Retrogradation of Concentrated Starch Systems;
Mechanism and Consequences for Product Properties

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C.J.A.M. Keetels

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Stellingen

1   Een geconcentreerd zetmeelgel kan worden beschouwd als een driedimensionale legpuzzel waarin de puzzelstukjes aan elkaar zijn geplakt, met de gezwollen zetmeelkorrels als puzzelstukjes en een dun laagje amylosegel als het plakmiddel.
Dit proefschrift

2   De onregelmatigheid van de vorm van gezwollen zetmeelkorrels bepaalt in grote mate de breukeigenschappen van geconcentreerde zetmeelgelen.
Dit proefschrift

3   De waarneming van Svegmark en Hermansson dat gezwollen aardappelzetmeelkorrels gevoeliger zijn voor afschuiving dan gezwollen tarwezetmeelkorrels kan worden verklaard door het grotere aantal warpunten in tarwezetmeelkorrels.
K. Svegmark and A.-M. Hermansson, *Carbohydr. Polym.* (1991) 15, 151-169.

4   De sensorisch waargenomen veroudering van brood wordt niet alleen bepaald door de eigenschappen van het vaste materiaal maar ook door de macroscopische structuur van het brood, met name door variaties in de grootte van de gascellen en in de dikte van de staafjes tussen de cellen.
Dit proefschrift

5   In de theorie van Ashby en Gibson worden de mechanische eigenschappen van vaste materialen met een sponsstructuur gerelateerd aan de volumefractie en de mechanische eigenschappen van de matrix. Deze theorie is niet zonder meer toepasbaar op brood gezien de onregelmatigheid van de sponsstructuur.
Gibson and Ashby, "Cellular Solids", Pergamon Press.
Dit proefschrift

6   De term retrogradatie heeft voor verschillende onderzoekers een verschillende betekenis, vaak afhankelijk van het systeem en de methode waarmee dit verschijnsel is bestudeerd. Het is daarom zaak om de term retrogradatie goed te definiëren. Nog beter zou het zijn om de term in het geheel niet te gebruiken.

7   Om het effect van amylosegelering op de gelering van een 20% zetmeelgel te bestuderen gingen Miles et al. uit van een 4% amylose-oplossing. In werkelijkheid is de effectieve concentratie tussen de gezwollen zetmeelkorrels veel hoger (~ 20%). Aangezien de stijfheid van een amylosegel veel sterker dan evenredig toeneemt met de concentratie en ook de snelheid waarmee een gel wordt gevormd sterk afhankelijk is van de concentratie, geven de resultaten van Miles et al. slechts een indicatie.
M.J. Miles et al. (1985) *Carbohydr. Res.*, 135, 271-281
H.S. Ellis and S.G. Ring (1985) *Carbohydr. Polym.*, 6, 201-213.
A.H. Clark et al. (1989) *Macromolecules*, 22, 346-351.

8   Het effect van hoogpasteurisatie van de ondermelk op de weerstand tegen vervorming van aangezuurde ondermelkgelen is toe te schrijven aan een verandering in de fysische structuur van het gel.

9   De heterogene structuur van snoepjes gemaakt van gellan gom is belangrijk voor het vrijkomen van smaakstoffen bij consumptie ervan. Om het inzicht in de structuur van materialen op basis van dit verdikkingsmiddel te vergroten zouden permeabiliteitsmetingen een goede aanvulling zijn op de reologische metingen.
A. Tsiami et al. (1994) In: Gums and Stabilisers for the Food Industry 7, pp. 157-165.

10   Het aankopen van grond voor stadsuitbreiding en natuurgebieden drijft de grondprijs op en remt de groei van bestaande boerenbedrijven.

11   Een grotere mobiliteit spoort niet met het milieubeleid.

Stellingen behorende bij het proefschrift "Retrogradation of concentrated starch systems; mechanism and consequences for product properties" door C.J.A.M. Keetels.
Wageningen, 24 mei 1995.

voor mijn ouders

Abstract

Keetels, C.J.A.M. (1995) Retrogradation of Concentrated Starch Systems; Mechanism and Consequences for Product Properties. Ph.D. thesis, Wageningen Agricultural University (165 pp., English and Dutch summaries).

Keywords: Starch, gelation, retrogradation, gels, starch bread, rheology, fracture, thermal analysis, microscopy, amylose, amylopectin, GMS, SSL.

The mechanical properties of concentrated starch + water systems were studied during heating, cooling and storage. Methods used were a small-amplitude dynamic rheological test and compression between parallel plates. The mechanical properties were related to the structure of the gels. Information about the structure of the gels was obtained by electron and light microscopy and DSC. Starches used were from wheat and potato.

During heating of starch suspensions at rest, storage moduli first increased and subsequently decreased. This result is related to swelling of starch granules, melting of crystallites, separation of amylose and amylopectin, and loss of entanglements between starch molecules. Concentrated starch gels formed during heating at rest consist of partly swollen, irregularly shaped granules, which are tightly packed, with a thin amylose gel layer in between. The mechanical properties of these gels at large deformations are determined by the stiffness of the swollen granules, their shape and the mechanical properties of the thin amylose gel layer. Observed changes in Young modulus, and in the stress and strain at fracture during ageing are primarily ascribed to the increase in stiffness of the swollen granules. This increase is due to the formation of (semi) crystalline domains consisting of clusters of ordered double helices of short branches of amylopectin molecules.

The mechanical properties of starch breads were measured in two successive compression/decompression cycles and the results were discussed by applying a theory developed for cellular solids. The mechanical properties of starch bread are determined by the mechanical properties and the dimensions of the condensed lamellae and beams, which have a structure comparable with that of concentrated starch gels, as well as the size and size distribution of the gas cells. GMS and SSL affect the mechanical properties of starch bread in two opposite ways; by affecting the properties of the lamellae and beams forming the bread structure and by making the crumb structure finer (gas cell size distribution) and more even. They hardly affect amylopectin recrystallization in starch bread.

Contents

Chapter 1

General Introduction

1.1   Starch: origin and production

Nearly all green plants produce starch as a form of storage energy. After cellulose, starch is the most abundant carbohydrate present in food and feed, and most of it is consumed without being separated from the rest of the plant material. On an industrial scale, starch is purified by separating it from other plant materials, such as fibre, proteins, sugars and salts. The most common sources from which starch is isolated are potato tubers, kernels from wheat, maize and waxy maize, cassava roots (tapioca) and the trunk of the sago-palm. The estimated world production of these starches is given in Table 1.1.

Table 1.1 *World production of commercial starches.*[1]

Botanical source	Starch production in 10^6 kg/year
Maize	19.9
Potato	2.3
Cassava	1.8
Wheat	1.3
Waxy maize	0.3

The properties of the starches prepared from different sources vary considerably, *i.e.* each type of starch is unique. In industry, several modification techniques are used to provide starch products with the properties needed for specific uses. In Western Europe two thirds of the total starch production is used in the food and beverage industries.[2] It is applied as thickener in sauces, custards, pie fillers and desserts. After enzymic hydrolysis, it is also used as sweetener in drinks and

confectionary. Other applications include the use of maltodextrins in dietetic and low-calory foods. Of the total starch production in Western Europe, approximately one third is used for non-food products, mainly in the paper, packaging and textile industries. A small part is used as a raw material in the chemical industry. Fermented products are applied in, e.g., cosmetics, pharmaceuticals and biodegradable plastics.

1.2 The native starch granule

1.2.1 Appearance of starch granules

Starch occurs in partially crystalline granules that are insoluble in water at room temperature. Starch is produced through photosynthesis via glucose in chloroplasts and amyloplasts. Inside the chloroplasts, starch granules remain very small. In the living plant, the energy accumulated through photosynthesis by the chlorophyll is used for energy during the night. Amyloplasts are organelles specialized in the storage of starch.[3] Starch biosynthesis is initiated at a recognizable site in the later granule called the hilum, which is usually less organized than the rest of the granule. A starch granule grows by apposition; newly synthesized oligomers are linked to starch molecules at the surface; as a consequence, the granule grows. The size of the granules varies from 2 to 100 μm. The size and shape of the granules depend on species and maturity of the plant. Granules of tuber and root starches are generally larger than those of cereal starches. Potato starch granules are largest (15-100 μm diameter), ellipsoidal in shape, and asymmetric with respect to the position of the hilum.[4,5] Maize starch granules have a more polyhedral shape, varying in size from 5 to 25 μm.[4] In wheat starch two types of granules occurs; lenticular 'A' granules with a diameter of 10-45 μm and polyhedral 'B' granules with a diameter up to 10 μm.[5] Both the composition and gelatinization characteristics of A and B-type granules differ.[6]

Most native granules exhibit concentric growth rings, which are, in fact, alternating concentric shells of high and low refractive index, density, crystallinity and resistance to attack by enzymes or chemicals.[7] In wheat starch, the number of growth rings appears to correspond to the number of days of granule development.[8] If wheat is grown under constant environmental conditions, growth rings may be absent. In potato starch granules, growth rings are produced even when the potatoes are continuously exposed to light at a constant temperature.

1.2.2 Composition and structure of starch

Starch granules are composed mainly of a mixture of two large polysaccharide

molecules, amylose and amylopectin. Amylose contents of most reserve starches are rather similar: 20-30%.[4,9] It is about 28% for wheat and maize starch, and 23-26% for potato starch. In many plant species mutants are known with an altered starch composition. In amylomaize, the amylose content is very high (about 50 - 80%) and in waxy starches very low (<1%).[9]

Amylose is an essentially linear molecule consisting of α-D-glucopyranose residues linked together by (1→4) bonds (Figure 1.1). The amylose molecule never can be stretched; it therefore has the tendency to form a helix. The size of the amylose molecules varies according to the plant source. For instance, the average degree of polymerization (DP) of amylose from potato starch is much larger than those of wheat and maize starches.[10,11] It has been known for some time that, as a result of the presence of a few branching points, amylose molecules may not be completely hydrolysed to maltose by the enzyme β-amylase. Recently, the heterogeneity of amyloses has been investigated in more detail.[10,11] It was shown that amylose from various sources contains, on average, 2 to 8 branch points per molecule. Amylose molecules from potato starch have, on average, more branch points than those from wheat starch. The chains showed broad distributions of molar mass, varying from short (maltotetraose) to long (DP > 100) chains. A more branched polysaccharide molecule with, on average, 20 chains per molecule has been fractionated from maize starch.[11] This molecule contained short (DP = 18), long (DP > 230) and very long chains (DP > 2730). This polysaccharide seemed to have a structure that locally resembles that of amylopectin.

amylose
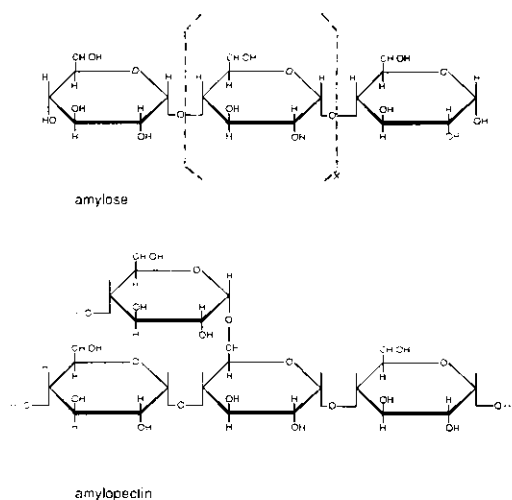
amylopectin

Figure 1.1   *Linkage of the α-D-glucopyranose residues in amylose and amylopectin molecules.*

Amylopectin is the highly branched component of starch. On average, 4-5% of the glucose residues carry, besides the $(1\rightarrow4)$-bond, a $(1\rightarrow6)$-bond to an adjacent residue (Figure 1.1). The molar mass of amylopectin varies between 10^6 and 10^9 Da.[4] The value is dependent on the botanical origin of the starch, the conditions of the fractionation of amylose and amylopectin, and the method used to determine the molar mass. The chain profile of amylopectin has been investigated by enzymic debranching and gel permeation chromatography. Two main populations were observed, one with a DP $\approx$ 60 and another, more abundant, population with DP $\approx$ 17.[12] By improving the separation techniques a polymodal chain distribution with periodic peaks at multiple lengths was obtained.[13] It is generally agreed that amylopectin has a structure, in which the short chains are arranged in clusters on the longer chains[4,7,14] (Figure 1.2). Hizukuri[13] has proposed that 80-90% of the chains are present in only one single cluster. The remaining 10-20% form inter-cluster connections, most of which connect two successive clusters.
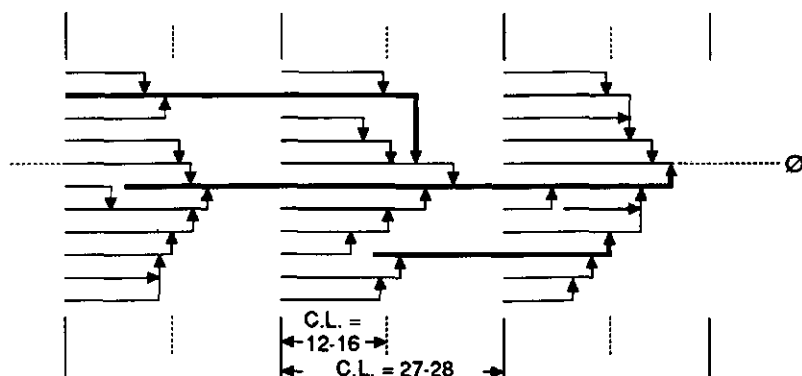
Figure 1.2   *Cluster model of amylopectin proposed by Hizukuri.[13] ϕ, reducing chain-end; —, $(1\rightarrow4)$-α-D-glucan chain; $\rightarrow$, α-$(1\rightarrow6)$ linkage; c.l. = chain length = number of glucopyranose residues.*

Besides amylose and amylopectin, small amounts of non-carbohydrate components are present, particularly lipids, proteins, and minerals, among which phosphates. Tuber and root starches contain only a small amount of lipids ($<$ 0.1%) compared with cereal starches.[9] The amount of lipids in normal maize and wheat starch granules is about 0.8%.[15,16] The protein content also varies among sources of starch. Cereal starches contain a considerable amount of proteins (0.25 - 0.5%), whereas the protein content in tuber and root starches is less than 0.1%.[4,17] In potato starch, negatively charged phosphate groups are present.[18] Most of these

groups are esterified onto glucose residues in amylopectin (about 1 phosphate group per 300 glucose units).

The minor components in starch may affect the behaviour of starch in various applications. For instance, amylose readily complexes with the hydrocarbon chain of monoacyl lipids.[19] It is, however, still unknown whether internal lipids are complexed with amylose in the native granules or whether a complex is formed during heating of starch in water.[20] Amylose-lipid complexes reduce the swelling capacity of cereal starches.[21] The mutual repulsion of negatively charged phosphate groups probably accounts partly for the high degree of swelling of potato starch granules in pure water.[18] This will be discussed in section 1.3.3.

1.2.3 Granule organization

The structure of starch has been subject of many investigations. Well-documented reviews on the organization of the granule are given by French[22] and Blanshard.[7] Here a short description is given.

When starch granules are observed under a polarization microscope a characteristic dark cross, known as a 'Maltese cross', is seen, which implies that there exists a high degree of molecular organization within the granule. The sign of the birefringence is positive, indicating that the polysaccharide molecules are radially oriented in the granule.[7] Wide angle X-ray scattering (WAXS) has shown that starch granules are partially crystalline; the overall crystallinity of starch is about 20-45%.[23,24] Amylose and branching regions of amylopectin form the amorphous regions in the starch granule. The crystallinity arises mainly from ordered linear segments of amylopectin. These are present in the form of double helices with a length of approximately 5 nm. The chains in the double helices are left-handed and parallel to each other. The double helices are crystallized into thin (~ 5 nm) lamellar domains (Figure 1.3A), which are visible in transmission electron micrographs (TEM).[25-28] WAXS has revealed the presence of three forms of packing of the double helices, the so-called A-, B-, and C-forms. The A-form is found in most cereal starches, the B-form in tuber starches, and the C-form in legume starches. The crystals of both the A- and B-form are made up of a hexagonal arrangement of double helices, which are packed in a parallel register.[29,30] Water molecules are integral parts of the starch polymorphs A and B, the amount of water varying for the different types. The amounts of water of crystallization are estimated at 0.1 and 0.25 kg per kg dry starch for the A- and B-form, respectively.[31] The C-form is probably a mixture of the A- and B-type crystallites.[32] From results of electron microscopy and small-angle X-ray scattering (SAXS), Oostergetel and van Bruggen[27,33,34] suggested that the crystalline lamellae of starches with more than 50% amylopectin are helically arranged (Figure 1.3B). In this model the amylopectin segments in the crystalline regions are all parallel to

the axis of the large helix. The diameter of this helix is ~18 nm, and the spacing (distance) between adjacent turns of the helix is approximately 10 nm. The diameter of the central cavity of the helix inside the helix is ~8 nm; it is not clear until now what material is present in the cavity. The large helices form a more or less continuous super helical structure, in which the left-handed helices are packed in a tetragonal array.[34]
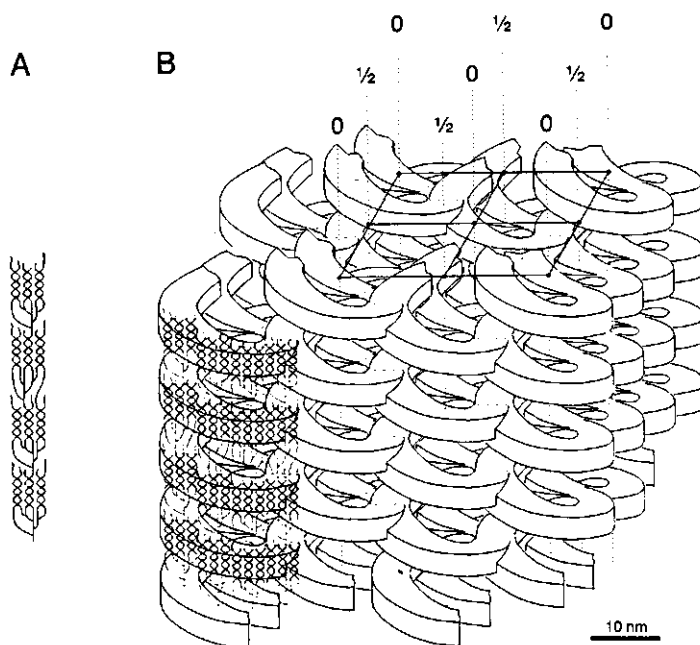
Figure 1.3   *Schematic model for the arrangement of amylopectin in potato starch. (A) Model showing the clustering of the double helical linear chains. (B) Model showing layers of crystalline lamellae containing double helical linear chains alternated with amorphous layers containing the branch points. The crystalline layers form a continuous network consisting of left handed helices packed in a tetragonal array. Neighbouring helices are shifted relative to each other by half the helical pitch (indicated by 0 and ½). (From: Oostergetel and van Bruggen[34])*

1.2.4 Starch biosynthesis

In starch biosynthesis three distinct enzymic processes are involved: initiation, chain elongation and branching. It has been proposed that the initial step consists of elongation of "primer" chains on a glucoprotein.[35-38] These chains can serve as an acceptor for glucose residues transferred from ADPG (adenosine diphosphate glucose) and UDPG (uridine diphosphate glucose). The enzymes ADPG- and UDPG-glycosyltransferases (starch synthases) transfer the glucose units from ADPG or UDPG to an acceptor substrate $[G]_x$; these enzymes catalyze the formation of α-1,4-bonds between the D-glucopyranosyl units and the non-reducing ends of the chain molecules.[39,40]

$$UDPG + [G]_x \xrightarrow{\text{synthase}} G\text{-}(1{\rightarrow}4)\text{-}[G]_x + UDP$$

$$ADPG + [G]_x \xrightarrow{\text{synthase}} G\text{-}(1{\rightarrow}4)\text{-}[G]_x + ADP$$
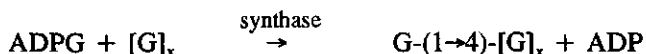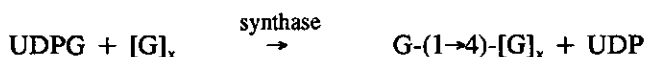
Multiple starch synthases are known:[37] one or two associated with the starch granule and one, but more often two, in solution. The granule-bound starch synthase seems to be responsible for the synthesis of amylose, since this enzyme is absent in waxy mutants.[41,42] The synthesis of amylopectin would result from a combined action of one or more soluble starch synthases and branching enzymes.[37,40] The branching enzyme (or Q-enzyme) can synthesize the α-1,6 cross-links in amylopectin.[39,40] Various plants have been shown to contain more than one branching enzyme.[40] Amylopectin is therefore not a product formed from a simple two-enzyme complex of a starch synthase and a branching enzyme, but it is a product of various pairs of these enzymes.

Various hypotheses have been developed to explain the side-by-side synthesis of amylose and amylopectin. Recently, Ponstein[37] suggested that the more soluble 'granule-bound' starch synthases compete with the branching enzyme for complexation of the linear amylose-like fragments. The formation of complexes with starch synthase protects linear fragments against branching enzyme activity. Thus, the ratio of amylose and amylopectin is probably affected by the ratio of branching enzyme and granule bound starch synthase and their affinities for linear chains.

1.3   Structural changes during heating

1.3.1 Introduction

When native starch granules are suspended in water at room temperature, a small amount of water is reversibly taken up.[43] For example, potato starch granules absorb water until the water content is approximately 35%.[44,45] This results in a minor swelling of the granules; the granules keep their shape and birefringence. When heat is applied to such a system, starch undergoes a series of processes known as gelatinization. There is a drastic increase in swelling, which is no longer reversible. Nearly simultaneously, the granules lose their birefringence and X-ray pattern, which indicates that the crystallites melt. These processes are accompanied by a (partial) leaching of amylose from the granules. The order-disorder transition occurs over a temperature range characteristic for the type of starch. For starches of normal amylose content (20-30%) and in the presence of enough water, this transition occurs somewhere in the temperature range 55-70 °C.[46,47] After all crystallites are disrupted, the swelling of the granules and the leaching of amylose continue.

Below, I will discuss the different aspects of starch gelatinization more comprehensively. Moreover, I will describe the effect of gelatinization on rheological properties of starch suspensions.

1.3.2 The order-disorder transition

Several instrumental methods have been applied to study changes that occur in the ordering of starch molecules during heating; these include polarization microscopy,[48-52] small angle X-ray scattering (SAXS),[53-55] wide angle X-ray scattering (WAXS),[51,56-58] differential scanning calorimetry (DSC)[44,46,47,49-52,56,59-67] and ^{13}C-NMR spectroscopy.[43,56,68,69] These techniques are sensitive to chain organization at various length scales, and changes in it during heating in water. For instance, decreases in crystallinity as measured with WAXS occur over a much broader temperature range than the loss of birefringence; they start before the birefringence of granules starts to disappear and continue after all birefringence has been lost.[51] On the other hand, with several starches that had been pretreated thermally, the decreases in crystalline order (as monitored by WAXS) and molecular order (as followed by ^{13}C-NMR spectroscopy) follow the same relative quantitative pattern.[56]

In recent years, several workers have used DSC to study phase transitions that occur when an aqueous suspension of starch granules is heated. DSC registrates both first order (melting) and second order (glass) thermal transitions. These studies show that at relatively high water contents, roughly at more than 65%,[44,61] a

single endotherm occurs at about 60 °C (Figure 1.4). The position of the peak depends on the type of starch studied,[46,47,67] and on the DSC heating rate.[60,61] As the amount of water available per unit amount of starch is reduced, the enthalpy change in this transition is progressively reduced, with a concomitant development of a second transition at a higher temperature. At low water contents, about less than 35%, only the second endotherm is present.[44,61] Thus, at intermediate water levels the disorganization of the ordered structure of starch shows itself in the form
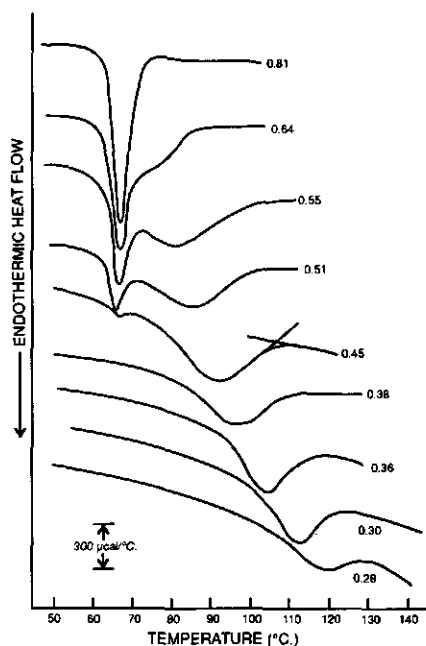
Figure 1.4 *DSC thermograms of potato starch with different water contents. The volume fractions of water are indicated. The heating rate was 10 K·min⁻¹. (From: Donovan[61])*

of two endothermic transitions. Moreover, in lipid containing starches a third endothermic transition can be observed at higher temperatures, which has been attributed to the dissociation of amylose-lipid complexes.[63,66] The temperature of the first endothermic transition does not vary significantly with the water content, whereas the temperatures of the second and third endotherms increase with decreasing water content.

The molecular mechanism responsible for the biphasic endotherm is still uncertain. Several workers[44,59,61] have given an explanation for this behaviour, that

of Evans[44] being a plausible one. It is based on the hypothesis that the crystalline zones would have different stabilities. Each granule contains crystallites that have a range of stabilities, and with the application of heat, the least stable crystallites would melt first. As some crystallites melt, the granule can absorb some water. The water content of the granule thus increases successively, and therefore the melting point of the remaining crystallites decreases. Due to this, a single granule gelatinizes over a narrow temperature interval. At low water contents there is a reduction in the effective water content for the remaining ungelatinized granules or regions in a granule, which therefore melt at a higher temperature.

In view of the partially crystalline nature of the starch granule, attempts were made by several workers[44,49,59-61,66,67] to treat starch gelatinization as a melting process by applying the Flory-Huggins equation.[70] This expression relates the melting temperature of a semi-crystalline polymer to the diluent concentration under equilibrium conditions. However, the validity of this approach has recently been questioned because of the non-equilibrium character of the melting process.[60,71,72]

1.3.3 Swelling and solubilization

The order-disorder transition is accompanied by swelling of the granules. Moreover, during these processes part of the amylose molecules become solubilized in the water phase surrounding the granules. The leaching out of amylose is due to amylose and amylopectin being incompatible in a fairly concentrated solution and the higher mobility of amylose compared to amylopectin. At temperatures of 70-90 °C, mixed aqueous systems of purified samples of amylose and amylopectin separate into phases enriched to about 70-80% of the preponderant component.[73] This means that during gelatinization always a certain amount of amylose would remain in the granules.

Morphological changes that take place during gelatinization can be observed by scanning electron microscopy[74-76] and light microscopy.[77,78] Wheat, barley and rye starches show a characteristic two-stage swelling.[74,76] After the first swelling step, which occurs between 60 and 80 °C (thus at temperatures at which the crystallites melt), the starch granules are only slightly swollen. They exhibit radial swelling in one plane, resulting in the formation of flattened discs. During the first stage of swelling, solubilized amylose was observed in the centre of the swollen granules and, to some extent, outside the granules.[77] The release of amylose outside the granules is thus low, as was also observed by others.[79,80] During the second swelling stage (80-85°C), changes occurred rapidly. The granules displayed a tangential swelling, yielding a characteristic geometrical structure.[74,76,77] The solubilization of amylose occurred mainly during this step. Doublier[81] suggests that the solubilization is related to the dissociation of amylose-lipid complexes, which in

dilute systems occurs at about the same temperatures.[82-84] It has been postulated that most of the amylose is transported from the centre of the granules to the outside through pores in the granular structure.[77]

The swelling of potato starch granules follows a different pattern. The expansion of these granules occurs in all directions, resulting in swollen granules that have about the same shape as the native starch granules.[76] With increasing temperature, the granules swell very rapidly and to a much greater extent than do wheat starch granules.[76,85] The high swelling power of potato starch may be due to repulsion between the negatively charged phosphate groups.[5,18] It has also been reported that amylose-lipid complexes present in cereal starches restrict swelling of granules.[5,18,21] Another difference between potato and wheat starch is the separation of amylose and amylopectin. During heating of potato starch dispersions (4-10%) most of the amylose molecules remain within the swollen granules together with the amylopectin.[78] At a starch concentration of 4% or more, the swollen granules fill all of the volume; this makes leaching out of amylose virtually impossible. On heating to higher temperatures, separation of amylose and amylopectin takes place in the granules, resulting in amylose-rich and amylopectin-rich domains.[78]

Shearing of starch suspensions during heating may lead to a breakdown of swollen granules. In a study of potato starch systems, rigorous shearing completely altered the microstructure.[78] The granules were disrupted into fragments and extensive solubilization took place. Both amylose and amylopectin were solubilized and formed a continuous phase containing dispersed fragments of granules. In the continuous phase a demixing into amylose-rich and amylopectin-rich regions took place, which was enhanced by increasing the temperature. The amylopectin-rich phase seemed to be continuous and enclosed amylose-rich domains. When wheat starch suspensions were heated under shear, only the outer layer of the swollen granules was disrupted.[77] The amylopectin fragments were dispersed into the continuous phase. Thus, the effect of shear on the microstructure of wheat starch systems is small compared to the changes observed in potato starch systems.

1.3.4 Rheological changes

Structural changes that occur during gelatinization, such as swelling of granules and melting of crystallites, result in interesting changes in rheological properties. In this section the rheological properties of starch suspensions during heating will be discussed. The Brabender viscograph is commonly used for characterizing the mechanical properties of various types of starch during a heating cycle. Often, this method is also utilized for quality control. With this apparatus "viscosity" is measured in instrument-linked units. In the viscograph the deformations are so large as to induce irreversible changes in the structure of starch systems except, maybe, at low concentrations. These alterations always affect the results and

complicate interpretation of the results. Therefore, more recently, dynamic mechanical analysis has been used to study the rheological properties during heating.[80,86-91] Dynamic mechanical tests can be performed at such small deformations that changes in structure due to shear are negligible.

With the Brabender viscograph as well as with dynamic mechanical analysis an increase in viscosity and modulus can be observed at temperatures of about 60 °C, which is due to swelling of the starch granules. At low concentrations (for instance 5%), the viscosity and modulus of potato starch systems increases more rapidly than that of cereal starches.[18,91] Moreover, its peak viscosity and modulus are higher,[18,80,91] which has been attributed to the higher swelling ability of potato starch granules. In more concentrated systems (for instance 30%), the modulus increases rapidly for both potato and wheat starch systems.[88,89] As the concentrations are high, the granules have to swell only slightly for close-packing to occur. The peak modulus of a concentrated wheat starch system is higher than that of a concentrated potato starch system,[88,89] which has been attributed to the greater stiffness of the swollen wheat starch granules (see also Chapter 2). Both in dilute and concentrated starch systems, heating to still higher temperatures results in a decrease of viscosity and modulus,[18,80,88-91] as a result of softening of the granules. The viscosity as measured with the Brabender viscograph decreases also due to breakdown of the swollen granules. Shear affects the rheological properties of potato starch systems significantly.[90,91] The rheological properties of wheat starch systems are considerably less affected by shear.[91] This agrees with microscopic observations, which show that the structure of swollen potato starch granules is affected to a much higher extent[78] (see also section 1.3.3).

1.4   Gelation and retrogradation

During heating and cooling of sufficiently concentrated starch suspensions, an elastic gel is formed.[88-92] Starch gels are metastable; both amylose and amylopectin partly recrystallize during storage, which results in an increase in firmness of the gel.[92] Retrogradation is a term that is often used to describe the changes that occur on cooling and storage, although it has been defined in various ways. In this study retrogradation is defined as recrystallization of starch molecules. In order to separate the roles played by amylose and amylopectin, several studies have been performed on the gelation and retrogradation of both pure amylose and amylopectin. This will be described in section 1.4.1. In section 1.4.2, the gelation and retrogradation of starch systems will be discussed.

1.4.1 Amylose and amylopectin

Aqueous solutions of amylose at neutral pH are unstable. After cooling a dilute solution to room temperature, a precipitate forms in time. More concentrated solutions form an opaque elastic gel. Ellis and Ring[93] and Miles *et al.*[94] have proposed that amylose gelation takes place above the so called coil overlap concentration, c^*. This is the minimum concentration at which the collective molecules can occupy the available volume completely. For amylose with a molar mass of 500000, a coil overlap concentration of about 1.5% was observed.[93,94] Gidley and coworkers,[95-97] however, observed that gelation is possible from "dilute" non-entangled amylose solutions, and that no correlation is found between the critical gelling concentrations and the coil overlap concentrations.[97] Surprisingly, the minimum concentration for gel formation (0.8-1.1%) varied only slightly with the molar mass, provided the degree of polymerization was larger than 300.[95]

In the gelation of amylose, several stages can be distinguished. First, a short range ordering, as has been observed by Fourier transform infrared spectroscopy,[98] takes place. In this stage some double helices are formed, which does not increase the gel modulus.[99] At the same time or very soon afterwards, a phase separation takes place, giving rise to polymer-rich and polymer-poor phases.[94,100] An interconnected network is formed by the polymer rich phase, resulting in an increase in gel modulus.[93,94,99] Subsequent to gelation, the appearance of an WAXS-pattern shows the development of crystallinity.[93,94] Electron microscopy experiments have shown that amylose gels exhibit a macroporous structure with mesh sizes of 0.1 to 1 μm.[101] In these gels, crystalline filaments with a width of 20 $\pm$ 10 nm are interconnected by an amorphous fraction consisting of about 18-33% of the polysaccharide. These filaments consist of a large number of ordered double helical chains,[97,101] giving rise to the B-type WAXS-pattern. The amorphous fraction consists of mobile chain segments[97-101] with a chain length of 6 < DP < 30.[101]

The gelation of amylose takes minutes or at most a few hours to reach a plateau value in moduli.[94,96] The relative rates of increase in gel moduli and the final values of the moduli depend on amylose concentration,[95,99] chain length,[99] and ionic strength.[95,102] At small deformations, amylose gels behave as elastic solids. Shear moduli of amylose gels depend very much on their concentration, the more so the chains are longer. Their relationship can be described by $G \propto c^n$; the exponent n in the equation increases with increasing degree of polymerization, which is shown in Table 1.2.

At temperatures below 5 °C, concentrated amylopectin solutions form gels. The gelation of amylopectin is very different from that of amylose. Unlike amylose, amylopectin requires high polymer concentrations (>10%), well above the coil overlap concentration of this biopolymer (c^* ~ 0.9%).[103] The gelation of

Table 1.2   *The effect of the chain length of the amylose molecules on the dependence of the shear moduli on concentration.*

DP	exponent n	reference
300	4.4	96
660	4.7	96
1100	5.9	96
2500-3000	7.0	93

amylopectin occurs over several weeks,[103] as opposed to the gelation of amylose, which occurs over much shorter time scales.[94,96] The development of the modulus of amylopectin systems is closely related to the association of double helices, as monitored by WAXS.[103] The gelation and crystallization of amylopectin are preceded by an initial fast formation of double helices consisting of amylopectin side chains, as was observed by Fourier transform infrared spectroscopy.[98] The interchain association of amylopectin involves short chains of DP $\approx$ 15, whereas amylose association involves chain segments of DP $\approx$ 65.[101] The association of amylopectin chains is thermoreversible at temperatures below 100 °C,[103] whereas much higher temperatures (about 160 °C) are required for the melting of the amylose crystallites.[94] This is a result of the different length over which the double helices are associated.

The stiffness of amylopectin gels does not depend greatly on concentration. In the concentration range 10-25%, there is a linear relationship between modulus and concentration.[103] The rate of gelation of amylopectin is related to the length of the short branches of the molecule. Amylopectins with longer short chains (e.g. potato) gel at a faster rate than amylopectins with shorter short chains (e.g. wheat).[104] However, the plateau value of the shear modulus does not show any simple relationship with the molecular structure of the molecule.

1.4.2 Starch

Starch gels are formed when a sufficiently concentrated starch system is heated and subsequently cooled. Several authors[80,92,99,105,106] have recognized the composite nature of starch gels, in which swollen granules are embedded in and reinforce an amylose gel matrix. The rheology of starch gels has primarily been related to three factors: the rheological characteristics of the gel matrix (primarily amylose), the volume fraction of the swollen granules, and the deformability of the granules.

Gel formation has primarily been ascribed to gelation of amylose in the

continuous phase,[90-92] which occurs over relatively short storage times. The gelation of amylose would therefore contribute to the short time development of the gel moduli. Upon storage, a further increase in stiffness of the starch gel occurs.[47,88,89,92,107] This slow stiffening is accompanied by the formation of B-type crystals, which has been attributed to recrystallization of amylopectin within the granules.[47,92,107] Stiffening of the granules would reinforce the amylose gel.

Several techniques have been used to study the rate of recrystallization of starch gels. Over the last fifteen years, DSC has often been applied to study starch recrystallization.[47,62,66,92,107,108-112] Other techniques used are SAXS,[55,108] WAXS,[47,92,107] Fourier-transform infrared spectroscopy[113,114] and Raman spectroscopy.[115,116] Crystallization as measured by DSC and by WAXS show a similar increase with time.[47,92,107] According to Miles *et al.*[92] and Orford *et al.*,[47] the increase in crystallinity parallels the increase in stiffness of the gel, whereas Roulet *et al.*[107] concluded that recrystallization occurs faster than does strengthening of the gel.

Retrogradation has traditionally been treated as an equilibrium process. Often, the Avrami equation[117,118] has been applied to model the rate of starch recrystallization,[110,111,119-121] and also to describe the consequences of starch retrogradation for the rheological properties of the starch system.[122-124] The Avrami equation expresses the change in crystallinity of a system as a function of time. The fraction of uncrystallized material (θ) is related to time (t) by:

$$\theta = \exp(-kt^n)$$

where k is a growth parameter. According to the theory on which the Avrami equation is based, the exponent n depends on crystal shape and on the time dependence of the nucleation process; it may have values between 1 and 4. Several workers derived a value of unity from experiments with concentrated starch gels,[119,121,122,124] from which it was concluded that the nucleation in starch crystallization is instantaneous, and that the crystallites grow in one direction only. However, from some sets of experimental data, exponents smaller than unity have been derived;[110,111,120,123] this would make the application of the Avrami equation to starch retrogradation questionable.

Recent work has emphasized the role of water as a plasticizer and the importance of the glass transition in affecting starch retrogradation.[71,125] In this work, retrogradation is seen as a non-equilibrium process, consisting of three steps: nucleation, propagation and maturation. To describe the kinetics of starch retrogradation, several workers[71,125,126] have used the classical theory of crystallization kinetics as applied to partially crystalline polymers.[127] Partially crystalline polymers show two characteristic kinetic transitions. In the first place, the amorphous component shows a (second order) transition from the glassy solid

to the amorphous rubbery state. The temperature at which this transition occurs is called the glass transition temperature T_g. The other transition is shown by the crystalline component at the melting temperature T_m, which represents a transition from a crystalline solid to a liquid. Water as a plasticizer is known to affect both T_g and T_m of partially crystalline polymers. The effect of water on T_g and T_m can be illustrated in a state diagram. In Figure 1.5 a state diagram for a gelatinized starch-water system is given. In this figure, T_m is the melting temperature for potato starch crystals.

temperature (°C)

liquid

amorphous + crystalline

glass + crystalline

mass fraction of water
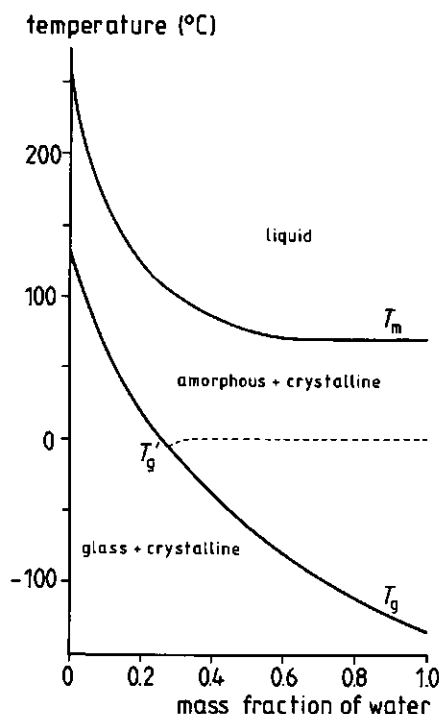
Figure 1.5   *State diagram showing the approximate* T_g *and* T_m *temperatures of starch-water mixtures as a function of mass fraction of water on total mass. After results of van den Berg,[31,128] Evans and Haisman[44] and Donovan.[61]*

When cooling an aqueous solution below 0 °C, water starts to crystallize (assuming that the water content is high enough). The ice formation will lead to an increase in solute concentration in the water that is still liquid and this depresses the freezing point (dashed line in Figure 1.5) and increases the viscosity. Eventually, ice crystallization becomes so slow that it is not observable on practical

time scales. Then, the solution is maximally freeze concentrated and has become glassy. The glass transition temperature of this concentrate is designated T_g'. A maximally freeze-concentrated amorphous suspension of gelatinized starch would contain about 27% non-freezing water.[71] For starch gels recrystallized from homogenous and completely amorphous sols or pastes containing ≥ 27 wt% water, T_g' is about -5 °C.[71,125]

Crystallization would only occur between the glass transition temperature T_g and the melting temperature T_m.[125,126] According to the classical theory, the nucleation rate is zero at T_m and increases with decreasing temperature over a relatively narrow interval (Figure 1.6).[126,127] At still lower temperatures, where nucleation depends on local mobility or local viscosity, the nucleation rate decreases with decreasing temperature and increasing local viscosity, to zero at T_g. At T_g, the propagation rate is near zero; it increases rapidly with increasing temperature until it drops precipitously to zero rate at T_m.[125,126] Theoretically, the rate of crystallization (the resultant of nucleation and growth rates) will have a maximum value at a temperature about midway between T_g and T_m. For instance, for 50% starch gels, which have a T_g of about -65 °C and a T_m of 75 °C, this would indicate that the maximum rate occurs at temperatures close to 5 °C. This agrees well with results obtained on starch gels.[107,108,110,119] The temperature at which starch gels are stored affects the melting temperature of the crystallites. Starch gels stored at low temperatures (for instance at 5 °C) had a lower melting temperature than those stored at room temperature.[108,125] This indicates that storage of starch gels at low temperatures results in crystalline regions with less perfect symmetry.
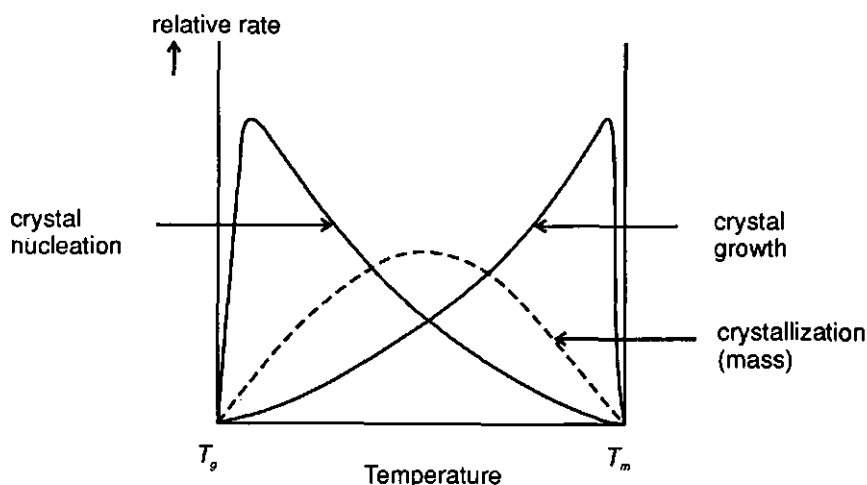
Figure 1.6 *Crystallization kinetics of partially crystalline polymers.*

An implicit requirement for starch recrystallization is the availability of sufficient water, both for mobilizing long polymer chains and for being incorporated into B-type crystal latices.[71,125] The minimum concentration required for nucleation is estimated to be about 27%.[125] In the range 27-50%, the rate of recrystallization increases with increasing percent total moisture,[110,125,129] which would be due to an increasingly effective mobility.[125] At higher water contents, the crystallization rate decreases,[47,110,129,130] presumably due to dilution.[125] Thus the rate of retrogradation shows a maximum at a water content of about 50%. At a water content below 10%, retrogradation of amylopectin is not observed by DSC.[110,129]

The rate of recrystallization and the increase in firmness of concentrated starch gels during storage are larger for potato than for wheat starch.[47,88,89,111,131] Several explanations have been proposed for this observation. As was discussed in section 1.4.1, the rate of recrystallization of amylopectin would be affected by the lengths of its short branches.[104] The slow rate of retrogradation of wheat starch gels may then be explained by the short average chain length of its short branches. Moreover, it is supposed that the lipids present in wheat starch retard the rate of retrogradation of wheat starch gels.[111] By adding free fatty acids[132] or lipids[120,133-135] like GMS, SSL, CSL and POES to starch gels, the rate of retrogradation appeared also to be reduced. This has been shown by both a slower increase in stiffness[132-135] and a slower rate of recrystallization of amylopectin, as determined by DSC.[120,133] Lipids can form inclusion complexes with amylose,[82,136,137] which may affect the gelation and retrogradation of amylose. Since the recrystallization of amylopectin appears to be mainly responsible for the increase in stifness of concentrated starch gels upon storage, it is not clear how lipids would retard the retrogradation process. It has been suggested recently that also amylopectin can to some extent form inclusion complexes with lipids;[138-143] this may then explain the reduced rate of retrogradation of starch gels in the presence of lipids.[140,141]

1.5   Bread

1.5.1 Making of bread

Wheat is the main cereal for the production of leavened bread. The reason for its dominance in breadmaking is its supreme baking performance in comparison with all other cereals. This is the result of the potential of wheat flour doughs to retain gas.

The essential ingredients for the production of bread are flour, water and small amounts of yeast and salt. Often, oxidants and/or emulsifiers are added. The first step in the breadmaking process is mixing and kneading of the ingredients into a dough. The mixing of the ingredients fulfils several functions.[144,145] An important

aim is the formation of a gluten network (dough development). This network would be formed by interactions between the monomeric gliadins and complexes of disulphide-linked subunits of the glutenins. The most important cross-links present in the gluten network would be disulphide bonds, hydrogen bonds and hydrophobic interactions.[144] Another important process in the mixing step is the occlusion of air forming the nuclei of the gas cells. Shortly after mixing, the gas cells are present as spherical holes with diameters between 10 and 100 μm.[146,147] At this stage, the relative volume fraction (the total volume of a dough divided by the volume of its liquid dough phase) of a bread dough is 1.1.[148,149]

Dough development proceeds during fermentation, in which dough pieces may undergo periods of rest, punching and moulding. During fermentation the yeast produces carbon dioxide, which diffuses to the gas cells; as a result they grow. At an advanced stage of fermentation the structure of the dough changes from a foam with spherical gas cells into a foam with polyhedral gas cells.[148,149]

After mixing and fermentation, the expanded dough is transferred to the oven and baked. During the initial stage of baking the gas cells expand further; the relative volume increases to between 5 and 7, because of evaporation of water, thermal expansion of the gas and enhanced production of carbon dioxide,[145] due to an ongoing yeast activity until the fermentation system of the yeast is inactivated at about 50 °C. In a later stage, when the temperature of the dough has become about 60 °C, starch partly gelatinizes resulting in the dough membranes becoming more solid. The setting of the membranes by starch gelatinization is an important process, as it is known that it is impossible to bake a bread without starch.[150] The expansion of the dough membrane will continue as a result of a further expansion of the gas. Due to this, the biaxial stresses in the membranes increase and, since the membranes are now solid and rather brittle, they may rupture. The structure of a dough with closed gas cells is transformed into a sponge structure with interconnected gas cells.

1.5.2 Dough stability

A high volume and a fine and even crumb structure are important quality attributes of bread. The use of different cereals or different wheat cultivars may lead to large differences in bread quality. The behaviour of gas cells in dough mainly determines the final appearance of the bread crumb and the bread volume. To obtain a high-quality bread, it is important that many gas cells are entrapped during the mixing step. At the start of the fermentation stage an appropriate number of gas cells with a narrow size distribution must be present. Moreover, the dough must retain the gas produced and the gas cells must stay apart from each other during fermentation and baking until the dough sets due to gelatinization of the starch. The most important types of instability in bread dough are disproportionation (growth of gas

cells at the expense of smaller ones) and coalescence (the merging of two gas cells caused by rupture of the film between them). Both surface and rheological properties of the dough are relevant in controlling disproportionation and coalescence. The relevance of these properties was studied in some detail by Kokelaar.[151]

The extent of disproportionation, which mainly occurs during the first stage of fermentation, is initially affected by surface rheological properties of the dough. These properties certainly affect the structure of the final product, but they cannot explain the differences in baking performance among cereal species or among wheat cultivars.[149,151] However, disproportionation of gas cells in dough can be retarded by wheat lipids and surfactants added in the right concentration.[151] If strain hardening of the dough is sufficiently large, the growth of the larger gas cells and, consequently, disproportionation will be retarded or stopped. A material is strain hardening if the resistance against deformation is higher for a relatively more extended test piece. This results in a more narrow size distribution of the gas cells and consequently a more even crumb in the final bread.

In more advanced stages of fermentation coalescence may occur, resulting in a coarse bread crumb. To retard coalescence, the following bulk rheological properties are required:[149,151] a high strain hardening, a high extensibility and a resistance against biaxial deformation in a restricted range. If the resistance is too high, only limited or no expansion of the dough will occur, which will lead to a low bread volume. If the resistance is too low, the gas cells will expand very fast leading to early rupture and the formation of large holes in the bread. The required properties are indeed found in doughs from wheat cultivars with a good breadmaking performance.[149,151,152] There is probably a relationship between the required properties and a high glutenin content as well as the tendency to form high molecular-mass assemblies by the gluten proteins in the dough.[152]

For a high resistance against coalescence during baking, the dough must also at elevated temperatures have the properties of a large enough strain hardening and a resistance to biaxial extension in the proper range. Moreover, the fracture strain of the dough membrane must be large enough. Possibly, surface rheological properties contribute to dough stability at the end of the oven rise when films have become much thinner than the diameter of the starch granules.[151]

1.5.3 Staling of bread

After a bread is removed from the oven, several changes may occur, which eventually lead to a deterioration of quality, *i.e.* decreasing consumer acceptance. These changes are commonly called staling and include all processes that occur in both crumb and crust during storage other than microbiological spoilage.[153] The crust, which in its fresh state is relatively dry, crisp and brittle, becomes soft and

leathery due to migration of moisture from the crumb to the crust.[153,154] Characteristic changes occurring in the crumb are an increase in firmness and crumbliness. Crumb firming and loss of crust crispness are accompanied by a loss of the typical aroma of fresh bread and the development of a stale flavour.

For more than a century scientists have tried to elucidate the mechanism of crumb staling. The first research on staling was reported in 1853 by Boussingault,[155] who demonstrated that staling of the crumb also occurs if loss of moisture is prevented. In 1928, Katz[156] concluded that recrystallization of starch was primarily responsible for bread staling. Starch recrystallization is still believed to be a major contributor to the staling process.[157-160] Other processes that contribute to the deterioration of quality are the migration of water from the crumb to the crust,[153,154] and possibly changes in the gluten matrix;[161,162] any role of an exchange of water between protein and starch[163,164] is quite uncertain.

Over the years numerous methods have been applied to follow the rate and degree of staling. The use of a sensory panel is perhaps the most direct method for detecting changes connected with staling,[159,160,165,166] as e.g. appearance of the crust and the crumb, taste and mouthfeel, firmness, and flavour. The disadvantage of these tests is the subjective assessment by the members of the panel, the high costs and the time needed for performing the tests. Therefore, several so-called objective methods have been used to follow staling of bread, especially that of the crumb. Rheological methods like dynamic mechanical analysis[167,168] and compression tests[165,166,169-176] have often been used, which show that crumb stiffness increases in time. It has been concluded that changes in crumb firmness and organoleptic staling rate are closely related.[177] Other methods used are WAXS,[178-180] thermal analysis,[175,176,181-183] Fourier transform infrared spectroscopy[184] and near infrared reflectance spectroscopy.[184] These methods show an increase in the ordering of starch molecules in bread crumb during storage.

Over the years the roles of the various factors affecting the rate of bread staling have been investigated extensively, as indicated by the number of reviews written.[158-160,185] For the greater part, these factors affect the rate of starch recrystallization and/or the bread structure.

The storage temperature of bread affects the rate of recrystallization of amylopectin and the increase in firmness[167,170,180,186] in the same way that it affects these properties of starch gels (section 1.4.2). By choosing the appropriate storage temperature, the shelf life of bread can be extended. Room temperature or freezing temperature is preferred over refrigerator temperature. If stale bread is heated to temperatures of 60-100 °C, it will, as a consequence of the melting of amylopectin crystallites (section 1.4.1), become as soft as it was immediately after baking.[168,182,187]

The flour protein level is an important factor in affecting the rate of staling. Generally, a higher protein content gives a higher bread volume and would retard

the increase in firmness.[186,188-190] The anti-firming effect of gluten could be due to the higher specific loaf volume of high-protein breads, because it is known that the rate of increase in crumb firmness is lower for breads with a high specific volume.[191]

Addition of several lipid surfactants to the dough has several effects. At first, the loaf volume is enhanced[188-198] and the stiffness of the bread crumb directly after baking is lower.[143,199-203] Moreover, addition of certain lipid surfactants decreases the staling rate[143,176,199-202] and retards the rate of recrystallization of amylopectin.[143,176,202] Among the substances used in breadmaking, especially monoglycerides are effective in retarding bread staling and starch retrogradation. DATEM and SSL only decrease the initial crumb softness, but they hardly affect the relative increase in bread staling and the recrystallization rate of amylopectin.[143,202-204] As was described in section 1.4.2, it is still not clear how lipids retard amylopectin recrystallization. Complexation of lipids with amylose may result in an initially softer crumb,[200] but it may be more logical to ascribe the initially softer crumb and the lower staling rate to a higher loaf volume and a more even crumb structure in the presence of emulsifiers[203,204] (see also Chapter 7).

Other methods that have received attention as a means of retarding staling are incorporation of low levels of bacterial α-amylases[205,206] and addition of pentosans.[207]

1.6   Aim and outline of this thesis

Although it has already been known for more than several decades that starch recrystallization is a major contributor to the staling of bread, it is still not exactly clear how changes in the structure of the gelatinized starch are related to a decrease in the eating quality of bread, in which an increase in firmness and crumbliness are important factors. In the past, rheological measurements at small deformations were often used to discuss the effect of starch recrystallization on the change in eating quality of starch based products, like e.g. bread. However, the consumers' perception of product properties is usually related to large deformation properties, including fracture or yielding of the product. Thus, to obtain a better understanding of the decrease in eating quality of products containing a high amount of starch, it is more relevant to study large deformation properties.

The primary aim of the investigations described in this thesis is to gain insight into the relation between the structure of concentrated starch systems and their rheological and fracture properties as a function of storage time. Therefore, I started to study the mechanical properties of relatively simple concentrated starch gels in relation to their structure. A secondary aim is to get a better understanding of the consequences of it for the staling of bread. Bread structure is very complex,

because there are several different structures at different length scales; *i.e.* from macromolecular scale to "sponge" scale. Moreover, recrystallization of starch may be affected by various components present in bread crumb, like gluten, lipids, pentosans, etc.

Chapter 2 describes the rheological properties of concentrated starch systems during heating and cooling. Knowledge about the gelation properties of starch is required for interpreting the relation between structure and mechanics of concentrated starch gels during storage, to which attention is paid in Chapter 3. Measurements were performed on starch systems prepared from several types of starch. Most of the experiments were done with potato and wheat starch; wheat starch because it is the starch present in bread, and potato starch because it is known that its properties are greatly different from those of wheat starch. The effect of one process variable, heating temperature, was studied. The effect of shear, another process variable, was left out of consideration, because it was studied in detail by Svegmark and Hermansson.[78,90,91] I found that the main factors that determine the large deformation properties of concentrated starch gels are the irregularity and the stiffness of the swollen granules. The rate at which the stiffness of the swollen granules changes during storage is determined largely by the number of naturally present entanglements or artificially induced chemical cross-links in the granules. Chapter 4 focuses on the underlying mechanism of starch recrystallization. Two additional techniques, differential scanning calorimetry and transmission electron microscopy, were applied and their results compared.

A direct translation of the results obtained on concentrated starch gels to the structure of bread crumb in relation to its mechanical properties is impossible, because the composition as well as the structure are different; *i.e.* a compact gel and a sponge, respectively. Therefore, it was decided to vary only the structure, and, consequently, staling was studied in starch breads prepared from potato and wheat starch. The results obtained on starch breads are presented and discussed in Chapter 5. For ideal sponge structures with uniform gas cell sizes and beam thicknesses, a theory that describes the relation between the structure of these cellular solids and their mechanical properties has been developed.[204] In Chapter 6 it will be shown that this theory cannot be applied to starch breads without due consideration of the inhomogeneous structure of its crumb.

In this thesis I will only deal with one group of additives which are commonly used in bread making, that is lipid surfactants. As was discussed before, these substances are, among others, added to dough to retard the staling of bread. Application of these substances is mainly based on experience; the underlying mechanism is still not well understood, although much has been published on this subject. In Chapter 7 attention is paid to the mechanism by which lipid surfactants affect bread staling.

A general discussion of the mechanism of starch retrogradation and its

consequences for product properties will be given in Chapter 8. In this final
chapter the main conclusions of this thesis will also be summarized.

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Chapter 2

Gelation of Concentrated Starch Suspensions

Summary

Small deformation properties of potato and wheat starch suspensions were studied during heating and cooling at rest by a small amplitude dynamic rheological test method. Starch concentrations used were 10 to 30 wt%. The temperature to which the suspensions were heated varied from 65 to 90 °C. During heating, the moduli of the starch systems at first increase and subsequently decrease. On prolonged heating, the moduli depend greatly on heating temperature; the higher the temperature, the lower the moduli. Properties of concentrated starch systems during heating are related to swelling of granules, melting of crystallites, separation of amylose from amylopectin, and disentanglement of non-covalent bonds. The differences between the rheological properties of potato and wheat starch suspensions are discussed, and compared with those of chemically cross-linked potato starches. Our hypothesis for the explanation of the observed phenomena is that physical entanglements or chemical cross-links, naturally or artificially present in starch granules, reduce the swelling capacity of the granules and increase the stiffness of the swollen granules; as a consequence, they affect the rheological properties of concentrated starch suspensions.

2.1   Introduction

Starch is an important structural component in many foods. Most starch containing foods are prepared by cooking or baking in the presence of water, which causes large changes in the structure of starch. These changes are jointly designated "gelatinization". They depend on temperature, amount of water present, agitation during heating, etc. By varying composition and/or process conditions, one can affect the structure of a starch-based product, and with it, its handling and eating quality. During storage, the structure of most products with a high starch content also changes, mostly resulting in a decrease in quality. For instance, bread stales; the crumb firms and becomes more crumbly, which makes it less attractive to eat. Due to its

commercial importance, bread staling has been the subject of extensive research (see for example some review papers[1-5]). There is considerable evidence suggesting that the decrease in eating quality of many starch-based products is at least partly caused by changes in the structure of the processed starch.[1-5] These changes are jointly known as "retrogradation". To obtain a better understanding of the role of starch in the structure of starch based products in relation to retrogradation, experiments are often done on relatively simple starch-water systems.[6-13]

In plant tissues starch is present in the form of water-insoluble granules. The granules contain ordered regions, which are semi-crystalline, and show birefringence. Most starches consist of a mixture of two polysaccharides, amylose and amylopectin. The amylose content is mostly between 20 and 35%. Amylose is an essentially linear molecule consisting of (1→4)-α-linked D-glucan chains. Amylopectin is a highly branched molecule. It contains short chains of (1→4)-α-linked D-glucan chains with (1→6)-α-linked branches, which may be branched again.[14] The molecular masses and the fine structures of amylose and amylopectin vary with the botanical source.[15,16]

When dry native starch granules are suspended in water at room temperature, a small amount of water is reversibly absorbed. Heating starch in excess of water results in melting of the starch granules, with loss of X-ray crystallinity. Nearly simultaneously, a loss of birefringence can be observed. At the same time, the starch granules may swell to many times their original size, depending on the space available. The swelling of granules and the melting of crystallites are semi-cooperative processes.[17] These changes in the structure of the starch granules are accompanied by the separation of amylose and amylopectin, which results in leaching of amylose out of the granules. At temperatures below 100 °C and without appreciable shear forces the granules maintain their integrity.

On cooling sufficiently concentrated starch dispersions, amylose molecules rearrange to form an elastic gel.[8] Heating and subsequent cooling of starch systems under conditions as mentioned above thus results in a transition of a liquid system with dispersed granules to a gel that consists of swollen granules in an amylose gel.[18,19] Such a material, in which a continuous phase is interspersed with filler particles, may be regarded as a composite material. The mechanical properties of such a material mainly depend on the rheological properties of the continuous phase, the volume fraction of the particles, the deformability of the particles, and the interactions between the dispersed and the continuous phase.[20,21] For concentrated starch gels it was found that swollen granules increase the modulus of the amylose gel.[18] Heating at high temperatures (>120 °C) and/or in the presence of shear changes the rheological and structural character of starch systems.[22] (Partial) fragmentation of the granules occurs, and in the resulting gels fragments of granules dispersed in a continuous phase can be observed.

During storage of concentrated starch gels the starch molecules rearrange, and stiffness increases. The short term development of the gel structure and crystallization

(local ordering) was found to be dominated by gelation and crystallization of amylose within the continuous phase.[8] Over longer periods (days), the increase in the modulus of starch gels was attributed to reordering of amylopectin, occurring at a much slower rate than amylose gelation.[8,23] Thus, as a result of reordering of amylopectin, the rigidity of the granules increases; this enhances the reinforcement of the amylose matrix gel.

The mechanical properties of starch systems have been extensively studied by using dynamic mechanical analysis; both during gel formation[13,19,24,25] and storage.[8-11] This type of measurement yields information about the stiffness of the material and its viscoelastic behaviour. The advantage of this technique is that it can be performed at such a small deformation that the effect of the test on the structure of the gel is negligible. However, during eating or handling of solid like foods, large deformations often occur, which may result in fracture or yielding of the product. To obtain a better understanding of the decrease in eating quality of starch based products, experiments at large deformations are more relevant. Little has been published on this, despite of its importance.

The aim of this study was to investigate the structure of concentrated starch gels and their formation in relation to retrogradation as expressed in rheological and fracture properties. In this chapter small deformation properties of these systems during heating and cooling will be presented and discussed in relation to the mechanism of starch gelatinization. In Chapter 3 the relation between the structure of concentrated starch gels and their mechanical properties at small and large deformations, and changes therein during storage will be discussed.

2.2   Materials and Methods

2.2.1 Materials

Potato starch, slightly and highly cross-linked potato starch, and waxy potato starch were supplied by AVEBE (Foxhol, the Netherlands). Waxy potato starch was obtained by suppression of the expression of the granule-bound starch synthase gene by means of antisense RNA technology.[26] The cross-linking was achieved by reaction with trimetaphosphate: it reacts with two hydroxyl groups and forms covalent bonds between two starch molecules or within a starch molecule. Amylose as well as amylopectin may be involved in this cross-linking process. Wheat starch was supplied by Latenstein BV (Nijmegen, the Netherlands). The chemical composition of the starches is given in Table 2.1.

Particle size distributions of potato and wheat starch were determined using a Coulter Laser LS 130 particle size analyzer. The determination with the Coulter Laser is based on analysis of the forward light scattering by the particles. The diffraction

patterns were converted into particle size distributions by use of Fraunhofer theory. Results of the particle size distributions are given in Figure 2.1. For both potato and wheat starch a bimodal size distribution was found.

NaCl was of analytical grade (Merck). Degassed, deionized water was used in the experiments.

Table 2.1 *Chemical composition of potato, wheat, slightly cross-linked (cr.-l.) potato, highly cross-linked potato and waxy potato starch. Dry matter, protein, lipids and ash contents are given in % of total weight, and amylose content in % of starch weight.*

starch	dry matter	protein	lipids	ash	amylose
potato	84.4	0.09	0.02	0.43	21.1
wheat	88.7	0.23	0.50	0.24	19.0
slightly cr.-l. potato	84.7	0.05	0.03	0.32	
highly cr.-l. potato	85.7	0.05	0.04	0.40	
waxy potato	87.4	0.07	0.27	0.59	< 1
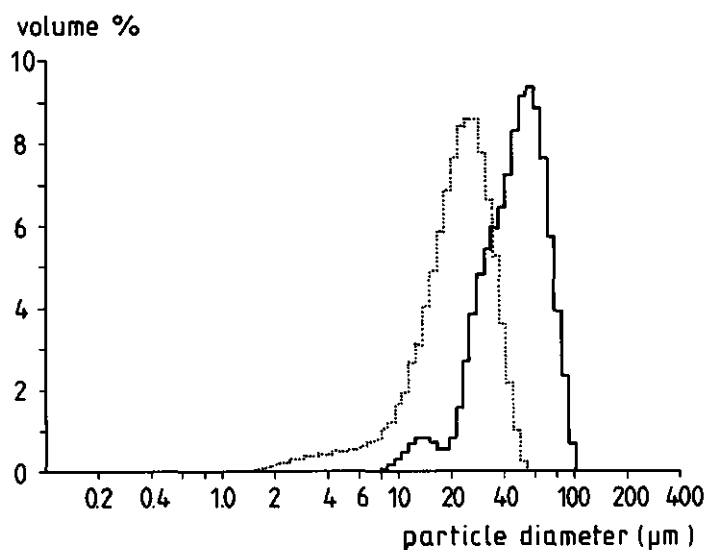
Figure 2.1 *Volume frequency distributions of native wheat (---) and potato (—) starch granule size.*

2.2.2 *Small deformation experiments*

Dynamic rheological properties were determined by applying a small oscillating shear deformation using a Bohlin VOR Rheometer, equipped with concentric cylinders made of stainless steel. The radius of the inner cylinder was 14.00 mm, the radius of the outer cylinder was 15.25 mm. A controlled sinusoidal oscillating strain is applied to the sample by oscillating the outer cylinder; the resulting torque on the inner cylinder is measured via a torque bar. Torque bars of 2.0, 8.8, 19.7 mN·m were used.

From these dynamic measurements two independent parameters can be obtained (e.g. Ferry[27]). The storage modulus G' ($N·m^{-2}$) is a measure of the energy stored and subsequently released per cycle of deformation and per unit of volume, *i.e.* the elastic response. The second parameter is the loss modulus G'' ($N·m^{-2}$), which is a measure of the energy dissipated as heat per cycle of deformation, *i.e.* the viscous response. The ratio of these two moduli is the loss tangent (-):

$$\tan\delta = \frac{G''}{G'} \tag{2.1}$$

A lower loss tangent means that the material behaves more solid-like. Care was taken that measurements were made in the linear region, in which the amplitudes of stress and strain are proportional to each other. A comprehensive description of the calculations of G' and G'' from experimental data is given by Ferry.[27]

2.2.3 *Sample preparation*

Potato and wheat starch gels

Two different sample preparation procedures, A and B, were followed. Both procedures were aimed at preventing sedimentation of starch granules and indeed resulted in homogeneous starch gels. Procedure A was the common one, whereas procedure B was only followed for some wheat starch systems. In procedure A, a 3 wt% potato starch or a 8 wt% wheat starch suspension was heated to 65 °C, while being gently stirred. After cooling to room temperature, sufficient starch was added to obtain suspensions with 15 or 30 wt% dry matter. These starch suspensions were transferred to a Bohlin VOR Rheometer, and heated to the required temperature. The starch suspensions were kept at these temperatures for a certain time, and then cooled to and kept at 20 °C. Heating and cooling were performed at a rate of 2 K·min^{-1}. Sequential measurements were made every 30 s during heating and cooling, and every 300 s when temperature was constant. Oscillation frequency was 0.1 Hz and strain 0.01. At this strain all samples showed linear behaviour. To prevent evaporation, samples were covered with paraffin oil.

Procedure B was used especially for concentrated wheat starch systems, because

then the chance of slippage occurring between the gel and the measuring body during the cooling regime was observed to be less. In this procedure 15 and 30 wt% starch suspensions were heated to about 55 to 60 °C while being gently stirred, until the starch suspension had a viscosity, at which it was still just possible to pour it. The heated starch suspensions were directly transferred to the measuring body of the Bohlin VOR Rheometer, which was at a temperature of 50 °C. The suspensions were heated from this temperature to the required temperature, kept at this temperature, and subsequently cooled according to procedure A. No difference in rheological properties between gels made according to both methods was observed.

Effect of heating temperature
This was investigated for 15 and 30 wt% potato and wheat starch systems by applying sample preparation procedure A, and, in addition, for 15 and 30 wt% wheat starch systems prepared by procedure B. The 15% suspensions were heated to 70, 80 or 90 °C, whereas 30% starch suspensions were also heated to 65 °C. The starch suspensions were kept at these temperatures for 120 minutes.

Effect of starch concentration
10, 15, 20, 25, and 30 wt% starch suspensions were made as mentioned before. The suspensions were heated to 90 °C, kept at 90 °C for 15 minutes, cooled to 20 °C, and finally kept at this temperature. Sequential measurements were done every 30 s.

Effect of chemical cross-links and absence of amylose
The effect of cross-linking of starch molecules was studied by investigating the rheological behaviour of 30 wt% potato starch, slightly cross-linked potato starch and highly cross-linked potato starch suspensions during heating and cooling following the procedure as described for the effect of starch concentration. This heating and cooling procedure was also used for studying the rheological properties of a 30 wt% waxy potato starch system. Since waxy potato starch contains hardly any amylose, the effect of amylose on the rheological properties can be studied by comparing the results of potato and waxy potato starch systems.

Reheating of starch gels
15 and 30 wt% potato and wheat starch gels were made as mentioned before. The suspensions were heated to 90 °C, kept at this temperature for 15 minutes, and cooled to 20 °C. After 15 minutes the same temperature cycle was followed. Sequential measurements were done every 30 s.

Frequency dependence
The effect of frequency was studied from 0.001 to 20 Hz for 15 and 30 wt% potato and wheat starch systems at a temperature of 90 °C. The gels were kept at this

temperature for one hour before determining the frequency dependence, because this made G' less dependent on storage time.

2.2.4 *Swelling capacity*

0.5 wt% potato and 4 wt% wheat starch suspensions, and 2 wt% potato starch and 4 wt% wheat starch in 0.1 M NaCl solutions were heated in a water bath to 80 °C, while being gently stirred. Immediately after heating, the suspensions were poured into graduated cylinders, and kept at room temperature. The height of the sediment was measured after 24 hours. The volume (ml) occupied by the swollen granules per gram dry starch was calculated.

2.2.5 *DSC measurements*

Differential scanning calorimetry was performed using a Mettler DSC-30. 15 and 30 wt% potato and wheat starch suspensions were weighted into aluminium pans. After one hour, the suspensions were heated from 5 °C to 125 °C at a scanning rate of 2 or 10 K·min^{-1}. Immediately after heating, the pans were cooled to 5 °C at a rate of about 100 K·min^{-1}.

An aluminium pan filled with small aluminium discs was used as a reference. For each endotherm, the melting enthalpy ΔH and the onset (T_o), peak (T_p) and melting (T_m) temperatures were measured according to a standard procedure described by e.g. Biliaderis *et al.*[28]

2.3 Results and Discussion

2.3.1 *Rheological behaviour of starch systems during heating and cooling*

Changes in the storage moduli G' of 15 and 30 wt% potato and wheat starch systems, determined during heating and cooling, are shown in Figure 2.2. The storage moduli start to increase strongly at temperatures of about 55 °C and 60 °C for the wheat and potato starch systems, respectively. The storage moduli of both 30% starch systems reach a maximum at a temperature of about 63-64 °C, whereas the maximum was observed at 69 °C for the 15 wt% potato starch system and at 82 °C for the 15 wt% wheat starch system. The higher the starch concentration, the lower the temperature at which a maximum in G' during heating of the starch system was reached in the concentration range of 10 to 30 wt% (Figure 2.3). For most systems the storage moduli decreased during prolonged heating to the maximum temperature. The decrease was stronger as the heating temperature was higher. Moreover, G' also decreased slightly when the starch systems were kept at the maximum temperatures. Exceptions
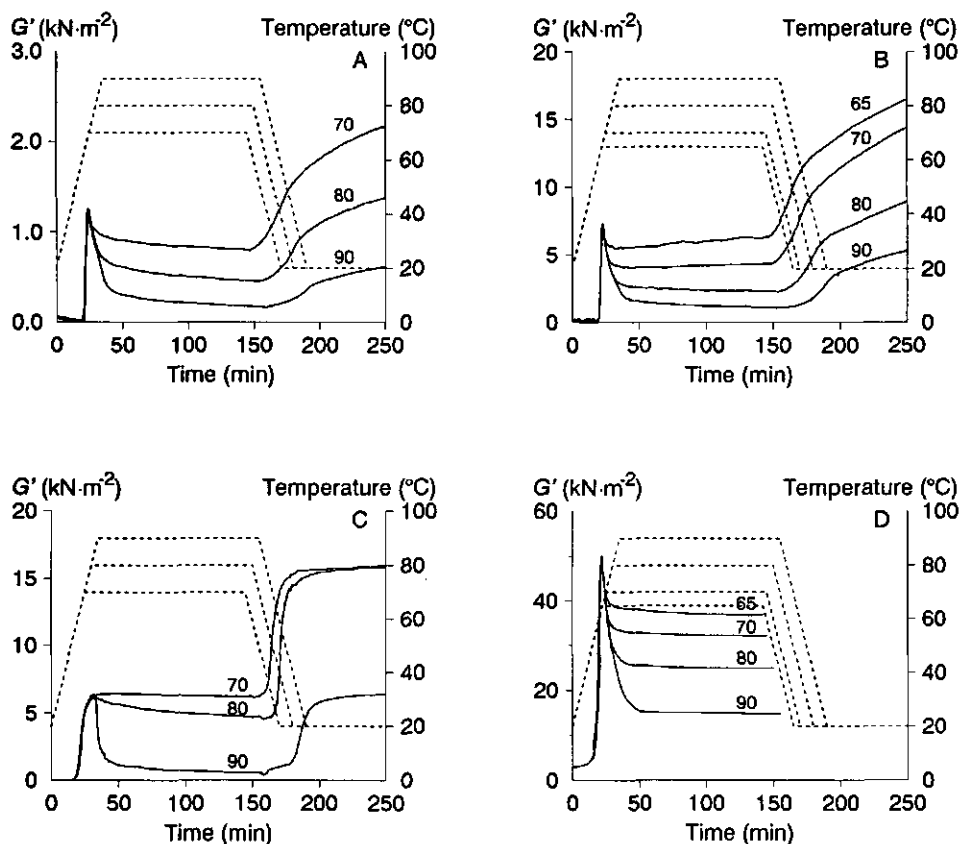
Figure 2.2 *The storage modulus of concentrated starch suspensions as a function of time during a heating and cooling cycle: (A) 15 wt% potato starch; (B) 30 wt% potato starch; (C) 15 wt% wheat starch; (D) 30 wt% wheat starch. The maximum heating temperature is indicated. Note the differences in scale. The dashed lines show temperature against time.*

are 30 wt% potato starch suspensions at temperatures of 65 °C and 70 °C, the storage moduli of which slowly increased with time, and 15 wt% wheat starch suspensions at 70 °C, the modulus of which was constant. Cooling to 20 °C caused the stiffness of the starch systems to increase. The storage moduli of the systems after a heating and cooling cycle were lower for higher heating temperatures. Exceptions are 15 wt% wheat starch suspensions after being heated at 70 or 80 °C; they reached the same modulus. The moduli of 30 wt% wheat starch suspensions during cooling are not shown, because they were not reliable as a result of slippage.
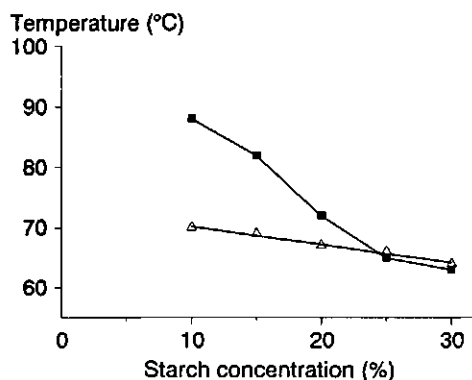
Figure 2.3 *Temperatures at which G' reaches its maximum during heating of potato* (▲) *and wheat* (■) *starch systems as a function of concentration.*

For either suspension tan δ ($= G''/G'$) sharply decreased in the region where G' increased strongly during the heating stage, as shown in Figure 2.4 for the 30% starch systems. The decrease of tan δ of the 30% wheat starch gel to 0.05 during heating implies that then an elastic gel is already formed. The somewhat larger tan δ of the potato starch system may be due to the granules exhibiting a more viscous behaviour. This more viscous behaviour may also be a result of a less developed amylose network between the granules. However, my working hypothesis is that for potato starch an
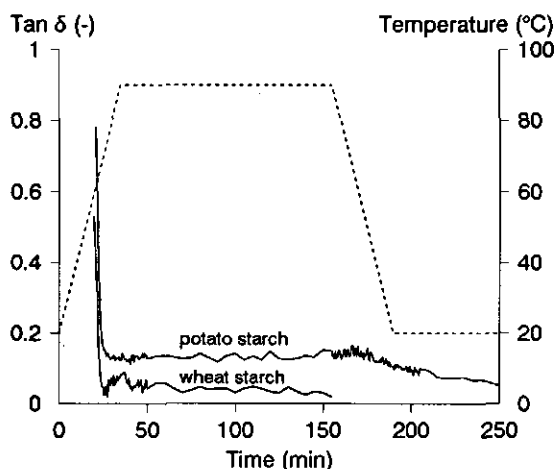
Figure 2.4 *The loss tangent of 30 wt% potato and wheat starch suspensions as a function of time during a heating and cooling cycle. The dashed line shows temperature against time.*

amylose network has already been formed (at least partly) at 90 °C. The decrease in tan δ continued during cooling and storage, probably as a result of rearrangements involving amylose and amylopectin (see further on).

DSC thermograms of 30 wt% potato and wheat starch suspensions at a heating rate of 10 K·min⁻¹ are given in Figure 2.5. In Table 2.2 T_o, T_p, T_m and ΔH are given for both starch suspensions. Measurements at a heating rate of 2 K·min⁻¹ were also performed (results not shown). ΔH was about the same as at the higher heating rate, whereas T_o, T_p, and T_m were about 2 to 4 °C lower. However, the noise level was high and therefore the data obtained were less precise. Thermograms of 15 wt% potato and wheat starch suspensions (results not shown) were virtually identical to those of 30 wt% potato and wheat starch suspensions, respectively.
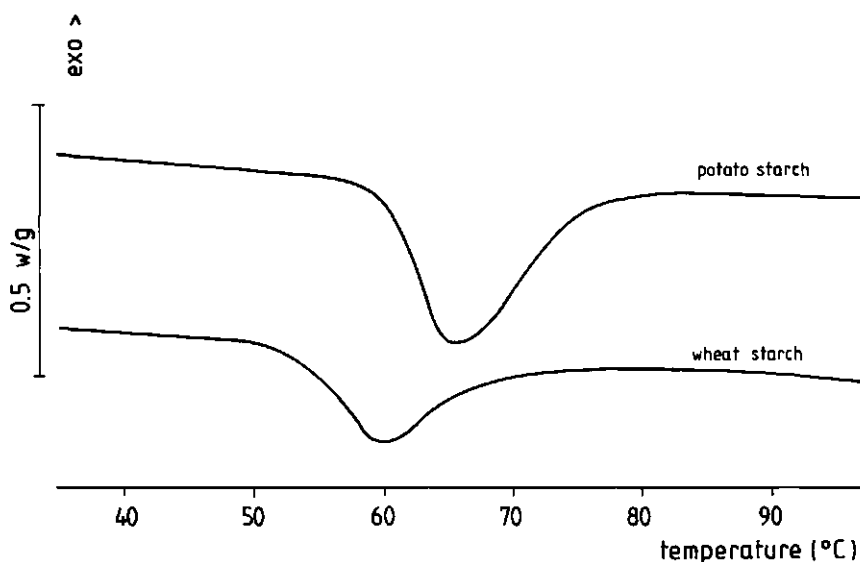
Figure 2.5 *DSC thermograms of 30 wt% potato and wheat starch suspensions at a heating rate of 10 K·min⁻¹.*

Table 2.2 *Differential scanning calorimetry data for potato and wheat starch; 30% suspensions. Heating rate 10 K·min⁻¹.*

starch	T_o (°C)	T_p (°C)	T_m (°C)	ΔH (J·g⁻¹)
potato	58	64	74	17.8
wheat	52	59	68	9.6

Several processes may occur in the starch granules during heating and so may cause a change in the rheological behaviour of the starch system; several of these changes are more or less strongly related. Schematically, it concerns:

1. swelling of granules (e.g., French[17]),
2. melting of crystallites in the granules (e.g., French[17]),
3. (partial) separation of amylose and amylopectin,[29]
4. changes in the extent of entanglement of starch molecules in the granules (see below),
5. (partial) disruption of the swollen starch granules.[22]

I will discuss these phenomena more comprehensively below.

The initial increase of the storage moduli, which was observed at temperatures of about 55 to 60 °C, can be ascribed to the fact that the starch granules swell progressively and start to fill the whole sample volume, *i.e.* a transition from a liquid system with dispersed particles to a system nearly packed with deformable particles. This initial increase in G' coincides with the first stages of crystallite melting, as observed with DSC (Figure 2.5). As the starch concentrations of the systems used are high, the swelling of the individual granules would be restricted by the space available. It is supposed that the granules would become tightly packed at temperatures close to that at which a maximum in the modulus plotted as a function of temperature was observed. The higher the starch concentration, the lower the temperature at which the system would be tightly packed.

The decrease in G' on a further increase in temperature coincides with the later part of the gelatinization endotherm. The melting of remaining crystallites as such would cause the swollen starch granules to become softer, so that the dynamic moduli would decrease. However, in Figure 2.5 it can be seen that the crystallites have melted completely at a temperature of about 75 to 85 °C for wheat starch and potato starch, respectively. This implies that the melting of remaining crystallites cannot be the only· cause of the decrease of the storage moduli during heating and during the time the gel is kept at a high temperature. Moreover, the higher the temperature at which the system is stored, the stronger the extent to which the moduli decrease during that time (Figure 2.2). Therefore, it is assumed that another phenomenon is involved in this decrease.

Another process occurring when heating a starch suspension is the separation of amylose and amylopectin. The separation of amylose and amylopectin on heating would be due to these large molecules being thermodynamically incompatible.[30,31] Inside a native starch granule separation of amylose and amylopectin can hardly occur, because the native granule contains many crystalline domains. Therefore it may be considered as a kind of frozen system, in which the molecules are hardly mobile. Motion of parts of the molecules would certainly be possible if the water content is high enough for the starch granules not being in the glassy state (roughly at > 20%

water at room temperature[32]). On heating a starch suspension, the crystallites melt and the granules take up water, which would result in an increased motion of the large molecules, and amylose and amylopectin would start to separate. Several factors may affect the separation of macromolecules, as described, e.g., by the model of de Gennes,[33] which is based on a snake-like motion of the molecule inside a kind of tube formed by the molecules surrounding it. Comparing this model with the structure of the starch granule,[34] the amylopectin framework may be considered as a matrix, with the amylose molecules in between. The most plausible mode of motion of the amylose molecules is lengthways, because rotational motion would be very unlikely. The amylose molecules would thus reptate out of the granules. The extent to which this process occurs would depend strongly on temperature: the higher the temperature, the faster and more extensive the motion. Furthermore, the molecular mass and the extent of branching of the amylose molecules and the structure of the amylopectin framework would affect the rate of separation. However, it is questionable whether there is a direct relation between the extent of demixing of the amylose and amylopectin molecules and the changes in the rheological properties of the starch granules. Therefore, the rheological properties of a 30 wt% waxy potato starch suspension during heating and cooling were studied. This starch suspension also showed an increase followed by a decrease in G' during heating, which continued after all crystallites have melted (Figure 2.6). Thus, a decrease in G' on prolonged heating is also found if no 'demixing' of amylose and amylopectin can occur.
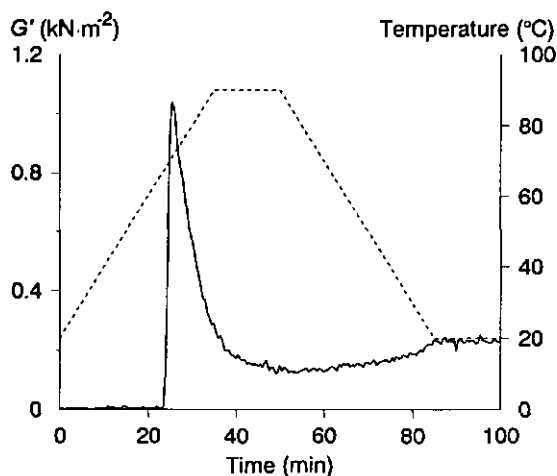
Figure 2.6 *The storage modulus of a 30 wt% waxy potato starch suspension during a heating and cooling cycle. The dashed line shows temperature against time.*

A fourth process that, in my opinion, may affect the rheology of the starch systems is a slow breakdown of the amylopectin gel matrix in the starch granules. A swollen starch granule may be considered as an amylopectin gel with a certain modulus, due to bonds between the different molecules. An alternative, but in my opinion very unlikely, possibility would be that the stiffness of a swollen starch granule is due to a skin, which is extended in equilibrium with an osmotic pressure inside the granule (similar to the primary cause of the stiffness of many animal and plant cells). In the native starch granules most bonds between the starch molecules are probably non-covalent. Because starch is very hydrophilic, the presence of hydrophobic bonds is unlikely. Moreover, at high temperatures these are very weak or absent.[35] It is also unlikely that hydrogen bonds are present between starch molecules at conditions where all ordered (semi-crystalline) regions have melted and quite some water is present. Therefore, I feel that entanglements are present between the amylopectin molecules in a swollen starch granule.

Most of the short branches of the amylopectin molecules in the native starch granule probably are present in double helices.[34] These may be considered entanglements that would thus already be formed during starch synthesis. Part of these double helices may contribute to the stiffness of the swollen granule. However, results from NMR-experiments have shown that all double helices are already disordered at temperatures below 80 °C.[36] It is therefore presumed that entanglements are present at a larger scale, e.g. between branches that themselves contain subbranches. It is also presumed that these entanglements are formed during starch synthesis. This is speculative, because until now no research has been done on the formation and the presence of such "bonds" in starch granules and on their disentanglement at temperatures around 90 °C. However, the formation of these entanglements would be compatible with the ideas about starch synthesis as ascribed by, e.g., Manners[37] and Ponstein.[38]

Various enzymes are involved in the synthesis of amylopectin, among which a soluble starch synthase and a branching enzyme.[37,38] Labelling techniques combined with electron microscopy have shown that these enzymes are located on the surface of the starch granules,[39] from which it may be concluded that the growth of the amylopectin molecules occurs at the surface of the granule. This process is highly ordered, because a certain periodicity in the distribution of the chain lengths of the amylopectin branches is observed.[40] Moreover, a highly ordered structure develops in the granule.[41,42] It is plausible that the new branches are formed in a disordered state and are only ordered upon incorporation into the ordered, crystalline structure. Entanglements between branches may be formed in the temporarily disordered state. However, it is unknown for how long the outer part of the granule is disordered. This may depend, among other things, on the botanical source of the starch.

Due to the presence of such entanglements, starch granules would maintain their integrity when heated to 90 °C at rest. However, further heating to high temperatures

may cause the amylopectin chains to (further) disentangle, due to the faster and more extensive motion of the starch molecules. It is supposed that such a breakdown of the amylopectin matrix causes a severe weakening of the remaining granule structure. Disentanglement of amylopectin chains would thus result in softer starch granules; this explains why the storage modulus of concentrated starch systems eventually decreases if their heating temperature and/or time is increased. Moreover, the separation of amylose and amylopectin would be enhanced by a disentanglement of chains. The puckering of wheat starch granules observed at temperatures above 80 °C was also supposed to be a result of disruption of non-covalent bonds.[43] Starch granules would also become softer due to breakage of covalent bonds, which would certainly occur at high temperatures (roughly above 120 °C) as well as under shear.

During cooling to 20 °C the rigidity of the gel increases (Figure 2.2). Changes in rheological properties during storage will be discussed in Chapter 3. It was suggested by Miles *et al.*[8] that the increase in modulus during cooling and storage consists of two separate reactions with different time scales. The short time change, which is heat irreversible (T < 100 °C), has been ascribed to gelation of solubilized amylose in the continuous phase, whereas the long-term increase was ascribed to a heat-reversible crystallization involving amylopectin within the swollen granules.[8] This description assumes an ideal situation in which amylose and amylopectin are fully separated during the heating process. It is known that the leaching out of amylose is due to the incompatibility of amylose and amylopectin and the higher mobility of amylose compared with amylopectin. It was reported that mixed aqueous systems of purified samples of amylose and amylopectin are separated into phases enriched to about 70-80% of each component at 70-90 °C.[30] This implies that during gelatinization always a certain amount of amylose would remain in the granules.

Gels in which amylose and amylopectin are almost fully separated exist, but it was observed in only a few cases. By using light microscopy in combination with iodine staining of the starch systems (results not shown), such systems were observed for wheat starch gels in the concentration range of 5% to approximately 15%. It was observed visually that wheat starch suspensions in this concentration range were liquid during heating at high temperatures, and that they rapidly formed a gel during cooling. This observation is reflected by the sharp increase of G' of a 15 wt% wheat starch suspension during cooling (Figure 2.2), which is primarily due to gelation of amylose between the swollen granules. Thus in a "diluted" gel (Figure 2.7A), the volume fraction of the granules is low enough for most of the amylose molecules to leach out of the granules and the amylose concentration is high enough to form what is essentially a matrix gel.

Potato starch systems at concentrations higher than 4 wt% and wheat starch systems at concentrations higher than 15 wt% were found to be tightly-packed systems of partly swollen granules (Figure 2.7B). As the concentrations are high, amylose and
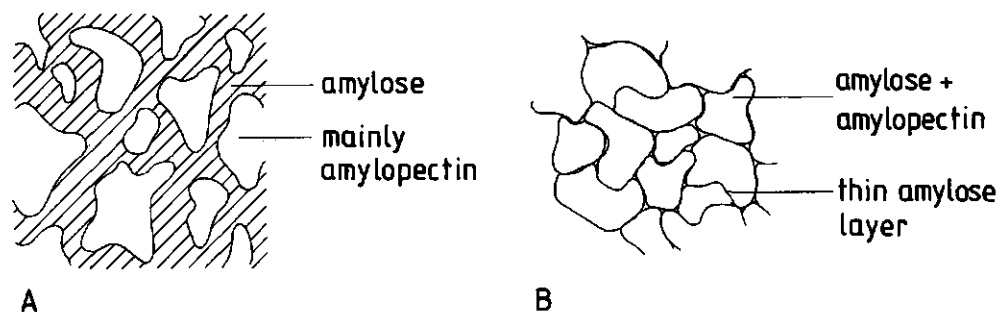
Figure 2.7 *Schematic presentation of starch gel structures: (A) Fully swollen granules acting as a filler in an amylose matrix gel; (B) Partly swollen granules that are tightly packed with a thin amylose gel layer in between.*

amylopectin become only slightly separated during heating. Consequently, the swollen granules consist of amylose and amylopectin. Moreover, there is only a thin layer of an amylose "gel" between the granules. Due to the high amylose concentration, some rearrangements between amylose molecules are enough to form an amylose gel between the swollen granules. It was observed visually that in such concentrated systems a gel had already been formed during heating at 90 °C.

Concentrated starch systems also show an increase in stiffness during cooling (Figure 2.2). The increase in modulus of the potato starch gels proceeded over the whole temperature range during cooling, and the increase continued for some time during storage. These results do not permit an unequivocal distinction between rearrangements involving amylose or amylopectin. To study the relative contributions of amylose and amylopectin, 15 and 30 wt% potato starch gels were reheated to 90 °C almost immediately after cooling to 20 °C. Figure 2.8 shows that G' decreased upon reheating a 15 wt% potato starch gel, but it did not completely return to its original value at 90 °C. If amylose gelation would be irreversible and the rearrangements of amylopectin reversible at 90 °C, it may be concluded that the increase in G' of a 15 wt% potato starch gel during cooling and short storage times is partly due to recrystallization of amylopectin. Consequently, amylose and amylopectin would both rearrange during heating and short storage times. Similar results were found for 30 wt% potato starch gels. When reheating a concentrated wheat starch gel, slippage occurred in the rheometer; consequently, the contribution of rearrangements of amylopectin to the increase in G' during cooling could not be established.

For concentrated wheat starch gels a sharper increase in gel modulus was observed during cooling. The temperature at which this sharp increase occurred depended on concentration, the increase starting at higher temperatures, *i.e.* earlier in time, for
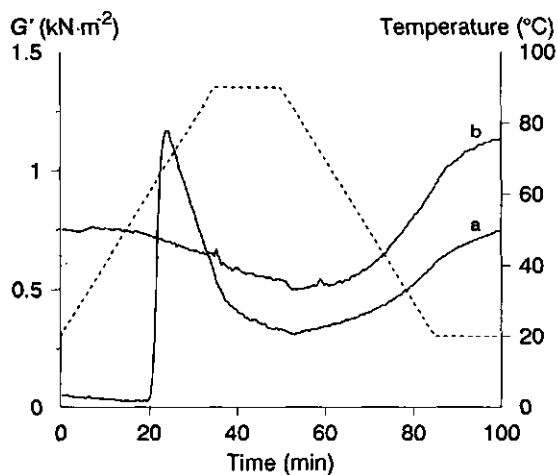
Figure 2.8 *The storage modulus of a 15 wt% potato starch suspension during a heating and cooling cycle (a). After 15 minutes at 20 °C the gel was heated and cooled again (b), according to the same temperature cycle. The dashed line shows temperature against time.*
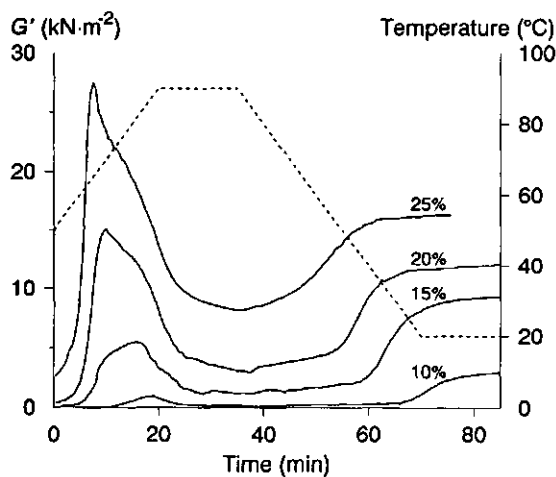
Figure 2.9 *The storage modulus of 10, 15, 20, and 25 wt% wheat starch suspensions during a heating and cooling cycle. The dashed line shows temperature against time.*

more concentrated wheat starch systems (Figure 2.9). Moreover, the increase was less sharp for more concentrated wheat starch systems. The effect of concentration on the temperature of the sharp increase in modulus can be explained by the higher amylose concentration between the granules in the more concentrated systems. It has been observed that amylose gels form and age faster at higher concentrations.[44] Mixed potato starch and amylose systems, containing 2 wt% amylose and 3 to 10 wt% starch, showed similar results as presented for the wheat starch systems in Figure 2.9,[45] *i.e.* the increase in gel modulus occurring at a higher temperature for more concentrated gels.

2.3.2 *Frequency dependence at higher temperature*

The storage modulus G' $(N \cdot m^{-2})$ and the loss tangent tan δ (-) as functions of frequency (Hz) for 15 and 30 wt% potato and wheat starch systems at 90 °C are given in Figure 2.10. For all systems G' increases with increasing frequency, while tan δ has a (shallow) minimum at intermediate frequencies studied. The lower the stiffness of the starch system, the stronger the dependence of G' on frequency. The starch concentration as well as the number and the relaxation time (lifetime) of the entanglements may affect this behaviour. Unfortunately, no definite conclusions on the structure of the system can be drawn from these results. For instance, without other information no difference can be made between the contributions due to the rheological properties of the amylose matrix and those of the swollen granules.

2.3.3 *Differences between potato and wheat starch*

From the results presented in the previous section it is clear that potato and wheat starch systems show a different rheological behaviour on heating and cooling. Figure 2.3 shows that, in the range of concentrations examined, the temperature at which the modulus has its maximum depends more strongly on concentration in wheat starch suspensions than in potato starch suspensions. This would be a result of their different swelling capacity. Potato and wheat starch are known to behave differently as to the rate and extent of swelling;[29,46] potato starch granules swell much more than do wheat starch granules. This is especially clear at low concentrations, because at a lower concentration a larger extent of swelling of the granules is required to attain a tightly-packed system. Up to a wheat starch concentration of approximately 15%, wheat starch systems are probably not completely tightly packed. For 11% wheat starch systems this was shown microscopically.[47]

The results of Figure 2.2 show that the 30% wheat starch systems were much stiffer than 30% potato starch systems. G' of these concentrated systems would be mainly determined by the stiffness of the swollen granules, because these systems are almost completely filled with swollen granules. Therefore, it is plausible that, at the
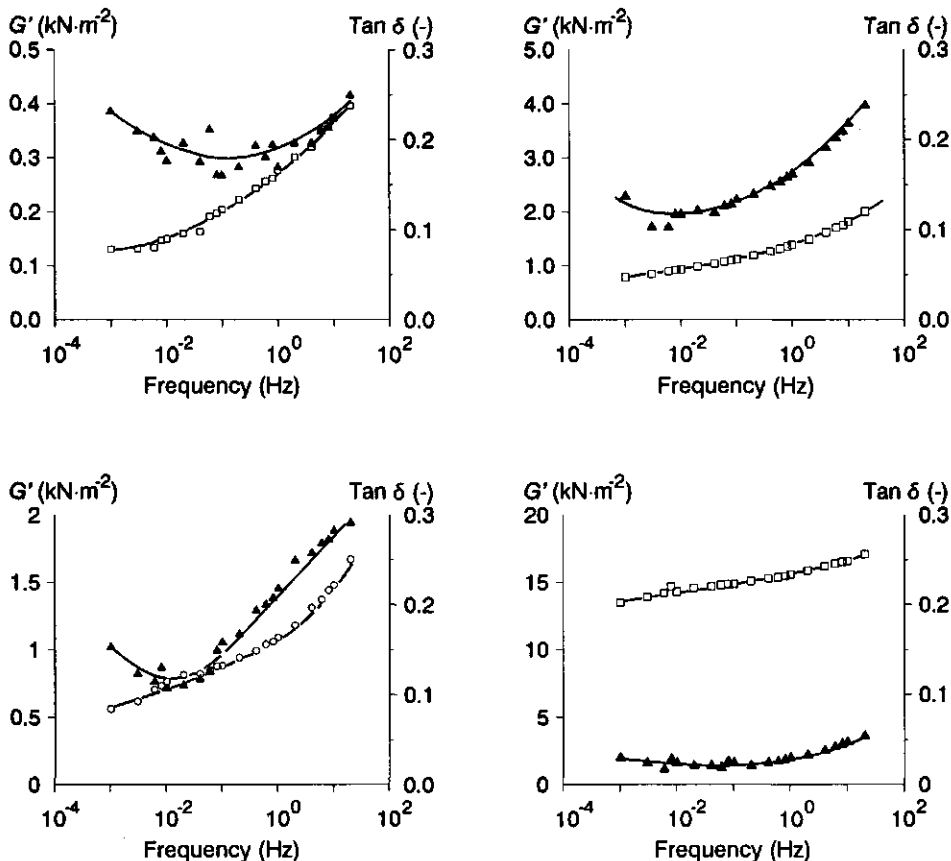
Figure 2.10 *The storage modulus G' (□) and tan δ (▲) as a function of frequency for concentrated starch gels at a temperature of 90 °C after about 1 hour: (A) 15 wt% potato starch; (B) 30 wt% potato starch; (C) 15 wt% wheat starch; (D) 30 wt% wheat starch.*

same starch concentration, swollen wheat starch granules are much stiffer than swollen potato starch granules. This points to a relation between the swelling capacity of starch granules and the stiffness of the swollen granules in tightly-packed systems, *i.e.* the lower the swelling capacity, the higher the stiffness of a tightly-packed system. Such a relation was also found by Steeneken.[46]

It is known that phosphate groups, which are esterified onto some of the glucose residues of amylose and especially amylopectin, greatly affect the swelling behaviour of potato starch.[48] Repulsion between these negatively charged groups would be largely responsible for the more rapid and the higher extent of swelling of potato starch granules, as compared with most other starches. To investigate whether the

presence of negatively charged phosphate groups completely explains this difference, diluted potato and wheat starch suspensions, either in demineralized water or in a 0.1 M NaCl solution, were heated to 80 °C, and poured into graduated cylinders. In the concentration of NaCl applied, the electrical double layer around charged groups is effectively compressed, the Debye length decreasing from, say, 30 nm to about 1 nm. After sedimentation for 24 hours it was clear that the potato starch granules in 0.1 M NaCl swelled less than those in the absence of salt (Table 2.3), whereas the added salt had no noticeable effect on the swelling of the wheat starch granules. This confirms that phosphate groups greatly affect the swelling capacity of the potato starch granules. However, in the presence of added salt the swelling capacity of potato starch granules was still substantially greater than that of wheat starch granules.

Table 2.3 *The volume (ml) occupied by sedimented swollen granules per gram starch. Measured after one day at room temperature.*

Botanical source of starch	Potato	Wheat
Solvent	Volume ml per g starch	
demineralized water	140	11.3
0.1 M NaCl	30.6	11.3

It has also been reported that starch lipids may have a reducing effect on the swelling of the individual granules.[48] Since wheat starch granules contain lipids, contrary to potato starch granules, this may possibly explain part of the difference in the swelling capacity of these starches.

In my opinion, a larger number of entanglements between starch molecules in wheat starch also contributes to the difference in swelling behaviour of potato and wheat starch. The possibility of the presence of such entanglements in starch granules has been discussed above. Such entanglements would restrict the granules to swell. Moreover, the presence of more entanglements in swollen wheat starch granules would explain their greater stiffness.

It is to be expected that the presence of entanglements also affects the sensitivity of swollen granules to shear, since entanglements would provide an increased resistance against their disruption. The sensitivity to shear was thoroughly studied by Svegmark and Hermansson by light microscopy[29] and rheology;[25] they found that swollen potato starch granules were much more sensitive to shear than swollen wheat starch granules. This result supports my hypothesis. Since it is supposed that more entanglements become disentangled during prolonged heating, the sensitivity to shear

would also depend on the time of heating at a high temperature. Until now, this latter factor has not been studied.

2.3.4 The effect of chemical cross-links on the rheological behaviour of starch systems

In the preceding section, the hypothesis was posed that a difference in the number of entanglements between amylopectin chains explains the difference in behaviour between potato and wheat starches. These entanglements act as non-covalent cross-links between chains. For a test of this hypothesis, it is interesting to study the effect of covalent cross-links in starch on its rheological behaviour. For practical applications the introduction of such cross-links, in particular in potato starch, is used to modify its properties. For my experiments, potato and slightly and highly cross-linked potato starches were used.

The effect of chemical cross-linking on the rheological properties of 30 wt% potato starch systems is presented in Figure 2.11. A large number of covalent bonds indeed has an enormous effect on the stiffness of the 30% starch system. Moreover, it is known that the swelling capacity of starch granules is strongly reduced by chemical cross-linking of starches,[46] although the swelling obviously is sufficient to result in a tightly-packed system in the present case (30% starch). These results are in accordance
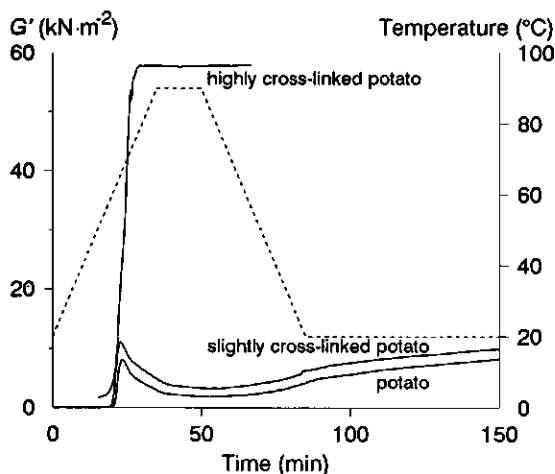
Figure 2.11 *Changes in the storage moduli of 30 wt% potato starch and slightly and highly cross-linked potato starch suspensions during a heating and cooling cycle. The dashed line shows temperature against time.*

with the idea that more entanglements are present in wheat than in potato starch granules. The fact that G' of the highly cross-linked potato starch systems did not decrease on prolonged heating, may be due to the covalent bonds in cross-linked potato starch being more stable than the chain entanglements in wheat starch. In addition, these covalent cross-links may prevent the disruption of naturally present chain entanglements.

2.4 Conclusions

Small deformation properties of concentrated starch systems heated at rest depend strongly on heating temperature; the higher the temperature, the lower the elastic shear modulus G'. Phenomena that may affect the decrease in G' on prolonged heating are:
- melting of remaining crystallites
- loosening of non-covalent bonds by disentanglement
- (partial) separation of amylose and amylopectin.
Generally, concentrated starch gels are tightly-packed systems, and the stiffness of the swollen granules (*i.e.* the rheological properties of the starch gel in the granules) primarily determines the stiffness of the gel as a whole. The properties of the matrix between the swollen granules are of lesser importance for the small deformation properties of concentrated starch gels.

Differences between the gelation properties of concentrated potato and wheat starch systems could be explained by the hypothesis that wheat starch granules contain more chain entanglements. Such bonds would reduce the swelling capacity of granules and increase the stiffness of swollen granules. Cross-links of a covalent nature affect gelation properties of concentrated starch systems in a similar way, as was shown by comparing the rheological properties of a highly cross-linked potato starch system with those of a potato starch system. Moreover, potato starch granules may swell to a greater extent due to their phosphate groups, provided that the ionic strength is very low.

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Chapter 3

Effect of Retrogradation on the Mechanical Properties of Concentrated Starch Gels

Summary

Concentrated starch gels were prepared by heating starch suspensions at rest. Large deformation properties of these gels were studied by compressing cylindrical samples between parallel plates. It was observed that the properties of concentrated starch gels at large deformations depend on the age of the gels, the type of starch, the heating temperature and the starch concentration. The mechanical properties of the gels are related to their structure, consisting of partly swollen, irregularly shaped granules with a thin amylose matrix gel in between. Observed changes in the Young modulus, and in the fracture stress and strain during storage are ascribed primarily to the increase in stiffness of the swollen granules. The rate at which the mechanical properties change during storage is at least partly affected by the number of entanglements between the starch molecules or the number of chemical cross-links present. Moreover, the extent to which amylose and amylopectin become separated during heating of the starch suspensions affect the mechanical properties of concentrated starch gels.

3.1 Introduction

In Chapter 2 the gelation of concentrated starch systems was described. Small deformation properties of concentrated potato and wheat starch systems during heating and cooling were presented. The changes occurring in the rheological properties of the starch systems were discussed and related to changes in the starch granules, such as the swelling of the granules, the melting of crystallites, the separation of amylose and amylopectin, and disentanglement of entanglements between starch molecules. Differences between the rheological properties of potato and wheat starch were discussed, and it was suggested that the number of entanglements in wheat starch is higher. A concentrated starch gel was considered an amylose matrix in which swollen

granules are dispersed. Because of the high starch concentrations, the granules occupy almost the whole volume and are only partly swollen. Consequently, the volume fraction of the amylose matrix is very small. Therefore, the mechanical properties at small deformations depend mainly on the mechanical properties of the swollen granules.

When eating or handling solid foods large deformations occur, which may result in fracture or yielding of the product. Thus to obtain a better understanding of starch retrogradation and its consequences for product properties, for instance the role of starch in the staling of bread, it is relevant to know more about the mechanical properties at large deformations. By studying these properties, a far better understanding of structure and interactions between structural elements can be obtained. However, despite its importance, only little is published about large deformation properties of starch gels.[1-3]

There are several methods to study large deformation properties of food materials, such as compression, tension and bending tests. A comparison of various methods to study large deformation properties of some food materials, among which 10% potato starch gels, has been made by Luyten *et al.*[1] In this study, compression tests were chosen, because of the relatively easy performance.

In this chapter large deformation properties of 30 wt% starch gels as a function of storage time will be presented. Since in bread the starch concentration is higher, measurements at starch concentrations above 30% might have yielded information more directly applicable to the staling of bread. However, at concentrations higher than 30%, and certainly higher than 40%, inhomogeneous gels are formed and the repeatability becomes poor. Results of the experiments performed on concentrated starch gels will be discussed in relation to their structure. First, some theoretical aspects of fracture of a material will be discussed.

3.2   Fracture theory

For the fracture and yielding behaviour of engineering materials, the theory has satisfactorily been developed.[4,5] The basic assumption in this theory is that the energy applied during deformation is stored in the material and can become available for fracture. In most food materials, however, this is not true; part of the energy applied is dissipated.[6,7] As a consequence, other theories must be used, taking the loss of energy into account. Extensive discussions have been published by van Vliet *et al.*[6] and Luyten *et al.*[7] Here, a brief summary of this theory will be given.

A material fractures when all bonds between structural elements in a certain macroscopic plane break within a certain time interval, resulting in breakdown of the structure of the material over length scales much larger than the structural elements; this finally results in the falling apart of the material into smaller pieces. Fracture of

a material starts if the local stress is higher than the adhesion or cohesion forces between structural elements, multiplied by the number of bonds between these elements per unit surface area. In the theory of fracture mechanics one usually assumes that all materials are inhomogeneous. The inhomogeneities or defects may be small cracks; weak spots also act as inhomogeneities. Near a defect, the local stress is higher than the overall stress in the material. Therefore, the maximum stress that a material can sustain is first reached near defects, and fracture will start there (fracture initiation). The extent to which defects affect the fracture properties depends on the size and the shape of the crack as well as on the notch sensitivity of the material. A material is notch sensitive if there are strong bonds between the stress bearing elements. If such a material contains defects, even if they are small, the overall stress at fracture is much smaller than that of the same material without defects.

A crack propagates spontaneously if the amount of energy released due to crack growth exceeds the energy required to create new surfaces. When a material fractures, the stress in the material at the sides of the newly formed crack will relax and the stored deformation energy is released. This energy can be used for further crack growth if it is transported to the tip of the crack. In order to deform a material, a certain amount of energy has to be supplied to it. This deformation energy can be stored elastically, dissipated, and used for fracture. An energy balance can be written:

$$W = W' + W_m'' + W_c'' + W_f \qquad (3.1)$$

where W is the amount of energy supplied to the material, W' is the part of the deformation energy that is elastically stored, W_m'' is the energy dissipated due to matrix flow, W_c'' is the energy dissipated by friction between structural elements of the material, and W_f is the fracture energy. W_f can only increase at the expense of W'. In purely elastic materials all deformation energy is stored ($W_m'' + W_c'' = 0$), until it is released when fracture occurs; then, W' decreases and W_f increases. The energy needed is then independent of the deformation (strain) rate.

In purely viscous materials (*i.e.* liquids) all deformation energy is dissipated ($W = W_m''$), and no fracture occurs. Most solid food materials are not purely elastic. It then depends on the properties of the material whether the rate dependence of W_m'' or W_c'' is overriding. In viscoelastic materials, part of the energy input is dissipated in flow of the material. Normally the viscous properties are relatively more important at a lower strain rate. Then, the energy dissipation is relatively higher, and thus W' is lower at a certain strain. The material must be deformed further before W' is high enough for fracture propagation to occur. This would cause the fracture strain to be higher for a lower strain rate.

Friction between elements due to inhomogeneous deformation when an inhomogeneous system is deformed would also result in energy dissipation. Normally,

these sliding processes dissipate more energy at higher speeds. This results in a larger W_c^m as the strain rate is higher. Consequently, W needed for fracture and the fracture stress are larger. Moreover, extensive local energy dissipation will slow the rate at which the crack propagates. This explains, why a larger fracture strain is observed at a higher strain rate.[6-8]

3.3   Materials and Methods

3.3.1 Materials

Potato, highly cross-linked potato and waxy potato starches were supplied by AVEBE (Foxhol, the Netherlands), and wheat starch was supplied by Latenstein BV (Nijmegen, the Netherlands). The chemical composition of the starches, and the size distributions of the potato and wheat starch are given in Chapter 2. Thiomersal (BDH Chemicals) was used as a preservative. Degassed, deionized water was used in the experiments.

3.3.2 Small deformation experiments

A comprehensive description of small deformation experiments has been given in Chapter 2.

3.3.3 Large deformation experiments

Large deformation properties of concentrated starch gels were measured in uniaxial compression, by using a Zwick material testing machine, fitted with a 50 or 2000 N load-cell. A cylindrical test piece was compressed between two parallel perspex plates by lowering the upper plate at a constant displacement speed v. The diameter of the plates was 75 mm. The plates were much larger than the diameter of the test piece, which was 15 mm. Conditions were chosen so that there was a negligible friction between the plates and the test piece. The force needed for compression was recorded as function of displacement. From this curve stress and strain can be calculated.

The relative deformation at a certain stage is expressed as a true or Hencky strain ϵ_h (-), which is defined as:

$$\epsilon_h = \ln \frac{h(t)}{h_o} \tag{3.2}$$

where h_o is the original height of the test piece, and $h(t)$ the height after a certain deformation at time t. For compression, the Hencky strain is negative, but it is mostly

expressed as a positive figure. As the height of the test piece decreases during compression at a constant displacement rate v, the strain rate $\dot{\epsilon}_h$ ($= \mathrm{d}\epsilon_h/\mathrm{d}t$) (s^{-1}) increases:

$$\dot{\epsilon}_h = \frac{\mathrm{d}h}{h(t)\,\mathrm{d}t} = \frac{v}{h(t)} \tag{3.3}$$

The average stress σ (N·m^{-2}) in the test piece at a certain deformation at time t is equal to the force $F(t)$ (N) per unit of area $A(t)$ (m^2):

$$\sigma(t) = \frac{F(t)}{A(t)} \tag{3.4}$$

Assuming that the volume of the cylindrical test piece does not change during compression, the area of the test piece at time t is:

$$A(t) = \frac{h_o}{h(t)} \cdot A_o \tag{3.5}$$

From the stress-strain curve the Young modulus E (N·m^{-2}) can be calculated:

$$E = \left[\frac{\mathrm{d}\sigma}{\mathrm{d}\epsilon}\right]_{\epsilon \to 0} \tag{3.6}$$

3.3.4 Sample preparation

30 wt% potato, wheat, waxy potato and highly cross-linked potato starch suspensions were made according to procedure A, heating part of the starch first, as was described in Chapter 2. Teflon cylindrical moulds with an inner length of 100 mm and an inner diameter of 15 mm were filled with these suspensions. The suspensions were heated in an oil bath at 95 °C for 90 minutes. The course of the temperature in the centre of the mould of a suspension heated at 95 °C is given in Figure 3.1. After cooling in a water bath to 7 or 20 °C the gels were stored at these temperatures in the teflon moulds, or were removed from the moulds and kept in paraffin oil to prevent them from drying out. No difference was observed between storage in teflon moulds or in paraffin oil. The cylindrical samples with a diameter of 15 mm were cut into test pieces of 20 mm height. Cutting of test pieces was performed with a wire of stainless steel, which had a diameter of 0.3 mm. Large deformation properties were determined after various storage times up to 19 weeks. The initial strain rate was 1.7×10^{-2} s^{-1}; the measurement temperature was 20 °C.
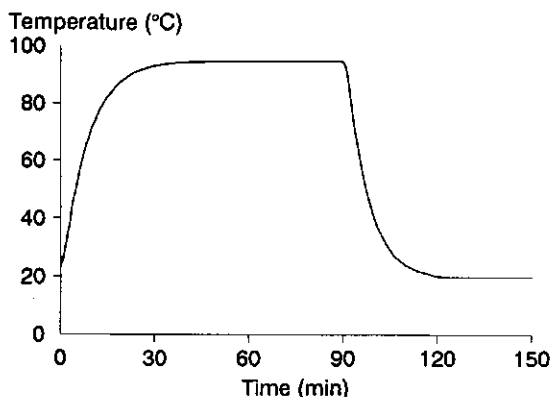
Temperature (°C)

Figure 3.1 *The course of the temperature of a starch suspension in the centre of a teflon cylindrical mould during heating in an oil bath of 95 °C, and cooling in a water bath of 20 °C.*

Effect of heating temperature

In order to study the effect of heating temperature, 30 wt% potato and wheat starch suspensions were heated in an oil bath at 70 or 90 °C for 90 minutes, or in a pressure cooker at 120 °C for 60 minutes. After cooling, the gels were stored in the teflon moulds at 20 °C.

Effect of starch concentration

In order to determine the concentration dependence of the moduli of starch systems at 90 °C, 10-40 wt% potato starch suspensions were made according to procedure A, whereas 10-40 wt% wheat starch suspensions were prepared according to procedure B (Chapter 2). The suspensions were transferred to the Bohlin VOR Rheometer. The suspensions were heated to 90 °C at a rate of 2 K·min^{-1}. The moduli were measured immediately after the suspensions had reached a temperature of 90 °C.

In order to study the effect of concentration on the mechanical properties of starch gels at large deformations, 10-40 wt% potato and wheat starch gels were prepared according to procedure A (Chapter 2). For preparation of the 10% gels, moulds with a diameter of 20 mm were used, whereas moulds with a diameter of 15 mm were used for preparation of the other gels. The suspensions were heated at 95 °C, and the gels were stored at 20 °C. All samples were cut into test pieces of 20 mm height.

3.4   Results

3.4.1 Experimental setup

When determining large deformation behaviour of food material, it is important to ensure that the results obtained reflect true material properties. Results of large deformation experiments may depend on the way the tests are performed. Several factors can affect the results obtained by a large deformation experiment. A comprehensive discussion of these factors has been given before.[1] Here I discuss only factors of importance when studying the change in large deformation properties of concentrated starch gels during storage.

a) *Shape of the test piece*. Samples of concentrated starch gels were made using teflon cylindrical moulds. Firstly, too large a diameter of the mould results in inhomogeneous heating of the suspension and, consequently, an inhomogeneous gel. Moulds with a diameter of 15 mm were used; with this diameter, the gels were homogeneous. Also the height-to-diameter ratio of the test pieces must be considered. If this ratio is too small, friction between the test piece and the compression plates is no longer negligible. Too large a height-to-diameter ratio may lead to buckling during compression. Test pieces with a height of 20 mm were used in most of the experiments. Virtually identical stress-strain curves for test pieces with a height of 10 mm were obtained (Figure 3.2). The effect of height-to-diameter ratio on the stress needed for fracture has also been studied for 10% potato starch gels by Luyten *et al.*;[1] they did not observe an effect of the height-to-diameter ratio in the range between 0.3 and 1.15.
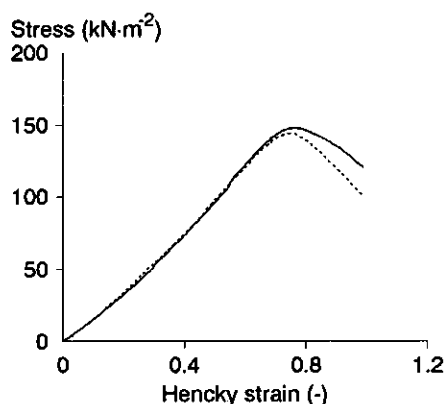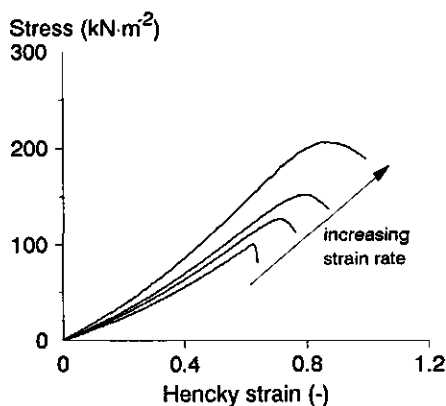

Figure 3.2 *Stress-strain curves of 30 wt% potato starch gels with a diameter of 15 mm and heights of 10 mm (---) and 20 mm (—).*

b) *Defects*. During deformation, local stresses and strains are highest near inhomogeneities; fracture starts near or at such places. Thus, both fracture initiation and fracture propagation are affected by inhomogeneities. Starch gels are found to be very sensitive to inhomogeneities.[1,2] Therefore, it is important that no macroscopic defects, like air bubbles, are present in the starch gel, because they would highly affect stress and strain at fracture. Starch gels prepared according to the procedure described above did not show any visible defects.

c) *Rate of deformation*. Concentrated starch gels are almost purely elastic at small deformations: the loss tangent is lower than 0.05 (Chapter 2) and the gels exhibit time independent behaviour (no effect of deformation speed). However, at large deformations time dependent behaviour was observed. At a higher rate of compression, fracture stress and strain were higher. An example is shown in Figure 3.3. This behaviour was observed for both 30 wt% potato and wheat starch gels, whether freshly made or aged. Time dependent behaviour of starch gels at large deformations was also observed by applying other deformation modes.[8] Therefore, it is important to keep the strain rate constant. However, the deformation at which fracture occurs varied slightly with the type of starch used and with ageing time; as a result the relative strain rate at the moment of fracture varies slightly. However, it was checked that differences in fracture parameters caused by this effect were small compared to those due to the variables tested.

Figure 3.3 *Stress-strain curves of 30 wt% potato starch gels at various initial relative strain rates: 1.0×10^{-4} s^{-1}, 1.7×10^{-3} s^{-1}, 1.7×10^{-2} s^{-1}, 1.7×10^{-1} s^{-1}. The gels had been stored at 20 °C for 24 hours.*

3.4.2 Large deformation properties of 30 wt% starch gels

Potato, wheat, highly cross-linked potato and waxy potato starch suspensions were heated in teflon cylindrical moulds at 95 °C. Directly after cooling, some gels were removed from the moulds. Potato and waxy potato starch gels were transparent, whereas wheat and highly cross-linked potato starch gels were opaque. After storage for some hours, the potato and waxy potato starch gels also had become opaque. Freshly made potato and waxy potato starch gels were highly deformable. They did not fracture at a Hencky strain up to 1.7 as measured in compression at the strain rate studied; I could even tie a knot in a long cylindrical test piece. This was not possible with freshly made wheat or highly cross-linked potato starch gels. After short storage times, the latter gels were more brittle than the potato and waxy potato starch gels.

If large deformations are imposed on starch gels, fracture occurs around the granules. This was observed by examination under a light microscope of thin slices of starch gel that had been extended beyond fracture. There were some exceptions, namely freshly made potato and waxy potato starch gels. Microscopic observation showed that the swollen granules of freshly made potato and waxy potato starch were damaged upon extension of these gels; the granules were also damaged upon making a smear of the gel on a glass plate. This did not occur with swollen wheat, highly cross-linked potato, retrograded potato and retrograded waxy potato starch granules.

In Figure 3.4 the stress-strain curves of 30 wt% potato, wheat, highly cross-linked potato and waxy potato starch gels are given for various ageing times. The curves represent the average of at least six replicates from each of at least two batches of gels. The curves of the waxy potato starch gel are an average of six replicates from one batch. The repeatability of the results was good, *i.e.* the variation was mostly less than 5% and never more than 10%. Wheat starch gels had a lower stress and strain at fracture than potato starch gels. Stress and strain at fracture were lowest for the highly cross-linked potato starch gels. As described earlier, waxy potato starch gels are, directly after cooling, very "long". A one day old waxy potato starch gel is smooth. Its fracture strain was slightly smaller than that of a one day old potato starch gel, but its fracture stress was much smaller. The fracture stress of waxy potato starch gels increased strongly during storage.

For all types of gels, fracture stress increased and fracture strain decreased during storage. The relative magnitude of the changes increased in the order wheat starch, highly cross-linked potato starch, potato starch, waxy potato starch. This is also shown by the increase of *E* as a function of ageing time (Figure 3.5).

In Figure 3.6 the fracture stress is given as a function of the Young modulus for 30% potato and wheat starch gels stored at 7 or 20 °C. The rate of recrystallization of amylopectin is more rapid at 7 °C than at 20 °C.[9] Thus highly retrograded starch gels can be studied within a relatively short period by storing them at 7 °C. The storage temperature did not affect the mechanical properties at such; at a certain
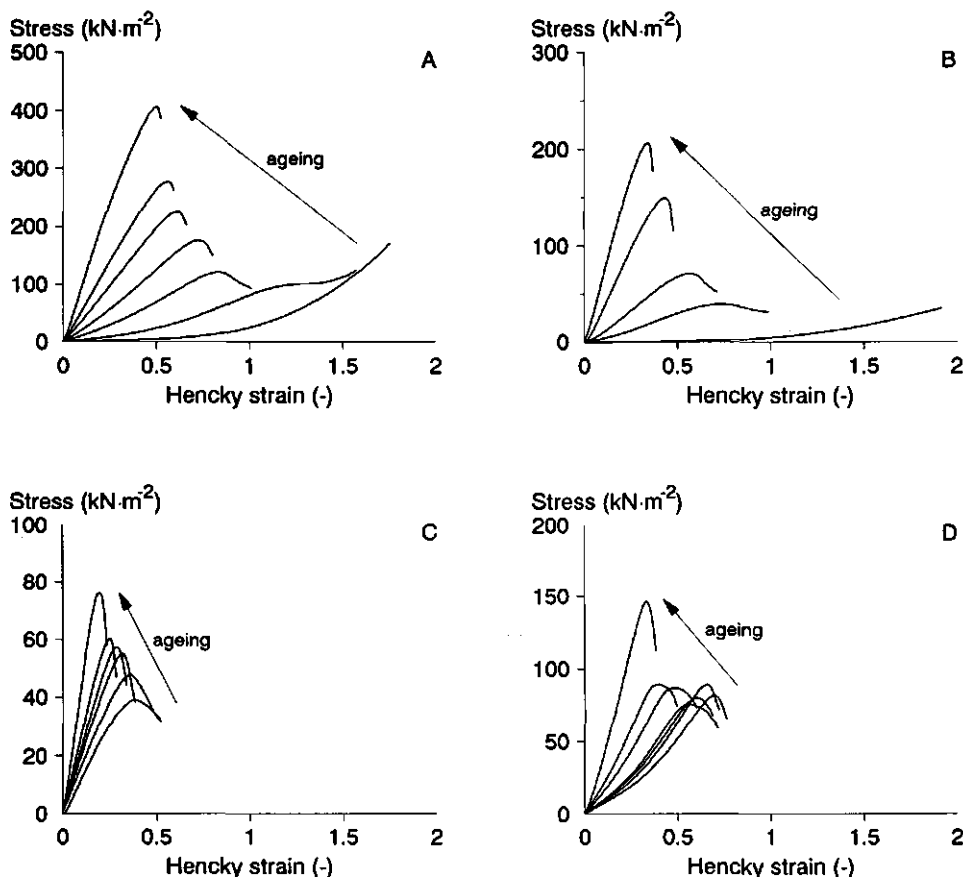
Figure 3.4 *Stress-strain curves of 30 wt% starch gels: (A) potato starch; (B) waxy potato starch; (C) highly cross-linked potato starch; (D) wheat starch. Measurements were made after storage times of 0 (only A, C and D) and 4 hours, and 1, 2, 6, 16 and 130 (only A and D) days. The gels had been stored at 20 °C. The initial strain rate was 1.7×10^2 s^{-1}. Fracture occurred at the maximum of the stress-strain curve. Note that the vertical axes differ.*

stiffness of the gel, the stress-strain curves were similar (Figure 3.7). Figure 3.6 shows that there is a high positive correlation between the fracture stress and the Young modulus. The increase in fracture stress as a function of the Young modulus is more pronounced for 30% potato starch gels than for 30% wheat starch gels; the slopes of the lines are 0.30 and 0.11, respectively.
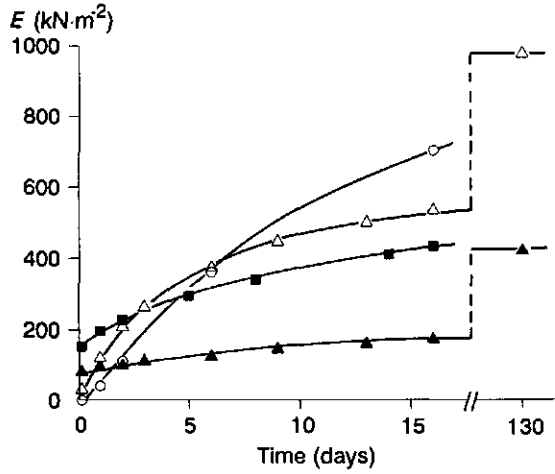
Figure 3.5 *The Young modulus of 30 wt% potato (▵), waxy potato (o), highly cross-linked potato (■) and wheat starch gels (▴) as a function of storage time at 20 °C.*
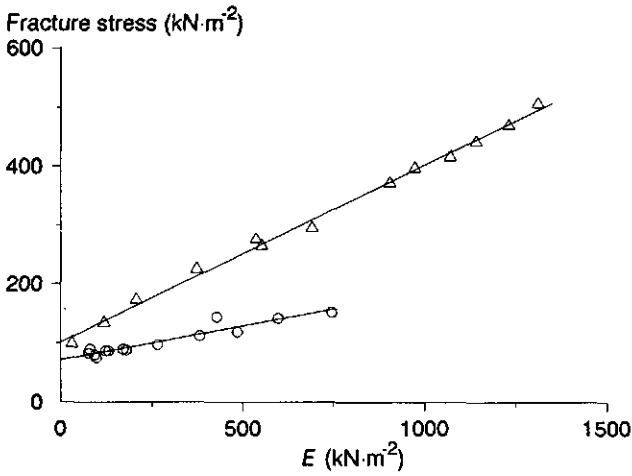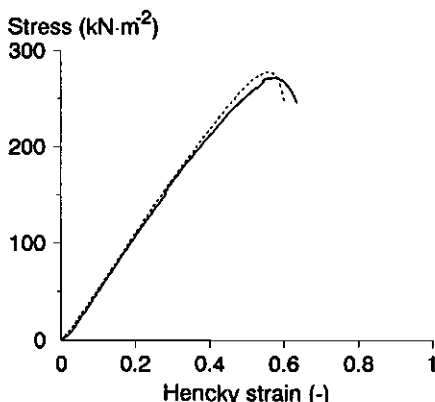

Figure 3.6 *The fracture stress as a function of the Young modulus for 30 wt% potato (▴) and wheat (o) starch gels stored at 7 and 20 °C.*

Figure 3.7 *Stress-strain curves for 30 wt% potato starch gels stored at 7 ˚C for 1 day (—) and at 20 ˚C for 16 days (---).*

3.4.3 Effect of heating temperature

Heating temperature affects the properties of both 30 wt% potato and wheat starch gels. Freshly made 30% potato starch gels that had been heated during gelatinization to 70, 90, or 120 °C, all were transparent and highly deformable. After 4 hours the gels heated to 120 °C were more opaque than the gels heated to 70 and 90 °C. This difference was still visible after 15 days of storage. The large deformation properties of these potato starch gels are shown in Figure 3.8. The differences between the large deformation properties of potato starch gels heated to 70 and 90 °C were rather small; gels heated to 70 °C had a slightly larger stress at fracture. Even in gels heated to 120 °C granules were still visible. These gels were very smooth during the first days of storage; they showed yielding rather than fracture. After some days these gels also fractured. The stress and strain at fracture of the gels heated to 120 °C were much smaller than of potato starch gels heated to 70 or 90 °C.

The effect of the heating temperature on the large deformation properties of the 30% wheat starch gels was less pronounced than for the 30% potato starch gels (compare Figure 3.8 with Figure 3.9). 30% wheat starch gels heated to 70 °C had a slightly smaller fracture stress than had the wheat starch gels heated to 90 °C. The gels heated to 120 °C had a much smaller fracture stress. They were more opaque than the other wheat starch gels.

The moduli of the potato and wheat starch gels heated to 70, 90 and 120 °C are shown in Figure 3.10. The relative increase in E with time was most pronounced with starch gels heated to 120 °C. This was more obvious for the potato starch gels than for the wheat starch gels.
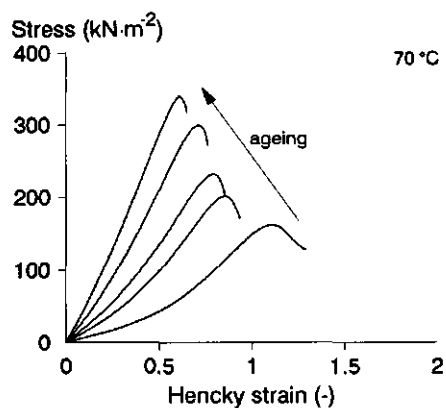
Figure 3.8

Stress-strain curves of 30 wt% potato starch gels heated to 70 °C, 90 °C and 120 °C after a storage time of 4 hours and 1, 2, 6 and 16 days. The initial strain rate was 1.7×10^2 s^{-1}. The gels had been stored at 20 °C.

Stress (kN·m^{-2})

Stress (kN·m^{-2})

Stress (kN·m^{-2})
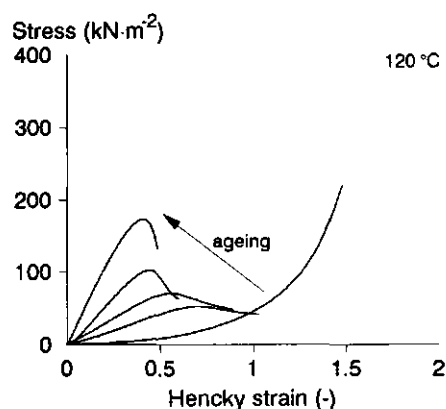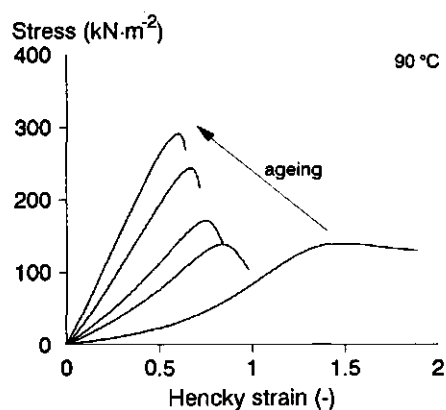
Figure 3.9
Stress-strain curves of 30 wt% wheat starch gels heated to 70 °C, 90 °C and 120 °C after a storage time of 4 hours and 1, 2, 6 and 16 days. The initial strain rate was 1.7×10^2 s^{-1}. The gels had been stored at 20 °C.
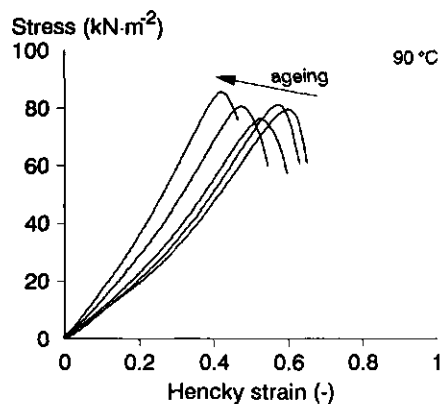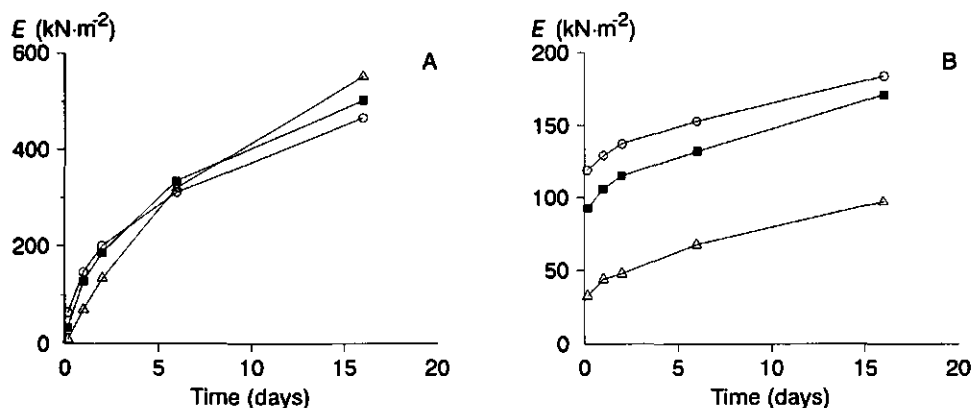
Figure 3.10   *The Young moduli of 30 wt% potato (A) and wheat (B) starch gels heated to 70 (o), 90 (■) and 120 °C (▲), as a function of storage time at 20 °C.*
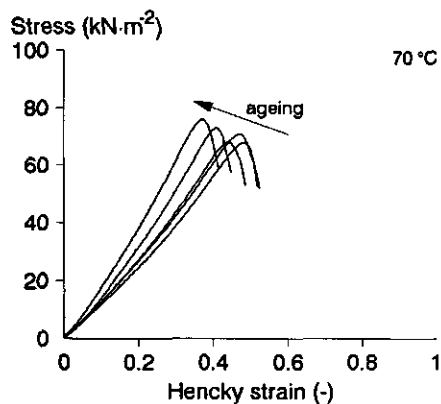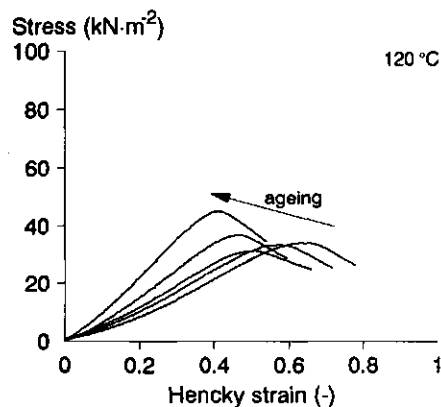
3.4.4 Effect of starch concentration

The effect of starch concentration (10-40%) on the moduli of potato and wheat starch gels is shown in Figure 3.11. The relative increase in modulus during the first days of storage was most pronounced for the 40% starch gels, and decreased with decreasing starch concentration. Among the potato starch gels, the relative increase in modulus after some days of storage was strongest for the 20% gels. 40% potato starch gels had almost reached their maximum modulus after about 10 days. For wheat starch gels the relative increase in the modulus was still most pronounced for the 40% starch gels.

The concentration dependence of the moduli of potato and wheat starch gels is shown in Figure 3.12. The moduli are given for gels at a temperature of 90 °C, and for gels stored at 20 °C for one and sixteen days. The moduli at 90 °C were determined with a Bohlin VOR Rheometer, whereas the other moduli were determined in uniaxial compression. To compare the moduli determined in shear and compression, the storage moduli were multiplied by three ($3 \times G' \approx E$; G'' was small compared to G'). For potato as well as wheat starch gels at a temperature of 90 °C the logarithm of the modulus as a function of the concentration can be fitted by a linear relation. The slope of the lines is approximately the same for potato starch gels and wheat starch gels at a concentration higher than 15%. For potato starch gels stored for one day and wheat starch gels stored for one and sixteen days at 20 °C the results can also be fitted by a linear relation. Such a relation is not valid for potato starch gels after sixteen days of storage.
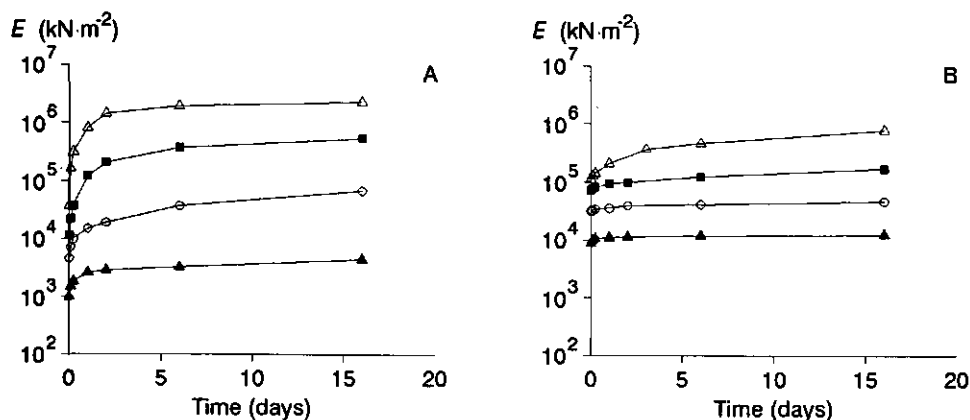
Figure 3.11   *The Young moduli as a function of storage time for 10% (▲), 20% (o), 30% (■) and 40% (△) potato (A) and wheat (B) starch gels stored at 20 °C.*
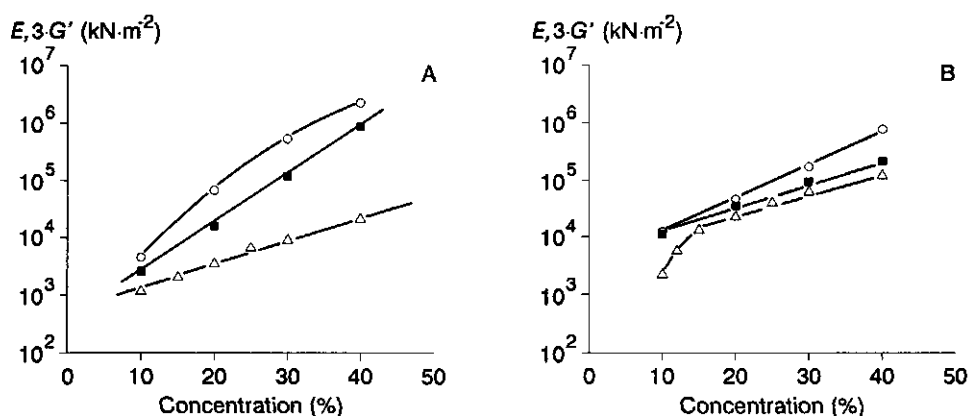
Figure 3.12   *The logarithm of the modulus (E or 3×G') as a function of starch concentration for potato (A) and wheat (B) starch gels. The moduli were determined during heating at 90 °C (△) (= 3×G') and after one (■) and sixteen (o) days (= E). The storage temperature was 20 °C.*

The large deformation properties of 10-40% potato starch gels for varying ageing times are shown in Figure 3.13. At all starch concentrations, fracture stress increased and fracture strain decreased during storage. The increase in fracture stress was most pronounced for the 30 and 40% starch gels. For fresh potato starch gels the strain at fracture was higher as the concentration was lower. For the 20, 30 and 40% gels, the strain at fracture approached a value of 0.5 to 0.6 after a storage time of 16 days. The
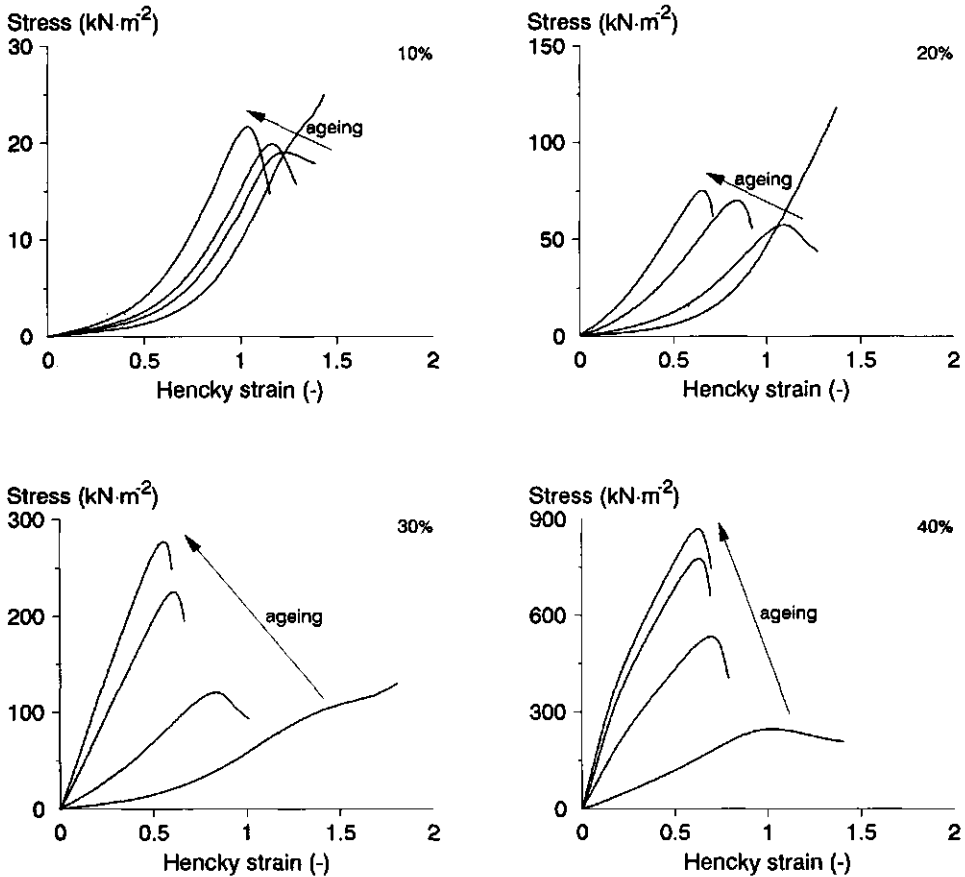
Figure 3.13   *Stress-strain curves of 10-40 wt% potato starch gels after a storage time of 4 hours and 1, 6 and 16 days. The initial strain rate was 1.7×10^2 s^{-1}. The gels had been stored at 20 °C.*

increase in fracture stress was much less for 10-40% wheat starch gels (Figure 3.14). The effect of starch concentration on the fracture strain of wheat starch gels was also different from that of potato starch gels. Fresh 20, 30 and 40% wheat starch gels had a fracture strain of about 0.7. During storage the strain at fracture decreased strongest for the 40% wheat starch gel. The 10% wheat starch gels showed a small increase in fracture stress as a function of ageing time, whereas the modulus (Figure 3.11) and the fracture strain of these gels hardly changed.
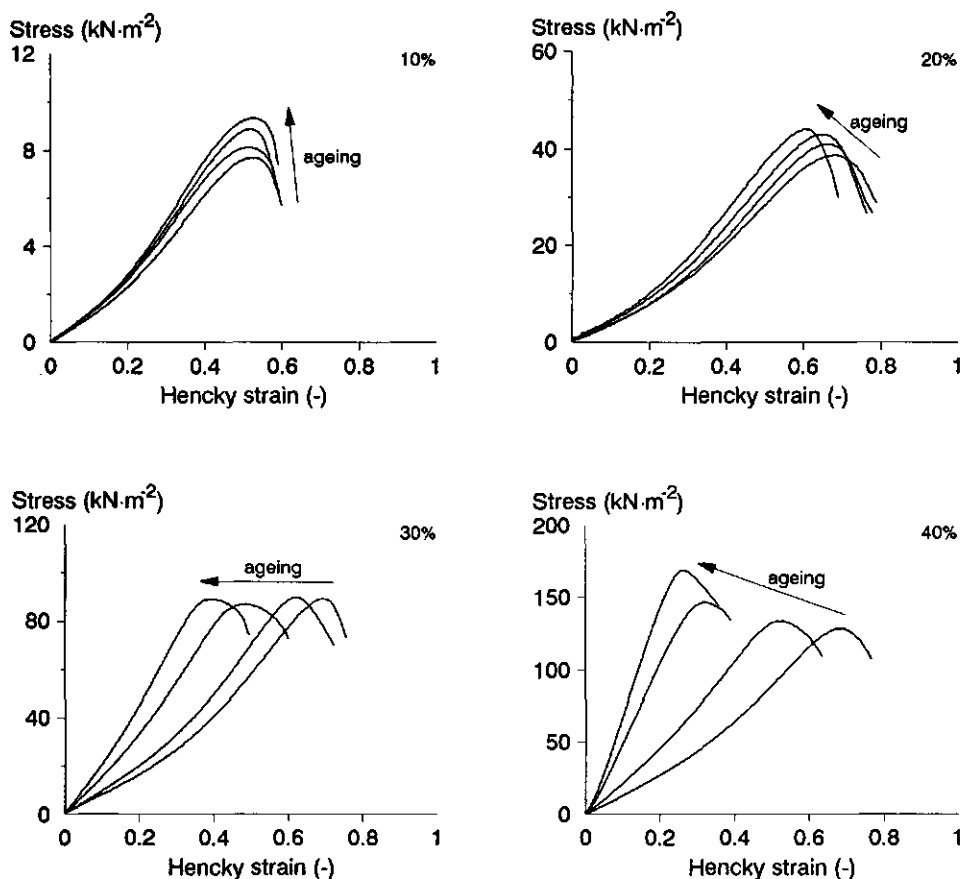
Figure 3.14   *Stress-strain curves of 10-40 wt% wheat starch gels after a storage time of 4 hours and 1, 6 and 16 days. The initial strain rate was 1.7×10^2 s^{-1}. The gels had been stored at 20 °C.*

3.5   Discussion

3.5.1   *Large deformation properties of concentrated starch gels in relation to their structure*

It was observed visually that concentrated starch gels consist of swollen granules that are presumably glued together. In these systems, the glue layer between the swollen granules is hardly visible with a light microscope; therefore, it must be thin. It was seen that fracture of the gels occurred around the starch granules, except in fresh
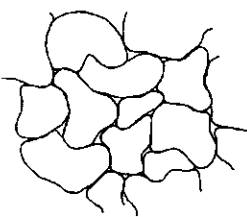
potato and fresh waxy potato starch gels. This implies that mainly the 'glue' or the matrix between the granules is ruptured, and that the granules stay intact. Therefore, the glue is weaker than the granules. As described before, fracture of a material starts from defects. For 30% potato starch gels it was found that the size of the inherent defects is about 0.13 mm;[2] this is roughly equal to the size of the largest swollen granules. Thus most probably the glue layer between two granules act as a defect in a starch gel.

Several parameters can be obtained from the stress-strain curves, such as the stress at fracture, the strain at fracture and the Young modulus. Below I will relate these parameters to the structure of a concentrated starch gel. First, I will consider a starch gel as a system of interacting cubes (Figure 3.15A). In this system, the cubes would occupy almost the whole volume, the glue layer being very thin. The Young modulus of such a system depends primarily on the Young modulus of the cubes. If in this system the glue is weaker than the cubes, the fracture stress of the system depends primarily on the fracture stress of the glue. The fracture stress of the system as a whole is thus larger if the fracture stress of the glue is larger. In this model, the strain at fracture is roughly the strain of the cubes if the stress in the glue equals its fracture stress. It will be larger for a smaller Young modulus of the cubes and for a larger fracture stress of the glue.

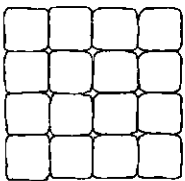Above, I represented the interaction between the cubes by a glue. What is the glue in a concentrated starch gel? In Chapter 2 it was described that, when a concentrated starch suspension is heated, the crystalline regions of the starch granules melt and the granules start to swell at a temperature of about 55 to 60 °C. At about 65 °C the starch system is tightly packed with swollen granules. At further heating, the remaining crystallites melt, the starch molecules disentangle, and amylose and amylopectin (partly) separate, because they are incompatible.[10,11] It was assumed that due to this separation, a small amount of amylose will be leached out of the swollen granules. However, most of the amylose will still be present in the swollen granules (Chapter 2). This assumption is in accordance with observations of Svegmark and Hermansson[12] on 10% potato starch systems. The amylose molecules between the starch granules can form cross-links with each other and probably also with amylose molecules still present (partly) in the granules. In addition, amylose molecules may possibly form cross-links with amylopectin molecules. The amylose network thus acts as a glue. It was observed that, directly after heating, a starch gel has already developed (Chapter 2). Therefore, the amylose molecules would start to cross-link and form a network already during heating.

In the model given above I represented swollen granules by cubes that are glued together. The swollen granules in concentrated starch systems consist of both amylose and amylopectin, because amylose and amylopectin are not fully separated. The swollen granules have maintained their integrity during heating, presumably due to the presence of entanglements between the starch molecules (Chapter 2). Swollen starch

model   structure   structure characteristics
that determine mechanical
properties

A.

E :stiffness of cubes

σ_f: fracture stress of glue

ε_f: deformability of cubes
in relation to fracture
stress of glue

B.

E: stiffness of granules

σ_f: fracture stress of thin
amylose layer and / or
stiffness of granules

ε_f: deformability of granules
in relation to fracture
stress of gel

Figure 3.15 *Highly schematic presentations of: (A) Cubes that are glued together; (B) Partly swollen granules that are tightly packed with a thin amylose gel layer in between them.*

granules can thus be considered a network of entangled amylopectin molecules interspersed with amylose.

The mechanical properties of concentrated starch gels are affected by the storage time. Figure 3.4 shows that, during storage, the stress at fracture increases and the strain at fracture decreases. The Young modulus also increases during storage (Figure 3.5). The increase in the Young modulus is mainly due to an increase in stiffness of the swollen granules. It was shown before that the increase in modulus is highly correlated with the reordering of amylopectin molecules as measured by DSC or wide-angle X-ray scattering.[13,14] Thus, the increase of the Young modulus is mainly due to a slow reordering of amylopectin molecules. It would be possible that some amylose molecules in the swollen granules rearrange during storage, and that the time scale of

this process is longer than that of the ordering of leached out amylose molecules, which occurs very fast (Chapter 2). Consequently, ordering of amylose molecules in the swollen granules may also contribute to the increase in stiffness of the swollen granules during storage. According to the model of glued cubes, the increase in fracture stress of the starch gels during storage would be due to an increase in the fracture stress of the thin amylose gel layer between the granules. It is well-known that amylose molecules form ordered regions during storage, but this happens fast, *i.e.* usually within one day.[15,16] Consequently, the increase in fracture stress of starch gels on longer time scales can hardly be due to reordering of amylose molecules. Therefore, a more complicated model has to be considered.

Light microscopy showed that the shape of the swollen granules is far from cubic; they are very irregularly shaped (Figure 3.15B). This was also shown earlier for 10% potato starch systems[12] and 11% wheat starch systems.[17] Swollen granules are hooked into each other, almost like pieces of a three-dimensional jigsaw puzzle. I assume that the stiffer the granules are, the more difficult it is to fracture the gels, because the hooks are more rigid. Hence, as the granules become stiffer during storage, the fracture stress increases. Consequently, the fracture stress of concentrated starch gels would not only depend on the fracture stress of the thin amylose layer between the granules, but also on the stiffness of the swollen granules; of these two factors, the stiffness of the granules is the more important one. This conclusion is supported by the observation that, during storage of 30% potato and wheat starch gels, there is a high positive correlation between the fracture stress and the modulus (Figure 3.6). That the increase in fracture stress is relatively less than the increase in stiffness of the granules, may be because the fracture stress of the amylose matrix also contributes to the fracture stress of the gels; this contribution would be constant after one day.

The decrease in the strain at fracture is explained by the granules becoming less deformable as a result of reordering of amylopectin.

The shape of the stress-strain curve will be affected by the stiffness of the granules as well as the interaction between the granules. Figure 3.4 shows that the shape of the stress-strain curves, especially those of 30% potato starch gels (A), changes during storage. Fresh starch gels show a stress-strain curve that is concave with respect to the stress axis, whereas the curves of aged gels are convex. In fresh starch gels, especially of potato starch, the interaction forces between the granules are relatively strong compared with the forces resisting deformation of the granules. When compression of these gels is started, the stress increases only gradually. As the deformation increases, the deformability of the starch granules decreases (non-linear behaviour), because the chain sections between entanglements become increasingly stretched (hence stiffer), but there is still no (visible) fracture because of the relatively strong interaction between the swollen granules. The non-linear properties of the granules result in a strong increase of the stress at large deformations. In more retrograded starch gels, the granules are much stiffer, implying that the interaction between the granules is

relatively weak. Consequently, fracture between the granules already starts at a small deformation of the granules, resulting in a lower strain at fracture and a different shape of the curves in more retrograded starch gels.

The large deformation properties of starch gels also depended on the type of starch. It was observed that the strain at fracture is much higher for fresh potato starch gels than for fresh wheat starch gels (Figure 3.4). This would be mainly caused by the higher deformability of freshly gelatinized potato starch granules. However, if the fracture stresses are extrapolated to zero modulus (Figure 3.6), the fracture stress of a potato starch gel is still higher than that of a wheat starch gel. This may indicate that the fracture stress of the thin amylose gel layer in potato starch gels is higher, which would then imply that the potato starch granules are more strongly glued to each other. In agreement with this is the observation that in very fresh 30% potato starch gels fracture occurred through the swollen granules instead of around the swollen granules, which means that the amylose matrix gel is even stronger than the swollen granules. It was found earlier that the rate of gelation and the gel properties at small deformations of monodisperse amylose depend on the chain length.[16] It is further known that the mean chain length of potato amylose molecules is much larger than for wheat amylose.[18] This may have an effect on the amylose matrix gel properties, and consequently on the large deformation properties of potato and wheat starch gels. Another factor that can affect the mechanical properties of starch gels is the presence of lipids. Lipids are known to form a complex with amylose.[19-21] Probably, this results in an incomplete network formation, and thus a weaker interaction between the granules. Wheat starch contains lipids contrary to potato starch. This may partly explain the differences in the mechanical properties between gels of these starches.

Figure 3.6 also shows that the increase in fracture stress as a function of modulus is more pronounced for 30% potato starch gels. There are some indications that swollen potato starch granules have a more irregular shape than swollen wheat starch granules.[12] Swollen potato starch granules are thus more hooked into each other than are swollen wheat starch granules; *i.e.* potato starch gels behave more strictly like model B (Figure 3.15). This would explain why the fracture stress of potato starch gels increases more rapidly with increasing modulus than that of wheat starch gels.

Highly cross-linked potato starch gels were found to be very brittle (Figure 3.4). In the cross-linking process, covalent bonds between starch molecules are formed. The presence of covalent bonds would result in a very incomplete separation of amylose and amylopectin during heating of an aqueous suspension of such a starch. The low fracture stress and strain may be due to the fact that there is hardly any amylose matrix ('glue') formed between the starch granules. Moreover, due to the presence of covalent bonds, the swollen granules would be less irregularly shaped. Consequently, they are less hooked into each other.

Waxy potato starch gels were highly deformable directly after heating. This can be explained by the high deformability of the swollen granules. Since waxy potato starch

hardly contains amylose, I expected only a weak interaction between the granules. However, waxy potato starch gels are not brittle. Therefore, I speculate that amylopectin molecules from adjacent starch granules interact one with another. Another possibility is that the shape of the swollen starch granules is very irregular, so that the friction between the swollen granules is relatively high.

Figure 3.4 shows that the changes in the stress-strain curves during storage are more pronounced for potato starch than for wheat starch. This can also be observed in Figure 3.5, which shows a cross-over of the Young moduli of these starches. The higher rate of retrogradation of potato starch has also been observed by other authors.[13,22,23] An explanation of this observation is far from simple. In the literature several causes have been proposed. First, it is reported that the rate of recrystallization of amylopectin is affected by the length of its short branches.[24] The reduced rate of ordering of wheat amylopectin is explained by the shorter average length of the short branches. It is also supposed that lipids present in wheat starch may retard the rate of recrystallization of the wheat amylopectin molecules.[22] In my opinion, the number of entanglements or chemical cross-links present in the starch granules may also affect the rate of retrogradation. In Chapter 2 the presence of entanglements in potato and wheat starch was extensively discussed, and it was suggested that wheat starch contains more entanglements than does potato starch. Figure 3.5 shows that the relative rates of increase of the Young moduli of gels from wheat starch and highly cross-linked potato starch gels are roughly the same. These results support my hypothesis that wheat starch granules contain more entanglements than potato starch granules, and that this is at least a partial explanation of the lower rate of retrogradation of wheat starch gels. Thus, the more entanglements or chemical cross-links are present, the lower the rate of retrogradation.

In waxy potato starch hardly any amylose is present. This may result in the starch molecules disentangling more readily during heating. With waxy potato starch a strong increase in the Young modulus is observed after some days of storage. Presumably, some time is needed for nuclei of ordered regions to form.

3.5.2 Effect of heating temperature

Apart from the type of starch, the heating temperature affected the mechanical properties of concentrated starch gels after cooling down to 20 °C. The mechanical properties were especially affected if the starch suspension was heated to 120 °C (Figures 3.8 and 3.9). At this high temperature, the amylopectin molecules in the swollen granules will disentangle to a greater extent than at 90 °C. This results in a breakdown of the granule matrix. Granules were, however, still visible in systems heated to 120 °C at rest. If more entanglements are lost, the deformability of the granules would increase. In addition to disentanglement of amylopectin chains, the separation of amylose and amylopectin proceeds during heating. It is supposed that

both disentanglement and separation are important for the smoothness of the starch gels heated to 120 °C.

The heating temperature also affected the rate of retrogradation (Figure 3.10). The relative rate of increase of the modulus was highest for starch gels heated to 120 °C, and lowest for those heated to 70 °C. I suppose that the higher extent of disentanglement of amylopectin molecules and separation of amylose and amylopectin during heating allows a faster rate of rearrangements of amylopectin molecules after cooling, which will cause the swollen granules or amylopectin-rich regions to become relatively more firm after longer ageing times. This explanation agrees with the suggestion that the relatively stronger increase of the modulus of potato starch gels compared with wheat starch gels is partly due to the swollen potato starch granules containing less entanglements (see above). The assumption of the higher rearrangement rates would have to be checked by performing DSC experiments on starch gels as a function of storage time after heating to various temperatures.

3.5.3 Effect of starch concentration

A linear relation between the logarithm of the moduli and the starch concentration at a temperature of 90 °C was shown for potato starch gels in the concentration range of 10 to 40 wt% and wheat starch gels in the range of 15 to 40 wt% (Figure 3.12). Under these circumstances the concentration dependence was the same for the potato and wheat starch gels. All these gels consist of partly swollen granules that are tightly packed (Chapter 2). The swollen granules contain entangled amylopectin molecules with amylose molecules in between. In these swollen granules no crystalline regions are present, because all crystallites have melted completely during heating. These results thus show that the concentration dependence is the same for tightly-packed potato and wheat starch systems in which the granules consist of an entangled network of starch molecules. The absolute values differ by an order of magnitude at the same concentration. The difference in moduli of potato and wheat starch gels at 90 °C is a result of the different number of entanglements present in the swollen granules (Chapter 2). The concentration dependence of the modulus of wheat starch systems drastically changes at concentrations below 15 wt%; then the concentration dependence is higher. At these concentrations, the wheat starch systems consist of swollen granules in an amylose solution (Chapter 2).

During storage of concentrated starch gels amylopectin recrystallizes. In starch gels containing crystalline regions there is also a linear relation between the logarithm of the modulus and the concentration. The slope of the line increases during storage; this increase is more pronounced for potato than for wheat starch gels. The steeper slope after a certain storage time is due to the more rapid retrogradation at higher starch concentrations. For wheat starch systems it is known that the rate of retrogradation shows a maximum at a starch content of approximately 50%.[25,26] The decrease in

crystallization rate at lower starch contents is due to a dilution effect.[27] The stronger increase in the slope on storage for potato starch gels is a result of their higher retrogradation rate, which has been discussed before. After storage for 16 days, the logarithm of the moduli versus concentration of potato starch gels could not be fitted by a linear relation. Then, the relative increase in E is most pronounced for the 20% potato starch gels; probably the 40% potato starch gel has almost reached its maximum modulus (Figure 3.11).

An exponential dependence of the modulus on concentration for starch gels has, as far as I know, never been published before. A linear or almost linear concentration dependence was observed for potato, wheat and maize starch gels at a concentration up to 10%,[28] for pea, maize and potato starch gels in the concentration range from 6 to 30%[29] and for waxy-maize amylopectin gels in the concentration range 10-25%.[30] The moduli of amylose gels were reported to show a strong power law dependence of the modulus on concentration,[15,16,31] with the exponent ranging from 4.4 to 7, depending on the molecular mass of the amylose molecules (see also Chapter 1).

It was discussed before that the stress at fracture of concentrated starch gels would depend on two factors. First, it is affected by the rigidity of the hooks between the swollen granules, which would depend on the irregularity of the shape of the granules and the stiffness of the granules. Moreover, the properties of the amylose matrix gel in between the swollen granules would play a role. By changing the starch concentration, all these factors change, which makes a discussion of the results presented in Figures 3.13 and 3.14 complicated. The stiffness of the granules is of course higher for more concentrated starch gels. It would be expected that the shape of the granules is more irregular at lower starch concentrations, due to the fact that in this case the granules swell more. This is accompanied by a more extensive separation of amylose and amylopectin and a more pronounced disentanglement of starch molecules during heating of less concentrated starch systems. Since the fracture stress of all concentrated starch gels increased during storage and because this increase would be due to an increase in the rigidity of the hooks between swollen granules, it was concluded that even at high concentrations the swollen granules have an irregular shape. The importance of the mechanical properties of the amylose matrix gel for the value of the fracture stress of concentrated starch gels then depends on the rigidity of the hooks between the swollen granules. For instance, in 10% potato starch gels, the granules would be rather irregularly shaped, but they are not very stiff, especially after short storage times. Consequently, the rigidity of the hooks in this gel is supposed to be so low that the properties of the amylose matrix gel may be of importance. Assuming hooks between adjacent starch granules also to be present in rather concentrated, for instance 40%, starch gels, the fracture stress would mainly depend on the stiffness of the granules, the properties of the amylose matrix gel being not very important. The increase in fracture stress with increasing starch concentration would thus mainly be due to an increase in stiffness of the swollen granules.

The fracture strain of concentrated starch gels depends on the deformability of the swollen granules in relation to the fracture stress of the gels (see above). As it is not possible to make an unequivocal distinction between the factors affecting the large deformation properties of concentrated starch gels, it is hardly possible to explain the effect of starch concentration on the absolute values of the fracture strain.

3.6   Conclusions

It was shown that large deformation properties of concentrated starch gels change during storage: the stress at fracture and the Young modulus increase, and the strain at fracture decreases. The increase in the Young modulus is ascribed to an increase in stiffness of the swollen granules, which is a result of reordering of amylopectin. The increase in fracture stress is a result of two processes. First, the fracture stress of the starch gel is affected by the fracture stress of the amylose matrix gel between the swollen granules and the latter increases due to rearrangement of the amylose molecules. Probably, the rearrangement is finished within the first day of storage, implying that the mechanical properties of the amylose matrix gel do not change after longer storage times. Another process contributing to the increase in fracture stress is an increase in the rigidity of the hooks between adjacent, irregularly shaped swollen granules during deformation of the gel. This is due to an increase in stiffness of the swollen granules. On ageing, the interaction between the granules becomes relatively weak compared to the stiffness of the granules, resulting in a decrease in the strain at fracture.

The large deformation properties of starch gels were found to depend on the type of starch. This is due to differences in the deformability of the granules and in the interaction between the granules. Potato starch granules were more irregular in shape than wheat starch granules and therefore more hooked into each other. The rate of retrogradation, too, depends on the type of starch. From the results presented it is concluded that the number of entanglements or chemical cross-links present in starch granules not only affects the gelation properties, as shown before (Chapter 2), but also the rate of retrogradation.

The heating temperature of a starch suspension clearly affects the mechanical properties of the resulting gel and the rate of retrogradation. This is mainly caused by further disentanglement of the starch molecules and a more pronounced separation of amylose and amylopectin at a higher temperature.

Finally, the contributions of the strength of the amylose matrix gel and the rigidity of the hooks between the swollen granules to the large deformation properties of concentrated starch gels are supposed to be affected by the starch concentration.

Summarizing, retrogradation affects small and large deformation properties of starch in a partly different way.

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Chapter 4

Recrystallization of Amylopectin in Concentrated Starch Gels

Summary

The relation between the recrystallization of amylopectin and the increase in stiffness of starch gels during storage was studied by various techniques. The crystalline structure present in retrograded 30 wt% potato starch gels was investigated by transmission electron microscopy. The size of the observed crystalline domains was about 5 nm, much smaller than those present in native starch. The super-helical structure formed by the crystalline domains in native starch granules was not seen in retrograded starch. It may thus be concluded that in retrograded starch gels the long range ordering is not regained during retrogradation. The relation between the degree of recrystallization, as determined with differential scanning calorimetry (DSC), and . the increase in stiffness, as measured in compression, was found to be closely related. Two mechanisms will be discussed which may explain these observations.

4.1 Introduction

Nearly all green plants produce starch to store energy. The starch is present in the form of granules that are insoluble in water at room temperature. Most starches consist of a mixture of two large polysaccharide molecules, amylose and amylopectin. Amylose is an essentially linear molecule consisting of α-D-glucopyranose residues linked together by (1→4) bonds. Amylopectin is the highly branched component of starch. On average, it contains about 4-5% (1→6)-α-D linked branch points. It is generally agreed that amylopectin has a cluster type structure,[1,2] in which short chains are arranged in clusters on longer chains. About 80-90% of the chains is present in only one single cluster.[3] The remaining 10-20% form inter-cluster connections, most of which connect two successive clusters.

Starch granules contain ordered regions, which are semi-crystalline and show birefringence. The overall crystallinity is about 20-45%. Amylose and the residues

around the branch points of amylopectin form the amorphous regions in the starch granule. The crystallinity arises mainly from ordered linear segments of amylopectin. These are present in the form of double helices with a length of approximately 5 nm. These double helices are crystallized into thin ($\sim$ 5 nm) lamellar domains (Figure 4.1), which are visible in transmission electron micrographs (TEM).[4-7] Wide-angle X-ray scattering (WAXS) has revealed the presence of three different forms of packing arrangements of the amylopectin double helices,[8,9] the so-called A, B and C forms. Results of TEM, optical analysis of electron micrographs and small-angle X-ray scattering (SAXS) suggested that the crystalline lamellae are helically arranged[6,10,11] (Figure 1.3). In this model the amylopectin segments in the crystalline regions are all parallel to the axis of the large helix. The diameter of the helix is $\sim$ 18 nm, and the spacing between successive turns of the helix is approximately 10 nm, which corresponds with the 10 nm periodicity observed with SAXS, TEM and optical diffraction. The large helices form a more or less continuous super-helical structure, in which left-handed helices are packed in a tetragonal array.[11] For a more comprehensive description see Chapter 1.
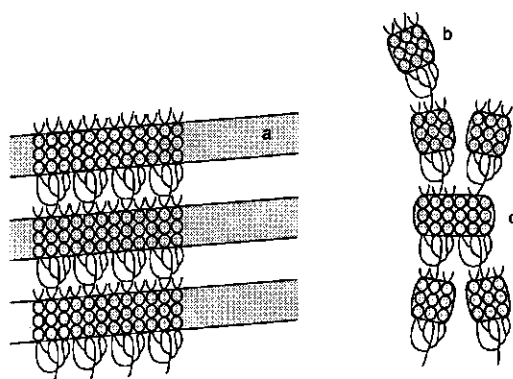
Figure 4.1   *Highly schematic model for the arrangement of amylopectin in native and retrograded starch. In native starch, the double helices are arranged into thin lamellar domains (a). In retrograded starch, the double helices form small ordered regions being the clusters of linear α-(1→4) glucan chains (b). Probably, adjacent clusters of ordered chains form physical cross-links between amylopectin molecules (c).*

When heat is applied to a starch suspension, starch undergoes a process known as gelatinization. The starch granules swell to many times their original size, and amylose and amylopectin separate. In the course of this process at a fairly well defined

temperature, the starch granules lose their birefringence and X-ray pattern, which indicates that the ordered regions are disrupted. It has been observed that decreases in crystalline order (as monitored by WAXS) and molecular (double helical) order (as followed by ^{13}C-NMR spectroscopy) followed the same relative quantitative pattern.[12] Moreover, after complete gelatinization, the periodicity observed by small-angle X-ray scattering is no longer present,[13] which indicates that also the large helical structures have been disrupted. Thus during gelatinization, the small and long range orders both disappear.

During storage, starch gels regain some of their structural order (retrogradation). The short amylopectin side chains undergo a rapid coil to helix transition.[14-16] WAXS has shown a slow development of crystallinity of the B-form in time, which is closely related to the development of an endothermic transition observed by DSC.[17-19] These changes are due to a slow association of the double helices. Recrystallization of amylopectin in starch gels results in an increase in stiffness of the gels. Until now there is no agreement about the relation between the recrystallization of amylopectin and the stiffness of the starch gels. According to Miles *et al.*[17] and Orford *et al.*,[18] the increase in crystallinity of starch gels parallels the increase in stiffness, whereas Roulet *et al.*[19] propose that the recrystallization of amylopectin proceeds faster than the stiffening of the starch gel.

The aim of this chapter is to discuss the relation between the increase in stiffness, as measured in uniaxial compression, and the recrystallization of amylopectin, as measured with DSC, of 30% potato and wheat starch gels during storage. To make an unequivocal comparison, both compression and calorimetry experiments were performed after approximately the same storage time on the same batches of gels. To gain a better understanding of reordering of amylopectin and its consequences for the increase in stiffness of starch gels, transmission electron microscopy was applied to 30% retrograded potato starch gels. A comparison will be made between the structure of amylopectin in native and retrograded starch granules.

4.2   Materials and Methods

4.2.1 *Preparation of concentrated starch gels*

In order to make homogeneous starch gels, dispersions of 3 wt% potato starch (AVEBE, the Netherlands) or 8 wt% wheat starch (Latenstein BV, the Netherlands) in demineralized water were heated to 65 °C, while being gently stirred. After cooling to room temperature, sufficient starch was added to obtain suspensions with 30 wt% dry matter. Teflon cylindrical moulds were filled with these suspensions and then heated in an oil bath at 95 °C for 90 minutes. After cooling the gels formed were stored at 4, 7 or 20 °C.

4.2.2 *Large deformation experiments*

Uniaxial compression tests were performed with a Zwick material testing machine equipped with a 2000 N load-cell. Test pieces with a height of 20 mm and a diameter of 15 mm were compressed between parallel perspex plates. The initial strain rate was 1.7×10^{-2} s^{-1}, and the measurement temperature was 20 °C. From the force-displacement curve, the compressive stress σ and the Hencky strain ϵ_h were determined (Chapter 3). The Young modulus E [$= (d\sigma/d\epsilon)_{\epsilon \to 0}$] was calculated from the stress-strain curve. Large deformation measurements were performed on 30% potato and wheat starch gels stored at 7 °C for 1, 2, 4, 8, 16, 32 and 65 days.

4.2.3 *DSC*

DSC was performed in a TA Instrument DSC-2910. Just before measurement, approximately 20 mg starch gel was weighed in an aluminium-coated low pressure cup (25 μl). The gels were heated from 20 to 120 °C at a scanning rate of 5 K·min^{-1}. Immediately after heating the gels were rapidly cooled to 5 °C. The experiments were performed in two- or threefold after approximately the same storage times as in the compression tests.

4.2.4 *Transmission electron microscopy*

Transmission electron microscopy experiments were performed on 30 wt% potato starch gels that had been stored at 4 or 20 °C for 13 days. Small fragments were prepared by first rubbing starch gels into small pieces with a mortar and a pestle. After this treatment, the swollen granules were still intact. They were fragmented by wet mashing in a Potter-Elvehjem tissue homogeniser. Specimens of these fragments for transmission electron microscopy were prepared by negative staining with uranyl acetate as described previously.[6] For ultramicrotomy small (~ 0.1 mm^3) cubes of a starch gel were infused overnight with 2.3 M sucrose, mounted on a copper stub and frozen in liquid nitrogen. Thin (~ 100 nm) frozen sections were cut with a Diatom cryo knife in a Reichert FC 4D/Ultracut E cryo-ultramicrotome at -110 °C. Sections were thawed, washed in water, stained with 1% uranyl acetate and air dried. Electron micrographs were recorded at 25,000 or 30,000 x magnification and 80 kV in a Jeol 1200-EX transmission electron microscope.

4.3 Results and Discussion

In transmission electron micrographs (Figure 4.2) of small negatively stained fragments of a retrograded 30 wt% potato starch gel, worm-like particles with a diameter of approximately 6 nm and varying lengths are visible. Occasionally, a subdivision into small globular domains can be seen. These worm-like particles are presumably the individual amylopectin molecules, the domains being the clusters of linear α-(1→4) glucan chains (Figure 4.1). Similar images were obtained from ultra-thin cryo-sections of a starch gel (Figure 4.3). In the presented images there is no indication for the presence of helically arranged lamellar domains as seen in electron micrographs of native starch granule fragments and sections.[10] Apparently, the crystallization of amylopectin during retrogradation is limited to individual clusters of linear glucan chains along the amylopectin molecules. The resulting structure is not regular enough to give a discrete reflection in SAXS experiments, consistent with the results of Cameron and Donald.[13] These results thus show that the long range ordering is not regained during starch retrogradation.

Figure 4.2 *Electron micrograph (negative staining) of small fragments of a 30 wt% potato starch gel stored at 4 °C for 13 days. Similar micrographs were obtained for a gel stored at 20 °C. Bar 200 nm.*
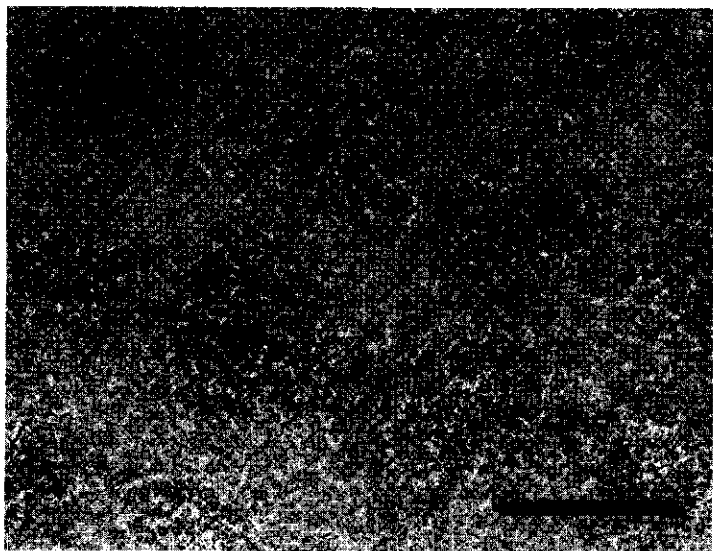
Figure 4.3   *Electron micrograph of a thin cryo section of a 30 wt% potato starch gel
stored at 4 ˚C for 13 days. Cryo sections were thawed and stained with 1%
uranyl acetate. Bar 200 nm.*

In concentrated starch gels the partly swollen granules occupy almost the whole
volume (Chapter 3). Therefore, the stiffness of the gel depends mainly on the stiffness
of the swollen granules. Directly after heating, the swollen granules consist of a
network of entangled amylopectin molecules. During storage of the starch gels
amylopectin recrystallizes, resulting in an increase of the Young modulus.

To study the relation between the increase in stiffness of concentrated starch gels
and the recrystallization of amylopectin, 30 wt% potato and wheat starch gels were
stored at 7 ˚C. At this temperature, the rate of recrystallization is more rapid than at
higher temperatures.[13] However, at a lower temperature the crystals formed are less
perfect,[13] which may to some extent affect the relation between the increase in stiffness
and the recrystallization of amylopectin. In Figure 4.4 $(E_t - E_0)$ is shown as a function
of the melting enthalpy change ΔH, where E_t is the modulus at time t and E_0 is the
modulus directly after cooling to 7 ˚C. The relation between the increase in stiffness
and the recrystallization of amylopectin is not linear, although they are closely related.
The results fit with $(E_t - E_0) = 31(\Delta H)^{1.6}$ for 30 wt% wheat starch gels and
with $(E_t - E_0) = 32(\Delta H)^{1.5}$ for 30 wt% potato starch gels. Thus, the relation between
the increase in stiffness and the recrystallization of amylopectin is approximately the
same for potato and wheat starch gels.
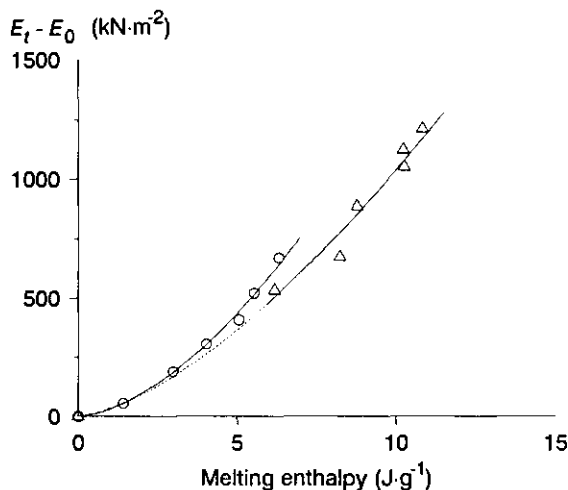
Figure 4.4 *The increase in stiffness* (E_t - E_0) *plotted as a function of the melting enthalpy change* ΔH *as determined with DSC for 30 wt% potato* ($\triangle$) *and wheat* (o) *starch gels. The storage temperature was 7 °C.*

There are two possible mechanisms to explain this relation. In the first place, the close relation between ΔH and (E_t - E_0) and the results obtained from TEM (Figures 4.2 and 4.3) suggest that formation of clusters of ordered chains along the amylopectin molecules results in stiffening of chains between the entanglements, and with this in an increase of stiffness of the gels. If this were the case, it is to be expected that also the linear range obtained from mechanical measurements will decrease with the increase in stiffness of the gels, *i.e.* during storage of the gels or with increasing starch concentration. Such changes were observed indeed in large deformation experiments performed on concentrated starch gels (compare for instance the stress-strain curve of a fresh 10% potato starch gel with that of an aged 40% potato starch gel shown in Figure 3.13).

Another possibility is that during storage of concentrated starch gels ordered regions between adjacent clusters of ordered double helices are formed, resulting in (physical) cross-links between amylopectin molecules (Figure 4.1). The close relation between ΔH and (E_t - E_0) would then suggest that the increase in crystallinity is roughly proportional to the increase in the number of cross-links in the swollen granules, assuming that the cross-links are rather homogeneously distributed. The formation of cross-links would thus result in further stiffening of granules. The deviation from linearity may be due to some inhomogeneity in the distribution of the ordered regions. Assuming the cross-links to be microcrystalline regions of parallel chains, the average length of such cross-links would affect the melting enthalpy more

than the Young modulus, whereas the number of cross-links would affect the Young modulus more than the melting enthalpy.

The assumption that crystalline regions form cross-links during storage cannot be supported by the transmission electron micrographs, which do not show the formation of ordered regions between adjacent clusters resulting in cross-links. However, the formation of cross-links between two adjacent molecules cannot be excluded.

Summarizing, due to recrystallization of amylopectin, stiffening of strands between entanglements as well as formation of cross-links between adjacent molecules would result in stiffening of concentrated starch gels. Both mechanisms may play a role simultaneously, although results of TEM suggest that the first mechanism is more prominent. Further research would be required to establish which of the two mechanisms is the more important one.

4.4   Conclusions

During storage of fully disordered starch gels, the short branches of the amylopectin molecules form double helices that become ordered in (semi-)crystalline clusters. The size of these crystalline domains is smaller than those in native starch, and probably mostly limited to the side branches of one main chain. The super-helical structure present in native starch is not regained during retrogradation.

A close relation between the recrystallization of amylopectin, as measured with DSC, and stiffening of concentrated starch gels was observed. Two different processes may explain the increase in stiffness that accompanies recrystallization. The first one is the formation of crystalline clusters along the glucan chains in the amylopectin molecule, which results in stiffening of strands between entanglements. The second process is the formation of cross-links between adjacent clusters. Both processes may occur simultaneously.

4.5   References

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Chapter 5

Structure and Mechanics of Starch Bread

Summary

The mechanical properties of breads made of potato or wheat starch were measured in two successive compression/decompression cycles. From the shape of the stress-strain curves the initial modulus, and the critical stress and strain could be derived; the critical stress and strain are those at which the crumb structure starts to collapse. The magnitude of the stress-strain related parameters changed markedly during storage. The initial modulus as well as the critical stress for structural collapse increased and the critical strain decreased. Moreover, the resistance of bread crumb to collapse of structure in compression decreased. The mechanical properties of potato starch bread changed more rapidly than those of wheat starch bread. The results are discussed in relation to the structure of starch bread on different levels, *i.e.* from molecular (amylopectin recrystallization) to macroscopic (sponge structure). It is concluded that the mechanical properties of starch bread are determined by the mechanical properties of the condensed lamellae and beams consisting of irregularly shaped, partly swollen starch granules as well as by the distribution of the thickness of the lamellae and beams.

5.1 Introduction

When fresh bread is stored several changes occur, which eventually lead to a reduced appeal to the consumer. These changes are commonly called staling; staling then includes all processes that occur during storage in crust and crumb, other than microbial spoilage.[1] The firming of bread crumb and its increase in crumbliness are recognized as essential factors in reducing the eating quality of bread. These processes have traditionally been explained by the partial recrystallization of amylopectin.[2-5] Several techniques, such as X-ray diffraction[6-8] and differential scanning calorimetry (DSC)[9-12] have been applied to study recrystallization processes in bread and the role of so-called bread improvers. To obtain a better understanding of the consequences of

starch recrystallization for the eating quality of bread it is also relevant to study changes in the mechanical properties of bread crumb. In some studies, the mechanical properties of bread and other baked foods have been evaluated by determining stress-strain relationships at large deformations.[13-16]

Many cereal foods, including bread, cakes and biscuits, have a porous structure, which can be described as a cellular solid. A theoretical relationship between the structure of cellular solids and their mechanical properties was published by Ashby and Gibson[17,18] and successfully applied to sponge cake by Attenburrow *et al.*[13] The theory of Ashby and Gibson mainly deals with the effect of film material (elastic, plastic or brittle) and film geometry. Moreover, they made a distinction between cellular solids with open cells (like in a sponge) and closed cells (like in a foam). In cellular solids with open cells, the solid material is distributed in columns or beams which form the cell edges. In materials with closed cells, much of the solid material is distributed in plates or lamellae which form the faces of the cells. In this theory, the most important characteristic of the structure is the relative density, *i.e.* the ratio of the bulk density of the foam/sponge and the density of the solid of which the lamellae and beams are made.

The theory of Ashby and Gibson, however, has been developed for ideal sponge structures with uniform gas cell sizes and thicknesses of the beams and the lamellae. In the case of bread crumb, the structure is far from ideal because of large variations in gas cell sizes and local densities. Despite these inhomogeneities in structure, the theory provides a useful relation between microstructure and the overall shape of the stress-strain curve. A typical stress-strain curve of a cellular solid exhibits three regions,[17,18] indicated schematically in Figure 5.1. When an ideal sponge is compressed, the lamellae and beams bend at first and the sponge deforms in a linearly elastic way (region I). Upon further compression, buckling, plastic deformation or brittle fracture of the lamellae and beams occur. Buckling of the lamellae and beams is partly recoverable, whereas plastic deformation and brittle fracture result in irreversible severe changes in the lamellae (structural collapse). Buckling, plastic deformation and fracture give rise to a plateau region over which the compressive stress is almost independent of deformation (region II). Finally, there is a region where the stress increases rapidly with increasing deformation due to strong compaction of the material (region III).

Not only the geometry of the sponge, but also the mechanical properties of the material forming the lamellae and beams determine the overall properties of the sponge. In bread crumb, these lamellae and beams are mainly composed of a bicontinuous system of gluten and gelatinized starch. Due to the limited amount of water present in bread dough, the starch granules are only partly gelatinized and swollen. Moreover, only a part of the amylose molecules has leached out of the granules. In the swollen granules both amylose and amylopectin are present. It is on this level that structural changes occur during storage, namely recrystallization of

Stress

I   II   III

Cauchy strain
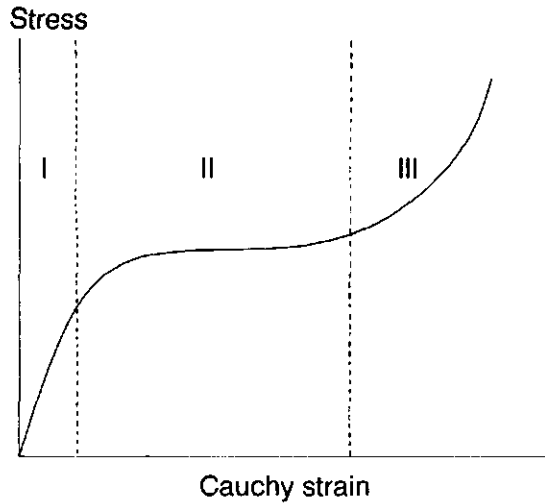
Figure 5.1   *Schematic representation of the typical shape of stress-strain curves of cellular solids. I elastic bending of the lamellae, II collapse of the lamellae, III compaction.*

the amylopectin molecules (Chapter 4).

The above description shows that bread structure is very complex, because there are different specific structures at different length scales. This makes it difficult to relate amylopectin recrystallization to changes in mechanical properties of bread crumb during staling. Moreover, bread staling may be affected by various components present in bread crumb, like gluten, lipids, pentosans, etc. Therefore, the mechanical properties of relatively simple concentrated starch gels in relation to the structure of the gels were studied first (Chapter 3). A direct translation of the results obtained on starch gels into the mechanical properties of bread crumb in relation to its microstructure is difficult, because both the composition of the system and its structure (a compact gel and a sponge, respectively) are different. Therefore, staling was studied in starch breads prepared from potato or wheat starch, from the same starches that were studied in gels (Chapter 3). To obtain a good crumb structure in the absence of gluten, xanthan, a hydrophillic polysaccharide of microbiological origin, was added to the starch suspension. In the preparation of gluten-free bread, xanthan has been found to make its crumb structure more like that of bread made from normal wheat flour.[19,20] Xanthan acts probably by improving the strain hardening properties of the "dough". A material shows strain hardening if the resistance against deformation is higher for relatively more extended test pieces.[21,22]

The aim of this chapter is to determine the role of starch in the mechanical properties of starch bread and the changes therein during storage. These properties

will be related to the structure of this bread at different scales, *i.e.* from molecular to macroscopic. Moreover, the structure and mechanical properties of starch bread and those of the concentrated starch gels will be compared.

5.2   Materials and Methods

5.2.1 Materials

Potato starch was supplied by AVEBE (Foxhol, the Netherlands), and wheat starch by Latenstein BV (Nijmegen, the Netherlands). The chemical composition of the starches is given in Table 2.1. Xanthan was a gift from Suiker Unie (Roosendaal, the Netherlands).

5.2.2 Breadmaking

Starch bread was made from potato or wheat starch. The compositions of the doughs used are given in Table 5.1. The viscosity of the doughs was rather low; their appearance was more like that of a batter. The actual compositions of the doughs were chosen such that the final starch breads and their crumb structure were as close in appearance to normal wheat flour bread as possible.

Table 5.1   *Composition of "doughs" used for preparation of potato and wheat starch breads.*

ingredients	mass (g)	
	potato starch bread	wheat starch bread
starch	4375	4375
water	3440	3876
yeast	138	155
sugar	69	78
xanthan	69	78

Water and xanthan were mixed thoroughly in a Hobart mixer for 30 min at position 2. Immediately thereafter the other ingredients were added and mixed for 3 min,

followed by a bulk fermentation of 10 min at 28 °C, after which the dough was mixed again for 3 min. Portions of 500 g of mixed doughs were deposited directly into baking tins with a size of 17 × 9.5 × 7.5 cm. After a final proof of approximately 50 min at 30 °C, the dough was baked at 240 °C for 30 min. The loaves were cooled for 3 hours at room temperature. Before packaging, the loaves were treated with 2% propionic acid to avoid mould growth during storage. Each loaf was packed in two PVC bags that were carefully sealed to minimize loss of water during storage, and stored at 20 °C for up to 11 days. The mechanical and thermal properties of the crumb of each type of loaf were determined after various storage times.

5.2.3 Dry matter content and crumb density

The dry matter content of the crumb was determined at least in duplicate by oven drying of 3-g samples of bread crumb for 2 h at 120 °C. The density of the crumb was calculated from the length, diameter and mass of cylindrical test pieces.
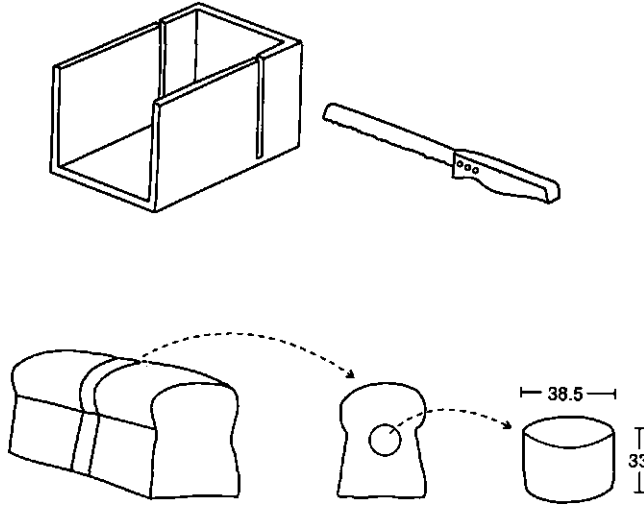
5.2.4 Mechanical testing

Bread crumb is not homogeneous; one may readily find greater differences in mechanical properties within a single loaf than at identical positions in different loaves. The mechanical properties of a test piece differ with the position in the loaf as well as by the orientation of the test piece.[14] Therefore, for any comparative test, standardization of the preparation of the test piece is necessary. It is also important that the test piece is large with respect to the structural elements like the gas cells. It goes without saying that the test piece must be significantly smaller than the loaf from which it is taken.

The loaves of bread were cut in slices with an electric knife. To keep the sides perpendicular to the axis of the loaf and their thickness constant, the bread was placed in a box with a small slit at a certain distance from the end of the box, through which the knife could be moved (Figure 5.2). From both ends of the loaf at least 3.5 cm was discarded. Cylindrical test pieces were taken with a borer[23] from the centre of the slices. The test pieces had a height of 33 mm and a diameter of 38.5 mm. The test pieces were taken so that the axes of the cylinders were parallel to the length axis of the loaf. In this way, three test pieces were taken from one loaf. At least the properties of six replicate test pieces were determined for each variable.

In one experiment height and diameter were varied between 27 and 33 mm, and between 28.5 and 38.5 mm, respectively. No significant differences were observed in the mechanical properties, expressed in stress and relative deformation.

Uniaxial compression tests were performed with a Zwick material testing machine, equipped with a 50 N load cell. Test pieces were compressed at constant displacement rate between parallel perspex plates. Each test piece was compressed twice, each

Figure 5.2   *Test piece preparation of bread crumb for mechanical testing.*

compression being followed by decompression. The time interval between the end of the first decompression and the second compression was one minute, which was found to be the optimal rest period. After shorter rest times the height of the test pieces was still materially increasing at the start of the second compression, whereas during longer rest times drying of the test pieces became a factor not to be ignored. From the force-displacement curve, the stress-strain relation was calculated. The deformation at a certain point was expressed as Cauchy strain ϵ_c (-), which is defined as:

$$\epsilon_c = \frac{h_o - h(t)}{h_o} \tag{5.1}$$

where h_o is the original height of the test piece and $h(t)$ the height at time t. As the cross-sectional area of the test piece of bread crumb did not change significantly during compression, the stress $\sigma(t)$ ($N \cdot m^{-2}$) is equal to the force $F(t)$ (N) per unit initial area A_o (m^2):

$$\sigma(t) = \frac{F(t)}{A_o} \tag{5.2}$$

From the stress-strain curve the Young modulus E ($N \cdot m^{-2}$) was calculated:

$$E = \left[\frac{d\sigma}{d\epsilon} \right]_{\epsilon \to 0} \tag{5.3}$$

Because in practice the stress-strain curves show an initiating effect, resulting from a non-ideal flat surface of the samples, the Young modulus is taken from the increase in the stress-strain curve just after the initiating effect. Both initial strain rate and maximum deformation were varied; the initial strain rate from 1.7×10^{-3} to 1.7×10^{-1} s^{-1} and the maximum deformation from 0.1 to 0.7. Unless stated otherwise, the initial strain rate was 1.7×10^{-2} s^{-1} and the maximum deformation 0.4.

If bread crumb is compressed to a large extent, collapse of structure of the lamellae and beams between the gas cells may occur. The second compression provides information on the extent of collapse of structure during the first one. Typical stress-strain curves of bread crumb are given in Figure 5.3. Several parameters could be derived from these curves: σ_1^*, σ_2^* and ϵ_1^*, as indicated in the figure. σ_1^* and σ_2^* are defined as the critical values of stress at which buckling or fracture of the lamellae and beams occurs in the first and the second compression, respectively. The values of these critical stresses are determined by linear extrapolation of the initial linear increase in stress (region I) and of the plateau region (region II) (Figures 5.1 and 5.3). The ratio between σ_2^* and σ_1^* is considered to be a measure for the resistance against
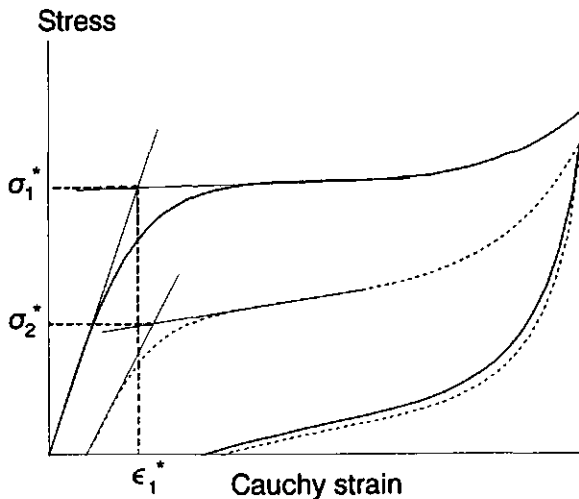
Figure 5.3 *Schematic representation of typical compression/decompression stress-strain curves of bread illustrating the derived parameters. (—) first compression/decompression; (---) second compression/decompression.*

mechanical collapse during the first compression. If σ_2^*/σ_1^* approaches 1, the resistance to structural collapse is high, which means that damage of the lamellae and beams is relatively slight. The deformation at which the initial region of the first compression changes into the plateau region is designated ϵ_1^*.

5.2.5 *Differential scanning calorimetry*

Differential scanning calorimetry was performed using a TA Instruments DSC-2910. Approximately 70 mg of bread crumb was weighed in a stainless steel pressure cup just before measuring. An empty cup was used as a reference. The scanning rate was 5 K·min⁻¹ in the temperature range of 20 to 160 ˚C. As the repeatability was very good, the experiments were performed singly or in duplicate.

In order to simulate amylopectin crystallization during the first hours of storage of potato starch bread, bread crumb was heated in the DSC apparatus to 120 ˚C and kept at this temperature for 20 min. Then the sample was cooled to 20 ˚C as fast as possible and kept at this temperature for a certain time (= storage time). Next, the sample was cooled to 0 °C, and immediately thereafter a DSC scan was performed under the same conditions as mentioned above, in order to determine the melting enthalpy. This procedure was repeated with the same sample after increasing storage times.

5.3   Results

The dry matter content and the density of the crumb of the potato and wheat starch breads are given in Table 5.2. The dry matter content of the crumb was approximately the same for potato and wheat starch breads, and it hardly increased during storage. The crumb of the wheat starch bread had a higher density and was more even in structure than the crumb of the potato starch bread (Figure 5.4).

Table 5.2 *Dry matter content and density of the crumb of potato and wheat starch breads.*

	Potato starch bread	Wheat starch bread
Dry matter (wt%)	47.0	47.2
Density (kg·m⁻³)	303 ± 26	388 ± 14

Directly after baking, the crumb of potato starch bread was more or less transparent. It was very soft and highly deformable, *i.e.* if the crumb was compressed

temporarily, it recovered almost completely, even if the compression had been considerable. In contrast with fresh potato starch bread, fresh wheat starch bread had a white appearance. Moreover, it was stiffer and more brittle; the crumb recovered to a lesser extent after being compressed. After some hours of storage, the potato starch bread was also white, the stiffness of its crumb had increased strongly and it was much more brittle.

1   2
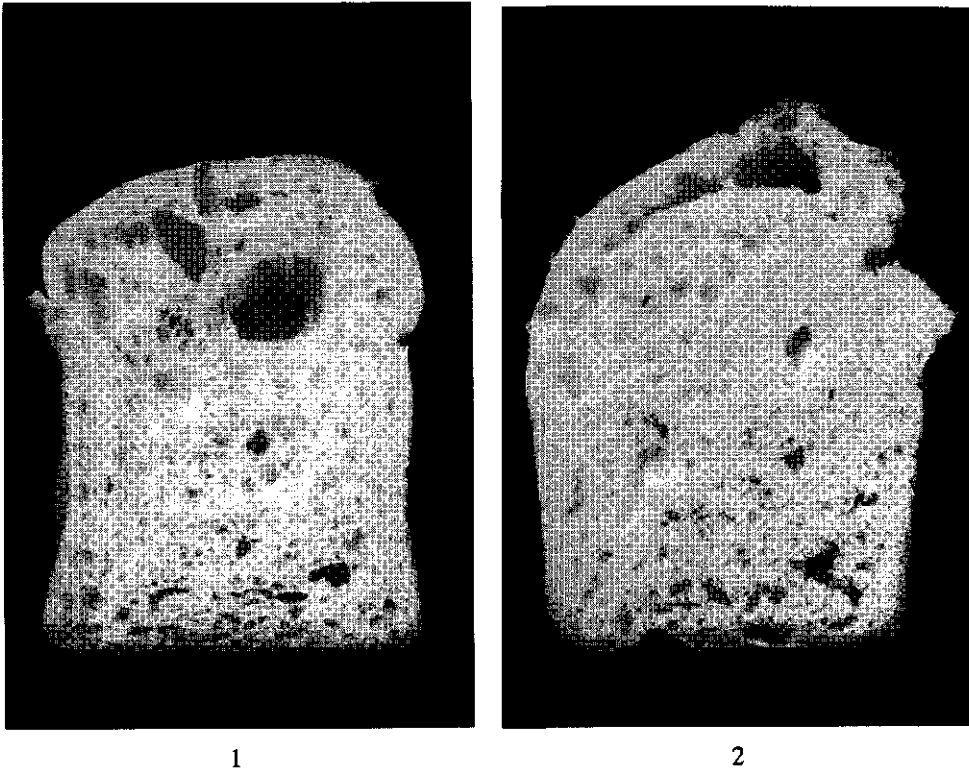
Figure 5.4   *Loaves of bread baked from potato (1) and wheat (2) starch.*

The first compression tests were started directly after cooling to 20 ˚C. By then, the modulus of the potato starch bread crumb was already higher than the modulus of the wheat starch bread crumb (Figure 5.5), which means that the potato starch bread stiffened much faster during cooling. The higher rate of stiffening of the potato starch bread continued during the first day of storage. After a few days, the moduli of the potato starch bread almost reached a plateau value, whereas the moduli of the wheat starch bread continued to increase.
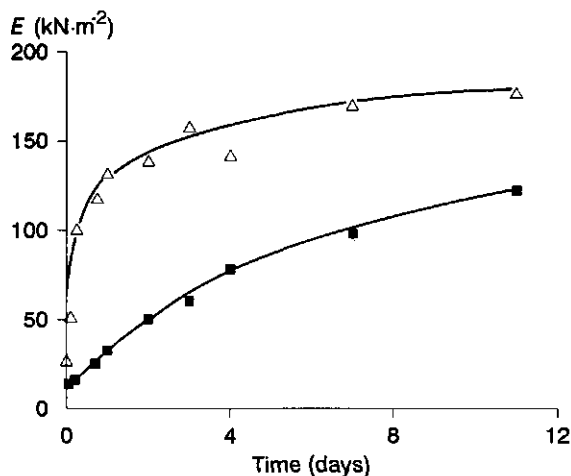
Figure 5.5   *Young moduli as a function of storage time for potato (△) and wheat (■) starch breads. The storage temperature was 20 °C.*

In Figure 5.6 melting enthalpies (ΔH) as a function of storage time of the potato and wheat starch breads are shown. Melting of the crystallites in samples of the wheat starch bread started at about 45 °C. The heat flow in the curve had its maximum at about 59 °C. Melting was completed at about 78 °C. For samples of potato starch bread these temperatures were 40, 69 and 90 °C, respectively. At the moment the measurements were started (about 4 hours after cooling) the crumb of the potato starch bread had a melting enthalpy of approximately 6 $J \cdot g^{-1}$. This means that in this bread a considerable amount of amylopectin had already crystallized during the first 4 hours of storage. By simulating the first hours of storage of bread crumb in the DSC apparatus, it was indeed observed that ΔH of potato starch bread crumb increased very fast during this period (Figure 5.6). Since the temperature histories in the regular DSC procedure and in the simulation experiments are different, it is not surprising that the values of ΔH were slightly different. After two to three days, ΔH of potato starch bread increased only very slowly. The increase in ΔH for wheat starch bread proceeded much slower during the first days of storage but still continued after about 12 days.

Figure 5.7 shows the relation between $(E_t - E_0)$ and ΔH, where E_t is the modulus at time t and E_0 is the modulus directly after cooling. This figure shows that for both types of bread the relation between the increase in stiffness and the recrystallization of amylopectin is not linear, although they are closely related.
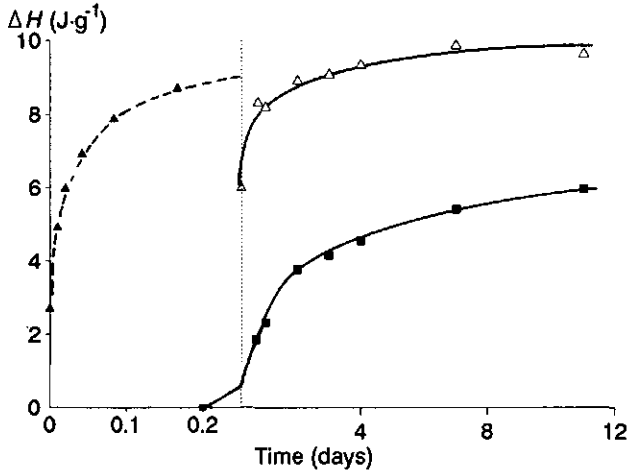
Figure 5.6   *Melting enthalpies per g bread crumb as a function of storage time of potato (▲) and wheat (■) starch breads, and of potato starch bread in simulation experiments (▲). The storage temperature was 20 ˚C. Note that the scale on the horizontal axis changes at 0.25 days.*
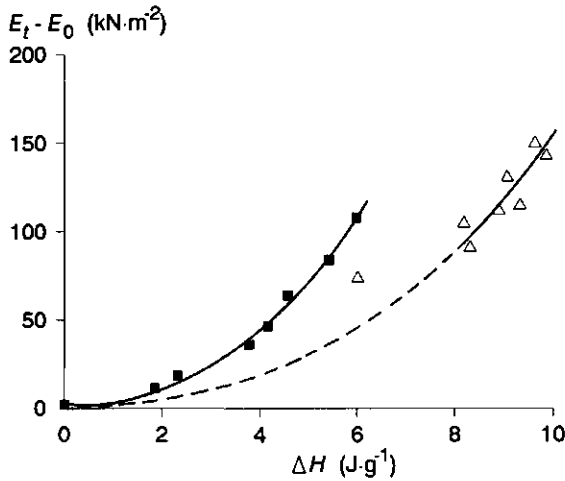
Figure 5.7   *Increase in stiffness (E_t - E_0) plotted as a function of the melting enthalpy for potato (▲) and wheat (■) starch breads. The storage temperature was 20 ˚C.*
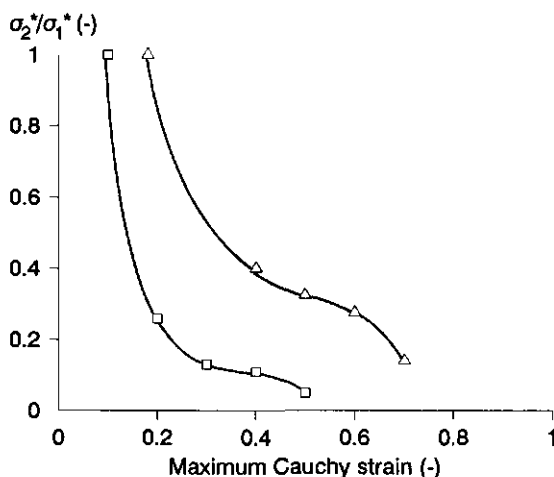
Figure 5.8 σ_2^*/σ_1^* *as a function of the maximum Cauchy strain applied during the first compression/decompression cycle for potato (□) and wheat (▲) starch breads stored for one day at 20 °C.*

Of the large deformation properties, the effect of the maximum deformation applied before decompression on the structural collapse as given by σ_2^*/σ_1^*, is shown in Figure 5.8. The larger the maximum deformation, the smaller is σ_2^*/σ_1^*. This means that the collapse of structure is stronger after a larger deformation, as is to be expected.

In Figure 5.9, stress-strain relationships of potato and wheat starch breads after storage times of 4 hours, 1 day and 7 days are given. For potato as well as for wheat starch bread σ_1^* increased while ϵ_1^* decreased during storage. During storage the shape of the first compression curve also changed. For fresh bread the stress continued to increase beyond σ_1^*, whereas for aged bread a decrease in stress was observed after σ_1^* had been reached. Thus, more aged bread showed more collapse of structure and this bread behaved very brittle (low strain at fracture). This is confirmed by the decrease in σ_2^*/σ_1^* during storage (Figure 5.10). Because ϵ_1^* decreased during storage, aged bread is compressed further after the critical stress σ_1^* has been reached than fresh bread. This presumably results in more damage to the lamellae and beams of this bread (see also effect of maximum deformation). In principle, one could correct for this by adapting the maximum deformation to variations in ϵ_1^*. However, this is impractical, because ϵ_1^* can only be estimated after completion of the experiment and because of variation in ϵ_1^* among test pieces.

Some striking differences between the large deformation properties of potato and wheat starch breads could be observed. As described above, shortly after baking wheat starch bread was somewhat more brittle than potato starch bread. After some hours,

when the first stress-strain curves were obtained, this was already reversed (Figure 5.10), which means that during the first hours the change in the large deformation properties occurred much faster in potato starch bread. The faster change in the large deformation properties continued during the first days of storage. After about a week, the collapse of structure at large deformation was very severe in both types of starch bread. Other differences between potato and wheat starch breads are their values of
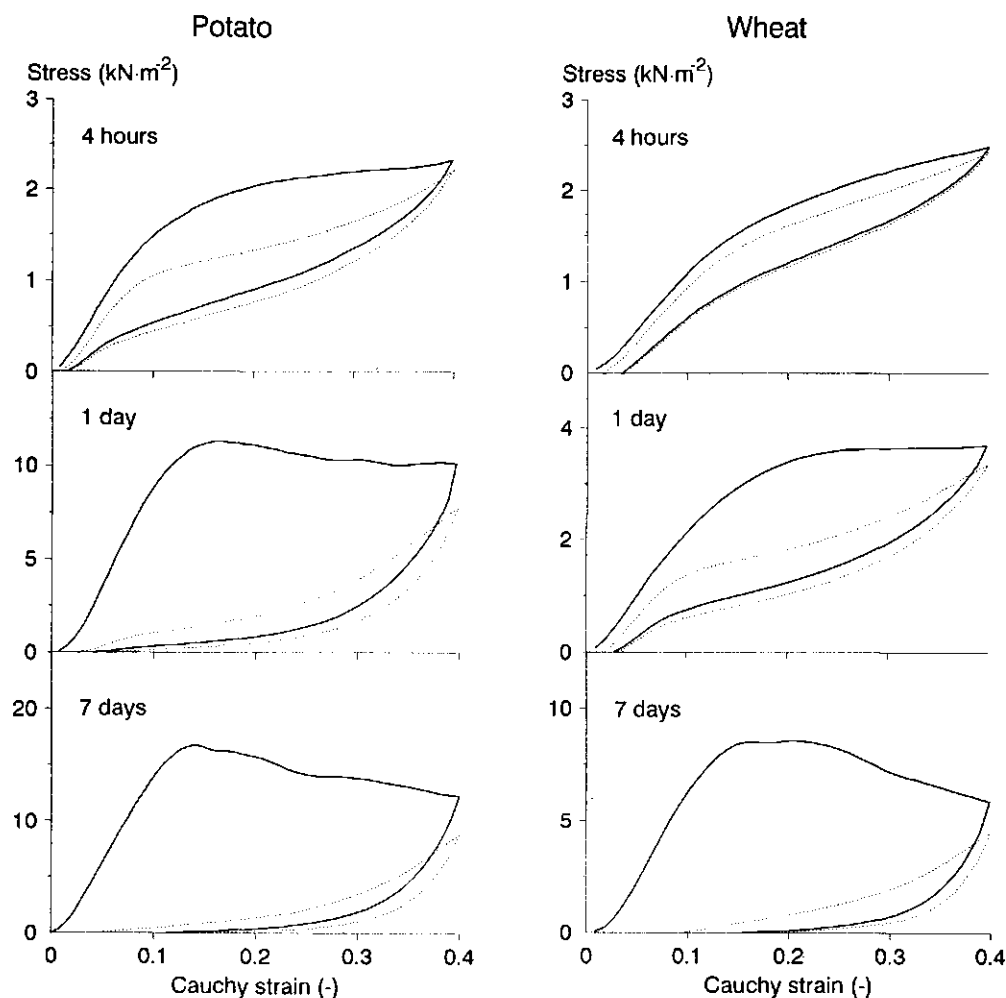
Figure 5.9   *Stress-strain curves of potato and wheat starch breads stored for 4 hours, 1 day and 7 days at 20 °C. The solid line shows the first compression and decompression and the dashed line the second ones. The initial strain rate was $1.7 \times 10^2\ s^{-1}$.*
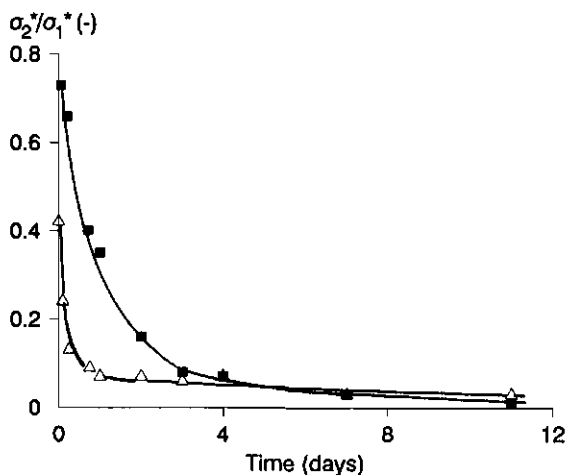
Figure 5.10 σ_2^*/σ_1^* *as a function of storage time for potato* ($\triangle$) *and wheat* ($\blacksquare$) *starch breads. The storage temperature was 20 °C.*

σ_1^* and ϵ_1^*: generally, potato starch bread exhibited a higher σ_1^* and a lower ϵ_1^* than wheat starch bread (Figure 5.9).

There was a clear effect of the strain rate on the large deformation properties of the starch bread (Figure 5.11). Increasing the strain rate resulted in a higher σ_1^* and a larger ϵ_1^*. Moreover, σ_2^*/σ_1^* was higher at higher strain rates, *i.e.* the collapse of structure during the first compression was less.

5.4   Discussion

Bread dough (batter) can be considered as a foam; during mixing of bread dough small air cells are entrapped. During the fermentation stage and the oven rise these cells grow. The foam with more or less spherical gas cells is transformed into a foam with elongated polyhedral gas cells. In a later stage of the oven rise, starch eventually gelatinizes and the highly viscous dough is transformed into an elastic bread crumb. Rupture of most lamellae between the gas cells occurs and the structure with separated gas cells is transformed into a sponge structure with interconnected cells.[24] Bread crumb thus has a porous structure with mainly open polyhedral cells; it can be described as a solid elastic sponge. Probably, some very small cells are still closed.

The structure of the lamellae and beams, which build starch bread, is assumed to be similar to the structure of concentrated starch gels (Chapter 3). Because of the limited amount of water present during gelatinization, the granules in these gels are

Potato　　　　　　　　　　Wheat

Figure 5.11 *Stress-strain curves of potato and wheat starch breads that had been compressed and decompressed twice. The initial strain rates of compression were 1.7×10^3 s^{-1} (low) and 1.7×10^1 s^{-1} (high). The solid line shows the first compression and decompression and the dashed line the second ones. The starch breads had been stored for 1 day at 20 °C.*

partly swollen. The granules have retained their integrity, but they are highly deformed and their shape is irregular. Within the swollen granules amylose as well as amylopectin are present, which are in an amorphous state directly after gelatinization. Presumably, amylose and amylopectin partly separate within the swollen granules, because they are thermodynamically incompatible at the prevailing water content.[25] This would also result in a small amount of amylose leaching out of the granules and forming a thin layer of amylose gel in between the granules. During storage of concentrated starch gels, amylopectin in the swollen granules recrystallizes. Thus, the molecular structure is changing.

In starch bread the starch concentration is 10 to 40% higher than that of the starch gels studied (Chapter 3). This may affect the structure somewhat. Firstly, the granules would be less swollen and maybe somewhat less irregular in shape. Moreover, the separation of amylose and amylopectin is expected to be less, resulting in an even

thinner amylose layer between the granules. Another difference between the concentrated starch gels and the starch bread is a small amount of xanthan in the latter material, which is supposed to have hardly any effect on the gelation and retrogradation of starch.

For concentrated starch gels it was concluded that recrystallization of amylopectin results in the swollen granules becoming stiffer (Chapter 3). As these gels are almost completely filled with swollen granules, the modulus of the gel would be approximately equal to the modulus of the granules. The modulus of the starch bread will depend on the modulus of the lamellae and beams as well as on the large scale structure, of which the most relevant variable presumably is the ratio of the density of the bread and that of the lamellae and beams.[17,18] Due to recrystallization of amylopectin (Figure 5.6) the modulus of the swollen granules and, consequently, the modulus of the lamellae and beams increase during storage. This will result in an increase of the moduli of the starch bread (Figure 5.5). It was observed that the relation between the increase in modulus of starch bread and the increase in recrystallization of amylopectin was closely related. The shape of the curves was very similar to those observed for the concentrated starch gels (Chapter 4). However, the relation between the increase in modulus and ΔH was approximately the same for potato and wheat starch gels (Figure 4.3), whereas this relation was different between potato and wheat starch bread (Figure 5.7). Probably, the higher values of $E_t - E_0$ of wheat starch bread are mainly due to the higher density of its crumb. A more extensive discussion of such a behaviour for concentrated starch gels has been given in Chapter 4. For starch bread the same mechanism would be applied.

As described before, a typical stress-strain curve of a cellular solid, such as e.g. starch bread, has three regions (Figure 5.1); (i) elastic deformation as a result of bending of the lamellae and beams, (ii) collapse of structure as the lamellae and beams buckle or fracture and (iii) compaction as the lamellae and beams are pressed together. The shape of the stress-strain curves of fresh starch breads (Figure 5.9) was for the greater part similar to those of elastically deformable cellular solids, the deformation of which is recoverable even after applying large strains.[17,18] This would mean that at large deformations buckling predominated over fracture of the lamellae and beams. In contrast, the stress-strain curves of the aged bread showed a very irregular shape after σ_1^* was reached (Figure 5.9); generally, beyond ϵ_1^* the stress decreased. These observations would indicate that in aged starch bread fracture of the lamellae and beams predominated. This increase in brittleness is also illustrated by the decrease in σ_2^*/σ_1^* during storage (Figure 5.10), indicating that the susceptibility of structure to collapse had increased. Moreover, it was observed that the bread fell into crumbs at large deformations. Therefore, σ_1^* may be considered a kind of fracture stress. To gain more insight in the changes in this parameter during storage, this will be compared with the change in the fracture stress, σ_f, of concentrated starch gels during storage (Chapter 3).

During storage of concentrated starch gels its fracture stress increased (Figure 3.4). As was described before, concentrated starch gels consist of tightly packed, partly swollen granules with a thin layer of amylose gel in between. The shape of the granules is very irregular; they are hooked into each other like pieces of a jigsaw puzzle. Fracture of the gels occurs around the swollen granules. The fracture stress of such a gel may to some extent depend on the fracture stress of the thin amylose layer between the granules. It is known that gelation of concentrated amylose systems is usually completed within a few hours.[26] Therefore, it is unlikely that the increase in fracture stress of the concentrated starch gels is a result of gelation of amylose. The main cause for the increase in fracture stress must be stiffening of the swollen granules (Chapter 3); then the "hooks" become more rigid and it is more difficult to fracture the gels. This presumption was confirmed by the observation that, during storage of concentrated starch gels, there was a high positive correlation between the fracture stress and the modulus (Chapter 3). By comparing the fracture behaviour of concentrated potato and wheat starch gels, it was observed that the increase in fracture stress with modulus was much more pronounced in the potato starch gels (see also Table 5.3). It was suggested that this is a result of the more irregular shape of the swollen potato starch granules.

Table 5.3 *The relative increases in fracture stresses, σ_f, of 40 wt% potato and wheat starch gels and in σ_1^* of potato and wheat starch breads with the increase of the Young moduli* E.

Material	$d\sigma_f/dE$ (-)	$d\sigma_1^*/dE$ (-)
40% potato starch gel	0.30	-
40% wheat starch gel	0.11	-
Potato starch bread	-	0.11
Wheat starch bread	-	0.10

During storage of potato as well as wheat starch bread σ_1^* increased. As was concluded above, the structure of the lamellae and beams of the starch-bread crumb is similar to that of concentrated starch gels. Therefore, it is plausible that the increase in σ_1^* during storage is due to stiffening of the swollen granules by the same mechanism causing the increase in fracture stress of concentrated starch gels. For starch bread a positive correlation between σ_1^* and modulus was observed (results not shown). The ratio of σ_1^* and E in bread from wheat starch is about the same as the

ratio of σ_f and E in wheat starch gels (Table 5.3). Contrary to this, the ratio of σ_1^* and E in bread of potato starch is much smaller than the ratio of σ_f and E in potato starch gels. This difference may be due to the difference in the structure on a millimeter scale between breads from wheat and potato starch. It may be the consequence of the larger variation in the thickness of the lamellae and beams in potato starch bread than in wheat starch bread. For very thin lamellae and beams, *i.e* lamellae and beams that are only a few granules thick, the granules will hardly be hooked into each other; the fracture stress of a thin lamella or beam would thus be much lower and increases less with further stiffening of the granules than that of a thick lamella or beam, because it would mainly depend on the fracture stress of the thin amylose layer. The importance of the variation in the thickness of the lamellae and beams will be further discussed in Chapter 6.

For concentrated starch gels it was observed that the fracture strain decreased during storage (see e.g. Figure 3.4), which was ascribed to the granules becoming less deformable, *i.e.* stiffer (Chapter 3). The decrease in ϵ_1^* of starch breads may also be due to this phenomenon. Potato starch gels had a higher strain at fracture than wheat starch gels, even in those cases where the granules were stiffer. This may be due to the more irregular shape of the swollen potato starch granules. For the starch bread the opposite behaviour was observed: ϵ_1^* of potato starch bread was smaller than ϵ_1^* of wheat starch bread. Here, the larger variation in the thickness of the lamellae and beams of potato starch bread may play a role.

Figures 5.5, 5.6 and 5.10 show that the increase in E and ΔH, and the decrease in σ_2^*/σ_1^* are more pronounced in potato starch bread, especially during the first days of storage. Generally, it is known that concentrated potato starch gels recrystallize and stiffen at a faster rate than concentrated wheat starch gels (Chapter 3 and Ref. 27-29). Several explanations have been offered for this difference. First, it has been suggested that lipids present in wheat starch retard the rate of recrystallization of amylopectin.[29] It has also been supposed that the length of the short branches of amylopectin affects its recrystallization rate. The reduced rate of recrystallization of wheat amylopectin would then be explained by its shorter average chain length.[30] Furthermore, it has been supposed that the number of entanglements or cross-links between the starch molecules in the swollen granules may affect the rate of recrystallization of amylopectin (Chapter 3). It was presumed that wheat starch granules contain more entanglements than potato starch granules, which would be a factor of importance for the lower rate of recrystallization of wheat amylopectin.

At small deformations, bread crumb behaves as an elastic solid; the deformation behaviour was independent of time scale. However, there was a large effect of the strain rate on the large deformation properties of starch bread. In general, increasing the strain rate from 1.7×10^{-3} to 1.7×10^{-1} s^{-1} resulted in a higher σ_1^* and a higher ϵ_1^* (Figure 5.11) and this was also observed for crumb of commercial loaves of bread.[31] Moreover, σ_2^*/σ_1^* was higher at higher strain rates. To my knowledge, the mechanism

of the rate dependent behaviour of bread crumb has not been discussed before.

Rate or time dependent mechanical behaviour is not uncommon for food materials.[32,33] Time dependency may be explained in terms of energy dissipation processes. At least two different mechanisms leading to time dependency may act in a food material. First, a material may be viscoelastic, causing energy dissipation due to flow of the material. If the strain rate is smaller, the amount of the deformation energy that is dissipated is smaller and the proportion of the dissipated deformation energy is generally higher. Second, friction between structural elements of different mechanical properties may occur, which also causes energy dissipation. Such friction processes commonly dissipate more energy if they take place at faster rates.

It was observed that not only starch bread but also concentrated starch gels exhibit time independent behaviour at small deformations and time dependent behaviour at large deformations (Chapter 3 and Ref. 32). The time dependency observed at large deformations presumably is a result of energy dissipation due to friction between the swollen granules. Energy dissipation due to friction will increase with the strain rate, resulting in a higher stress and strain at fracture.[32] Such a mechanism will also be possible in starch bread, provided that parts of the lamellae and beams in which fracture occurs are at least more than several granules thick. Then, as a result of friction between the swollen granules in the lamellae and beams, σ_1^* and ϵ_1^* would be larger for higher strain rates.

5.5   Conclusions

It is clear that the mechanical properties of starch bread are determined for a large part by the mechanical properties of the lamellae and beams from which it is built. The structure of these lamellae and beams is similar to that of concentrated starch gels. Consequently, knowledge about the mechanical properties of concentrated starch gels in relation to their structure is required for an understanding of the mechanical properties of starch bread. However, knowledge of the role of the structure, especially of the effect of the variation in the thickness of the lamellae and beams, is also required to obtain a complete understanding of the mechanical properties of starch bread and the changes therein during storage. In Chapter 6 this will be discussed further.

5.6   References

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Chapter 6

The Importance of Structural Imperfections in Starch Bread for its Mechanical Properties

Summary

In order to obtain a better understanding of the importance of structural imperfections for the mechanical properties of starch bread I applied a theory that had been developed for other cellular solids. This theory describes the relation between the mechanical properties (Young modulus, critical stress) and cell geometry. The data obtained for the Young modulus fitted well with the theory. However, data for the critical stress, in particular for fracture, showed less agreement with the theory. From these results it is concluded that the large variations in sizes of the cells and thicknesses of the beams strongly affect the fracture behaviour of 'beams' building up the starch bread.

6.1   Introduction

In Chapter 5, the mechanical properties of the crumb of potato and wheat starch breads were described and discussed in relation to their structure. Starch bread was considered a cellular solid with mainly open cells (sponge structure); the solid material is distributed in 'beams' forming the cell edges. Probably, very small cells are still closed (foam structure); most of the solid material is present in lamellae that form the cell faces. The stress-strain curves of starch bread showed the typical stress-strain curve of a cellular solid (Figure 5.1), exhibiting three specific regions. From the linear elastic part of the curve (region 1), the Young modulus E was obtained. The transition from the first to the second region was characterized by a dramatic decrease in the rate of increase of the stress, due to buckling or fracture of the lamellae and beams. Two parameters were derived from this point: the critical stress, σ_1^*, and the critical strain, ϵ_1^*.

The mechanical properties of the crumb of potato and wheat starch breads changed markedly during storage; E and σ_1^* increased, whereas ϵ_1^* decreased. These changes

were ascribed to changes in the properties of the beams. The beams consist of irregularly shaped, partly swollen granules; their structure is comparable with the structure of concentrated starch gels. In the previous chapter, the structure and mechanical properties of concentrated starch gels and starch bread were compared. It was concluded that the mechanical properties of starch bread can to a considerable extent be explained on the basis of those of concentrated starch gels. However, there were some discrepancies, like e.g. the relation between σ and E, especially for the systems made of potato starch. Probably, these result from variations in the thickness of the lamellae and beams.

The aim of the study described in this chapter is to obtain a more quantitative understanding of the importance of the inhomogeneity, *i.e.* the distributions of the dimensions of the gas cells and the lamellae and beams around them. For this purpose, the model of Ashby and Gibson[1,2] was used. This model predicts the relation between the three-dimensional structure of cellular solids and their modulus and fracture strength; it is applicable to materials with uniform gas cell sizes and thicknesses of beams and lamellae. The next section briefly describes the conclusions of the theory of Ashby and Gibson that are most relevant for my purpose. Subsequently, this theory will be applied to the results obtained from experiments with concentrated starch gels (Chapter 3) and starch bread (Chapter 5).

6.2   Theory

According to Ashby and Gibson,[1,2] the salient structural features of a cellular solid are the shape of the cells (isotropic or anisotropic), the degree to which the cells are open or closed and the relative density ρ^*/ρ_s, where ρ^* is the density of the cellular solid and ρ_s that of the material which the cellular solid is made of. As described above, bread crumb has a porous structure with mainly open cells; it can be described as a solid elastic sponge. Therefore, the formulae developed for cellular solids with open cells will be used in this study.

When a sponge is compressed, the beams at first bend. From the linear elastic part of the stress-strain curve, the Young modulus can be determined. The Young modulus E^* of a cellular solid is related to the modulus of the beams E_s, the density of the sponge ρ^* and the density of the beams ρ_s, as follows:

$$\frac{E^*}{E_s} = C_1 \left[\frac{\rho^*}{\rho_s} \right]^2 \tag{6.1}$$

where C_1 is a constant. Results obtained for various cellular solids show that $C_1 \approx 1$.

When an elastomeric sponge is compressed further, the beams will start to buckle.

The stress at which buckling occurs, σ_{el}^*, is given by:

$$\frac{\sigma_{el}^*}{E_s} = C_2 \left[\frac{\rho^*}{\rho_s} \right]^2 \tag{6.2}$$

where C_2 is a constant. Results for the elastic collapse stress of elastic sponges have shown that $C_2 \approx 0.05$.

When a brittle sponge is compressed, fracture of the beams happens at σ_f^*. The following relation between σ_f^* and the fracture stress of the beams, σ_{fs}, ρ^* and ρ_s was obtained:

$$\frac{\sigma_f^*}{\sigma_{fs}} = C_3 \left[\frac{\rho^*}{\rho_s} \right]^{3/2} \tag{6.3}$$

where C_3 is a constant. The few experimental results available suggest $C_3 \approx 0.65$.

6.3 Materials and Methods

The formulae given above were applied to starch bread kept for various storage times. σ_{el}^* and σ_f^* were taken to be equal to σ_1^*. E^*, σ_1^* and ρ^* were obtained from results presented in Chapter 5.

The solid material of the beams consists of a 47 wt% (= dry matter content of bread crumb) starch gel. Therefore, E_s and σ_{fs} would have to be determined from 47 wt% starch gels. However, at this high concentration, starch gels were inhomogeneous; the centres of the gels were more rigid than their outer parts. Consequently, results obtained from 10, 20, 30 and 40 wt% starch gels (Chapter 3) were extrapolated to a starch concentration of 47 wt%. With the extrapolated values of E_s and σ_{fs}, calculations were performed. The density of a 47 wt% starch gel was calculated from the densities of dry starch and water, assuming that their volumes are cumulative.

6.4 Results and Discussion

In Table 6.1, ρ^*, E^*, and σ_1^* of potato starch bread crumb and ρ_s, E_s and σ_{fs} of 47% potato starch gels are given as a function of storage time. The properties of the gels were obtained by extrapolation (section 6.3). Calculated values of C_1, C_2, and C_3 are also given in this table. Results obtained for the wheat starch systems are given in Table 6.2. It is shown that C_1, C_2, and C_3 hardly vary with time for both the potato

Table 6.1 Densities and parameters obtained for the mechanical properties of potato starch bread crumb and potato starch gels, and calculated values of C_1, C_2 and C_3 as a function of storage time.

Time (days)	ρ^* (kg·m⁻³)	ρ_s (kg·m⁻³)	E^* (kN·m⁻²)	E_s (kN·m⁻²)	σ_1^* (kN·m⁻²)	σ_{fs} (kN·m⁻²)	C_1	C_2	C_3
1	—	—	131	3.84×10^3	11.5	9.47×10^2	0.64	0.055	0.10
2	—	—	138	6.00×10^3	12.9	1.32×10^3	0.39	0.036	0.081
3	303	1240	157	6.28×10^3	14.9	1.56×10^3	0.42	0.039	0.079
7	—	—	169	6.46×10^3	16.1	1.83×10^3	0.44	0.041	0.073
11	—	—	176	6.48×10^3	16.6	1.97×10^3	0.45	0.043	0.070

Table 6.2 Densities and parameters obtained for the mechanical properties of wheat starch bread crumb and wheat starch gels, and calculated values of C_1, C_2 and C_3 as a function of storage time.

Time (days)	ρ^* (kg·m⁻³)	ρ_s (kg·m⁻³)	E^* (kN·m⁻²)	E_s (kN·m⁻²)	σ_1^* (kN·m⁻²)	σ_{fs} (kN·m⁻²)	C_1	C_2	C_3
1	—	—	33	4.66×10^2	4.72	1.79×10^2	0.73	0.10	0.15
2	—	—	50	6.71×10^2	6.89	1.85×10^2	0.77	0.11	0.21
4	388	1240	78	8.85×10^2	9.19	1.96×10^2	0.90	0.11	0.27
7	—	—	98	1.16×10^3	11.4	2.14×10^2	0.86	0.10	0.30
11	—	—	122	1.50×10^3	13.1	2.40×10^2	0.83	0.09	0.31

and wheat starch systems. Only for storage times of one day some values of the constants calculated were slightly different from those determined after longer ageing times.

During storage, starch bread becomes more crumbly. Shortly after baking, starch bread crumb recovers to a large extent after compressing it considerably for a short period, whereas staled bread does not. Presumably, deformation of fresh bread results in buckling of the beams. In stale bread, deformation causes mainly fracture of the beams. The mechanical properties of potato starch bread crumb changes very fast during the first day of storage. Directly after baking it is highly elastically deformable, but after one day it is already very crumbly. Then, the resistance to mechanical collapse of the structure is already very small, indicating that fracture of the beams already predominates. After one day, the mechanical properties change much more slowly. This may explain why C_2 and C_3 do not change markedly after the first day. It would be interesting to determine C_2 and C_3 for very fresh potato starch systems. However, this is not possible. Firstly, very fresh potato starch gels do not fracture. Consequently, it is not possible to determine σ_{fs}, and thus C_3 for very fresh potato starch systems. A second problem is that it is not possible to obtain identical cooling conditions for starch gels and starch breads. Especially for starch bread it was observed that stiffness increased markedly during cooling. Moreover, the mechanical properties change very fast during the first hours of storage. Therefore, an accurate estimate of C_1 and C_2 cannot be made. The mechanical properties of wheat starch bread change less rapidly. After one day of storage, wheat starch bread crumb is somewhat crumbly, but to a much lesser extent than potato starch bread crumb. Then, the resistance to collapse of its structure is larger than that of potato starch bread crumb, and after one day of storage, it continues to decrease. This may explain the slight increase of C_3 of wheat starch systems.

Ashby and Gibson[1,2] compared data from various cellular solids with uniform gas cell sizes and thicknesses of the beams. They found that C_1, which depends on the relation between the moduli and the structure, was approximately 1. For wheat starch bread, C_1 was slightly lower, whereas for potato starch bread, C_1 was lower by about a factor two. Figure 5.4 in Chapter 5 shows that the structures of potato and wheat starch bread crumb are rather uneven; the lengths and thicknesses of the beams vary to a large extent, especially those of potato starch bread. Probably, the structural imperfections account for the lower values of C_1.

For many cellular solids, C_2 was observed to be approximately 0.05. It is remarkable that C_2 of wheat starch systems is larger. In the first place, it is possible that this is due to the presence of some closed cells, which results in σ_1^* being somewhat larger. However, if closed cells would be present in wheat starch bread crumb, C_1 is expected to be larger than 1, but this is not the case. Another explanation of C_2 being larger than 0.05 is that some of the beams are too short to buckle, resulting in a higher σ_1^*, and, consequently, in a higher C_2. As wheat starch bread

crumb showed a decreased recoverability during storage, it was not expected that C_2 would remain constant. In potato starch bread crumb, the cells are much larger and moreover less uniform. Probably, by chance, C_2 of this system is approximately 0.05. As described above, after one day of storage deformation of potato starch bread crumb causes fracture rather than buckling of the beams. It is therefore remarkable that C_2 for potato starch bread crumb agrees so well with the theoretical value.

C_3 of potato starch systems is much smaller than that of some model systems,[1,2] whereas C_3 of the wheat starch systems is slightly lower. This confirms the suggestion given in Chapter 5 that the collapse of structure in potato starch bread crumb is much stronger than would be expected from the results obtained from the mechanical properties of the starch gels and the relative density. Attenburrow *et al.*[3] observed also for sponge cake that the critical stress for fracture showed poorer agreement with theory. As described in the previous chapter, this would be due to the large variations in the thicknesses of the beams in potato starch bread. Thick beams contain several layers of swollen granules (Figure 6.1). It is expected that the swollen granules in the thick beams are hooked into each other. The structure of thick beams is comparable with concentrated starch gels. As was described in Chapter 3, the fracture stress of such a system depends mainly on the stiffness of the swollen granules and only slightly on the properties of the thin amylose layer in between the swollen granules. It is expected that the fracture stress of thick beams is approximately equal to that of 47% starch gels. Thin beams are only a few granules thick (Figure 6.1). The fracture stress of this system probably is approximately equal to the fracture stress of the thin amylose layer in between the swollen granules, and it is therefore lower than the fracture stress of thick beams. Consequently, in starch bread crumb with both very thin (some granules thick) and thick beams, the critical stresses for fracture of the beams within one piece of bread crumb may vary considerably. This may explain that C_3 of potato starch bread crumb is much lower than the theoretical value.

A   B
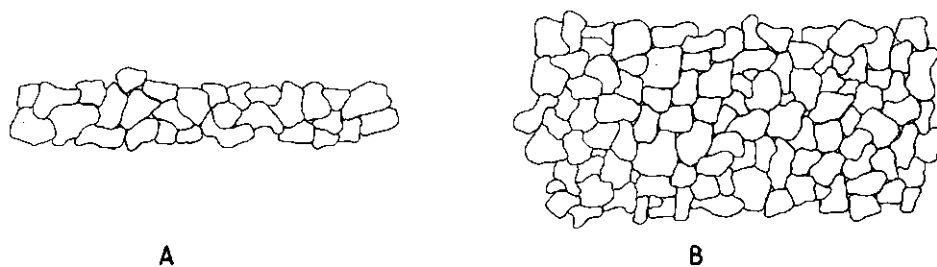
Figure 6.1   *Highly schematic presentations of the structure of thin (A) and thick (B) beams in starch bread, consisting of swollen granules. The fracture stress of the thin beams is lower than that of the thick beams. For an explanation see text.*

Several explanations can be thought of for C_3 of wheat starch bread crumb being higher than that of potato starch bread crumb. Firstly, wheat starch bread crumb shows less variations in thicknesses of the beams. Secondly, it was proposed (Chapter 3) that swollen wheat starch granules are much less hooked into each other than are swollen potato starch granules. The difference in the critical stresses for fracture of thin and thick beams may therefore be less. Moreover, the mean size of wheat starch granules is lower by about a factor 2 than that of potato starch granules (Chapter 2). Consequently, one passes twice as many granules when travelling from one side to the other side of the beam, which results in a behaviour more related to structure B with a higher σ_f.

6.5   Conclusions

Application of the theory developed for cellular solids with a homogeneous structure to starch bread contributes to the understanding of their mechanical properties. The Young modulus of starch bread is only slightly affected by structural inhomogeneities. However, structural imperfections, like variations in cell sizes and thicknesses of the beams, highly affect large deformation properties of starch bread, in particular fracture behaviour. This would be caused by differences in the fracture behaviour between very thin and thick beams. Therefore, the ratio of particle size to beam thickness is extremely important.

6.6   References

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Chapter 7

The Effect of Lipid Surfactants on the Structure and Mechanics of Concentrated Starch Gels and Starch Bread

Summary

The effect of lipid surfactants (emulsifiers) on changes in the mechanical properties of concentrated starch systems during heating, cooling and storage was studied. In the presence of glycerol monostearate (GMS) or sodium stearoyl-2-lactylate (SSL), the dynamic moduli of potato and wheat starch systems during heating and cooling were somewhat higher and the increase in stiffness of the gels during storage was slightly retarded. Furthermore, the fracture stresses and strains of these starch gels were lower if GMS or SSL had been added to the systems. These changes in mechanical properties were ascribed to complex formation between amylose and the lipid surfactants. No effect of GMS and SSL on the mechanical properties of waxy maize starch systems was observed. The effect of GMS and SSL on the mechanical properties of wheat starch bread was also studied. It was found that neither the increase in the Young modulus during storage nor the recrystallization of amylopectin were significantly affected by these substances. However, the large deformation properties of the breads were affected. The effect of lipid surfactants on the mechanical properties of starch gels and starch bread are compared.

7.1   Introduction

It is well established that the shelf-life of bread can be increased by the addition of some lipid surfactants (emulsifiers), such as monoglycerides and sodium stearoyl-2-lactylate, to the dough.[1-3] These substances are known to interact with amylose during gelatinization; they form a helical inclusion complex, the emulsifier occupying the central axis of the amylose helix.[4] The amylose-lipid complex can be obtained in crystalline form, which gives rise to a V-type X-ray diffraction pattern.[5] These crystals

have a lamellar structure in which the amylose chains are folded.[6,7] The amylose-lipid complexes dissociate at high temperatures; differential scanning calorimetry (DSC) reveals this dissociation by an endothermic transition. This transition occurs at higher temperatures than starch gelatinization. The temperature depends on the water content[8,9] and the type of lipid surfactant in the complex.[10-13] In bread, dissociation of the complexes occurs at temperatures near 120 °C.[1]

Provided that there is enough water present for the granules to swell fully, lipid surfactants present during gelatinization of starch drastically change the swelling power of the granules and the leaching out of amylose. Most substances cause a decrease of swelling power and amylose leaching.[14-18] The rheological properties of starch-water systems during heating are also affected by addition of lipid surfactants. Rheological properties of starch-water systems have been studied by the use of a Brabender viscograph or by the use of dynamic rheological measurements. When heating starch-water systems, an increase in consistency can be observed above a certain temperature; upon further heating this increase is followed by a decrease. It has been observed that in the presence of lipid surfactants, the consistency starts to increase at higher temperatures and the peak values are reached at higher temperatures.[14,16,18,19] Lipid surfactants generally increase the consistency of starch systems kept at a high temperature (e.g. 95 °C) and during their cooling after gelatinization.

The increase in stiffness of starch gels[20-23] and bread[1,3,24] during storage is said to be retarded by addition of lipid surfactants. This is remarkable, as it has been accepted for a long time that amylopectin, and not amylose, is the main component causing the increase in stiffness.[25] Until now it is not clear how the amylose-lipid complexes would affect the recrystallization of amylopectin. It has been suggested that also amylopectin forms complexes with lipid surfactants,[1,20,26-29] which would result in a slower rate of recrystallization of amylopectin.

In general, lipid surfactants in bread do not only prolong shelf-life, but also improve loaf volume and texture of bread. In this respect, it has been suggested that these substances may act as dough conditioners in two ways. Firstly, they may interact with gluten resulting in a "reinforcement" of the gluten network.[30] However, addition of lipid surfactants to dough does not affect the rheological properties of dough.[31] A second possibility is, that the lipid surfactants improve the gas cell stability in the dough and, consequently, the loaf volume and the texture of bread.[30,31]

Much work has been done on the effect of lipid surfactants on the mechanical properties of bread as well as of model systems, such as starch gels, during storage. Most of it concerned rheological measurements at small deformations.[1,20,22,24] However, knowledge of large deformation properties of these systems is far more relevant, because consumers' perception of product properties is usually related to large deformation properties. The present investigation was undertaken to gain understanding of the role of lipid surfactants in the structure and large-deformation properties of bread. To this end, concentrated starch gels and starch bread were

studied. The structure and mechanics of concentrated starch gels and starch breads were discussed in detail in the Chapters 3 and 5. In this chapter, first the effect of two widely used lipid surfactants, glycerol monostearate (GMS) and sodium stearoyl-2-lactylate (SSL), on the mechanical properties of concentrated starch systems during heating, cooling and storage will be discussed, as well as the relation between these properties and gel structure. Three different starches were used; potato starch (no lipids by nature), wheat starch (lipids by nature) and waxy maize starch (no lipids and no amylose by nature). Subsequently, the effect of GMS and SSL on the mechanical properties of wheat starch bread will be discussed.

7.2   Materials and Methods

7.2.1 Materials

Potato starch and waxy maize starch were obtained from AVEBE (Foxhol, the Netherlands) and wheat starch from Latenstein BV (Nijmegen, the Netherlands). The emulsifiers used were commercial samples of glycerol monostearate and sodium stearoyl-2-lactylate (Quest International, Zwijndrecht, the Netherlands). Xanthan was obtained from Suiker Unie (Roosendaal, the Netherlands).

7.2.2 Preparation of concentrated starch gels

Suspensions of GMS or SSL in water were prepared by first adding the emulsifiers to the water and heating them to 68 °C, while being gently stirred. The amount of emulsifier added to the water was such that its concentration at the end of the preparation procedure was 0.25 wt% on dry starch, unless mentioned otherwise. After cooling the suspensions to approximately 50 °C, a small amount of potato, wheat or waxy-maize starch was added, and these dispersions were heated again to 68 °C under gentle stirring. After cooling to room temperature, sufficient starch was added to obtain suspensions with 30 wt% starch. In this way, sedimentation of the starch granules during preparation of the gels was avoided. For small deformation experiments on changes in the mechanical properties during heating and cooling, the starch suspensions were transferred to a Bohlin VOR Rheometer (see below). Test pieces for the large deformation experiments were prepared by filling teflon cylindrical moulds with the suspensions. The moulds had an inner diameter of 15 mm and an inner length of 100 mm. The filled moulds were heated in an oil bath at 95 °C for 90 min. Next they were cooled to and stored at 20 °C, except for the waxy maize starch gels, which were cooled to and stored at 7 °C. In this way, homogeneous samples without visible defects could be made. The cylindrical samples were cut into test pieces of 20 mm height.

7.2.3 Preparation of wheat starch bread

A comprehensive description of the formula and breadmaking process of wheat starch bread has been given in Chapter 5. GMS and SSL, 0.25% on dry starch, were added in the form of a 9% suspension to the xanthan solution simultaneously with the other ingredients. Test pieces with a height of 33 mm and a diameter of 38.5 mm were taken from the bread crumb according to the procedure described in Chapter 5.

7.2.4 Small deformation experiments

Dynamic mechanical properties of concentrated starch suspensions during heating and cooling were determined by applying a small oscillating shear deformation using a Bohlin VOR Rheometer, equipped with concentric cylinders and a torsion bar of 8.8 or 20 mN·m. The starch suspensions were heated to 90 °C, kept at this temperature for 15 minutes, cooled to 20 °C, and kept at this temperature. Heating and cooling were performed at a rate of 2 K·min^{-1}. Sequential measurements were made every 30 s. Oscillation frequency was 0.1 Hz and strain amplitude 0.01. At this strain all samples showed linear behaviour. To prevent evaporation, samples were covered with paraffin oil.

7.2.5 Large deformation experiments

Large deformation properties of concentrated starch gels and starch breads were measured in uniaxial compression in a Zwick material testing machine, fitted with a 2 kN or 50 N load cell. Cylindrical test pieces were compressed between perspex plates at an initial strain rate of 1.7×10^{-2} s^{-1}. From the force-displacement curve, compressive stress σ versus strain curves were calculated (Chapters 3 and 5). For the concentrated starch gels the Hencky strain ϵ_h, and for starch breads the Cauchy strain ϵ_c was used as a strain measure. From the stress-strain curve the Young modulus E = $(d\sigma/d\epsilon)_{\epsilon \to 0}$ could be determined. For starch bread this was corrected for onset effects (Chapter 5). Test pieces of concentrated starch gels were compressed up to fracture. Each test piece of starch bread was compressed twice (Chapter 5), each compression being followed by decompression. The time interval between the end of the first decompression and the second compression was one minute. The maximum deformation was 0.4. From the stress-strain curves of the starch breads the parameters σ_1^*, σ_2^* and ϵ_1^* could be derived (Chapter 5). σ_1^* and σ_2^* are defined as the critical values of the stress at which the increase in stress with increasing deformation decreases dramatically during the first and second compression, respectively (Chapter 5). The ratio between σ_2^* and σ_1^* is considered a measure of the resistance of the bread to collapse of structure during the first compression. The deformation at which the initial region of the first compression changes to a plateau region is called ϵ_1^*.

Measurements were made after various storage times up to 11 days for starch breads and 16 days for concentrated starch gels. For a comprehensive discussion of the determination of the large deformation properties of concentrated starch gels and starch breads see Chapters 3 and 5.

7.2.6 Differential scanning calorimetry

DSC measurements were performed using a TA Instrument DSC-2910. Approximately 70 mg of bread crumb was weighed out in stainless steel pressure cups just before measuring. An empty cup was used as a reference. The cups were heated from 20 to 160 °C at a scanning rate of 5 $K \cdot min^{-1}$.

7.3 Results

7.3.1 Gels

On heating 30% potato, wheat and waxy maize starch suspensions, the storage moduli started to increase strongly at temperatures of approximately 55 to 65 °C, reached a maximum at somewhat higher temperatures, and subsequently decreased (Figure 7.1). On cooling, the moduli increased again. In the presence of 0.25% GMS and 0.25% SSL the stiffness of 30% potato starch systems was considerably higher. Additions of 0.25% GMS and 0.25% SSL to 30% wheat starch systems also resulted in an increase in G', although their effect on the mechanical properties of these systems was less than that on 30% potato starch systems. The temperature at which the maximum in the storage moduli of potato and wheat starch systems was reached did not significantly change in the presence of GMS and SSL. No significant effect of 0.25% GMS or 0.25% SSL on the mechanical properties of a 30% waxy maize starch system during heating was observed. The moduli of this system with GMS and SSL seemed to be somewhat higher during cooling, but the curves were very irregular.
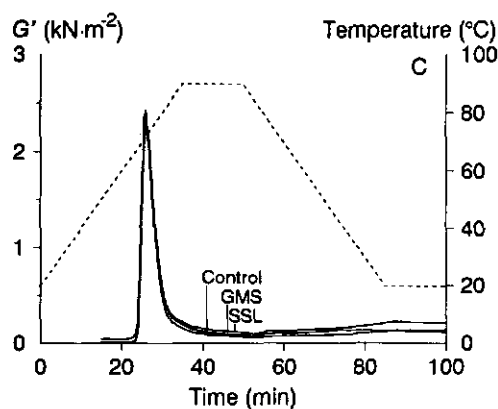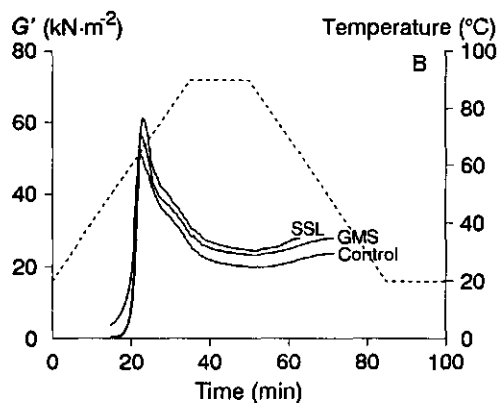
The effect of the GMS concentration on the mechanical properties of 30% potato starch systems during heating and cooling is shown in Figure 7.2. Increasing the GMS concentration from 0 to 0.25% resulted in an increase of the storage moduli over the whole temperature range after the moduli started to increase. At still higher GMS concentrations, the peak modulus was hardly larger. However, during further heating to temperatures above approximately 70 °C, during the cooling stage and during short storage times, higher GMS concentrations resulted in higher moduli.

Concentrated 30% potato starch, wheat starch and waxy maize starch suspensions were heated in teflon cylindrical moulds at 95 °C for 90 minutes. Directly after cooling, some gels were removed from the moulds. Then, the potato starch and waxy
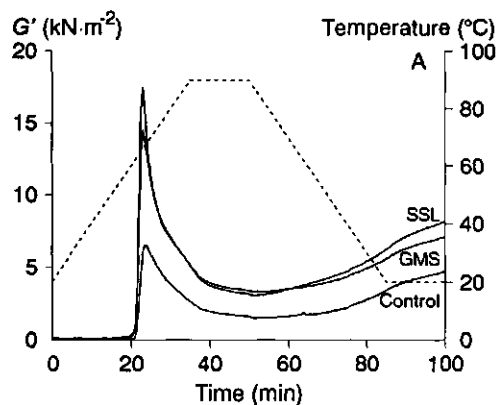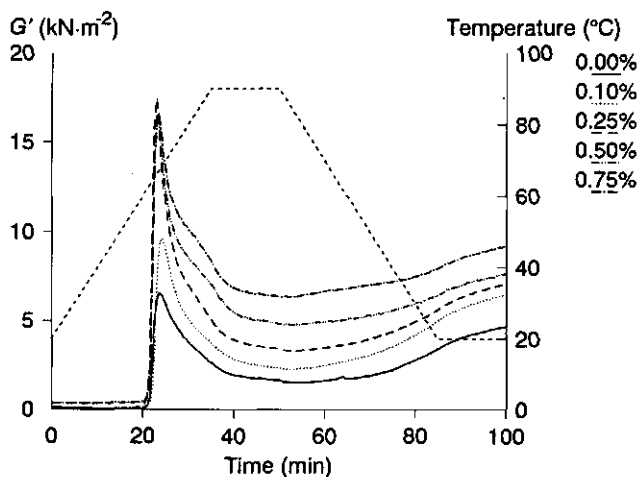
Figure 7.1
The effect of 0.25% SSL and 0.25% GMS on the storage moduli of 30% starch suspensions as a function of time during a heating and cooling cycle: (A) potato starch; (B) wheat starch; (C) waxy maize starch. The dashed lines show temperature against time.

Figure 7.2 *The effect of the GMS concentration (wt% on dry matter) on the storage moduli of 30% potato starch suspensions as a function of time during a heating and cooling cycle. The dashed line shows temperature against time.*

maize starch gels were transparent, whereas wheat starch gels were opaque. After some hours, the potato and waxy maize starch gels also became opaque. Addition of GMS and SSL did not affect the appearance of the starch gels.

The effect of 0.25% GMS and 0.25% SSL on the mechanical properties of 30% potato, wheat and waxy maize starch gels during storage is shown in the Figures 7.3, 7.4 and 7.5, respectively. The results are averages of at least six replicates. The repeatability was good, *i.e.* the variation was mostly less than 5% and never more than 10%. Fracture of the gels occurred around the granules at the maximum of the stress-strain curve. During storage, the Young moduli and the fracture stresses of the gels increased and the fracture strains decreased. In the presence of GMS and SSL, the increase in stiffness of potato starch gels was somewhat retarded (Figure 7.3A). GMS and SSL had hardly any effect on the moduli of wheat starch and waxy maize starch gels (Figures 7.4A and 7.5A). Addition of GMS and SSL to the concentrated potato and wheat starch systems resulted in a decrease of the fracture stresses and fracture strains (Figures 7.3B-D and 7.4B-D), except for the very fresh potato starch gels. Generally, GMS had a somewhat more pronounced effect than SSL. The effects of GMS and SSL on the mechanical behaviour at large deformations of waxy maize starch gels were negligible (Figures 7.5B-D).
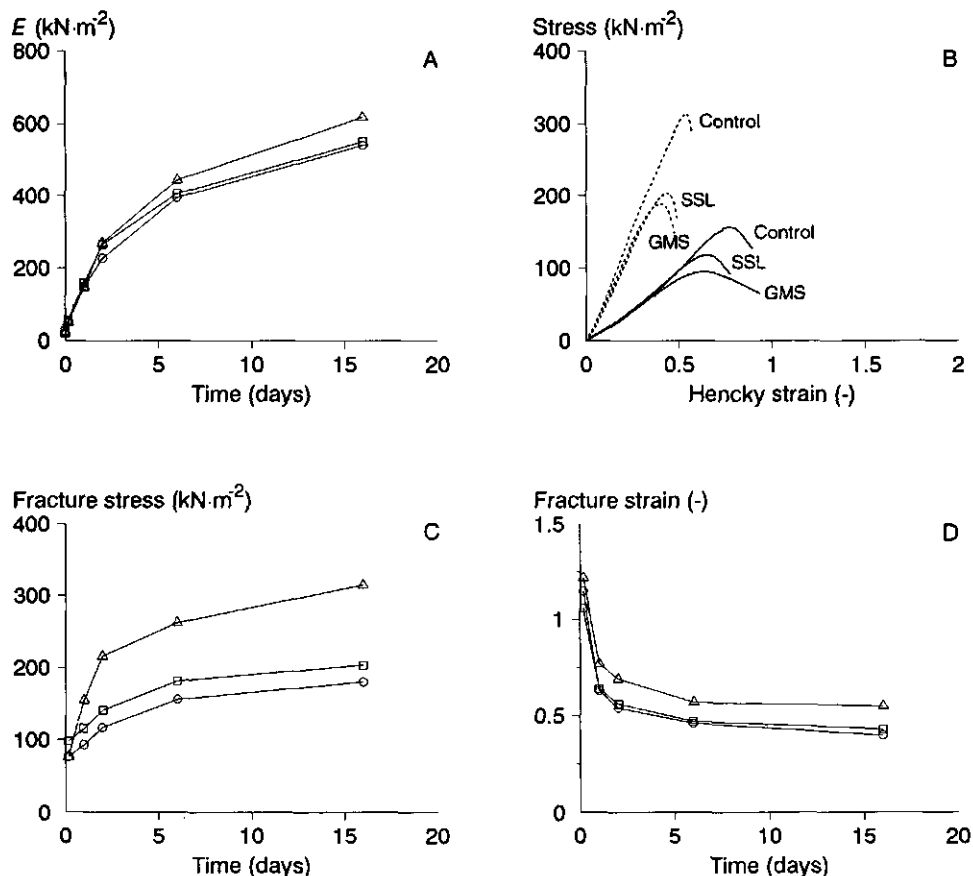
Figure 7.3 *The mechanical properties of 30% potato starch gels during storage at 20 ˚C:*
(▲) control; (□) 0.25% SSL; (o) 0.25% GMS.
A. Young moduli versus time,
B. Stress-strain curves determined after 1 day (solid lines) and 16 days
*   (dashed lines),*
C. Fracture stresses versus time,
D. Fracture strains versus time.
The initial strain rate was $1.7\times10^{2}\ s^{-1}$.

7.3.2 Starch bread

   The structure of wheat starch bread with and without lipid surfactants is shown in
Figure 7.6. Bread with 0.25% SSL was somewhat more regular than the control, but
the effect was not very pronounced. However, the structure of the wheat starch breads

Figure 7.4 *The mechanical properties of 30% wheat starch gels during storage at 20 °C:*
(▲) control; (□) 0.25% SSL; (○) 0.25% GMS.
A. Young moduli versus time,
B. Stress-strain curves determined after 1 day (solid lines) and 16 days
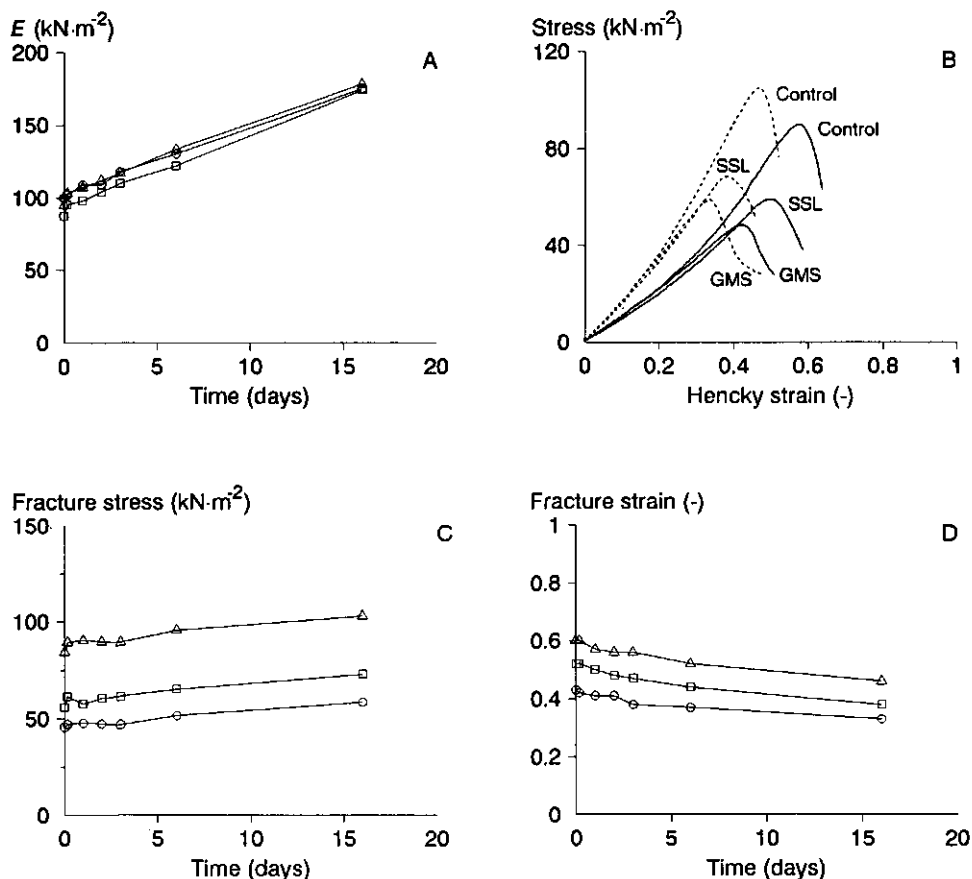*(dashed lines),*
C. Fracture stresses versus time,
D. Fracture strains versus time.
The initial strain rate was 1.7×10² s⁻¹.

was significantly different in the presence of 0.25% GMS; the crumb structure of this bread was much finer and more homogeneous. The dry matter contents of the various wheat starch breads were approximately the same (Table 7.1). The density of the wheat starch breads with GMS was somewhat higher.

Figure 7.5   *The mechanical properties of 30% waxy maize starch gels during storage at 20 °C: (▲) control; (□) 0.25% SSL; (o) 0.25% GMS.*

A. Young moduli versus time,

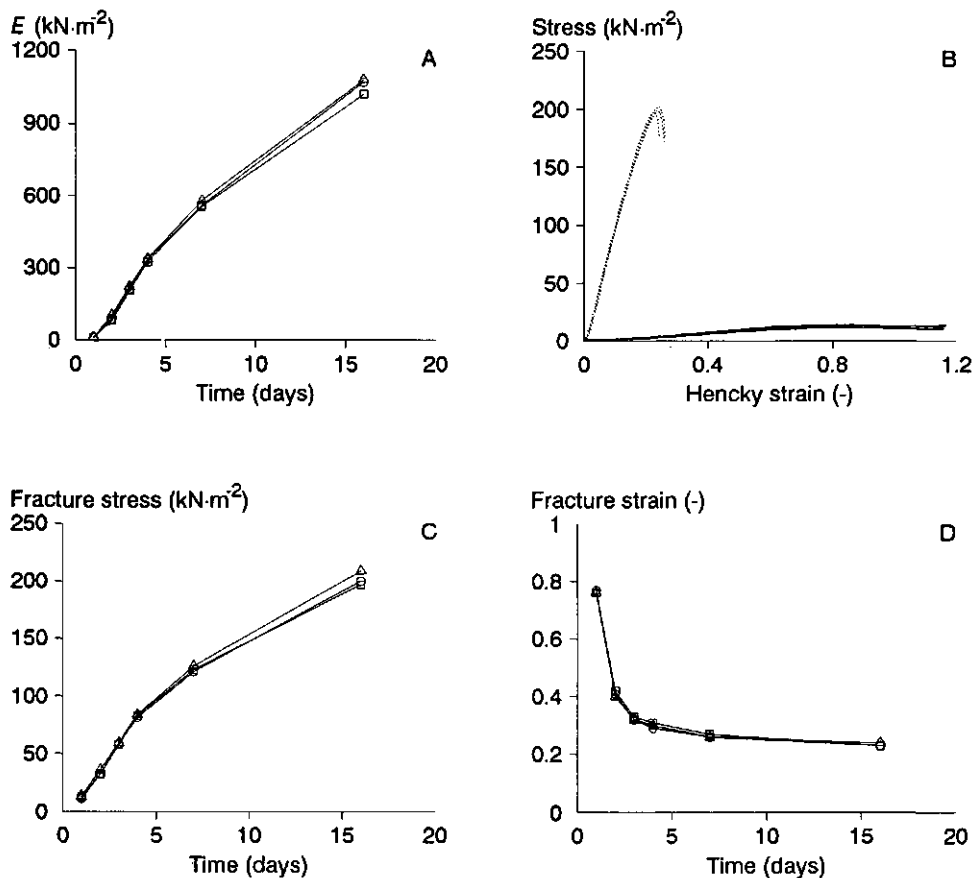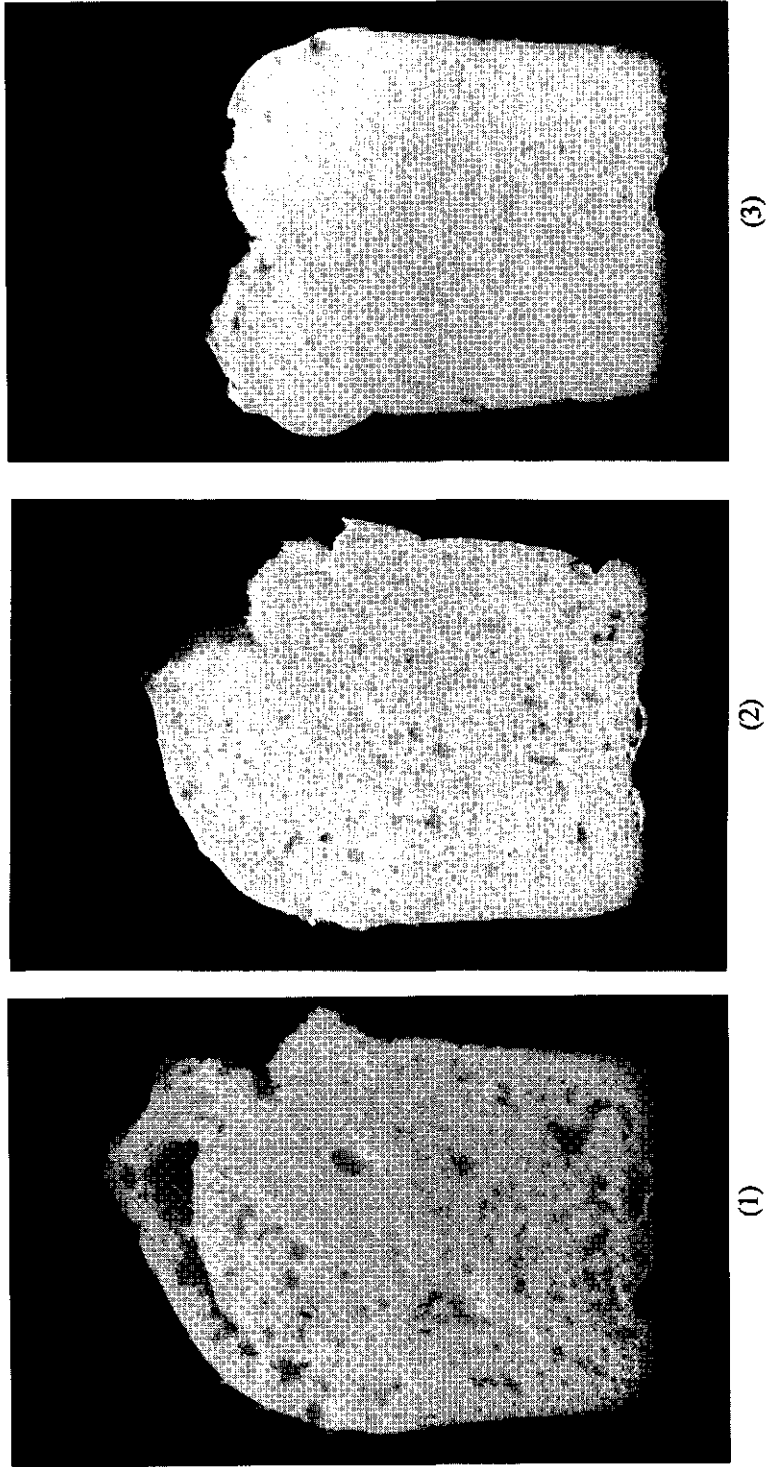*B. Stress-strain curves determined after 1 day (solid lines) and 16 days (dashed lines),*

C. Fracture stresses versus time,

D. Fracture strains versus time.

*The initial strain rate was $1.7 \times 10^2 s^{-1}$.*

Figure 7.6 *Bread baked from wheat starch without a lipid surfactant (1), with 0.25% SSL (2) and with 0.25% GMS (3).*

Table 7.1 *Dry matter content and density of the crumb of wheat starch breads without a lipid surfactant, with SSL and with GMS.*

	without a lipid surfactant	with SSL	with GMS
Dry matter (wt%)	47.2	46.9	46.5
Density (kg·m⁻³)	388 ± 14	389 ± 12	424 ± 12

The effects of 0.25% SSL and 0.25% GMS on the change in enthalpy by melting of amylopectin crystallites, ΔH, of wheat starch bread as a function of storage time are shown in Figure 7.7. Addition of GMS and SSL had no effect on the recrystallization of amylopectin during storage. When testing wheat starch bread crumb with DSC, an endothermic transition at approximately 113 °C can also be observed. This transition is ascribed to dissociation of the amylose-lipid complex, the lipid part of which originated from the wheat starch. Addition of SSL, and in particular of GMS, increased the high-temperature enthalpy change (Table 7.2). This would imply that GMS has greater complex-forming ability than SSL. The thermal transitions of the amylose-lipid complexes did not change during storage.
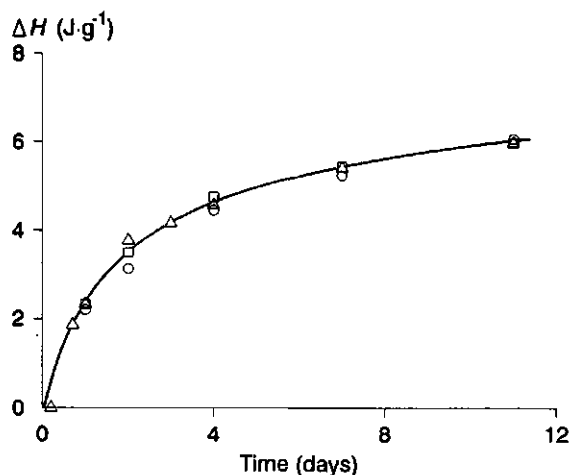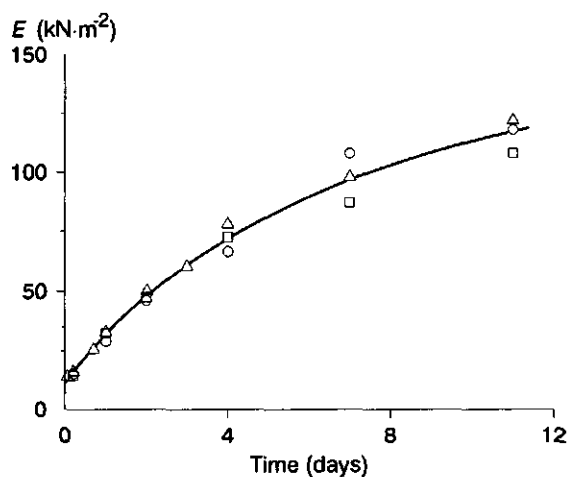

Figure 7.7   *The melting enthalpies per g dry bread crumb, ΔH, as a function of storage time for wheat starch bread without a lipid surfactant (▵), with 0.25% SSL (□) and with 0.25% GMS (o). The storage temperature was 20 °C.*

Table 7.2 *Effect of SSL and GMS on the high-temperature transition enthalpy (J/g dry matter) in wheat starch bread.*

	transition enthalpy $(J \cdot g^{-1})$
without a lipid surfactant	2.12
with SSL	2.33
with GMS	3.13

The effects of 0.25% SSL and 0.25% GMS on the Young moduli, E, of wheat starch bread as a function of storage time are shown in Figure 7.8. The results of the mechanical tests are the averages of at least six replicates. The repeatability of the moduli of the wheat starch breads was rather good in view of the natural variation in structure of these products, *i.e.* the relative standard deviation was 10% on average. No significant effect of GMS or SSL on the stiffness of the breads during storage could be observed.

Figure 7.8 *The Young moduli as a function of storage time for wheat starch bread without a lipid surfactant (▲), with 0.25% SSL (□) and with 0.25% GMS (o). The storage temperature was 20 °C.*

Stress (kN·m^2)
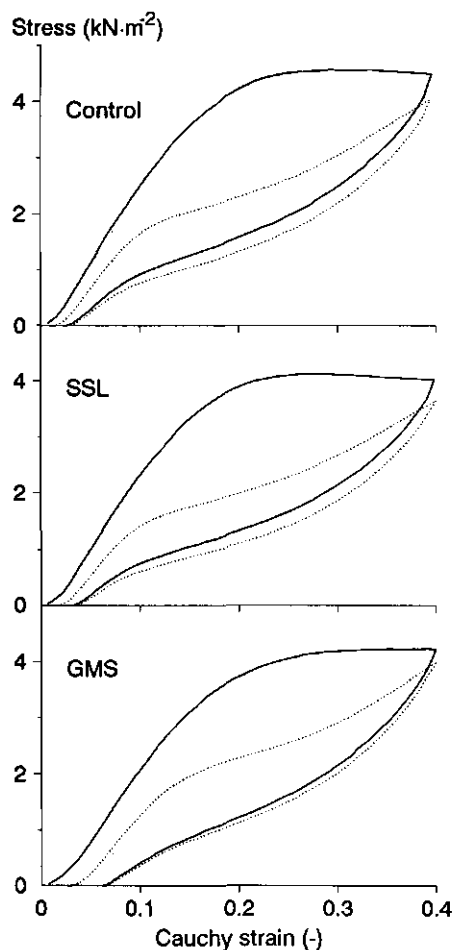
Figure 7.9
Stress-strain curves of wheat starch bread stored for one day at 20 °C. The solid lines show the first compression and decompression and the dashed lines the second ones. The initial strain rate was 1.7×10^2 s^{-1}.

The stress-strain curves of wheat starch breads with and without lipid surfactants after one day of storage are shown in Figure 7.9. No significant difference was observed for the values of σ_1^* and ϵ_1^*. The relative standard deviation of the values of σ_1^* was approximately 10 to 15%. Again this is reasonable considering the inhomogeneous nature of the product. Nevertheless, values of σ_2^*/σ_1^* observed for wheat starch bread with GMS were significantly higher than those for wheat starch breads with SSL and without an lipid surfactant; the latter two were approximately the same (Figure 7.10). This means that the resistance against collapse of structure during the first compression became higher by addition of GMS. This difference in σ_2^*/σ_1^* was observed during the whole storage period, except for the very fresh bread. This may be due to a slight, but visible damage of the test pieces of this bread during sample preparation, due to the crumb being sticky.
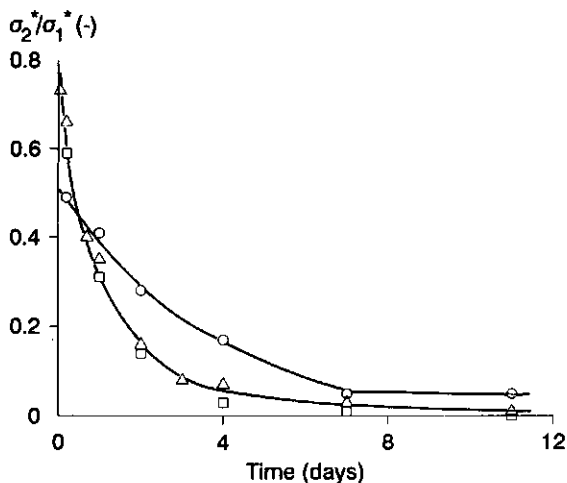
Figure 7.10 σ_2^*/σ_1^* *as a function of storage time for wheat starch bread without a lipid surfactant* ($\triangle$), *with 0.25% SSL* ($\square$) *and with 0.25% GMS* (o). *The storage temperature was 20 °C.*

7.4 Discussion

7.4.1 Concentrated starch suspensions during heating and cooling

When heating concentrated suspensions of starch in water, several changes in the structure of the granules occur. These processes are mutually related and they cause a change in the rheological behaviour of the starch system. The effect of starch gelatinization on the storage modulus of concentrated starch systems is shown in Figure 7.1. The initial increase of the moduli can be ascribed to the starch granules swelling progressively and starting to fill the whole sample volume. It has been observed (Chapter 2) that the initial increase of the moduli coincides with the first stages of crystallite melting. As the amount of water present in the system is limited, the granules are only partly swollen. They fully occupy the available volume at the moment the moduli reach their maximum. From then on, the stiffness of the starch system approximately equals the stiffness of the granules. The decrease in the moduli on prolonged heating is the resultant of two different processes (Chapter 2). In the first place, melting of remaining crystallites in the granules will result in the granules becoming softer and therefore cause a decrease in the modulus. Secondly, in areas that have already lost their crystallinity, the number of entanglements between starch molecules decreases, resulting in a further breakdown of the amylopectin matrix.

Another process occurring during heating of starch suspensions is a partly separation of amylose and amylopectin, since these materials are thermodynamically incompatible in solution.[32] In concentrated starch systems, amylose and amylopectin separate only partly and, consequently, only a small amount of the amylose molecules leaches out of the granules (Chapter 2).

During heating of starch suspensions amylose molecules can form inclusion complexes with added lipid surfactants. This complex formation occurs only after the granules start to swell, because from that point on the mobility of the amylose molecules rapidly increases. Probably, the lipid surfactants primarily form a complex with amylose molecules leached out of the granules. However, it is supposed that these substances may also form a complex with amylose molecules still present in the swollen granules, because the lipid surfactant molecules are small enough to diffuse into the swollen granules. Complex formation of lipid surfactants with amylose molecules in the swollen granules may retard the separation of amylose and amylopectin, because the flexibility of the complex is much smaller than that of free amylose molecules. As the separation of amylose and amylopectin and the loss of entanglements are probably coupled processes, complex formation of lipid surfactants with amylose molecules would indirectly retard weakening of the amylopectin matrix inasfar as the weakening is due to loss of entanglements between the starch molecules within the swollen granules. In my opinion, this mechanism would explain the increase in the moduli of potato and wheat starch systems by the addition of GMS and SSL (Figure 7.1). The effect of GMS and SSL on the moduli of wheat starch suspensions during heating (Figure 7.1B) is somewhat smaller than the effect on those of the potato starch systems (Figure 7.1A). This may be due to the fact that swollen wheat starch granules contain lipids by nature. Probably, in a gelatinized wheat starch system, a considerable amount of amylose molecules would already have formed a lipid complex with the naturally present lipids, and therefore the addition of GMS and SSL would have a smaller effect. This explanation is supported by the observation that the additional increase in the modulus of 30% potato starch systems by the addition of GMS decreases with an increasing amount of GMS already present (Figure 7.2). Waxy maize starch hardly contains amylose. Consequently, no formation of amylose-lipid complexes would occur if GMS or SSL is added to this system. This may explain the observation that addition of GMS or SSL did not affect the rheological properties of this system.

If all crystallites have melted, the degree of swelling of starch granules most likely is affected by the number of entanglements present in the swollen granules (Chapter 2). A larger number of entanglements would then result in a smaller swelling capacity. Addition of lipid surfactants would retard the loss of entanglements within the granules during heating (see preceding paragraph) and, consequently, reduce the swelling capacity of the granules. Consequently, the peak modulus would shift to higher temperatures. At high starch concentrations, as is used in this study, a slight swelling

of the granules is sufficient to make the system tightly packed; therefore, the predicted effect of lipid surfactants is masked and cannot easily be observed. In addition, at the moment the granules will become tightly packed, crystallites are still present, and this may further curtail the effect of lipid surfactants. The predicted effect of lipid surfactants will, on the other hand, be obvious in dilute starch systems, which are the systems that have mostly been studied in the past.[14,16,18,19]

7.4.2 *Concentrated starch gels during storage*

During storage of concentrated starch gels, an increase in the stiffness of the gels occurred, which must mainly be due to recrystallization of amylopectin. Addition of GMS and SSL to 30% potato starch gels somewhat retarded the increase in the moduli (Figure 7.3A). It is not clear how the recrystallization of amylopectin could be affected by the complex formation of the lipid surfactants with amylose. My hypothesis is that, in the presence of lipid surfactants, the disentanglement of the starch molecules during heating is somewhat retarded (see above). Following my earlier suggestion that the relative increase in stiffness as a result of retrogradation would be less if more entanglements between the starch molecules are already present (Chapter 3), the formation of amylose-lipid complexes may retard the increase in stiffness during storage due to a decreased local mobility. It has also been suggested that recrystallization of amylopectin is retarded by complex formation of lipid surfactants with amylopectin.[1,20,26-29] The results presented in this chapter do not support this hypothesis. No effect of lipid surfactants was observed on the increase of the moduli of waxy maize starch gels during storage (Figure 7.5A). Also, hardly any effect of SSL or GMS on the moduli of wheat starch gels during storage was observed (Figure 7.4A), maybe because a considerable amount of the amylose molecules had already formed a complex with the natural lipids in wheat starch. Addition of lipid surfactants would therefore have little effect. This supports my suggestion that any effect on the recrystallization of amylopectin would be due to complex formation of lipid surfactants with amylose and it belies substantial complex formation between amylopectin and the lipid surfactant used.

It has been concluded before that concentrated starch gels consist of partly swollen granules, which almost fully occupy the available volume. Moreover, the granules have a very irregular shape. Between the swollen granules a thin amylose gel layer is present. If large deformations are imposed on concentrated starch gels, any fracture occurs around the granules, in the thin amylose gel layer. The fracture stress of a concentrated starch gel would thus depend both on the fracture stress of the thin amylose gel layer, and, since the granules have a very irregular shape, on the stiffness of the granules (Chapter 3). The fracture strain of a concentrated starch gel then would be roughly the strain of the granules at fracture of the gel. Addition of GMS and SSL to 30% potato and wheat starch gels reduces their fracture stress and strain

(Figure 7.3B-D and 7.4B-D). The most plausible explanation for this behaviour is that the formation of the thin amylose gel in between the swollen granules is disturbed by the complex formation between leached out amylose molecules and the lipid surfactants. This may result in a lower fracture stress of the thin amylose gel layer and, consequently, in a lower fracture stress and strain of the starch gels. No effect of GMS and SSL on the large deformation properties of 30% waxy maize starch gels was observed (Figures 7.5B-D); this supports the idea that lipid complexes act only via amylose.

Some differences were observed to exist between the properties of potato and wheat starch gels (Chapters 2 and 3), the amylose level in these starches being approximately the same (Table 2.1). The moduli during heating of 30% potato starch systems were much lower than those of 30% wheat starch systems. Furthermore, the increase in the moduli of 30% potato starch gels during storage was much more pronounced, resulting in a cross-over of the moduli of 30% potato and wheat starch gels. It has been suggested that these differences may be due to the presence of lipids in wheat starch.[33] However, my results show that, although the mechanical properties of 30% potato starch systems were somewhat affected by the presence of SSL or GMS, they certainly became not similar to those of 30% wheat starch systems (compare Figures 7.1A and 7.2 with Figure 7.1B, and Figure 7.3A with Figure 7.4A). Moreover, the relative increase in stiffness of the 30% potato starch gels with GMS and SSL was far higher than the increase in stiffness of 30% wheat starch gels (compare Figure 7.3A with Figure 7.4A). Consequently, the differences between concentrated potato and wheat starch systems can not solely be ascribed to lipids present in wheat starch. Other explanations for these different properties have been suggested before in Chapters 2 and 3.

7.4.3 Starch bread

In Chapter 5, the structure and mechanical properties of concentrated starch gels and starch breads were compared. It was argued that the inner structure of the lamellae and beams of which the starch breads are built is closely similar to the structure of concentrated starch gels. The mechanical behaviour of these lamellae and beams as a function of storage time would therefore be like that of concentrated starch gels. However, it has been shown in Chapter 5 and 6 that the mechanical properties of starch breads do not only depend on the mechanical properties of the lamellae and beams, but also on the size distribution of the lamellae and beams and on the local densities in the structure of the breads. The effect of GMS and SSL on the structure and mechanics of wheat starch breads will now be discussed. It has been shown (Figures 7.7 and 7.8) that these substances have hardly any effect on the moduli of the starch breads nor on the recrystallization of amylopectin, as reflected in the change in ΔH. Thus, no retardation of these processes has been observed, which seems to be

in contradiction to the effects observed with bread from wheat flour.[1,3,24] Maybe the higher lipid surfactant concentrations used in the latter studies explain this difference. It is also notable that addition of lipid surfactants to wheat starch bread hardly affected the loaf volume, whereas loaf volume usually increases by the addition of lipid surfactants.

The large deformation properties of wheat starch bread as a function of storage time were affected by addition of GMS, but the effect of SSL on the mechanics of the bread was rather small (Figures 7.9 and 7.10). The most remarkable change is the increase in σ_2^*/σ_1^*, which means that the resistance against collapse of structure during the first compression is increased by addition of GMS. The question then is how the lipid surfactants affect the mechanical properties of the starch bread. It has been observed that the fracture stress and the fracture strain of concentrated wheat starch gels decreased by addition of lipid surfactants (Figure 7.4B-D). This would imply that the lamellae and beams of the starch breads containing lipid surfactants would be more brittle and consequently, σ_2^*/σ_1^* of the starch breads would be lower instead of higher. Thus, if the lipid surfactants would only act due to the complex formation of amylose affecting the mechanical properties of the lamellae and beams, the starch breads would be more crumbly and softer. However, not only the mechanical properties of the lamellae and beams forming the sponge structure are affected by addition of lipid surfactants to the starch breads. Such an addition also affects (normally improves) gas cell stability in the dough during breadmaking and with this the crumb structure of the bread. This latter effect may have caused the observed increase in σ_2^*/σ_1^* by the addition of GMS. Possibly, in the case of breads with added SSL, the consequence of the changes in the properties of the lamellae and beams and the changes in the overall structure of the bread balance each other, which may explain that hardly any effect of SSL on the mechanical properties of the bread crumb was observed.

7.5   Conclusions

It has been shown that the lipid surfactants GMS and SSL have hardly any effect on the increase in stiffness of concentrated starch gels and starch breads nor on the rate of recrystallization of amylopectin. However, the large deformation properties of concentrated starch gels and starch breads are affected by these substances. It is concluded that the action of lipid surfactant on the mechanical properties of bread crumb acts in two opposite ways; by affecting the properties of the lamellae and beams forming the bread structure and by making the crumb structure finer and more even.

7.6   References

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Chapter 8

General Discussion

The aim of this study was to describe the structure of concentrated starch systems and to understand the relation between this structure and their mechanical properties as a function of ageing time, to obtain a better understanding of the consequences of starch recrystallization on the staling of bread. The research described in this thesis started with relatively simple starch gels as model systems, in which the effects of gas cells and various bread components, like gluten and lipids, were excluded. Knowledge of the structure-mechanics relationship of the gels resulted in a better understanding of the effect of starch recrystallization on the mechanical properties of starch bread crumb, in which especially the presence of gas cells complicates the structure. However, the structure of starch bread crumb is still relatively simple compared to commercial bread.

In this chapter, a general discussion of the effect of starch recrystallization on the structure and mechanics of starch bread crumb will be given. Firstly, I will focus on the structure of starch bread crumb. Then the importance of the structural elements for the mechanical properties will be discussed. Finally, I will give some ideas about the consequences of starch recrystallization on the product properties of commercial loaves of bread.

8.1   Structure of starch bread crumb

In the structure of starch bread crumb, several different structures at different length scales should be distinguished, *i.e.* from (macro)molecular scale to "crumb" scale. Starting at the largest scale, bread crumb can be considered a sponge, in which the gas cells are interconnected. Probably, some gas cells, especially the smallest ones, are still closed. The size distribution of the gas cells is rather broad; it varies from, say, 10 μm to about 5 mm.

The solid material building up starch bread crumb is mainly distributed in beams forming the cell edges, and also in some lamellae forming the faces of the (closed) cells. The thicknesses of the beams in starch bread crumb varies

considerably, from some microns to about 1 mm.

Starch is the important structural component of the beams. It occurs in swollen granules, which are rather irregular in shape. They are as it were hooked into each other, like pieces of a three-dimensional jigsaw puzzle. The swollen granules are approximately 2 to 200 μm in size, depending on the type of starch used. Generally, in wheat starch bread crumb the swollen granules are about a factor of 2 smaller than in potato starch bread crumb.

In between the swollen granules, a thin amylose gel layer presumably is present, forming a continuous matrix. The primarily linear amylose molecules forming this gel layer have been leached out of the granules during baking of the starch bread, as a result of incompatibility of amylose and amylopectin.[1] In Chapter 2 it was concluded that in a concentrated starch system (as in bread), only a small amount of amylose molecules can have leached out. Amylose gel formation is due to a crystallization process, which occurs within minutes or at most a few hours,[2,3] and it would thus be finished directly or shortly after baking. Consequently, the recrystallization of amylose hardly contributes to bread staling.

In the swollen granule, amylopectin as well as amylose are present. The amylose molecules probably are present in an amorphous mass in the swollen granules. Presumably, their conformation hardly changes during baking, cooling and storage of bread, while that of the highly branched amylopectin molecules is subject to changes during baking, cooling and storage of bread. In native starch granules amylopectin is ordered on various length scales[4] (Table 8.1). From a small to a large scale, these are:

1. The short side chains of the amylopectin molecules with a length of about 12 to 20 glucose units, which are arranged on the longer chains, are present in the form of double helices.
2. The double helices form layers of crystalline lamellae (see Figure 1.3). These lamellae consist of several clusters of double helices.
3. The crystalline lamellae form large helices, which are packed in a tetragonal array (see also Figure 1.3).

Directly after heating in the presence of enough water, amylopectin is completely amorphous, both the short[5,6] and long[7,8] range ordering disappears. In the case of commercial loaves of bread probably not all ordering disappears during baking of the dough, because the water content may be too low. Consequently, the conformation of starch in bread is somewhat different from that in starch gels.

During storage of concentrated starch systems, amylopectin regains some of its structural order. The short side chains form double helices, which slowly associate. By using transmission electron microscopy it was observed (Chapter 4) that the double helices are associated in crystalline domains, and not in larger crystalline lamellae as in native starch granules. The crystalline domains are formed by double helices of individual clusters of linear glucan chains along the amylopectin

molecules (Figure 4.1). Thus, during storage of fully disordered starch systems, the short range ordering is comparable to that of native starch, whereas the long range ordering is not regained.

Table 8.1 *Ordering of amylopectin in native, gelatinized and retrograded starch granules*

system	extent of ordering		
	small scale ⟶		large scale
native starch granule	double helices	crystalline lamellae	superhelices
gelatinized starch granule	-	-	-
retrograded starch granule	double helices	crystalline domains	-

- is no 'clear' ordering

8.2 The importance of the structural elements for the mechanical properties of starch bread crumb

In this thesis both small and large deformation properties of concentrated starch systems were studied and these were related to the structure of these systems. The emphasis was on large deformation properties, because the eating quality of food is usually related to fracture and yielding properties of the product.

The mechanical properties of starch bread crumb were studied by compressing and decompressing test pieces of bread crumb twice (see, for instance, Figure 5.3). In this way, information was obtained about stiffness, buckling or fracture behaviour and resistance to collapse of the structure at large deformations of starch bread crumb as a function of ageing time.

As mentioned, starch bread crumb has a sponge structure and can be described as a cellular solid. According to the theory of Ashby and Gibson,[9,10] which was developed for ideal sponge structures with uniform gas cell sizes and thicknesses of the beams and the lamellae, the mechanical properties of a sponge depend on the mechanics of the material building up the sponge and the density ratio of this material and the sponge. In Chapter 6, it has been shown that this theory was not completely applicable to starch bread crumb, mainly because the bread crumb

structure was inhomogeneous; the size of the air cells and the thicknesses of the beams varied considerably. Moreover, it was argued that the mechanical properties of thick beams are different from those of thin beams, which makes interpretation of the relation between the structure and the mechanical properties more complicated.

Structure and mechanical properties of thick beams in starch bread crumb are comparable with those of concentrated starch gels, which were discussed comprehensively in Chapter 3. In Figure 8.1, a short summary of the relation between the structure and mechanics of thick beams is given. Thick beams consist of swollen granules, which are hooked into each other, with a thin amylose gel layer in between. As the volume fraction of the amylose gel layer is very small, the stiffness of the beams depends mainly on the stiffness of the swollen granules. If large deformations are imposed on a concentrated starch system, fracture occurs around the granules, which implies that the amylose gel layer is fractured and that the granules stay intact. Thus, the granules are stronger than the amylose gel layer. Consequently, the fracture stress of a concentrated starch gel would depend on the fracture stress of the thin amylose gel layer. However, it was concluded in Chapter 3 that the stiffness of the swollen granules also affects the fracture stress of a concentrated starch system; stiffer granules result in more rigid hooks, and consequently in a higher fracture stress. The relative contribution of the swollen granules to the fracture stress is higher if the granules are stiffer and more irregularly shaped. For instance, it was shown in Chapter 3 that the contribution of the granules to the fracture stress is more pronounced in potato starch than in wheat starch systems, the latter having less irregularly shaped granules. The strain at fracture of thick beams depends on the stiffness of the granules in relation to the stress at fracture.

The mechanical properties of thin beams in starch bread are somewhat different from those of thick beams (Figure 8.1). The mechanical properties of thin beams are more comparable with those of the model of interacting cubes presented in Chapter 3. As the thin beams are only a few granules in thickness, they are hardly hooked into each other. Consequently, the fracture stress of thin beams mainly depends on the mechanics of the thin amylose gel layer; the stiffness of the swollen granules has hardly any effect on it and neither has amylopectin recrystallization. The strain at fracture therefore depends on the mechanical properties of the amylose gel layer in relation to the stiffness of the swollen granules. It goes without saying that the stiffness of thin beams is determined by the stiffness of the swollen granules.

In this study, the mechanical properties of the thin amylose gel layer were not studied, for instance via the mechanics of amylose gels. Recently, the small deformation properties of amylose gels were studied thoroughly by others.[2,3,11,12] They concluded that the time scale of gel formation is rather short; the modulus
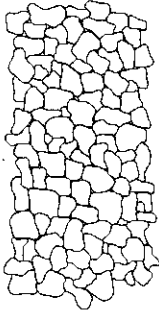
Structure

Thin beams
1-2 granules

Thick beams
> 2 granules

A

B

Mechanical properties

E: stiffness of swollen granules
σ_f: mechanical properties of the amylose gel matrix

ϵ_f: stiffness of the granules in relation to the mechanical properties of the amylose gel matrix

E: stiffness of swollen granules
σ_f: stiffness of the swollen granules in relation to the mechanical properties of the amylose gel matrix

ϵ_f: stiffness of the granules in relation to the stress at fracture

Effect of amylopectin
(re)crystallization

E: increases
σ_f: hardly any change
ϵ_f: strongly decreases

E: increases
σ_f: increases
ϵ_f: decreases

Figure 8.1 *The relation between structure and mechanical properties of thin and thick beams, and the effect of amylopectin (re)crystallization on it.*

hardly changed after some hours of storage. The gel properties and the rate of gel formation would depend on the amylose concentration as well as on the length of the molecules.

The stiffness of the swollen granules depends on their internal structure. Directly after baking, when all structural ordering in the granules has disappeared, the stiffness of the swollen granules depends on the number of entanglements present. During storage of starch systems, amylopectin recrystallizes. This would result in the formation of cross-links between adjacent molecules or in stiffening of strands between entanglements (Chapter 4). Probably, both mechanisms are involved in stiffening of the granules, which was found to be closely related with the recrystallization of amylopectin.

The consequences of the increase in stiffness of the swollen granules for the mechanical properties of thick and thin beams is summarized in Figure 8.1. For both thin and thick beams, stiffening of the swollen granules results in stiffer beams. The effect on the large deformation properties is however different for thin and thick beams. In the case of thick beams, stiffening of the swollen granules results in more rigid hooks and consequently the fracture stress increases. As the swollen granules in thin beams are hardly hooked into each other, stiffening of the granules has little effect on the fracture stress. Due to stiffening of the granules, the fracture strain of the beams decreases, and this would be more pronounced for thinner beams, because their fracture stress remains about constant. As a result of the increase in fracture stress of the thick beams, their fracture strain is less affected.

Summarizing, recrystallization of amylopectin results in a bread crumb that is stiffer, stronger and more crumbly. In theory, it is possible that only the stiffness and crumbliness of starch bread crumb increases; this would be the case if either the crumb is mainly built up of very thin beams or if the swollen granules have a rather regular shape. In both cases the swollen granules are hardly hooked into each other.

8.3   Consequences for product properties

The structure of the crumb of commercial loaves of bread is even more complicated than that of the crumb of starch bread, which was studied here. In commercial loaves of bread, besides water and starch, also some other components, like gluten and lipids, are present. It is supposed that especially the presence of gluten would make interpretation of results obtained on commercial loaves of bread more difficult. Besides amylose, gluten may be present in between the swollen granules. Probably, even two matrices are present: a continuous amylose gel phase as well as a continuous gluten phase. Therefore, besides starch gluten may affect

the mechanical properties of bread crumb.

In the crumb of commercial loaves of bread, recrystallization of amylopectin would result in an increase in the stiffness of the swollen granules and, consequently, the stiffness, strength and crumbliness of bread crumb would increase. As the consumers' acceptance of a product is mainly related to large deformation properties, especially the increase in strength and crumbliness result in a decrease in eating quality.

It is generally believed that bread staling can be retarded by addition of some lipid surfactants (see for instance the review paper of Kulp and Ponte[13]). Several researches[14,15,16] have suggested that lipid surfactants form complexes with amylopectin, resulting in a decrease in the rate of recrystallization of amylopectin. Consequently, they would retard bread staling. It has been shown in Chapter 7 that addition of GMS or SSL, two commercial lipid surfactants commonly used in the bakery industry, hardly affected the recrystallization of amylopectin, whereas the latter is responsible for the staling of bread. Lipid surfactants thus do not act via retarding amylopectin recrystallization. However, in the presence of GMS in particular, wheat starch bread was less crumbly than the control bread; it seems to be "fresh" for a longer period. It was suggested that this improving effect is mainly a result of a finer and more even crumb structure.

Based on this result it appears that the evenness of the crumb structure would be an important factor for the eating quality of bread. By improving the crumb structure, the consumers' acceptance of bread would remain higher during storage of bread, despite of the continuing recrystallization of amylopectin. Therefore, it is suggested that improving bread crumb structure is an important tool for tackling the bread staling problem.

8.4 References

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List of symbols

A	area	m^2
A_o	original area	m^2
c	concentration	%
c^*	coil overlap concentration	%
C_1	constant in equation 6.1	-
C_2	constant in equation 6.2	-
C_3	constant in equation 6.3	-
E	Young modulus	$N \cdot m^{-2}$
E^*	Young modulus of a cellular solid	$N \cdot m^{-2}$
E_s	Young modulus of beams and lamellae building up a cellular solid	$N \cdot m^{-2}$
F	force	N
G'	storage modulus	$N \cdot m^{-2}$
G''	loss modulus	$N \cdot m^{-2}$
h	height	m
h_o	original height	m
ΔH	melting enthalpy	$J \cdot g^{-1}$
k	rate constant in the Avrami equation	s^{-1}
n	exponent	-
T_g	glass transition temperature	°C
T_g'	glass transition temperature of a maximally freeze concentrated solution	°C
T_m	melting or termination temperature	°C
T_o	onset temperature	°C
T_p	peak temperature	°C
t	time	s
W	total energy input	$J \cdot m^{-3}$
W'	stored energy	$J \cdot m^{-3}$
W_m''	dissipation energy due to network flow	$J \cdot m^{-3}$
W_c''	dissipation energy due to friction between structural elements	$J \cdot m^{-3}$
W_f	energy used for fracture	$J \cdot m^{-3}$

δ	loss angle	rad
ϵ	strain	-
ϵ_c	Cauchy strain	-
ϵ_h	Hencky strain	-
$\dot{\epsilon}_h$	Hencky strain rate	s^{-1}
ϵ_1^*	critical strain in the first compression (see Figure 5.3)	-
θ	fraction of uncrystallized material	-
ρ^*	density of a cellular solid	$kg \cdot m^{-2}$
ρ_s	density of beams and lamellae building up a cellular solid	$kg \cdot m^{-2}$
σ	stress	$N \cdot m^{-2}$
σ_1^*	critical stress in the first compression (see Figure 5.3)	$N \cdot m^{-2}$
σ_2^*	critical stress in the second compression (see Figure 5.3)	$N \cdot m^{-2}$
σ_{el}^*	elastic buckling stress of a cellular solid	$N \cdot m^{-2}$
σ_f^*	fracture stress of a cellular solid	$N \cdot m^{-2}$
σ_{fs}	fracture stress of beams and lamellae	$N \cdot m^{-2}$

Abbreviations

ADP	adenosine diphosphate
ADPG	adenosine diphosphate glucose
cl	chain length
cr-l	cross-linked
CSL	calcium stearoyl-2-lactylate
Da	Dalton
DATEM	diacetyl tartaric acid esters of monoglycerides
DP	degree of polymerization
DSC	differential scanning calorimetry
G	glucose
GMS	glycerol monostearate
NMR	nuclear magnetic resonance
POES	polyoxyethylene monostearate
SAXS	small angle X-ray scattering
SSL	sodium stearoyl-2-lactylate
TEM	transmission electron microscopy
UDP	uridine diphosphate
UDPG	uridine diphosphate glucose
WAXS	wide angle X-ray scattering

Summary

The eating quality of bread decreases during storage. The most important aspect of bread staling is the increase in firmness and crumbliness of the crumb. As early as 1853, Boussingault demonstrated that staling of bread also occurs if no loss of water can occur; bread staling is thus not only due to drying out of bread crumb. In the twenties, Katz showed by use of X-ray diffraction that changes in the structure of the starch matrix are primarily responsible for bread staling. From that time on, much research has been done on the molecular structure of starch and the ordering of starch molecules in the granule on the one hand and on improving bread quality on the other hand. This is reviewed briefly in Chapter 1. When the research described in this thesis was started, it was not exactly clear how changes in the structure of starch during storage affect the eating quality of bread. This applies also for other products containing a high starch level, like some custards. The aim of this study was therefore to obtain a better understanding of the role of starch in the decrease in eating quality of food products with a high starch content. Since during eating of solid foods large deformations occur, which may result in fracture or yielding of the product, large deformation properties of the products were studied in particular. To simplify the systems, the study started with relatively simple starch-water systems. Later on, starch bread, which has a more complicated structure, was studied. Especially potato and wheat starch were used in this study; wheat starch because it is the starch in bread, and potato starch because its properties differ considerably from that of wheat starch.

In Chapter 2 mechanical properties of concentrated starch-water systems during heating and cooling are presented and discussed in relation to the mechanism of starch gelatinization. The mechanical properties were determined by applying a small oscillating shear deformation to the material. During heating of the starch-water system it was observed that the storage modulus started to increase strongly at temperatures of about 60 ˚C. This increase was ascribed to swelling of the granules as a result of the fact that the crystallites in the granules start to melt. Then, a material developed existing of tightly packed granules. At a further increase of temperature, the modulus decreased, which was ascribed to further melting of the crystalline regions and disentanglement of molecules in the starch granules; as a consequence, the swollen granules became less stiff. Probably, the separation of amylose and amylopectin played also a role in this decrease. As a

result of the separation of amylose and amylopectin, a small amount of amylose leached out of the granules. During heating and cooling the leached-out amylose molecules are presumed to form ordered crystalline regions, resulting in the formation of a gel consisting of swollen granules with a thin amylose gel layer in between. The swollen granules contain both amylose and amylopectin. They stayed intact during gel preparation, probably due to the presence of extensive entanglements.

During cooling and storage of concentrated starch gels, part of the amylopectin in the swollen granules recrystallize, which process is often designated retrogradation. The consequences of starch retrogradation for the mechanical properties of concentrated starch gels are the subject of Chapter 3. Starch gels were prepared by filling teflon cylindrical moulds with starch suspensions and heating these in an oil bath at 95 °C. After cooling the gels were stored at 20 °C. Test pieces of these gels were compressed between parallel plates until fracture occurred. The parameters obtained from the stress-strain curves were related to the structure of the gels. As the starch gel was almost completely filled with swollen granules, the modulus or stiffness of the gels would approximately equal the stiffness of the swollen granules. Fracture of the gels occurred around the granules; the thin amylose gel layer in between the swollen granules fractured. As a consequence, the fracture stress of the gels would depend on the fracture stress of the thin amylose gel layer. However, also the stiffness of the swollen granules would affect the fracture stress of the gels. The swollen granules were namely very irregular in shape; they were hooked into each other like pieces of a jigsaw puzzle. The stiffer the granules are, the more difficult it would be to fracture the gels and the higher the fracture stress. The fracture strain of the gels would be determined by the deformability of the granules.

During storage of the gels the modulus and the fracture stress increased and the fracture strain decreased. This was ascribed to the swollen granules becoming stiffer as a result of recrystallization of amylopectin. The type of starch, the heating temperature and the starch concentration affected the mechanical properties of the gels and the rate at which these properties changed during storage. It was supposed that the number of entanglements or cross-links in the swollen granules play a role in this.

In Chapter 4 attention was paid to the mechanism of amylopectin recrystallization and to the relation between this process and the stiffening of concentrated starch gels. First of all, the ordering of amylopectin in aged starch gels was compared with the ordering of amylopectin in native starch granules. In retrograded starch gels crystalline domains of 5 nm in size were observed by transmission electron microscopy. Similar domains are also present in native starch granules, but these are ordered in crystalline lamellae, which themselves form superhelices. These latter structures are absent from retrograded starch. It was

already known that the crystalline regions in both materials are built of double helices formed by the short amylopectin chains. It was therefore concluded that in retrograded starch gels the short range ordering is the same as in native starch, whereas the long range ordering is not regained.

The relation between the degree of recrystallization, as measured by differential scanning calorimetry (DSC), and the stiffness of the gels was closely related. It was concluded that two different processes can be involved in the stiffening of the granules. In the first place, recrystallization of amylopectin would result in stiffening of amylopectin chains between entanglements. Moreover, it may cause the formation of cross-links, consisting of ordered regions, between two adjacent clusters of short amylopectin side chains.

In Chapter 5 the structure and the mechanical properties of starch bread are discussed. Starch bread was prepared from starch, water and a small amount of xanthan. Test pieces of starch bread crumb were compressed and decompressed twice with a rest time of 1 minute in between the two cycles. The Young modulus, the critical stress and critical strain for collapse of structure (presumably due to buckling or fracture of the beams) and the resistance of bread crumb to this collapse could be derived from the stress-strain curves. During storage, the shape of the stress-strain curves changed markedly; the modulus and the critical stress increased and the critical strain decreased. Moreover, the resistance of bread crumb against further collapse of its structure during ongoing compression decreased. The mechanical properties of bread crumb were related to its structure. Starch bread crumb has a sponge structure, which means that the gas cell are interconnected. The solid material building up starch bread crumb is mainly present in beams consisting of tightly-packed swollen starch granules. The structure of the material in the beams is thus comparable with that of concentrated starch gels. The mechanical properties of bread crumb were therefore compared with those of concentrated starch gels. It was concluded that the mechanical properties of the material in the beams as well as variations in the thickness of the beams and in the size distribution of the gas cells are important for the mechanical properties of starch bread crumb.

To gain better, albeit largely qualitative understanding of the mechanical properties of bread, a theory relating mechanical properties (Young modulus, critical stress) of cellular solids to cell geometry was applied to starch bread crumb. The relation between Young modulus and density agreed well with theory. However, the results for the critical stress, in particular for fracture, showed poorer agreement with theory, especially for potato-starch bread crumb. This discrepancy was mainly ascribed to variations in thickness of the beams. Beams that are several granules thick would have a fracture behaviour comparable with that of concentrated starch gels; here, the fracture stress depends on the properties of the thin amylose gel layer as well as on the stiffness of the swollen granules. As

the swollen granules in beams of a few granules thick are hardly hooked into each other, the fracture stress of the thin beams would only depend on the properties of the thin amylose gel layer between the swollen granules. The results obtained on potato starch bread crumb agreed poorer with theory than those on wheat starch bread crumb, presumably because (i) potato starch bread crumb had a less even structure, and (ii) swollen potato starch granules were much irregularly shaped, and thus more hooked into each other. As a consequence, the fracture stress of thick beams in potato starch bread crumb would be determined to a larger extent by the stiffness of the swollen granules, resulting in larger differences in the fracture stress between thin and thick beams than in wheat starch bread crumb.

In Chapter 7, effects of the lipid surfactants GMS and SSL on changes in the mechanical properties of concentrated starch systems during heating, cooling and storage are discussed. In the presence of GMS and SSL, fracture stress and fracture strain of potato and wheat starch gels were lower than in the absence of these substances. Moreover, the Young moduli of potato starch gels with the lipid surfactants increased slightly less during storage. As GMS and SSL hardly affected the mechanical properties of a waxy maize starch gel, it was concluded that these substances affect the mechanics by complex formation with amylose. Due to this complex formation, GMS and SSL may indirectly affect amylopectin recrystallization slightly. Based on the results obtained on starch gels, it might be expected that starch bread crumb is more crumbly in the presence of GMS and SSL. However, in particular starch bread crumb with GMS showed less collapse of its structure during compression. It was therefore supposed that GMS and SSL also improve gas cell stability in the dough during breadmaking, thereby making the bread crumb structure more even.

In Chapter 8, the most important conclusions of this thesis are summarized. It is discussed how the starch network structure at different length scales and the changes therein during storage, affect the eating quality of bread. It is concluded that besides the mechanical properties of the starch matrix, the bread crumb structure plays an important role in the eating quality of bread, and in the extent to which it decreases during storage.

Samenvatting

De kwaliteit van brood gaat achteruit bij bewaren. Het belangrijkste aspect van het oudbakken worden van brood is dat het stugger en kruimeliger wordt. Vaak wordt dit geassocieerd met het uitdrogen van de kruim. Maar in 1853 werd al door de Fransman Boussingault waargenomen dat brood ook stugger wordt indien het wordt bewaard onder omstandigheden waarbij het niet kan uitdrogen. In de jaren twintig was het Katz die met Röntgendiffractie aantoonde dat verandering in de structuur, d.w.z. de moleculaire ordening, van het zetmeel verantwoordelijk is voor het oudbakken worden van brood. In de daaropvolgende decennia is er veel onderzoek verricht naar de structuur van zetmeel enerzijds, en het verbeteren van de kwaliteit van brood anderzijds. Een overzicht hiervan is beschreven in hoofdstuk 1. Op het moment dat met het onderzoek beschreven in dit proefschrift werd begonnen, was het niet precies duidelijk hoe veranderingen in de structuur van zetmeel tijdens bewaren van brood de eeteigenschappen ervan beïnvloeden. Doel van dit onderzoek was daarom een beter inzicht te verkrijgen in de rol van zetmeel voor de eeteigenschappen van brood. Aangezien desbetreffende eeteigenschappen i.h.a. gerelateerd zijn aan breukeigenschappen, werd in dit onderzoek met name gebruik gemaakt van technieken om de breukeigenschappen van het produkt te bestuderen. In eerste instantie werden relatief eenvoudige geconcentreerde zetmeel-watersystemen bestudeerd. Daarna kwamen meer gecompliceerde zetmeelbroden aan bod. In dit onderzoek werd met name het gedrag van aardappel- en tarwezetmeel bestudeerd. Voor tarwezetmeel werd gekozen omdat brood daarvan wordt gebakken. Aardappelzetmeel was interessant omdat de eigenschappen van dit zetmeel aanzienlijk verschillen van die van tarwezetmeel.

Zetmeel komt in de natuur voor in de vorm van korrels met een grootte van ongeveer 1 tot 125 μm. Deze korrels zijn onoplosbaar in water en ze zijn gedeeltelijk kristallijn. De zetmeelkorrels bevatten twee soorten macromoleculen: amylose en amylopektine. Amylose is vrijwel een lineair molecuul, terwijl amylopektine sterk vertakt is. Alleen amylopektine draagt bij aan de kristallijne gebiedjes.

Wanneer zetmeel in aanwezigheid van water wordt verhit, bijvoorbeeld bij het bakken van brood, treden een aantal veranderingen op die tezamen de verstijfseling worden genoemd: de kristallijne gebiedjes in de zetmeelkorrels gaan smelten, de zetmeelkorrels zwellen op, amylose en amylopektine gaan deels ontmengen, en het aantal warpunten tussen de moleculen neemt af. Dit resulteert in grote veranderingen

in de eigenschappen van het produkt. In hoofdstuk 2 worden de mechanische eigenschappen van geconcentreerde zetmeel-watersystemen tijdens verhitten en afkoelen beschreven en bediscussieerd in relatie tot het mechanisme van zetmeelverstijfseling. Daartoe werd gebruik gemaakt van dynamische metingen bij kleine vervormingen. Een belangrijke variabele die hierbij wordt bepaald is de opslagmodulus, die iets zegt over de stijfheid van het materiaal. Er werd waargenomen dat tijdens verhitten van zetmeel-watersystemen de opslagmodulus eerst sterk toenam bij een temperatuur van ongeveer 60 °C. Deze toename werd toegeschreven aan het zwellen van de zetmeelkorrels als gevolg van het smelten van de kristallijne gebiedjes in de korrels. Er ontstaat dan een materiaal dat bestaat uit gezwollen korrels die zeer dicht gepakt zijn en dus vrijwel geheel het volume vullen. Bij verdere stijging van de temperatuur daalde de modulus. Dit werd toegeschreven aan het verder smelten van de kristallijne gebiedjes en het ontwarren van de zetmeelmoleculen, waardoor de gezwollen korrels zachter worden. Mogelijk speelt ontmenging van amylose en amylopektine hierin ook een rol. Door ontmengen van amylose en amylopektine kan een beetje amylose tussen de gezwollen korrels komen. Tijdens verhitten en afkoelen ordenen de amylosemoleculen zich lokaal in kristallijne gebiedjes waardoor een gel ontstaat; het geheel bestaat dan uit gezwollen korrels met daartussen een dun laagje amylosegel. De gezwollen korrels bestaan uit zowel amylose als amylopektine. Ze blijven intact door de aanwezigheid van warpunten tussen de moleculen.

Tijdens afkoelen en bewaren van de zetmeelgelen gaat een deel van de amylopektine in de gezwollen korrels herkristalliseren. Dit proces wordt vaak aangeduid als retrogradatie. De gevolgen van retrogradatie voor de mechanische eigenschappen van geconcentreerde zetmeelgelen worden beschreven in hoofdstuk 3. De zetmeelgelen werden gemaakt door teflon cilinders te vullen met zetmeelsuspensies. Deze werden verhit in een oliebad bij 95 °C, afgekoeld en bewaard bij 20 °C. De verkregen gelen werden tot cilindervormige proefstukken gesneden en deze werden met een duw-trekbank gecomprimeerd tot breuk, waarbij de kracht werd geregistreerd als functie van de indrukking. Parameters die hieruit werden verkregen, waren de Young-modulus, de breukspanning en de breukvervorming. Deze parameters werden gerelateerd aan de structuur van de geconcentreerde zetmeelgelen. De modulus van de gelen werd gelijk gesteld aan de modulus (stijfheid) van de gezwollen korrels, omdat de gelen vrijwel geheel gevuld zijn met gezwollen korrels. Er werd waargenomen dat breuk optrad om de gezwollen korrels heen; het dunne laagje amylosegel breekt en is dus de zwakste plaats. De breukspanning is daarmee afhankelijk van de eigenschappen van het amylosegel. Maar ook de stijfheid van de korrels heeft grote invloed op de breukspanning. De korrels bleken namelijk zeer onregelmatig van vorm; ze grijpen in elkaar als de stukjes van een legpuzzel. Hoe steviger die korrels nu zijn, des te moeilijker ze uit elkaar te krijgen zijn en des te hoger de breukspanning is. De breukvervorming wordt bepaald door de vervormbaarheid van de korrels op het moment dat het gel breekt.

Er werd waargenomen dat tijdens bewaren de modulus en de breukspanning van de gelen toenamen en dat de breukvervorming afnam. Dit werd toegeschreven aan het steviger worden van de gezwollen zetmeelkorrels als gevolg van herkristallisatie van amylopektine. Het soort zetmeel, de verhittingstemperatuur en de zetmeelconcentratie bleken effect te hebben op de mechanische eigenschappen en de snelheid waarin deze veranderen tijdens bewaren. Verondersteld werd dat de hoeveelheid warpunten of knooppunten in de gezwollen zetmeelkorrels hierbij een rol spelen.

In hoofdstuk 4 wordt aandacht besteed aan het mechanisme van herkristallisatie van amylopektine en de relatie tussen dit proces en het steviger worden van de gelen. Allereerst werd de ordening van amylopektinemoleculen in verouderde gelen vergeleken met de ordening van amylopektinemoleculen in natieve zetmeelkorrels. Met behulp van elektronenmicroscopie werd waargenomen dat in geretrogradeerde zetmeelgelen kristallijne gebiedjes (5 nm) aanwezig zijn. Deze zijn kleiner dan de in eerder onderzoek waargenomen kristallijne lamellen in natieve zetmeelkorrels, die zelf grote helices vormen (zie ook hoofdstuk 1). Er was reeds bekend dat het kristallijne materiaal zowel in natieve zetmeelkorrels als in geretrogradeerd zetmeel is opgebouwd uit geordende dubbele helices gevormd door de korte ketens van amylopektinemoleculen. Uit de resultaten werd dus geconcludeerd dat de ordening op kleine schaal wel, maar die op grote schaal niet terugkeert tijdens bewaren van de zetmeelgelen.

De mate waarin de herkristallisatie in geconcentreerde zetmeelgelen optrad werd bepaald met DSC, en werd vergeleken met de toename van de modulus. De toename van de modulus bleek nauw gerelateerd te zijn aan de hoeveelheid kristallijn materiaal. Hieruit en uit resultaten verkregen met elektronenmicroscopie werd geconcludeerd dat door het ontstaan van kristallijne gebiedjes de amylopektineketens tussen de warpunten stijver worden en dat hierdoor de stijfheid van de gezwollen korrels wordt vergroot. Bovendien werd het mogelijk geacht dat aangrenzende kristallijne gebiedjes knooppunten kunnen vormen, waardoor de modulus van de gelen ook zou toenemen.

In hoofdstuk 5 worden de structuur en mechanische eigenschappen van zetmeelbroden beschreven. Deze broden werden bereid van zetmeel en water met een beetje van het verdikkingsmiddel xanthaan. Cilindervormige proefstukken genomen uit de broden werden met de duw-trekbank tweemaal achtereen gecomprimeerd met daartussen een rusttijd van 1 minuut. Uit de spanning-vervormingscurven werden een aantal parameters gehaald: de Young-modulus, de kritieke spanning en de kritieke vervorming waarbij afbraak van structuur optreedt (zeer waarschijnlijk als gevolg van knikken of breuk van het staafvormige vaste materiaal tussen de gascellen) en de weerstand tegen deze structuurafbraak. De vorm van de curves veranderde aanzienlijk gedurende bewaren; de modulus en de kritieke spanning namen toe, terwijl de kritieke vervorming afnam. Bovendien nam de weerstand tegen de structuurafbraak af. Met andere woorden, de broden werden steviger en kruimeliger. De gemeten eigenschappen werden gerelateerd aan de structuur van zetmeelbroden. Zetmeelbroden

hebben een sponsachtige structuur, hetgeen betekent dat de gascellen in open verbinding met elkaar staan. De broden zijn opgebouwd uit met elkaar verbonden staafjes, die weer bestaan uit dicht tegen elkaar gepakte gezwollen zetmeelkorrels. De structuur van deze staafjes is dus vergelijkbaar met de structuur van de zetmeelgelen. Uit vergelijking van de mechanische eigenschappen van de broden en de eigenschappen van geconcentreerde zetmeelgelen bleek dat niet alleen de eigenschappen van de staafjes van belang zijn voor de eigenschappen van de broden, maar dat ook variaties in de dikte van de staafjes en in de grootte van de gascellen een belangrijke rol spelen.

Om in kwalitatieve zin meer inzicht te verkrijgen in dit aspect werd een theorie die de mechanische eigenschappen van cellulaire vaste stoffen relateert aan hun structuur, toegepast op de zetmeelbroden. Dit wordt beschreven in hoofdstuk 6. In deze theorie wordt het verband gegeven tussen de mechanische eigenschappen van de cellulaire vaste stof, de mechanische eigenschappen van het materiaal waaruit deze is opgebouwd, en de verhouding van de dichtheden van beide materialen. De cellulaire vaste stof was in dit geval het zetmeelbrood, dat is opgebouwd uit de staafjes bestaande uit de gezwollen zetmeelkorrels. De mechanische eigenschappen van de staafjes werden gelijk verondersteld aan die van geconcentreerde zetmeelgelen. De waarden van de moduli bleken redelijk te kloppen met de theorie. De waarden voor de kritieke spanning, met name voor breuk van de staafjes, klopten veel minder goed met de theorie, vooral bij de aardappelzetmeelbroden. Dit werd toegeschreven aan spreiding in de dikte van de staafjes. Staafjes die meerdere korrels dik zijn hebben een breukgedrag vergelijkbaar met dat van geconcentreerde zetmeelgelen; de breukspanning wordt zowel bepaald door de eigenschappen van het amylosegel als door de stijfheid van de gezwollen korrels. Aangezien in staafjes van 1 of enkele korrels dikte de gezwollen korrels nauwelijks in elkaar grijpen, is de breukspanning ervan alleen afhankelijk van de eigenschappen van de amyloselaag tussen de korrels. De mechanische eigenschappen van dunne staafjes kunnen dus niet gelijk gesteld worden aan die van geconcentreerde zetmeelgelen. De gevonden grootheden van de aardappelzetmeelbroden klopten minder goed met de theorie dan die van de tarwezetmeelbroden, omdat (i) de aardappelzetmeelbroden een veel onregelmatigere structuur hadden en (ii) gezwollen aardappelzetmeelkorrels een veel onregelmatigere vorm bleken te hebben, waardoor ze sterker in elkaar grijpen. Hierdoor wordt bij aardappelzetmeelbroden de breukspanning van de dikke staafjes grotendeels bepaald door de stijfheid van de gezwollen korrels, met als gevolg dat de verschillen in breukspanning tussen dikke en dunne staafjes extremer zijn dan in tarwezetmeelbroden.

In de bakkerij worden hulpstoffen gebruikt om de houdbaarheid van brood te verbeteren. Een belangrijke groep hulpstoffen zijn de emulgatoren; veel toegepast worden GMS en SSL. Het is bekend dat deze stoffen een complex kunnen vormen met amylose. Maar amylopektine en niet amylose is verantwoordelijk voor het oudbakken

worden van brood. Sommigen suggereren daarom dat GMS en SSL ook kunnen complexeren met amylopektine. In hoofdstuk 7 wordt ingegaan op het effect van deze emulgatoren op de structuur en mechanische eigenschappen van zetmeelgelen en zetmeelbroden. In aanwezigheid van GMS en SSL bleken aardappel- en tarwezetmeelgelen een lagere breukspanning en breukvervorming te hebben dan in afwezigheid van deze stoffen. Bovendien bleek de modulus van aardappelzetmeelgelen iets minder sterk toe te nemen gedurende bewaren. Aangezien GMS en SSL geen effect hadden op de mechanische eigenschappen van amylosevrije waxy-maiszetmeelgelen, werd geconcludeerd dat GMS en SSL werken via complexering met amylose. Door complexering met amylose in de korrel zouden GMS en SSL indirect de herkristallisatie van amylopektine in geringe mate kunnen beïnvloeden. Op basis van de resultaten van de zetmeelgelen zou verwacht worden dat de zetmeelbroden kruimeliger zouden worden in aanwezigheid van GMS en SSL. Het tegendeel werd echter waargenomen; met name zetmeelbroden met GMS vertoonden juist minder structuurafbraak tijdens compressie. Daarom werd verondersteld dat GMS en SSL de kwaliteit van brood vooral verbeteren doordat bij toevoegen ervan aan het deeg de sponsstructuur van het brood regelmatiger is.

In hoofdstuk 8 zijn de belangrijkste conclusies van dit proefschrift op een rij gezet. Aangeven werd hoe zetmeel en de veranderingen die daarin optreden de eetkwaliteit van brood bepalen. Echter, ook de structuur van de kruim van brood blijkt een belangrijke rol te spelen in de eetkwaliteit van brood, en in de mate waarin die kwaliteit afneemt tijdens bewaren.

Curriculum vitae

Christel Keetels werd op 21 januari 1966 in Waalwijk geboren. In 1984 behaalde zij het Atheneum-B diploma aan het Dr. Mollercollege in Waalwijk. In datzelfde jaar begon zij met de studie levensmiddelentechnologie aan de toenmalige Landbouwhogeschool in Wageningen. Zij koos voor de afstudeervakken zuivelkunde en levensmiddelennatuurkunde. In november 1989 slaagde zij voor het doctoraalexamen. Van 1 november 1989 tot 1 februari 1994 was zij werkzaam als onderzoeker in opleiding bij de sectie Zuivel en Levensmiddelennatuurkunde van de Landbouwuniversiteit. De resultaten van het onderzoek dat in deze periode werd uitgevoerd zijn beschreven in dit proefschrift. Van 1 juli 1991 tot 1 januari 1992 was zij tevens part-time werkzaam als toegevoegd docente levensmiddelentechnologie. Ook vervulde zij deze functie van 1 april 1994 tot 6 juli 1994. In samenwerking met de vakgroep Plantenveredeling verrichte zij van 1 augustus 1994 tot 1 november 1994 onderzoek naar functionele eigenschappen van nieuw ontwikkelde aardappelzetmelen. Vanaf 30 januari 1995 is zij als wetenschappelijk medewerkster in dienst bij DOMO Food Ingredients te Beilen.

Nawoord

(Foxhol) en Latenstein (Nijmegen). Medewerkers van AVEBE hebben deze monsters geanalyseerd.

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Christel