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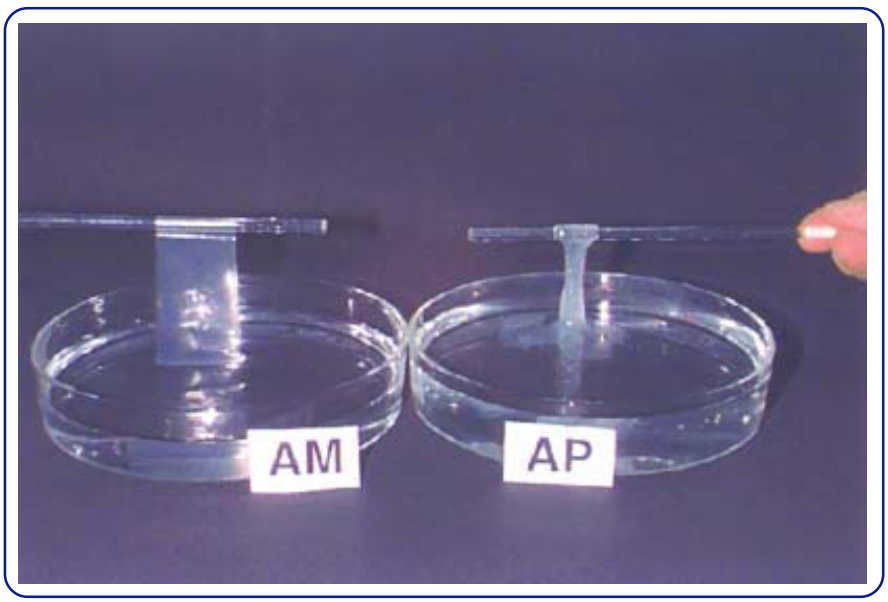

Päivi Myllärinen

Starches – from granules to novel applications

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VTT Biotechnology

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Abstract

In this study, the effects of growth temperature, starch composition and granule size on the gelatinisation and solubility properties of barley starch were studied. The mechanical properties and structures of amylose and amylopectin film, with and without added glycerol, were investigated. A novel starch-based microencapsulation method was developed for probiotic bacteria by combining native starch granules with amylose coating.

The suboptimal growth conditions influenced the properties of two-rowed malting barley cultivars "Kustaa" and "Kymppi" as well as Kustaa′s large A-granules and small B-granules. The gelatinisation temperature was lower and the rate of retrogradation less in starch grown in cold and rainy conditions. Swelling and solubility were greater and the starch was more susceptible to amylases. The small B-granules had a higher lipid:amylose ratio than the large A-granules. The B-granules also had a higher dissociation enthalpy of the amylose–lipid complex than the A-granules. Furthermore, the gelatinisation temperature was 4°C higher for B-granules than for A-granules. During heating in excess water up to 90°C amylopectin was mainly leached from the B-granules, whereas amylose was solubilised from the A-granules. At 95°C, B-granules were completely solubilised but A-granules remained partially particulate.

Amylose produced good quality films from water solutions in the presence of glycerol. Film formation was successful even at a 70% glycerol content, whereas films could not be prepared from amylopectin above a 30% glycerol content. Based on calorimetric glass transition (Tg) analysis, pure glycerol was observed to decrease the Tg. However, water had a stronger plasticisation effect. No difference between the Tgs of two starch polymers could be observed. The Tg was at room temperature at a water level of 21%. Phase separation to starch-rich and starch-poor regions occurred in glycerol plasticised systems. Fresh amylose

films with 0–30% glycerol at an RH of 0–91% had B-type crystallinity at a level of 6–32%, depending on the amount of water and glycerol. No changes were observed in the crystallinity of the amylose films during 2 months of storage. The fresh amylopectin films were amorphous. After a storage period of 2 months at RH 91%, the amylopectin film with 30% glycerol showed a crystalline structure but all other amylopectin films remained amorphous after ageing. Crystallisation occurred in the rubbery phase of the system. Amylose film was not fragmented or dissolved in water, and part of its structure was resistant to amylases and acid.

Freeze-drying of native potato starch made the granules accessible to α-amylase, and enzymatic hydrolysis appeared to occur from the inside out, producing porous granules. The porous material was used as a bacteria carrier in a fermentation process. Dissolved high amylose maize starch solution was added after the growth period. Finally, the product was freeze-dried to produce powder.

Academic dissertation

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Preface

This study was carried out at VTT Biotechnology during the years 1992–2002. I sincerely thank professor Juha Ahvenainen for providing excellent working facilities. I want to thank the "cereal power" professor, Hannu Salovaara, for his interest and help during these years. I also wish to thank professor Yrjö Mälkki, the former head of the VTT Food Laboratory, for his enthusiasm for new ideas that encouraged young students to look to the future. I would like to thank the reviewers, professor Eric Bertoft and Professor Yrjö Roos, for their constructive criticism. Kaisa Vesivalo from Noodi Oy is gratefully acknowledged for her help with English language corrections.

I am most grateful to professor Kaisa Poutanen. She carries food and life science in her heart. To my supervisor, Dr. Pirkko Forssell, I owe my deep gratitude for her wise and intelligent advice. I want to thank Alan Schulman, PhD, for showing me the world of starch biosynthesis. My first working partner, Erkki Pessa, is thanked from my heart. I am grateful to professor William Morrison and Dr. Richard Tester from Strathclyde University, Scotland, for their guidance on starch analysis methods in July 1992. I also thank Dr. Steve Ring and Dr. Roger Parker from the Institute of Food Research in Norwich for their kind supervision of starch rheology in June 1996. Sincere thanks to Dr. Alain Buléon and his team at INRA, Nantes, for teaching me about interesting X-rays in autumn 1998.

My working group, HITEK, is fantastic with laughter and diligence. I warmly thank Jaana, Heljä, oat-Olli, Teija, Riitta, Piia, Ritva, Tuomo, Sirpa, Eeva, Leena, Tapani, and Shamekh. I thank Marianna and her family for helpful discussions and their kindness. Very special thanks are due to all my colleagues and co-workers at VTT: Dr. Karin Autio and her baking and microscopy group, especially Helena Lukka for her excellent microscopy work, and professor Tiina Mattila-Sandholm and her bacteria team. I am also grateful to the entire staff at VTT Biotechnology for creating a pleasant working environment.

I wish to thank the Finnish food industry for the support and interest in my work. Risto Lampinen from Kesko Hahkiala is acknowledged for providing the barley samples. Järviseudun Peruna is acknowledged for providing the potato starch granules. I would also like to thank the Nordic Industrial Fund for

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My student, skiing, and singing friends, I want to thank you all. Many thanks to the members of SYFS, "life is food and fun".

I want to thank my parents and family for their support and encouragement over the years ♥

Helsinki 26.6.2002

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 Myllärinen, P., Schulman, A. H., Salovaara, H. & Poutanen, K. 1998. The effect of growth temperature on gelatinization properties of barley starch. *Acta Agriculturae Scandinavica* 48, pp. 85–90.

II Myllärinen, P., Autio, K., Schulman, A. H. & Poutanen, K. 1998. Heat-induced structural changes of small and large barley starch granules. *Journal of the Institute of Brewing* 104, pp. 343–349.

III Myllärinen, P., Partanen, R., Seppälä, J. & Forssell, P. 2002. Effect of glycerol on behaviour of amylose and amylopectin films. Accepted for publication in *Carbohydrate Polymers*.

IV Myllärinen, P., Buléon, A., Lahtinen, R. & Forssell, P. 2002. The crystallinity of amylose and amylopectin films. *Carbohydrate Polymers* 48, pp. 41–48.

V Myllärinen, P., Forssell, P., von Wright, A., Alander, M., Mattila-Sandholm, T. & Poutanen, K. 2000. Starch capsules containing micro-organisms and/or polypeptides or proteins and a process for producing them. WO9952511. FI104405.

The author of the present thesis had the main responsibility for planning the research, practical work and interpretation of the results in all publications. Light microscopy photographs were produced by technician Helena Lukka at VTT Biotechnology. X-ray diffraction analyses in publication IV were carried out in INRA by Dr Alain Buléon and the interpretations were made together.

Contents

Appendices of this publication are not included in the PDF version. Please order the printed version to get the complete publication (http://www.vtt.fi/inf/pdf/)

List of symbols

ADPglucose	adenosinediphosphate glucose
ADPGPPase	ADP-glucose pyrophosphorylase
AFM	atomic force microscopy
AM	amylose
AP	amylopectin
ATP	adenosine triphosphate
13C-NMR	solid-state nuclear magnetic resonance
DAPI	4´,6 diamidino-2-phenylindole
DETA	dielectric thermal analysis
DMTA	dynamic mechanical thermal analysis
DSC	differential scanning calorimetry
ESEM	environmental scanning electron microscopy
FAM	free amylose
GBSS	granule bound starch synthase
LAM	lipid complexed amylose
LPL	lysophospholipid
MPa	mega pascal (tensile strength)
Mw	molecular weight
RH	ambient humidity (%)
SBE	starch branching enzyme
SS	starch synthase
SSS	soluble starch synthase
SF	swelling factor
Tc	gelatinisation conclusion temperature by DSC
Tg	glass transition temperature by DSC
Tm	gelatinisation temperature by DSC
To	gelatinisation onset temperature by DSC
TPS	thermoplastic starch

1. Introduction

Starch is a plant reserve polysaccharide synthesised in green plants via a complicated photosynthetic pathway (Martin and Smith 1995). In plants such as potato or barley, starch exists as partially crystalline granules, which are insoluble in water at room temperature. The biosynthetic pathway is genetically regulated, but environmental conditions, especially growth temperature, have an effect on the activity of biosynthesis and the enzymes involved in it. Starch granules have different sizes and shapes depending on the plant origin. Amylose and amylopectin are the polymers that build up the granule architecture. Amylose is a long and linear polymer with very few branches, while amylopectin is a huge and highly branched molecule. The monomer building block of starch is glucose. Glucopyranosyl residues are held to each other by α-1,4 bonds and branches are formed by α-1,6-linkages.

From 1969 to 2001, there have been over 23,000 scientific articles in the FSTA database concerning starch; 2960 of these articles concentrated on amylose, while 1416 papers focused on amylopectin. The early papers described methodologies utilised in studying starch chemical compositions and starch structures. Many papers dealt with the microstructure and functional properties of starches. Lately the interest has focused on starch biosynthesis and the modification of the starch structure by genetic methods. Many new techniques have been developed and used to find correlations between observed properties and compositions.

Of all plants, the most important as starch sources, commercially and globally, are cereals – corn, rice and wheat – and tubers – potato, tapioca and arrowroot. Of the total world starch production, approximately 83% is derived from corn; the next most important sources are wheat (7%), potatoes (6%) and tapioca (4%) (De Baere 1999). Specific sources are sorghum, barley, oat, rye, amaranth and banana. The USA produces more than 60% of the world's starch. In 1997 in Europe the total production of native starches was 7.3×10^6 t (Bergthaller et al. 1999).

Starch is used both in food and non-food applications. The largest user is the paper industry (Röper 1996), which accounts for approximately 20% of all of Europe′s starch production. Of total starch production, food applications use

55% and non-food applications use 45%. Native starch is usually modified mechanically or chemically in order to induce special properties. Fifty years ago, it was suggested that starches could be used as edible films or coatings (Wolff et al. 1951, Langlois and Wagoner 1967). Recently, much research activity has focused on the use of starches as bioplastics (van Soest and Essers 1997, Della Valle et al. 1998, Hulleman et al. 1998, Forssell et al. 1999). Additionally, recent studies have aimed at understanding the structural/functional properties of amylose and amylopectin films (Lourdin et al. 1995, Rindlav-Westling et al. 1998). Special starch products include maltodextrins, cyclodextrins and resistant starches. Resistant starch is used as a nutrition compound (Röper 1996), and maltodextrins (Che Man et al. 1999) and β-cyclodextrin (Dziezak 1988, Brazel 1999) are used for microencapsulation or as binders for active compounds, such as flavours. The new applications are starch aggregates and starch granule hydrolysates, which have been suggested to function as carriers or binders (Whistler 1989, 1997), and amylose, which has been used as a component in controlling drug delivery (Ring et al. 1994).

Finland is located at 60° N, and has a hard climate. Summers are short, and some years they can be very wet and cold. Growing conditions are often difficult. Barley and potato can be grown over the whole country, and barley is the most cultivated cereal, used widely for food and feed. The Finnish company Primalco uses barley for ethanol production, especially the small B-granules with their high protein content. Finland produces 189,000 t of malting barley annually, which is about 2.5% of all malt production in the European Union (Home 2002, personal communication). The potato is also an important plant in Finland, especially in northern areas. Potato starch is used both in the food and non-food industries, especially in the paper industry.

1.1 Starch biosynthesis

Starch synthesis occurs in green plants in two steps. Transitory starch, leaf starch, is synthesised during daylight at the same time as photosynthesis occurs in the chloroplast (Visser and Jacobsen 1993). During the dark period when photosynthesis is inhibited, leaf starch is degraded, and sucrose is formed, which is then transported to sink tissues (Ghiena et al. 1993, Matheson 1996).

Starch granule synthesis in green plants occurs in so-called amyloplasts, which are organelles containing the enzymes needed to biosynthesise starch polymers (Keeling et al. 1988, Martin and Smith 1995). The mechanism of starch biosynthesis is not known; in particular, the regulation of the reactions is mostly not understood. The enzymes involved are ADP-glucose pyrophosphorylase (ADPGPPase; EC 2.7.723), starch synthase (SS; EC 2.4.1.21) and starch branching enzyme (SBE; EC 2.4.1.28). The synthesis begins in the stroma, where the sucrose from transitory starch is degraded to fructose and glucose. The deposition of starch within endosperm amyloplasts starts within a few days of fertilisation.

Amylose and amylopectin molecules are synthesised from ADP-glucose, which is synthesised from glucose-1-phosphate and ATP in a reaction that is catalysed by ADPGPPase (Martin and Smith 1995). Starch synthesis is facilitated by the enzyme SS, which catalyses the synthesis of an α-1,4-linkage. The enzyme SS has two different forms, one bound to the starch granule and the other in the soluble phase of the amyloplast. During the maturation, both starch polymers are synthesised simultaneously (Visser and Jacobsen 1993), but at the beginning of the synthesis the amount of amylopectin is higher than amylose (Shannon and Garwood 1984). Martin and Smith (1995) postulated that the amylose molecule is synthesised by the granule-bound starch synthase (GBSS), which is located in the amylopectin molecule. The amylopectin molecule is synthesised by very complex enzyme collaboration. The α-1,6-branches are made by SBE, which hydrolyses an α-1,4-linkage within a chain and then catalyses the formation of an α-1,6-linkage between the reducing end of the "cut glucan chain" (Martin and Smith 1995). Recently Ball et al. (1996) suggested that the building of the amylopectin molecule structure starts from highly branched glycogen prestarch (Fig. 1).

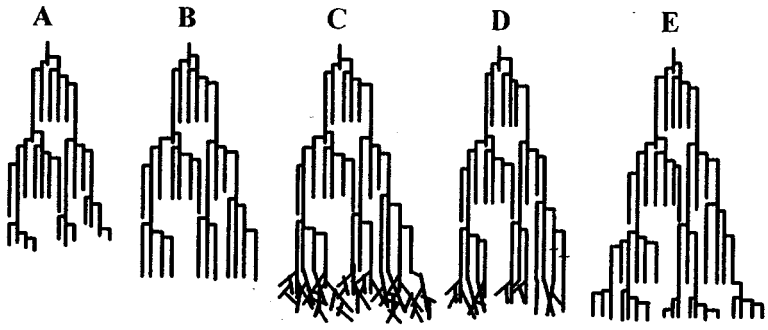

Fig. 1. The Ball et al. (1996) suggestion for amylopectin molecule synthesis. A and B: elongation from amorphous lamella until critical size for branching enzymes is reached. C: random branching. D: debranching trims down the loosely branched glucans. E: next amorphous lamella is generated.

It is not only the genetic background of a plant but also the growth conditions that affect starch biosynthesis. Excessively high growth temperatures have been observed to reduce the conversion of sucrose to starch in the endosperm, cleavage of sucrose by sucrose synthase is reduced and soluble starch synthase activity decreases (Keeling et al. 1988, Jenner 1991, Hawker and Jenner 1993, Keeling et al. 1993, Savin et al. 1997).

1.1.1 Starch granule

The size and morphology of starch granules vary depending on the plant origin, and the shapes can be round, elliptical, oval, lenticular, polyhedral or polygonal and irregular sausage shaped (Lineback 1984, Jane et al. 1994). The smallest granules are rice and amaranth starches (1 μm in diameter) and the largest granules are potato and tapioca starch granules, with a 100-μm diameter (Seidemann 1966, Bello-Pérez et al. 1998). The maize starch granule surface can be porous, or starch granules may have pinholes on the surface or near the equatorial groove (Evers et al. 1971, Fannon et al. 1992), as in large wheat and barley starch granules. In general, the surface structure has been studied very little. Starch contains around 10% water at RH 54% and 20°C.

Two different sizes of granules are observed in barley and wheat starches. Starch granules from mature barley kernels can be separated into two clearly defined populations: large A-granules, ca. 10–15 μm in mean diameter, and small B-granules, 2–5 μm in mean diameter (Morrison et al. 1986). During maturation, the large A-granules are formed first, while around 15 days after anthesis, small B-granules are developed in barley (Karlsson et al. 1983). Following this, large granules only grow in size but more and more B-granules are synthesised. The regulation of the two granule populations in wheat and barley is unknown.

The amount of amylose and amylopectin in starch granules varies depending on the plant origin. Normal starch is composed of 25% amylose and 75% amylopectin. The amylose polymer is a long and linear molecule with very few branches (less than 1%) (Ball et al. 1996) and its molecular weight is approximately 500,000 g/mol. Glucose units are linked with α-1, 4 bonds in the amylose molecule. Buléon et al. (1998a) estimated that the number of amylose molecules in one starch granule (diameter 20 μm, density 1.5 g/cm^3) was 1.8×10^9. In the starch granule, amylose exists in a helical form, the interior of which is hydrophobic (Thomas and Atwell 1999). Most studies have demonstrated a higher amylose content in large barley granules (MacGregor and Morgan 1984, Gudmunsson and Eliasson 1989, Palmer 1989, Morrison et al. 1993), whereas in some others the amylose content was not dependent on the granule size (Evers et al. 1974, Kano 1977, Kang et al. 1985). Walker and Merritt (1969) developed an amylose variety in barley with 40% amylose. Granules in this mutant were smaller than in both its parents and the shape was uniform. Whistler and Kramer developed high-amylose corn in 1946, and they were able to increase the amylose content from 25 to 65%. Later, the amount was increased up to 85% (Whistler 1984). A plant cultivar with 100% pure amylose does not exist. A novel high-amylose potato with 60–89% amylose was developed using gene modification, by the simultaneous inhibition of starch branching enzymes SBE A and B. In this potato variety, an approximately 5-fold increase in phosphorus levels was observed (Schwall et al. 2000).

Amylopectin has a very branched molecular structure. Its molecular weight can reach from 10^7 to 10^9 g/mol depending on the plant. Amylopectin chains are short A- and B-chains with a DP of 14–18, mid long B-chains with a DP of 45–55, and long B-chains with a DP of above 60 (Buléon et al. 1998a). Starch varieties with almost 100% amylopectin have been known for a long time. The

first was found in China among the corn varieties and it was brought to the USA in the first years of the 20th Century. Then the Iowa State geneticists developed waxy corn (Whistler 1984). Since then, waxy barley, rice, wheat and potato have been developed. By studying the shrunken barley mutant Bomi *shx*, with only 31% of the normal dry weigh of starch (the activity of SSS is reduced), it was observed that the structures of amylose and amylopectin were similar to that of normal Bomi (Schulman et al. 1995). It was concluded that the structure of amylopectin depends on the distinct roles of the SSS and SBE forms present. The location of amylose and amylopectin molecules in starch granules is still unknown.

Morrison et al. (1986) claimed that in native non-waxy barley, starch amylose occurs as two amorphous fractions, lipid complexed amylose (LAM) and free amylose (FAM), which are not homogenously distributed. Jane and Shen (1993) postulated that amylose was more concentrated at the periphery of the potato starch granule. Morrison (1995) reported that in the cereal starches the number of amylose–lipid complexes is higher on the granule surface (Morrison 1995). The lysophospholipid content of low-amylose starches was approximately 0.1–0.5% and for non-waxy starches approximately 0.8–1%. Vasanthan and Bhatty (1996) showed that there were more lipids and true (total) amylose in the small barley starch granules, but a lower content of free (apparent) amylose than in large granules. The results indicate that there are more internal lipids and amylose–lipid complexes in small granules. Tester et al. (1991) observed that the lipid contents of barley starches were much higher at a higher growth temperature.

1.1.2 Crystalline structure

Starch granules are partially crystalline particles (French 1984, Buléon et al. 1998b). According to Zobel (1988b), the native starch granule structure has crystallinity levels from 15 to 45%, depending on the starch origin. Starch granules in their native form have a complex structure, with parts that are very densely packed and others that are loosely packed. It was shown that the crystalline parts in a starch granule are formed by short outer chains (DP 15-18) of amylopectin molecules (French 1984). The densely packed areas are measurable by X-ray techniques. By this method, different types of crystal

structures can be analysed and the amount of crystalline and amorphous material measured. In starch granules, the crystal types are called A and B and their mixture C, which are formed in cereals, potato and legumes, respectively (Colonna et al. 1982, Zobel 1988a, Buléon et al. 1998a).

Water content is also essential when investigating the crystallinity of starches. Dry starch has a completely amorphous X-ray pattern, and the crystallisation of B-type starches has been shown to vary with varying water contents (Cleven et al. 1978, Buléon et al. 1982, Buléon et al. 1998a). The B-type is converted to A-type by means of heat-moisture treatment at 100–120°C (Sair 1967, Kulp and Lorenz 1981).

Imberty et al. (1988) proposed a crystal model of the A-type starch. Left-handed double helices are packed in monoclinic units, with eight water molecules. The crystal unit cell is 2,124 nm wide, 1,172 nm thick and 1,069 nm high. In the B-type crystal model, the double helix is packed in a hexagonal unit, with 36 water molecules (Imberty and Perez 1988). The width and thickness of the B-crystal unit cell is 1.85 nm and the height is 1.04 nm (Buléon et al. 1998a). There is an amorphous area between the crystal clusters, which is mainly composed of the branched points of the amylopectin chains (Morrison 1995). Single helices, called V-type crystals, are formed by treating amylose with iodine, alcohols, or fatty acids (Sarko and Zugenmaier 1980). Bear (1942) demonstrated that A- and B-type double helices cannot bind iodine. Amylose in mature starch granules is suggested to be in an amorphous form, because of its higher degradation rate by alpha-amylase in maize starch (Zobel 1988b). The main reason why a plant creates different crystal structures is unknown.

1.2 Starch properties

1.2.1 Starch gelatinisation and retrogradation

The partially crystalline granule structure of starch is stable in water at room temperature. Below 50°C, only minor swelling of the amorphous parts occurs, and this process is reversible. Above 50°C, the amorphous parts absorb more water and the granule structure begins to change. This process is irreversible and the original structure is disrupted. With further heating of the granule, swelling

continues, while at the same time the amorphous linear amylose diffuses from the granule to the surrounding water phase. This temperature is called gelatinisation or pasting temperature. By increasing the temperature, starch polymers, mainly amylose, are further dissolved. Finally, the granules lose their crystalline structure, and will be fragmented at 100–150°C (van den Berg 1981, Blanshard 1987). Different starches have their own special gelatinisation temperatures, oat has the lowest (60°C), and high-amylose maize varieties have the highest (86°C) (Lineback 1984, Zobel 1988b). The main structural difference that influences the measured gelatinisation temperatures has been widely studied, but to date there is no exact answer.

Growth temperature and conditions have been reported to affect the amount, chemical composition, granule size and physical properties of starch in different cereal crops (Asaoka et al. 1984, 1985 a and b, 1986, 1991, MacLeod and Duffus 1988 a and b, Shi et al. 1994, Tester et al. 1991, 1995). It was found that rice plants grown at 30°C had higher onset (T_o) and conclusion (T_c) temperatures, and a greater heat of gelatinisation (ΔH) than those grown at 25°C, as analysed by differential scanning calorimetry (DSC) (Asaoka et al. 1984, 1985b, MacLeod and Duffus 1988a and b, Tester et al. 1991). It was assumed that higher gelatinisation temperatures were due to greater perfection of starch crystals (Tester et al. 1991).

The gelatinisation enthalpy of small B-granules in barley was 15–20% lower than that of large granules, but the gelatinisation temperature range (T_c–T_o) was higher for small granules. Furthermore, the gelatinisation temperature of small granules has been shown to be higher than that of large granules, as estimated from the loss of birefringence (Evers et al. 1974, MacGregor 1980).

A gel is formed upon cooling of hot starch dispersion. B-type crystallinity is usually observed when storing starch gels at ambient temperatures (Blanshard 1987, Colonna et al. 1987). In amylograph studies, it is possible to see how the gelatinised starch makes gel while cooling (Zobel 1984). This gelling property of starch is widely exploited in many food applications, including puddings, jellies, confectionery, sausages and drinks. The native starch gels are unstable because of recrystallisation (retrogradation) of the amylopectin molecule. During long-term storage, the gel forms a rigid structure that is not favourable for food products. A good example of the retrogradation process is the ageing of bread

(Russell 1983), which has been studied by following the retrogradation kinetics of gelatinised wheat starch gels (Roulet et al. 1987).

1.2.2 Glass transition temperature

The glass transition temperature (T_g) is the most important parameter in determining the mechanical properties of amorphous polymers and in controlling the kinetics of the crystallisation of amorphous materials (Biliaderis et al. 1986, Levine and Slade 1986, Orford et al. 1989, Roos 1995, Schenz 1995). For dry amylose and amylopectin, the T_g has been estimated to be at 227°C, and the presence of 13% water has been observed to decrease the T_g to 56°C (Orford et al. 1989). The T_g of gelatinised wheat starch containing 22% water was detected to be at room temperature (Zeleznak and Hoseney 1987). Many studies have demonstrated the plasticisation effect of water on starches, and the various techniques for analysing the glass transition have been compared. When examining different techniques, the most frequently used DSC was observed to give 10–30°C higher T_gs than pulsed NMR (Kalichevsky et al. 1992).

The effect of water on the T_g of amylose and amylopectin was recently analysed using DSC (Bizot et al. 1997). The much-branched amylopectin had a somewhat lower T_g than the amylose polymer. Studies of the effect of glycerol and other plasticisers on the T_g of potato starch showed that the plasticisation of starch follows Couchman's model (Lourdin et al. 1997). When amylose and maltose were plasticised by glycerol, phase separation above 25% glycerol was found, based on dielectric thermal (DETA) analysis (Lourdin et al. 1998). In a combination of water and glycerol as plasticisers of barley starch, two calorimetric glass transition temperatures were measured. The two transitions observed were an indication of phase separation (Forssell et al. 1997). The dynamic mechanical, the dielectric thermal and the calorimetric behaviour of a binary amylose–glycerol system, assessed by DMTA, DETA and DSC, respectively, indicated that the system was composed of amylose-rich and glycerol-rich phases (Moates et al. 2001).

1.2.3 Enzymatic hydrolysis

Starch-degrading enzymes were classified by Banks and Greenwood (1975) as exo-acting, endo-acting, or de-branching. The main products from the α-amylolysis of amylose using alpha-amylase are glucose and maltose. α-Amylases are endo-acting. Exo-acting enzymes, such as β-amylase, remove low-molecular-weight products from the non-reducing chain-end (Schwar 1958, Whistler et al. 1984). Starch hydrolysis enzymes, such as amylases, are digestive enzymes that also process enzymes when starch is industrially modified into syrups, and in malting or fermentation processes (Oates 1997). The hydrolysis mechanisms are not well understood. It has been shown that raw starch granules hydrolyse slower than pregelatinised starch (Lauro et al. 1993, 2000, Lauro 2001). It has also been postulated that easily accessible parts for the α-amylase in the starch granules varied depending on the structure/organisation of the starch source rather than the type of crystallinity (Bertoft and Manelius 1992, Bertoft et al. 1993, Manelius 2000). According to Leach et al. (1959), MacGregor (1980), and MacGregor and Morgan (1984), small ungelatinised barley starch granules were hydrolysed faster than the large granules by malt alpha-amylases at 18–35°C. A similar result was also shown by Bertoft and Kulp (1986). After hydrolysis by α-amylase, large granules from normal barley contained many pinholes on the surface and it was postulated that hydrolysis occurred from the inside out (MacGregor 1980). No characteristic pinholes were observed in degraded small starch granules, which were hydrolysed by surface erosion. A similar observation was made by Bertoft and Henriknäs (1982).

During the malting process, Bathgate and Palmer (1973) studied the attack of malt alpha-amylase on barley starch granules at 65°C. They noticed that the small granules from barley were most resistant to malt alpha-amylase, even after complete gelatinisation. Small granules, which had a higher gelatinisation temperature than large granules, required a longer time to gelatinise at 65°C, and would therefore be degraded more slowly.

The beta-amylolysis limit value of small granules has been shown to be slightly higher (43–65%) than that of large granules (39–54%) (Evers et al. 1974). The amylopectin molecules of small and large starch granules are reported to have similar structures (MacGregor and Ballance 1980). Large granules were shown

to contain a greater amount of long amylopectin B chains (Morrison and Laignelet 1983, Gudmunsson and Eliasson 1989).

1.3 Starch functionality

Native and modified starches are widely used materials in the food and non-food industries (Osman 1967, Petersen 1975, Anon 1976, Moore et al. 1984, Whistler et al. 1984, Schoch 1985, Rapaille 1986, Röper 1996). The possibility of producing special new "designed" starch varieties with novel properties has also been discussed (Visser and Jacobsen 1993, Ring 1995). Industry uses mostly modified starches, such as pregelatinised, extruded, acid-converted and cross-linked starches or starch derivatives with various substituent groups (Wurzburg 1986). Amylose-rich starch varieties have been little used (Senti 1967), but there is an interest in developing slowly digesting starches (Thompson 2000), and in exploiting the film formation ability of starches.

1.3.1 Resistant starch

Starch is an everyday dietary component in human and animal nutrition (Ring et al. 1988). It also plays an important role in colonic physiology and functions, and provides potential protective effects against colorectal cancer (Cassidy et al. 1994, Silvi et al. 1999). Resistant starch is not digested by pancreatic amylase in the small intestine and thus reaches the colon (Thompson 2000). Three types of resistant starches have been classified by Englyst et al. (1992): RS1 is a starch entrapped within a food matrix, RS2 is granular starch, and RS3 is retrograded starch formed in food processing. Chemically modified starch was characterised as type RS4 (Thompson 2000). Resistant starch can be fermented by human gut microflora, thus providing a source of energy for bacteria. In a study where rats were fed with native potato starch (RS2), an increase in the intestinal population of bifidobacteria, lactobacilli, streptococci and enterobacteria was demonstrated. In the fermentation of resistant carbohydrates by anaerobic bacteria, acetic, propionic and butyric acids were produced, and the pH decreased in the lumen (Macfarlane and Cummings 1991, Kleessen et al. 1997, Le Blay et al. 1999).

1.3.2 Starch films

The film-forming properties of starch have been widely studied (Wolff et al. 1951, Rankin et al. 1958, Langlois and Wagoner 1967, van Soest et al. 1996a and b, van Soest and Essers 1997, Della Valle et al. 1998, Hulleman et al. 1998, Forssell et al. 1999). Thermoplastic starch (TPS) is prepared by extrusion in the presence of glycerol. Different mechanical properties can be obtained depending on the glycerol content and botanical origin of starch (van Soest and Essers 1997, Della Valle et al. 1998, Hulleman et al. 1998). High-amylose starch has been shown to form stronger and stiffer thermoplastic films than high amylopectin starch. The hydrophilic nature of the TPS makes it sensitive to environmental humidity (Rankin et al. 1958), and the presence of a high level of glycerol strengthens this behaviour. During storage, TPS made of potato starch has a tendency to change its structures, becoming more brittle (van Soest et al. 1996a). The changes observed in TPS were similar to retrogradation of normal starch gels, indicating that this process may be linked to the crystallisation of amylopectin (Forssell et al. 1999). In one commercial product, the water resistivity is improved by adding synthetic polycaprolactone (PCL/starch, MaterBi^{TM,} Novamont, Italy) (Vikman et al. 1999). These materials can be used in the production of applications such as the waste bag (Bioska^{TM}, Ylöjärvi, Finland), which can be composted. Pure corn-based TPSs have some applications as a filler in packages and golf tees.

The film-forming properties of amylose and amylopectin were studied by Wolff et al. (1951) and Langlois and Wagoner (1967). Amylose was observed to be an excellent film former, and was claimed to have similar properties to cellulose films. Amylose and amylopectin films have been shown to have very good oxygen barrier properties at humidities below 81% (Forssell et al. 2002). Shamekh et al. (2002) found that after removing sugars from potato starch hydrolysates (DE 10, 15 and 20), good quality films were formed. Studies on starch films prepared by water-casting and plasticised by glycerol have attempted to elucidate in more detail the effects of starch structure on plasticisation and properties (Lourdin et al. 1995, Rindlav et al. 1997, Rindlav-Westling et al. 1998). The structures of the starch films were observed to be entirely amorphous (Lourdin et al. 1995). Amylose films were crystalline and the structure of amylopectin films depended on the preparation conditions (Rindlav-Westling et al. 1998). Films dried at 50°C or above were almost

amorphous. When the drying temperature was 20°C, the amount of B-type crystallinity detected depended on the RH during drying, The highest humidity produced the highest crystallinity (23%) (Rindlav-Westling et al. 1998). X-ray diffraction was applied to investigate the effect of RH on the structures of amylose and amylopectin films with and without glycerol at 23°C. B-type crystallinity in the amylose film was approximately 34% and it did not depend on either the conditions or the presence of glycerol. The amylopectin film without glycerol was amorphous under all conditions, but when glycerol was the plasticiser, B-type crystallinity gradually formed with increasing humidity (Rindlav-Westling et al. 1998). Bader and Göritz (1994 a and b) observed that the drying temperature during the film preparation affected the diffraction pattern observed, and the B-type pattern was present at lower temperatures, whereas the A-type pattern was detected above 80°C. It was concluded that amylopectin was more sensitive than amylose to plasticisation caused by glycerol. Amylose was also found to form mechanically stronger films. In a study of enzyme susceptibility of amylose films and gels, their crystallinities were analysed by X-ray diffraction (Cairns et al. 1995). Amylose was isolated from pea starch, and films were prepared by drying amylose gels at 50°C. Amylose films were more resistant to α-amylase than amylose gels, but only the gels were characterised by X-ray diffraction. A typical B-type pattern was observed. Hydrolysis of the gels resulted in a significant increase in the crystallinity. B-type crystalline starches are usually believed to cause the resistivity of the retrograded starches (RS3), such as amylose gels and fibres.

Starch is the cheapest biopolymer that can be used in various applications, such as a protective barrier or carrier of sensitive compounds in food, pharmaceutical and non-food products (Trubiano and Kasica 1986, Whistler 1989, Kobayashi et al. 1993, Whistler 1997). In the special field of colon drug delivery, polysaccharide-based dosage forms have been suggested to be very promising (Vandamme et al. 2002). Amorphous amylose mixed with ethyl cellulose (Ethocel®) is one example of exploiting amylose functionality for the delayed-release of drugs (Ring et al. 1994, Newton 1996). The basic understanding of starch granule structure, the functionality of amylose and amylopectin, and the modifications caused by enzymes and physical treatments, are of crucial importance when developing the novel applications. Vandamme et al. (2002) concluded that the mechanisms of degradation of film coatings designed for

specific drug delivery in the colon need more in-depth investigation in future research.

1.4 Aims of the present study

* To understand the factors influencing the gelatinisation properties of barley starch granules.

* To study the plasticisation mechanism of amylose and amylopectin by glycerol, and the relationship between starch film structural and mechanical behaviour.

*To develop a novel, microencapsulation method for probiotic bacteria by means of the combined use of starch granules and films.

2. Materials and methods

2.1 Starch materials

The 2-row malting barley varieties Kustaa and Kymppi were field-grown in Hahkiala in 1987 and 1991 (Publication I) and Kustaa in 1989 (Publication II). Summer 1987 in Finland was cold, and the sum of an effective growing temperature was only 994 (base temperature 5°C) in the period from April 28th to October 19th. The summer in 1987 was also 1.5–2 times more rainy than average (Aikasalo 1989). In 1991, the sum of the effective growth temperature was 1173 (April 30th–October 31st).

Isolated amylose from potato starch (Sigma A 0512, Type III, 4.7% of residual butanol), and granular waxy maize starch (National Starch, USA) were used for the cast film preparation (Publications III and IV).

Native potato starch granules for bioencapsulation of probiotic bacteria were from Järviseuden Peruna, Vimpeli, Finland. The high amylose maize starch was Hylon™ VII, from National Starch, USA (Publication V).

2.2 Separation of starch

Starch was isolated from the grains using the method of McDonald and Stark (1988), with minor modifications. To increase yield, the isolation procedure was modified so that the bran fraction was also treated with protease enzyme (Fig. 2) to release more small granules from the outer seed layer. Barley grains (6–20 g) were first husked with a special blocking machine, then milled and suspended in 0.02 M HCl to denature the enzymes in the seeds. The separated fibre fraction and the brown protein/starch layer were treated separately with protease (Sigma XIV no. 5147, from *Streptomyces griseus*). At the end of the separation, all starch fractions were combined and treated twice in a 0.2 M NaCl solution (7 vol) with added toluene (1 vol), washed twice with water and twice with acetone and finally air-dried (Fig. 2). The powder was very smooth and white in colour, which indicated that only a very small amount of impurities were left. This was also observed when the total starch content was measured.

Small and large granules from the barley starch were separated by the sedimentation method of Decker and Höller (1962). The sedimentation was repeated 15 times. Finally, the small granule fraction was further purified by sedimenting it twice overnight (16 h) at 5°C. Both fractions were washed twice with acetone and dried in air. The large (>50 μm) granules were separated from potato starch in cold water by sieving through a sieving cloth. The granules over the cloth were then frozen and freeze-dried. The separation of small and large starch granules from barley starch was very time consuming, but both fractions obtained were pure. The cut-off point between small B-granules and large A-granules was 10 μm, based on Coulter counter analysis.

2.3 Preparation of starch films

In order to dissolve amylose and granular amylopectin (waxy maize starch), a starch-water dispersion (1% or 2%w/w) was heated to 140°C in a pressure heater equipped with a stirrer (VTT Automation, Protolab, Espoo) and kept constant for 30 min. The system was then cooled to 100°C and the vessel was opened. Glycerol (10 or 30%, dry weight basis) was added with a syringe and mixed for 5 min. The hot solution (35 ml) was poured into pre-warmed (70°C) Teflon moulds (10 × 10 cm). Films were dried in a climate-testchamber (Weiss Technik, Reiskirchen, Germany) at 70°C for 3–4 h. For the mechanical tests, the film was dried in an 11 × 3 cm mould (10 ml of starch solution).

2.4 Analytical methods

2.4.1 Chemical composition

Starch content was determined enzymatically using the method of Karkalas (1985), with some modifications. Apparent and total amylose content were determined colorimetrically (Morrison and Laignelet 1983). The phospholipid content was determined by measuring the phosphorus content using the method of Morrison (1964), and using a conversion factor of 16.5 (Tester and Morrison, 1990a). Protein content was determined as Dumas-protein using a Carlo Erba Nitrogen analyser 1500 (Milan, Italy).

2.4.2 Granule size distribution

The granule size distribution was measured using a Coulter Counter Multisizer analyser. Starch samples were first suspended in a 2% NaCl solution and pretreated with ultrasonification (30 s). Number-average diameters and the weight average granule diameters for starches (about 500,000 granules/measurement) were calculated using the formula described by Schoch and Maywald (1956).

2.4.3 Water sorption

Amylose and amylopectin film samples were cryomilled prior to the water sorption test. Film samples were frozen using liquid N_2 and milled using a Fritsch pulverisette mill (Germany) equipped with a sieve (100-μm mesh opening). Two hundred milligrams of powder (duplicate samples) were weighed into ceramic dishes (which were dried over the P_2O_5 in a desiccator). The RHs were adjusted in glass chambers by saturated salt solutions: 11 (LiCl), 33 ($MgCl_2$), 54 ($Mg(NO_3)_2$), 81 ($(NH_4)_2SO_4$) and 91% (KNO_3). The samples were kept in RH chambers at 20°C for 7 days. The water content was calculated based on the weight change and on the initial water content of the powder, which was measured using a Karl Fisher Titrator (Mettler DL18).

2.4.4 Microscopy

The microstructure of starch dispersions was studied by light microscopy using the smear technique and iodine staining (Autio 1990, Autio et al. 1992). Starch suspensions (8%) were heated to the appropriate temperature (90 and 95°C) and incubated 5 min prior to preparation of the samples. The starch dispersion was smeared onto an object glass and stained by iodine (0.33% iodine and 0.67% potassium iodide). The samples were then immediately examined using an Olympus BH-2 microscope.

In order to study the microstructure of the native and hydrolysed starch granules and starch microcapsules, starch powder was fixed in 5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.0), dehydrated with ethanol, and embedded in Historesin (Jung, Germany), following the method of Parkkonen et al. (1994).

Cross-sections (2–4 μm thick) were cut with a rotary microtome (Leica Jung RM 2055, Germany) and stained with iodine solution or by DAPI (4′,6 diamidino-2-phenylindole, Sigma Chemicals, USA).

The native and hydrolysed potato starch granules were examined by ESEM (Environmental Scanning Electron Microscopy, Electro Scan, Kebo Lab.) in collaboration with The Finnish Pulp and Paper Research Institute (Espoo, Finland). The dry starch powder was put on a holder, which had a black surface. The surface was taped with Scotch tape and a thin layer of sample powder was added. The sample was put inside the ESEM and immediately examined at room temperature, at varying magnifications, ranging from 500 to 3800×. The pictures were saved on computer.

2.4.5 Swelling power and solubility

The swelling power (hydration capacity) and solubility of the barley starches at different temperatures were determined using the procedure of Leach et al. (1959), with some modifications. The starch sample (100 mg ± 0.1 mg) was weighed (triplicate samples) into small screw-capped test tubes; 5 ml distilled water were added and tubes were incubated at varying temperatures, ranging from 78 to 100°C for 30 min, with occasional manual stirring. Cooled tubes were centrifuged for 15 min and the phases were separated immediately after the centrifugation. The solubilised starch was determined as total carbohydrates using the method of Dubois et al. (1956), with glucose as the standard. The swelling power was calculated based on the amount of water absorbed and corrected for the amount of carbohydrate solubilised during the gelatinisation process in excess water (Zobel 1984). The soluble fractions were freeze-dried and the amylose content was measured using the method of Morrison and Laignelet (1983).

2.4.6 Enzymatic and acid hydrolysis, water dispersibility

The effect of preheating on the hydrolysis level of barley starch was studied by heating a 7.5% starch–water dispersion in a water bath for 3 h at 50 and 55°C (triplicate samples). The starch was separated by centrifugation (15,000 rpm/10

min). Enzymatic hydrolysis of the preheated starch granules was performed at 30°C, as described by Lauro et al. (1993) using *Bacillus licheniformis* α-amylase (Sigma type XII A, A-3403), and the enzyme was inhibited by adjusting to pH 2 for 30 min. The total carbohydrates of hydrolysis supernatants were assayed using the phenol-sulphuric acid method, with glucose as a standard (Dubois et al. 1956).

The accessibility of amylose and amylopectin film to α-amylase and hydrochloric acid, as well as water dispersibility, were analysed for the fresh films which were conditioned at RH 50% (at 20°C) for 7 days (duplicate samples). Film samples were powdered by grinding the frozen films with liquid N_2 using a Fritsch pulverisette mill (Germany) equipped with a sieve (100 μm mesh opening), and drying the powders over P_2O_5 in a desiccator. For enzymatic treatment, dry samples (containing 450 mg of starch) were weighed in tared centrifuge tubes and suspended in 10.8 ml distilled water. Following this, 1.2 ml of pancreatic α-amylase (8000 U/g starch, SIGMA P-1750) dissolved in 150-mM $NaHCO_3$ buffer (pH 9.7) were added. The samples were then incubated for 3 h at 37°C, with magnetic stirring. After hydrolysis, the suspension was centrifuged for 10 min (10800 × g). The total carbohydrate content of the solution was determined using the phenol–sulphuric acid method (Dubois et al. 1956). The extent of hydrolysis was calculated as the quantity of glucose (×0.9) divided by the amount of starch in the original sample. Gravimetric analysis was performed for the insoluble residue. The residue was washed with distilled water, re-centrifuged and dried over P_2O_5 in a desiccator. The amount of insoluble carbohydrates was calculated as the quantity of residue divided by the amount of starch in the original sample. As confirmed earlier, glycerol was dissolved during hydrolysis.

An analysis of acid hydrolysis was performed using hydrochloric acid at 35°C for 7 days (Leloup et al. 1991, Leloup et al. 1992). Film powders (duplicate samples) were suspended in 2.2 M HCl (100 mg in 20 ml HCl). The extent of hydrolysis was expressed as the soluble carbohydrates divided by the quantity of starch in the original sample. Water dispersibility was monitored in distilled water at 37°C for 3 h. The samples were treated in the same way as the samples in the enzymatic hydrolysis, except that only the total carbohydrates in the liquid fraction were analysed.

2.5 Thermal properties

2.5.1 Gelatinisation temperature and retrogradation rate

The DSC measurements were performed with a Mettler DSC-30S instrument equipped with a Mettler TC 11 analysis data station connected to a computer. The gelatinisation and retrogradation of barley starches were studied in starch dispersions with 50% or 70% (w/w) water. The samples were prepared by weighing 3–5 mg of starch in DSC standard aluminium sample pans (ME-26763, Al crucibles 40 µl), 15 µl of distilled water were added by pipette and the contents were mixed well with a needle. Pans were hermetically sealed after a 50% moisture level was reached through the evaporation of excess water from the mixture at room temperature. The samples were heated from 10 to 100°C (heating rate 10°C/min). A pan with Al_2O_3 was used as a reference. The gelatinisation temperature was taken from the maxima of the endotherm peaks. Transition enthalpies (ΔH, J/g) were calculated from the area of the endotherm curve. The extent of recrystallisation was studied by reheating the pans after 1, 2, 4, 7, 11 and 15 days of storage at +4°C. The dissociation of the amylose–lipid complex in starch was studied at 70% moisture, using medium pressure pans (Mettler, ME-26929, with centring pins, 120 µl), with water as a reference. The samples were heated from 10 to 130°C, at a rate of 10°C/min. The dissociation temperature was taken from the maxima of the endotherm peak. Transition enthalpies (ΔH, J/g) were calculated from the area of the endotherm curve. Each sample was run in triplicate; and standard deviations were ±0.2°C for T gelatinisation and for the dissociation of the amylose–lipid complex. The standard deviation for the gelatinisation and dissociation enthalpy (ΔH) was ±0.5 J/g.

2.5.2 The glass transition temperature

Glass transition temperatures of the amylose and amylopectin films with and without glycerol were measured using a Mettler Toledo DSC820 (TA 8000 system, Switzerland) with an automatic opening and closing sample chamber and robot for 34 crucibles. The system was connected to a computer. Sealed pressure pans (Mettler, ME-26929, with centring pins, 120 µl) were used, with empty pans as the reference. The scanning rate was 10°C/min, and the scan was

performed from 20 to 170°C, followed by rapid cooling to –150°C using liquid N_2. It was then heated to 170°C at a rate of 10°C/min. The glass transition temperature was taken as the midpoint of the change in the heat capacity observed in the second heating scan. The changes in heat capacities (Δc_p) were evaluated using the Mettler system. Each sample was run in duplicate.

2.6 Tensile properties

The mechanical properties of amylose and amylopectin film samples (20 × 80 mm, cut from the film sample made in a Teflon mould, 3 × 11 cm) were measured using a Texture Analyzer TX. (TA, XT.2, England) following standard methods (ISO 1184-1983). The experiments were performed under controlled conditions at 20°C and RH 50%, where the film samples were stored for 7 days in open polyethylene bags. The thicknesses of the films were measured with a microtome (Mitoyo, Japan) prior to testing. Five measurements of each test sample were taken, and the variations were ±0.4–7 MPa and ±0.3–6% for stress and strain, respectively.

2.7 X-ray analysis

2.7.1 Sample preparation for X-ray diffraction

The structures of the amylose and amylopectin films were investigated using X-ray. For X-ray analysis, the amylose and amylopectin films with glycerol content of 0, 10 or 30% of the total dry weight, were prepared at VTT Biotechnology, Finland and then sent by DHL-express to INRA, Nantes, France. The films (10 × 10 cm) were packed into polyethylene bags and soft filler was placed around the bags in order to protect them during the journey. Films were X-rayed in film form, and not as powder. It was found that at least 16 film pieces (25 μm thick) were required in order to obtain enough "crystalline" material for the X-ray test. The film samples (10 × 10 cm) were initially cut into five strips (2 cm width). Each strip was then cut into four pieces and 16 of these pieces were packed into an Al-foil envelope (2 × 2 cm). A thin layer of vacuum paste was added to the other side of the envelope. A sample piece (5 mm in diameter) was cut with a paper punch (from the side where the vacuum paste was added). The foil piece

was removed and the sample was then put inside the X-ray copper ring (with 6-mm diameter hole). The copper ring and sample were then covered with Al-foil with a 4-mm diameter hole. The prepared samples were kept at RH 0% (vacuum desiccator with P_2O_5), 54% $(MgNO_3)_2$ and 91% (KNO_3) at room temperature, for 7 days, 1 month and 2 months.

2.7.2 X-ray diffraction

X-ray diffractions were recorded by means of a transmission technique using an XRG 3000 X-ray generator (Inel, Orleans, France) operating at 40 kV and 30 mA. $CuK\alpha_1$ radiation ($\lambda = 0.15405$ nm) was selected using a quartz monochromator. A curved position sensitive detector (Inel CPS120) was used to monitor the diffracted intensities using 2-h exposure periods. The recorded diffraction curves were normalised at the same total scattering between 3 and 30° (2θ). Crystallisation was determined using a technique derived from the methods of Wakelin et al. (1959) with recrystallised amylose (B-type crystals) and extruded potato starch as amorphous standards.

3. Results and discussion

3.1 Properties of barley starch (Publications I & II)

A starch isolation procedure (Fig. 2) from barley seeds and various analytical methods were developed. The purity of starch was the most important criterion (at least 95%), in addition to the yield (90% of the total starch in the seeds). It was noted that the most difficult step of the isolation procedure was the removal of impurities, mostly proteins, which is in agreement with the findings of Richter et al. (1968) and of McDonald and Stark (1988).

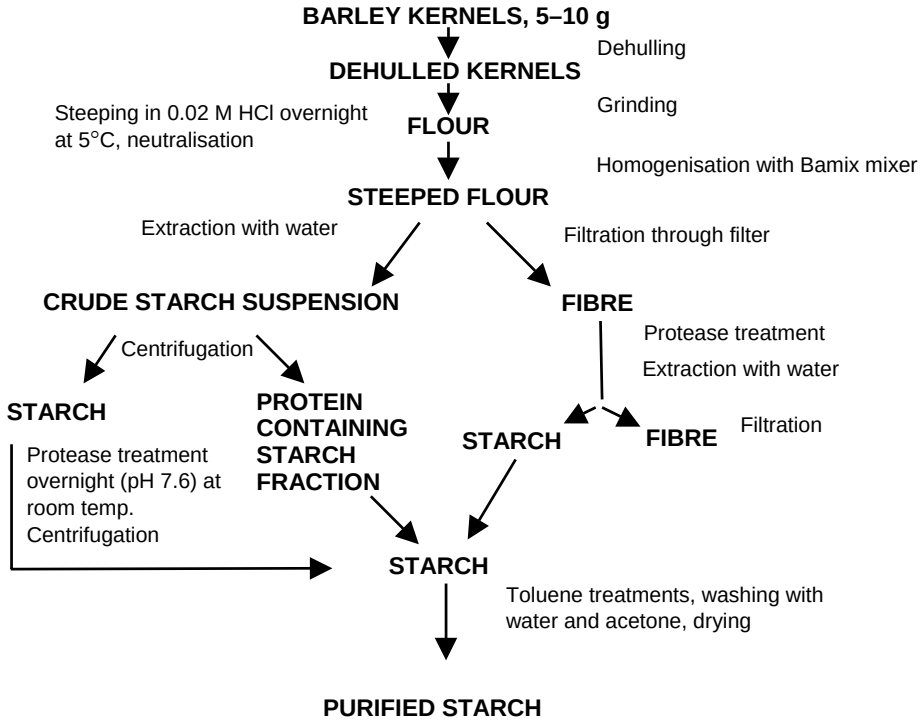

Fig. 2. The starch isolation procedure developed after McDonald and Stark (1988).

The growth conditions of barley influenced the properties of starch. The starch from a very cold summer (1987) was clearly different from that of a more normal summer (1991). The gelatinisation temperature of the 1987 cold-summer starch

was 4°C lower and the recrystallisation level was lower than for starches from 1991. When examining the effect of heating in the presence of excess water, it was noticed that at 50°C, Kustaa 1987 and 1991 and Kymppi 1991 starch samples were only partially gelatinised but Kymppi-starch 1987 was completely gelatinised. At 55°C, all of the starches were wholly gelatinised. Furthermore, the cold summer starches were more easily hydrolysed by α-amylase (Fig. 3). All of these differences might be explained by a less ordered structure due to the cold weather. Starches from 1987 were more sensitive to water (swelling and solubility) than 1991 starches, which may have been caused by a lower quantity of lipids present in the 1987 starches. Lipids have been shown to inhibit swelling (Morrison et al. 1993, Tester and Morrison 1990a, b, 1992, Tester et al. 1991).

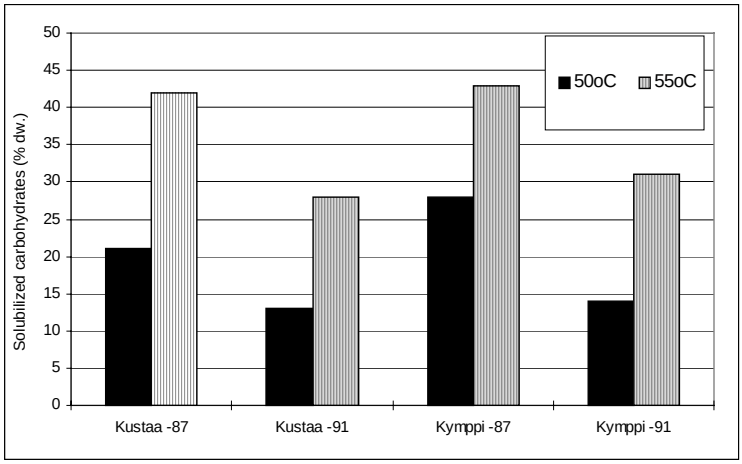

*Fig. 3. α-amylase (*Bacillus Licheniformis*, Sigma type XII A) hydrolysis of Kustaa and Kymppi barley (grown in the summers of 1987 and 1991) starches after 3 h of treatment at 50 and 55 °C.*

Normal or superoptimal growth temperatures have previously been associated with higher starch lipid contents, higher gelatinisation temperatures, and a higher extent of retrogradation of barley, wheat and rice starches (Asaoka et al. 1984, 1985 a, b, 1986, 1991, MacLeod and Duffus 1988 a, b, Shi et al. 1994, Tester et al. 1991, 1995). High growth temperatures are also known to reduce soluble starch synthase activity, and consequently to reduce starch granule size and number. Granule size and number were not affected under the suboptimal conditions investigated herein. Because there are no reports on the potential effects of low growth temperatures on

the soluble starch synthase, it is not possible to speculate on the effect of biosynthesis on starch structure under low growth temperatures.

When the properties of Kustaa barley starch were examined in more detail, the small and large granules were separated from three different Kustaa-barley samples grown in 1987, 1989 and 1991. In Kustaa, the average sizes of the two populations were approximately 4 μm and 18 μm. It was observed that the growth conditions affected gelatinisation temperature in a similar manner in both granule populations. B- and A-granules grown during a normal summer had higher gelatinisation temperatures than their cold-summer counterparts.

It was found that the lipid content (1.30%) of B-granules was higher than that of A-granules (1.30 and 0.90%, respectively). The endotherm describing the dissociation of the amylose/lipid complex was larger for B-granules than for A-granules. Below 90°C, amylose leached out from the A-granules while amylopectin solubilised from the B-granules. The higher content of the amylose–lipid complex possibly inhibited swelling and increased the gelatinisation temperature of the small granules (Tester and Morrison 1990a, 1992). At 95°C, small granules solubilised completely, but some of the large granules remained as particles, indicating a more stable structure.

3.2 Amylose and amylopectin films (Publications III & IV)

Rankin et al. (1958) reported that amylose formed very good self-supporting films, but because of its polar nature, the amylose film had high water permeability. The effects of water and glycerol on the properties of amylose and amylopectin films were reported in publications III and IV. Based on water sorption isotherms, the equilibrium water content at low humidities for both amylopectin and amylose was lower in the presence of glycerol than without, and the effect increased with increasing glycerol content (Fig. 4). The phenomenon can be explained by a replacement of the very strongly immobilised structural water with glycerol (van der Berg 1981, Gaudin et al. 1999). At 50% RH, the moisture contents of all films were approximately 10%, while at above 50% RHs, the highest glycerol content showed the highest water content. Furthermore, at higher RHs the equilibrium water contents of the

glycerol plasticised powders were higher than those without glycerol, which was most likely caused by the reorganisation of the phases.

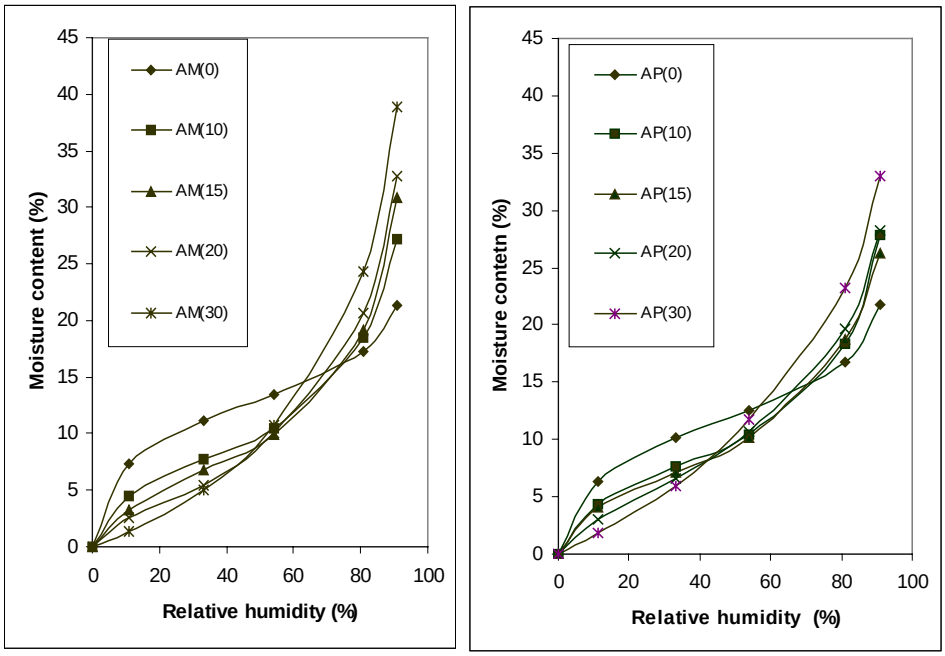

Fig. 4. Moisture contents of amylose (AM) and amylopectin (AP) films with and without glycerol (0–30%) *conditioned at different humidities at 20℃ for 7 days.*

The calorimetric T_gs observed were similar for amylose and amylopectin (Fig. 5). In the absence of glycerol, both amylose and amylopectin showed only one glass transition above 0°C. The glass transition occurred at room temperature when the water content was 21%. It was noted that Tg was not only affected by water but that glycerol also had a plastisicing effect; however, water was more effective. At a level of 21% glycerol (no water), the glass transition was at 93°C. It was estimated that a level of 35% glycerol would be needed to reduce the Tg to room temperature.

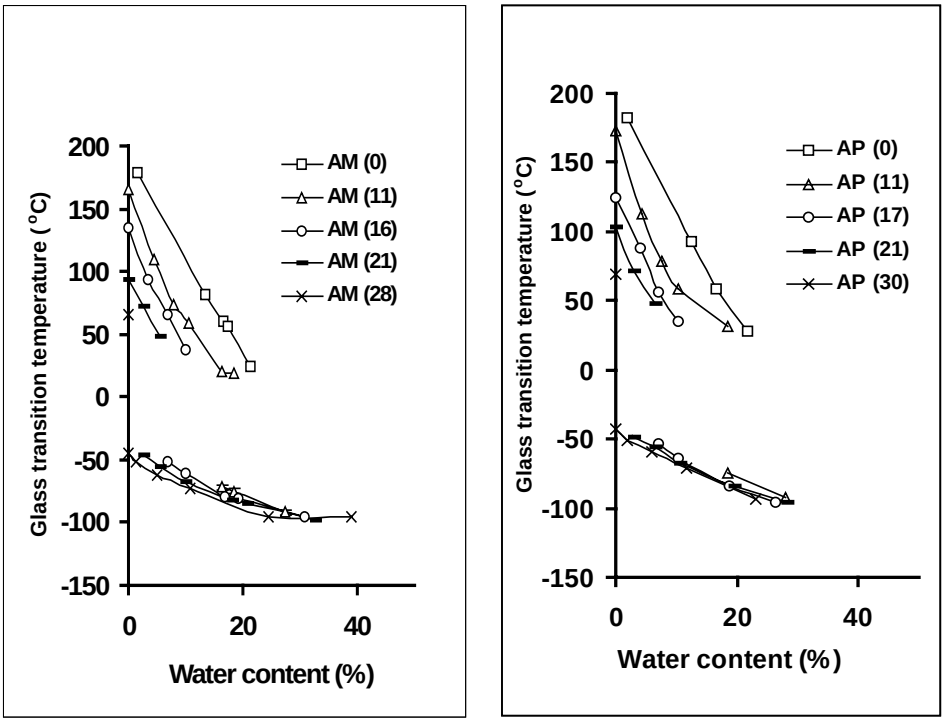

Fig. 5. The glass transition temperatures of amylose (AM) and amylopectin (AP) films with and without glycerol (0–30%) conditioned at different humidities at 20℃ for 7 days.

The effect of water on the mechanical properties was not studied. Tensile failure behaviour was examined only under RH 50% (T = 20°C). This RH was chosen because it is "ambient" and the amount of water was approximately 10% for all films. A film was produced even from amylose mixed with 70% glycerol, indicating the excellent film formation ability of the linear starch polymer. Amylopectin films were brittle (Fig. 6) and difficult to handle; film with 10% glycerol was particularly brittle. Above 30% glycerol, amylopectin showed liquid-type behaviour (Fig. 6).

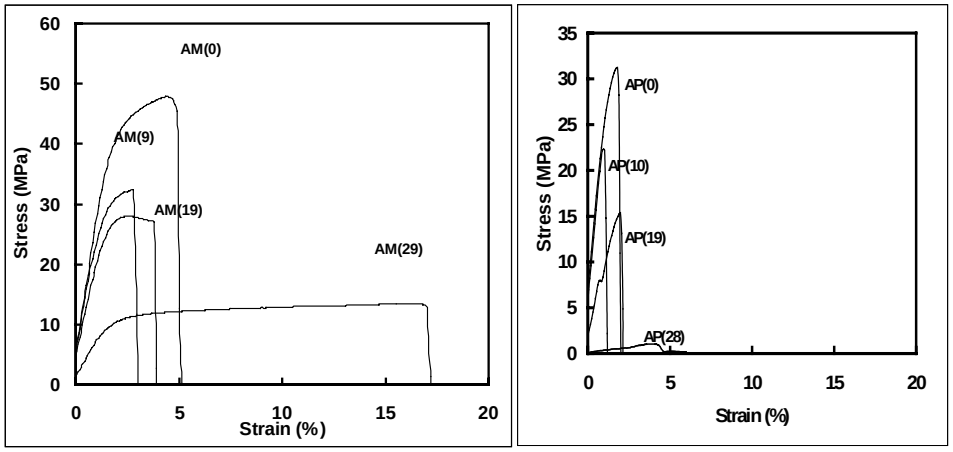

Fig. 6. The strain/stress curves of amylose (AM) and amylopectin (AP) films with and without glycerol (0–29%) at 20 ℃ and 50% RH.

Solubility and crystallinity studies were performed in order to understand the film structures. Amylose film had many good properties: it did not fragment in water, 35% of it was resistant to α-amylase, and 50% of the amylose film was resistant to acid. Conversely, amylopectin film fragmented in aqueous solution and dissolved entirely and rapidly in the presence of α-amylase or acid (Fig. 7).

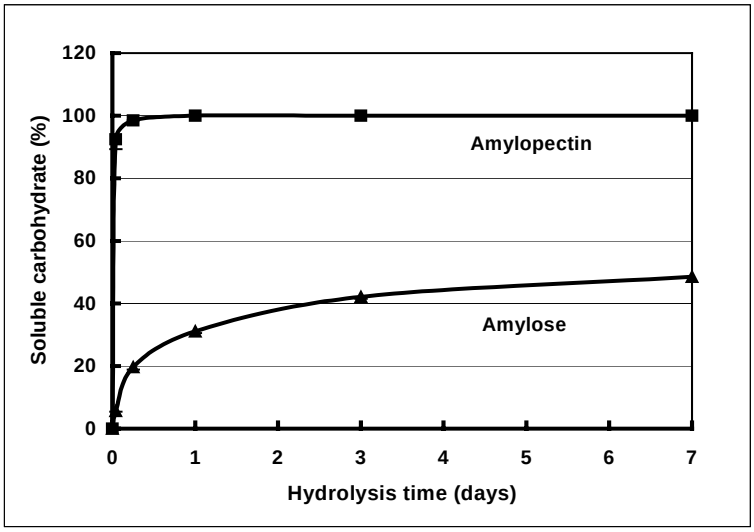

Fig. 7. The rate of acid (2.2 M HCl) hydrolysis of fresh amylose and amylopectin films without glycerol at 35 ℃.

The films were stored at relative humidities of 11, 54 and 91% for 7 days, 1 to 2 months at room temperature, and the structure of the films was followed using X-ray. Amylose films plasticised with water or plasticised with water and glycerol were partially crystalline with B-type structures. The crystallinity of the dry amylose films varied from 6 to 14%. Under ambient humidity the crystallinity was about 20%, and in the presence of more water at RH 91% the crystallinity reached a value of 32%. Upon ageing at 20^{O}C and at RHs of 0 to 91%, the crystallinities of the amylose films did not change. Similarly prepared films of amylopectin were amorphous. The only change observed among amylopectin films was the most plasticised film (30% glycerol and RH 91%), which showed a predominant B-type crystallinity developing upon storage during the first month of storage (Fig. 8). These results demonstrated that the crystallinity of the amylose films was stable. On the other hand, highly plasticised amylopectin films were unstable, and changed their amorphous structures to B-type crystalline forms when aged for several weeks. Similar crystallisation was observed previously for extruded barley and oat starch films (Forssell et al. 1999). The behaviour of amylopectin films demonstrated that the changes observed in the extruded starches were due to the crystallisation in amylopectin in the rubbery state. The structures of the starch films were reported to vary from amorphous (Lourdin et al. 1995) to crystalline, depending on the preparation conditions (Rindlav et al. 1997, Rindlav-Westling et al. 1998).

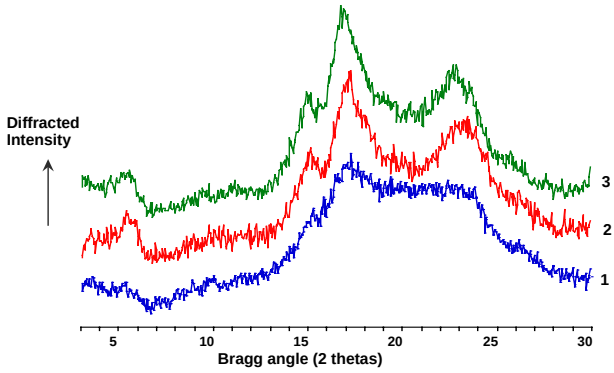

Fig. 8. X-ray diffraction diagrams of amylopectin film with 30% glycerol after storage at RH 91% and 20℃: 1, 7 days; 2, 1 month; 3, 2 months.

Fresh amylopectin films dissolved completely and rapidly in acidic solution. Partially crystalline amylose film did not dissolve in acid (Fig. 7), even though less than 50% was crystalline. Solubility demonstrated the different material properties of amylose and amylopectin, as was observed in the tensile stress results (Fig. 6). It is difficult to predict the functional behaviour of starchy material based only on X-ray crystalline structures.

3.3 Microencapsulation (Publication V)

Probiotics are live microorganisms that are used as dietary supplements with the aim of benefiting the health of consumers by positively influencing the intestinal microbial balance (Fuller 1989). *Lactobacillus* or *Bifidobacteria* products such as yoghurts, milks and juices are widely consumed in Europe and Japan because of their health-promoting image. Living bacteria may be used not only in foods but also in novel drugs for the therapeutic management of colon diseases (Mattila-Sandholm et al. 1999, Collins 2000, Shanahan 2000, Steidler et al. 2000) or in the treatment of children′s atopic eczema by *Lactobacillus rhamnosus*, LGG™ (Isolauri and Salminen 2000). The viability of probiotics in various food systems is provided only for short storage periods and in most cases under chilled temperatures. Brown et al. (1996) have patented a method in which high amylose starch granules are used as carriers for *Bifiobacteria*, to transport them to the large bowel. They also postulated that amylose-rich starch could promote the growth of microorganisms in the large bowel. Crittenden et al. (2001) found that *Bifidobacteria*, which have strong adhesive properties, were also able to hydrolyse the native raw starch. It was concluded that the adhesion appeared to be mediated by cell-surface protein(s).

The method developed in the present study is based on using partially hydrolysed potato starch granules as bacteria carriers and resistant starch as a coating material for the bacteria-carrier matrix. The main objective was to develop a one-step batch fermentation process (Fig. 9). In the process, large potato starch granules (50–100 μm) were used as a carrier, which became porous due to α-amylase (Fig. 10). After fermentation, the slurry was filtered. For coating, high amylose maize starch was solubilised in hot water (at 170°C) in a pressure heater equipped with a stirrer (VTT Automation, Protolab, Espoo),

cooled and precipitated over the starch granules filled with bacteria. *Lactobacillus rhamnosus* has been used in this study (VTTE800, human origin).

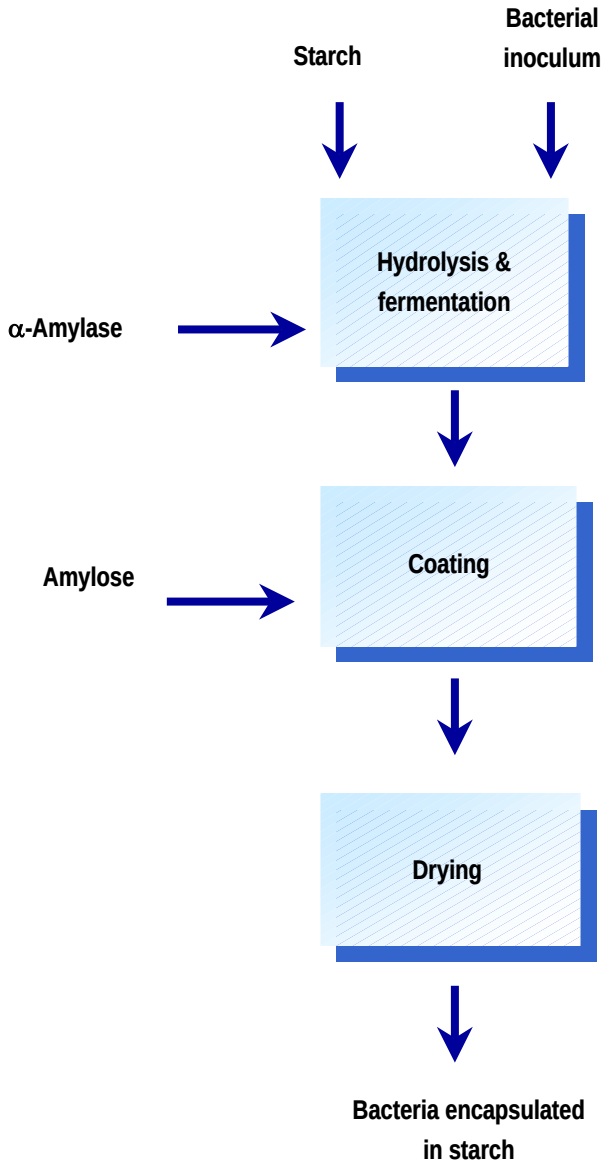

Fig. 9. Starch encapsulation method developed for probiotics.

For the people of the Andes in Peru, in the early 1550s, living up to 12,000 ft above sea level, only the potato could be grown. Keeping and cooking food were difficult, because fuel was less abundant, and frost could ruin anything in storage. Potatoes, which contain 80% water, were particularly susceptible, but the highland people turned that to advantage. They let part of the harvest freeze overnight and squeezed the water out to obtain a freeze-dried potato, which was easily and quickly cooked (the first fast-food -product!), and they named the product *chuño* (Zuckerman 2000). Eliasson et al. (1981) have shown that the gelatinisation temperature of potato starch was decreased as a result of freeze drying. Raw potato starch is very resistant to amylases (Thompson 2000). Freeze-dried potato starch granules were hydrolysed up to 70% and native granules only 25% by bacterial α-amylase at 37°C, which was the optimal temperature for the growth of bacteria. The degree of hydrolysis, however, depends on the origin of the amylase. It was observed that the enzyme attacked the freeze-dried granules from the inside out, making the granules porous (Fig. 10). The granular size distribution was not affected by the hydrolysis.

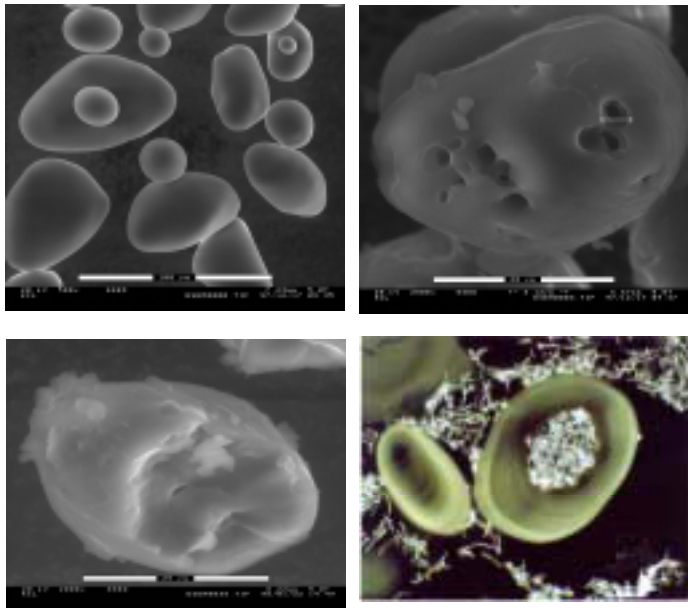

Fig. 10. Potato starch granules (top left, 500×, by ESEM), hydrolysed granules with pores (top right, 2500×, by ESEM), amylase-coated potato starch granules bottom left, 1000×) by ESEM and light microscopy (DAPI colour) cross-section of bacteria-filled potato starch granule (bottom right, 1450×).

The coating formation was demonstrated by applying amylose solution to the waxy maize starch granules (Fig. 11). It was also observed, using light microscopy, that partially hydrolysed potato starch granules were filled with amylose solution (Fig. 12).

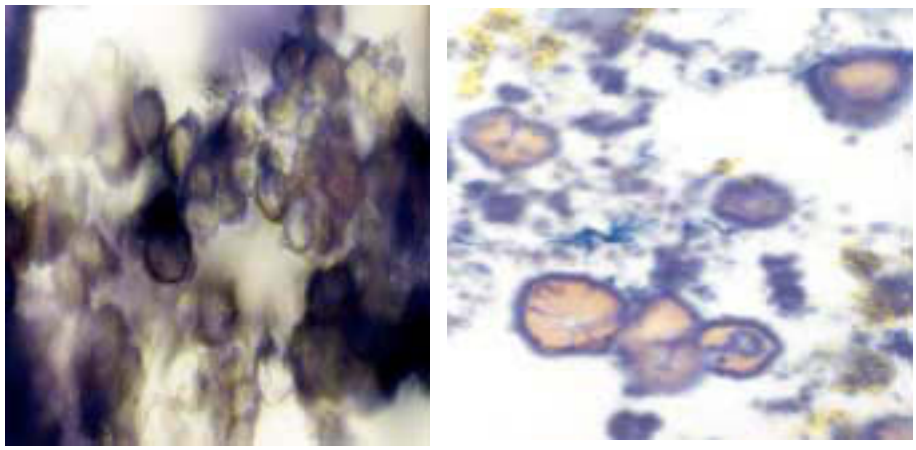

Fig. 11. Amylose-coated waxy maize starch granules (light microscopy and microscopy cross-section, iodine colour, thickness 4 μm, 725×).

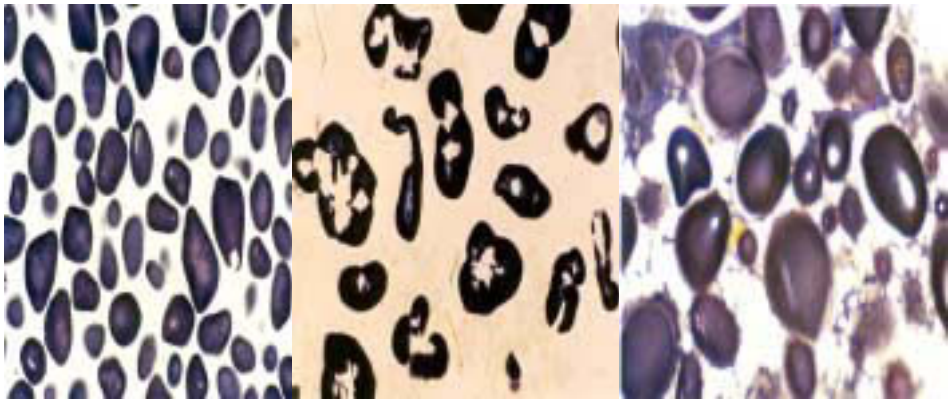

Fig. 12. Native raw potato starch granules (left), hydrolysed granules (middle) and amylase-coated hydrolysed granules (microscopy cross-sections, iodine colour, thickness 4 μm, 290×).

The stability of encapsulated bacteria stored at room temperature and humidity was at least half a year. There are no reports in the literature concerning starch as an encapsulation material for probiotic bacteria. The encapsulation technology developed in the present study may be used not only for bacteria but also for other bioactive compounds such as plant extracts or drugs, in order to stabilise them against oxygen or light, or to mask the bitter taste. The most challenging target is to achieve a controlled release system, as described by Pereswetoff-Morath (1998), who described the nasal drug delivery system by using microspheres made from starch.

4. Conclusions

Both growth conditions and granule type influenced the functional properties of starch. The barley starch granular structure was less ordered and more susceptible to amylases when grown in cold and rainy conditions. Small granules of barley starch differed from the large grains in chemical composition and heat-induced functional properties. The lipid:amylose ratio was higher, which increased the gelatinisation temperature and inhibited the amylose diffusion during heating in the water. This was demonstrated by light microscopy observations. The gel prepared from small granules had a slower retrogradation rate than the gel prepared from the large granules, possibly because of its higher lipid content.

Films prepared from amylose and amylopectin had different mechanical and solubility properties, but their glass transition temperatures were similar. Amylose produced much better films than amylopectin, especially in the presence of high amounts of glycerol (30%). Based on calorimetric glass transitions, both polymers were plasticised with water and glycerol, but water was more effective. Starch-rich and glycerol-rich phases were formed in the presence of glycerol. Fresh amylose film was not soluble or degradable in water, 50% of the material was very resistant against 2.2 M HCl, and 35% was not dissolved by α-amylase. Fresh amylopectin film was completely and rapidly dissolved in 2.2 M HCl. X-ray analysis revealed that fresh amylopectin films were amorphous. The amylose films showed B-type crystallinity structures, but the amount of crystallinity was not more than 32%. When comparing the mechanical properties with glass transition temperatures it may be concluded that the glass–rubber transition perhaps does not always describe the mechanical behaviour of amorphous polymers. Furthermore, the more stable and flexible character of the amylose film could not be explained based only on crystalline structures. It may be concluded that, based on mechanical and solubility results, the amylose film network structure differed considerably from that of amylopectin.

Freeze-drying changed the enzymatic accessibility of potato starch granules, and porous granules, for encapsulation of probiotic bacteria, could be produced using partial α-amylolysis. A simple coating technique based on water-dissolved amylose was applied, and a fine, starchy powder was produced.

5. References

Aikasalo, R. 1989. Ohran sadonmuodostus äärikesinä 1987 ja 1988. Käytännön Maamies, Vol. 8, pp. 42–43. (In Finnish)

Anon. 1976. The food industry. Industrial Uses of Starch and its Derivatives. Applied Sci. Publishers Ldt. London, Pp. 51–115.

Asaoka, M., Blanshard, J. M. V. & Rickard, J. E., 1991. Seasonal effects on the physico-chemical properties of starch from four cultivars of cassava. Starch/Stärke, Vol. 43, pp. 455–459.

Asaoka, M., Okuno, T., Sugimoto, J., Kawakami, J. & Fuwa, H. 1984. Effect of environmental temperature during development of rice plants on some properties of endosperm starch. Starch/Stärke, Vol. 36, pp. 189–193.

Asaoka, M., Okuno, K. & Fuwa, H. 1985a. Effect of environmental temperature at the milky stage on amylose content and fine structure of amylopectin of waxy and nonwaxy endosperm starches of rice (*Oryza sativa* L.). Agric. Biol. Chem., Vol. 49, pp. 373–379.

Asaoka, M., Okuno, K., Sugimoto Y. & Fuwa, H. 1985b. Developmental changes in the structure of endosperm starch of rice (*Oryza sativa* L). Agric. Biol. Chem., Vol. 49, pp. 1973–1978.

Asaoka, M., Okuno, K., Sugimoto, Y., Yano, M., Omura, T. & Fuwa, H. 1986. Characterization of endosperm starch from high-amylose mutants of rice (*Oryza sativa* L.). Starch/Stärke, Vol. 38, pp. 114–117.

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.

Bader, H. G. & Göritz, D. 1994a. Investigations on high amylose corn starch films. Part 1: Wide angle X-ray scattering (WAXS). Starch/Stärke, Vol. 46, pp. 229–232.

Bader, H. G. & Göritz, D. 1994b. Investigations on high amylose corn starch films. Part 3: stress strain behaviour. Starch/Stärke, Vol. 46, pp. 435–439.

De Baere, H. 1999. Starch policy in the European community. Starch/Stärke, Vol. 51, pp. 189–193.

Ball, S., Guan, H.-P., James, M., Myers, A., Keeling, P., Mouille, G., Buléon, A., Colonna, P. & Preiss, J. 1996. From glycogen to amylopectin: a model for the biogenesis of plant starch granule. Cell, Vol. 86, pp. 349–352.

Banks, W. & Greenwood, C. T. (eds.) 1975. Starch and its Components. Edinburgh University Press, Edinburgh. Aberdeen University Press. Pp. 1–342.

Bathgate, G. N. & Palmer, G. H. 1973. The in vivo and in vitro degradation of barley and malt starch granules. Journal of the Institute of Brewing, Vol. 79, pp. 402–406.

Bear, R. S. 1942. The significance of the "V" X-ray diffraction patterns of starches. J. Am. Chem. Soc., Vol. 64, pp. 1388–1391.

Bello-Pèrez, L. A., de Léon, Y. P., Agama-Acevedo, E. & Paredes-Lòpez, O. 1998. Isolation and partial characterization of amaranth and banana starches. Starch/Stärke, Vol. 50, pp. 409–413.

van den Berg, C. B. 1981. Water sorption equilibria and other water-starch interactions; a physico-chemical approach. Dissertation. Agricultural University Wageningen, the Netherlands. Pp. 186.

Bergthaller, W., Witt, W. & Goldau, H.-P. 1999. Potato starch technology. Starch/Stärke, Vol. 51, pp. 235–242.

Bertoft, E. & Henriksnäs, H. 1982. Initial stages in α-amylolysis of barley starch. Journal of the Institute of Brewing, 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. Journal of the Institute of Brewing, 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., Quin, 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.

Biliaderis, C. G., Page, C. M., Maurice, T. J. & Juliano, B. O. 1986. Thermal characterization of rice starches: a polymeric approach to phase transitions of granular starch. J. Agric. Food Chem., Vol. 34, pp. 6–14.

Bizot, H., Le Bail, B., Leroux, J., Davy, P. Parker, P. & Buleon, A. 1997. Calorimetric evaluation of the glass transition in hydrated, linear and branched polyanhydro glucose compounds. Carbohydrate Polymers, Vol. 32, pp. 33–50.

Blanshard J. M. 1987. Starch granule structure and function: a physicochemical approach. Crit. Rev. App. Chem., Vol. 13, pp. 16–54.

Brazel, C. S. 1999. Microencapsulation: offering solutions for the food industry. Cereal Foods World, Vol. 44, pp. 388–393.

Brown, I. L, McNaught, K. J., Ganly, R. N., Conway, P. L., Evans, A. J., Topping, D. L. & Wang, X. 1996. Probiotic compositions. WO96/08261A1.

Buléon A., Bizot, H., Delage, M. M. & Multon, J. L. 1982. Evolution of crystallinity and specific gravity of potato starch versus ad- and desorption. Starch/Stärke, Vol. 34, pp. 361–366.

Buléon, A., Colonna, P., Planchot, V. & Ball, S. 1998a. Starch granules: structure and biosynthesis. Int. J. Biol. Macromol., Vol. 23, pp. 85–112.

Buléon, A., Gerard, C., Riekel, C., Vuong, R. & Chanzy, H. 1998b. Details of the crystalline ultrastructure of C-starch granules revealed by synchroton microfocus mapping. Macromolecules, Vol. 31, pp. 6605–6610.

Cairns, P., Sun, L., Morris, V. J. & Ring, S. G. 1995. Physicochemical studies using amylose as an in vitro model for resistant starch. J. Cereal Sci., Vol. 21, pp. 37–47.

Cassidy, A., Bingham, S. A. & Gummings, J. H. 1994. Starch intake and colorectal cancer risk: an international comparison. British J. of Cancer, Vol. 69, pp. 119–125.

Che Man, Y. B., Irwandi, J. & Abdullah, W. J. W. 1999. Effect of different types of maltodextrin and drying methods on physico-chemical and sensory properties of encapsulated durian flavour. J. Sci. of Food and Agriculture, Vol. 79, pp. 1075–1080.

Cleven, R., van den Berg, C. & van der Plas, L. 1978. Crystal structure of Che hydrated potato starch. Starch/Stärke, Vol. 30, pp. 223–228.

Collins, K. 2000. Probiotics for adults. Functional Foods for EU Health in 2000. 4th Workshop Demonstration of the Nutritional Functionality of Probiotic Foods. FAIR CT96-1028. VTT Technical Research Centre of Finland. Espoo. VTT Symposium 198. Pp. 33–34.

Colonna, P., Buleon, A. & Mercier, C. 1987. Physically modified starches. Crit. Rev. Appl. Chem., Vol. 13, pp. 79–114.

Colonna, P., Buleon, A., Lemaguer, M. & Mercier, C. 1982. *Pisum Sativum* and *Vicia Faba* carbohydrates: Part IV - granular structure of wrinkled pea starch. Carbohydr. Polym., Vol. 2, pp. 43–59.

Crittenden, R., Laitila, A., Forssell, P., Mättö, J., Saarela, M., Mattila-Sandholm, T. & Myllärinen, P. 2001. Adhesion of bifidobacteria to granular starch and implications in probiotic technologies. Applied and Environmental Microbiology, Vol. 67, pp. 3469–3475.

Decker, P. & Höller, H. 1962. Ein Zeitgradientenverfahren zur Fraktionierung von körnigen Materialen, insbesondere Ionentauscherharzen, durch Sedimentation. J. Chromatogr., Vol. 7, pp. 392–399.

Della Valle, G., Buleon, A., Carreau, P. J., Lavoie, P. A. & Vergnes, B. 1998. Relationship between structure and viscoelastic behavior of plasticized starch. J. Rheol., Vol. 42, pp. 507–525.

Dziezak, J. D. 1988. Microencapsulation and encapsulated ingredients. Food Technology, April, pp. 136–148, 151–153.

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.

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. 7, pp. 231–235.

Englyst, H. N., Kingman, S. M. & Gummings, J. H. 1992. Classification and measurement of nutritionally important starch fractions. European J. of Clinical Nutrition, Vol. 46, (Suppl. 2), pp. S33–S50.

Evers, A. D., Gough, B. M. & Pybus, J. N. 1971. Scanning electron microscopy of wheat starch. IV. Digestion of large granules by glucoamylase of fungal (*Aspergillus niger*) origin. Starch/Stärke, Vol. 23, pp. 16–18.

Evers, A. D., Greenwood, C. T., Muir, D. D. & Venables, C. 1974. Biosynthesis of starch granules. 8. Comparison of properties of small and large granules in mature cereal starches. Starch/Stärke, Vol. 26, pp. 42–46.

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

Forssell, P., Hulleman, S. H. D., Myllärinen, P. J., Moates, G. K. & Parker, R. 1999. Ageing of rubbery thermoplastic barley and oat starches. Carbohydrate Polymers, Vol. 39, pp. 43–51.

Forssell, P., Lahtinen, R., Lahelin, M. & Myllärinen, P. 2002. Oxygen permeability of amylose and amylopectin films. Carbohydrate Polymers, Vol. 27, pp. 125–129.

Forssell, P., Mikkilä, J., Moates, G. & Parker, R. 1997. Phase and glass transition behaviour of concentrated barley starch-glycerol-water mixtures, a model for thermoplastic starch. Carbohydrates Polymers, Vol. 34, pp. 275–282.

French, D. 1984. Organization of starch granules. In: Whistler, R. L., BeMiller, J. N. & Paschall, E. F. (eds.) Second edition. Starch Chemistry and Technology. Academic Press, New York. Pp. 183–247.

Fuller, R. 1989. Probiotics in man and animals. J. Appl. Bacteriol., Vol. 66, pp. 365–378.

Gaudin, S., Lourdin, D., Le Botlan, D., Ilari, J. L. & Colonna, P. 1999. Plasticisation and mobility in starch-sorbitol films. J. Cereal Science, Vol. 29, pp. 273–284.

Ghiena, C., Schulz, M. & Schnabl, H. 1993. Starch degradation and distribution of the starch-degrading enzymes in *Vicia faba* leaves. Plant Physiol., Vol. 101, pp. 73–79.

Gudmundsson, M. & Eliasson, A.-C. 1989. Some physico-chemical properties of oat starches extracted from varieties with different oil content. Acta Agric. Scand., Vol. 39, pp. 101–111.

Hawker, J. S. & Jenner, C. F. 1993. High temperature affects the activity of enzymes in the committed pathway of starch synthesis in developing wheat endosperm. Aust. J. Plant Physiol., Vol. 20, pp. 197–209.

Hulleman, S. H. D., Janssen, F. H. P. & Feil, H. 1998. The role of water during plasticization of native starches. Polymer, Vol. 39, pp. 2043–2048.

Imberty, A., Chanzy, H., Perez, S., Buléon, A. & Tran, V. 1988. The double helical nature of the crystalline part of A-starch. J. Mol. Biol., Vol. 201, pp. 365–378.

Imberty, A. & Perez, S. 1988. A revisit to the three-dimensional structure of B-starch. Biopolymers, Vol. 27, pp. 308–325.

Isolauri, E. & Salminen, S. 2000. Probiotics for children-the Probdemo view. Functional Foods for EU Health in 2000. 4th Workshop Demonstration of the Nutritional Functionality of Probiotic Foods. FAIR CT96-1028. VTT Technical Research Centre of Finland. Espoo. VTT Symposium 198. Pp. 31–32.

Jane, J.-L., Kasemsuwan, T., Leas, S., Zobel, H. & Robyt, J. F. 1994. Anthology of starch granule morphology by scanning electron microscopy. Starch/Stärke, Vol. 46, pp. 121–129.

Jane, J. & Shen, J. J. 1993. Internal structure of the potato starch granule revealed by chemical gelatinization. Carbohydrate Research, Vol. 247, pp. 279–290.

Jenner, C. F. 1991. Effects of exposure of wheat ears to high temperature on dry matter accumulation and carbohydrate metabolism in the grain of two cultivars. I. Immediate responses. Aust. J. Plant Physiol., Vol. 18, pp. 165–177.

Kalichevsky, M. T., Jaroszkiewicz, E. M., Ablett, S., Blanshard, J. M. V. & Lillford, P. J. 1992. The glass transition of amylopectin measured by DSC, DMTA and NMR. Carbohydrate Polymers, Vol. 18, pp. 77–88.

Kang, M. Y., Sugimoto, Y., Kato, I., Sakamoto, S. & Fuwa, H. 1985. Some properties of large and small starch granules of barley (*Hordeum vulgare* L) endosperm. Agricultural and Biological Chemistry, Vol. 49, pp. 1291–1297.

Kano, Y. 1977. A comparison of the amylose content of large and small starch granules from barley and malt. Bulletin of Brewing Sci., Vol. 23, pp. 1–8.

Karkalas, J. 1985. An improved enzymatic method for the determination of native and modified starch. J. Sci. Food Agric., Vol. 36, pp. 1019–1027.

Karlsson, R., Olered, R. & Eliasson, A.-C. 1983. Changes in starch granule size distribution and starch gelatinization properties during development and maturation of wheat, barley and rye. Starch/Stärke, Vol. 35, pp. 335–340.

Keeling, P. L., Bacon, P. J. & Holt, D. C. 1993. Elevated temperature reduces starch deposition in wheat endosperm by reducing the activity of soluble starch synthase. Planta, Vol. 191, pp. 342–348.

Keeling, P. L., Wood, J. R., Huw Tyson, R. & Bridges, I. G. 1988. Starch biosynthesis in developing wheat grain. Plant Physiol., Vol. 87, pp. 311–319.

Kleessen, B., Stoof, G., Proll, J., Schmiedl, D., Noack, J. & Blaut, M. 1997. Feeding resistant starch affects fecal and cecal microflora and short chain fatty acids in rats. J. Animal Sci., Vol. 75, pp. 2453–2462.

Kobayashi, S., Miwa, S. & Tsuzuki, W. 1993. Method of preparing modified starch granules. EU0539910A1.

Kulp, K. & Lorenz, K. 1981. Heat-moisture treatment of starches. I. Physicochemical properties. Cereal Chem., Vol. 58, pp. 46–48.

Langlois, D. P & Wagoner, J. A. 1967. Production and use of amylose. In: Whistler, R. L. & Paschall, E. F. (eds.) Starch: Chemistry and Technology, Vol. II. Academic Press. New York. Pp. 451–496.

Lauro, M. 2001. α-Amylolysis of barley starch. Dissertation. VTT Technical Research Centre of Finland, Espoo. VTT Publications 433, Pp. 45.

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

Lauro, M., Suortti, T., Autio, K. & Poutanen, K. 1993. Accessibility of barley starch granules to alpha-amylase during different phases of gelatinization. J. Cereal Sci., Vol. 17, pp. 125–136.

Leach, H. W., McCowen, L. D. & Schoch, T. J. 1959. Structure of the starch granule. I. Swelling and solubility patterns of various starches. Cereal. Chem., Vol. 36, pp. 534–544.

Le Blay, G., Michel, C., Blottière, H. M. & Cherbut, C. 1999. Enhancement of butyrate production in the rat caecocolonic tract by long-term ingestion of resistant potato starch. British J. of Nutrition, Vol. 82, pp. 419–426.

Leloup, V., Colonna, P. & Buleon, A. 1991. Influence of amylose-amylopectin ratio on gel properties. J. Cereal Sci., Vol. 13, pp. 1–13.

Leloup, V. M., Colonna, P., Ring, S. G. Roberts, K. & Wells, B. 1992. Microstructure of amylose gels. Carbohydr. Polym., Vol. 18, pp. 189–197.

Levine, H. & Slade, L. 1986. A polymer physico-chemical approach to the study of commercial starch hydrolysis products (SHPs). Carbohydrate Polymer. Vol. 6, pp. 213–244.

Lineback, D. R. 1984. The starch granule organisation and properties. Bakers Digest, Vol. 13, pp. 16–21.

Lourdin, D., Coignard, L., Bizot, H. & Colonna, P. 1997. Influence of equilibrium relative humidity and plasticiser concentration on the water content and glass transition of starch materials. Polymer, Vol. 38, pp. 5401–5406.

Lourdin, D., Della Valle, G. & Colonna. P. 1995. Influence of amylose content on starch films and foams. Carbohydrate Polymers, Vol. 27, pp. 261–270.

Lourdin, D., Ring, S. G. & Colonna, P. 1998. Study of plasticizer-oligomer and plasticizer-polymer interactions by dielectric analysis: maltose-glycerol and amylose-glycerol-water systems. Carbohydrate Research, Vol. 306, pp. 551–558.

Macfarlane, G. T. & Gummings, J. H. 1991. The colonic flora, fermentation and large bowel digestive function. In: Phillips, S. F., Pemberton, J. H. & Shorter, R. G. (eds.) The Large Intestine: Physiology, Pathophysiology, and Disease. New York Foundation, Raven Press. Pp. 51–92.

MacGregor, A. W. 1980. Action of malt α-amylases on barley starch granules. MBAA Technical Quarterly, Vol. 17, pp. 215.

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

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

MacLeod, L. C. & Duffus, C. M. 1988a. Temperature effects on starch granules in developing barley grains. J. Cereal. Sci., Vol. 8, pp. 29–37.

MacLeod, L. C. & Duffus, C. M. 1988b. Reduced starch content and sucrose synthase activity in developing endosperm of barley plants grown at elevated temperatures. Aust. J. Plant Physiol., Vol. 15, pp. 367–375.

Manelius, R. 2000. Enzymatic and acidic hydrolysis of native and modified starch granules. Dissertation. Department of Biochemistry and Pharmacy, Åbo Akademi University, Åbo, Finland. Pp. 51.

Martin, C. & Smith, A. M. 1995. Starch biosynthesis. The Plant Cell, Vol. 7, pp. 971–985.

Matheson, N. 1996. The chemical structure of amylose and amylopectin fractions of starch from tobacco leaves during development and diurnally-nocturnally. Carbohydrate Research. Vol. 282, pp. 247–262.

Mattila-Sandholm, T., Blum, S., Collins, K., Crittenden, R., de Vos, W., Dunne, C., Fondén, R., Grenov, B., Isolauri, E., Kiely, B., Marteau, P., Morelli, L., Ouwenhand, A., Reniero, R., Saarela, M., Salminen, S., Saxelin, M., Schiffrin, E., Shanahan, F., Vaughan, E. & von Wright, A. 1999. Probiotics: towards demonstrating efficacy. Trends in Food Science & Technology, Vol. 10, pp. 393–399.

McDonald, A. M. L. & Stark, J. R. 1988. A critical examination of procedures for the isolation of barley starch. J. Inst. Brew., Vol. 94, pp. 125–132.

Moates, G. K., Noel, T. R., Parker, R. & Ring, S. G. 2001. Dynamic mechanical and dielectric characterisation of amylose-glycerol films. Carbohydrate Polymers, Vol. 44, pp. 247–253.

Moore, C. O., Tuschhoff, J. V., Hastings, C. W. & Schanefelt, R. V. 1984. Applications of starches in foods. In: Whistler, R. L., BeMiller, J. N. & Paschall, E. F. (eds.). Starch: Chemistry and Technology. Second edition. Academic Press, Inc. Orlando, San Diego, New York, London, Toronto, Montreal, Sydney, Tokyo. Pp. 575–591.

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

Morrison, W. R., Tester, R. F., Snape, C. E., Law, R. & Gidley, M. J. 1993. 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. & 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. 1995. Starch lipids and how they relate to starch granule structure and functionality. Cereal Foods World, Vol. 40, pp. 437–446.

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.

Newton, J. M. 1996. In vivo studies of amylose coated microsphres for drug delivery to the colon. Controlled Release, Vol. 40, pp. 123–131.

Oates, C. G. 1997. Towards an understanding of starch granule structure and hydrolysis. Trends in Food Science & Technology, Vol. 8, pp. 375–382.

Orford, P. D., Parker, R., Ring, S. G. & Smith, A. C. 1989. Effect of water as a diluent on the glass transition behaviour of malto-oligosaccharides, amylose and amylopectin. Int. J. Biol. Macromol., Vol. 11, pp. 91–96.

Osman, E. M. 1967. Starch in food industry. In: Whistler, R. L. & Paschall, E. F. (eds.). Starch: Chemistry and Technology, Vol. II. Academic Press, Inc. Pp. 163–215.

Palmer, G. H. 1989. Cereals in malting and brewing. In: Palmer, G. H. (ed.). Cereal Science and Technology. Aberdeen Scotland, Aberdeen University Press. Pp. 61–242.

Parkkonen, T., Härkönen, H. & Autio, K. 1994. Effect of baking on the microstructure of rye cell walls and protein. Cereal Chem., Vol. 71, pp. 58–63.

Pereswetoff-Morath, L. 1998. Microspheres as nasal drug delivery systems. Advanced Drug Del. Rev., Vol. 29, pp. 185–194.

Petersen, N. B. 1975. Edible starches and starch-derived syrups. Noyes Data Corporation. Park Ridge, New Jersey, London, England. Pp. 1–426.

Rankin, J. C., Wolff, I. A., Davis, H. A. & Rist, C. E. 1958. Permeability of amylose film to moisture vapor, selected organic vapors, and the common gases. Industrial and Engineering Chemistry, Vol. 3, pp. 120–123.

Rapaille, A. 1986. Starch applications in the food industry. CPC Europe Industrial Products Research and Development. Center Belgium. 28.5.1986.

Richter, M., Augustat, S. & Schierbaum, F. 1968. Ausgewählte Methoden der Stärkechemie. Isolierung, Charakterisierung und Analytik von Stärke-polysacchariden. Wissenschaftliche Verlagsgesellschaft MBH Stuttgart. Pp. 1–208.

Rindlav, Å., Hulleman, H. D. & Gatenholm, P. 1997. Formation of starch films with varying crystallinity. Carbohydr. Polym., Vol. 34, pp. 25–30.

Rindlav-Westling, Å., Stading, M., Hermansson, A.-M. & Gatenholm, P. 1998. Structure, mechanical and barrier properties of amylose and amylopectin films. Carbohydr. Polym., Vol. 36, pp. 217–224.

Ring, S. 1995. Stiff tests for designer starches. Chemistry in Britain, April, pp. 303–307.

Ring, S., Archer, D., Allwood, M. & Newton, J. 1994. Delayed release formulations. US5294448.

Ring, S. G., Jennifer, M. G., Whittam, M., Orford, P. & Johnson, I. T. 1988. Resistant. starch: its chemical form in foodstuffs and effect on digestibility *in vitro*. Food Chemistry, Vol. 28, pp. 97–109.

Roos, Y. 1995. Phase Transitions in Foods. In: Taylor, S. L. (ed.). Academic Press, San Diego, New York, Boston, London, Sydney, Tokyo, Toronto. Pp. 1–360.

Roulet, P. H., Raemy, A. and Wuersch, P. 1987. Retrogradation kinetics of concentrated gelatinized wheat starch gels or powders; a comparative study by rigidity modulus, differential scanning calorimetry (DSC) and X-ray diffraction. Food Hydrocolloids, Vol. 1, pp. 575–578.

Russell, P. L. 1983. A kinetic study of bread staling by differential scanning calorimetry and compressibility measurements. The effect of added monoglyceride. J. Cereal Sci., Vol. 1. pp. 297–303.

Röper, H. 1996. Starch: present use and future utilization. In: vanBekkum, H., Röper, H. & Voragen, F. (eds.). Carbohydrates as Organic Raw Materials III. CRF, Carbohydrate Research Foundation, Wageningen, The Netherlands. Pp. 17–35.

Sair, L. 1967. Heat-moisture treatment of starch. Cereal Chem., Vol. 44, pp. 8–26.

Sarko, A. & Zugenmaier, P. 1980. Crystal structures of amylose and its derivatives. ACS Symp. Series, American Chem. Soc. Washington DC, Vol. 141, pp. 459–482.

Savin, R., Stone, P. J., Nicolas, M. E. & Wardlaw, I. F. 1997. Grain growth and malting quality of barley. I. Effects of heat stress and moderately high temperature. Aust. J. Agric. Res., Vol. 48, pp. 615–624.

Schenz, T. W. 1995. Glass transitions and product stability-an overview. Food Hydrocolloids, Vol. 9, pp. 307–315.

Schoch, T. J. 1985. Carbohydrates: Starch. In: Pomeranz, Y. (ed.). Functional Properties of Food Components. Orlando FC, Academic Press. Pp. 25–90.

Schoch, T. J. & Maywald, E. C. 1956. Microscopic examination of modified starches. Analytical Chemistry, Vol. 28, pp. 382–387.

Schulman, A. H., Tomooka, S., Suzuki, A., Myllärinen, P. & Hizukuri, S. 1995. Structural analysis of starch from normal and shx (shrunken endosperm) barley (*Hordeum vulgare* L.). Carbohydrate Research, Vol. 275, pp. 361–369.

Schwall, G. P., Safford, R., Westcott, R. J., Jeffcoat, R., Tayal, A., Shi, Y.-C., Gidley, M. J. & Jobling, S. A. 2000. Production of very-high-amylose potato starch by inhibition of SBA A and B. Nature Biotechnology, Vol. 19, pp. 551–554.

Schwar, C. 1958. Stärke. A. Ziemsen Verlag. Wittenberg Lutherstadt. Pp. 1–104.

Seidemann, J. 1966. Stärke-Atlas. Grundlagen der Stärke-Mikroskopie und Bescbreibung der wichtigsten Stärkearten. Seidemann, J. (ed.). Paul Parey in Berlin und Hamburg. Pp. 1–360.

Senti, F. 1967. High amylose corn starch: its production, properties, and uses. In: Whistler, R. L. & Paschall, C. F. (eds.). Starch: Chemistry and Technology, Vol. II. Industrial Aspects. Academic Press, N.Y. Pp. 499–522.

Shamekh, S., Myllärinen, P., Poutanen, K. & Forssell, P. 2002. Film formation of potato starch hydrolysates. Starch/Stärke, Vol. 54, pp. 20–24.

Shanahan, F. 2000. Therapeutic manipulation of gut flora. Science, Vol. 289, pp. 1311–1312.

Shannon, J. C. & Garwood, D. L. 1984. Genetics and physiology of starch development. In: Whistler, R. L., BeMiller, J. N. & Paschall, E. F. (eds.) Starch: Chemistry and Technology. Academic Press, Inc. Orlando, San Diego, New York, London, Toronto, Montreal, Sydney, Tokyo. Pp. 25–86.

Shi, Y.-C., Seib, P. & Bernardin, J. E. 1994. Effects of temperature during grain-filling on starches from six wheat cultivars. Cereal Chem., Vol. 71, pp. 369–383.

Silvi, S., Rumney, C. J., Cresci, A. & Rowland, I. R. 1999. Resistant starch modifies gut microflora and microbial metabolism in human flora-associated rats inoculated with faeces from Italian and UK donors. J. Applied Microbiology, Vol. 86, pp. 521–530.

van Soest, J. J. G. & Essers, P. 1997. Influence of amylose-amylopectin ratio on properties of extruded starch plastic sheets. J. M. S. - Pure Appl. Chem., A34 (9), pp. 1665–1689.

van Soest, J. J. G., Hulleman, S. H. D., de Wit, D. & Vliegenthart, J. F. G. 1996a. Changes in the mechanical properties of thermoplastic potato starch in relation with changes in B-type crystallinity. Carbohydrate Polymers, Vol. 29, pp. 225–232.

van Soest, J. J. G., de Wit, D. & Vliegenthart, J. F. G. 1996b. Mechanical properties of thermoplastic waxy maize starch. J. Applied Polymer Sci., Vol. 61, pp. 1927–1937.

Steidler, L., Hans, W., Schotte, L., Neirynck, S., Obermeier, F., Falk, W., Fiers, W. & Remaut, E. 2000. Treatment of murine colitis by *Lactococcus lactis* secreting interleukin-10. Science, Vol. 289, pp. 1352–1355.

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

Tester, R. F. & Morrison, W. R. 1990b. Swelling and gelatinization of cereal starches. II. Waxy rice starches. Cereal Chem., Vol. 67, pp. 558–563.

Tester, R. F. & Morrison, W. R. 1992. Swelling and gelatinization of cereal starches. III. Some properties of waxy and normal nonwaxy barley starches. Cereal Chem., Vol. 69, pp. 654–658.

Tester, R. F., South, J. B., Morrison, W. R. & Ellis, R. P. 1991. The effects of ambient temperature during the grainfilling period on the composition and properties of starch from four barley genotypes. J. Cereal Sci., Vol. 13, pp. 113–127.

Tester, R. F., Morrison, W. R., Ellis, R. H., Piggott, J. R., Batts, G. R., Wheeler, T. R., Morrison, J. I. L., Hadley, P. & Ledward, D. A. 1995. Effects of elevated growth temperature and carbon dioxide levels on some physicochemical properties of wheat starch. J. Cereal Sci., Vol. 22, pp. 63–71.

Thomas, D. J. & Atwell, W. A. (eds.) 1999. Starches. Eagan Press, St. Paul, Minnesota, USA. Pp. 1–94.

Thompson, D. B. 2000. Strategies for the manufacture of resistant starch. Trends in Food Science & Technology, Vol. 11, pp. 245–253.

Trubiano, P. C. & Kasica, J. J. 1986. Compressible starches as binders for tablets or capsules. US Patent B14551177.

Vandamme, Th. F., Lenourry, A., Charrueau, C. & Chaumeil, J.-C. 2002. The use of polysaccharides to target drugs to the colon. Carbohydrate Polymers, Vol. 48, pp. 219–231.

Vasanthan, T. & Bhatty, R. S. 1996. Physicochemical properties of small- and large-granule starches of waxy, regular, and high-amylose barleys. Cereal Chemistry, Vol. 73, pp. 199–207.

Vikman, M., Hulleman, H. D., Van Der Zee, M., Myllärinen, P. & Feil, H. 1999. Morphology and enzymatic degradation of thermoplastic starch-polycaprolactone blends. J. Applied Polymer Sci., Vol. 74, pp. 2594–2604.

Visser, R. G. F. & Jacobsen, E. 1993. Towards modifying plants for altered starch content and composition. Trends in Biotechnology, Vol. 11, pp. 63–68.

Wakelin J. H., Virgin H. S. & Crystal E. 1959. Development and comparison of two X-ray method for determining the crystallinity of cotton cellulose. J. Appl. Phys., Vol. 30, pp. 1654–1662.

Walker, J. T. & Merritt, N. R. 1969. Genetic control of abnormal starch granules and high amylose content in a mutant of glacier barley. Nature 221, pp. 482–483.

Whistler, R. L. 1984. History and future expectation of starch use. In: Whistler, R. L., BeMiller, J. N. & Paschall, E. F. (eds.). Starch: Chemistry and Technology. Second Edition. Academic Press, Inc. Orlando, San Diego, New York, London, Toronto, Montreal, Sydney, Tokyo. Pp. 1–9.

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

Whistler, R. L. 1997. Porous particle aggregate and method therefor. US Patent 5670490.

Whistler, R. L & Paschall, E. F. (eds.) 1965. Starch: Chemistry and Technology. Vol. 1. Academic Press, New York and London. Pp. 1–579.

Whistler, R. L., BeMiller, J. N. & Paschall, E. F. 1984. Starch: Chemistry and Technology. Second Edition. Whistler, R. L., BeMiller, J. N. & Paschall, E. F. (eds.). Academic press, Inc. Orlando, San Diego, New York, London, Toronto, Montreal, Sydney, Tokyo. Pp. 1–718.

Wolff, I. A., Davis, H. A., Cluskey, J. E., Gundrum, L. J. & Rist, C. E. 1951. Preparation of films from amylose. Industrial and Engineering Chemistry, Vol. 43, pp. 915–919.

Wurzburg, O. B. (ed.) 1986. Modified Starches: Properties and Uses. CRC Press, Inc. Boca Raton, Florida. Pp. 1–277.

Zeleznak, K. J. & Hoseney, R. C. 1987. The glass transition in starch. Cereal Chem., Vol. 64, pp. 121–124.

Zobel, H. F. 1984. Gelatinization of starch and mechanical properties of starch pastes. In: Whistler, R. L., BeMiller, J. N. & Paschall, E. F. (eds.). Starch: Chemistry and Technology. Second Edition. Academic Press, Inc. Orlando, San Diego, New York, London, Toronto, Montreal, Sydney, Tokyo. Pp. 285–309.

Zobel, H. F. 1988a. Starch crystal transformations and their industrial importances. Starch/Stärke, Vol. 40, pp. 1–7.

Zobel, H. F. 1988b. Molecules to granules: a comprehensive starch review. Satrch/Stärke, Vol. 40, pp. 44–50.

Zuckerman, L. 2000. Treasure of the Andes. Peru and Europe, 1550-1650. In: Zuckerman, L. The Potato. London, Pan Books. Pp. 3–15.

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Title

Starches – from granules to novel applications

Abstract

In this study, the effects of growth temperature, starch composition and granule size on the gelatinisation and solubility properties of barley starch were studied. The mechanical properties and structures of amylose and amylopectin film, with and without added glycerol, were investigated. A novel starch-based microencapsulation method was developed for probiotic bacteria by combining native starch granules with amylose coating.

The suboptimal growth conditions influenced the properties of two-rowed malting barley cultivars "Kustaa" and "Kymppi" as well as Kustaa′s large A-granules and small B-granules. The gelatinisation temperature was lower and the rate of retrogradation less in starch grown in cold and rainy conditions. Swelling and solubility were greater and the starch was more susceptible to amylases. The small B-granules had a higher lipid:amylose ratio than the large A-granules. The B-granules also had a higher dissociation enthalpy of the amylose–lipid complex than the A-granules. Furthermore, the gelatinisation temperature was 4°C higher for B-granules than for A-granules. During heating in excess water up to 90°C amylopectin was mainly leached from the B-granules, whereas amylose was solubilised from the A-granules. At 95°C, B-granules were completely solubilised but A-granules remained partially particulate.

Amylose produced good quality films from water solutions in the presence of glycerol. Film formation was successful even at a 70% glycerol content, whereas films could not be prepared from amylopectin above a 30% glycerol content. Based on calorimetric glass transition (Tg) analysis, pure glycerol was observed to decrease the Tg. However, water had a stronger plasticisation effect. No difference between the Tgs of two starch polymers could be observed. The Tg was at room temperature at a water level of 21%. Phase separation to starch-rich and starch-poor regions occurred in glycerol plasticised systems. Fresh amylose films with 0–30% glycerol at an RH of 0–91% had B-type crystallinity at a level of 6–32%, depending on the amount of water and glycerol. No changes were observed in the crystallinity of the amylose films during 2 months of storage. The fresh amylopectin films were amorphous. After a storage period of 2 months at RH 91%, the amylopectin film with 30% glycerol showed a crystalline structure but all other amylopectin films remained amorphous after ageing. Crystallisation occurred in the rubbery phase of the system. Amylose film was not fragmented or dissolved in water, and part of its structure was resistant to amylases and acid.

Freeze-drying of native potato starch made the granules accessible to α-amylase, and enzymatic hydrolysis appeared to occur from the inside out, producing porous granules. The porous material was used as a bacteria carrier in a fermentation process. Dissolved high amylose maize starch solution was added after the growth period. Finally, the product was freeze-dried to produce powder.

Keywords

starch, amylose, amylopectin, granule, structure, gelatinisation, film, crystallisation, x-ray analysis, glass transition, microencapsulation

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