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VTT Biotechnology

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Abstract

The susceptibility of native barley starch granules and granules at different stages of gelatinization to α-amylolysis was studied by analyzing the amounts of solubilizing carbohydrates. The subsequent changes in the structure and properties of the insoluble residue were analyzed by various methods. The early stages of α-amylolysis of gelatinized barley and waxy barley starches were also followed. The gelation behavior of enzymatic hydrolysates of waxy barley starch with different molecular sizes was studied.

In the α-amylolysis of both gelatinized and ungelatinized barley starch, the molecular weights of both amylose and amylopectin decreased. Amylopectin hydrolysis started between the clusters without shortening of the external chains. In the early stages of α-amylolysis of barley starch granules, lipid-complexed amylose was less accessible and concentrated in the insoluble granule residue and the solubilizing carbohydrates originated from free amylose and amylopectin. Amorphous and crystalline regions of granules solubilized equally and with more extensive hydrolysis, the granular structure and crystallinity were destroyed.

Partial gelatinization changed the α-amylolysis pattern and the pinholes typical of α-amylase-treated large barley starch granules could not be seen. With regard to the leaching of lipid-complexed amylose, the α-amylolysis was similar to that of native barley starch granules. Additional lipid binding to starch during partial gelatinization stabilized the granular structure. Along with lipid complexed amylose, the small amount of free amylose remaining also concentrated in the residue, indicating that free amylose no longer existed as separate molecules but rather as part of otherwise complexed and thus insoluble molecules.

Partial α-amylolysis increased the solubility of barley starch and changed the mechanism of swelling of the granules; granules became more transparent and no twisting was observed.

Preface

Most of the present work was carried out at VTT Biotechnology during the years 1992–1998. I spent the year 1993 at the Institute of Food Research in Norwich, England. Financial support was provided by the Finnish Academy and the Finnish Cultural Foundation and is gratefully acknowledged.

I am most grateful to Prof. Kaisa Poutanen and Dr. Pirkko Forssell for supervising my work at VTT and to Dr. Steve Ring for his guidance at the Institute of Food Research. I also wish to thank them for encouragement and support during my studies and for providing excellent working facilities.

Very special thanks to my colleagues and co-workers at VTT; Dr. Karin Autio for useful discussions on light microscopy; Dr. Tapani Suortti for helping with the interpretation of HPLC results; Sirpa Karppinen and Eeva Manninen for helping with some of the ion exchange chromatography analysis; Päivi Myllärinen for fruitful discussions about work and other things; and Heljä Heikkinen, Leena Öhrnberg and Liisa Änäkäinen for their skillful technical assistance as well as Pirkko Wolin (KCL) for her assistance with ESEM-pictures. Similarly I want to thank my co-workers at the Institute of Food Research; Vicky Bull, Yvonne Gunning, Dr. Louisa Botham, Timothy Noel and Dr. Roger Parker.

I am furthermore grateful to the whole staff at VTT Biotechnology and the Institute of Food Research for creating a pleasant working atmosphere. And last, but not least, I thank the members of SYFS for proving that food science can be fun.

List of original publications

The present thesis is based on the following publications, which will be referred to in the text by their Roman numerals. Additional unpublished data are also presented.

I Lauro, M., Forssell, P., Suortti, T., Hulleman, S. and Poutanen, K. 1999. α-Amylolysis of large barley starch granules. *Cereal Chem.* 76, pp. 925–930.

II Lauro, M., Suortti, T., Autio, K., Linko, P. and Poutanen, K. 1993. Accessibility of barley starch granules to α-amylase during different phases of gelatinization. *J. Cereal Sci*. 17, pp. 125–136.

III Lauro, M., Poutanen, K. and Forssell, P. 2000. Effect of partial gelatinization and lipid addition on α-amylolysis of barley starch granules. *Cereal Chem.* 77, pp. 595–601.

IV Poutanen, K., Lauro, M., Suortti, T. and Autio, K. 1996. Partial hydrolysis of gelatinized barley and waxy barley starches by alpha-amylase. *Food Hydrocolloids* 10, pp. 269–275.

V Lauro, M., Ring, S., Bull, V. and Poutanen, K. 1997. Gelation of waxy barley starch hydrolysates. *J. Cereal Sci.* 26, pp. 347–354.

The author of the present thesis had the main responsibility for planning the research, practical work and interpretation of the results in all publications, except for the X-ray diffraction analysis in publication I, which was carried out in ATO by Frank Janssen and the interpretations were made together with Stephan Hulleman. Publications II and IV are based on the author's Master's Thesis and the author also contributed to the writing of Publication IV.

Contents

Appendices of this publication are not included in the PDF version. Please order the printed version to get the complete publication (http://otatrip.hut.fi/vtt/jure/index.html)

1. Introduction

Starch is the major energy reserve in plants. The most important sources of starch are cereal grains, pulses and tubers. Barley is, in terms of production, among the most important cereals in the world. Barley is produced all over the world since it can be grown in many different environments. In Finland, barley is the most produced cereal with an annual yield of about 1500 million kg. Barley grains contain 63–65% starch (MacGregor and Fincher 1993). The commercial uses of starch are numerous since it is abundant and renewable. Starch can be used as such or it can be chemically, physically and/or enzymatically modified. In starch processing for food purposes, enzymes are widely used because of their ability to catalyze specific hydrolysis under moderate conditions and without formation of by-products. More than 70 % of starch produced is intended for food and feed purposes. Starches and their derivatives are used to improve the functional properties of foods and to provide a source of numerous oligo-, di- and monosaccharides.

α-Amylases have long been used for starch liquefaction in the production of dextrose syrups. The demand for such starch-based sweeteners is growing, mostly for use as food ingredients. Maltodextrins with specific functional properties are also widely used nowadays. Some of the most important functional properties of maltodextrins are low hygroscopicity, bland flavor, low sweetness, high viscosity and the ability to work as ice crystal growth retarders (Guzmán-Maldonado & Paredes-López 1995). Maltodextrins have the ability to form gels and retain water. They are used for instance as replacements of fat, gelatin and hydrocolloids, and as adhesives and coatings (Pszczola 1999).

Furthermore, amylase-treated granular starches can be used as fat replacers (Whistler 1996, 1997a). They provide a microporous matrix material that can also be used to adsorb aromatic components, peptides, proteins, drugs, flavoring agents or enzymes (Trubiano & Kasica 1986; Whistler 1989, 1997b; Kobayashi *et al.* 1992; Forssell *et al.* 1999) and even live microbes (Myllärinen *et al.* 1999, 2000). These can be used, for example, in the preparation of instant foods and controlled release drugs.

1.1 The composition of barley starch

Barley starch consists of two polysaccharides: amylose and amylopectin. In normal starches, the amylose content is 20–30 % and the amylopectin content 70–80%. However, barley cultivars containing waxy- and high-amylose starches are also available. Amylose content in barley starches may thus vary from less than 1 to 45% in waxy and high-amylose starches, respectively (Morrison *et al.* 1986). Besides amylose and amylopectin, barley starch consists of minor components, such as lipids and proteins. Barley starch granules, and other cereal starches have lipids inside the granules instead of having only surface lipids. The amount of lipids in normal barley starch is about 1% and they are mostly lysophospholipids (Morrison 1988).

1.1.1 Amylopectin

Amylopectin is one of the largest molecules in nature; the reported molecular weights are in the range of 10^6–10^8 g/mol. Very few results on molecular weights of cereal amylopectins have been reported because cereal starches are difficult to dissolve in aqueous solutions and they may be easily degraded by shear forces in chromatographic systems. DMSO treatment and solubilization in water by microwave heating has proved to be an effective method for sample preparation for SEC-MALLS-RI chromatography (Bello-Pérez *et al.* 1998). With this method, a molecular weight of 2.2×10^8 g/mol has been obtained for maize starch.

Amylopectin is a highly branched polymer consisting of relatively short α-(1,4)-D-glucan chains that are joined together with α-(1,6)-linkages. French (1972) proposed a cluster model for the structure of amylopectin. This model is now widely accepted. Models for the fine structure of amylopectin have been reported for waxy maize (Bertoft 1989), waxy barley (Bertoft & Åvall 1992) and smooth pea starch (Bertoft *et al.* 1993) using amylopectin β-limit dextrins as substrates for α-amylolysis in which case only the endo-attack at internal chains occurs.

Amylopectin external chains can be characterized as short and long chains with average chain lengths of 11–20 glucosyl units and 38–50 glucosyl units,

respectively (Lii & Lineback 1977; MacGregor & Morgan 1984; Hizukuri 1986). For at least some amylopectins, these groups can be divided into sub-groups (Lii & Lineback 1977; Manners & Matheson 1981; MacGregor & Morgan 1984; Hizukuri 1985; Hizukuri 1986; Ong *et al.* 1994). Amylopectins from large and small granules of barley and waxy barley starch contained three groups of unit chains: DP 45–50, 18–20 and DP 10–12 (MacGregor & Morgan 1984). Amylopectins of A-type cereal starches have shorter chains in both the long- and short-chain fractions and larger amounts of the short-chain fractions than those of B-type root and tuber starches (Hizukuri 1985; Ong *et al*. 1994; Hanashiro *et al*. 1996).

1.1.2 Amylose

Although amylose is an essentially linear glucose polysaccharide, some molecules are slightly branched. The amount of branched molecules range from 25 to 55% on a molecular basis (Takeda *et al*. 1987). Hizukuri *et al.* (1997) found the average number of sidechains to be in the range of 2–8 per molecule, and the side chains to to be from four to over 100 glucosyl units in length. The molecular sizes of the branched molecules are 1.5–3 times higher than those of linear molecules. No significant differences have been observed between average molecular sizes of amyloses of cereal starches on the one hand and those of tuber starches on the other hand (Buléon *et al*. 1998). Reported values for the MW of amylose are in the magnitude range of 10^5–10^6 g/mol, recent values obtained for barley amylose being 7×10^5 g/mol (Suortti *et al.* 1998).

Amylose is capable of forming helical complexes with various components such as lipids, alcohols and iodine. The amylose content of starch can be determined based on complex formation with iodine (Morrison and Laignelet 1983). In an amylose-lipid complex, amylose forms two or three turns of the helix around the nonpolar part of a monoacyl lipid. The polar head of the lipid is located outside the helical cavity. In native barley starches amylose occurs in two forms, as lipid-free amylose (FAM) and lipid-complexed amylose (LAM) (Morrison 1988; Morrison *et al.* 1993b). LAM content is determined by iodine staining and calculated as the difference between the amylose content before (FAM) and after lipid extraction (total amylose). The amount of free amylose in barley starches is 23–25% and the amount of lipid-complexed amylose is 6–7% (Morrison *et al*.

1993a and b). The existence of amylose-lipid complexes in native starch granules has been proven using CP/MAS-NMR (Morrison *et al*. 1993b and c). FAM and LAM exist in starch granules as separate molecules (Morrison *et al*. 1993b). Amylose-lipid complexes are suggested to be enriched near the granule surface (Morrison & Gadan 1987; McDonald *et al*. 1991).

1.2 Granular structure of starch

Native starches exist as semi-crystalline granules, the degree of crystallinity being 20–40% (Hizukuri 1996). The shape and size of a granule is typical of its botanical origin. Barley starch granules exist in two size populations, large granules being the major fraction, 9 % by weight. Large barley starch granules are 10–25 μm in diameter and lenticular shape; small ones are more irregular in shape and their diameter is < 10 μm. Large and small granules appear to contain similar proportions of amylose and amylopectin (MacGregor and Ballance 1980). And amylopectins of small and large barley starch granules appear to have also similar structures (MacGregor & Morgan 1984). On the other hand, the small granules have a higher lipid:amylose ratio than large granules (Myllärinen *et al.* 1998).

The center of the granule, the hilum, contains a large proportion of the reducing ends of starch molecules and is less organized than the rest of the granule (Blanshard 1987). Light and electron microscopy of acid hydrolyzed starch granules show that granules consist of concentric layers (growth rings) of amorphous and semi-crystalline regions. In the semi-crystalline stack, the crystalline lamellae contain the double helices of the outer chains of amylopectin and the amorphous lamellae contain amylopectin branches (Jenkins *et al.* 1994). Detailed knowledge of the structure of the starch granule at the level of amorphous and crystalline domains is still lacking. It has been proposed, for potato starch, that the crystalline domains form continuous networks of left-handed helices with empty voids roughly 8 nm wide; the interpenetrating superhelices are assumed to form a skeleton on which the granule is developed (Oostergetel & van Bruggen 1993).

The location of amylose in the granule has been a matter of speculation. Amylose molecules are thought to occur in the granule as individual molecules,

randomly interspersed among the amylopectin molecules in both the crystalline and amorphous regions (Jane *et al.* 1992). According to Jenkins *et al.* (1994), most of the amylose is located in the amorphous growth rings.

The amylose-lipid complexes in cereal starches occur in distinct regions of the starch granule (Morgan *et al.* 1995). The X-ray evidence shows that the amylose-lipid complexes in starch granules are not crystalline, but they appear to be more ordered than the amorphous branching regions of amylopectin. Non-waxy cereal starches thus consist of three distinct components: (i) highly crystalline regions from double-helical amylopectin chains, (ii) solid-like regions formed from lipid-complexed amylose and (iii) completely amorphous regions, associated with the amylopectin branches and possibly the lipid-free amylose (Morgan *et al.* 1995).

Not much is known about the molecular composition and arrangement at the starch granule surface. The current model is based on Lineback's "hairy billiard ball" model (1986), in which the granule surface is not smooth but rather is characterized by protruding chains. These chains are suggested to be ends of amylose chains and protruding amylopectin clusters (Stark & Lynn 1992; Baldwin *et al.* 1997, 1998). Because of the tight packing of the amylopectin chains, the granule surface is relatively smooth and impenetrable to large molecules such as amylases, which can enter the granule through the possible pores or channels, which have been shown to exist in some starches (Fannon *et al*. 1992, 1993; Huber & BeMiller 1997; Huber & BeMiller 2000). The presence of surface pores has been challenged because of the possible artefactual nature of the techniques used (Baldwin *et al.* 1994). On the other hand, the model of Oostergetel and van Bruggen (1993) supports the presence of surface openings, since in the superhelical structures there is a central cavity of about 8 nm in diameter.

1.3 The properties of starch

Native starch granules are relatively inert and insoluble in cold water. When heated above a critical temperature range in water, starch granules gelatinize. During gelatinization, both the crystalline and molecular (double helical) orders of starch granules are disrupted, the enthalpy of gelatinization primarily

reflecting the loss of molecular order (Cooke & Gidley 1992). Some irreversible changes occur; granules swell and some polysaccharide leaches out of the granules (Atwell *et al*. 1988). The gelatinization of barley starches occurs normally in the temperature range 56–62 °C (MacGregor & Fincher 1993). The small barley starch granules have higher gelatinization temperature than the large granules (Myllärinen *et el.* 1998).

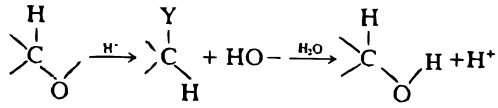
The changes that occur in cooled gelatinized starch, from an amorphous state initially to a more ordered or crystalline state, are called retrogradation. At starch concentrations higher than about 6%, retrogradation leads to formation of a gel. Below this concentration, only weak viscoelastic pastes are formed. Starch gels can be considered as composites containing gelatinized granules embedded in a continuous amylose matrix (Ring & Stainsby 1982). Retrogradation consists of two separable processes: (1) gelation and crystallization of amylose, and (2) recrystallization of amylopectin. Recrystallization of amylopectin is also one of the reasons for the staling of bread.

The gelatinization properties, as well as other physicochemical properties of starch, can be modified without destroying the granular structure. This can be achieved by annealing, heat-moisture treatment, chemical modification and addition of other components, such as lipids, to starch. Incubating starch with excess water below gelatinization temperature causes annealing. Annealing increases the gelatinization temperature and narrows the gelatinization temperature range. At water contents lower that that required for gelatinization but at higher temperatures, the starch is exposed to heat-moisture treatment. This also causes an increase in the gelatinization temperature but a broadening of the endotherm. The X-ray pattern of starch changes from B- and C-type to A-type. Lipids are used to modify the rheological and textural properties of starch pastes, the effect being widely attributed to complex formation with amylose.

1.4 The action of α-amylases on starch

Amylases are enzymes that hydrolyze starch. α-Amylases (E.C. 3.2.1.1.) are found in plants, animals and micro-organisms. The α-amylases have a molecular weight ranging from 50 000 to 60 000 g/mol. They are mostly endoenzymes that

cleave α-1,4-linkages of amylose and amylopectin. α-Amylases catalyze the following reaction:



The reaction proceeds by a double displacement mechanism with a transient covalent bond, of β-configuration, formed between group Y of the enzyme and C1 of the glucose ring at which the reaction takes place (Tao et al. 1989; MacGregor 1993). The action of α-amylases is not inhibited by α-1,6-linkages although such bonds are not split by them. The products of hydrolysis are oligosaccharides of various chain lengths depending on the source of amylase. The optimum pH and temperature are also dependent on amylase origin.

1.4.1 Hydrolysis of amylose and amylopectin

The initial stages of the α-amylolysis of amylose proceed predominantly by random attack (Greenwood & MacGregor 1965; Banks *et al*. 1970). The attack pattern changes and more specific products start to form when the chain lengths have been shortened to about 10 (MacGregor 1978; Beleia & Varriano-Marston 1981). This two-stage hydrolysis pattern has been explained by the concept of subsites. The active sites of α-amylases are considered to be composed of a catalytic site and several contiguous subsites, where a subsite is an area of the active site capable of interacting with one glucose residue. The number of subsites and their affinity for a glucose residue differs between α-amylases and therefore different end-products are obtained.

The action of α-amylases on amylopectin is a non-random process (Bertoft 1986). In the initial stages, preferential hydrolysis of glucosidic bonds between the unit cluster occurs. In addition to glucose, maltose and maltotriose, branched α-limit dextrins with different chain stubs attached to the branches are obtained. The main end -products obtained by hydrolysis of starch with *Bacillus licheniformis* α-amylase are DP5, DP3 and DP2 oligomers (Inglett 1987, Ivanova *et al.* 1991).

1.4.2 Hydrolysis of granular starch

The action of α-amylase on granular starch consists of the following three steps: (1) diffusion of the enzyme towards the substrate, (2) the adsorption of the enzyme on the substrate surface and possible pores, and (3) the catalytic event. The action of α-amylases on granular starch is widely studied since this type of hydrolysis plays a major role in many biological functions, such as digestion and germination. Due to the complexity of the substrate, however, it is still not a very well known process.

Most of the earlier studies on the hydrolysis of native starch granules have concentrated on detecting the changes of the granule surface structure by scanning electron microscopy. The amylase either erodes the granule surface or digests channels from selected points on the surface toward the center of the granule. Five patterns of attack have been identified: pin-holes/pepper-potting, sponge-like erosion, many medium-sized holes, distinct loci leading to single holes in individual granules and surface erosion (Evers 1979). Below gelatinization temperatures, large barley starch granules have been shown to hydrolyze via pinholes from the inside out (MacGregor & Ballance 1980). Hydrolysis appears to be rather uneven, while many granules are hydrolyzed extensively, others show only minor damage (MacGregor & Morgan 1986).

α-Amylases of various origin attack starch granules with different efficiencies and also the susceptibility of starch granules of different origin to α-amylolysis varies widely. Generally, cereal starch granules are more susceptible than tuber starches and there is a relationship between the crystalline type and accessibility. Small granules solubilize faster than large granules due to the larger surface area (MacGregor & Ballance 1980; Bertoft & Kulp 1986). And although similar hydrolytic products are obtained from large and small barley starch granules, the two types are degraded by different mechanisms. Degraded small granules exhibit roughened surfaces rather than pinholes, indicating that hydrolysis takes place through surface erosion.

The effect of other parameters, such as morphology and crystal defects and the interrelations of the crystals, should also be taken into consideration (Planchot *et al*. 1997). Amylopectins of A-type cereal starches have more short A-chains (dp 6–12) than B-type starches. The short A-chains are likely attached to a B-chain

with the branch linkage located in the crystalline region. It has been suggested that the branch linkages present in the crystalline region and the short double helices derived from the short A-chains provide the "weak points", which are more susceptible to enzymatic hydrolysis and to the generatation of pinholes and pits in the A-type starches (Jane *et al*. 1997).

In all the stages, from initial to later digestion, some regions of the starch granules are more readily digested than others. Unlike mineral acids, α-amylases can solubilize both amorphous and crystalline regions of starch granules. Solubilization of crystalline regions occurs through disentanglement of crystalline chains by α-amylase attacking the amorphous parts nearby (Colonna *et al*. 1988).

During hydrolysis of granular starch, no preferential attack on either amylose or amylopectin has been detected (Leach and Schoch 1961; Colonna *et al*. 1988). In solution, however, lipid-complexed amylose is more resistant to α-amylase than free amylose (Holm *et al*. 1983; Galloway *et al*. 1989; Biliaderis & Galloway 1989). Also, in the α-amylolysis of granular wheat starch, the lipid-complexed amylose is concentrated in the granule residues (Anger *et al*. 1994).

1.5 Aims of the present study

The aim of the investigation was to learn about the role of the physical state of barley starch on its hydrolysis by α-amylase. The mechanism of α-amylolysis of native starch granules, granules at different stages of gelatinization and gelatinized starch was studied. Furthermore, the aim was to find out about the effect of lysophospholipids on granule structure and starch properties, particularly on the accessibility to α-amylase.

The aim was to reveal the effect of α-amylase treatment on the granular and molecular structure of barley starch and also to clarify the effect of α-amylolysis on the microstructure of barley starch and to establish the influence of the enzyme treatment on the heat-induced changes of the partially hydrolyzed starch.

2. Materials and methods

2.1 Substrates and enzymes

Barley A-starch and waxy barley starch used as substrates were obtained from the Raisio Group (Raisio, Finland) and Primalco Ltd (Rajamäki, Finland).

Two preparations of *Bacillus licheniformis* α-amylase were used in the experiments: a product of Sigma (St. Louis, MO, U.S.A.) (type XII A,A-3404) and a product of Megazyme (Bray, Ireland) (E-BLAAM). *Pseudomonas amyloderamosa* isoamylase was obtained from ICN Pharmaceuticals (Costa Mesa, CA, U.S.A). Lysophospholipid (from egg yolk) was obtained from Sigma (Deisenhofen, Germany).

2.2 α-Amylolysis of starch

2.2.1 α-Amylolysis of starch granules (Publications I–III)

The method used for hydrolysis of barley starch granules is presented in Figure 1. In some cases, prior to α-amylolysis starch-water suspensions were incubated in a water bath at 50, 55 and 60 ºC in order to obtain granules at different stages of gelatinization. When the effect of added lipids was studied along with the partial gelatinization, starch-water suspensions were incubated at 54 ºC with and without added LPL for 3 h with minimum magnetic stirring to prevent sedimentation. Incubation at this temperature caused the gelatinization enthalpy to be about half that of the original. In order to remove any unbound LPL from the granules, the suspensions were centrifuged at 10 844 g for 10 min and the insoluble residue was washed once with ethanol and twice with water and then freeze dried.

The starch-buffer (0.1 M ammonium acetate, pH 6) suspensions were tempered to the hydrolysis temperature of 30 ºC. α-Amylase solution was added, so that the enzyme dosage was 0–1500 U/g of starch, and final starch concentration was 5%. Minimal magnetic stirring was used to prevent sedimentation. After 24 h, the hydrolysis was stopped by adjusting to pH 2 with 1 M HCl for 30 min and

then back to pH 6 with 1 M NaOH. Insoluble starch was separated by centrifugation (10 844 g, 10 min) and freeze dried.

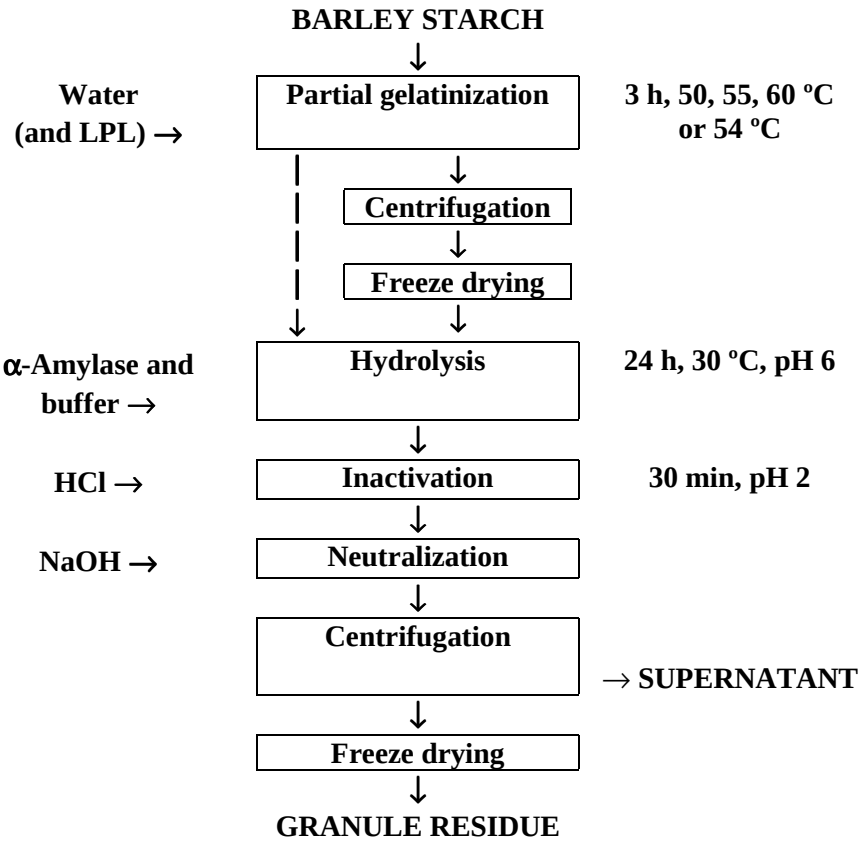


Figure 1. Scheme for preparation of hydrolyzed barley starch granules.

2.2.2 α-Amylolysis of gelatinized starch (Publications IV & V)

Figure 2 summarizes the steps used in preparing the hydrolysates from gelatinized barley and waxy barley starches. The starch-water suspension (7% starch) was gelatinized in a boiling water bath for 20 or 30 min, or in one case at 120 ºC in a pressure reactor to study the effect of a more uniform microstructure of the substrate on hydrolysis. Suspensions were then cooled to the hydrolysis temperature of 70 ºC. The α-amylase solution and 0.1 M ammonium acetate, pH 6, were added to reach a final starch concentration of 5% and an α-amylase

dosage of 0–500 mU/g. After incubation (0–24 h), the hydrolysis was stopped by keeping the samples in a boiling water bath for 10 to 30 min.

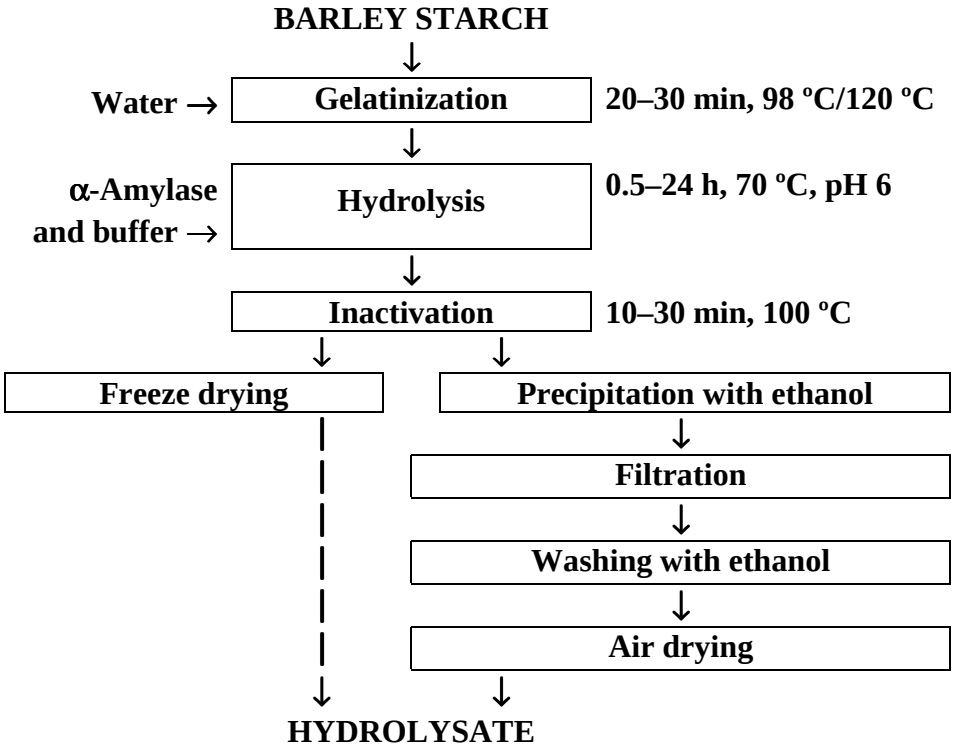


Figure 2. Scheme for gelatinization and hydrolysis of barley and waxy barley starches.

2.3 Analyses

Details of the analytical methods are given in the original papers, and the methods are only briefly described below.

2.3.1 Chemical analyses

The amounts of carbohydrates solubilized due to the α-amylolysis as well as the solubilities of the hydrolysates at 85 and 90 ºC were determined using the phenol-sulphuric acid method using glucose as standard (Dubois *et al.* 1956;

Leach *et al.* 1961). The amounts of reducing sugars were determined by the 3,5-dinitrosalisylic acid (DNS) method using glucose as standard (Sumner 1924). Total and apparent amylose contents were determined colorimetrically according to the method of Morrison and Laignelet (1983). Phospholipid content was analyzed by phosphorous analysis (Morrison 1964, Tester & Morrison 1990).

2.3.2 Analysis of molecular structure

Molecular weight distributions of starches were analyzed after dissolving the samples in NaOH either by SEC-HPLC with the column combination μHydrogel 2000, 500 and 250 (Suortti & Pessa 1991) or gel permeation chromatography on Sepharose CL 2B. The molecular weight of starch was determined by laser-light scattering and the molecular weight of amylose was determined using post-column iodine coloring and spectrophotometric detection as described by Suortti *et al.* (1998). For the analysis of lower molecular weight saccharides μHydrogel columns 250 and 120 and, in some cases two Fast Fruit Juice columns were also used. The chain length distribution of amylopectin was studied by hydrolyzing the samples with isoamylase and analyzing the formed dextrins by ion exchange chromatography with pulsed amperometric detection. Along with the one used in publication V, another gradient was also used. In this case, the samples were eluted with a linear gradient of 48–300 mM sodium acetate in 100 mM sodium hydroxide over 50–62.5 minutes. Also, in this case, the flow rate was 1 ml/min.

2.3.3 Analysis of granular structure

Starch granules and granule residues were examined with environmental scanning electron microscopy. Particle size distributions of the granule residues suspended in water were analyzed using a particle size analyzer based on laser diffraction. The results were shown as the total volume of particles as a function of diameter.

2.3.4 Gelatinization behavior and crystallinity

The gelatinization behavior of barley starch granules was analyzed with differential scanning calorimetry at 70% moisture content and a scanning rate of 10 ºC/min. The melting of waxy barley starch hydrolysate gels was also followed with DSC. Changes in the crystallinity of starch granules due to the α-amylolysis were studied with X-ray analysis.

Effects of α-amylolysis on the heat-induced microstructural changes of barley starch suspensions were studied by light microscopy using the smear technique and iodine staining (Autio 1990).

3. Results and discussion

3.1 Accessibility and hydrolysis mechanism

3.1.1 Native starch granules (Publication I)

Susceptibility of large barley starch granules to hydrolysis by *Bacillus licheniformis* α-amylase at 30 ºC was studied by measuring the amount of carbohydrates solubilized during hydrolysis. Measurement of solubilized carbohydrates does not give a direct measure of hydrolyzed linkages but it is convenient in estimating the extent of hydrolysis. During 24 h of hydrolysis, up to 94% solubilization occurred with the enzyme dosages used (Fig. 3). Until about 50% of the starch was solubilized, the amount of solubilized carbohydrates increased dramatically with the amount of enzyme used. For further solubilization, the relative amount of enzyme needed was clearly increased. After 94% of the starch had solubilized due to the hydrolysis, an increase in the enzyme dosage did not further solubilize the granules.

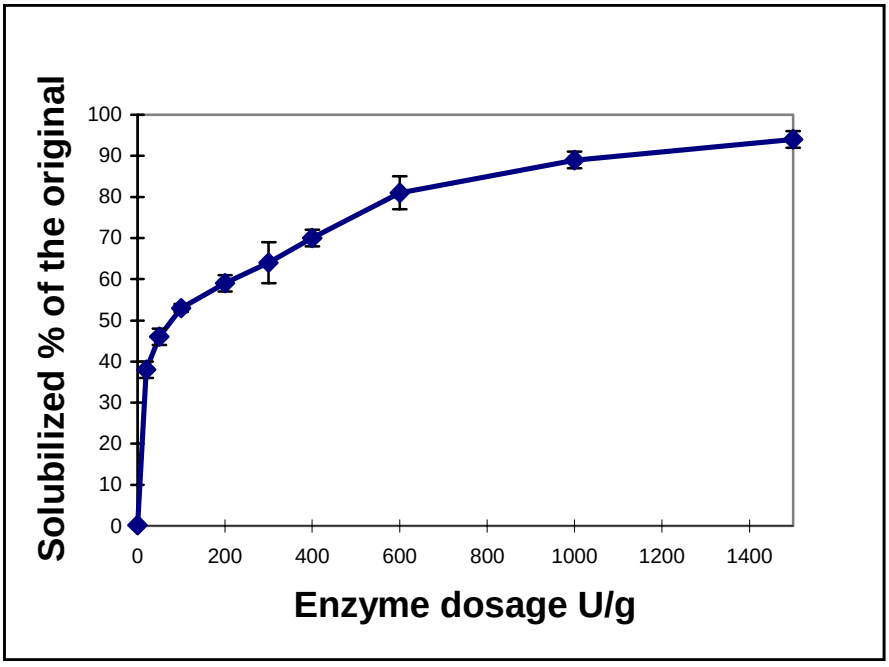


Figure 3. The extent of solubilization of large barley starch granules due to α-amylolysis.

Changes in molecular structure

In the insoluble hydrolysis residue, the molecular weights of both amylose and amylopectin were decreased due to the α-amylolysis. The MW of barley starch (the reference sample) was 180×10^6 g/mol as determined with laser-light scattering. The MW of amylose was 880×10^3 g/mol. When half of the starch had solubilized due to the amylolysis, all of the original amylopectin had degraded and its MW in the insoluble residue had decreased to 31×10^6 g/mol and the MW of amylose had decreased to 230×10^3 g/mol. According to Colonna *et al*. (1988) and Manelius *et al*. (1997), in the α-amylolysis of wheat A-starch granules the molecular weight profile of the residue is similar to the original, except for a small peak representing short dextrins. This was explained by a granule-by-granule hydrolysis mechanism. In the study of Colonna *et al*. (1988), large granules were hydrolyzed preferentially and in our study, only large barley starch granules were used; thus the hydrolysis could be expected to be more uniform. According to Bertoft & Manelius (1992) the changes in the molecular weight within the granules are more marked when higher concentrations of enzyme are used instead of a longer hydrolysis time, and in our study, the enzyme dosage was increased whereas in the study of Colonna *et al*. (1988) and Manelius *et al*. (1997) the hydrolysis time was varied.

The distribution of amylopectin chain lengths was studied by treating the granule residues with isoamylase and analyzing the formed dextrins with ion exchange chromatography. There were no changes in the chain length profile even when half of the starch had solubilized due to the amylolysis (Fig. 4). According to Bertoft & Henriksnäs (1982) and Bertoft (1986) the distinct intermediate products formed from amylopectin due to the α-amylolysis represent clusters or part of clusters suggesting a highly ordered structure of amylopectin. The highly ordered structure of amylopectin forces the amylase to initially attack some glucosidic bonds in preference to others. Amylopectin was thus first hydrolyzed mainly between the clusters near branch points in the amorphous regions.

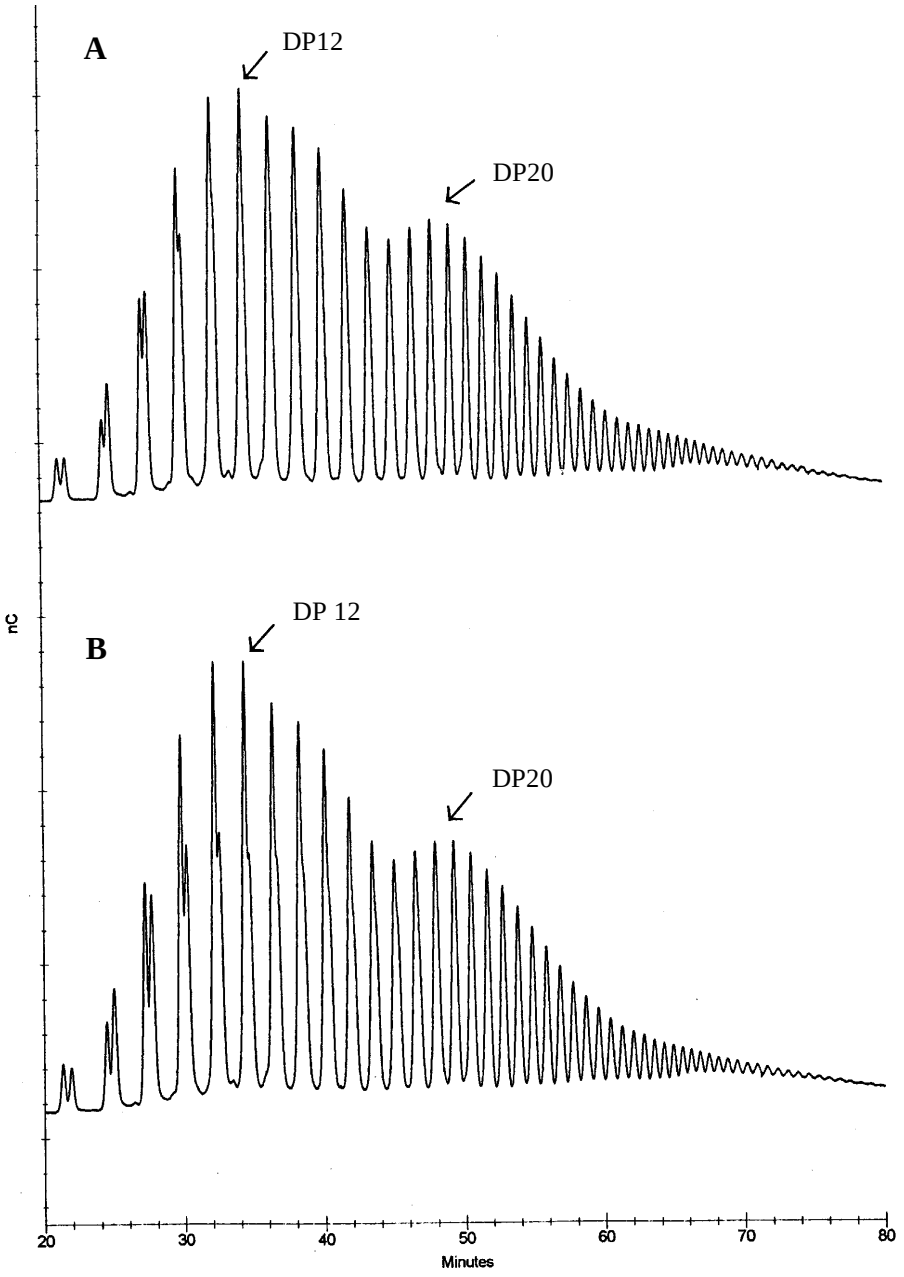


Figure 4. The effect of α-amylolysis on the chain length profile of amylopectin from large barley starch granules. (a) native barley starch, (b) hydrolysis residue (58 % left).

The accessibility of starch components was further studied by analyzing the amounts of amylose, free amylose (FAM) and lipids in the residue. The amount of lipid complexed amylose (LAM) was obtained by subtracting free amylose from total amylose. An improved method for the colorimetric determination of amylose as its polyiodide complex was used (Morrison & Laignelet 1983). The procedure may be used to measure amylose with DP > 230 which was the case in this study despite the shortening of amylose chains due to the amylolysis.

The FAM content of the granule residues had alreadydecreased in the early stages of the hydrolysis whereas LAM and lipids concentrated in the granule residues until 40–50% of the total starch was solubilized. While the original LAM content was 6%, the maximum LAM content was 16% when the residue represented 30% of the original starch. Also, the lipid-complexed amylose may have been hydrolyzed in between the complexed parts, but the products were insoluble. With higher enzyme dosages LAM and lipids also leached out of the granule residues. No preferential solubilization of either amylose or amylopectin has been observed in the case of large wheat starch granules with total solubilization of 74% (Colonna *et al.* 1988) and with total solubilization of 15% (Manelius *et al.* 1997). However, an increase in the amount of short amylose chains in large wheat starch granule residues is observed suggesting that part of the amylose is hydrolyzed into non-soluble dextrins (Manelius *et al.* 1997). From the small wheat starch granules, slightly more amylose than amylopectin was was hydrolyzed into solubilizing material when total solubilization was 15% (Manelius *et al.* 1997). For oat starch granules, it has been suggested that free amylose is preferentially attacked by α-amylase (Manelius & Bertoft 1996). All types of starch granules thus appear to be unique.

Changes in granular structure

The changes in granular structure of the hydrolysates were studied by environmental scanning electron microscopy, particle size analysis, X-ray analysis and differential scanning calorimetry (DSC). At low degrees of solubilization due to hydrolysis, pinholes in the granules were seen with ESEM. With more extensive hydrolysis, partially hollow granule residues and granule fragments were observed. This formation of pinholes and hydrolysis from inside out is typical for large barley starch granules (MacGregor and Ballance 1980).

Helbert *et al*. (1996) investigated the diffusion of *Bacillus licheniformis* α-amylase into corn starch granules by direct electron microscopic observation with a technique involving immuno-gold labeling of the enzyme and cross-sectioning of hydrated starch granules. According to them, *B. licheniformis* α-amylase randomly adsorbs at the surface of the granules and hydrolysis then proceeds radially resulting in the formation of pores. The pores prevent the free diffusion of the enzymes and entraps them inside the granule leading to inside-out hydrolysis.

Even when the residue represented only 6% of the original starch, it contained apparently intact granules when observed with ESEM. The evenness of the hydrolysis of granular starch is dependent on the starch origin, enzyme origin and the conditions used in the hydrolysis. According to Colonna *et al.* (1988), wheat starch granules were not equally susceptible to α-amylolysis, and even after 91% solubilization, most of the residual granules were intact. On the other hand, Bertoft *et al.* (1993) showed that in the α-amylolysis of granular pea starch, almost all granules were already fragmented after 7% solubilization. There are also reports of similar results from corn starch (Leach & Schoch 1961; Planchot *et al.* 1995).

α-Amylolysis caused only a slight decrease in the average size of large barley starch granules, from 16 μm to 14 μm. However, the amount of both small (<10 μm) and large (>30 μm) particles increased when only 6 % of the starch was left in the hydrolysis residue. The small particles were granule fragments also seen with ESEM. The increase in the large particles was most likely an artifact caused by the aggregation of granule fragments when suspended in water for the particle size analysis, since no large particles were observed with ESEM.

According to X-ray data, the degree of crystallinity remained constant during the early stages of α-amylolysis of large barley starch granules, indicating that both amorphous and crystalline parts of the granules were equally solubilized. More extensive hydrolysis (> 50% solubilized) caused a gradual decrease in total crystallinity and degradation of the granular structure. This was also seen as a more dramatic decrease in the gelatinization enthalpy measured with DSC. α-Amylolysis thus occurs in a different manner than acid hydrolysis of starch granules as acid preferentially attacks the amorphous areas of the granule, leaving a highly crystalline residue. It has been postulated that the larger size of

the amylase molecule compared to the H_3O^+ molecule prevents its diffusion throughout the granule, thus forcing the breaking of the crystalline structures. Solubilization of crystalline regions during amylolysis is said to occur through disentanglement of crystalline chains by α-amylase attacking amorphous parts nearby (Colonna *et al.* 1988).

3.1.2 Partially gelatinized granules (Publications I & III)

During gelatinization, both the crystalline and molecular order of starch granules are disrupted. When compared with accessibility of native starch, incubation of starch for 3 hours below 50 ºC did not increase its enzymatic accessibility. With higher pre-heating temperatures of 54, 55 and 60 ºC, the amount of solubilized carbohydrates increased during subsequent α-amylolysis at 30 ºC. When starch was pre-heated at 54 ºC and higher temperatures, a decrease in the gelatinization enthalpy was observed with DSC. The increased enzymatic accessibility can well be explained by the reduced order and crystallinity of starch due to partial gelatinization.

The dissociation of the amylose-LPL complex occurs at temperatures above 100 ºC (Biliaderis & Galloway 1989). The pre-heating temperatures used were thus not high enough to cause the dissociation. With regard to the leaching of lipid complexed amylose, the α-amylolysis of partially gelatinized barley starch was similar to that of native barley starch granules: lipid complexed amylose hydrolyzed less into solubilizing material than free amylose and amylopectin.

Partial gelatinization changed the α-amylolysis pattern of barley starch granules and the pinholes typical of α-amylase treated large barley starch granules could not be seen with environmental scanning electron microscopy. The lack of pinholes suggests that changes in the granule surface structure had occurred during partial gelatinization. The effect of partial gelatinization on the microstructure of barley starch granule dispersion during heating was studied using light microscopy (smear technique, iodine staining). The preincubation of starch at 54 ºC and 55 ºC caused the granules to be less swollen at 80 ºC and 90 ºC, and the center of the granules to be more disrupted. However, the resolutions of ESEM and light microscopy are not high enough to allow detailed

study of the granule surface structure. This could be done by using atomic force microscopy (AFM) combined with SEM (Baldwin *et al*. 1997, 1998).

3.1.3 Gelatinized starch (Publications IV & V)

Gelatinized starch is highly susceptible to amylolysis and the enzyme dosages and/or times needed for starch solubilization are much smaller than in the case of granular starch. Barley starch was gelatinized in a boiling water bath. In order to prevent retrogradation of starch, the temperature used in amylolysis of gelatinized barley and waxy barley starches was 70 ºC instead of 30 ºC which was used in the hydrolysis of granular starch. Therefore, direct comparisons of the enzyme dosages and incubation times could not be done in this study.

During α-amylolysis of gelatinized barley starch, the molecular weight of both amylose and amylopectin decreased quickly and simultaneously. As was the case with granular barley starch, the initial attack of amylopectin occurred mainly between the clusters here also, without significant hydrolysis of the external chains. The formation of low molecular weight hydrolysis products, as indicated by an increase in reducing power, was only observed when all the original high molecular weight amylopectin had degraded. This was verified for barley starch amylopectin with GPC analysis and for waxy barley starch amylopectin by isoamylolysis and ion exchange chromatography. Using gelatinized waxy maize amylopectin, α-amylase has also previously been found to hydrolyze amylopectin molecules in a non-random manner, preferentially attacking the glucosidic bonds between the unit clusters (Bertoft 1986).

3.1.4 Role of lipids (Publication III)

As stated in the introduction (1.1.2), in native barley starch, about 20% of amylose is complexed with lysophospholipids. In the α-amylolysis of large barley starch granules, the lipid complexed amylose appeared to be more resistant than free amylose. The effect of lipid addition on accessibility was studied.

Lysophospholipid (3% of the amount of starch) was added during partial gelatinization. LPL was bound to the starch granules since it could not be removed even by washing with ethanol in which LPL is highly soluble. To achieve the best conditions for the formation of a complex between amylose and LPL, both should be in solution (Eliasson & Krog 1985). When lipids are added to starch, the conditions for complex formation are less favorable. Despite the binding of LPL to the granules, the complexation of amylose and added lipids did not necessarily take place during this treatment. It could also have occurred during gelatinization of starch during DSC analysis, and when analyzing amylose contents, starch is solubilized in DMSO, in which the complex formation also occurs. The lipid content of the granules increased from 0.9% to 3.1% and LAM content from 7.6% to 22.1%. The dissociation enthalpy of the amylose-lipid complex increased from 1.1 J/g to 4.5 J/g.

Swelling of starch granules is considered to be a property of amylopectin. The fraction of free amylose that does not leach out during heating contributes to the swelling whereas lipid complexed amylose inhibits the swelling (Morrison *et al.* 1993b). Binding of lipids to the starch granules during partial gelatinization stabilized the granular structure. The swelling was prevented and solubility at 90 ºC was decreased. The gelatinization properties of starch can thus be modified by added lipids. Also, Eliasson *et al.* (1981) observed decreased disruption of potato starch granules with an increasing amount of lipids present on the surface.

The solubilization during α-amylolysis at 30 ºC also decreased due to the added lipids. In the case of partially gelatinized starch, with an enzyme dosage of 0.5 U/g of starch, 53% of the starch remained in the insoluble residue. To achieve a similar degree of solubilization when lipids had been added during partial gelatinization, about ten times more enzyme was required. LAM and lipids concentrated in the insoluble residue and also the remaining FAM (6%) did not solubilize. In this case in the early stages of amylolysis, mostly amylopectin was hydrolyzed into soluble carbohydrates. In native barley starch, FAM and LAM exist as separate molecules (Morrison *et al.* 1993b). It is possible that at this point, the free and lipid complexed amylose were not separate molecules. The small amount of FAM could have existed as parts of otherwise complexed molecules.

These results suggest that complexation occurred either during pre-treatment at 54 ºC between amylose and added LPL or after hydrolysis at 30 ºC between linear hydrolysis products of amylose and LPL. In both cases, insoluble products were formed.

3.2 Properties of hydrolysates

3.2.1 Solubility (Publications II & III)

Partial α-amylolysis increased the solubility of granular barley starch and had a large influence on the microstructure of barley starch dispersion during heating. When about 3% of carbohydrates had solubilized during amylolysis, solubility of the residue at 85 ºC had already increased by about 20%, and when the residue represented half of the original starch granules, the solubility at 90 ºC was doubled. Also, acid-hydrolysis increases the solubility of barley starch granules (Autio *et al*. 1992). Since acid and enzymatic hydrolysis affect the granules in different ways, acid preferentially hydolyzing the amorphous areas of the granule, the increase in solubility might be caused more by the decreased molecular weight than by changes in the granular structure. A similar increase in the solubility was observed for the partially gelatinized starch after α-amylolysis. Partial gelatinization itself did not affect the solubility of barley starch. Lipid addition during partial gelatinization, however, decreased the solubility at 90 ºC from 15% to 2%. This was because the added lipids formed insoluble complexes with amylose molecules during the heating of starch and lipids in water. α-Amylolysis increased the solubility of LPL-treated starch to 10%. In this case, the soluble carbohydrates were branched and originated from amylopectin and not from amylose as in the case of partially gelatinized starch without added lipids (Fig. 5).

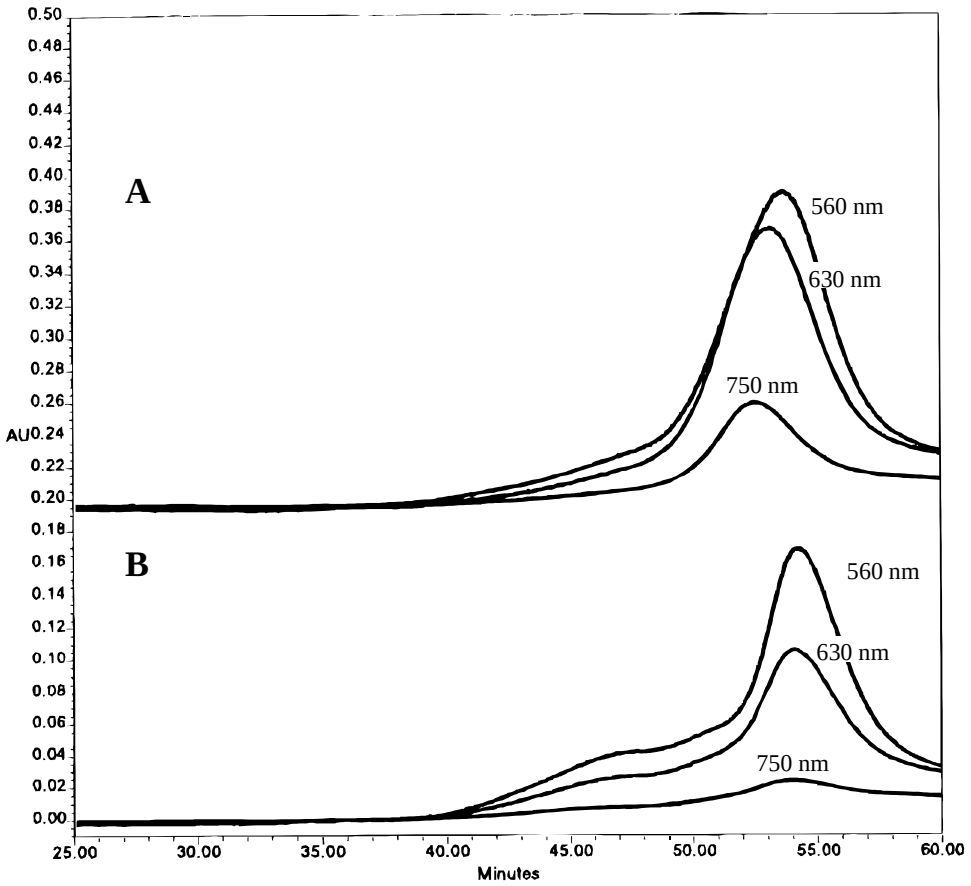


Figure 5. The size-exclusion chromatography of solubilized material at 90°C from (a) partially gelatinized and hydrolyzed (residue 53%) large barley starch granules and (b) partially gelatinized with added lipids and hydrolyzed (residue 55%) large barley starch granules.

3.2.2 Microstructure (Publications III & IV)

During heating of dilute (2–8%) starch suspensions up to 90 ºC, native barley starch granules swell and amylose leaches out leaving twisted and folded amylopectin-rich granule residues, "ghosts". Mild α-amylolysis (less than 1 % of starch solubilized) caused no visible difference in this behavior. More extensive hydrolysis however caused visible changes in the microstructure of starch

suspensions. α-Amylase-treated starch granules (58% of original starch left) in 2% suspensions at temperatures of 80 and 90 ºC were more transparent than native starch granules. Although the extent of swelling appeared to be similar, the swelling mechanism of amylase-treated granules was different, partially hydrolyzed granule residues swelled more evenly without the twisting typical of native granules. The pinholes seen in SEM pictures were also visible by light microscopy.

The transparency and different swelling behavior was also observed with starch partially gelatinized prior to hydrolysis (53% of original starch left). As also seen with SEM, no pinholes were seen. After the addition of LPL during partial gelatinization, the α-amylase-treated starch granules (55% of the original starch left) were partly fragmented and they did not swell during heating in water at temperatures below 90 ºC. The granules stained violet with iodine and no leaching of amylose from the granules was observed.

Heating barley starch suspensions to ~100 ºC without shear produced a paste containing granule residues, ghosts. The addition of α-amylase caused degradation of these ghosts and phase separation of amylose and amylopectin. Barley starch heated in a pressure reactor to 120 ºC was more homogeneous, only a few ghosts were observed, with small amylose-rich domains dispersed in an amylopectin network. After α-amylolysis, however, amylose-rich domains were separated in a way similar to hydrolysis of starch gelatinized at 100 ºC. The microstructure resulting from depolymerization of gelatinized barley starch by α-amylase was thus independent of the different preheating conditions and substrate microstructure. The extent and pattern of α-amylolysis was the same with both substrates.

3.2.3 Gelation (Publication V)

Starch and starch hydrolysis products are widely used in the food industry mostly because of their ability to form viscous solutions, gels, films and encapsulating matrices. Applications for starch hydrolysis products could be further developed by understanding the mechanisms of their enzymatic hydrolysis and also their structure-function relationships. In order to get more information on the dependence of amylopectin association on size and structure,

the gelation behavior of enzymatic hydrolysates of waxy barley starch (amylopectin) with different molecular sizes was studied.

Concentrated solutions of amylopectin can form opaque, elastic gels. The rate of gelation is slow. With increasing concentration and decreasing temperature, gelation is more rapid but even at +1 ºC the stiffness of the gel takes several weeks to reach the plateau value. The stiffness of gels (20% w/w of amylopectin) prepared from the polymeric products decreased with increasing hydrolysis of the amylopectin. A linear relationship between the shear modulus and the original high-molecular weight amylopectin content was observed. Although the branched degradation products of amylopectin (average DP < 6 x 10^4) were not involved in network formation, they retained their ability to recrystallize as analyzed by DSC. For more extensively hydrolyzed products (average DP < 5000) no recrystallization or gel forming was observed under the conditions tested. These results are in agreement with the results of Durrani and Donald (1995) in that both ΔH and G' increase with increasing storage time and increasing molecular weight; but there was no linear correlation between the melting enthalpy and the shear modulus like Durrani and Donald (1995) observed for acid-hydrolyzed waxy maize amylopectins. Also Ring *et al.* (1987) found no simple relationship between the melting enthalpy and the shear modulus while studying the 20% gels of amylopectins from different origin.

Information on the gel structure was obtained by acid hydrolysis of the gels and ion exchange chromatography of the obtained dextrins. The structure of the acid-resistant gel residues was complicated, showing a five-peak distribution when analyzed with ion-exchange chromatography. No differences were observed between the original and hydrolysate gels. The gel residues were resistant to isoamylase. In conclusion, the approximately linear relationship between the original amylopectin content and shear modulus of the waxy barley starch gels, and the similarity in the structure of acid resistant regions of the reference gel and hydrolysate gels indicate that high molecular weight amylopectin is needed for gel formation to occur in these gels.

4. Conclusions

In the batch-wise α-amylolysis of large barley starch granules, the molecular weights of both amylose and amylopectin decrease simultaneously. Amylopectin hydrolysis starts between the clusters without shortening of the external chains. Also during α-amylolysis of gelatinized barley starch, the molecular weight of both amylose and amylopectin decrease simultaneously. As in the case of granular starch, the initial attack of amylopectin occurs mainly between the clusters, without significant hydrolysis of the external chains. It can thus be concluded that, regardless of the physical state of barley starch, hydrolysis of amylopectin starts in between the clusters.

It was shown that at the early stages of amylolysis of barley starch granules, the solubilizing carbohydrates originate from free amylose and amylopectin. Lipid-complexed amylose is less accessible and concentrates in the insoluble granule residues until about half of the original starch has solubilized.

Partial gelatinization changes the α-amylolysis pattern of large barley starch granules and the pinholes typical of α-amylase-treated large barley starch granules cannot be seen. With regard to the leaching of lipid-complexed amylose, the α-amylolysis of partially gelatinized barley starch is similar to that of native barley starch granules. When lipids are added to starch during partial gelatinization, the small amount of remaining free amylose concentrates in the residue along with lipid complexed amylose during subsequent α-amylolysis. This indicates that free amylose no longer exists as separate molecules but as part of otherwise complexed and thus insoluble molecules.

Partial gelatinization has no effect on the solubility of barley starch but it increases the accessibility of starch components to α-amylase. Additional lipid binding to starch at the early phases of gelatinization stabilizes the granular structure. The swelling is prevented and solubility at 90 ºC, as well as solubilization during α-amylolysis at 30 ºC, is decreased. It was shown that starch structure and its properties can be changed with partial gelatinization and lipid addition without destroying the granule structure.

References

Anger, H., Richter, M., Kettlitz, B. & Radosta, S. 1994. Hydrotermische Behandlung von Stärke in Gegenwart von α-Amylase. Teil 1: Hydrolyse von Stärke mit α-Amylase in heterogener Phase. Starch/Stärke, Vol. 46, pp. 182–186.

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

Autio, K. 1990. Rheological and microstructural changes of oat and barley starches during heating and cooling. Food Structure, Vol. 9, pp. 297–304.

Autio, K., Poutanen, K., Suortti, T. & Pessa, E. 1992. Heat-induced structural changes in acid-modified barley starch dispersions. Food Structure, Vol. 11, pp. 315–322.

Baldwin, P.M., Adler, J., Davies, M.C. & Melia, C.D. 1994. Holes in starch granules: confocal, SEM and light microscopy studies of starch granule structure. Starch/Stärke, Vol. 46, pp. 341–346.

Baldwin, P.M., Davies, M.C. & Melia, C.D. 1997. Starch granule surface imaging using low-voltage scanning electron microscopy and atomic force microscopy. Int. J. Biol. Macromolecules, Vol. 21, pp. 103–107.

Baldwin, P.M., Adler, J., Davies, M.C. & Melia, C.D. 1998. High resolution imaging of starch granule surfaces by atomic force microscopy. J. Cereal Science, Vol. 27, pp. 255–265.

Banks, W., Greenwood, C.T. & Khan, K.M. 1970. Studies on starch-degrading enzymes. Carbohydr. Res., Vol. 12, pp. 79–87.

Beleia, A. & Varriano-Marston, E. 1981. Pearl millet Amylases. II. Activity toward intact and heated starch granules. Cereal Chem., Vol. 58, pp. 437–440.

Bello-Pérez, L.A., Roger, P., Colonna, P. & Paredes-López, O. 1998. Laser light scattering of high amylose and high amylopectin materials, stability in water after microwave dispersion. Carbohydr. Pol., Vol. 37, pp. 383–394.

Bertoft, E. 1986. Hydrolysis of amylopectin by the alpha-amylase of *B. subtilis*. Carbohydr. Res., Vol. 149, pp. 379–387.

Bertoft, E. 1989. Partial characterization of amylopectin alpha-dextrins. Carbohydr. Res., Vol. 189, pp. 181–193.

Bertoft, E. & Henriksnäs, H. 1982. Initial stages in α-amylolysis of barley starch. J. Inst. Brew., Vol. 88, pp. 261–265.

Bertoft, E. & Kulp, S.-E. 1986. A gel filtration study on the action of barley α-amylase isoenzymes on granular starch. J. Inst. Brew., Vol. 92, pp. 69–72.

Bertoft, E. & Manelius, R. 1992. A method for the study of the enzymatic hydrolysis of starch granules. Carbohydr. Res., Vol. 22, pp. 269–283.

Bertoft, E., Qin, Z. & Manelius, R. 1993. Studies on the structure of pea starches. Part 3: Amylopectin of smooth pea starch. Starch/Stärke, Vol. 45, pp. 377–382.

Bertoft, E. & Åvall, A.-K. 1992. Structural analysis on the amylopectin of waxy-barley large starch granules. J. Inst. Brew., Vol. 98, pp. 433–437.

Biliaderis, C.G. & Galloway, G.I. 1989. Crystallization behavior of amylose-V complexes: structure-property relationships. Carbohydr. Res., Vol. 189, pp. 31–48.

Blanshard, J.M.V. 1987. Starch granule structure and function: a physico-chemical approach. In: Galliard, T. (ed.) Starch: Properties and Potential. Chichester: John Wiley & Sons. Pp. 16–54.

Buléon, A., Colonna, P., Planchot, V. & Ball, S. 1998. Starch granules: structure and biosynthesis. Biological Mocramolecules, Vol. 23, pp. 85–112.

Colonna, P., Buléon, A. & Lemarié, F. 1988. Action of *Bacillus subtilis* α-amylase on native wheat starch. Biotechnol. Bioeng. Vol. 31, pp. 895–904.

Cooke, D. & Gidley, M.J. 1992. Loss of crystalline and molecular order during starch gelatinization: origin of the enthalpic transition. Carbohydr. Res., Vol. 227, pp. 103–112.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. & Smith, F. 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem., Vol. 28, pp. 350–356.

Durrani, C.M. & Donald, A.M. 1995. Physical characterization of amylopectin gels. Polymer Gels and Networks, Vol. 3, pp. 1–27.

Eliasson, A.-C. & Krog, N. 1985. Physical properties of amylose-monoglyseride complexes. J. Cereal Sci., Vol. 3, pp. 239–248.

Eliasson, A.-C., Larsson, K. & Miezis, Y. 1981. On the possibility of modifying the gelatinization properties of starch by lipid surface coating. Starch/Stärke, Vol. 33, pp. 231–235.

Evers, A.D. 1979. Cereal starches and proteins. In: Vaughan, J.G. (ed.) Food Microscopy. London: Academic Press. Pp. 139–191.

Fannon, J.E., Hauber, R.J. & BeMiller, J.N. 1992. Surface pores of starch granules. Cereal Chem., Vol. 69, pp. 284–288.

Fannon, J.E., Shull, J.M. & BeMiller, J.N. 1993. Interior channels of starch granules. Cereal Chem., Vol. 70, pp. 611–613.

Forssell, P., Mylläri nen, P., Heinämäki, J., Palviainen, P., Yliruusi, J. & Poutanen, K. 1999. Coated starch capsules and a process for producing them. WOPatent 9952512 A1.

French, D. 1972. Fine structure of starch and its relationship to the organization of starch granules. J. Jpn. Soc. Starch Sci., Vol. 19, pp. 8–25.

Galloway, G.I., Biliaderis, C.G. & Stanley, D.W. 1989. Properties and structure of amylose-glyceryl monostearate complexes formed in solution or on extrusion of wheat flour. J. Food Sci., Vol. 54, pp. 950–957.

Greenwood, C.T. & MacGregor, A.W. 1965. The isolation of α-amylase from barley and malted barley, and a study of the properties and action-patterns of the enzymes. J. Inst. Brew., Vol. 71, pp. 405–417.

Gusmán-Maldonado, H. & Paredes-López, O. 1995. Amylolytic enzymes and products derived from starch: A review. Critical Reviews in Food Science and Nutrition, Vol. 35(5), pp. 373–403.

Hanashiro, I., Abe, J. & Hizukuri, S. 1996. A periodic distribution of the chain length of amylopectin as revealed by high-performance anion-exchange chromatography. Carbohydr. Res., Vol. 283, pp. 151–159.

Helbert, W., Schülein, M. & Herrissat, B. 1996. Electron microscopic investigation of the diffusion of *Bacillus licheniformis* α-amylase into corn starch granules. Int. J. Biol. Macrom., Vol. 19, pp. 165–169.

Hizukuri, S. 1985. Relationship between the distribution of the chain-length of amylopectin and the crystalline structure of starch granules. Carbohydr. Res., Vol. 141, pp. 295–306.

Hizukuri, S. 1986. Polymodal distribution of the chain lengths of amylopectins, and its significance. Carbohydr. Res., Vol. 147, pp. 342–347.

Hizukuri, S. 1996. Starch: analytical aspects. In: Eliasson, A.-C. (ed.). Carbohydrates in Food. New York: Dekker. Pp. 347–429.

Hizukuri, S., Takeda, Y., Abe, J., Hanashiro, I., Matsunobu, G. & Kiyota, H. 1997. Analytical developments: molecular and microstructural characterization. In: Frazier, P.J., Richmond, P. & Donald, A.M. (eds.) Starch: Structure and Functionality. London: Royal Society of Chemistry. Pp. 121–128.

Holm, J., Björck, I., Ostrowska, S., Eliasson, A.-C., Larsson, K. & Lundquist, I. 1983. Digestibility of amylose-lipid complexes *in-vitro* and *in-vivo.* Starch/Stärke, Vol. 35, pp. 294–297.

Huber, K.C. & BeMiller, J.N. 1997. Visualization of channels and cavities of corn and sorghum starch granules. Cereal Chem., Vol. 74, pp. 537–541.

Huber, K.C. & BeMiller, J.N. 2000. Channels of maize and sorghum starch granules. Carbohydr. Pol., Vol. 41, pp. 269–276.

Inglett, G.E. 1987. Action pattern of *Bacillus licheniformis* alpha-amylase on ordinary, waxy and high-amylose corn starches and their hydroxypropyl derivatives. J. Food Biochem., Vol. 11, pp. 249–258.

Ivanova, V., Emanuilova, E., Sedlák, M. & Pazlarová, J. 1991. HPLC study of starch hydrolysis products obtained with α-amylase from *Bacillus amyloliquefaciens* and *Bacillus licheniformis.* Appl. Biochem. Biotechnol., Vol. 30, pp. 193–202.

Jane, J.-L., Xu, A., Radosavljevic, M. & Seib, P.A. 1992. Location of amylose in normal starch granules. I. Susceptibility of amylose and amylopectin to cross-linking reagents. Cereal Chem., Vol. 69, pp. 405–409.

Jane, J.-L., Wong, K.-S. & McPherson, A.E. 1997. Branch-structure difference in starches of A- and B-type X-ray patterns revealed by their Naegeli dextrins. Carbohydr. Res., Vol. 300, pp. 219–227.

Jenkins, P.J., Cameron, R.E., Donald, A.M., Bras, W., Derbyshire, G.E., Mant, G.R. & Ryan, A.J. 1994. In situ simultaneous small and wide-angle X-ray scattering: a new technique to study starch gelatinization. J.Polym. Sci., Polym. Phys. Ed., Vol. 32, pp. 1579–1583.

Kobayashi, S., Miwa, S. & Tsuzuki, W. 1992. Method of preparing modified starch granules. European Patent 0 539 910 A1.

Leach, H.W. & Schoch, T.J. 1961. Structure of the starch granule, II. Action of various amylases on granular starches. Cereal Chem., Vol. 38, pp. 34–46.

Lii, C.-Y. & Lineback, D.R. 1977. Characterization and comparison of cereal starches. Cereal Chem., Vol. 54, pp. 138–149.

Lineback, D.R. 1986. Current concepts of starch structure and its impact on properties. J. Jpn Soc. Starch Sci., Vol. 33, pp 80–88.

MacGregor, A.W. 1978. α-Amylase I from malted barley-physical properties and action pattern on amylose. Cereal Chem., Vol. 55, pp. 754–765.

MacGregor, A.W. 1993. Relationships between structure and activity in the α-amylase family of starch-metabolising enzymes. Starch/Stärke, Vol. 45, pp. 232–237.

MacGregor, A.W. & Ballance, D.L. 1980. Hydrolysis of large and small starch granules from normal and waxy barley cultivars by alpha-amylases from barley malt. Cereal Chem., Vol. 57, pp. 397–402.

MacGregor, A.W. & Fincher, G.B. 1993. Carbohydrates in the barley grain. In: MacGregor, A.W. & Bhatty, R.S. (eds.) Barley. Chemistry and technology. St. Paul: American Association of Cereal Chemists. Pp. 73–130.

MacGregor, A.W. & Morgan, J.E. 1984. Structure of amylopectins isolated from large and small starch granules of normal and waxy barley. Cereal Chem., Vol. 61, pp. 222–228.

MacGregor, A.W. & Morgan, J.E. 1986. Hydrolysis of barley starch granules by alpha-amylases from barley malt. Cereal Foods World, Vol. 31, pp. 688–693.

Manelius, R. & Bertoft, E. 1996. The effect of Ca^{2+}-ions on the α-amylolysis of granular starches from oats and waxy-maize. J. Cereal Sci., Vol. 24, pp. 139–150.

Manelius, R., Qin, Z., Åvall, A.-K., Andtfolk, H. & Bertoft, E. 1997. The mode of action on granular wheat starch by bacterial α-amylase. Starch/Stärke, Vol. 49, pp. 142–147.

Manners, D.J. & Matheson, N.K. 1981. The fine structure of amylopectin. Carbohydr. Res., Vol. 90, pp. 99–110.

McDonald, A.M.L., Stark, J.R., Morrison, W.R. & Ellis, R.P. 1991. The composition of starch granules from developing barley genotypes. J. Cereal Sci., Vol. 13, pp. 93–112.

Morgan, K.R., Furneaux, R.H. & Larsen, N.G. 1995. Solid-state NMR studies on the structure of starch granules. Carbohydr. Res., Vol. 276, pp. 387–399.

Morrison, W.R. 1964. A fast, simple and reliable method for the micro-determination of phosphorous in biological materials. Anal. Biochem., Vol. 7, pp. 218–224.

Morrison, W.R. 1988. Lipids in cereal starches: A review. J. Cereal Sci., Vol. 8, pp. 1–15.

Morrison, W.R. & Gadan, H.J. 1987. The amylose and lipid contents of starch granules in developing wheat endosperm. J. Cereal Sci., Vol. 5, pp. 263–275.

Morrison, W.R., Laignelet, B. 1983. An improved colorimetric procedure for determining apparent and total amylose in cereal and other starches. J. Cereal. Sci., Vol. 1, pp. 9–20.

Morrison, W.R., Scott, D.C. & Karkalas, J. 1986. Variation in the composition and physical properties of barley starches. Starch/Stärke, Vol. 38, pp. 374–379.

Morrison, W.R., Tester, R.F., Gidley, M.J. & Karkalas, J. 1993a. Resistance to acid hydrolysis of lipid-complexed amylose and lipid-free amylose in lintnerised waxy and non-waxy barley starches. Carbohydr. Res., Vol. 245, pp. 289–302.

Morrison, W.R., Tester, R.F., Snape, C.E., Law, R. & Gidley, M.J. 1993b. Swelling and gelatinization of cereal starches. IV. Some effects of lipid-complexed amylose and free amylose in waxy and normal barley starches. Cereal Chem., Vol. 70, pp. 385–391.

Morrison, W.R., Law, R.V. & Snape, C.E. 1993c. Evidence for inclusion complexes of lipids with V-amylose in maize, rice and oat starches. J. Cereal Sci., Vol. 13, pp. 93–112.

Myllärinen, P., Autio, K., Schulman, A.H. & Poutanen, K. 1998. Heat-induced changes of small and large barley starch granules. J. Inst. Brew., Vol. 104, Pp. 343–349.

Myllärinen, P., Forssell, P., von Wright, A., Alander, M., Mattila-Sandholm, T. & Poutanen, K. 1999. Starch capsules containing micro organisms and/or polypeptides or proteins and a process for producing them. WO 9952511A1.

Myllärinen, P., Forssell, P., von Wright, A., Alander, M., Mattila-Sandholm, T. & Poutanen, K. 2000. Mikro-organismeja sisältävät tärkkelyskapselit ja niiden valmistusmenetelmä. FI 104405B. [In Finnish]

Ong, M.H., Jumel, K., Tokarczuk, P.F., Blanshard, J.M.V. & Harding, S.E. 1994. Simultaneous determinations of the molecular weight distributions of amyloses and the fine structure of amylopectins of native starches. Carbohydr. Res., Vol. 260, pp. 99–117.

Oostergetel, G.T. & van Bruggen, E.F.J. 1993. The crystalline domains in potato starch granules are arranged in a helical fashion. Carbohydr. Pol., Vol. 21, pp. 7–12.

Planchot, V., Colonna, P., Gallant, D.J. & Bouchet, B. 1995. Extensive degradation of native granules by *alpha*-amylase from *Aspergillus fumigatus*. J. Cereal Sci., Vol. 21, pp. 163–171.

Planchot, V., Colonna, P. & Buleon, A. 1997. Enzymatic hydrolysis of α-glucan crystallites. Carbohydr. Res., Vol. 298, pp. 319–326.

Pszczola, D.E. 1999. Starches and Gums move beyond fat replacement. Food Technology, Vol. 53(8), pp.74–80.

Ring, S.G., Colonna, P., l'Anson, K.J., Kalichevsky, M. T., Miles, M.J., Morris, V.J. & Orford, P.D. 1987. The gelation and crystallization of amylopectin. Carbohydr. Res., Vol. 162, pp. 277–293.

Ring, S.G. & Stainsby, G. 1982. Filler reinforcement of gels. Prog. Food Nutr. Sci., Vol. 6. pp. 323–329.

Stark, J.R. & Lynn, A. 1992. Biochemistry of plant polysaccharides: starch granules large and small. biochem. Soc. Trans., Vol. 20, pp. 7–12.

Sumner, J.B. 1924. The estimation of sugar in diabetic urine, using dinitrosalicylic acid. J. Bio.l Chem., Vol. 62, Pp. 287–290.

Suortti, T. & Pessa, E. 1991. The GPC analysis of starches with alkaline eluents. J. Chromatogr., Vol. 536, pp. 251–254.

Suortti, T., Gorenstein, M.V. & Roger, P. 1998. Determination of the molecular mass of amylose. J. Chromatogr. A., Vol. 828, pp. 515–521.

Takeda, Y., Hizukuri, S., Takeda, C. & Suzuki, A. 1987. Structures of branched molecules of amyloses of various origins, and molar fractions of branched and unbranched molecules. Carbohydr. Res., Vol. 165, pp. 139–145.

Tao, B.Y., Reilly, P.J. & Robyt, J.F. 1989. Biochim. Biophys. Acta, Vol. 995, pp. 214–220.

Tester, R.F. & Morrison, W.R. 1990. Swelling and gelatinization of cereal starches. I. Effects of amylopectin, amylose and lipids. Cereal Chem., Vol. 67, pp. 551–557.

Trubiano, P.C. & Kasica, J.J. 1986. Compressible starches as binders for tablets or capsules. US Patent B! 4,551,177.

Whistler, R.L. 1989. Microporous granular starch matrix composition. WO Patent 89/04842.

Whistler, R.L. 1996. Subgranular crystalline starch as fat substitute. US Patent 5580390.

Whistler, R.L. 1997a. Fat substitute for processed foods. US Patent 5651828.

Whistler, R.L. 1997b. Porous particle aggregate and method therefor. US Patent 5,670,490.

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α-Amylolysis of barley starch

Abstract

The susceptibility of native barley starch granules and granules at different stages of gelatinization to α-amylolysis was studied by analyzing the amounts of solubilizing carbohydrates. The subsequent changes in the structure and properties of the insoluble residue were analyzed by various methods. The early stages of α-amylolysis of gelatinized barley and waxy barley starches were also followed. The gelation behavior of enzymatic hydrolysates of waxy barley starch with different molecular sizes was studied.

In the α-amylolysis of both gelatinized and ungelatinized barley starch, the molecular weights of both amylose and amylopectin decreased. Amylopectin hydrolysis started between the clusters without shortening of the external chains. In the early stages of α-amylolysis of barley starch granules, lipid-complexed amylose was less accessible and concentrated in the insoluble granule residue and the solubilizing carbohydrates originated from free amylose and amylopectin. Amorphous and crystalline regions of granules solubilized equally and with more extensive hydrolysis, the granular structure and crystallinity were destroyed.

Partial gelatinization changed the α-amylolysis pattern and the pinholes typical of α-amylase-treated large barley starch granules could not be seen. With regard to the leaching of lipid-complexed amylose, the α-amylolysis was similar to that of native barley starch granules. Additional lipid binding to starch during partial gelatinization stabilized the granular structure. Along with lipid complexed amylose, the small amount of free amylose remaining also concentrated in the residue, indicating that free amylose no longer existed as separate molecules but rather as part of otherwise complexed and thus insoluble molecules.

Partial α-amylolysis increased the solubility of barley starch and changed the mechanism of swelling of the granules; granules became more transparent and no twisting was observed.

Keywords
barley, starch, amylopectin, amylose, solubilisation, enzymatic hydrolysis, alpha-amylolysis, composition, structure, gelation

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