

Properties of Starch and Cell Wall Components and their Effects on Processing

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Introduction

Starch is the major component in all cereals. It is the energy source as such in food and feed applications and in the hydrolysed form for microbes in fermentation processes. High starch content is the universal target in breeding programmes both for feed, starch and malting barleys. Starch properties of different cereals, such as granule size, gelatinisation and recrystallisation, vary and cause differences in their processing behaviour. The amount of proteins in different cereals are about equal but the composition and quality of the proteins are characteristic of each species. Cell walls are the third major group of components. They are mainly composed of β -glucans and pentosans, the ratio of which varies depending on the cereal. These compounds have a marked role both in baking and brewing processes. Lipids form a minor part of cereals and are mainly located in the embryo but partly bound to starch as amylose-lipid complexes. This presentation focuses on the role of starch and cell wall components in brewing. Synergism between brewing and baking research and possibilities to transfer knowledge and analysis techniques will be discussed.

Comparison of brewing and baking processes

The major targets in malting and brewing are to get maximum extract yield, enough nutrients for yeast growth, fermentable sugars for alcohol production and a balanced combination of high molecular weight compounds to have a beautiful and stable foam and smooth mouthfeel in beer and to avoid haze formation during storage. Particularly the latter beer quality targets are still difficult to define and are partly contradictory. The aim of malting is to induce or activate enzymes that during germination degrade cell wall components and proteins and release starch granules from the endosperm matrix. Starch remains nearly intact in malting because ungelatinised starch is not susceptible to enzyme attack. Wort and beer filtration difficulties are the most frequently encountered beer processing problems. They are often caused by insufficient hydrolysis of cell wall components and by proteins that may form gels and retain water in the mash cake.

The target in breadmaking is to form a viscoelastic dough by mixing, molding and sheeting a mixture made of water and flour and to transform the dough into an elastic bread by fermentation and baking. In wheat baking the ability to retain gas is primarily associated with gluten, which forms the continuous, immiscible aqueous phase, whereas in rye baking the gas retention is attributable to the high viscosity of soluble arabinoxylans (Chen and Hosney 1995). Starch has a minor effect on the structural properties of bread, although damaged starch granules may absorb more water leading to a weak loaf and sticky crumb. The extent of starch gelatinisation affects the rate of digestion and storage behaviour. Particularly recrystallization of the amylopectin fraction is believed to be one of the major reasons for bread staling during storage.

The targets in these two applications of cereals are totally opposite. The brewer wants to gelatinize and hydrolyse starch, to avoid gels and to improve the drainage of insoluble material. The baker wants to have a gel-like structure to retain gas bubbles and water in the dough and to have limited gelatinisation of starch to improve the functionality of baked products. The reactions between cereal components leading either to the wanted behaviour in the dough or to problems in wort separation are rather similar, which means that baking and brewing researchers may benefit from each others' experiences, knowledge and monitoring methods.

Degradation of starch in brewing

β -glucans and their effects on processing problems have been extensively studied in brewing. The major carbohydrate, i.e. starch has been considered an easy component that at least in all-malt brewing does not cause problems. Due to increasing demands set by process automation and process economy it has been necessary to reconsider this assumption. In general, malt extract yield depends on protein content. When protein content increases by a percentage unit, the extract yield decreases by about 0.8 % (Schildbach 1974). This does not always apply and thus some other factors must also affect malt extract. Instead of talking only about the total extract yield of raw materials, brewers nowadays emphasise the amount of fermentable extract, which is directly linked to the potential alcohol yield. Factors affecting the amount of fermentable extract are the amount, availability and state of starch and the spectrum of amylolytic enzymes available under hydrolysis conditions. We have studied modification of starch during malting and the effects of quality characteristics such as gelatinisation of small and large granules and amylose-lipid complexes on the degradation of starch and on the release of fermentable extract in mashing.

Modification of starch in malting

Samples were taken during malting and starch was purified and analysed. The aim of the study was to detect changes in starch structure and evaluate their effects on hydrolysis in mashing. Total amount of starch was determined by an enzymatic method (Megazyme), the ratio of small and large starch granules by Coulter Multisizer Analyzer, gelatinisation curves by Differential Scanning Calorimetry (DSC), total and apparent amylose contents by the iodine method (Morrison and Laignelet, 1983) and lipid content based on the amount of phosphorus (Morrison, 1964).

The changes are summarized in Table 1. Total starch content decreased about 5 % in the course of malting. The proportion of small starch granules based on volume decreased by about 5 % but no significant differences were found in gelatinisation temperatures. Total amylose content steadily increased during malting indicating that amylopectin was degraded to some extent. The amount of amylose-lipid complexes slightly increased in the beginning of germination but later on the lipid content decreased and the difference between barley and malt was negligible. This study showed that the changes that occur in starch during malting do not deteriorate its hydrolysis performance.

Amylolytic enzymes in mashing

Enzymes taking part in starch hydrolysis vary in their thermostability. β -Amylase, which is mainly responsible for maltose production, is rather thermolabile and is inactivated close to the typical gelatinisation temperatures of barley starch. Limit dextrinase which hydrolyses α -1,6-linkages of amylopectin is somewhat more thermostable. It is readily inactivated at

temperatures above 65 °C. α -Amylase is the most thermostable and retains the major part of its activity still at 70 °C. Activities of these enzymes in isothermal mashings are shown in Figure 1 (Stenholm et al. 1996, Stenholm 1997). The temperature programme in mashing is normally designed to favour both β - and α -amylase activities and includes a rest at temperatures around 62 °C to promote formation of fermentable sugars and another rest at about 68 °C to maximise extract yield and solubilisation of starch. Hydrolysis of amylopectin yields a mixture of branched dextrans in addition to fermentable sugars. Limit dextrinase is required to convert these to linear maltosaccharides which can then be degraded to smaller sugars.

Table 1. Summary of the changes in starch quality during malting.

Total starch content	↓
Proportion of small starch granules by volume	↓
Gelatinization, temperature range and peak temperature	±
Total amylose content	↑
Amylose-lipid complexes	±

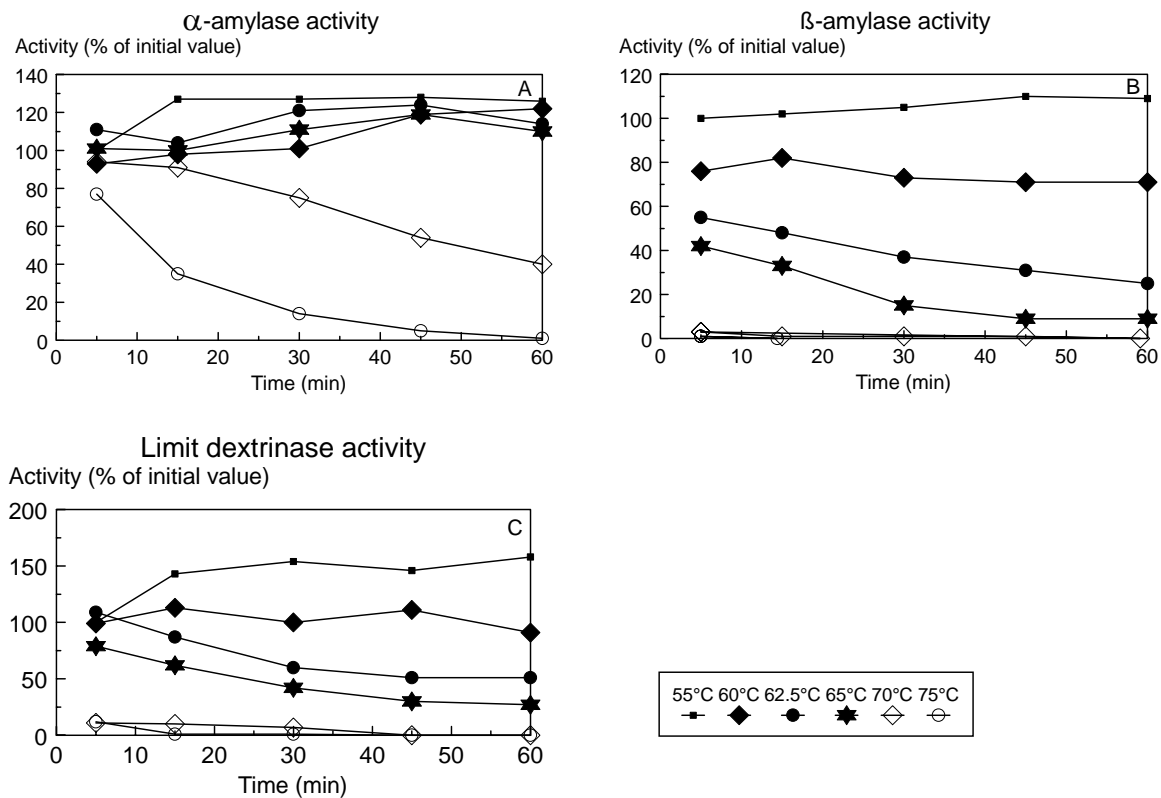


Figure 1. Thermostability of α -amylase, β -amylase and limit dextrinase in isothermal mashings.

The so-called High Gravity Mashing procedure (48 °C for 30 min, 63 °C for 30 min, 72 °C for 30 min and 80 °C for 10 min, temperature increase rate 2 °C/min) was developed by VTT to mimic brewery practice and to study enzymatic degradation of malt components in laboratory scale (Sjöholm et al. 1994). β -Amylase is very rapidly inactivated when the mashing

temperature reaches 63 °C. If starch is not sufficiently released at this temperature from the endosperm matrix and gelatinized, the fermentability target may not be reached. Although β -amylase is responsible for the formation of maltose, the free limit dextrinase activity appears to be a more limiting factor in hydrolysis of starch to fermentable sugars (Stenholm 1997, Stenholm and Home 1998).

Susceptibility of starch to enzymatic hydrolysis

Cereal starches vary in their gelatinisation properties. Maize and rice are widely used as cereal adjuncts in brewing for economic reasons and for their positive contributions to beer quality. Maize and rice starches are gelatinized at much higher temperatures than those favourable for malt enzymes. These kinds of raw materials have to be precooked before hydrolysis with malt enzymes (Home, Lauren and Autio, 1994). Wheat and barley starches are easier substrates, although in some cases problems may be encountered. Growth temperatures and conditions affect starch accumulation during kernel development, the ratio between small and large starch granules and the lipid content of starches (Tester et al. 1991). High growth temperatures have been shown to reduce starch yield and average granule size. The composition of isolated barley starches from the crops of a very cold summer (1987) and of a normal summer (1991) were compared (Myllärinen et. al 1998). Results are summarized in Table 2. Differences were observed in the lipid content of starch and in gelatinisation temperatures. Both of these phenomena affect the extent of amylolysis in mashing.

Table 2. Total amylose and lipid contents, granule size distribution and gelatinisation properties of starches isolated from cultivars Kustaa and Kymppi in 1987 and 1991.

sample	Total Amylose % dm	Lipid % dm	Small granules % (vol)	Average diameter μm	Gelatini-zation range, °C	Gel. tempe rature °C
Kustaa, 1987	28.2	0.79	16.9	15.1	53 - 79	59
1991	28.7	0.94	16.2	15.9	55 - 85	63
Kymppi, 1987	25.1	0.82	12.4	17.1	53 - 83	57
1991	27.1	0.94	12.8	17.6	55 - 89	60

A clear drop in fermentability of wort was noticed in Finnish breweries when malt prepared from the 1994 barley crop was taken into use. Traditional malt analysis did not indicate any problems. Comparison of gelatinisation temperatures between the crops 1993 and 1994 by DSC showed an increase of gelatinisation peak temperature from 62 to 65 °C. The use of the High Gravity Mashing procedure revealed a clear difference in the rate of saccharification between the malts made from these crops (Stenholm, Wilhelmson and Home 1998). The activities of starch-degrading enzymes were also slightly lower in the malts from the 1994 crop. The combination of higher starch gelatinisation temperatures and lower amylolytic activities resulted in decreased fermentability of wort. To improve fermentability the brewer can slightly increase the temperature of the first saccharification rest but at the same time he must assure that the level of β -amylase does not limit the liberation of maltose. Since the crop 1994, analysis of starch gelatinisation temperature is part of the crop-specific quality data which are generated soon after harvesting to get early information for maltsters and brewers.

The gelatinisation behaviour of small and large starch granules differ from each other. Small starch granules, especially those located close to the aleurone layer are degraded already

during malting. Some small granules still exist in malt. The hydrolysis of the purified small granule fraction of barley starch was studied under mashing conditions using a model system where starch was incubated in malt enzyme extract at temperatures similar to those used in the High Gravity Mashing procedure. A commercial barley starch preparation (large granules) was used as comparison (Table 3). The same extract yield was reached. Saccharification was completed somewhat later and the amount of fermentable sugars was lower in the case of small starch granules. This could be expected based on the gelatinisation temperature which was 68 °C for the small starch granule fraction, 5 °C higher than that of large granules.

Table 3. Differences between the type of starch granules in saccharification and release of extract yield and fermentable sugars.

Starch fraction	Saccharification	Extract content %	Fermentab. sugars g/l
Small granules (B starch)	10 min / 72 °C	17.1	87
Large granules (A starch)	5 min / 72 °C	17.1	99

Another quality characteristic that affects the dissolution and hydrolysis of starch is the existence of amylose-lipid complexes. These can be disrupted by heating at over 100 °C. Lipid complexed amylose is enzymatically hydrolysed more slowly than free amylose (Holm et al. 1983). Thus the complexes may be incompletely hydrolysed during mashing leading to a decreased extract yield. Amylose-lipid complexes do not stain with iodine and therefore unhydrolyzed complexes cannot be detected in a mash. A similar model mashing system as for the hydrolysis of small starch granules was used and the total and free amylose contents were analysed in the liquid phase and in the insoluble precipitate (Wilhelmson, Vainikka and Home 1997). The results revealed that the complexed amylose concentrated in the precipitate and the amount of unhydrolyzed amylose-lipid complex depended on the enzyme activity. In the model system released lipids seemed to remain in the liquid phase and contributed to the turbidity of the liquid but in all-malt mashing lipid material was found in the spent grain fraction.

Control of amylolysis in mashing

Uniform and extensive modification of malt is a first criterion to guarantee maximum extract yield from malt. It is a prerequisite for release of starch granules from the cell-wall and protein matrix and subsequent hydration, gelatinisation and enzymatic hydrolysis in mashing. The required degree of fermentability of wort may not be reached if the gelatinisation temperature of starch is increased due to the growing season or due to the proportion of small starch granules. Low levels of free limit dextrinase may also be a reason for insufficient fermentability. A shortage of enzymes may also limit the hydrolysis of amylose-lipid complexes and thus decrease the extract yield.

A simulation program was developed based on these studies. The models describe dissolution, activation and denaturation of α - and β -amylases, starch gelatinisation and hydrolysis of starch. As inputs the amounts of malt and starch adjunct, starch content of malt, moisture, amount of fermentable sugars and dextrans, α -amylase activity and diastatic power are needed (Koljonen et. al 1993, Koljonen 1995). The simulation program has successfully been used for design of a suitable mashing programme for new crops and new products. The simulation program is now commercially available from LP Research Centre Ltd., Finland.

Role of cell walls in brewing

Degradation products of the major cell wall component, the β -glucans, may cause several processing problems in breweries, including reduced rates of wort separation, poor filterability of beer and formation of hazes and gels. The amount and quality of β -glucans vary depending on the barley variety and on growth conditions. In breeding programmes varieties having thin cell walls and soft endosperm, leading to rapid cell wall modification are preferred (Aastrup 1979, Munck 1987, Home and Elamo 1993). During malting β -glucanases are produced and they hydrolyse cell walls into soluble low-molecular weight β -dextrins. Hydrolysis reactions continue in mashing. Malt β -glucanases are rather thermolabile and are rapidly inactivated in mashing. Analogous to the studies on the thermostability of amylolytic enzymes, β -glucanase activity and the accumulation of soluble β -glucans in the liquid phase of the mash were studied using isothermal mashings (Home, Pietilä and Sjöholm 1993). β -Glucanase is rapidly inactivated at temperatures above 50 °C. However, solubilization of β -glucans from intact cell walls continues and leads to the accumulation of soluble β -glucans in wort (Figure 3). Several enzymes, eg. ferulic acid esterase and carboxypeptidases, may be involved in the release of β -glucans from cell wall matrices (Bamforth et al. 1997). Determination of wort viscosity or β -glucans does not give enough information for the brewer about wort separation performance. High molecular weight compounds might remain in the mash cake and retard wort separation and are not necessarily found in wort. The speed of filtration in normal malt analysis is only a rough measure of mash separation. The above mentioned High Gravity Mashing procedure including monitoring of filtration speed gives a better prediction of the lautering behaviour of malt (Sjöholm et al. 1994, Stenholm et al. 1996).

The effects of the other cell wall components, the pentosans, on separation processes in brewing have not been extensively studied. These compounds have the ability to bind large amounts of water, form gels and increase the viscosity of aqueous solutions. In a preliminary study mash filtration performance was positively correlated with the amount of xylanase and arabinosidase activities. The effects of pentosans on the structure of mash cake and wort volume were not clearly shown. Soluble β -glucans and pentosans may form gels and cause problems in beer filtration. Total β -glucan content does not indicate these problems because very low amounts of β -glucan gels formed have been shown to retard filtration (Krüger, Wagner and Esser 1989). Addition of xylanase to beer after fermentation slightly improved filterability, indicating that pentosans also play a role in beer filtration.

Analysis techniques

Traditional malt analysis is empirical and based on a standardized laboratory mashing procedure and analyses of filtered wort. Processing or quality problems that might be encountered in practice cannot always be predicted from the results. Changes in wort analysis figures do not give specific information on the quality or availability of substrates or on the amount and spectrum of enzymes. For example high viscosities may be caused by high molecular weight compounds or gels from different origin, β -glucans, pentosans, proteins and starch degradation products or by their interactions. In the case of slow mash separation, chemical analysis of wort is not very informative because the compounds causing problems are retained in the mash cake that normally is not analysed.

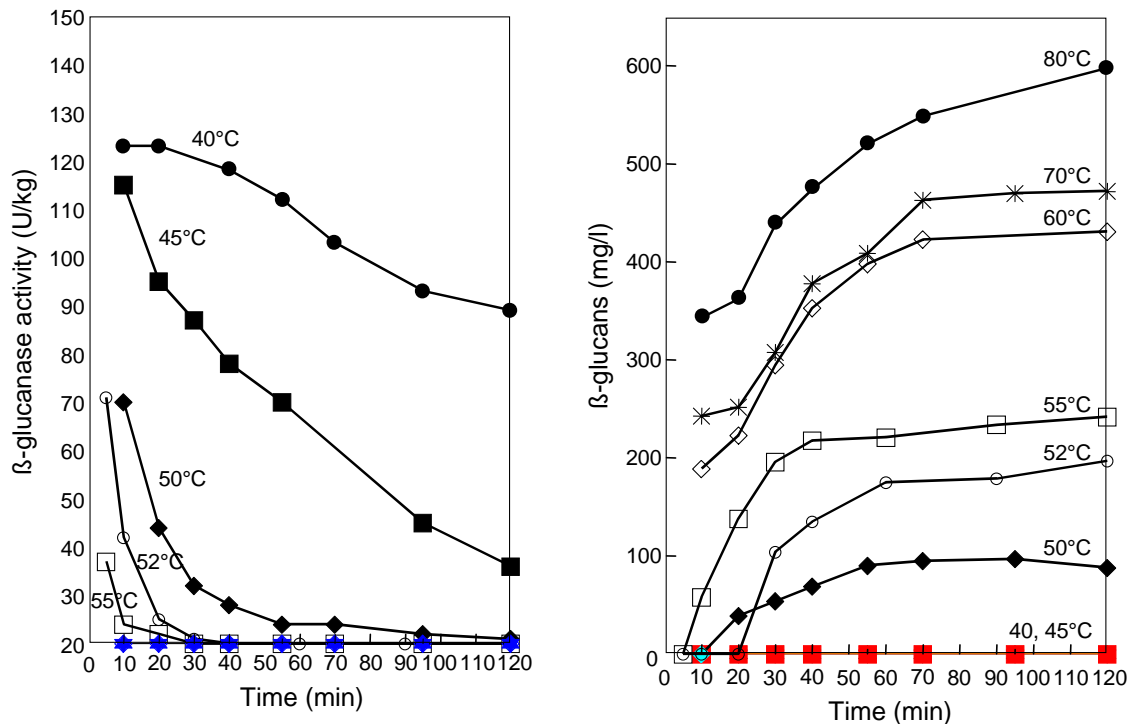


Figure 3. Thermostability of malt β -glucanase and accumulation of soluble β -glucans in isothermal mashing.

Microscopic methods based on sanding of barley or malt kernels and staining with specific fluorescent dyes are used to evaluate cell wall structures in barley and the degradation pattern of endosperm structure during malting (Aastrup, Gibbons and Munck, 1981). In baking research similar methods are used to study the structural changes in dough. A variety of specific dyes are available to monitor starch, proteins and cell wall components (Munck, 1989, Autio, Fabritius and Kinnunen, 1998). Application of similar techniques to the mash cake could give more information on its structure and the nature of complexes retarding filtration. Specific microscopic methods have also been applied to study the structure of cell walls using purified enzymes (Autio et.al 1996). In baking research dough rheology plays an important role. The effects of processing parameters and enzymes on viscoelastic properties are studied by rheological measurements (Autio, Fabritius and Kinnunen, 1998). Rheometry could also be used for studying the gelling properties of barley and malt. Possible applications could be found in evaluation of the susceptibility of cell walls to enzyme attack in breeding programmes and in the optimisation of processing parameters on viscometric properties during mashing and lautering.

At VTT, the research in the food sector is focused on cereals. Brewing research has benefited from research on starch properties and on modified starches for food and non-food applications as well as from the research on baking and food structure in general. Development of enzyme technology including purification and characterization of microbial and cereal based enzymes helps in understanding the enzymatic changes and solving problems in malting and brewing processes.

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