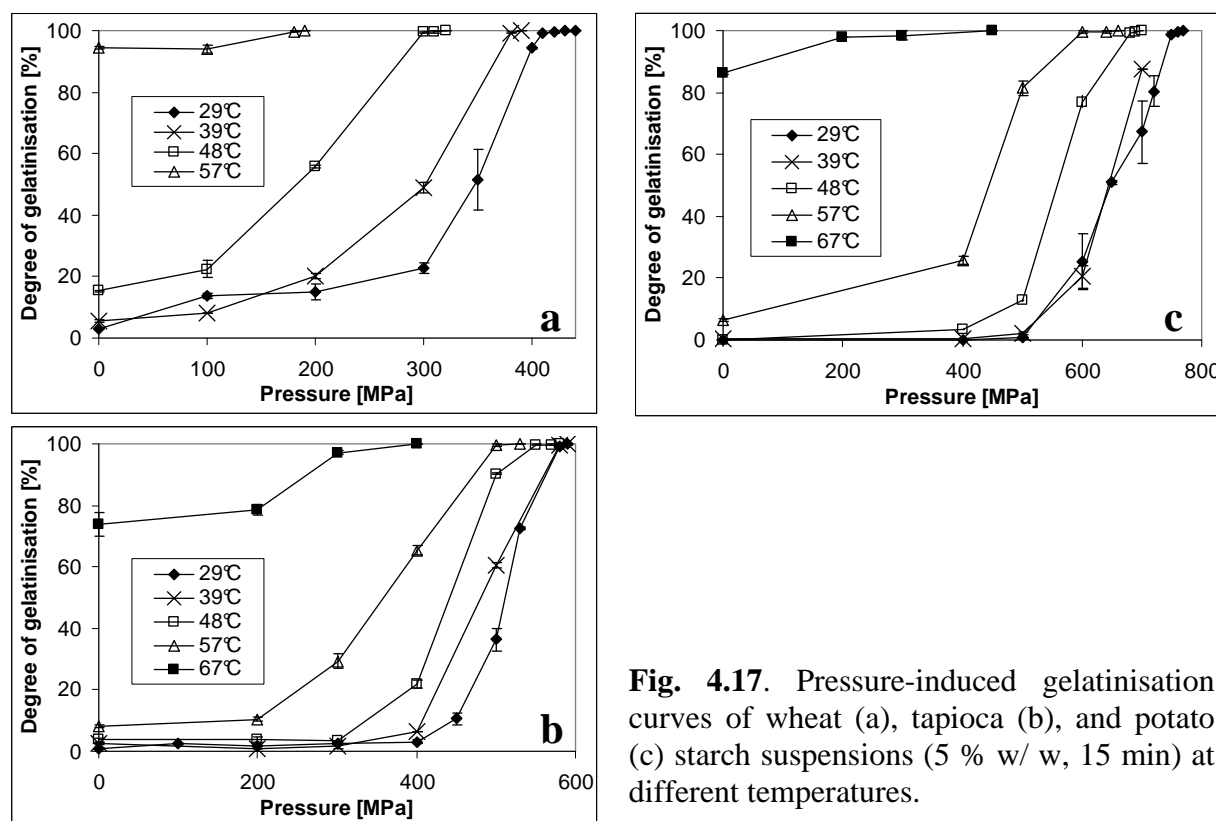
Fig. 4.17. Pressure-induced gelatinisation curves of wheat (a), tapioca (b), and potato (c) starch suspensions (5 % w/ w, 15 min) at different temperatures.

The starch suspensions were also pressurised at higher temperatures (39°C, 48°C, 57°C, and 67°C) in order to examine the influence of increasing temperatures (Fig. 4.17). The higher the temperature, the lower was the pressure of complete gelatinisation at the temperatures investigated. At 39°C the pressure-induced gelatinisation curve did not differ considerably from the curve at 29°C. At 48°C, 57°C and 67°C, the effect of temperature was much more distinct and the pressure-induced gelatinisation took place over a far smaller

pressure range. Nevertheless, a pressure dependence was noticeable at higher temperatures (below the gelatinisation temperature). In correspondence to the phase diagram of complete gelatinisation (Fig. 4.15), figure 4.17 shows the higher sensitivity of wheat starch towards pressure also at temperatures higher than 29°C in comparison to tapioca and potato starches. At 57°C, the "initial" degree of gelatinisation at atmospheric pressure is above 90 % and only a low pressure is required to achieve complete gelatinisation of wheat starch suspensions, whereas pressures above 400 MPa are needed for tapioca and potato starch suspensions.

In figure 4.18 the relation between pressure, temperature and degree of gelatinisation of wheat starch suspensions after a treatment of 15 min is recapitulatorily outlined in a three-dimensional diagram. At constant temperature the degree of gelatinisation increases with increasing pressure and at constant pressure it rises with increasing temperature. Furthermore, at a consistent degree of gelatinisation the points for the appendant temperatures and pressures form a parabolic-shaped graph. Additionally, the sigmoidal course of the thermal gelatinisation becomes clearly visible.
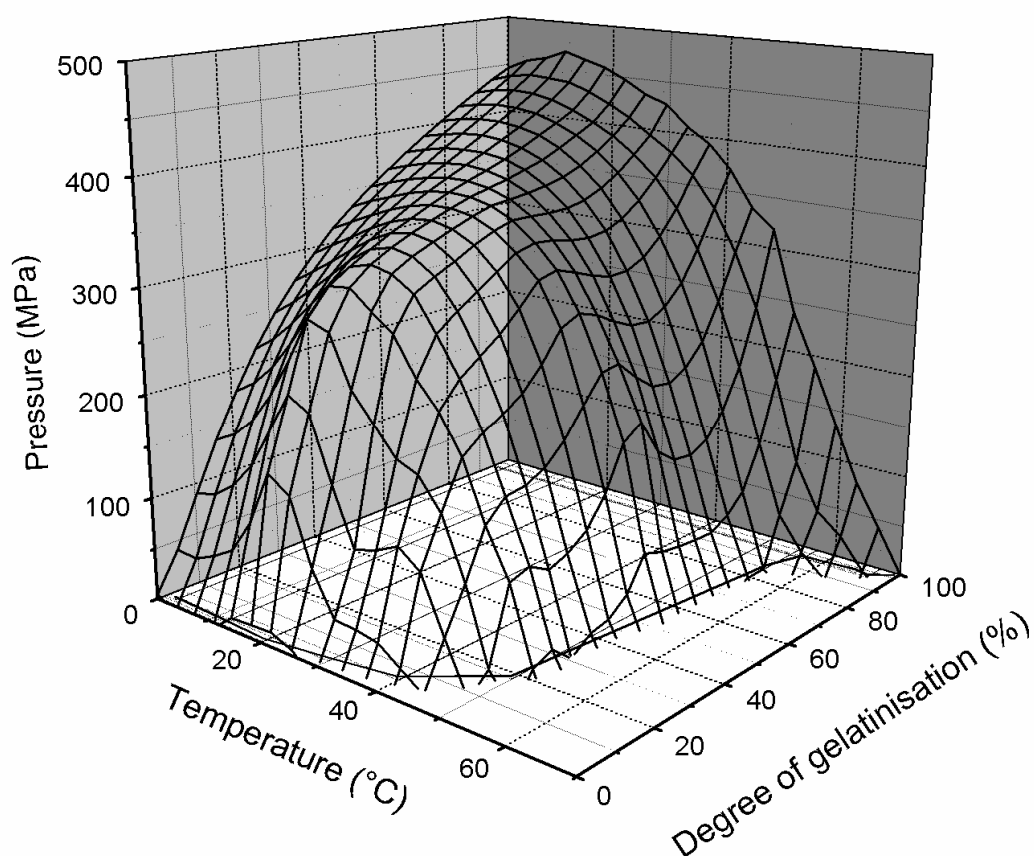
Fig. 4.18. Correlation of pressure, temperature, and degree of gelatinisation of wheat starch suspensions (5 % w/ w) treated for 15 min.

4.4.3 Pressurisation time

At constant temperature and pressure the treatment time was varied between 5 min and 4 h (Fig. 4.19) to gain insight into the relation between the degree of gelatinisation and pressurisation time (see also chapter 4.3.2). The constant pressures chosen resulted in a medium degree of gelatinisation at 15 min for each starch and hence permitted noticing a possible rise or decrease in starch gelatinisation at different treatment times. For all three starches examined the degree of gelatinisation increased with increasing treatment time during the first hour of treatment. At treatment times longer than 1 h, there was only a minor change in degree of gelatinisation in the case of potato starch. For wheat starch and tapioca starch the degree of gelatinisation continued to increase at pressure treatments up to four hours but the slope of the gelatinisation curve was reduced and the curve asymptotically approached 100 %.
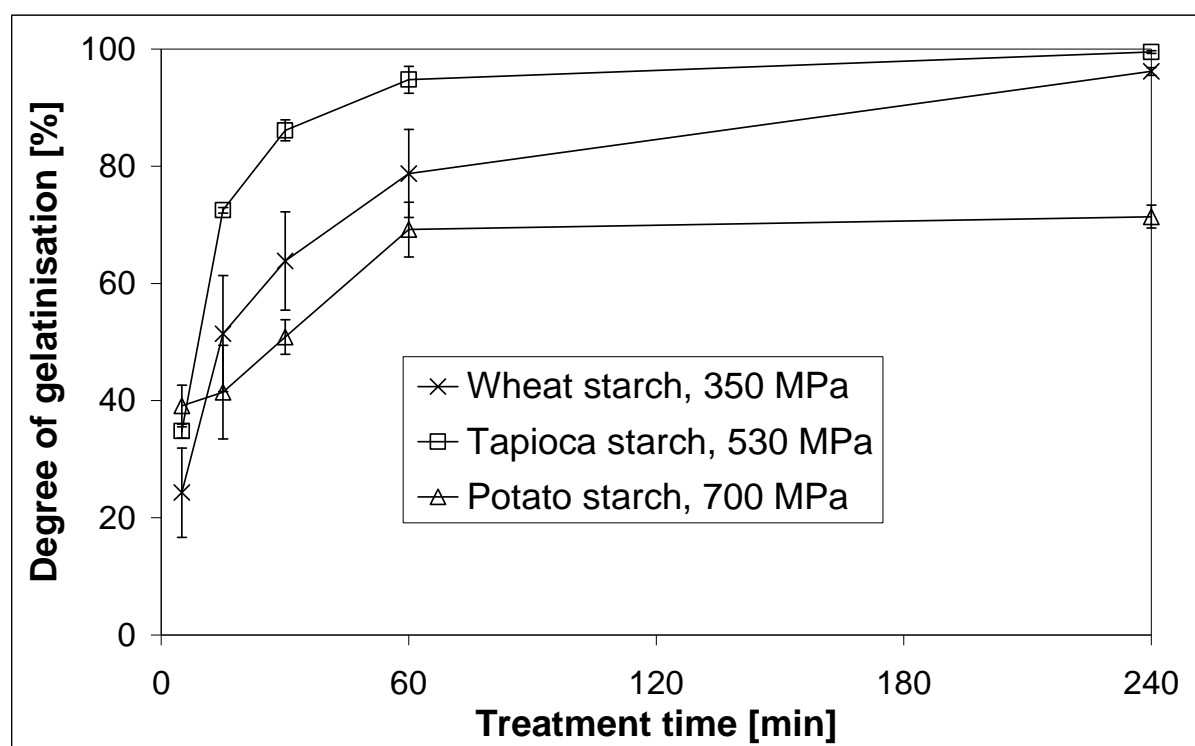
Fig. 4.19. Degree of gelatinisation of wheat, tapioca, and potato starch suspensions (5 % w/ w) after a pressure treatment at 29°C and a constant pressure of 350, 530, and 700 MPa, respectively, depending on the pressurisation time.

It can be concluded that the degree of gelatinisation of all three starches investigated strongly depended on the treatment time applied during the first hour of treatment. At longer treatment times the influence of pressurisation time diminishes and only wheat starch and

tapioca starch fulfilled the requirements of a PTTI, potato starch yielded no significant change in degree of gelatinisation upon pressurisation for more than 1 h. Because pressure treatments lasting longer than 1 h are not economical at an industrial scale, potato starch could well function as a PTTI. The apparently lacking influence of pressurisation time above 1 h on the degree of gelatinisation of potato starch is inexplicable, but it is possibly a result of the aforementioned inhomogeneity of the sample due to sedimentation. The experiment was repeated eight times, and each time no further rise in degree of gelatinisation of potato starch could be observed.
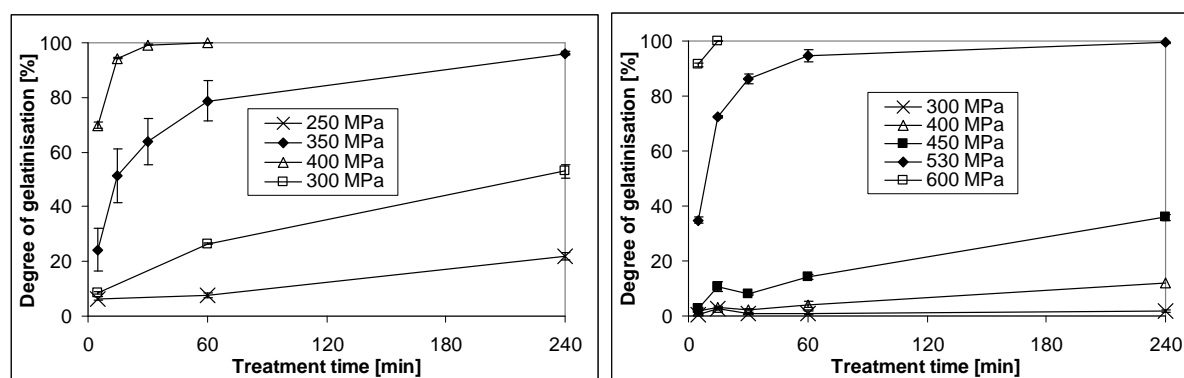
Fig. 4.20. Degree of gelatinisation of wheat (left) and tapioca (right) starch suspensions (5 % w/ w) after a pressure treatment at 29°C and a constant pressure depending on the pressurisation time.

To ensure that the time dependency of starches was not only valid in the intermediate pressure range, pressures at the initiation and shortly before completion of pressure-induced wheat and tapioca starch gelatinisation (after 15 min) have also been investigated (Fig. 4.20). At all pressures investigated, the degree of gelatinisation increased with increasing treatment time. The velocity rate of gelatinisation also increased with increasing pressure which was in accordance with results obtained by Stolt *et al.* (2001). Nonetheless the time dependency of pressure-induced starch gelatinisation persisted at higher or lower pressures. It also appeared that at any given pressure and temperature after an appropriate treatment time a complete gelatinisation of wheat starch and tapioca starch could be achieved but this cannot be concluded.

Pressure-time phase diagrams of wheat and tapioca starch suspensions (5 % w/ w) have also been generated (Fig. 4.21), where the relationship between pressure and treatment

time at a constant temperature (29°C) and achieved total gelatinisation is shown. As observed before, tapioca starch pressure-gelatinises at higher pressures than wheat starch at the same pressurisation time. The pressure required in order to attain complete gelatinisation decreases with increasing pressurisation time. The phase transition lines proceed logarithmic, the slopes of rise in gelatinisation pressure decrease with increasing treatment time. Additionally, both phase transition lines asymptotically approach a particular pressure, respectively. This leads to the conclusion that a threshold pressure is required to obtain total pressure-induced gelatinisation at indefinite treatment time and disproves the assumption that at any given pressure and temperature after an appropriate treatment time a complete gelatinisation of wheat starch and tapioca starch could be achieved. Nevertheless in order to achieve complete pressure-induced wheat and tapioca starch gelatinisation, the treatment time can be reduced to some extent by increasing the pressure and the pressure can be reduced to a certain degree by prolonging the pressurisation time.
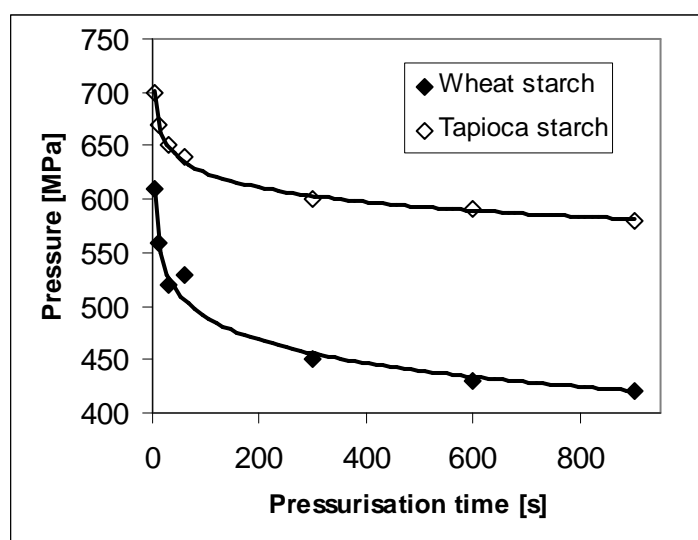
Fig. 4.21. Pressure-time phase diagrams of complete gelatinisation of wheat and tapioca starch suspensions (5 % w/ w) pressurised at 29°C.

4.4.4 Starch suspensions as pressure time temperature integrator

It can be concluded that the degree of gelatinisation of pressurised starch suspensions is highly dependent on the pressure, temperature and treatment time applied. As a result, pressure-induced starch gelatinisation qualifies as an adequate extrinsic PTTI. With its sensitivity to pressure, temperature and treatment time it enables to quantitatively and directly

measure the impact of the process as long as two process parameters are known. Due to its varying resistance to pressure depending on the type of starch, a starch can be selected meeting the precise requirements needed for a certain pressurisation process. If a pressure indicator for a higher pressure range is desired, a B-type starch being more resistant to pressure like potato starch would be suitable. However, the exact behaviour of the starch towards changes in pressure, temperature, and treatment time has to be thoroughly investigated and descried before it can be applied as a reliable pressurisation process indicator. For example, the tapioca starch examined in this work would not be applicable for temperatures below 50°C if the temperature was not held constant and the potato starch would be inadequate as a PTTI at pressures below 500 MPa. The most pronounced changes in degree of gelatinisation could be detected if starches were about midway of complete gelatinisation at the process conditions applied i.e. where the slope of the pressure-induced gelatinisation curve was the steepest. As a PTTI, a starch being in the intermediate range of gelatinisation at the process conditions would detect even slightest changes in pressure at known temperature characteristics and treatment time.

A quicker and easier possibility would be not to look at the degree of gelatinisation but to aim for complete gelatinisation. Then a starch would have to be chosen which achieved total gelatinisation at the desired process conditions. For the future it is desirable to develop a very quick and sophisticated way of measuring complete gelatinisation e.g. by a colour indicator which changes its colour when complete pressure-induced gelatinisation is achieved. For example, a decolouration of iodine stained starch granules during a pressure treatment has been observed and found to correspond with starch gelatinisation as confirmed by DSC (Douzals *et al.*, 1996). However, these results could not be confirmed in our laboratory (see chapter 4.2.2) and further research is required.

Since starch is widely used in food products it suggests itself to be applied as an intrinsic PTTI. In spite of this, the application of starch gelatinisation as an intrinsic PTTI is not recommendable. Pressure-induced starch gelatinisation is highly dependent on the free water content available (Stute *et al.*, 1996) and is e.g. influenced by the starch concentration of the suspension and the presence of salts and carbohydrates (see chapter 4.5). If there is a slight variation in the ingredients in a production process, the gelatinisation behaviour of starch under pressure would be altered. Accordingly, the use of starch gelatinisation as an extrinsic indicator demands a pure starch suspension with an identical water content.

4.4.5 Statistical evaluation

In order to investigate the statistically significant effect of temperature and pressure on the degree of gelatinisation of starches in 5 % (w/ w) suspensions treated for 15 min, multifactor analyses of variance (ANOVA) were performed for wheat, tapioca, and potato starch suspensions (5 % w/ w) with the assistance of the computer software Statgraphics 4.1. In a two-factor model, temperature and pressure functioned as independent factors and the degree of gelatinisation represented the dependent response variable. The Type III sums of squares have been chosen, where the contribution of each factor is measured having removed the effects of all other factors. Tables 4.2 – 4.4 depict the resulting ANOVA tables.

Tab. 4.2. ANOVA table for the degree of gelatinisation of wheat starch suspension (5 % w/ w) treated for 15 min

Source	Sum of squares	Degree of freedom	Mean squares	F-ratio	P-value
Temperature	30407.60	6	5067.94	9.05	0.0000
Pressure	21998.50	6	3666.42	6.55	0.0001
Residual	19592.50	35	559.79		
Total (corrected)	72690.80	47			

All F-ratios are based on the residual mean square error.

Tab. 4.3. ANOVA table for the degree of gelatinisation of tapioca starch suspension (5 % w/ w) treated for 15 min

Source	Sum of squares	Degree of freedom	Mean squares	F-ratio	P-value
Temperature	20923.10	5	4184.61	11.69	0.0000
Pressure	35299.80	5	7059.95	19.73	0.0000
Residual	8945.59	25	357.82		
Total (corrected)	65168.40	35			

All F-ratios are based on the residual mean square error.

Tab. 4.4. ANOVA table for the degree of gelatinisation of potato starch suspension (5 % w/ w) treated for 15 min

Source	Sum of squares	Degree of freedom	Mean squares	F-ratio	P-value
Temperature	34008.70	5	6801.74	12.36	0.0000
Pressure	11183.10	4	2795.78	5.08	0.0055
Residual	11009.20	20	550.46		
Total (corrected)	56201.10	29			

All F-ratios are based on the residual mean square error.

The F-Test showed that the means of the factor levels differed, since all F-ratios (Tab. 4.2. - 4.4) were larger than the corresponding F-value derived from an F-distribution table (Neter & Wasserman, 1974). As an example, the F-Test for effects of the factor pressure on potato starch gelatinisation at the 95.0 % confidence level given in the following equation

$$F\text{-ratio} = 5.08 < F (0.95, 5, 20) = 2.87$$

demonstrates that not all factor level means of the factor temperature are equal, or that some definite effects of pressure on the degree of gelatinisation of potato starch exist (Neter & Wasserman, 1974). This can be transferred to both factors of all three starches investigated.

The P-values test the statistical significance of each of the factors. Because all P-values are less than 0.05, the factors temperature and pressure have a statistically significant effect on gelatinisation at the 95.0 % confidence level for all three starches investigated. Even at a confidence level of 99.0 % the effects of the factors investigated on the response variable are statistically significant, since all P-values are below 0.01 (Neter & Wasserman, 1974).

4.5 Effect of water content and additives on pressure-induced starch gelatinisation

The influence of water content as well as the effect of additives such as sugars and salts on pressure-induced starch gelatinisation has also been investigated. Wheat, tapioca, and potato starch suspensions with concentrations of one to at least 55 % (w/ w) were pressurised at constant pressures for 15 min, respectively, to examine the impact of the water content. For the investigation of the effect of additives, the starch concentration was kept constant at 5 % (w/ w), as additives several mono-, di-, and trisaccharides, chlorides, sodium and potassium salts have been chosen. For the examination of the impact of salts on pressure-induced starch gelatinisation, emphasis was put on the effect of sodium chloride due to its importance in food products and its interesting effects on pressure-induced potato starch gelatinisation.

The effect of numerous chlorides and sodium salts on pressure-treated (800-1,200 MPa for 60 and 300 s) dried potato starch (2-22 % moisture content) has already been determined by Kudla and Tomasik (1992), they only obtained synergistic effects of the additives iodine, ferric and cobaltous chlorides and high pressure on deterioration of the

starch matrix. Since the water content was extremely low, the results could not be transferred due to the entirely different experimental set-up chosen for this work.

4.5.1 Impact of water content

Starch suspensions with different starch concentrations and hence different water contents were pressure-treated. At a constant pressure, it was observed that the degree of gelatinisation decreased with increasing starch concentration (Fig. 4.22). This was in accordance with results obtained by Kudla and Tomasik (1992) having obtained an accelerated destruction of the starch matrix of potato starch granules with increasing humidity. Below a water content of 50 % the influence of the pressure on starch gelatinisation decreased rapidly. Even an increase in pressure in the case of wheat starch suspensions from 350 to 500 MPa exhibited a decreasing influence of pressure at a water content below 50 % as represented by the abrupt decline in degree of gelatinisation. This clearly showed that water influences the gelatinisation pressure and furthermore indicated that a certain amount of water, i.e. 50 %, might be required for the pressure-induced starch gelatinisation and was in accordance with findings made by Hibi *et al.* (1993) and Stute *et al.* (1996).
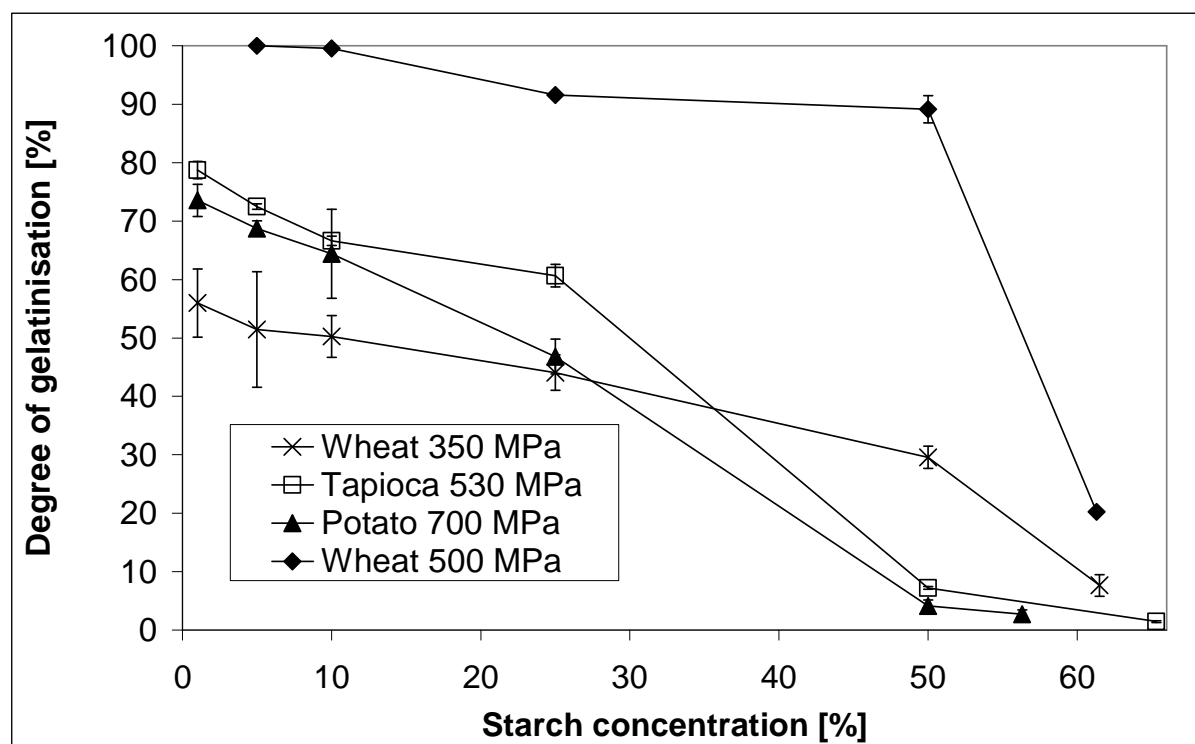
Fig. 4.22. Degree of gelatinisation of pressurised wheat, tapioca and potato starch suspensions at constant pressure (15 min, 29°C) with increasing starch concentration (% w/ w).

4.5.2 Effect of sugars

The addition of sucrose led to a reduction of the degree of gelatinisation of pressurised wheat, tapioca and potato starch suspensions and the degree of gelatinisation decreased with increasing sucrose content (Fig. 4.23). At a sucrose concentration of about 0.58 mol/ l (20 % w/ w) the pressure-induced gelatinisation was almost entirely suppressed for all three starches (at the pressures applied). Since the three starches differ in pressure sensitivity (see chapter 4.4) and different pressures had been applied (350 MPa for wheat starch, 530 MPa for tapioca starch, and 700 MPa for potato starch suspensions), the influence of the sucrose on the three starches was not comparable. Nonetheless it can be concluded that the presence of sucrose suppresses pressure-induced starch gelatinisation. This can be explained by a reduction of the water availability with increasing sucrose concentration and thus decreasing water activity. The slight plateau or reduced slope in the decrease of the degree of gelatinisation of the wheat and tapioca starches at low sucrose concentrations up to around 0.03 mol/ l indicates that a critical sucrose concentration has to be exceeded for the water structure making effect. Potato starch appeared to be more sensitive towards the suppressing effect of sucrose on pressure-induced gelatinisation. Already small concentrations (0.003 mol/ l) of sucrose led to a substantial decrease in degree of gelatinisation. However, a plateau up to around 0.03 mol/ l sucrose could also be observed.
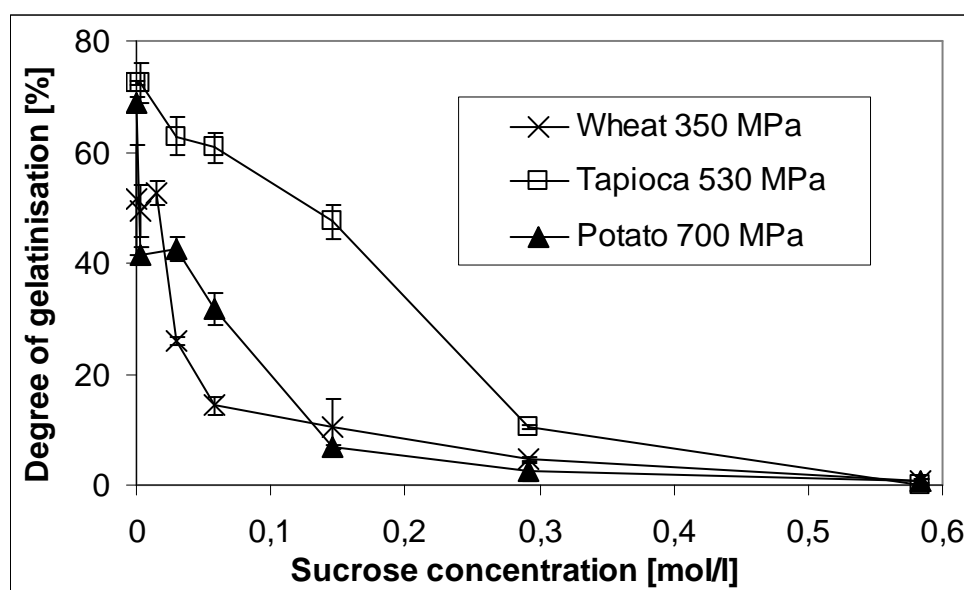
Fig. 4.23. Degree of gelatinisation of wheat, tapioca, and potato starch suspensions (5 % w/ w) pressurised at 350, 530, and 700 MPa, respectively, for 15 min at 29°C with increasing sucrose concentration.

At a constant sucrose content of 1 % (w/ w), a shift in gelatinisation pressure could be observed in comparison to native wheat starch suspension (Fig. 4.24). The gelatinisation pressure increased with increasing sucrose content. This correlates with the influence of sucrose on the thermal starch gelatinisation (Evans & Haisman, 1982; Spies & Hoseney, 1982; Chinachoti *et al.*, 1991; Ahmad & Williams, 1999; Jang *et al.*, 2001; Maaurf *et al.*, 2001). Consequently, sucrose could be used to alter and control the gelatinisation pressure and to impede gelatinisation.
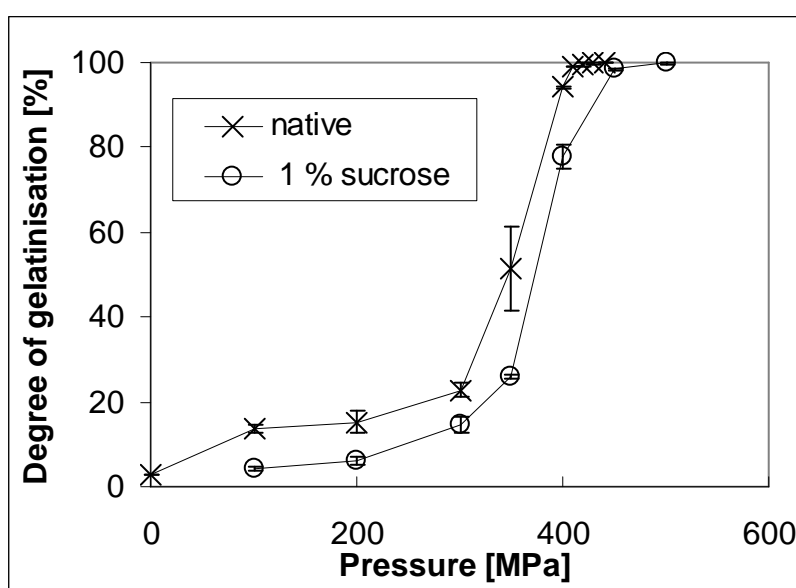
Fig. 4.24. Gelatinisation curves of wheat starch suspensions (5 % w/ w) without and with 1 % (w/ w) sucrose upon pressurisation (15 min, 29°C).

The effect of increasing concentration of fructose, glucose, sucrose, and trehalose on wheat, tapioca, and potato starch suspensions pressurised for 15 min at 29°C and 350, 530, and 700 MPa, respectively, is depicted in figure 4.25. All sugars led to a decrease in the degree of gelatinisation and hence interfered with the pressure-induced gelatinisation process. Disaccharides seemed to have a more pronounced effect than monosaccharides in terms of hindering pressure-induced gelatinisation. This is in agreement with the hypothesis that longer sugar molecules form more links with starch molecules than shorter sugar molecules leading to an increased restriction and reduced flexibility of the starch chains and thus requiring more energy for gelatinisation (Spies & Hoseney, 1982).

Fig. 4.25. Degree of gelatinisation of pressurised wheat starch suspension (a; 5 % w/ w, 15 min, 29°C, 350 MPa), tapioca starch suspension (b; 5 % w/ w, 15 min, 29°C, 530 MPa), and potato starch suspension (c; 5 % w/ w, 15 min, 29°C, 700 MPa) with increasing fructose, glucose, sucrose, and trehalose concentration, respectively.

For wheat starch suspensions, the influence of 0.15 mol/ l of a variety of sugars on the degree of gelatinisation after a pressure treatment was investigated (Fig. 4.26). It was discovered that the degree of gelatinisation linearly correlated with the number of equatorial hydroxyl groups of the sugars present. Additionally, it was observed that the degree of gelatinisation decreased with increasing molecular weight and number of hydroxyl groups. Since hydroxyl groups are presumably involved in forming bridges with starch molecules, sugars with a higher number of hydroxyl groups and consequently mostly also with a higher molecular weight were expected to lower starch gelatinisation under pressure. The correlation of starch gelatinisation in the presence of low-molecular sugars and their number of equatorial hydroxyl groups corroborates the theory that a reduction of water activity and decreased water availability reduces gelatinisation as well as sugar-starch interactions (Spies & Hoseney, 1982). The higher the number of equatorial hydroxyl groups of a sugar, the easier it forms hydrogen bonds with water (Kato *et al.*, 2001) because an equatorial hydroxyl group will be more strongly hydrated than an axial group (Kabayama & Patterson, 1958). Tamura (1999) found a linear correlation of thermotolerance and barotolerance of yeasts with mean number of equatorial OH groups of sugars, and Uedaira and Uedaira (1980) observed that the denaturation temperature of lysozyme increased linearly with increasing number of equatorial hydroxyl groups of sugars. Thus low-molecular sugars have a protective effect during heat

and pressure treatment that corresponds with their mean number of equatorial OH groups, not only on yeast vitality and protein denaturation, but also on starch gelatinisation during a pressure treatment.
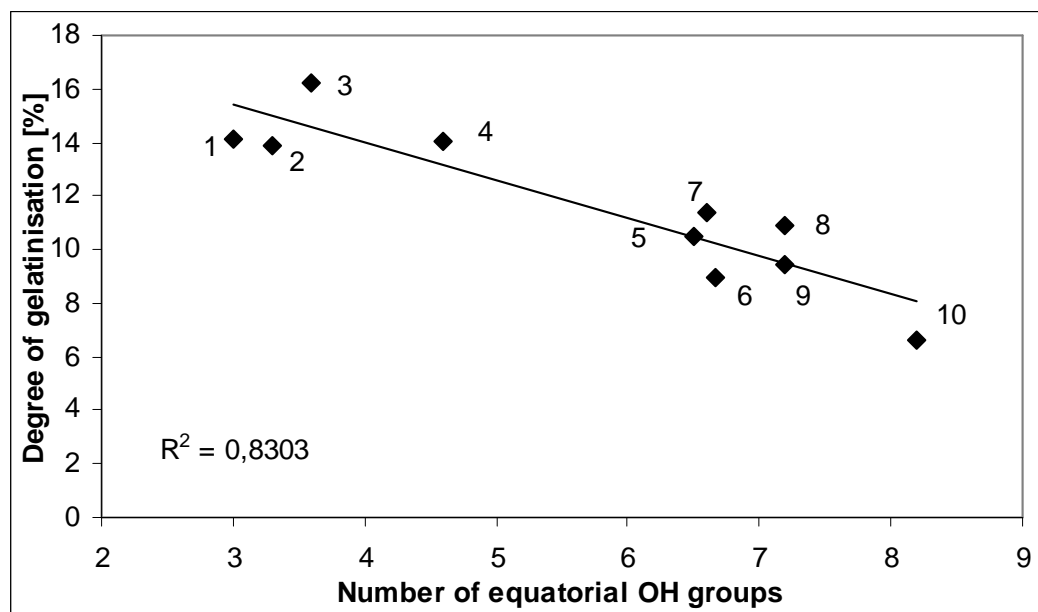
Fig. 4.26. Degree of gelatinisation of pressurised wheat starch suspensions (5 % w/ w, 15 min, 350 MPa, 29°C) with 0.15 mol/ l sugar (1 fructose, 2 mannose, 3 galactose, 4 glucose, 5 sucrose, 6 lactose, 7 melibiose, 8 maltose, 9 trehalose, 10 melezitose). The numbers of equatorial hydroxyl groups for the sugars were derived from Uedaira *et al.* 1980, 1985 & 1990, Gekko & Koga 1983, and Kato *et al.* 2001.

4.5.3 Impact of sodium chloride

The degree of gelatinisation of wheat, tapioca and potato starch suspensions with increasing sodium chloride concentration is depicted in figure 4.27.

For wheat and tapioca starches, NaCl reduced gelatinisation. Between a NaCl concentration of 0.5 and 2 M the gelatinisation was completely suppressed at the pressures applied, above 2 M the protective effect on starch crystallites subsided and the gelatinisation remained suppressed, but to a lesser extent. Consequently, sodium chloride can be utilised to suppress wheat and tapioca starch gelatinisation under high pressure

For potato starch suspensions, small concentrations of NaCl up to 2 M suppressed gelatinisation with a minimum at 0.7 M (4 %), above 3.4 M NaCl (20 %) the gelatinisation of potato starch was enhanced until complete gelatinisation was achieved at 5.1 M. As a result, small concentrations of NaCl can be used to impede potato starch gelatinisation, while the

gelatinisation pressure of potato starch can be decreased by high sodium chloride concentrations.
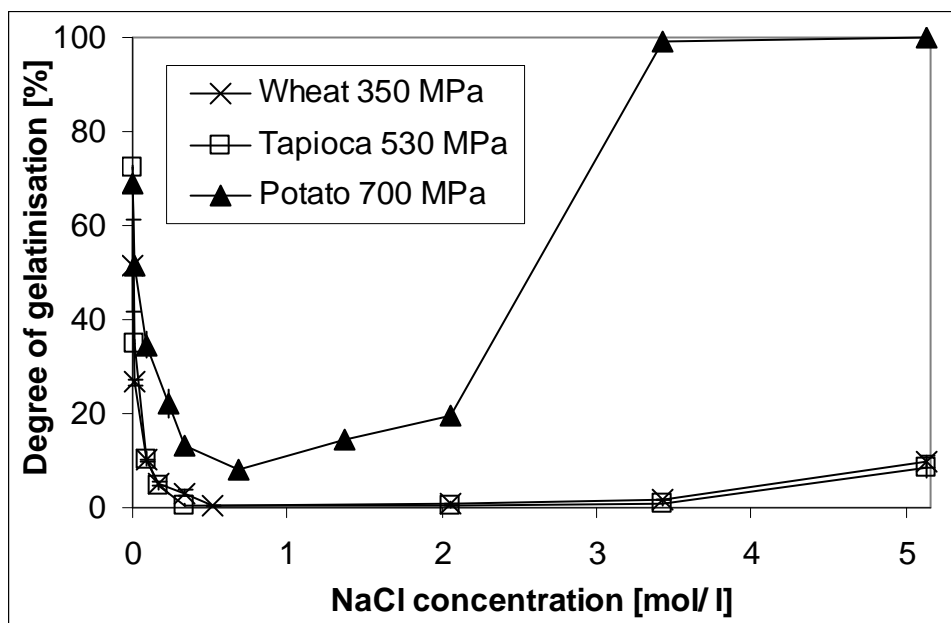
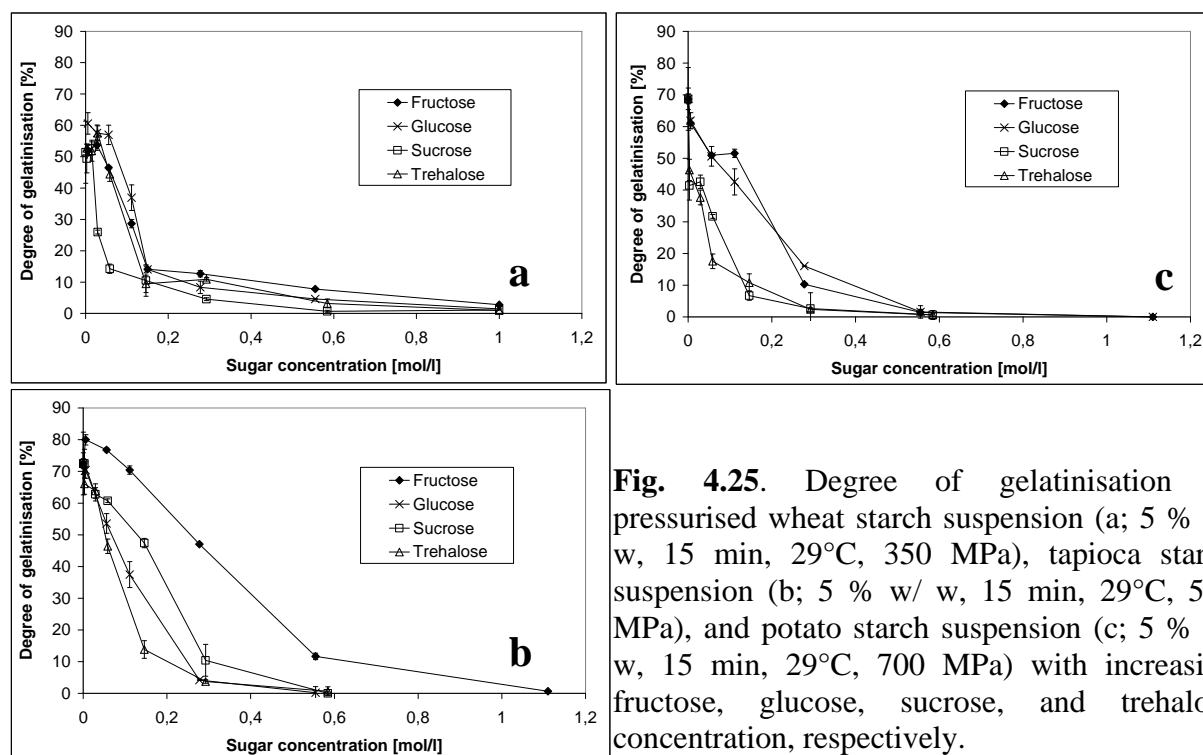**Fig. 4.27**. Degree of gelatinisation of wheat, tapioca, and potato starch suspensions (5 % w/ w) pressurised at 350, 530, and 700 MPa, respectively, for 15 min at 29°C with increasing sodium chloride concentration.
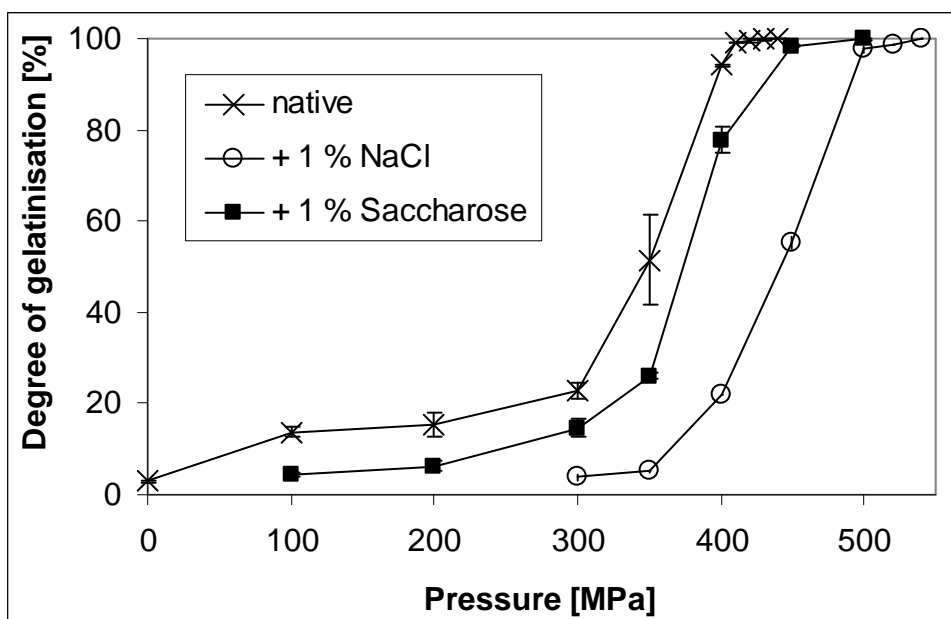
Fig. 4.28. Gelatinisation curves of wheat starch suspensions (5 % w/ w) without and with 1 % (w/ w) sucrose and sodium chloride, respectively, upon pressurisation (15 min, 29°C).

The inhibitory effect of NaCl on wheat starch gelatinisation under pressure is displayed in figure 4.28. In comparison to 1 % (w/ w) sucrose, 1 % NaCl has a more retarding effect on pressure-induced starch gelatinisation, which can partly be attributed to the higher molar concentration (0.17 M NaCl in contrast to 0.03 M sucrose). Nonetheless, at the same molar concentration the degree of gelatinisation of wheat starches is somewhat lower in the presence of NaCl than of sucrose.

As shown in fig. 4.27, small concentrations of NaCl (2 M) reduced gelatinisation of potato starch with a minimum at 0.7 M (4 %), above 3.4 M NaCl (20 %) the gelatinisation of potato starch was enhanced. This phenomenon was confirmed by pressurising potato starch suspensions with constant sodium chloride concentration and increasing pressure (Fig. 4.29). The gelatinisation pressure of potato starch suspensions was higher at a NaCl concentration of 4 % than without salt added, it was reduced when 20 or 30 % NaCl were dissolved. This corresponds roughly with observations made for thermal gelatinisation (Sandstedt *et al.*, 1960; Evans & Haisman, 1982; Maaurf *et al.*, 2001). It can be concluded that low concentrations of sodium chloride decreased the gelatinisation pressure of starches, at higher concentrations it increased again. This effect was more pronounced for potato starches than for tapioca and wheat starches where high NaCl concentrations led to complete gelatinisation. This cannot be explained so far, but it is assumed that differences in the molecular and the crystal structure and chemical composition of the starches might be responsible for that effect.
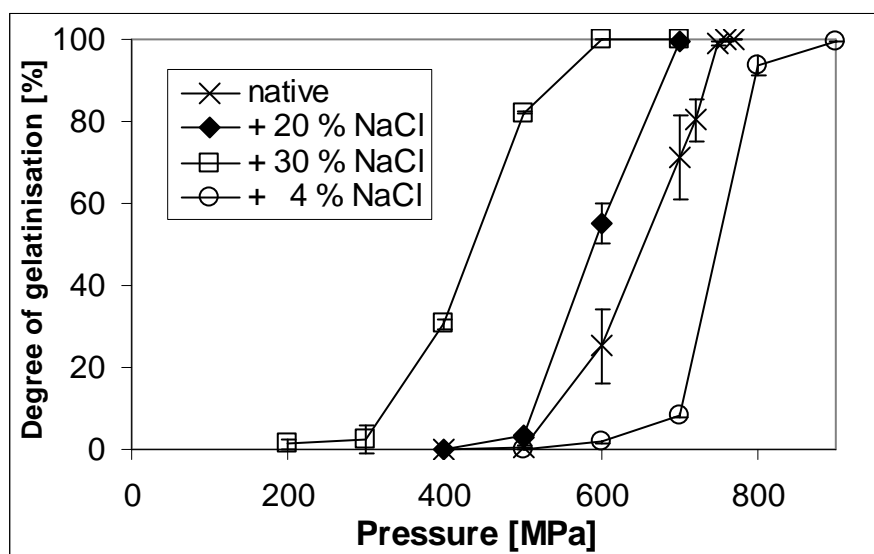
Fig. 4.29. Gelatinisation curves of potato starch suspensions (5 % w/ w) without and with 4, 20, and 30 % (w/ w) sodium chloride, respectively, upon pressurisation for 15 min at 29°C.

4.5.4 Influence of salts

The effect of several chlorides, sodium and potassium salts on the pressure gelatinisation of wheat, tapioca and potato starches was also examined (Fig. 4.30-32).

Results obtained for sodium, lithium, calcium and potassium chlorides in wheat starch suspensions and also for sodium chloride in potato and tapioca starch suspensions (Fig. 4.30) agreed with findings made by Sandstedt *et al.* (1960), Evans and Haisman (1982), and Jane (1993) for thermal starch gelatinisation. The gelatinisation pressure first increased and then decreased with increasing chloride concentrations as evidenced by a decrease and subsequent increase in degree of gelatinisation with increasing chloride concentration.

For other chlorides than NaCl in potato and tapioca starch suspensions a slight decrease in gelatinisation pressure was detected at low chloride concentrations (0.1 M) before the gelatinisation pressure increased and then decreased again with increasing chloride concentrations. This was not reported for the effect of these salts on the thermal gelatinisation. At high chloride concentrations (> 2 M) the impact of the salts on starch gelatinisation augmentation followed the order $Na^+ < K^+ < Li^+ < Ca^{2+}$, which corresponds to the order of the lyotropic series (v. Hippel, 1975).
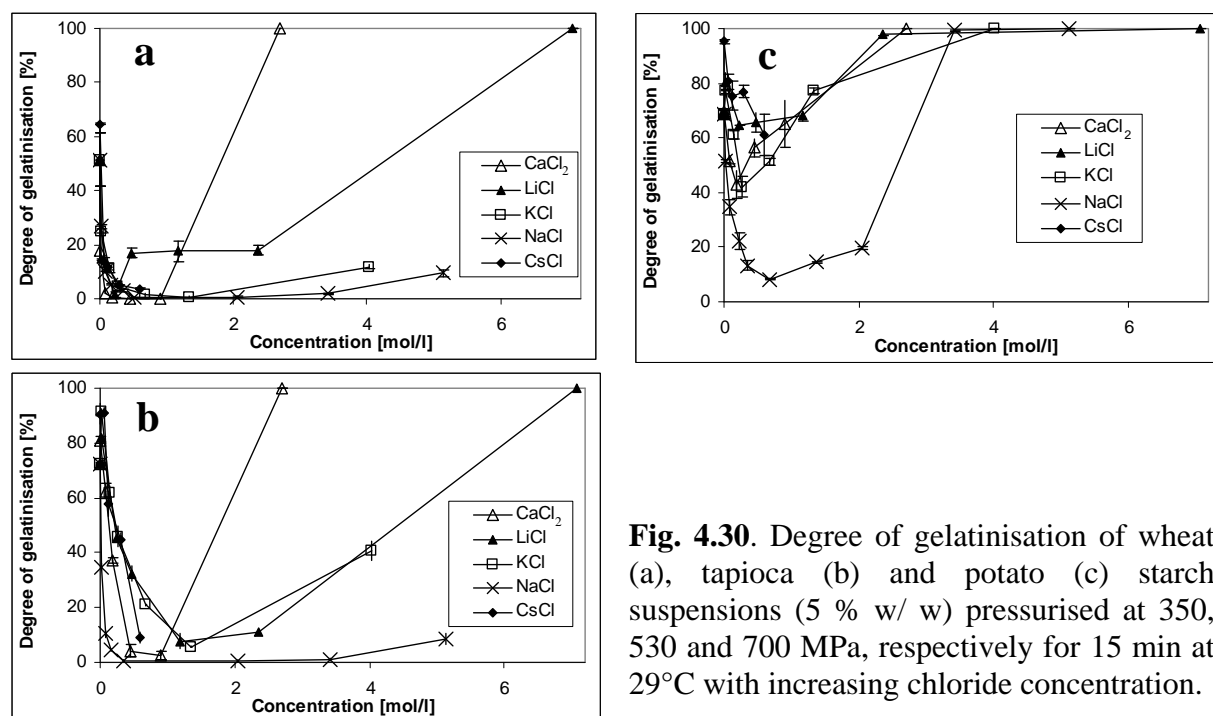
Fig. 4.30. Degree of gelatinisation of wheat (a), tapioca (b) and potato (c) starch suspensions (5 % w/ w) pressurised at 350, 530 and 700 MPa, respectively for 15 min at 29°C with increasing chloride concentration.

In the case of caesium chloride for all three starches investigated, there was first a decrease at very low CsCl concentrations followed by an increase of the gelatinisation pressure. The molar concentrations examined (0.6 M) were probably not high enough to show a subsequent decrease in gelatinisation. The effect of all chlorides examined on starch gelatinisation was the most pronounced for potato starch suspensions.

The investigation of the effect of potassium salts on pressure-induced starch gelatinisation included potassium thiocyanate, iodide, bromide and chloride (Fig. 4.31). Just as for chlorides, the effect of potassium salts on the degree of gelatinisation differed depending on the type of starch and on the counter ion. The effect of potassium salts on starch gelatinisation was again the most distinct for potato starch suspensions. For all starches an increase in the degree of gelatinisation could be detected above a salt concentration of 1.3 M.
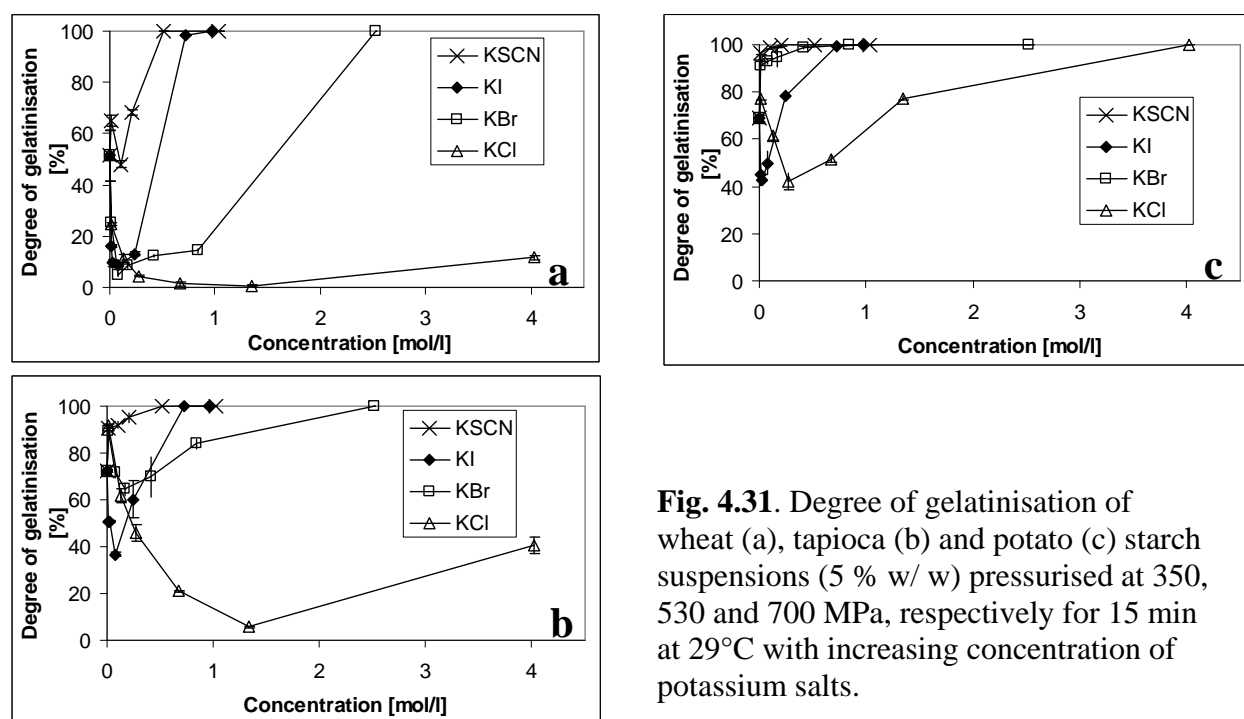
Fig. 4.31. Degree of gelatinisation of wheat (a), tapioca (b) and potato (c) starch suspensions (5 % w/ w) pressurised at 350, 530 and 700 MPa, respectively for 15 min at 29°C with increasing concentration of potassium salts.

Except for KCl in wheat and tapioca starch suspensions, a decrease in gelatinisation pressure could be observed at high concentrations. At concentrations above 1 M the effect of the potassium salts on starch gelatinisation upon pressurisation also followed the order of the lyotropic series ($Cl^-<Br^-<I^-<SCN^-$) (v. Hippel, 1975). Just as for the thermal gelatinisation, KI and KSCN reduced the energy required for starch gelatinisation. This is due to their low

viscosity, to their good hydrating properties by increasing the free water fraction and their low charge-density ions SCN^- and I_3^- (structure breakers) interacting with starch and facilitating dissociation by inducing single helical conformation (Jane, 1993). This could also be observed to a lower extent after 15 min at atmospheric pressure, where a degree of gelatinisation of wheat starch suspension of approximately 15 % was achieved at a potassium iodide concentration of 0.96 M (data not shown). The influence of high hydrostatic pressure treatment is much more pronounced, since complete gelatinisation was accomplished at the same KI concentration at 350 MPa.
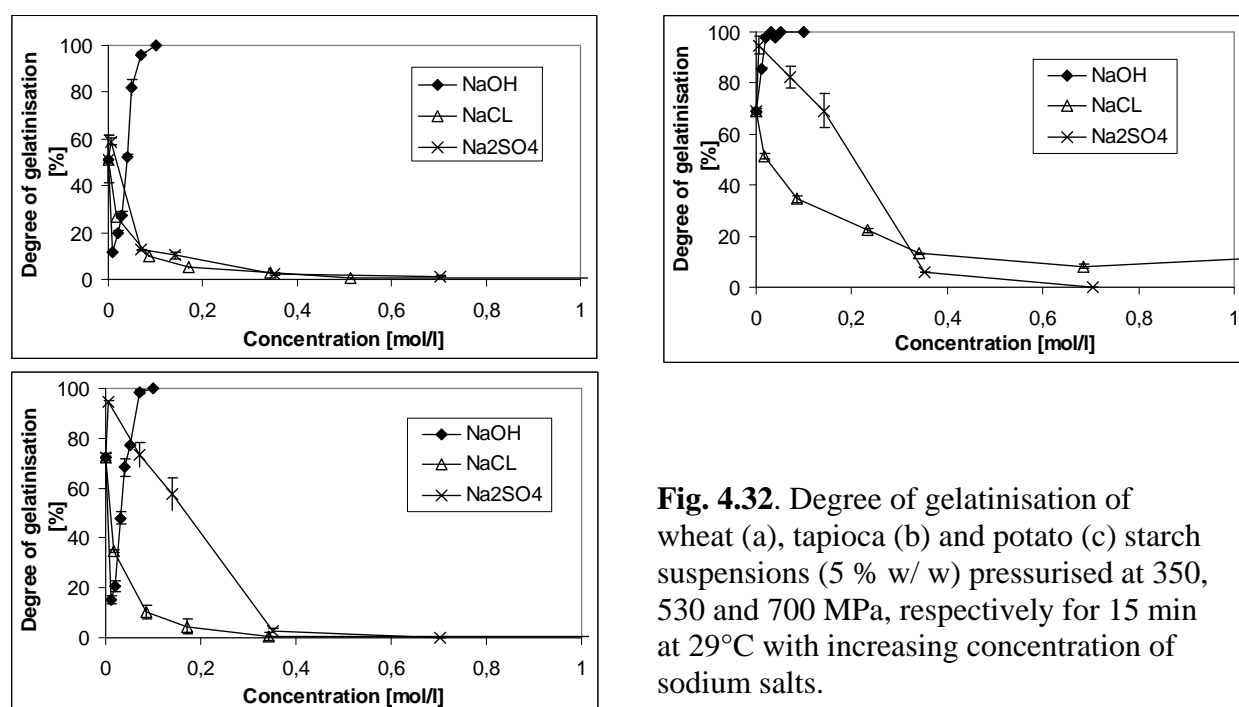

Fig. 4.32. Degree of gelatinisation of wheat (a), tapioca (b) and potato (c) starch suspensions (5 % w/ w) pressurised at 350, 530 and 700 MPa, respectively for 15 min at 29°C with increasing concentration of sodium salts.

Sodium hydroxide and sulphate in addition to sodium chloride have also been investigated (Fig. 4.32). Sodium sulphate causes a rise in gelatinisation pressure at concentrations below 0.07 M and a subsequent reduction with increasing sodium sulphate concentration above 0.07 M. At concentrations of around 0.7 M the gelatinisation process is almost entirely suppressed at the pressures applied. Sulphate ions are so-called structure makers, ions with a high charge density reducing the amount of free water and decreasing the viscosity of aqueous liquids by breaking or weakening hydrogen bonds between water molecules due to strong electrostatic interactions with water molecules (Jane, 1993). At concentrations above 0.07 M this appeared to be also applicable under pressure. Sodium

hydroxide led, in the case of wheat and tapioca starch, first to an increase in gelatinisation pressure, above 0.01 M the gelatinisation pressure decreased again until complete gelatinisation was obtained at 0.1 M NaOH. For potato starch, the degree of gelatinisation increased from the outset and complete gelatinisation was already achieved at 0.05 M NaOH at 700 MPa. Just as for other salts examined, potato starch appears to be the most susceptible to starch gelatinisation under pressure in the presence of sodium salts. The observed rise in degree of gelatinisation with increasing NaOH concentration was not solely due to an alkaline gelatinisation. At atmospheric pressure and 0.1 M NaOH in wheat starch suspensions, a degree of gelatinisation of 17 % was achieved (data not shown), whereas at 350 MPa complete gelatinisation was obtained. The influence of sodium chloride was described in detail above (Fig. 4.27).

4.6 Formation of resistant starch from pressurised wheat starch

It was investigated whether a pressure-gelatinisation of wheat starch suspensions in combination with additional treatment steps resulted in a formation of resistant starch (RS) in high amounts being advantageous to conventional resistant starch production without the use of high hydrostatic pressure. After determining process parameters such as starch concentration and storage temperature being favourable for the formation of RS, gelatinisation-retrogradation cycles according to Sievert and Pomeranz (1989) were adapted also after acid and enzyme hydrolysis and the RS content as well as the microscopic images and the X-ray diffraction patterns of the treated samples were determined and discussed. The RS amounts yielded were compared with RS yields obtained from thermally gelatinised wheat starch as stated in literature.

4.6.1 Preliminary tests

In preliminary tests, several process parameters were optimised in order to obtain an enhanced RS content in wheat starch by retrogradation subsequent to high hydrostatic pressure-induced gelatinisation. It was observed that the starch concentration ranging between 5 % and 20 % (w/ w) of pressure-treated suspensions did not have a notable influence on the RS content, whereas the RS content of heat treated wheat starch suspensions increased with increasing concentration (Fig. 4.33). This was presumably due to the increasing amount of released amylose molecules realigning with increasing starch concentration during thermal

gelatinisation in contrast to the low amylose release (Douzals et al, 1998) in the course of pressure-induced gelatinisation. A starch concentration of 5 % was chosen for all subsequent experiments.

Figure 4.33 also shows that the RS content in heat treated wheat starch suspensions immediately after the gelatinisation was clearly higher or about equal to pressure treated suspensions depending on the starch concentration. This is a strong indication that the rapid retrogradation immediately after pressurisation observed by DSC measurements by Stute *et al.* (1996) did not include the formation of RS.
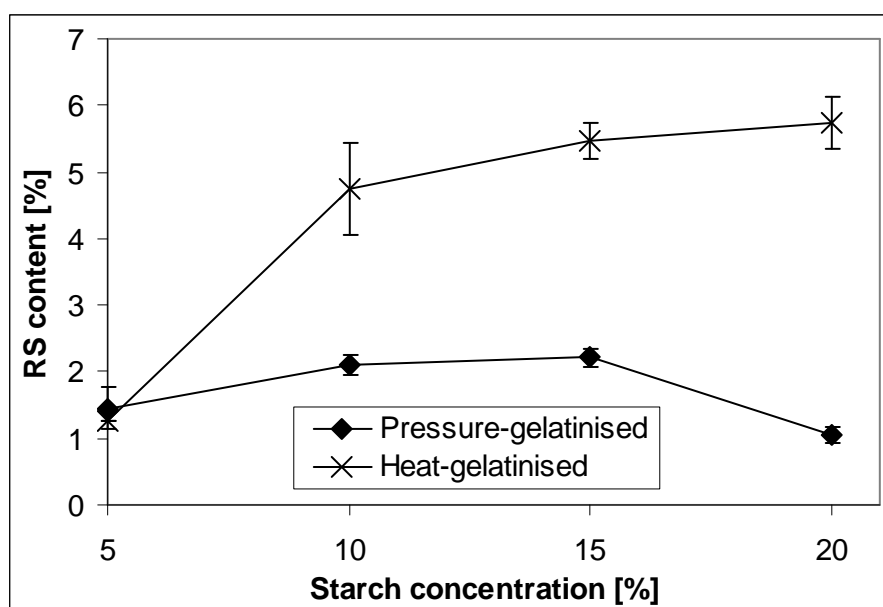
Fig. 4.33. Resistant starch content of gelatinised wheat starch suspensions immediately after a thermal gelatinisation (15 min, 0.1 MPa, 90°C) and a pressure-induced gelatinisation (15 min, 500 MPa, room temperature), respectively, in dependence on the starch concentration.

The storage temperature subsequent to pressurisation was also evaluated. As Douzals *et al.* (1998) already indicated analysing the retrogradation behaviour of pressure-gelatinised wheat starch, low storage temperatures (6°C and room temperature for 24 h) yielded lower RS contents compared to thermally gelatinised wheat starch (Fig. 4.34). Freeze-thawing (-20°C to room temperature) of pressurised starch did also not lead to an enhanced RS content in comparison to heat-treated or unfrozen pressure-treated starch , while freeze-thawing of thermally treated starch resulted in an enhancement of RS content. This was in accordance with findings made by Chung *et al.* (2003). Subsequent freeze-drying did not further enhance

the RS content of either heat or pressure-treated starch (data not shown). However, storage at 40°C and 52°C for 24 h following a pressure treatment resulted in higher RS yields in comparison to heat-gelatinised starch, as depicted in figure 4.34. The RS content of pressurised starch increased with increasing storage temperature. Therefore a storage temperature of 52°C was selected for further experiments. Storage slightly below the gelatinisation temperature is called annealing and is known to induce a molecular reorientation and reorganisation of the granule structure (Tester & Debon, 2000). Although annealing causes an increase in gelatinisation temperature, wide angle X-ray diffraction patterns of not gelatinised wheat starches are unaltered by annealing leading to the conclusion that crystallites remain unchanged. Nevertheless, a sharpening of DSC peaks due to annealing is a definite indication for a rise in homogeneity and for a modification of the embedding of starch crystallites in and their coupling forces to the amorphous matrix (Stute, 1992).
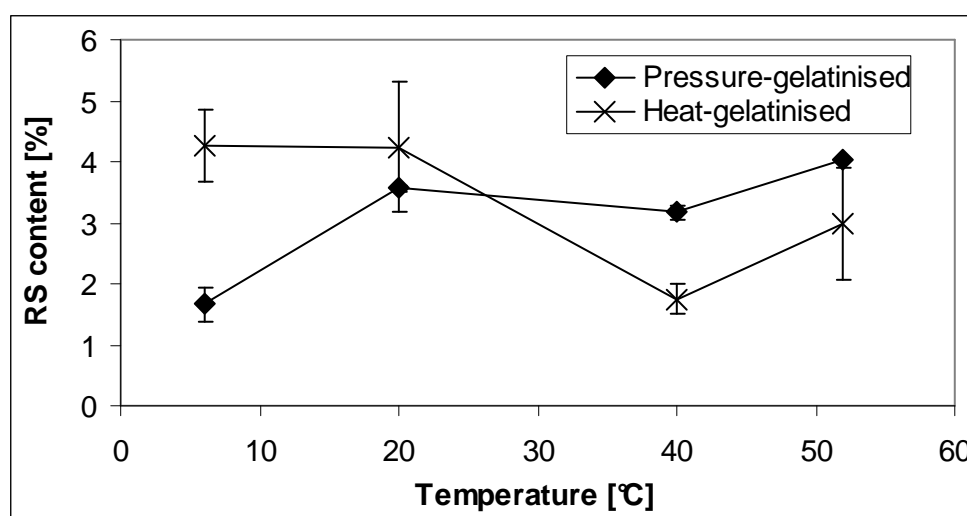
Fig. 4.34. Resistant starch content [%] of wheat starch suspensions (5 % w/ w) 24 h after a thermal gelatinisation (15 min, 0.1 MPa, 90°C) and a pressure-induced gelatinisation (15 min, 500 MPa, room temperature), stored at 6, 20, 40, and 50°C, respectively.

A pressure treatment of wheat starch suspensions was also performed at 53°C, 58°C, and 66°C, and compared to room temperature and a thermal treatment at 90°C to explore the effect of higher temperatures during pressurisation on the RS formation (Tab. 4.5). The RS content of pressurised suspensions appeared to increase slightly with increasing treatment temperature. But the determination method by McCleary and Monaghan (2002) is only designed for samples with a RS content above 2 %, and therefor a slight inaccuracy cannot be

excluded. In addition to that, the thermal influence seemed to prevail. Thus, in order to prevent a thermal impact, pressure treatments were performed at room temperature thereupon.

Tab. 4.5. Resistant starch content of wheat starch suspensions (5 % w/ w) pressurised at different temperatures in comparison to thermal gelatinisation

Pressure [MPa]	Time [min]	Temperature [°C]	RS content [%]
500	15	RT	1.46 ± 0.33
500	15	53	1.72 ± 0.09
500	15	58	1.81 ± 0.42
500	15	66	2.30 ± 0.46
0.1	15	90	2.25 ± 0.26

RS – resistant starch; RT – room temperature

4.6.2 Resistant starch formation after high pressure treatment

Based on all these experiences, high pressure-gelatinised wheat starch suspensions were continuously annealed at 52°C for up to ten days (Fig. 4.35). The RS content increased linearly up to 7.7 % the first192 h and remained about constant thereafter. However, after 528 h of annealing a RS content of approximately 9 % was measured (data not shown), so a slight increase in RS with continuous annealing proceeded. These findings were in agreement with Eerlingen *et al.* (1993a) stating that RS yields largely depended on storage temperature and storage time. Besides, it could be concluded that an enhancement in RS content after high hydrostatic pressure treatment is achievable.

Additionally, gelatinisation-retrogradation cycles according to Sievert and Pomeranz (1989) were adapted, i.e. wheat starch suspensions were pressure-gelatinised (500 MPa, 15 min) and annealed (23 h 45 min, 52°C) in 24 h-cycles repeated up to ten days. As depicted in figure 4.35, continuous annealing subsequent to a single pressure-treatment and pressure-annealing cycles were compared. The RS content increased more rapidly within the first 72 h of pressure-annealing cycles, then the curves ran more or less parallel exhibiting a similar RS formation rate. Overall, the cycling yielded a clearly higher RS content with 12.6 % after ten days.
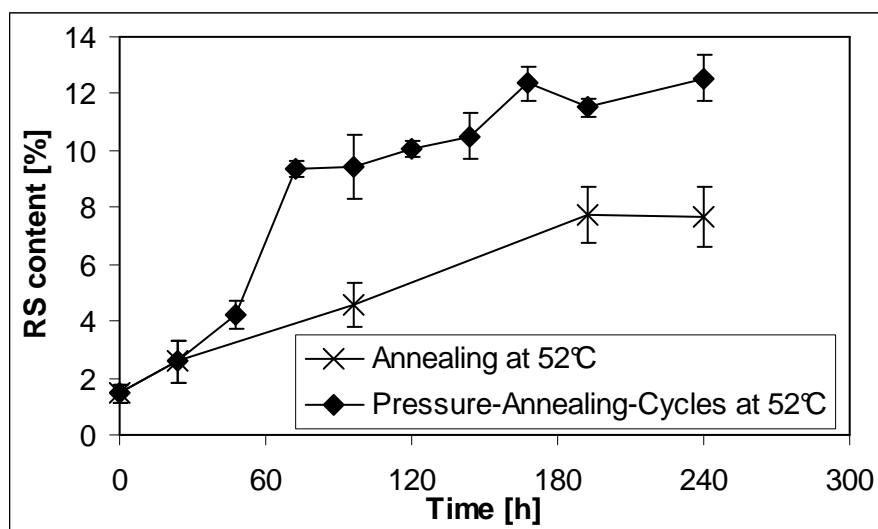
Fig. 4.35. Resistant starch content of wheat starch suspensions (5 % w/ w) pressurised at 500 MPa for 15 min at room temperature followed by continuous annealing at 52°C in comparison to annealing at 52°C interrupted by a pressurisation step every 24 h (Pressure-annealing-cycles).
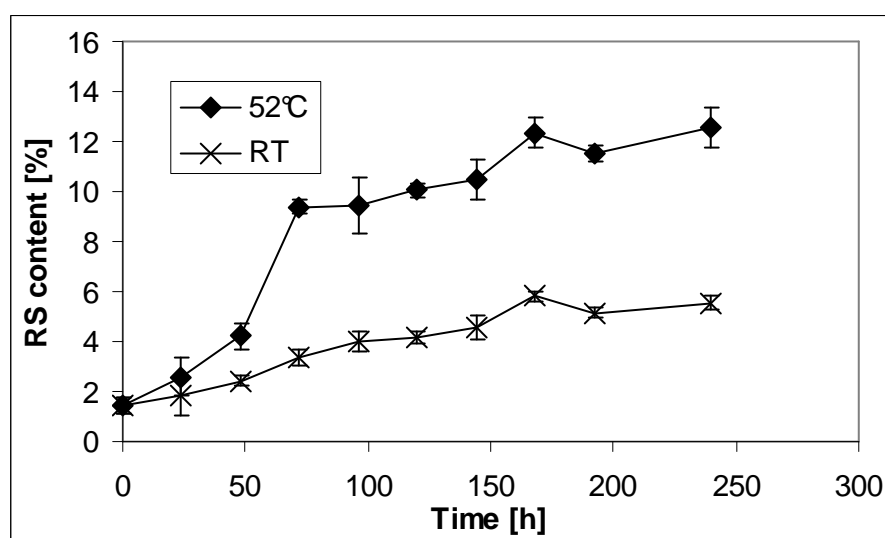
Fig. 4.36. Resistant starch content of wheat starch suspensions (5 % w/ w) alternately pressurised at 500 MPa for 15 min and stored at room temperature (RT) or at 52°C for 23 h 45 min (Pressure-storage-cycles and pressure-annealing-cycles).

Although the RS content of wheat starch suspensions was enhanced by pressure-annealing cycles respective to continuous annealing after a sole pressure-gelatinisation, the storage temperature nevertheless appeared to be determining for the increase in RS. Pressure-storage cycles at room temperatures resulted in much less RS formation in comparison to both pressure-annealing cycles and persistent annealing (Fig. 4.36). There is an increase in RS with

increasing treatment time but the RS formation is visibly slower and a RS content of about 5.6 % is reached after 10 cycles. However, a higher yield in RS is produced by cycles in comparison to continuous storage at room temperature (data not shown). Consequently, stored at the same temperature, the pressure-storage cycles improved the RS yield, while a higher temperature (below the gelatinisation temperature) leads to a further enhancement in RS content.
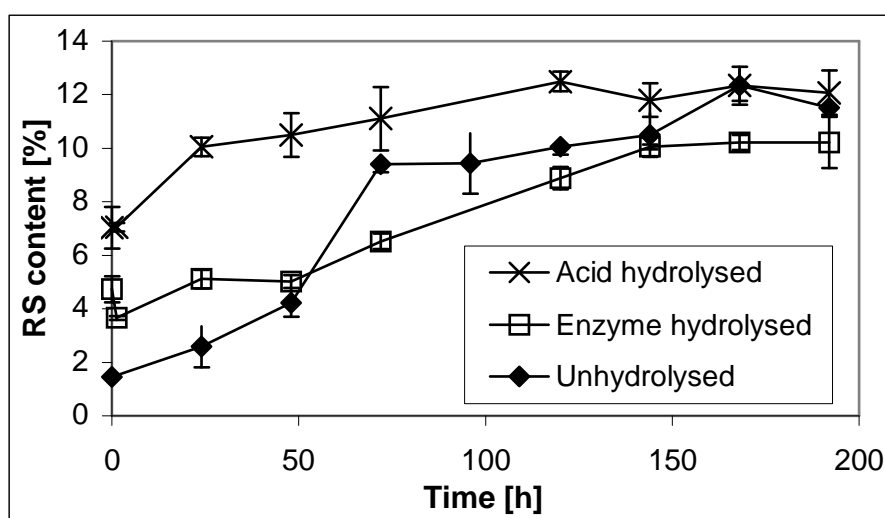
Fig. 4.37. Resistant starch content of pressure-gelatinised native, acid hydrolysed and enzyme hydrolysed wheat starch suspensions annealed at 52°C interrupted by a pressurisation step (500 MPa, 15 min, room temperature) every 24 h (Pressure-annealing-cycles).

For an additional increase in RS, high pressure gelatinised wheat starch suspensions were enzyme and acid hydrolysed prior to pressure-annealing cycles, respectively. Figure 4.37 shows the RS content of unhydrolysed, acid hydrolysed, and enzyme hydrolysed wheat starch suspensions during eight days of pressure-annealing cycles. For the hydrolysed samples, the RS content was also determined directly after the hydrolysis step and after the pressurisation following hydrolysis. Initially, a hydrolysis step increased the RS content, whereas acid hydrolysis led to a higher yield than enzyme hydrolysis. This could be explained by the maintaining of the granular form of the pressure-gelatinised starches which possibly impaired an enzyme attack (Fig. 4.38). However, it was surprising that the RS content after enzyme hydrolysis was lower than of unhydrolysed starch after four to eight cycles. Although leading to an initial increase in RS, the enzyme treatment obviously reduced the maximum yield at the conditions applied. This could possibly be due to the pH decrease by addition of

acetate buffer during pullulanase incubation. It is also possible that starch degradation products, being partially generated by enzyme hydrolysis subsequent to pressurisation, might sterically impede the formation of RS or that the association of linear starch chains to ordered structures due to enzyme hydrolysis was destroyed by the determination method utilised.

Nevertheless, it can be concluded that enzyme hydrolysis of pressure-gelatinised starch did not improve the RS yield as seen for heat treated starches (Vasanthan & Bhatty, 1998) which again indicated that thermal gelatinisation and pressure-induced gelatinisation differed. Generally, the acid hydrolysis yielded the highest amounts of RS, but a higher RS content than 12.1 % could not be achieved within 192 h.

4.6.3 Microscopic examination
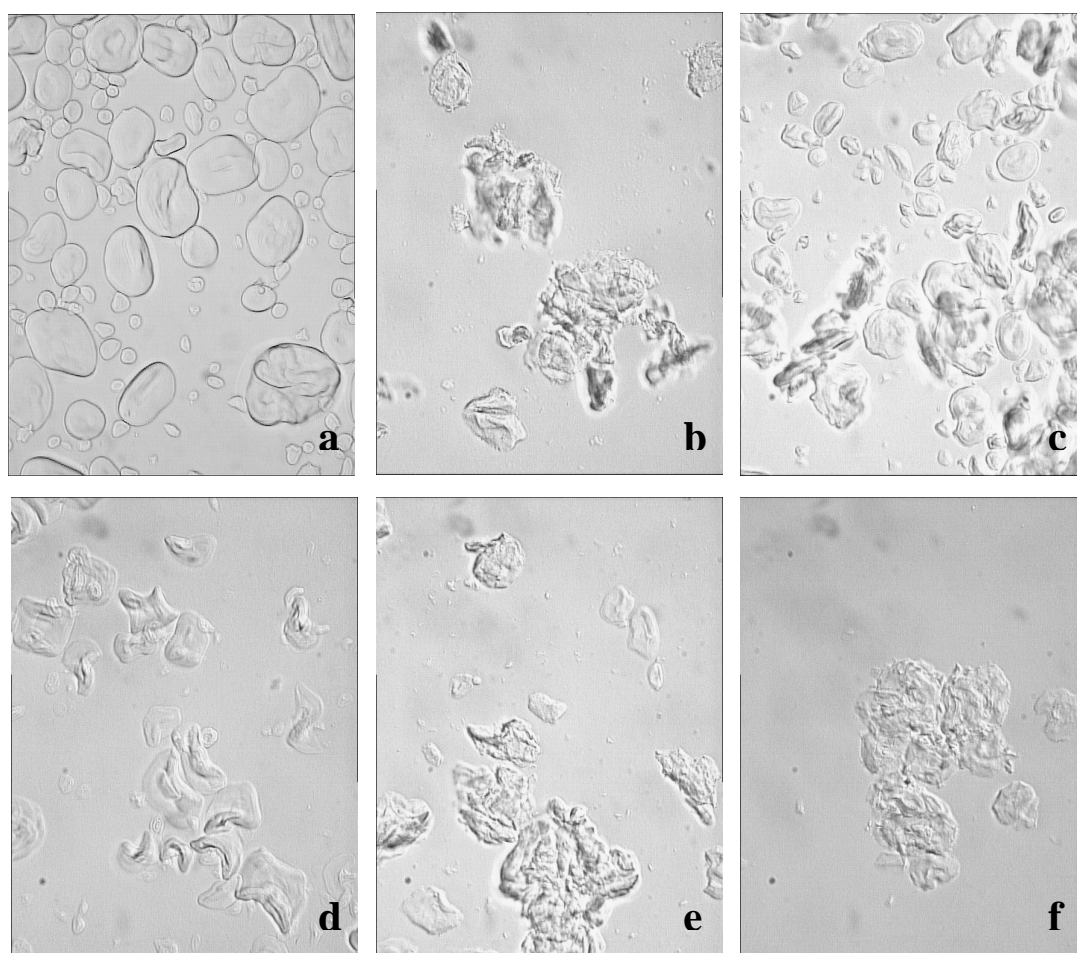
Fig. 4.38. Microscopic images (x 400) of pressure-gelatinised unhydrolysed, acid-hydrolysed and enzyme hydrolysed wheat starch suspensions (a-c; 500 MPa, 15 min) and of unhydrolysed, acid-hydrolysed and enzyme hydrolysed wheat starch suspensions after 192 h of pressure-annealing cycles (d-f; 500 MPa, 15 min, room temperature; 23 h 45 min at 52°C, atmospheric pressure).

Figure 4.38 depicts the optical appearance of non-hydrolysed, acid hydrolysed, and enzyme hydrolysed wheat starch suspensions after pressure-induced gelatinisation (Fig. 4.38 a-c) and after 8 pressure-annealing cycles (192 h) (Fig. 4.38 d-f).

It becomes clearly visible that the granular structure remained intact after all treatments. However, after acid hydrolysis the granule surfaces appeared to be slightly jagged and damaged suggesting a minor degradation which could possibly be responsible for realignment of degraded starch chains leading to retrogradation and crystal formation and the consequential rise in RS. After 8 pressure-annealing cycles, the starch granules seemed to be stressed as expressed by loss of shape reminding of ghost remnants. But a staining with iodine did not reveal a visible amylose leaching. Microscopic observations through polarised light featured absolutely no maltese crosses or any birefringency before the pressure-annealing cycles, however after 8 days of pressure-annealing cycles, regions of several starch granules exhibited the development of remote luminance indicating birefringence (data not shown).

4.6.4 X-ray analyses

X-ray diffraction patterns of native (a), pressure-gelatinised (b), pressurised and annealed (c), and pressurised unhydrolysed, acid hydrolysed and enzyme hydrolysed, respectively, prior to pressure-annealing cycled (d-f) wheat starches are shown in figure 4.39. Peaks at 9.5 °2Θ were caused by the aluminium sample carrier. The native wheat starch exhibited an evident A-type crystallinity as identified by strong peaks at 15.18, 17.13, 18.03, and 22.86 °2Θ (5.83, 5.17, 4.91, and 3.89 Å) and weaker peaks at 11.49, 20.06, 26.69, and 30.36 °2Θ (7.70, 4.42, 3.34, and 2.94 Å) (Fig. 4.39 a; Zobel, 1964). A transformation of the A towards a weak B-type pattern could be observed for all pressurised starches which was in agreement with results obtained by other research groups (Hibi *et al.*, 1993; Stute *et al.*, 1996; Katopo *et al.*, 2002). The intensity as well as the sharpness of the peaks of the X-ray patterns of pressure-gelatinised and pressurised, annealed wheat starch (Fig. 4.39 b-c) decreased as a result of loss of crystallinity. One blurred double peak (17.2 and 19.5 °2Θ, i.e. 5.16 and 4.54 Å) was obtained. When undergone pressure-annealing cycles after pressurisation, the peak intensity of the X-ray pattern slightly increased but was still to some extent lower in comparison to native wheat starch and a third peak around 21.82 °2Θ (4.07 Å) became visible (Fig. 4.39 d). This indicated a rise in B-type crystallinity. Enzyme hydrolysis prior to pressure-annealing cycles resulted in equal peak intensity and hence crystallinity as native wheat starch and a distinct B-type pattern could be observed (Fig. 4.39 f). In contrast, acid
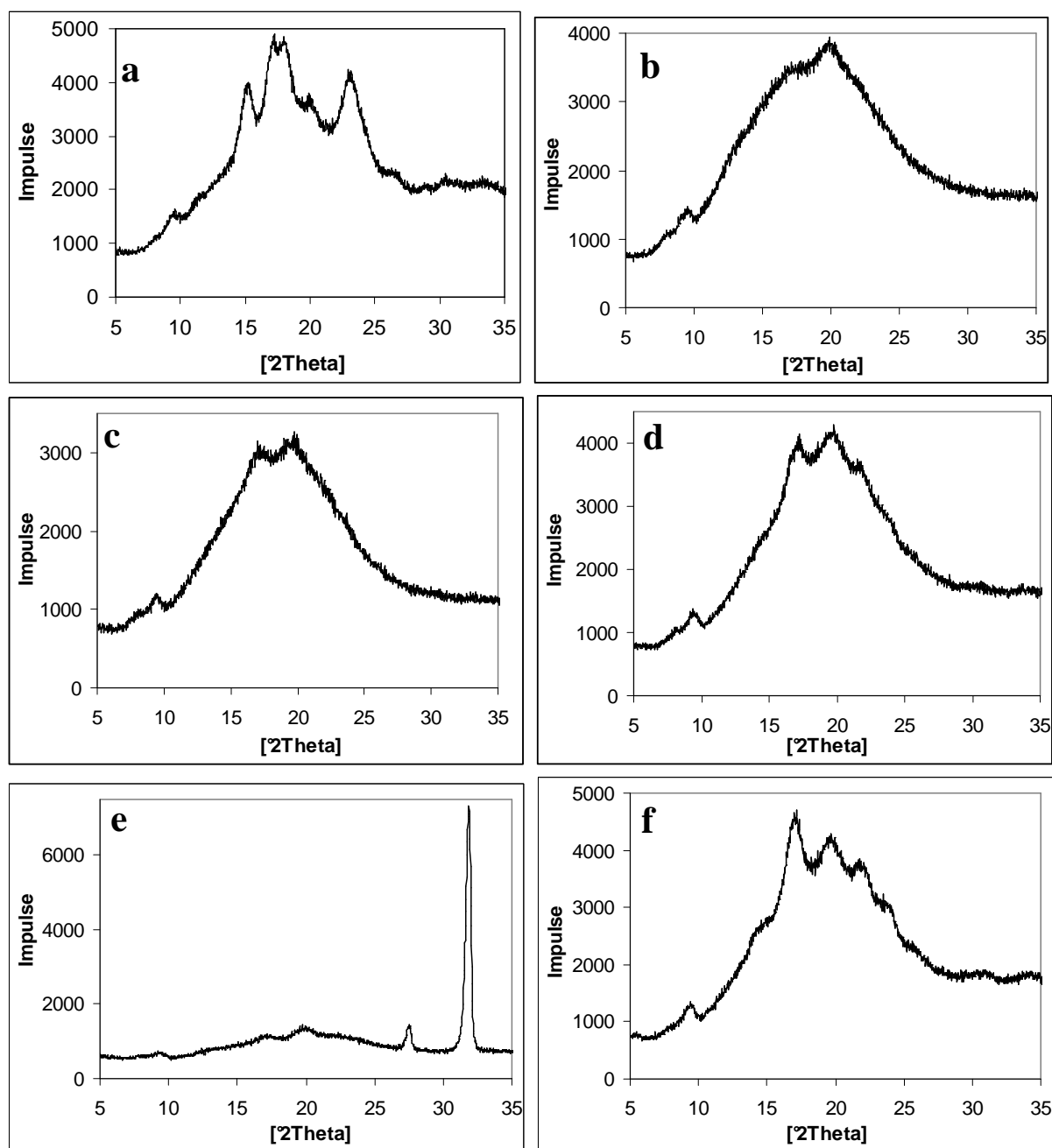
Fig. 4.39. X-ray diffraction pattern of native wheat starch (a), pressurised (500 MPa, 29°C, 15 min), freeze-dried wheat starch (b), pressurised, annealed (192 h, 52°C), freeze-dried wheat starch (c), and pressurised, unhydrolysed, acid-hydrolysed and enzyme hydrolysed wheat starch suspensions after 192 h of pressure-annealing cycles (500 MPa, 15 min, room temperature; 23 h 45 min at 52°C, atmospheric pressure) and a freeze-drying step (d-f).

hydrolysis prior to pressure-annealing cycles led to a major decrease in peak intensity of peaks representing B-type crystallinity. Therefor, a small peak at 27.57 °2Θ (3.24 Å) and an intense sharp peak around 31.86 °2Θ (2.8 Å) appeared (Fig. 4.39 e). It was unlikely that these peaks represented RS because they could not be found in the X-ray diffraction patterns of all

other samples. So it was assumed that they depicted crystalline regions of starch degradation products.

Except for the hydrolysed samples, the rise in peak intensity and consequently in crystallinity was accompanied by an increase in RS. But since the acid hydrolysis yielded the highest amount of RS, no association between crystallinity and RS content could be concluded. Furthermore, no peak was discovered that increased analogously with increasing RS content and thus represented RS crystallinity.

4.6.5 Résumé

Comparing the impact of gelatinisation process on enhancement in RS content, the influence of the starch type needed to be considered. High amounts of RS (up to 48 %) were yielded treating heat gelatinised high amylose starches (Berry, 1986; Sievert & Pomeranz, 1989; Chiu *et al.*, 1994; Vasanthan & Bhatty, 1998; Chung *et al.*, 2003) while Kettlitz *et al.* (2000) achieved a RS content of more than 55 % by producing an acid-thinned, enzymatically debranched, spray-dried starch product. This gives the impression that pressure-gelatinisation is not an effective method for the production of RS-rich starches considering the RS yields obtained. However, using wheat starch for the enhancement of RS content, the thermal gelatinisation did not appear superior to pressure-gelatinisation as can be seen in table 4.6.

Tab. 4.6. Resistant starch content of pressure-gelatinised wheat starch suspensions (5 % w/w) after 192 h of different treatments (annealing at 52°C, storage at room temperature) in comparison to resistant starch contents of wheat starch suspensions yielded after thermal gelatinisation as stated in literature

Pressure-gelatinised (192 h)	RS [%]
Continuous annealing	7.74
Pressure-storage-cycle	5.14
Pressure-annealing-cycle	11.52
Acid hydrolysed, pressure-annealing-cycle	12.08
Enzyme hydrolysed, pressure-annealing-cycle	10.22
Heat-gelatinised	
Boiling, autoclaving (1 h), cooling, freeze-drying (Siljeström *et al.*, 1989)	6.20
Autoclaving (1 h) once; cooling (1 h), boiling (30 min), 3 cyclesn (Björck *et al.*, 1987)	7.80
Autoclaving (1 h), cooling, freeze-drying (Sievert & Pomeranz, 1989)	7.80
Autoclaving (1 h), overnight at 4°C, 2 cycles(defatted) (Eerlingen *et al.*, 1994)	9.00
Autoclaving (1 h), 5000 min at 100°C (Eerlingen *et al.*, 1993a)	>10.00
Autoclaving (45 min), 24 h at 4°C, 5 cycles (Berry, 1986)	15.00

As stated in literature, the yields in RS obtained by thermal gelatinisation of wheat starches ranged between 6.2 and 15 %, whereas RS yields obtained after pressure-induced gelatinisation ranged between 5.14 and 12.08 %. Champ (1992) observed in an interlaboratory study that the method by Berry (1986) resulted in higher RS contents for cereal products in comparison to other determination methods. Consequently, the 15 % RS produced by Berry (1986) might be slightly inflated. Thus, the pressure-induced gelatinisation of starches produced comparable yields in RS at conditions optimised for pressure-gelatinised wheat starches. Further studies of the impact of high hydrostatic pressure treatment on the RS content of other starches, especially of high amylose starches, is required to confirm this observation for starches in general.

Although the pressure treatment of wheat starches does not appear to provide advantages regarding RS production in comparison to heat treated wheat starches, the resulting physico-chemical properties of the pressure-treated starch suspensions differ and thus offer opportunities for the development of novel food products with a supplementarily enhanced RS content. In contrast to thermal processing, high hydrostatic pressure treatment of starch suspensions leads to e.g. a limited retrogradation and a limited expansion of molten granules under pressure (Douzals *et al.*, 1998) and differing paste/ gel properties with lower viscosity and higher storage moduli (Stolt *et al.*, 2001). Therefore, products richer in starch but with the same viscosity can be obtained by high pressure treatment. Additionally, high pressure processing enables control of a desired degree of crystallinity by modulating process parameters such as pressure, temperature, water content and treatment time (Douzals *et al.*, 1998; see chapter 4.4 and 4.5).

4.7 Impact of pressurised wheat starch on bread

The influence of pressure-gelatinised wheat starch replacing 5 % (w/ w) of the flour in bread dough on the firmness and elasticity of the bread texture upon storage for one and five days was investigated. Since it was stated in literature that high hydrostatic pressure treated starches exhibited a slower retrogradation compared to thermally treated starches (Ezaki & Hayashi, 1992; Douzals *et al.*, 1998), it was aimed to obtain bread with better storage properties in terms of ageing. This was affirmed by a patent specification (Codovilli, 2004) proprietary of the manufacturer of pasta and bakery products Barilla, where it was claimed

that a replacement of 2-20 %, advantageously 5-10 %, high pressure treated starch (300-700 MPa, preferably 550-650 MPa, for 5-15 min) for flour resulted in a by at least 20 % decreased hardness measured after 30 days of storage in comparison to the reference bread without pressure treated starch.
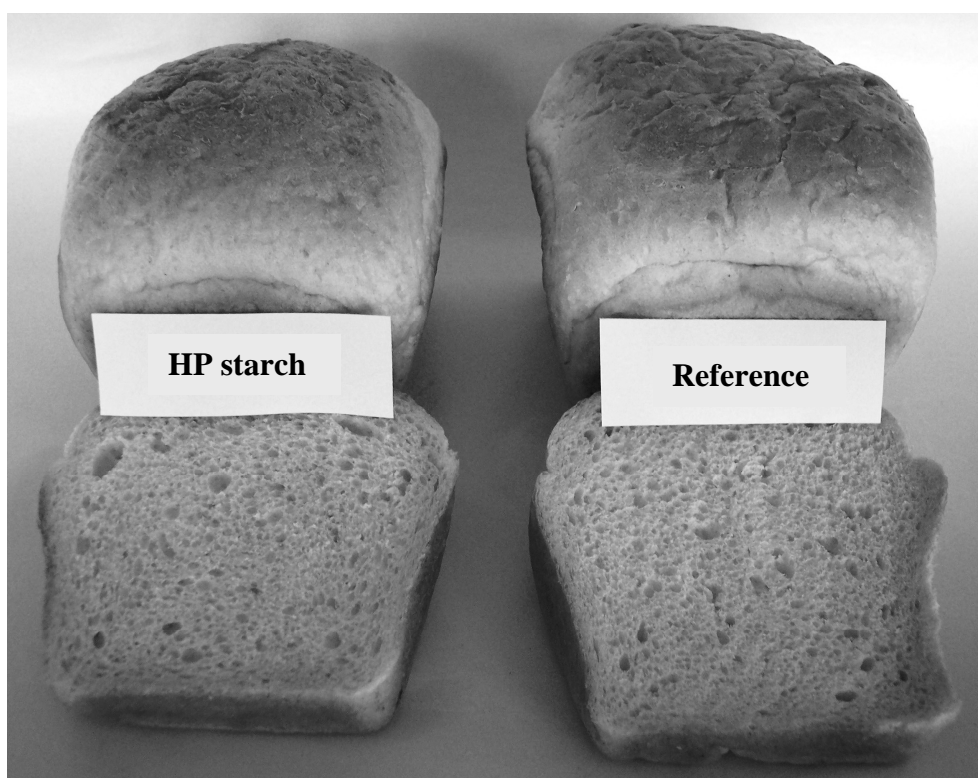
Fig. 4.40. Wheat bread with 5 % high pressure (HP) treated wheat starch (5 g/ 100 g flour) and reference wheat bread without pressurised starch after one day of storage.

However, it is unusual to determine textural properties of bread after a storage time as long as 30 days, since wheat bread is supposed to be consumed within approx. 3 days after baking. Additionally, contradictory statements concerning the retrogradation behaviour of pressurised starches could be found in literature. Stute *et al.* (1996) and Katopo *et al.* (2002) observed an additional DSC peak upon pressurisation of starches, which Stute *et al.* (1996) attributed to rapid retrogradation, and Katopo *et al.* (2002) concluded the peak to be an indication for the formation of B-type crystals out of A-type crystallinity due to the pressure treatment. Stolt *et al.* (2001) observed comparable retrogradation behaviour of heat-induced and pressure-induced starch gels. Except for the patent specification (Codovilli, 2004), all investigations regarding the retrogradation behaviour of pressure treated starches were

performed on mere starch suspensions without further treatment steps such as mixing or baking and without any solutes or acids such as sugar, salt and ascorbic acid. According to the patent specification mentioned before, the effect of pressure gelatinised wheat starch suspensions replacing flour (and water) on hardness and elasticity of bread was examined. The baking as well as the subsequent texture measurements of the breads were performed in the laboratory of food technology I at the University of Applied Sciences TFH Berlin, from where also the standard recipe and the baking procedure had been adopted. The baking temperature was chosen at 210°C according to the patent specification. Firmness and elasticity of the bread crumbs were determined after one and five days of storage at room temperature in a sealed plastic bag.

Generally, the pressurised starch containing breads had roughly the same size as the reference breads, only one was slightly smaller as can be seen exemplary in figure 4.40. The appearance of the breads as well as the crumb structure of the slices exhibited no visible discrepancies.
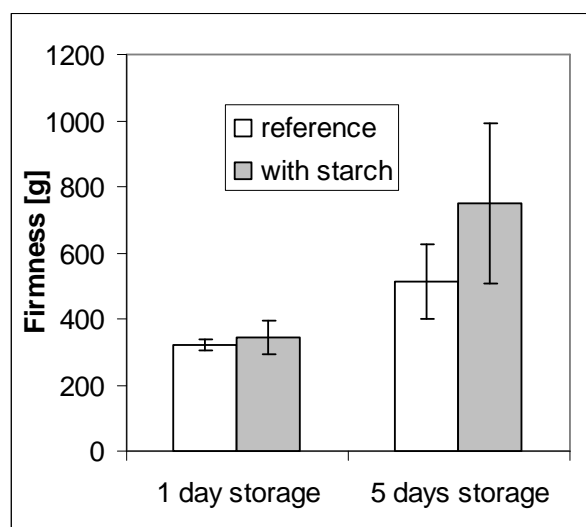
Fig. 4.41. Firmness of wheat bread baked after a standard recipe (reference) and after a standard recipe but with 5 % of the wheat flour replaced by high pressure treated wheat starch (10 % suspension, 15 min, 600 MPa).
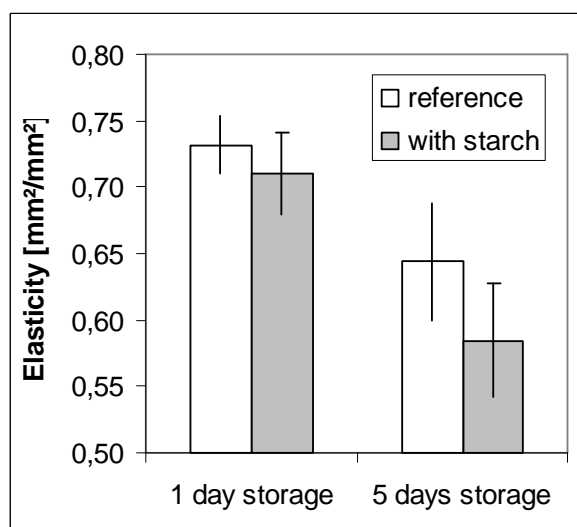
Fig. 4.42. Elasticity of wheat bread baked after a standard recipe (reference) and after a standard recipe but with 5 % of the wheat flour replaced by high pressure treated wheat starch (10 % suspension, 15 min, 600 MPa).

The firmness of the bread crumbs of the reference bread and of the bread containing pressure-gelatinised starch after one and five days of storage at room temperature is depicted

in the column chart in figure 4.41. The firmness was derived from the height of the first peak of the texture analysis (see appendix fig. A2).

In general, it can be concluded that the firmness of all breads increased after five days of storage with respect to one day of storage. One day after baking, the bread containing pressurised starch and the reference bread exhibited no difference in firmness. However, there appeared to be a trend that the bread containing pressurised wheat starch revealed a slightly higher firmness than the reference bread after one day of storage if the column heights were compared. This trend consisted five days after baking, where the mean firmness of pressurised starch containing bread was visibly higher than the mean firmness of the reference bread. Since the standards deviations of the measurements overlapped however, no clear statement can be made regarding the influence of pressure gelatinised starch on the firmness of bread crumbs.

The elasticity of the bread crumbs of the reference sample and of the bread containing pressure-gelatinised starch after one and five days of storage at room temperature is depicted in the column chart in figure 4.42. The elasticity was obtained from the ratio of the second peak area over the first peak area of the texture analysis (see appendix fig. A2).

Comparing the elasticity of the crumbs one and five days after baking, a decrease in elasticity can be observed with increasing storage time. After one day of storage, the elasticities of the bread containing pressurised starch and the reference bread showed no differences. In accordance with the firmness of the crumbs, there appeared to be a trend that the bread containing pressurised wheat starch revealed a somewhat lower elasticity than the reference bread after one day of storage if the column heights were compared. This trend enhanced five days after baking, where the average elasticity of pressurised starch containing bread was visibly lower than the average elasticity of the reference bread. Since the deviations of the measurements overlapped however, no explicit statement can be made regarding the influence of pressure gelatinised starch on the elasticity of bread crumbs at the conditions applied.

Recapitulatorily, it can be said that the expected reducing effect of pressurised starch on staling of bread and starch retrogradation as expressed by firmness and elasticity was not observed and the results of the patent specification (Codovilli, 2004) could not be confirmed. On the contrary, the firmness of the bread crumb showed an increasing trend for pressurised starch containing bread whereas the elasticity exhibited a decreasing trend. One reason for

this could be the lower content of flour ingredients besides starch such as wheat protein and pentosans due to a replacement of wheat flour by mere starch. Wheat proteins and pentosans have a high influence on bread quality e.g. pentosans cause better crumb properties and increased freshness (Ternes, 1994). However, Ottenhof and Farhat (2004) found no evidence for significant effects of gluten on amylopectin retrogradation, so a decreased gluten content should not alter the bread firmness. On the other hand it is assumed that bread firmness development and starch recrystallisation, although being related are separate events, because bread firmness was not determined by the total amount of crystalline fraction but the the type of crystallites formed (Osella *et al.*, 2005). Since an increase in B-crystallites in relation to V-crystallites enhanced bread firmness (Osella *et al.*, 2005) and A-type crystals underwent a transformation to B-type crystals upon pressurisation (Hibi *et al.*, 1993; Katopo *et al.*, 2002), it is therefore possible, that pressurised starch granules promote bread firmness.

Another possibility could be that the pressurised, swollen starch granules are disrupted during the mixing process leading to free amylose and amylopectin in the dough. Once released from the granular containment, the starch molecules might tend to retrogradation and thus increase the firmness and decrease the elasticity of the bread crumb.

5   CONCLUSIONS

With the development of the HPDS high pressure cell in combination with an inverse microscope it was possible to obtain in situ images with both high optical resolution and high quality up to pressures of 300 MPa. The maximum pressure of 300 MPa was not sufficient enough to observe swelling of potato and tapioca starch granules whereas wheat starch granules showed a thorough swelling of most granules during pressurisation. Further development is required to enable in situ microscopic observations at higher pressures.

A pressure treatment of potassium iodide-iodine stained wheat starch and tapioca starch suspensions resulted in a complete decolouration of the starch granules while pressurised potato starch granules discoloured only slightly. The decolouration was regarded as a cause of dilution due to water uptake as well as a possible amylose release from the amorphous regions in the process of pressure-induced starch gelatinisation where released amylose molecules formed complexes with iodine pushed out of the single helices by pressure and the complexes formed accumulated in the darkening liquid phase. More data derived from complementary analysis methods is required for explaining these experimental results.

The electrical conductivity of starch suspensions increased with increasing pressure which suggested an ion release during pressure-induced starch gelatinisation as well as an ion concentrating effect due to water uptake of the starch granules. Since the starch granules maintained their granular form, it was still uncertain if there was a release of ions during pressurisation or after pressure release. This needs to be clarified in future investigations.

There was a good linear relationship between the degree of gelatinisation and the electrical conductivity data for all three starches investigated. Since the electrical conductivity of wheat, tapioca and potato starch suspensions correlated well with the degree of gelatinisation of the starches after pressure treatment it could be applied as a tool for the quick and simple determination of pressure-induced starch gelatinisation. The same was valid for the degree of gelatinisation and the water binding capacity of pressurised starch suspensions.

Online-measurements of the electrical conductivity during a pressure treatment need to be developed and performed in order to ascertain if this method could be applicable for in situ monitoring and controlling the degree of gelatinisation obtained after a pressurisation step.

It was shown that pressure-induced starch gelatinisation was highly sensitive to changes in temperature, pressure and treatment time. The degree of gelatinisation of all three

starches examined increased with increasing temperature, pressure and treatment time. Hence, an application as an extrinsic PTTI seems possible. A major benefit of pressure-induced starch gelatinisation is the variation of pressure sensitivity depending on the type of starch. Starches can be systematically selected that gelatinise over a pressure range at the temperature and treatment time applied in the high hydrostatic pressure process that reflects the pressure range requested for ensuring process efficiency and safety. Furthermore, they can easily be encapsulated within the packaging material and thus provide, in conjunction with a colour indicator, a simple tool for the monitoring of high pressure processing conditions.

Additionally, it can be concluded that in general the effects of salts and sugars on starch gelatinisation were comparable for thermal and pressure treatments. However, the extent of influence of salts on pressure-induced starch gelatinisation seems to be dependent on the type of starch. Potato starch, although the most pressure resistant, appears to be the most susceptible to salts and gelatinises at lower salt concentrations under pressure in comparison to wheat and tapioca starches at the pressures applied. At high concentrations (> 2 M) the impact of chlorides on pressure-induced starch gelatinisation followed the order Na<K<Li<Ca, which corresponds to the order of the Hofmeister series. At concentrations above 1 M the effect of potassium salts on pressure-induced starch gelatinisation upon pressurisation also followed the order of the lyotropic series (Cl<Br<I<SCN). The gelatinisation pressure is increased by sugars and the degree of gelatinisation is linearly correlated with the number of equatorial hydroxyl groups. Since pressure-induced starch gelatinisation is strongly dependent on the water content and is decreased in the presence of sugars and water structure making ions (e.g. SO_4^{2-}) and increased by water structure breaking ions such as SCN^-, it is suggested that the availability of free water is crucial for starch gelatinisation under pressure.

Heat and pressure-gelatinised wheat starch suspensions exhibited differing resistant starch (RS) formation behaviours as expressed in e.g. optimum storage temperature and treatment steps such as enzyme hydrolysis not having improved the RS yield of pressure-gelatinised starch as seen for heat treated starches. This proves the differences in gelatinisation processes. It can be concluded that a RS enhancement after high hydrostatic pressure treatment is achievable, since RS yields of approx. 12 % were obtained. In comparison to patented RS formation via enzyme degradation subsequent to thermal gelatinisation yielding an RS content of above 55 % (Kettlitz *et al.*, 2000), pressure treatment

did not appear to be beneficial for the production of high amounts of RS. However, in comparison to heat gelatinised wheat starch, the RS yields achieved after pressure-induced gelatinisation were in the same range. This is an indication that high hydrostatic pressure-induced gelatinisation might well be used as an alternative for the production of high amounts of RS, though there is apparently no advantage in RS production from wheat starch by means of high pressure gelatinisation.

Further research should involve the influence of high pressure on high amylose starches and starches of different origins, pressure-temperature combinations above 66°C, enzymatic treatments during pressurisation, and the resistant starch formation due to long-time-pressurisation as suggested by Hayashi and Hayashida (1989).

Investigating the impact of pressure gelatinised wheat starch replacing 5 % of the flour on the storage stability of wheat bread no definite statement can be made. The expected improvements of the textural properties and the shelf life of the bread could not be detected. However, a trend towards a decrease in elasticity and increase in firmness of pressurised starch containing bread could be observed after five days of storage. This leads to the conclusion that pressurised wheat starch does not reduce retrogradation but shows a trend towards enhanced deterioration of quality in terms of textural attributes.

It can be concluded that pressure-induced starch gelatinisation offers a lot of potential for the development of novel food properties due to its differing physical and textural properties in comparison to heat-induced gelatinisation. More research is required to fully comprehend the differences in thermal and pressure-induced gelatinisation mechanisms in order to make use of the advantages of pressure-gelatinised starches for industrial products.

REFERENCES

Ahmad, F.B. and Williams, P.A. (1999). Effect of sugars on the thermal and rheological properties of sago starch. *Biopolymers* 50, 401-412.

Asp, N.-G. (1992). Resistant starch proceedings from the second plenary meeting of EURESTA: European FLAIR concerted action No. 11 on physiological implications of the consumption of resistant starch in man. *Eur. J. Clin. Nutr.* 46 (Suppl. 2), 1.

Asp, N.-G., Tovar, J., and Bairoliya, S. (1992). Determination of resistant starch *in vitro* with three different methods, and *in vivo* with a rat model. *Eur. J. Clin. Nutr.* 46 (Suppl. 2), 117-119.

Atwell, W.A., Hood, L.F., Lineback, D.R., Varriano-Marston, E., and Zobel, H.F. (1988). The terminology and methodology associated with basic starch phenomena. *Cereal Foods World* 33 (3), 306-311.

Baek, M.H., Yoo, B., and Lim, S.-T. (2004). Effects of sugars and sugar alcohols on thermal transition and cold stability of corn starch gel. *Food Hydrocoll.* 18, 133-142.

Banks, W. and Muir, D.D. (1980). Structure and chemistry of the starch granule. In: J. Preiss. *The biochemistry of plants Vol. 3* (pp. 321-369). Academic Press, New York, New York.

Begg, D.A., Salmon, E.D., and Hyatt, H.A. (1983). The changes in structural organization of actin in the sea urchin egg cortex in response to hydrostatic pressure. *J. Cell Biology* 97 (6), 1795-1805.

Behall, K.M. and Howe, J.C. (1996). Resistant starch energy. *J. Am. College Nutr.* 15 (3), 248-254.

References

Beleia, A., Miller, R.A., and Hoseney, R.C. (1996). Starch gelatinization in sugar solutions. *Starch/ Stärke* 48, 259-262.

Belitz, H.-D., Grosch, W., and Schieberle, P. (2001). *Lehrbuch der Lebensmittelchemie*. Springer Verlag, Berlin.

Bello-Pérez, L.A. and Paredes-López, O. (1995). Starch and amylopectin: effect of solutes on their calorimetric behavior. *Food Chem.* 53, 243-247.

Berry, C.S. (1986). Resistant starch: formation and measurement of starch that survives exhaustive digestion with amylolytic enzymes during the determination of dietary fibre. *J. Cereal Sci.* 4, 301-314.

Berry, C.S., l`Anson, K., Miles, M.J., Morris, V.J., and Russell, P.L. (1988). Physical chemical characterisation of resistant starch from wheat. *J. Cereal Sci.* 8, 203-206.

Björck, I., Nyman, M., Pedersen, B., Siljeström, M., Asp, N.-G., and Eggum, B.O. (1987). Formation of enzyme resistant starch during autoclaving of wheat starch: studies *in vitro* and *in vivo*. *J. Cereal Sci.* 6, 159-172.

Blanshard, J.M.V. (1987). Starch granule structure and function: a physicochemical approach. In: T. Galliard. *Starch: properties and potential* (pp.16-54). John Wiley and Sons, Chichester, Great Britain.

Brabender (2000). Starch measurement with the BRABENDER® Viscograph family. *http://www.brabender.com/89.0.html* (14.09.2004).

Bridgman, P.W. (1914). The coagulation of albumen by pressure. *J. Biol. Chem.* 19, 511-512.

Brouns, F., Kettlitz, B., and Arrigoni, E. (2002). Resistant starch and "the butyrate revolution". *Trends Food Sci. Technol.* 13, 251-261.

References

Brown, I.L., McNaught, K.J., and Moloney, E. (1995). Hi-maize: new directions in starch technology and nutrition. *Food Australia* 47 (6), 272-275.

Buléon, A., Colonna, P., Planchot, V., and Ball, S. (1998). Starch granules: structure and biosynthesis. *Int. J. Biol. Macromol*. 23, 85-112.

Cerestar (2003). Types of resistant starch. *http://www.cerestarpharmaexcipient.com/374.html* (07.05.2003).

Cerletti, P., Eynard, L., and Guerrieri, N. (1993). Resistant starch: a negative or positive entity in foods? *Agro-Food-Industry Hi-Tech* May/June, 27-29.

Chaiwanichsiri, S., Ohnishi, S., Suzuki, T., Takai, R., and Miyawaki, O. (2001). Measurement of electrical conductivity, differential calorimetry and viscosity of starch and flour suspensions during gelatinisation process. *J. Sci. Food Agric.,* 81, 1586-1591.

Champ, M. (1992). Determination of resistant starch in foods and food products: interlaboratory study. *Eur. J. Clin. Nutr.* 46 (Suppl. 2), 51-62.

Chinachoti, P., Kim-Shin, M.-S., Mari, F., and Lo., L. (1991). Gelatinization of wheat starch in the presence of sucrose and sodium chloride: correlation between gelatinization temperature and water mobility as determined by oxygen-17 nuclear magnetic resonance. *Cereal Chem*. 68, 245-248.

Chiu, C.-W., Henley, M., and Altieri, P. (1994). Process for making amylase resistant starch from high amylose starch. *U.S. Patent* 5,281,276.

Chung, H.-J., Jeong, H.-Y., and Lim, S.-T. (2003). Effects of acid hydrolysis and defatting on crystallinity and pasting properties of freeze-thawed high amylose corn starch. *Carbohydr. Polym.* 54, 449-455.

References
_____

Claeys, W.L., Indrawati, Van Loey, A.M., and Hendrickx, M.E. (2003). Review: are intrinsic TTIs for thermally processed milk applicable for high-pressure processing assessment? *Innov. Food Sci. & Emerging Technol.* 4, 1-14.

Codovilli, F. (2004). Bakery products containing high-pressure treated starch. *European patent EP 1 155 618 B1.*

D´Appolonia, B.L. (1972). Effect of bread ingredients on starch-gelatinization properties as measured by the amylograph. *Cereal Chem.* 49, 532-543.

Deplace, G. and Mertens, B. (1992). The commercial application of high pressure technology in the food processing industry. In: C. Balny, R. Hayashi, K. Heremans, P. Masson. *High pressure and biotechnology* (pp.163-165). Colloque INSERM / John Libbey Eurotext, Montrouge, France.

Douzals, J.P., Marechal, P.A., Coquille, J.C., and Gervais, P. (1996). Microscopic study of starch gelatinization under high hydrostatic pressure. *J. Agric. Food Chem.* 44 (6), 1403-1408.

Douzals, J.P., Perrier Cornet, J.M., Gervais, P., and Coquille, J.C. (1998). High-pressure gelatinization of wheat starch and properties of pressure-induced gels. *J. Agric. Food Chem.* 46 (12), 4824-4829.

Douzals, J.P., Perrier-Cornet, J.M., Gervais, P., and Coquille, J.C. (1999). Hydration and pressure-temperature phase diagrams of wheat starch. In: H. Ludwig. *Advances in high pressure bioscience and biotechnology* (pp. 333-336). Springer Verlag, Berlin, Heidelberg.

Douzals, J.P., Perrier-Cornet, J.M., Coquille, J.C., and Gervais, P. (2001). Pressure-temperature phase transition diagram for wheat starch. *J. Agric. Food Chem.* 49, 873-876.

References

Eerlingen, R.C., Cillen, G., and Delcour, J.A. (1994). Enzyme resistant starch. IV. Effect of endogenous lipids and added sodium dodecyl sulfate on formation of resistant starch. *Cereal Chem.* 71 (2), 170-177.

Eerlingen, R.C., Crombez, M., and Delcour, J.A. (1993a). Enzyme resistant starch. I. Quantitative and qualitative influence of incubation time and temperature of autoclaved starch on resistant starch formation. *Cereal Chem.* 70 (3), 339-344.

Eerlingen, R.C., Deceuninck, M., and Delcour, J.A. (1993b). Enzyme resistant starch. II. Influence of amylose chain length on resistant starch formation. *Cereal Chem.* 70 (3), 345-350.

Englyst, H.N. and Cummings, J.H. (1987). Digestion of polysaccharides of potato in the small intestine of man. *Am. J. Clin. Nutr.* 45, 423-431.

Englyst, H.N., Kingman, S.M., and Cummings, J.H. (1992). Classification and measurement of nutritionally important starch fractions. *Eur. J. Clin. Nutr.* 46 (Suppl. 2), 33-50.

Englyst, H.N., Kingman, S.M., Hudson, G.J., and Cummings, J.H. (1996). Measurement of resistant starch *in vitro* and *in vivo*. *B. J. Clin. Nutr.* 75, 749-755.

Englyst, H.N. and Macfarlane, G.T. (1986). Breakdown of resistant and readily digestible starch by human gut bacteria. *J. Sci. Food Agric.* 37, 699-706.

Englyst, H.N., Wiggins, H.S., and Cummings, J.H. (1982). Determination of the non-starch polysaccharides in plant foods by gas-liquid chromatography of constituent sugars as alditol acetates. *Analyst* 107, 307-318.

Escarpa, A., González, M.C., Mañas, E., García-Diz, L., and Saura-Calixto, F. (1996). Resistant starch formation: standardization of a high pressure autoclave process. *J. Agric. Food Chem.* 44, 924-928.

Evans, I.D. and. Haisman, D.R. (1982). The effect of solutes on the gelatinization temperature range of potato starch. *Starch/ Stärke* 34 (7), 224-231.

Ezaki, S. and Hayashi, R. (1992). High pressure effects on starch: structural change and retrogradation. In: C. Balny, R. Hayashi, K. Heremans, P. Masson. *High pressure and biotechnology* (pp.163-165). Colloque INSERM/ John Libbey Eurotext, Montrouge, France.

Faisant, N., Champ, M., Colonna, P., Buleon, A., Molis, C., Langkilde, A.-M., Schweizer, T., Flourie, B., and Galmiche, J.-P. (1993). Structural features of resistant starch at the end of the human small intestine. *Eur. J. Clin. Nutr.* 47, 285-296.

FoodStarch.com (2004). *http://www.foodstarch.com/index.asp?country=DE2* (29.10.2004).

Fredriksson, H., Björck, I., Andersson, R., Liljeberg, H., Silverio, J., Eliasson, A.-C., and Aman, P. (2000). Studies on α-amylase degradation of retrograded starch gels from waxy maize and high-amylopectin potato. *Carbohydr. Polym.* 43, 81-87.

French, D. (1984). Organization of starch granules. In: R.L. Whistler, J.N. Bemiller and E.F. Paschall. *Starch: chemistry and technology* (pp. 184-247). Academic Press, Inc., Orlando, Florida.

Fukuoka, M., Ohta, K.-I., and Watanabe, H. (2002). Determination of the terminal extent of starch gelatinization in a limited water system by DSC. *J. Food Eng.* 53, 39-42.

Gallant, D.J., Bouchet, B., and Baldwin, P.M. (1997). Microscopy of starch: evidence of a new level of granule organisation. *Carbohydr. Polym.* 32, 177-191.

Garcia-Alonso, A., Jimenez-Escrig, A., Martin-Carron, N., Bravo, L., and Saura-Calixto, F. (1999). Assessment of some parameters involved in the gelatinization and retrogradation of starch. *Food Chem.* 66, 181-187.

Gassner, G., Hohmann, B., and Deutschmann, F. (1989). *Mikroskopische Untersuchung pflanzlicher Lebensmittel.* Gustav Fischer Verlag, Stuttgart.

References

Gekko, K., and Koga, S. (1983). Increased thermal stability of collagen in the presence of sugars and polyols. *J. Biochem.* 94, 199-205.

Gerlsma, S.Y. (1970). Gelatinisation temperatures of starch, as influenced by polyhydric and monohydric alcohols, phenols, carboxylic acids and some other additives. *Starch/ Stärke* 22, 3-9.

Goñi, I., García-Diz, L., Mañas, E., and Saura-Calixto, F. (1996). Analysis of resistant starch: a method for foods and food products. *Food Chem.* 56 (4), 445-449.

Gough, B.M. and Pybus, J.N. (1973). Effect of metal cations on the swelling and gelatinization behaviour of large wheat starch granules. Starch/ *Stärke* 25, 123-130.

Hansen, L.M., Setser, C.S., and Paukstelis, J.V. (1989). Investigation of sugar-starch interactions using carbon-13 nuclear magnetic resonance. I. Sucrose. *Cereal Chem.* 66, 411-415.

Hartmann, M., Pfeifer, F., Dornheim G., and Sommer, K. (2003). HPDS-Hochdruckzelle zur Beobachtung mikroskopischer Phänomene unter Hochdruck. *Chemie Ingenieur Technik* 75 (11), 1763-1767.

Hayashi, R. and Hayashida, A. (1989). Increased amylase digestibility of pressure-treated starch. *Agric. Biol. Chem.* 53 (9), 2543-2544.

Heinz, V. and Knorr, D. (2002). Effects of high pressure on spores. In: M.E.G. Hendrickx, D. Knorr. *Ultra high pressure treatments of foods* (pp. 77-113). Kluwer Academic/ Plenum Publishers, N.Y., Boston, Dordrecht, London, Moscow.

Hemminger, W.F. and Cammenga, H.K. (1989). *Methoden der Thermischen Analyse*. Springer Verlag, Berlin.

Hibi, Y., Matsumoto, T., and Hagiwara, S. (1993). Effect of high pressure on the crystalline structure of various starch granules. *Cereal Chem.* 70 (6), 671-676.

References

v. Hippel, P.H. (1975). Neutral salt effects on the conformational stability of biological macromolecules. In: H. Sund, G. Blauer. *Protein-ligand interactions* (pp. 452-471). De Gruyter Verlag, Berlin, New York.

Hite, B.H. (1899). The effect of pressure in the preservation of milk. *Agricultural Experiment Station Bulletin,* 58, 15-35.

Hoseney, R.C. (1986). *Principles of cereal science and technology*. American Association of Cereal Chemists, Inc. Minnesota, U.S.A.

Jacobs, H., Mischenko, N., Koch, M.H.J., Eerlingen, R.C., Delcour, J.A., and Reynaers, H. (1998). Evaluation of the impact of annealing on gelatinisation at intermediate water content of wheat and potato starches: A differential scanning calorimetry and small angle X-ray scattering study. *Carbohydr. Res.* 306, 1-10.

Jacobson, M.R., Obanni, M., and BeMiller, J.N. (1997). Retrogradation of starches from different botanical sources. *Cereal Chem.* 74 (5), 511-518.

Jane, J. (1993). Mechanism of starch gelatinization in neutral salt solutions. *Starch/ Stärke* 45, 161-166.

Jang, J.K., Lee, S.H., Cho, S.C., and Pyun, Y.R. (2001). Effect of sucrose on glass transition, gelatinization, and retrogradation of wheat starch. *Cereal Chem.* 78 (2), 186-192.

Kabayama, M.A. and Patterson, D. (1958). The thermodynamics of mutarotation of some sugars II theoretical considerations. *Can. J. Chem.* 36, 563-573.

Karapantsios, T.D., Sakonidou, E.P., and Raphaelides, S.N. (2000). Electrical conductance study of fluid motion and heat transport during starch gelatinization. *J. Food Sci.* 65 (1), 144-150.

References
_____

Kato, N., Kamai, H., Sekiya, S., Sakai, Y., and Uyehara, T. (2001). Characterization of the UCST behavior of poly(N-acetylacrylamide) in sugar solutions. *Analytical Sciences* 17, i1129-i1132.

Katopo, H., Song, Y., and Jane, J.-L. (2002). Effect and mechanism of ultrahigh hydrostatic pressure on the structure and properties of starches. *Carbohydr. Polym.* 47, 233-244.

Keetels, C.J.A.M., Oostergetel, G.T., and van Vliet, T. (1996). Recrystallization of amylopectin in concentrated starch gels. *Carbohydr. Polym.* 30, 61-64.

Kettlitz, B.W., Coppin, J.V.J.-M., Röper, H.W.W., and Bornet, F. (2000). Highly fermentable resistant starch. *U.S. Patent* 6,043,229.

Knorr, D. and Heinz, V. (2001). Development of nonthermal methods for microbial control. In: S.S. Block. *Disinfection, sterilization and preservation* (pp. 853-877). Lippincott, Williams & Wilkins, Philadelphia.

Kolbe, E., Lester, L.A., and Hartel, R. (1999). A round robin evaluation of differential scanning calorimetry to measure transition enthalpy and temperatures. *J. Food Eng*. 40, 95-99.

Krüsi, H. and Neukom, H. (1984). Untersuchung über die Retrogradation der Stärke in konzentrierten Weizenstärkegelen. *Starch/ Stärke* 36 (2), 40-45.

Kudla, E. & Tomasik, P. (1992). The modification of starch by high pressure part II: compression of starch with additives. *Starch/ Stärke* 44 (7), 253-259.

Liljeberg Elmståhl, H. (2002). Resistant starch content in a selection of starchy foods on the Swedish market. *Eur. J. Clin. Nutr.* 56, 500-505.

Maaurf, A.G., Che Man, Y.B., Asbi, B.A., Junainah, A.H., and Kennedy, J.F. (2001). Gelatinisation of sago starch in the presence of sucrose and sodium chloride as assessed by differential scanning calorimetry. *Carbohydr. Polym.* 45, 335-345.

References

Masuda, M., Saito, Y., Iwanami, T., and Hirai, Y. (1992). Effects of hydrostatic pressure on packaging materials for food. In: C. Balny, R. Hayashi, K. Heremans, P. Masson. *High pressure and biotechnology* (pp.163-165). Colloque INSERM/ John Libbey Eurotext, Montrouge, France.

Mathers, J.C. (1992). Energy value of resistant starch. *Eur. J. Clin. Nutr.* 46 (Suppl. 2), 129-130.

McCleary, B.V., McNally, M., and Rossiter, P. (2002). Measurement of resistant starch by enzymatic digestion in starch and selected plant materials: collaborative study. *J. AOAC Int.* 85 (5), 1103-1111.

McCleary, B.V. and Monaghan, D.A. (2002). Measurement of resistant starch. *J. AOAC Int.* 85 (3), 665-675.

Medcalf, D.G. and Gilles, K.A. (1965). Wheat starches I. comparison of physicochemical properties. *Cereal Chem.* 42, 558-568.

Meuser, F., Klingler, R.W., and Niediek, E.A. (1978). Charakterisierung mechanisch modifizierter Stärke. *Starch/ Stärke* 30 (11), 376-384.

Michel, M. and Autio, K. (2002). Effects of high pressure on protein- and polysaccharide-based structures. In: M.E.G. Hendrickx, D. Knorr. *Ultra high pressure treatments of foods* (pp. 189-214). Kluwer Academic/ Plenum Publishers, N.Y., Boston, Dordrecht, London, Moscow.

Minerich, P.L. and Labuza, T.P. (2003). Development of a pressure indicator for high pressure processing of foods. *Innov. Food Sci. & Emerging Technol.* 4, 235-243.

Moorthy, S.N. (2004). Tropical sources of starch. In: A.-C. Eliasson. *Starch in food structure, function and applications* (pp.321-359). Woodhead Publishing Ltd., Cambridge, CRC Press, Boca Raton, Florida.

Muhr, A.H. and Blanshard, J.M.V. (1982). Effect of hydrostatic pressure on starch gelatinisation. *Carbohydr. Polym.* 2, 61-74.

Muhr, A.H., Wetton, R.E., and Blanshard, J.M.V. (1982). Effect of hydrostatic pressure on starch gelatinisation, as determined by DTA. *Carbohydr. Polym.* 2, 91-102.

Muhrbeck, P. and Svensson, E. (1996). Annealing properties of potato starch with different degrees of phosphorylation. *Carbohydr. Polym.* 31, 263-267.

Neter, J. and Wasserman, W. (1974). *Applied linear statistical models*. Richard D. Irwin, Inc., Homewood, Illinois.

Ochiai, S. and Nakagawa, Y. (1992). Packaging for high pressure food processing. In: C. Balny, R. Hayashi, K. Heremans, P. Masson. *High pressure and biotechnology* (pp.163-165). Colloque INSERM/ John Libbey Eurotext, Montrouge, France.

Ogawa, H., Fukuhisa, K., and Fukumoto, H. (1992). Effect of hydrostatic pressure on sterilization and preservation of citrus juice. In: C. Balny, R. Hayashi, K. Heremans, P. Masson. *High pressure and biotechnology* (pp.269-278). Colloque INSERM/ John Libbey Eurotext, Montrouge, France.

Osella, C.A., Sanchez, H.D., Carrara, C.R., de la Torre, M.A., and Pilar Buera, M. (2005). Water redistribution and structural changes of starch during storage of a gluten-free bread. *Starch/ Stärke* 57, 208-216.

Ottenhof, M.-A. and Farhat, I.A. (2004). The effect of gluten on the retrogradation of wheat starch. *J. Cereal Sci.* 40, 269-274.

Ottenhof, M.-A., Hill, S.E., and Farhat, I.A. (2005). Comparative study of the retrogradation of intermediate water content waxy maize, wheat, and potato starches. *J. Agric. Food Chem.* 53, 631-638.

References

Pfister, M.K.-H., Butz, P., Heinz, V., Dehne, L.I., Knorr, D., and Tauscher, B. (2001). Influence of high pressure treatment on chemical alterations in foods. A literature review. *BgVV-Hefte* 03/2001. BgVV-Hausdruckerei, Berlin.

Puupponen-Pimiä, R., Aura, A.-M., Oksman-Caldentey, K.-M., Myllärinen, P., Saarela, M., Mattila-Sandholm, T., and Poutanen, K. (2002). Development of functional ingredients for gut health. *Trends Food Sci. Technol.* 13, 3-11.

Ranhotra, G.S., Gelroth, J.A., and Glaser, B.K. (1996a). Energy value of resistant starch. *J. Food Sci.* 61 (2), 453-455.

Ranhotra, G.S., Gelroth, J.A., and Glaser, B.K (1996b). Effect of resistant starch on blood and liver lipids in hamsters. *Cereal Chem.* 73 (2), 176-178.

Rössler, C., Jacobasch, G., Schmiedl, D., and Gebhardt, E. (2002). Resistant starch type 3 from rye, a functional food. *Nutrition* 26 (7/8), 297-305.

Rubens, P., Snauwaert, J., Heremans, K., and Stute, R. (1999). In situ observation of pressure-induced gelation of starches studied with FTIR in the diamond anvil cell. *Carbohydr. Polym.* 39, 231-235.

Rubens, P. and Heremans, K. (2000). Pressure-temperature gelatinization phase diagram of starch: an in situ Fourier infrared study. *Biopolymers* 54, 524-530.

Sakonidou, T.D., Karapantsios, T.D., and Raphaelides, S.N. (2003). Mass transfer limitations during starch gelatinization. *Carbohydr. Polym.* 53, 53-61.

Sandstedt, R.M., Kempf, W., and Abbott, R.C. (1960). The effects of salts on the gelatinization of wheat starch. *Starch/ Stärke* 11, 333-337.

Sarko, A. and Wu, H.-C.H. (1978). The crystal structure of A-, B- and C-polymorphs of amylose and starch. *Starch/ Stärke* 30 (3), 73-78.

References

Sato, M. and Kobori, H. (1995). Ultrastructural effects of pressure stress to the nucleus in *Saccharomyces cerevisiae*: a study by immunoelectron microscopy using frozen thin sections. *Microbiology Letters* 132 (3), 253-258.

Saura-Calixto, F., Goñi, I., Bravo, L., and Mañas, E. (1993). Resistant starch in foods: modified method for dietary fiber residues. *J. Food Sci* 58 (3), 642-643.

Schulz, A.G.M., van Amelsvoort, J.M.M., and Beynen, A.C. (1993). Dietary native resistant starch but not retrograded resistant starch raises magnesium and calcium absorption in rats. *J. Nutr.* 123, 1724-1731.

Selmi, B., Marion, D., Perrier Cornet, J.M., Douzals, J.P., and Gervais, P. (2000). Amyloglucosidase hydrolysis of high-pressure and thermally gelatinized corn and wheat starches. *J. Agric. Food Chem.* 48 (7), 2629-2633.

Shamai, K., Bianco-Peled, H., and Shimoni, E. (2003). Polymorphism of resistant starch type III. *Carbohydr. Polym.* 54, 363-369.

Shimada, S., Andou, M., and Naito, N. (1993). Effects of hydrostatic pressure on the ultrastructure and leakage of internal substances in the yeast *Saccharomyces cerevisiae*. *Appl. Microbiol. Biotechnol.* 40 (1), 123-131.

Sievert, D. and Pomeranz, Y. (1989). Enzyme-resistant starch. I. characterization and evaluation of enzymatic, thermoanalytical, and microscopic methods. *Cereal Chem.* 66 (4), 342-347.

Sievert, D. and Pomeranz, Y. (1990). Enzyme-resistant starch. II. differential scanning calorimetry studies on heat-treated starches and enzyme-resistant starch residues. *Cereal Chem.* 67 (3), 217-221.

Sievert, D., Czuchajowska, Z., and Pomeranz, Y. (1991). Enzyme-resistant starch. III. X-ray diffraction of autoclaved amylomaize VII starch and enzyme-resistant starch residues. *Cereal Chem.* 68 (1), 86-91.

References

Siljeström, M., Eliasson, A.C., and Björck, I. (1989). Characterization of resistant starch from autoclaved wheat starch. *Starch/ Stärke* 41 (4), 147-151.

Silverio, J., Fredriksson, H., Andersson, R., Eliasson, A.-C., and Aman, P. (2000). The effect of temperature cycling on the amylopectin retrogradation of starches with different amylopectin unit-chain length distribution. *Carbohydr. Polym.* 42, 175-184.

Sivak, M.N. and Preiss, J. (1998). *Advances in food and nutrition research (Vol. 41) Starch: basic science and biotechnology.* Academic Press, San Diego, California.

Smelt, J.P., Hellemons, J.C., and Patterson, M. (2002). Effects of high pressure on vegetative microorganisms. In: M.E.G. Hendrickx, D. Knorr. *Ultra high pressure treatments of foods* (pp. 55-76). Kluwer Academic/ Plenum Publishers, N.Y., Boston, Dordrecht, London, Moscow.

Snauwaert, J. and Heremans, K. (1999). Pressure induced swelling kinetics of starch granules. In: H. Ludwig. *Advances in high pressure bioscience and biotechnology* (pp. 349-352). Springer Verlag, Berlin.

Snyder, E.M. (1984). Industrial microscopy of starches. In: R.L. Whistler, J.N. Bemiller, and E.F. Paschall. *Starch: chemistry and technology* (pp. 661-673). Academic Press, Inc., Orlando, Florida.

Spies, R.D. and Hoseney, R.C. (1982). Effect of sugars on starch gelatinization. *Cereal Chem.* 59 (2), 128-131.

Stolt, M., Oinonen, S., and Autio, K. (2001). Effect of high pressure on the physical properties of barley starch. *Innov. Food Sci. & Emerging Technol.* 1, 167-175.

Stolt, M., Stoforos, N.G., Taoukis, P.S., and Autio, K. (1999). Evaluation and modelling of rheological properties of high pressure treated waxy maize starch dispersions. *J. Food Eng.* 40, 293-298.

References

Stute, R. (1985). Vergleichende Betrachtungen von Stärken verschiedenen pflanzlichen Ursprungs. *Schriftenreihe des Fonds der chemischen Industrie* 25, 16-31.

Stute, R. (1992). Hydrothermal modification of starches: the difference between annealing and heat/moisture-treatment. *Starch/ Stärke* 44 (6), 205-214.

Stute, R., Klingler, R.W., Boguslawski, S., Eshtiaghi, M.N., and Knorr. D. (1996). Effects of high pressures treatment on starches. *Starch/ Stärke* 48, 399-408.

Svensson, E. and Eliasson, A.-C. (1995). Crystalline changes in native wheat and potato starches at intermediate water levels during gelatinization. *Carbohydr. Polym.* 26, 171-176.

Takahashi, T., Kawauchi, S., Suzuki, K., and Nakao, E. (1994). Bindability and digestibility of high-pressure-treated starch with glucoamylases from *Rhizopus* sp. *J. Biochem.* 116, 1251-1256.

Tamura, K. (1999). High-pressure stress response of microbes and the role of trehalose in their behaviour. *Rev. High Pressure Sci. Technol.* 9, 199-204 (Japanese with English abstract).

Tegge, G. (2004). *Stärke*. Behr's Verlag, Hamburg.

Ternes, W. (1994). *Naturwissenschaftliche Grundlagen der Lebensmittelzubereitung*. Behr´s Verlag, Hamburg.

Tester, R.F. and Debon, S.J.J. (2000). Annealing of starch – a review. *Int. J. Biol. Macromol.* 27, 1-12.

Tewari, G., Jayas, D.S., and Holley, R.A. (1999). High pressure processing of foods: an overview. *Science des aliments* 19, 619-661.

Thevelein, J.M., Van Assche, J.A., Heremans, K., and Gerlsma, S.Y. (1981). Gelatinisation temperature of starch, as influenced by high pressure. *Carbohydr. Res.* 93, 304-307.

References
_____

Tomasik, P., Wang, Y.-J., and Jane, J.L. (1995). Complexes of starch with low-molecular saccharides. *Starch/ Stärke* 47, 185-191.

Tovar, J., Björck, I.M., and Asp, N.-G. (1992). Incomplete digestion of legume starches in rats: a study of precooked flours containing retrograded and physically inaccessible starch fractions. *J. Nutr.* 122 (7), 1500-1507.

Tscheuschner, H.-D. (1996). *Grundzüge der Lebensmitteltechnik*. Behr´s Verlag, Hamburg.

Uedaira, H. and Uedaira, H. (1980). The effect of sugars on the thermal denaturation of lysozyme. *Bull. Chem. Soc. Jpn*. 53, 2451-2455.

Uedaira, H. and Uedaira, H. (1985). Sugar-water interaction from diffusion measurements. *J. Sol. Chem.* 14 (1), 27-34.

Uedaira, H., Ishimura, M., Tsuda, S., and Uedaira, H. (1990). Hydration of oligosaccharides. *Bull. Chem. Soc. Jpn*. 63 (12), 3376-3379.

Vasanthan, T. and Bhatty, R.S. (1998). Enhancement of resistant starch (RS3) in amylomaize, barley, field pea and lentil starches. *Starch/ Stärke* 50 (7), 286-291.

Wang, W.-C. and Sastry, S.K. (1997). Starch gelatinization in ohmic heating. *J. Food Eng.* 34, 225-242.

Watson, S.A. (1964). Determination of starch gelatinization temperature. In: R.L. Whistler. *Methods in carbohydrate chemistry* (pp. 240-242). Academic Press, New York and London.

Watzl, B. and Leitzmann, C. (1999). *Bioaktive Substanzen in Lebensmitteln*. Hippokrates Verlag GmbH, Stuttgart.

Wootton, M. and Ho, P. (1999). Alkali gelatinisation of wheat starch. *Starch/ Stärke* 41, 261-265.

References

Yen, G.-C. and Lin, H.-T. (1996). Comparison of high pressure treatment and thermal pasteurization effects on the quality and shelf life of guava puree. *Int. J. Food Sci. & Technol.* 31, 205-213.

Yook, C., Sosulski, F., and Bhirud, P.R. (1994). Effects of cationization on functional properties of pea and corn starches. *Starch/ Stärke* 46, 393-399.

Zobel, H.F. (1964). X-ray analysis of starch granules. In: R.L. Whistler. *Methods in carbohydrate chemistry* (pp. 109-113). Academic Press, New York and London.

Zobel, H.F. (1984). Gelatinization of starch and mechanical properties of starch pastes. In: R.L. Whistler, J.N. Bemiller, and E.F. Paschall. *Starch: chemistry and technology* (pp. 285-309). Academic Press, Inc., Orlando, Florida.

Zobel, H.F. (1988). Starch crystal transformation and their industrial importance. *Starch/ Stärke* 40 (1), 1-7.

APPENDIX

Tab. A1. Peak temperatures, inflexion points, and mass loss of DSC, TG, and DTG analyses of dry, native wheat, tapioca, and potato starches under anaerobic conditions at a heating rate of 10°K/ min in a temperature range from 0°C to 450°C

DSC curve	Wheat starch	Tapioca starch	Potato starch
Onset T [°C]	55.3	20.5	46.5
Peak T [°C]	114.4	109.6	120.5
Offset T [°C]	153.1	265.6	178.2
Onset T [°C]	262.6	265.6	253.0
Peak T [°C]	286.5	281.0	276.5
Peak T [°C]	294.9	290.2	280.5
Peak T [°C]	304.0	304.2	286.0
Peak T [°C]	310.3	315.2	-
Offset T [°C]	320.1	332.8	314.0
TG curve			
Inflexion point [°C]	308.9	313.8	303.2
mass loss [%]	77.35	81.11	71.68
DTG curve			
Onset T [°C]	293.0	289.7	285.5
Peak T [°C]	308.7	312.8	303.1
Offset T [°C]	319.4	331.7	314.1
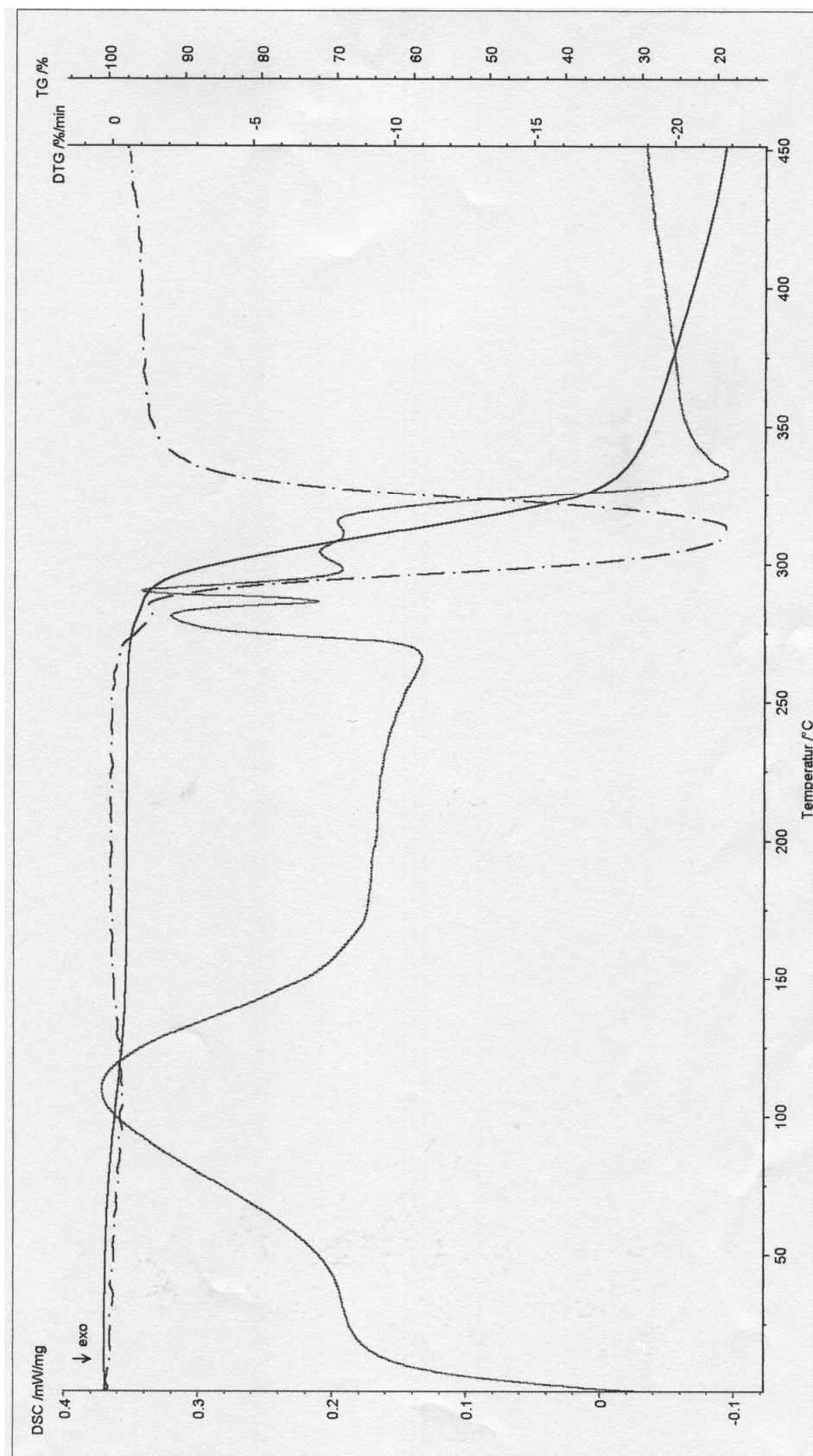
Fig. A1. DSC, TG, and DTG curves of dry, native tapioca starch under anaerobic conditions at a heating rate of 10 °K/min in a temperature range from 0°C to 450°C.
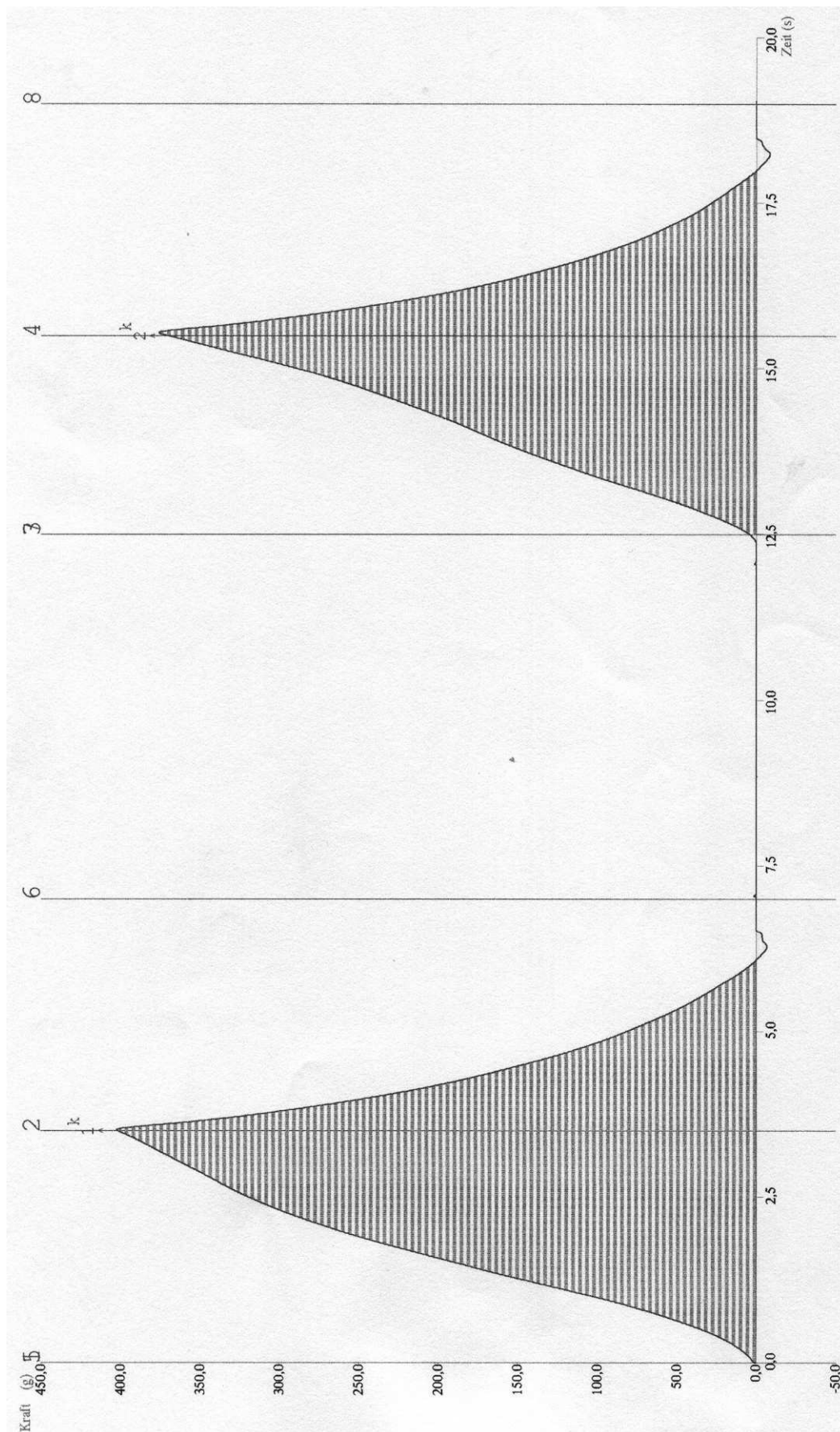
Fig. A2. Exemplary texture analysis diagram of bread containing pressurised wheat starch suspension after one day of storage.

SUMMARY

The impact of high hydrostatic pressure on wheat, tapioca, and potato starch suspensions at different pressures, temperatures and treatment times was investigated. The degree of gelatinisation as well as the electrical conductivity and the water binding capacity of the suspensions increased with increasing pressure and with increasing treatment time. The gelatinisation curves obtained with increasing pressure resembled sigmoid curves characteristic for thermal gelatinisation. The gelatinisation pressure of the three starches examined differed. Potato starch exhibited the highest pressure resistance, followed by tapioca and wheat starch. This was attributed to the crystalline structure of the starches.

Due to its sensitivity to pressure, temperature, and pressurisation time, it was suggested that starch could function as a pressure time temperature integrator (PTTI) in pressurisation processes. Additionally, the electrical conductivity was identified as a convenient tool for the quick measurement of pressure-induced starch gelatinisation.

Pressure treatment up to 300 MPa in a high pressure cell under a microscope enabled in situ observation of swelling of wheat starch granules during pressurisation without granule disintegration. This proved that the water uptake of the granules is associated with a volume decrease of the suspension and also emphasised the differing gelatinisation process under heat and under pressure. For tapioca and potato starches, 300 MPa was not sufficient to monitor any visible changes in volume. The starch suspensions were also stained with Lugol`s solution and a decolouration was observed upon pressurisation for 1 h at 300 MPa. This decolouration was the most pronounced for wheat starch granules, but also observable for tapioca and potato starch granules.

The effect of water content as well as sugars and salts on pressure-induced starch gelatinisation was also investigated. Generally, similar effects compared to thermal starch gelatinisation in the presence of sugars and salts were found. The gelatinisation pressure was increased by sugars and the degree of gelatinisation was linearly correlated with the number of equatorial hydroxyl groups of the sugars. Pressure-induced starch gelatinisation was strongly dependent on the water content of the starch suspension and the degree of gelatinisation was decreased in the presence of sugars and water structure making ions (e.g. SO_4^{2-}) and increased by water structure breaking ions such as SCN^-. Therefore, the availability of free water was crucial for starch gelatinisation under pressure. The extent of influence of salts on pressure-induced starch gelatinisation was dependent on the type of starch and regardless of the pressure sensitivity of the starch. At high salt concentrations (> 2

M), the impact of chlorides on pressure-induced starch gelatinisation of all three starches examined followed the order Na<K<Li<Ca, which corresponds to the order of the Hofmeister series. And at concentrations above 1 M, the effect of potassium salts on pressure-induced starch gelatinisation upon pressurisation also followed the order of the lyotropic series (Cl<Br<I<SCN).

Since functional food ingredients are becoming increasingly important, it was aimed to develop a process for resistant starch (RS) production from wheat starch including high hydrostatic pressure treatment. Of all treatment steps and combinations explored, acid hydrolysis of pressurised wheat starch prior to 192 h of pressure-annealing cycles resulted in the highest RS yield with 12.08 %. High pressure-induced starch gelatinisation did not prove to be superior to heat-induced gelatinsation for the production of RS from wheat starch. However, the RS yields obtained were in the same range as yields from heat-gelatinised wheat starches as stated in the literature.

In accordance with a patent (Codovilli, 2004) where it was claimed that pressurised starch suspensions replacing part of the flour in a wheat bread dough enhanced storage properties of bread in terms of ageing, 5 % of the flour in a wheat bread was replaced by pressure-gelatinised wheat starch. The elasticity and hardness of the baked breads were determined after one and five days of storage. The expected improvement of storage properties could not be confirmed, on the contrary a trend towards increased firmness and decreased elasticity of the bread was obtained.

It can be concluded that pressure-induced starch gelatinisation offers a lot of potential for the development of novel food properties due to its differing physical and textural properties in comparison to heat-induced gelatinisation. More research is required to fully comprehend the differences between temperature-induced and pressure-induced gelatinisation mechanisms in order to take optimum advantages of pressure-induced starch gelatinisation for industrial products.

ZUSAMMENFASSUNG

Der Einfluss von hohem hydrostatischem Druck auf Weizen-, Tapioka- und Kartoffelstärke bei verschiedenen Drücken, Temperaturen und Behandlungszeiten wurden untersucht. Der Verkleisterungsgrad, die elektrische Leitfähigkeit und die Wasserbindekapazität der Stärkesuspensionen erhöhten sich mit steigendem Druck und steigender Behandlungsdauer. Die erhaltenen Verkleisterungskurven mit steigendem Druck ähnelten dem sigmoiden Verlauf, der charakteristisch für die thermische Verkleisterung ist. Der Verkleisterungsdruck der drei untersuchten Stärken war verschieden. Kartoffelstärke wies die höchste Druckresistenz auf, gefolgt von Tapioka- und Weizenstärke. Dies wurde auf die kristalline Struktur der Stärken zurückgeführt.

Aufgrund ihrer Empfindlichkeit gegenüber Druck, Temperatur und Druckhaltezeit wurde vorgeschlagen, Stärke als Druck-Temperatur-Zeit-Integrator (PTTI) in Druckprozessen einzusetzen. Zusätzlich wurde die elektrische Leitfähigkeit als ein effektives Medium für die schnelle Messung des Verkleisterungsgrades aufgezeigt.

Druckbehandlungen bis 300 MPa in einer Hochdruckzelle unter einem Mikroskop ermöglichten in situ-Beobachtung von Quellung von Weizenstärkekörnern ohne Desintegration während einer Druckbehandlung. Dies bewies, dass eine Wasseraufnahme der Körner mit einer Volumenabnahme der Suspension verbunden ist, und bestärkte den Unterschied zwischen thermischem und druckinduziertem Verkleisterungsprozess. Für Tapioka- und Kartoffelstärken waren 300 MPa nicht ausreichend, um sichtbare Volumenveränderungen zu detektieren. Die Stärkesuspensionen wurden zusätzlich mit Lugol´scher Lösung angefärbt, und eine Entfärbung konnte nach einer Druckbehandlung für eine Stunde bei 300 MPa erfasst werden. Diese Entfärbung war für Weizenstärkekörner am deutlichsten, aber auch für Tapioka- und Kartoffelstärkekörner zu beobachten.

Auch der Einfluss vom Wassergehalt der Stärkesuspensionen, von Salzen und Zuckern auf die druckinduzierte Verkleisterung wurde ermittelt. Grundsätzlich wurden in der Anwesenheit von Salzen und Zuckern ähnliche Effekte wie bei der thermischen Stärkeverkleisterung gefunden. Der Verkleisterungsdruck stieg in der Gegenwart von Zuckern, und der Verkleisterungsgrad korrelierte linear mit der Anzahl der äquatorialen Hydroxyl-Gruppen der Zucker. Druckinduzierte Stärkeverkleisterung hing stark vom Wassergehalt der Stärkesuspensionen ab, und der Verkleisterungsgrad sank in der Gegenwart von Zuckern und Wasserstruktur gebenden Ionen (z.B. SO_4^{2-}) und stieg in der Anwesenheit von Wasserstruktur zerstörenden Ionen (z.B. SCN^-). Demzufolge war die Verfügbarkeit an

freiem Wasser entscheidend für die Verkleisterung von Stärke unter hydrostatischem Druck. Das Ausmaß des Einflusses von Salzen auf die druckinduzierte Stärkeverkleisterung hing von der Stärkesorte ab und war unabhängig von deren Druckresistenz. Bei hohen Salzkonzentrationen (> 2 M) folgte der Effekt von Chloriden auf die Druckverkleisterung von allen drei untersuchten Stärken der Reihenfolge Na<K<Li<Ca, was der Reihenfolge der Hofmeister-Reihe entspricht. Und bei Konzentrationen über 1M folgte der Effekt von Kaliumsalzen auf die Druckverkleisterung auch der Reihenfolge der Hofmeister-Reihe (Cl<BR<I<SCN).

Da funktionelle Lebensmittelinhaltsstoffe mehr und mehr in den Vordergrund rücken, wurde angestrebt, einen Herstellungsprozess von resistenter Stärke (RS) aus Weizenstärke zu entwickeln, der einen Hochdruckbehandlungsschritt einschließt. Von allen getesteten Behandlungsschritten und - kombinationen ergab eine Säurehydrolyse hochdruckbehandelter Stärke gefolgt von 192 h Druck-Temper-Zyklen den höchsten Ertrag an RS mit 12.08 %. Hochdruck hat sich damit gegenüber der thermischen Behandlung zur Produktion von RS aus Weizenstärke nicht als überlegen erwiesen, allerdings befanden sich die Ausbeuten an RS aus thermisch verkleisterter Weizenstärke, wie in der Literatur beschrieben, in derselben Größenordnung.

In Anlehnung an ein Patent (Codovilli, 2004), das beansprucht, dass durch den Einsatz von druckbehandelten Weizenstärke, die einen Teil des Mehls in Weizenbrotteig ersetzt, die Alterungseigenschaften von Brot verbessert werden, wurden 5 % des Mehls in Brotteig durch hochdruckbehandelte Weizenstärke ersetzt, und die Elastizität und die Festigkeit der gebackenen Brote nach einem und fünf Tagen Lagerung bestimmt. Die erwartete Verbesserung der Lagerungseigenschaften konnte nicht bestätigt werden, im Gegenteil, ein Trend zu erhöhter Festigkeit und sinkender Elastizität des Brotes wurde festgestellt.

Es kann geschlussfolgert werden, dass druckinduzierte Verkleisterung viel Potential für die Entwicklung neuartiger Lebensmitteleigenschaften aufgrund abweichender physikalischer und struktureller Eigenschaften im Vergleich zu thermisch induzierter Verkleisterung bietet. Um die Unterschiede der Mechanismen von temperaturinduzierter und druckinduzierter Verkleisterung voll zu verstehen, ist zusätzliche Forschung notwendig, damit die Vorteile der druckinduzierten Stärkeverkleisterung für industrielle Produkte ausgenutzt werden können.

LIST OF PUBLICATIONS

Bauer, B.A. and Knorr, D. (2004). Electrical conductivity: A new tool for the determination of high hydrostatic pressure-induced starch gelatinisation. *Innov. Food Sci. & Emerging Technol.* 5/4, 437-442.

Bauer, B.A., Hartmann, M., Sommer, K., and Knorr, D. (2004). Optical in-situ analysis of starch granules under high pressure with a high pressure cell. *Innov. Food Sci. & Emerging Technol.* 5, 293-298.

Bauer, B.A. and Knorr, D. (2005). The impact of pressure, temperature and treatment time on starches: Pressure-induced starch gelatinisation as pressure time temperature indicator for high hydrostatic pressure processing. *J. Food. Eng.* 68 (3), 329-334.

Bauer, B.A., Wiehle, T., and Knorr, D. (2005). Impact of high hydrostatic pressure treatment on the resistant starch content of wheat starch. *Starch/ Stärke* 57 (3-4), 124-133.

Rumpold, B.A. and Knorr, D. (2005). Effect of salts and sugars on pressure-induced starch gelatinisation of wheat, tapioca and potato starches. *Starch/ Stärke* 57 (8), 370-377.

Ananta, E., Bauer, B.A., Volkert, M., and Knorr, D. (2005). Sprühtrocknung von probiotischen Bakterien. *Deutsche Molkerei Zeitung* 2, 32-35.