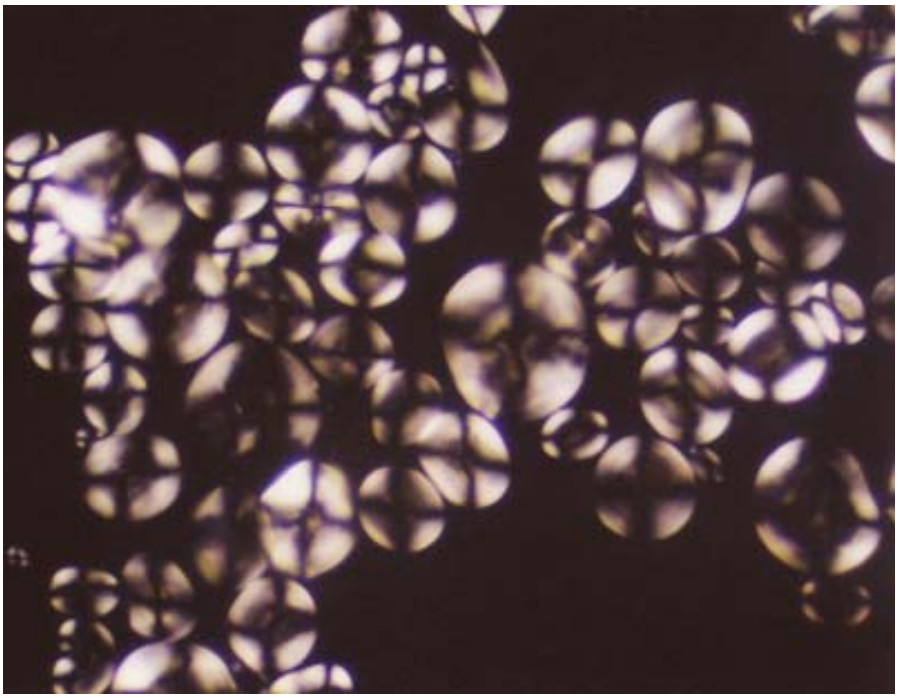
Salem Sassi Shamekh

Effects of lipids, heating and enzymatic treatment on starches



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Effects of lipids, heating and enzymatic treatment on starches

Salem Sassi Shamekh

VTT Biotechnology

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Abstract

The effects of heat treatment on oat and barley starch water dispersions were investigated. As compared with barley starch, the granules from oat starch were more degraded, but the fractionation patterns of both starches were not significantly different. Microscopic examination indicated that no evidence of amylopectin fragments was observed in the liquid fraction after starch dispersions were treated at 95°C even though chemical analysis demonstrated the solubility of both starch polymers. True solubility of oat starch was lower than that of barley starch, but the difference disappeared after removing the lipids from oat starch. By applying sequential centrifugation for starch dispersions, treated above 90°C, a fraction rich in an amylose-lipid complex could be produced.

Studies on crystallisation of starch gels were performed. The crystallisation rate of a gel prepared from oat starch was lower than those prepared from other cereal starches (barley and wheat). The effects of polar lipids separated from oats (oat lecithin) on crystallisation of wheat starch gel were investigated and compared with soya lecithin. Furthermore, the behaviour of polar lipids in the starch gel was compared with their effect on bread staling. Even though oat lecithin hydrolysate affected the crystallisation rate positively, soya lecithin hydrolysate was more effective both in gel as well as in bread.

The ability of esterases to hydrolyse lipids of barley starch at temperatures close to gelatinisation and of the gelatinised barley starch was examined. The extent of lipid hydrolysis of starch granules was only about 20%, but almost all of the lipids of the gelatinised starch were hydrolysed at 40°C. The effect of freeze-drying on α-amylolysis of potato starch was also investigated, and was observed to greatly enhance the enzyme accessibility of the granules.

The film formation property of hydrolysates prepared from potato starch was elucidated. Higher solids could be used in the film process due to a decrease in the molecular weight, and, after removing the water-soluble carbohydrates from the starch hydrolysates, good quality films were produced.

Preface

The present work was carried out at VTT Biotechnology during the years 1993–2000. I would like to express my sincere gratitude to Prof. Kaisa Poutanen and to Dr. Pirkko Forssell for complementing each other and for always being there to answer my endless questions. Without their support, great knowledge, professionalism, and kindness this work would not have turned out the way it did. I am most grateful to Prof. Simo Laakso and Prof. Katrina Nordström for their endless encouragement and support during my study and their kindness whenever we met.

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List of original publications

This dissertation is based on the following original articles, referred to as Publications I–V (Appendices I–V).

I Shamekh, S., Forssell, P. and Poutanen, K. 1994. Solubility pattern and recrystallization behavior of oat starch. Starch 46:129–133.

II Shamekh, S., Forssell, P., Suortti, T., Autio, K. and Poutanen, K. 1999. Fragmentation of oat and barley starch granules during heating. J. Cereal Sci. 30:173–182.

III Shamekh, S., Mustranta, A., Poutanen, K. and Forssell, P. 1998. Enzymatic hydrolysis of barley starch lipids. Cereal Chem. 75(5):624–628.

IV Forssell, P., Shamekh, S., Härkönen, H. and Poutanen. K. 1998. Effects of native and enzymatically hydrolysed soya and oat lecithins in starch phase transitions and bread baking. J. Sci. Food Agric. 76:31–38.

V Shamekh, S., Myllärinen, P., Poutanen, K. and Forssell, P. 2002. Film formation properties of potato starch hydrolysates. Starch 54:20–24.

The author of this thesis had the main responsibility for planning the research, practical work and interpretation of the results and wrote the manuscript in all publications, except the work related to bread in 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 in the form of water-insoluble granules is the major reserve polysaccharide in higher plants. The most important sources of starch are cereal grains, tubers and roots. Starch granules are mainly composed of two α-polyglucans, amylose and amylopectin, with different physico-chemical properties. Amylose is essentially linear, acting as an amorphous filler in the granules, whereas amylopectin is highly branched with shorter chains arranged as double helices in clusters of a partially crystalline character (Zobel 1988). The shape of the starch granules depends on botanical origin and many different forms are found in nature. Hence, the granular size varies from tiny granules in oat and rice to large ones in potato and banana starch. Because of the characteristic morphological properties of the granules, it is possible to identify most starches from their granular appearance under a light microscope.

When starch is heated in sufficient amounts of water, it is subjected to a number of irreversible changes known as starch gelatinisation (Atwell et al. 1988). Gelatinisation results with the formation of a gel with a dispersed phase of collapsed granules in an amylose-rich continuous phase. Gelatinisation significantly enhances the chemical reactivity of inert starch granules towards amylolytic enzymes; this has become the most acceptable method of enhancing starch hydrolysis and is widely adopted in the manufacture of starch syrups.

Starch is widely used as a food component and is sold on the basis of its functionality properties (Stockwell 1995). Starch is the main component of bread and its gelatinisation induces major structural changes during the baking of wheat bread. On cooling and ageing of starch gels or bread, rearrangements in the starch fraction lead to a series of changes including gelation and crystallisation (Zobel & Kulp 1996). Starch properties are often improved by chemical or enzymatic modifications (Fang et al. 2002). New food applications of starch are being developed continuously. There has been increased interest in the film formation properties of starches during the last years (Avérous et al. 2001). Starches may function as protective edible coatings and films due to their aroma and oxygen barrier characteristics.

1.1 Starch granular structure

Starch granules from different botanical sources possess a wide range of sizes, shapes and properties despite the similarities in their chemical composition and molecular structure (Stark & Lynn 1992). Barley and wheat have two types and size populations of starch granules; the large (25–40 μm) lenticular and small (5–10 μm) spherical granules. Oat starch has been shown to differ from other cereal starches in several ways. Oat starch granules are characterised by an angular or irregular shape, the granule size being mostly in the range of 3–10 μm (Paton 1977; Gudmundsson & Eliasson 1989; Hoover & Vasanthan 1992). However, individual granules have a marked tendency to aggregate into bundles or clusters (MacArthur & D´Appolonia 1979; Hoover & Vasanthan 1992). The granules of potato starch differ greatly in size and shape; the largest are often egg-shaped. The majority are flattened ellipsoids and the smallest may be perfectly spherical. The size of granules ranges from 15 to 100 μm. Several techniques are used for characterisation of starch granules. Light and scanning electron microscopy are widely applied to characterise the morphological structure and as well as the granule size distribution, while a coulter counter is also commonly used for starch granule analysis.

Starch granules consist of two structurally and functionally very different polymers: amylose and amylopectin. Starch functionality depends on the average molar mass of amylose and amylopectin as well as on their molecular structure and organisation within the granule. Their relative amounts, structures and molecular masses are determined by means of genetic and environmental control during biosynthesis, and hence a wide variation occurs among plant raw materials. The amylose contents of most starches, such as barley, wheat, oat, maize and potato starch, are all in the range of 20–30% (Morrison et al. 1993a,b; Morrison & Laignelet 1983). In amylomaize starch, the amylose content is higher (about 50–80%) and in waxy starches very low (<1%) (Morrison & Laignelet 1983). Amylose is an essentially linear polymer composed of α-D-glycopyranose units linked through α-(1→4) linkages. Amylopectin is a highly branched polymer; 5% of its structure is α-(1→6) branch links (Ball 1996). Amylose has a molar mass in the range 10^5–10^6 g/mol whereas amylopectin has a molecular mass of about 10^8 g/mol (Blanshard 1987). No significant differences have been found between the average molecular sizes of amylose of cereal starches and those of the tuber starches (Buléon et al. 1998).

Amylose and amylopectin form the amorphous and partially crystalline regions of the granules. The short chains of amylopectin are thought to be arranged in crystallites that would be responsible for the crystallinity of the native granule (Gallant et al. 1997). Crystallinity of starch granules is in the range of 20–40% (Hizukuri 1996) and the X-ray diffraction patterns are different for cereal and tuber starches (Zobel 1988). The two forms of X-ray diffraction patterns are associated with the two polymorph forms, the A-type in cereal starches (Zobel & Stephen 1995) and the B-type in tuber starches (Morrison et al. 1986; Imberty & Perez 1988; Zobel & Stephen 1995; Buléon et al. 1998). The structure of the A-type is denser than that of the B-type, which reflects the different behaviour of cereal and tuber starches in processing. A-type starches contain shorter average branch chain lengths of amylopectin, whereas B-type starches contain longer average branch chain lengths (Hizukuri 1985; Hanashiro et al. 1996). An additional pattern known as V-type crystallinity corresponds to structures of helical inclusion complexes of amylose (Godet et al. 1996). It is known that during complex formation, amylose changes from coil to helix and guest molecules (e.g. alcohols, monoglycerides or fatty acids) enter the central cavities of amylose helices, resulting in a partially crystalline amylose structure or V-amylose (Biliaderis 1992; Gernat et al. 1993).

1.2 Gelatinisation and gel formation

In most industrial applications, starches are heated in aqueous dispersions. The main events occurring during heating are swelling and solubilisation of starch polymers. The degrees of swelling and solubilisation depend mainly on the starch species. For example, amylose is preferentially leached from barley and wheat starches whereas amylose and amylopectin are reported to be co-leached from oat starch (Doublier et al. 1987; Autio 1990).

The term gelatinisation has become established in connection with starch and refers to irreversible physical changes taking place upon the heating of starch in water involving the loss of molecular order, the melting of crystallites, granular swelling and disruption and starch solubilisation (Atwell et al. 1988; Biliaderis 1998). These changes initially occur in the more accessible and amorphous regions. At the initial gelatinisation temperature, the granules swell and lose their birefringence, which indicates that the ordered regions are being disrupted.

The temperature of the starch gelatinisation is always a temperature range. For an individual starch granule in excess water this temperature range might be 0.5–1.5°C, whereas for the whole population of starch granules the range might be 10–15°C (Evans & Haisman 1982; Liu & Lelievre 1993).

Melting of the crystalline regions in the starch granule results in the disappearance of the birefringence of the starch granules observed in the polarising microscope and disappearance of the X-ray diffraction pattern. Differential scanning calorimetry (DSC) provides a facile procedure for the evaluation of starch gelatinisation. The gelatinisation enthalpy reflects primarily the loss of the molecular order of starch granules (Cooke & Gidley 1992). Single or double endothermic peaks are obtained depending on the water concentration during starch gelatinisation. Starch gelatinisation in excess water exhibits a single endothermic transition (typically with a peak temperature around 60°C), whereas when a starch-water dispersion is heated in the presence of a limited amount of water, two endothermic transitions were observed (Donovan 1979; Maaruf et al. 2001).

The properties of high and intermediate moisture materials, such as gels, cakes and bread, are mainly determined by gelation, i.e. the formation of an amylose and amylopectin network as well as retrogradation (Ring et al. 1987). Starch gels usually consist a complex system of swollen starch granules suspended in a matrix of amylose with the gelatinised granules reinforcing the gel (Figure 1). The rheological properties of starch gels are dependent on the type of starch (Shi & Seib 1992), on the characteristics of the degraded granule, and on the proportions of interacting constituents (Jan & Chen 1992; Lii et al. 1996). Amylose undergoes gelation at a faster rate and is dependent profoundly on water content (Longton & LeGrys 1981). Amylopectin has a high water-binding capacity and slowly undergoes retrogradation, thus forming clear gels that are soft and flow well (Yuan et al. 1993).

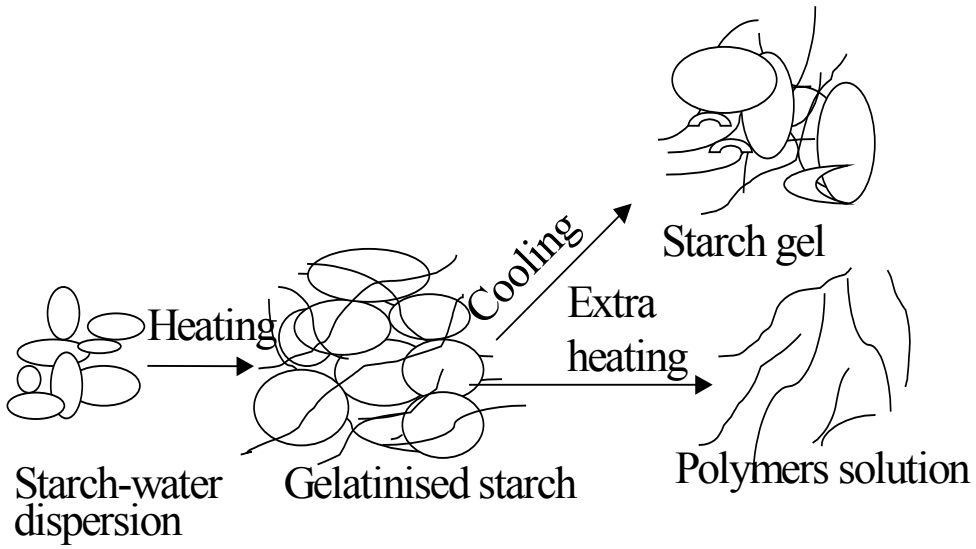

Figure 1. Scheme of starch gel and polymers solutions formation.

Starch retrogradation is a phenomenon that occurs during storage of starch gels and various starchy foods and is one of the main factors causing deterioration of food quality. Retrogradation causes changes in the amylopectin component that increases the firmness of a gel; a specific example is bread staling.

Starch retrogradation is a physical change of amylose and amylopectin in starch from a swollen gel-like state to a more crystalline state (Krog et al. 1989). During retrogradation, amylose may form double-helical associations of 40–70 glucose units (Leloup et al. 1992; Liu et al. 1997; Biliaderis 1998). Amylopectin, however, forms shorter double helices than amylose due to restrictions imposed by the branched structure of the molecules and the chain lengths of the branches at higher temperatures (Miles et al. 1985; Clark et al. 1989; Leloup et al. 1992). The retrogradation process takes place over several days, but the rate is dependent on the botanical source, hence the external chains length of amylopectin (Kalichevsky et al. 1990). The initial rate of retrogradation of

potato amylopectin is almost twice as fast as that of wheat and rice starches. The rate of starch crystallisation was also stated to depend on water content, storage temperature (Eliasson & Ljunger 1988; Roos 1995) and on temperature difference between the storage temperature and the glass transition temperature (Jouppila & Roos 1996). The changes in the extent of crystallisation of amylopectin retrogradation have been investigated frequently using different techniques such as DSC (Jouppila et al. 1998; Mua & Jackson 1998). Furthermore, DSC analysis provides a method for characterising the amorphous phase of starch by glass transition measurements. In starch solutions, the onset of retrogradation is accompanied by the development of turbidity, leading within a matter of hours or days to precipitation in dilute preparations. The cause is the aggregation of amylose molecules. The tendency of amylopectin to undergo such a change is inherently less in dilute solution (Ring et al. 1987). In concentrated solutions, linear segments do form helices that subsequently crystallise well. Thus, the amylose/amylopectin ratio (Leloup et al. 1991; Sievert & Würsch 1993) of the starch profoundly affects the rate of observable retrogradation which is also influenced by the molecular size distributions of the components (being rapid for chain lengths in the range of 75–100), the temperature and the presence of solutes other than starch in the dispersion (Chang & Liu 1991; Biliaderis & Juliano 1993).

1.3 Enzymatic modification of granular starch

Native starch has a low shear stress resistance, low decomposition, high retrogradation and high syneresis (Lillford & Morrison 1997). These shortcomings may be overcome by starch modification (Jacobs & Delcour 1998). The starch structure can be modified by chemical, physical and enzymatic methods. The modified starches generally show better paste clarity, better stability, increased resistance to retrogradation and increased freeze-thaw stability (Würzburg 1995; Zheng et al. 1999; Fang et al. 2002).

The enzymatic hydrolysis of native starches may be considered as one of the techniques to modify native starch by decreasing its average molecular weight. In addition to decreasing the size of the starch molecules and the viscosity of starch dispersions, the hydrolysis process may also modify other characteristics of starch dispersions. Amylases are often used for depolymerisation of starches

in the preparation of maltodextrins. α-Amylase occurs widely in human saliva, plants and microorganisms (Sogaard et al. 1993). The susceptibility of starch granules to α-amylolysis depends on their botanical origin (Madhusudhan & Tharanathan 1995), on the α-amylase source (Planchot et al. 1995) and on whether the starch is truly dispersed, partly gelatinised or suspended as intact granules. In general, cereal starch granules are much more readily hydrolysed by α-amylase than are potato starches, which is often attributed to the higher crystalline structure (Gallant et al. 1992). Gelatinisation is one of the procedures applied to increase the susceptibility of cereal and tuber starches to amylolysis (Kulp & Lorenz 1981; Bertoft & Manelius 1992; Lauro et al. 1993; Kawabata et al. 1995; Perera & Hoover 1998).

α-Amylases are mostly endoenzymes that are capable of cleaving α-1→4 linkages of both starch polymers. Moreover, α-amylase does not readily hydrolyse the α-1→4 linkages in maltose and maltotriose. Thus, maltose, trisaccharides and other low molecular-weight saccharides, especially those containing α-1→6 linkages, are present in the final hydrolysates. Amyloglucosidase and pullulanase are needed for the complete hydrolysis of starch to glucose. α-Amylase is also often employed for depolymerisation of starches when producing maltodextrins, which are starch hydrolysates with a dextrose equivalent (DE) below 20 (Chronakis 1998). Dextrose equivalent is a measure of the reducing power of starch-derived polysaccharides/ oligosaccharides compared with D-glucose on a dry-weight basis. The higher the DE, the greater the extent of starch hydrolysis.

1.4 Lipids and gels

Normal cereal starches contain lipids in quantities generally proportional to their amylose content (Morrison et al. 1986, 1993b; Morrison 1998). In barley, wheat and rye starches, the lipids are almost exclusively lysophospholipids which have been shown to exist as amylose lipid inclusion complexes in native starch granules rather than being formed during starch gelatinisation (Morrison et al. 1993a, b). Even though the lipid contents of cereal starches are low, around 1% on a dry weight basis, the lipids have been shown to affect many technologically important properties of starch-containing foods by changing the granule swelling, solubilisation, and crystallisation of starch polymers. It is well known

that when starch begins to gelatinise, amylose leaches out from the granules. If lipid is available for amylose complex formation at this stage, it is conceivable that the surface of the starch granule could be coated with an insoluble inclusion complex, preventing or diminishing further leaching of amylose. During a screening of starch-lipid interactions by DSC, it has been found that most monoglycerides will delay the gelatinisation process (Eliasson 1986, 1994). It was noticed also that the addition of a surfactant to wheat and potato starch caused a remarkable reduction in their gelatinisation enthalpies whereas their enthalpies of the endothermic transition of the amylose-lipid complex were significantly increased, indicating the formation of an amylose-lipid complex (Svensson et al. 1998).

The increase in stiffness of starch gels (Biliaderis & Tonogai 1991) during storage is said to be retarded by the addition of lipid surfactants. The presence of the amylose-lipid complex is observed in the DSC thermograms as an endothermic transition at a temperature above the melting endothermic of starch crystallites.

It has been assumed, for a long time, that amylopectin, because of its short outer chains, does not effectively complex with lipids. However, in recent publications, more and more evidence for the existence of the amylopectin-lipid complex has been reported (Gudmundsson & Eliasson 1990; Silverio et al. 1996). Calorimetry has provided evidence of such interaction, in which amylopectin-lipid complexes exhibited a transition at temperatures usually associated with the amylose-lipid complexes. Much effort has been made to slow down the retrogradation process of starchy foods, and various enzymes, emulsifiers and oligosaccharides have been used to retard the process.

Bread staling includes all processes that occur in both the crumb and the crust during storage. The crust becomes soft and leathery due to diffusion of water from the crumb to the crust (Eliasson & Larsson 1993). A characteristic change occurring in the crumb is an increase in firmness. Both the natural lipids of flour and added fats and emulsifiers are known to play an important role in the baking of wheat bread (Carr et al. 1992). It is well established that the shelf-life of bread can be increased by the addition of some surfactant, such as sodium stearoyl-2-lactylate, in the dough (Kulp & Ponte 1981; Krog et al. 1989). Today lecithins are alternative emulsifiers to the widely used synthetic compounds such as

monoglycerides and diacetyl tartaric ester of mono- and diglycerides (DATEM), because they can be modified in many ways for specific applications. The emulsifiers are known to interact with amylose during gelatinisation; they form a helical inclusion complex with the emulsifier occupying the central axis of the amylose helix. In general, surfactants in bread not only prolong shelf-life, but also improve loaf volume and the texture of bread. It has been suggested that surfactants may act as dough conditioner in two ways (Schuster & Adams 1984; Kokelaar et al. 1995) either by interaction with gluten resulting in a `reinforcement` of the gluten network or by improving the gas cell stability.

1.5 Starch films

Several biopolymer materials, including proteins, polysaccharides and lipids, either alone or in a mixture, have been proposed for preparing edible films and coatings (Kester & Fennema 1986; Krochta & De-Mulder-Johnston 1997). The potential properties of polysaccharides as edible films and coatings have long been recognised (Lourdin et al. 1995; Guilbert et al. 1996). The biodegradability, renewability, low cost (Röper & Koch 1990; Haugaard et al. 2001) and the excellent aroma retention barrier property of starch (Zeller et al. 1999) make it a promising raw material for the edible film formation. In general, the major drawback of starch films is water sensitivity (Zobel 1988) which results changes in mechanical properties (van Soest 1996).

In order to exploit the beneficial properties, starches should be processed economically which means using feasible technologies under proper processing conditions. Starch dissolution in water can be achieved only at low starch concentrations and at high temperatures (above 100°C). These kinds of conditions are not economical for industrial applications. Higher starch concentrations can be dissolved in water after partial depolymerisation of the starch polymers by acid or enzyme treatments. Starch films are usually prepared from the starch polymers obtained from the starch-water dispersions heated above 100°C (Figure 1). The origin of the starch, the amylose-amylopectin ratio, the molecular weight of its polymers, the type of modification, the heating temperature, and the type and quantity of plasticiser added affects the properties of starch films. Studies on the film formation properties of starch hydrolysis products have not been reported, but film formation is often claimed to be one of

the beneficial properties when using maltodextrins as the shell material in encapsulation applications (Qi & Xu 1999).

1.6 Aims of the present study

The aim of the investigation was to learn more about the effects of heat and enzyme treatments on the dissolution and hydrolysis of starch granules, and, as an example, the gelatinisation behaviour of oat and barley starches in dilute water dispersion was examined. Furthermore, the accessibility of potato starch granules to amylase and the hydrolysis of barley starch lipids by esterase treatments were studied.

The aim was also to examine the properties of gelatinised starches, particularly starch gels, and also the film formation ability of potato starch was elucidated. The properties of oat and wheat starch gels were studied, and the effects of emulsifiers on wheat starch gel crystallisation were analysed.

2. Materials and Methods

2.1 Materials

Commercial oat (I & II) and barley (I–III) starches (Primalco Ltd., Finland), wheat starch (IV) (Melia Ltd., Finland) and potato starch (V) (Järviseudun Peruna, Vimpeli, Finland) were used as substrates. Lysophosphatidylcholine (L-4129) was purchased from Sigma (St. Louis, MO, U.S.A.) (III). The commercial emulsifiers used were Panodan 10 and Amidan SDM-T (Grinsdstedt Products, Arhus, Denmark), Emulfluid E and Lecimultin 100 (Lucas Meyer, Hamburg, Germany). Crude soya (Kantvik, Finland) and oat lecithins (made at VTT) and their hydrolysates were used in this study (IV).

The enzyme preparations of phospholipase A1 and lipase (Biocatalysts Ltd., Pontypridd, England) were used for the hydrolysis of barley starch lipids (III). Thermostable α-amylase (Megazyme, Bray, Ireland) was used in the α-amylolysis of potato starch granules (V).

2.2 Methods

2.2.1 Heat-induced changes

The liquid and solid residue fractions of oat and barley starches were prepared as follows (II). The starch–water suspension (1% d.w.) made of oat or barley starch was kept in a water bath at the desired temperatures (85–97°C) for 15 min with minimal manual mixing sufficient to keep the starch completely suspended; the dispersions were cooled to room temperature and centrifuged at 10 800 *g* for 10 min to yield liquid and solid residue fractions. After that the liquid fractions of both starches were recentrifuged at 38 400 *g* for 15 min to produce a second liquid and solid residue fractions (II).

2.2.2 Hydrolysis of potato starch

Freeze-dried potato starch was prepared prior to hydrolysis by suspending the commercial potato starch (10% w/v) in water at room temperature. The solid fraction was separated by centrifugation (10 800 g/10 min) and freeze-dried (V). α-Amylolysis of potato starch granules was performed at 30°C under magnetic stirring. A thermostable α-amylase (Megazyme, Bray, Ireland) solution was added to freeze-dried and native potato starch-water dispersions, using enzyme dosages of 225–325 and 2500–3500 U/g of starch, respectively (V).

2.2.3 Hydrolysis of barley starch lipids

Prior to hydrolysis, barley starch was gelatinised and freeze-dried as shown in Figure 2 (III). The hydrolytic reaction was performed at 40, 50 and 60°C under magnetic stirring for 4 h using 0.1% lysophosphatidylcholine or 1% native or 1% gelatinised barley starch as the substrate in 50 mM acetic acid buffer, pH 5. The enzymes (phospholipase A1 and lipase) were administered as lysophospholipase activity units (100–20 000 nano katal/g LPL). The liberated free fatty acids were immediately quantified using an enzymatic test kit (Boehringer Mannheim 1383175) (III).

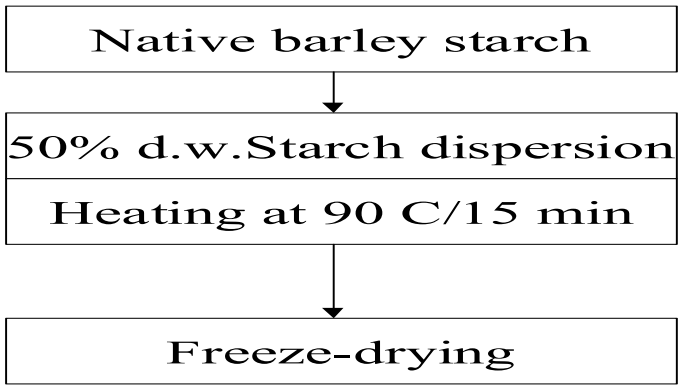

Figure 2. Scheme for the preparation of gelatinised barley starch.

2.2.4 Preparation of starch films

Films were made of native and freeze-dried potato starches and of their enzymatic hydrolysis products (Figure 3). A casting technique was applied to film formation in this study (V). The unhydrolysed starches were dissolved in water at 160°C using a 2% starch concentration. The concentrations used for the hydrolysates and solid residues were 10% and 20%, respectively, and both dispersions were dissolved at 120°C (V). After dissolution the water solutions were poured into prewarmed Teflon moulds and dried at 60°C. The drying time was 5 h for the native and freeze-dried starches as well as for the hydrolysates, and 24 h for the solid residues. The films were stored for 1 week at RH 50% and 20°C prior to testing (V).

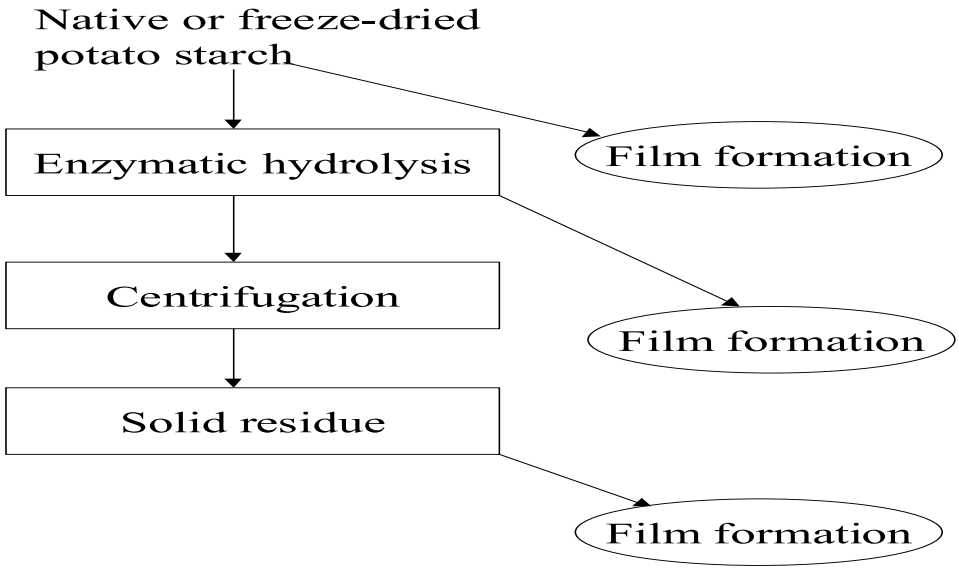

Figure 3. Scheme for film preparation of native and freeze-dried potato starches and their hydrolysates and solid residue fractions.

2.3 Analyses

Details of the analytical methods are given in the original publications (Appendices I–V) and the procedures are only briefly described below.

2.3.1 Chemical composition

Amylose content was determined colorimetrically according to the method of Morrison & Laignelet (1983) (I, II & V). The lysophospholipid (LPL) content was estimated by phosphorus analysis as described by Morrison (1964) and Tester & Morrison (1990) (I–II). The dry weight of the starch fractions (liquid and solid residue fractions) was determined by heating in an oven at 105°C overnight (II). Sugar content of the solid residue fractions of potato starch was quantitated by HPLC with pulsed amperometric detection (V). The water content of the starch films was determined by Karl-Fischer titration (V)

2.3.2 Starch solubility

Defatted oat starch was prepared prior to analysis from the native oat starch (I). In the first experiments (I), a modified method of Leach et al. (1959) was applied for determination of the solubility of native and defatted starches. After 30 min of incubation of the starch-water dispersion at 75–95°C under manual mixing, the tubes were cooled rapidly and centrifuged at 10800 *g* for 15 min. The liquid fraction was analysed for solubilised starch as total carbohydrates according to Dubois et al. (1956).

In further studies (II), the dry weights determined in liquid fractions obtained after first and second centrifugation of both starches, expressed as a percentage of the original weight of starch, were defined as "leachability" (i.e. apparent solubility) and "true solubility", respectively.

2.3.3 Microstructure of starch

The effects of thermal and enzymatic treatments of native barley and oat starch dispersions on their microstructure were studied by light microscopy using a smear technique and iodine staining (Autio 1990) (II & III).

2.3.4 Thermal behaviour of starch

A Mettler differential scanning calormetry (Mettler DSC30, Greifensee, Switzerland) was used to study the thermal properties of starch samples and starch films (I–V). The DSC measurements were performed with a scanning rate of 10°C/min and an empty pan as a reference. The thermograms were measured from 20 to 100°C or to 150°C for the dissociation of the amylose-lipid complex (AML) (I–IV). The retrogradation enthalpy was followed with DSC after storage of the gelatinised samples at 4°C (I) and 5°C (IV) for up to 7 days. The glass transition temperature (T_g) of the films was taken from the second heating thermogram as the midpoint of the transition (V). Three replicates were analysed for each sample (I–V).

2.3.5 Molecular weight distribution

Molecular weight distributions of oat, barley and potato starches were analysed (I, II & V) by size exclusion chromatography (SEC) as described by Suortti & Pessa (1991). A dual angle laser light scattering detector (PDI 2000, Precision Detectors, Amherst, MA, USA) was used to determine the absolute molecular weights. A diode detector was used for detection with post-column iodine staining; the wavelength employed for detection was in the range 540–760 nm.

3. Results and discussion

3.1 Heat-induced changes in starch–water dispersions (Publications I & II)

The study begun by investigating the effects of heating on the solubility of oat and barley starches (I & II). The heat treatment of oat and barley starch dispersions showed that oat starch had lower solubility at 85°C compared to that of barley starch. This result is consistent with the earlier results reported by Wang & White (1994). The lower solubility of oat starch at 85°C was probably due to the fact that at 85°C most of the lipids were inside the granules, which retarded the swelling and the leaching of oat starch polymers. Furthermore, the fractionation of oat and barley starch dispersions heated at 95°C was performed by sequential centrifugation (II). This was performed because, the liquid fractions of both starches after the first centrifugation (10 800 *g*) were opaque and developed precipitates after a few hours of storage, whereas the second centrifugation (38 400 *g*) produced clear liquid fractions and no precipitation occurred during storage.

The solubility of both starch dispersions (at 95°C) was determined from the liquid fractions produced after first and second centrifugation and expressed as apparent and true solubility, respectively (II). The apparent solubility of both starches was similar, but the true solubility of oat starch was lower than that of barley starch. The centrifugation conditions (10 800 *g*/15 min) applied in the method of Leach et al. (1959) for starch solubility determination were not enough to separate granular fragments from solubilised amylose. Therefore, ultracentrifugation should be applied when starch solubility is determined by this method.

Despite that, oat starch granules were more degraded than barley starch granules as a result of heat treatment, but the composition of the fractionations did not significantly differ. In agreement with the earlier results (Autio 1990; Virtanen et al. 1993), this study showed that the continuous phase of oat starch heated at 95°C contained both amylose and amylopectin. However, no microscopic evidence of amylopectin fragments in the liquid fractions of two starches obtained after the first centrifugation was observed (II). This was probably due

to a lower concentration of amylopectin in the liquid fractions of both starches or to the lower sensitivity of amylopectin to iodine staining.

The molecular weight of the soluble fraction of oat starch at different temperatures was analysed and observed to increase with increasing temperature (I & II). This was in accordance with the oat starch study of Mua & Jackson (1995). They showed that the lower molecular weight polymer amylose was solubilised at 65°C, and that the higher molecular weight polymer amylopectin was not solubilised before the temperature was raised to 120°C. In the present study (II), based on size exclusion chromatography (SEC), the leached polysaccharides at 90°C from oat starch were a mixture of linear and branched polymers, whereas the polysaccharides detected in barley starch were mostly linear. Furthermore, a laser scattering detector revealed that the weight average molecular weights (M_w) of liquid fractions (supernatants) of both starches obtained after the first centrifugation were similar (II). When the liquid fractions were recentrifuged, the M_w of the solubilised oat starch was somewhat higher than that of barley starch. In a recent study, ultracentrifugation of aqueous leached corn amylose was observed to decrease the M_w of the amylose fraction, indicating that the higher molecular weight polymers were not solubilised (Roger & Colonna 1996). This was also observed in the present study (II) as the result of the recentrifugation of the liquid fractions. The M_w of the polymers decreased from 4×10^6 to about 0.3×10^6 for both starches, indicating that insoluble high molecular weight polymers were removed by recentrifugation.

As the heating temperature increased from 85 to 97°C, the amount of leached carbohydrates of both starches increased and the lysophospholipid (LPL) of the granule residues decreased (I & II). The degree of LPL leaching of both starches was minor at 85°C but increased significantly when the temperature was raised from 90 to 95°C and reached a plateau at 97°C (II). This indicated that part of the lipid-bound starch had leached into the liquid fraction as a result of the granular fragmentation during heating. About 60% of the original LPL leached out from both starch granules in water at 95°C and, furthermore, precipitated after recentrifugation. This was an interesting result and showed that the AML complex could be isolated by sequential centrifugation of heated cereal starch dispersions above 90°C (II).

The role of lipids in oat starches in relation to heat-induced changes has been investigated by Paton (1987) and Gudmundsson & Eliasson (1989) using solvent extraction for the lipid removal. This method was also applied in the present work and observed to effect somewhat the solubility of oat starch above DSC-gelatinisation temperatures (I). Because the solvent removal (n-propanol-water at 100°C) caused very large changes to the granular organisation (gelatinisation enthalpy decreased from 10.5 to 3.2 J/g) the lipids were not necessarily inducing the solubility change.

3.2 Enzymatic hydrolysis of starch lipids and granules (Publications III & V)

Starch lipids greatly affect the heat-induced behaviour of starch-water dispersions as well as gel properties and modification of starch granules. This is why the ability of lipase and phospholipase A1 to hydrolyse barley starch lipids was investigated (III). The extent of hydrolysis of phospholipids in barley starch was analysed by measuring the amount of released fatty acids by the enzymatic treatment. At 40°C the granules were mostly intact and the extent of hydrolysis was low. When the temperature was raised to 60°C the extent of hydrolysis increased to about 20%. Microscopic examination of barley starch granules after the hydrolysis process showed that, at all reaction temperatures, the granules of the enzyme-treated starch were less swollen than those of the control starch. As the result of hydrolysis, the gelatinisation enthalpy of barley starch was also observed to decrease. However, the gelatinisation enthalpy of the non-treated starch was lower (1.4 J/g) than that of the enzymatically-treated starch (5.2 J/g). This indicated that the small amount of free fatty acids formed during the hydrolysis process retarded the granule swelling and as the cosequent gelatinisation (III). In the earlier studies, monoglycerides were shown to inhibit starch gelatinisation (Eliasson 1986, 1994).

AML complexes and free amylose have been assumed to exist in the amorphous regions of the granule, unhomogeneously distributed with an increasing amount from the hilum to the surface (Morrison 1995). The low accessibility of barley starch lipids to enzymatic hydrolysis was due to the solid substrate, which means that lipids were protected by the granular structure. When barley starch was gelatinised, the extent of hydrolysis at 40°C was almost as high as for free LPL

dispersed in water, indicating the flexible nature of the AML complex in water dispersion (III).

It is known that potato starch granules are much more resistant to α-amylolysis than cereal starch granules (Planchot et al. 1995, 1997; Kimura & Robyt 1996). The actual property which makes the potato starch granule more resistant is not known, but the arrangement of crystallites inside the granule has been suggested to be responsible for the behaviour (Gallant et al. 1992). In the present work (V), the effect of freeze-drying treatment on α-amylolysis of potato starch granules was studied. Freeze-drying treatment greatly improved the α-amylase accessibility of the granules, making them as easily accessible as barley starch granules. The enzyme dosage needed to produce hydrolysates with a dextrose equivalent of 10 to 20 from freeze-dried potato starch was 10% of that needed for the native starch. This was probably due to changes in the surface and/or in the granular structure. No reports of this kind of treatment were published, but heat treatment has always been applied to increase the α-amylase susceptibility of granular potato starch (Kimura & Robyt 1996; Farhat et al. 2001).

3.3 Properties of starch gels and films (Publications I, III, IV & V)

The crystallisation tendencies of oat, barley and wheat starches, as well as defatted oat starch, were examined during 1 week of storage under refrigerated temperature (4°C) (I). The extent of crystallisation was determined by analysing the melting endothermic transition of starch gels using differential scanning calorimetry. Oat starch gel was less susceptible to retrogradation than the gels produced from other cereal starches (I). The retrogradation rate of the defatted oat starch gel was, however, similar to that of the other starches studied (Table 1), indicating that the oat starch lipids prevent amylose from being involved in retrogradation (I). Gudmundsson and Eliasson (1989) have already shown that gels prepared from oat starches are more stable than those from wheat and maize starches, which is in agreement with the present study (I). They stated that the oat starch lipid is one of the major factors in lowering the retrogradation in oat starches, because the defatted oat starch variety, Chicauhua, showed a great increase in retrogradation compared to the native starch.

Table 1. Melting enthalpies (ΔH) of the crystallised starches in starch gels stored at 4°C for 1, 3 and 7 days (I).

Starch	ΔH J/g (1 day)	ΔH J/g (3 days)	ΔH J/g (7 days)
Barley	5.6	7.4	9.0
Wheat	5.2	6.3	7.5
Oat	1.7	3.9	6.1
Defatted oat	4.3	5.9	8.7

Starch is an essential structure builder of many food materials, such as breads and other baked products, and crystallisation of gelatinised starch usually means a more rigid structure and a decrease in quality. The problems related to bread staling are greatly linked to crystallisation of the gelatinised starch and have been studied for decades to better understand the mechanism (Zobel & Kulp 1996; Jacobson & BeMiller 1998), and often starch gels have been used as simple bread models.

In the present work (IV), the effectiveness of oat and soya lecithins in retarding crystallisation of wheat starch gel was elucidated. Together with the crude lecithins their hydrolysis products were also analysed because usually lysophospholipids are more effective. Baking experiments were also performed to get some idea of the correlation of the behaviour of the starch gel with the bread crumb in the presence of emulsifiers. The effects of the lecithins on dough and bread properties were compared with the commercial emulsifiers (Panodan and Amidan) (IV), which were known to affect positively bread staling.

The crystallisation of wheat starch gel stored at 5^{o}C for 1 week was retarded by both oat and soya lecithin hydrolysates, but the effects of the unmodified lecithins were not significant (IV). The presence of the lecithin hydrolysates also affected the gelatinisation and amylose-lipid complex behaviour especially in the case of soya lecithin hydrolysate; the gelatinisation enthalpy decreased and the AML complex transition enthalpy increased indicating that complex formation occurred during the heat treatment in the DSC run (IV). The reduction in gelatinisation enthalpy in the presence of polar lipids has been thought to be the

result of an exothermic effect from the formation of starch-lipid complexes occurring simultaneously as the granule gelatinises (Evans 1986; Eliasson 1986, 1994).

The result of the present study (IV) showed that the AML complex and amylopectin crystal formation are linked with each other. This was clearly demonstrated by examining the effect of the concentration of soya lecithin hydrolysate on gelatinisation and on AML complex formation in granular wheat starch and, furthermore, on crystallisation of the wheat starch gel (IV). The formation of the AML complex is said to retard the crystallisation of starch gels (Kulp & Ponte 1981). Furthermore, lysophospholipids were found to decrease the rate of amylopectin crystallisation in wheat, rice and pea starch gels (Biliaderis & Tonogai 1991). Recently, the results of Kweon et al. (1994) showed that at a concentration of 3% the phospholipid hydrolysate retards wheat starch gel crystallisation significantly during 5 days of storage at room temperature, which was in accordance with our results. On the contrary the results of Conde-Petit and Escher (1994) showed that the recrystallisation of potato starch gels during storage was not affected by the formation of AML complex. Furthermore when AML complex was added to waxy maize starch or amylose/amylopectin systems, the recrystallisation tendency of amylopectin was only reduced to small extent (Eliasson & Ljunger 1988; Gudmundsson & Eliasson 1990).

The effects of the lecithins on the staling of wheat bread crumb correlated with the retrogradation of wheat starch gel, except in the case of oat lecithin, which was as effective as the hydrolysate prepared of the oat lecithin. All lecithins increased crumb softness of the fresh bread similarly to the commercial emulsifiers (Amidan and Panodan) (IV). The soya lecithin hydrolysate was most effective, and was actually as good as the best commercial emulsifier used in the study, but both oat lecithins were also very good in retarding bread staling. The positive effective of soya lecithin hydrolysate on the firmness of the bread was due to its high content of lysophospholipids compared to that of oat lecithins (Aura et al. 1994). The beneficial effect of oat lecithin may be linked to its glycolipids.

Today, there is great interest in utilising biopolymer films in various food and non-food applications (Kester & Fennema 1986; Gennadios et al. 1993; Avérous et al. 2001; Martin et al. 2001). If starch is treated at a sufficiently high temperature, a true polymer solution is formed and, after evaporation, the solvent films can be produced. When preparing films from starches the essential step is the dissolution of the polymers, and if native starches are the raw materials only a very low solids content can be used in the dissolution process. This is of course not very feasible, and this is why, in the present work (V), preliminary experiments on the film formation ability of potato starch hydrolysates were performed.

When the film was produced from native potato starch 2% solids and 160°C were the conditions required for dissolution of starch in water (V). The potato starch hydrolysates used were maltodextrins with dextrose equivalents (DE) of 10, 15 and 20, and as a result of the hydrolysis as much as 20% solids could be used in the film preparation (V). Also the dissolution temperature needed for the hydrolysates was lower (120°C).

A semi-transparent and brittle film was produced from the native potato starch (V). When maltodextrins were used as raw materials for film formation, the film preparation was not easy because the materials produced were very sticky. After the most of the water-soluble carbohydrates were removed from the maltodextrins, good quality films were prepared easily. The best films were non-sticky, transparent and somewhat flexible. Calorimetric glass transition temperatures of the films were analysed and found to correlate with the sugar content of the films, and also with the actual behaviour (lower temperature-more flexible material). Thus, the more flexible nature of the films produced from maltodextrins was linked with the sugar content and not with the decrease in the molecular weight of the starch polymer. But higher solids could be used due to the decrease in the molecular weight (V). Glass transition temperatures of commercial maltodextrins with DE 10 and 15 reported by Roos & Karel (1991) were 30 and 13°C respectively which are very close to the temperatures observed in the present study (V) for solid residues prepared of native potato starch hydrolysates. A similar decrease of the glass transition temperature of commercial maltodextrins with increasing DE was also observed by Ruan et al. (1999).

4. Conclusions

Despite the fact that oat starch was more fragmented than barley starch during heat treatment in dilute water dispersion, no significant differences were found in the chemical composition of the dissolved fractions of these starches. The heating of the starch dispersion at 95°C caused granular fragmentation, and as a consequence, amylopectin and an amylose-lipid complex were observed, together with solubilised amylose, in the liquid fraction. The amylose-lipid complex was not really solubilised because it was precipitated after recentrifugation. Total solubility of oat starch was less than that of barley starch and defatting of oat starch was observed to increase the solubility above DSC-gelatinisation temperature.

The extent of hydrolysis of barley starch lipids was low under those temperatures at which starch granules were mostly intact. However, lipid hydrolysis inhibited swelling and gelatinisation of the granules even though the extent was small, about 20%. Most of the lipids of the gelatinised barley starch were hydrolysed at 40°C indicating perhaps the flexible nature of the amylose-lipid complex in water dispersion. Amylase accessibility of native potato starch granules was greatly improved after freeze-drying treatment of the starch granules in the presence of water. This may be due to changes in the surface and/or in the granular structures.

Gel prepared from oat starch showed a lower tendency to crystallisation than gels of barley and wheat starches under refrigerated temperature. Defatting of oat starch using solvent extraction for the lipid removal increased the rate of crystallisation of the gel. When investigating the effects of emulsifiers on starch gel properties, it was detected that the hydrolysed lecithin from oat and soya bound with amylose and retarded amylopectin crystallisation of wheat starch gel. This was in contrast to the native lecithin indicating the presence of lysophospholipids in the hydrolysed products. As compared with oat lecithin, the hydrolysate from soya was more effective due to its higher lysophospholipid content. The staling of wheat bread crumb correlated well with the starch gel crystallisation behaviour, except in the case of oat lecithin, which was as effective as oat lecithin hydrolysate. The beneficial effect of oat lecithin on bread crumb was most likely due to its glycolipid content.

Freeze-drying treatment of potato starch granules was observed to greatly increase its accessibility to α-amylolysis, because the required dose to produce hydrolysates with a specific dextrose equivalent was about ten times lower than that needed for native potato starch granules. Up to 20% solids could be used in the film preparation after the molecular weight was decreased due to α-amylolysis treatment. Good quality films were made after the small sugars were removed from the starch hydrolysates.

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Effects of lipids, heating and enzymatic treatment on starches

Abstract

The effects of heat treatment on oat and barley starch water dispersions were investigated. As compared with barley starch, the granules from oat starch were more degraded, but the fractionation patterns of both starches were not significantly different. Microscopic examination indicated that no evidence of amylopectin fragments was observed in the liquid fraction after starch dispersions were treated at 95°C even though chemical analysis demonstrated the solubility of both starch polymers. True solubility of oat starch was lower than that of barley starch, but the difference disappeared after removing the lipids from oat starch. By applying sequential centrifugation for starch dispersions, treated above 90°C, a fraction rich in an amylose-lipid complex could be produced.

Studies on crystallisation of starch gels were performed. The crystallisation rate of a gel prepared from oat starch was lower than those prepared from other cereal starches (barley and wheat). The effects of polar lipids separated from oats (oat lecithin) on crystallisation of wheat starch gel were investigated and compared with soya lecithin. Furthermore, the behaviour of polar lipids in the starch gel was compared with their effect on bread staling. Even though oat lecithin hydrolysate affected the crystallisation rate positively, soya lecithin hydrolysate was more effective both in gel as well as in bread.

The ability of esterases to hydrolyse lipids of barley starch at temperatures close to gelatinisation and of the gelatinised barley starch was examined. The extent of lipid hydrolysis of starch granules was only about 20%, but almost all of the lipids of the gelatinised starch were hydrolysed at 40°C. The effect of freeze-drying on α-amylolysis of potato starch was also investigated, and was observed to greatly enhance the enzyme accessibility of the granules.

The film formation property of hydrolysates prepared from potato starch was elucidated. Higher solids could be used in the film process due to a decrease in the molecular weight, and, after removing the water-soluble carbohydrates from the starch hydrolysates, good quality films were produced.

Keywords

oat, barley, wheat, potato, starch, amylopectins, amylose, lipids, solubility, gels, films, lecithins, enzymatic hydrolysis

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