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Carbohydrate Polymers 47 (2002) 169–179

Carbohydrate Polymers

www.elsevier.com/locate/carbpol

Gradual enzymatic modification of barley and potato amylopectin

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Received 14 July 2000; revised 20 November 2000; accepted 21 December 2000

Abstract

New potato and barley starches with no amylose were used in order to explore structural changes in amylopectin due to hydrolysis with α-amylase from *Bacillus amyloliquifaciens*. The degradation pattern was monitored with size exclusion, with light scattering and viscometry detectors for determination of molecular weight and radius of gyration. Fractions with relatively narrow molecular weight distributions at high yields were obtained as a result of the gradual enzymatic degradation and the subsequent sequential ethanol precipitation technique used. Generally the fractions obtained showed a pattern of both decreasing molecular weight and radius of gyration with increasing enzymatic hydrolysis. Fractions that precipitated with the lowest ethanol concentration had the highest values of both average molecular weight and radius of gyration. The unit chain length distributions of the fractions were analysed and the results showed that the chain length distribution varied with the time of hydrolysis. There was also a decreasing trend in average molecular weight with enzyme degradation time for fractions that precipitated at the same ethanol concentration, indicating that the fractionation was affected by structure and not only by molecular weight. Data from this study could be useful for the production of modified amylopectin molecules that vary in composition and characteristics.

Keywords: α-Amylase; Amylopectin; Barley; Ethanol precipitation; Hydrolysis; Molecular weight; Potato; Size; Unit chain distribution

1. Introduction

Normally starch contains amylose, the essentially linear α-D-(1 → 4) glucan, to a level of 20–30%. New tuber and cereal varieties with no amylose have been developed as a result of traditional plant breeding and modern gene technology (Bhatty & Rossnagel, 1997; Visser, Suurs, Steeneken &Jacobsen, 1997a). Amylopectin starch is constituted of a highly branched structure with α-(1 → 6) branch points in the α-(1 → 4) glucan chain and in comparison with amylose it has a higher molecular weight, 10^7–10^8 in contrast to 10^5–10^6 g/mol. The branches are arranged in clusters, allowing the formation of α-helices and giving the molecule a semi-crystalline nature in the granule (Smith, Denyer & Martin, 1997). Amylopectin, especially that from tuberous starches, also contains a small amount of covalently linked phosphate monoesters at the C3 and C6 positions of the glucose residues (Jane, Kasemsuwan, Chen & Juliano, 1997). Many unique physical and chemical properties have been reported for high amylopectin (~100 %) compared to amylose-containing potato starch. This includes a lower peak viscosity of gelatinised starch, improved gel stability and paste clarity together with a change of the lambda max and blue value in iodine staining (Visser, Suurs, Bruinenberg, Bleeker & Jacobsen, 1997b). Hull-less zero-amylose barley has been reported to have several beneficial properties useful for food and industrial applications such as low pasting temperature, high paste clarity and increased freeze-thaw stability (Zheng, Hang & Bhatty, 1998).

α-Amylase is an endo-specific enzyme commonly regarded as random acting and catalysing the hydrolysis of α-(1 → 4) linkages in amylose and amylopectin (BeMiller, 1997; Colonna, Leloup & Buleon, 1992). Chemical modifications such as enzymatic degradation of the polymers greatly affect the behaviour and functional properties of starch. Reduced viscosity and iodine coloration (blue value) and a gradual increase in reducing value is obtained with the action of α-amylases (Hizukuri, 1996). A study of enzymatic hydrolysis and the kinetics of α-amylase depolymerisation of commercial amylopectin suggests that the degradation mechanism is dependent on the substrate conformation in solution (Park & Rollings, 1994). Poutanen, Lauro, Surotti and Autio (1996) described the α-amylolytic activity on waxy barley amylopectin in two stages studied by gel permeation chromatography (GPC), the first producing larger molecules and the second producing smaller fragments. They also reported that the degradation was

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0144-8617/02/$ - see front matter
PII: S0144-8617(01)00161-8

independent of the preheating conditions and the microstructure before the degradation onset. Gelation properties of waxy barley starch after α-amylolytic attack were studied and gel stiffness was reduced with increased enzymatic hydrolysis time (Lauro, Ring, Bull & Poutanen, 1997). In another study, starches from different sources were treated with α-amylase at two different amounts (Bello-Perez, Paredes-López, Roger & Colonna, 1996). Two different fractions were obtained from treatment with a relatively low enzyme concentration, the first was referred to as a low molecular weight fraction and the second main fraction had DP 31–44. When the α-amylase concentration was increased glucose, maltose, DP 11–15 and 23–30 were detected as important hydrolysis products. Bertoft and Zhu (1996) applied α-amylase treatment, fractionation by methanol precipitation and GPC analysis to potato amylopectin. They obtained fractions that contained mixtures with different proportions of more high and low molecular weight dextrins. Further analysis suggested that structurally different domains built up this amylopectin.

More information is needed on structural and compositional characteristics of novel base starches suitable for large-scale production in Sweden. The action of enzymatic hydrolysis is dependent on, for example, the type of starch, amylose: amylopectin ratio, molecular associations and gelatinisation temperature (Zhang & Oates, 1999). The overall intention of this study was to modify the two amylopectins used and to investigate the course of events due to the hydrolytic action of the enzyme used. The functional properties of novel starches with a gradual change in mass and structure are studied in ongoing research.

2. Experimental

2.1. Starches

High amylopectin potato starch from Lyckeby, Stärkelsen, Sweden and amylose-free barley grain from the University of Saskatchewan, Saskatoon, Canada (Bhatty & Rossnagel, 1997) were obtained as raw material for these studies. The barley grain starch was isolated at Lyckeby Stärkelsen pilot plant using a procedure similar to that described by Andersson (1999). The starch yield was 48% of barley grain.

The total and apparent amylose contents were analysed by iodine staining (Morrison & Laignelet, 1983). The amylose content was also determined by GPC. Starch samples (1.5 mg/ml) were prepared essentially as described by Torneport, Salomonsson and Theander (1990). For debranching, however, 5 μl isoamylase was used, and the enzyme was inactivated in a boiling water-bath for 5 min. The samples were injected on a Sepharose CL-6B column (1.6 × 60 cm; Pharmacia, Uppsala, Sweden) using 0.25 M KOH as eluent at a flow rate of 13 ml/h. Two-ml fractions were collected, and the elution profile was detected by the phenol–sulphuric acid method of Dubois, Gilles, Hamilton, Rebers and Smith (1956).

2.2. Enzymes

α-Amylase from *Bacillus amyloliquifaciens* [(1 → 4)-α-D-glucan glucanohydrolase; EC 3.2.1.1] with an activity of 600 U/mg was purchased from Boehringer-Mannheim (Mannheim, Germany) and isoamylase from *Pseudomonas amyloderamosa* (glycogen-6- glucoanhydrolase; EC 3.2.1.68; 71 000 U/mg) was obtained from Hayashibara Biochemical Lab. (Okayama, Japan).

2.3. Partial degradation of amylopectin

A sample of 0.5 g of potato or 1 g of barley starch was dissolved in 50 ml water by boiling for 15 min and thereafter 50 ml 0.05 M sodium acetate buffer (pH 5.5) was added (Bertoft & Spoof, 1989; Nebesny, 1992; Poutanen et al., 1996). Mechanical treatment was carried out with an ultra-turrax for 0.5 (barley starch) and 1 min (potato starch) after which the samples were placed in a water-bath at 30°C and incubated with α-amylase (10 mU/g starch) for 5, 15, 30, 60 and 120 min. The enzyme was inactivated with 5 ml 5 M NaOH for 20 min, after which the pH of the solution was raised to 6–7 with 2 M acetic acid. Aliquots of this solution (25 ml) were transferred to centrifuge tubes and ethanol was added sequentially to 20, 40, 60 and 80% (w/v) concentration to precipitate the partially degraded amylopectin. Centrifugation at 2000 g for 10 min. was performed between each precipitation step and the pellet was then dispersed in 75 ml water after which the solution was dialysed, freeze-dried and weighed for determining the yield. This enzyme treatment was performed in triplicate.

2.4. Debranching and high performance anion exchange chromatography (HPAEC-PAD)

The native as well as the partially degraded amylopectin (~1.5 mg/ml) was dissolved in 0.0625 M NaOAc buffer (pH 3.6) in a boiling water-bath for 15 min. The mixture was cooled to room temperature and 5 μl isoamylase was added. The samples were debranched overnight in a shaking water-bath at 38°C, after which the enzyme was inactivated by boiling for 10 min. Analysis of the debranched amylopectin was performed by high performance anion exchange chromatography (HPAEC) consisting of a DX 500 chromatography instrument equipped with a pulsed amperometric detector (PAD) ED-40 (Dionex, Sunnyvale, CA, USA) under conditions described in Koch, Andersson and Åman (1998). The detector response of the PAD is not quantitative with respect to carbohydrate concentration. Therefore the area of each individual α-glucan peak between DP 6 and 55 was corrected for by its relative PAD response (Koch et al., 1998). Principal component analysis (PCA) on all individual unit chains was performed using The Unscrambler

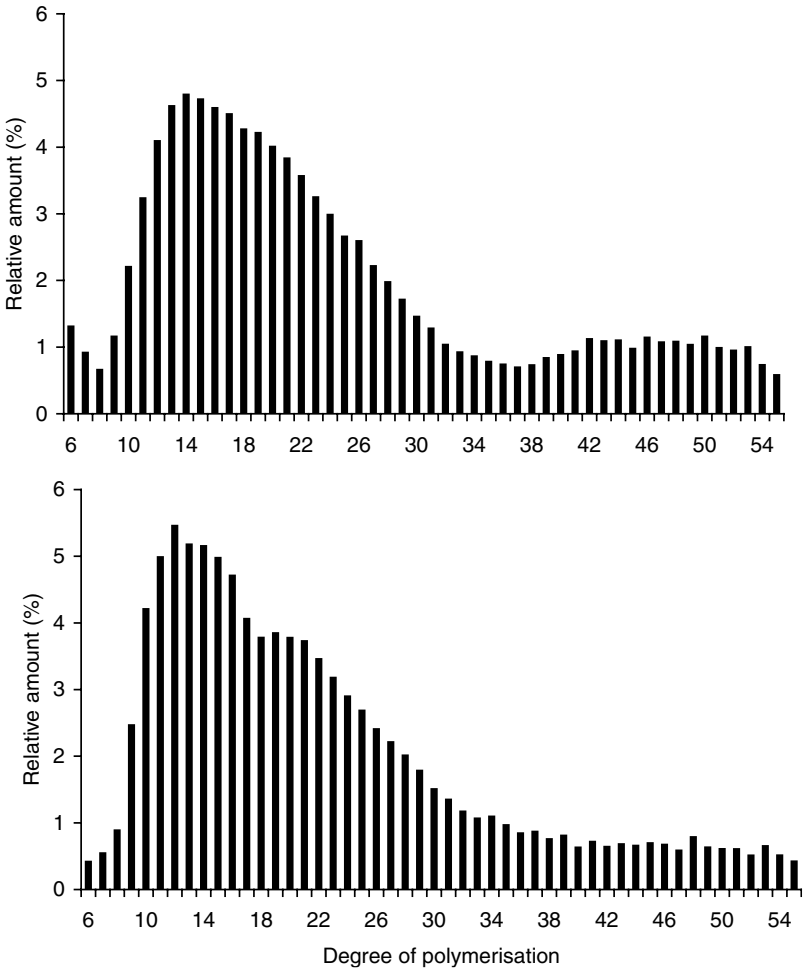

Fig. 1. The chain length distribution profiles of amylopectin starches from new varieties of Potato (upper) and Barley (lower) amylopectin.

software, version 7.5 (Camo ASA, Oslo, Norway). All variables were centred and scaled to equal variance before further analysis and the first two principal components were used to set ranges for unit chain classes.

2.5. High performance size exclusion chromatography, static light scattering and viscometry

The size exclusion chromatography system consisted of solvent delivery system LC-10AD attached with online degasser DGU-4A (Shimadtzu, Kyoto, Japan), thermostated (30°C) automatic injector model Waters 717 (Millipore Corp., Marlborough, MA, USA) and column oven (Microlab Universal thermostat, Aarhus, Denmark). A 0.1 μm online filter was added between the pump and injector. The columns used were a guard column Aquagel-OH Guard (Polymer Laboratories, Amherst, MA, USA), and size exclusion columns OH-Pak 803-HQ, 804-HQ and 806-M-HQ (Shoko CO., Ltd, Tokyo, Japan) in series at a temperature of 30°C. The mobile phase was 0.1 M sodium nitrate with 0.02% sodium azide. Samples were prepared by dissolving the amylopectin fractions (1–3 mg/ml) in the mobile phase in a boiling water-bath for 15 min. Prior to analysis the samples were filtered through a 0.45 μm syringe tip filter. The flow was set for 0.5 ml/min and the injection volume was 100 μl.

Light scattering measurements were performed at room temperature with a DAWN DSP multiangle laser light scattering detector (MALLS) in chromatography mode using a He–Ne laser light source operating at 632.8 nm (Wyatt Technology Corp., Santa Barbara, CA, USA). Toluene was used as the calibration standard at 90° scattering angle. Light intensity of the diodes at the other angles was normalised to the 90° angle using a monodisperse isotropic scatterer, pullulan P-20 (Macherey-Nagel, Düren, Germany). The online concentration and viscosity was measured with a Viscotek Model 250 combined refractometer and viscometer operated at 30°C and coupled to the 90° signal from the right angle light scattering detector. Again Pullulan P-20 was used as the standard for determining calibration constants. The softwares used were ASTRA version 4.72.03 (Wyatt Technology) and TriSEC version 3 (Viscotek). All calculations in Astra were carried out on unfitted data and using the Zimm formalism ($K \times c/R\theta$). The model of angular dependence (Manual

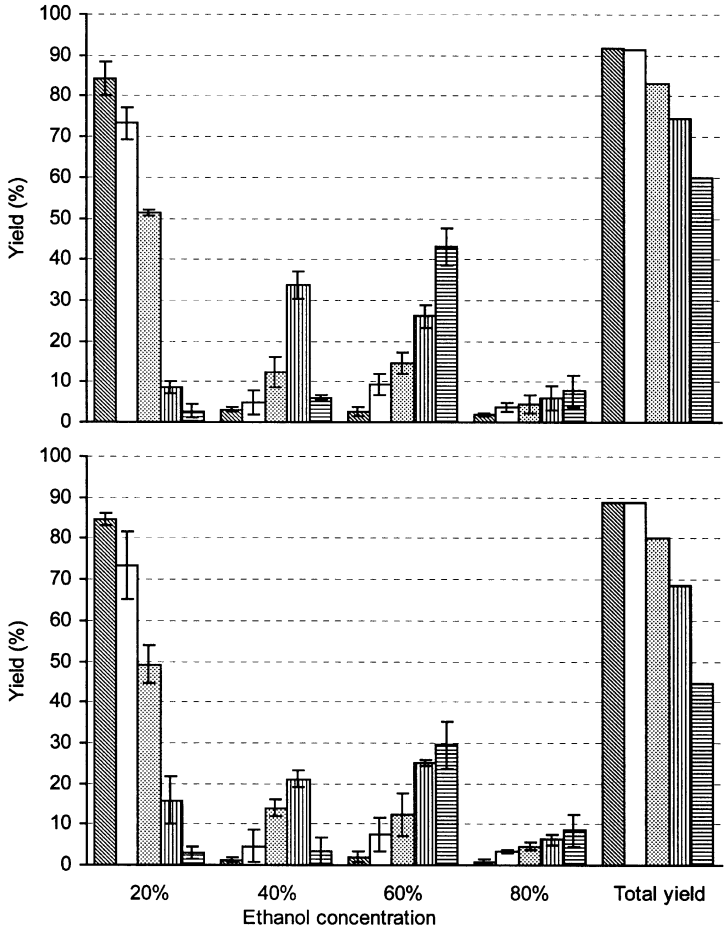

Fig. 2. Yield of Potato (upper) and Barley (lower) amylopectin fractions obtained from enzyme degradation with α-amylase for 5 (▨), 15 (□), 30 (■), 60 (▥) and 120 (▤) min of time and subsequent sequential ethanol (20–80 %) precipitation. Error bars denotes the standard deviations measured from the triplicate samples of the enzyme treatment procedure.

Viscotek) on unfitted data performed calculations in TriSEC. The dn/dc value used for amylopectin in water solutions (0.146 ml/g) was obtained from literature (Chanliaud, Roger, Saulnier & Thibault, 1996; You, Fiedorowicz & Lim, 1999).

3. Results and discussion

3.1. Characterisation of starch raw material

Amylopectin starch from potato and barley was used in this study. Debranching followed by gel filtration verified the 0% amylose content of both starches. However analysis with iodine staining of the potato amylopectin showed a value indicating a low amylose content. This could be due to the fact that longer unit chains are present in this material. Bradbury and Bello (1993) suggested that the linear amylose contents determined by a HPSEC procedure were lower than those determined by the iodine colorimetric analysis, indicating a contribution from the long linear chains of amylopectin to the amylose values determined colorimetrically. It is believed that outer chains of amylopectin are long enough to produce helices participate in the complex formation with iodine (Knutson, 1999). Other parts of the amylopectin molecule are sterically hindered from taking part in this complexing.

Debranching of the amylopectin samples with isoamylase and analysing individual unit chains by HPAEC-PAD revealed structural differences between the two samples. The unit chain length distribution showed some major differences in the fingerprint region (DP 6–8) (Fig. 1), as well as no obvious differences in chain lengths between populations of DP 12 and 20. However, a higher proportion of longer unit chains in potato amylopectin were found. The present results agreed well with earlier studies showing similar chain length distribution profiles (Jane, Chen, Lee, McPherson, Wong, Radosavljevia & Kasemsuwan, 1999; McPherson & Jane, 1999; Silverio, Fredriksson, Andersson, Eliasson & Åman, 2000). Structural differences between different starches are believed to give different physical characteristics measured as viscosity, gelatinisation and retrogradation behaviour and pasting properties (Jane & Chen, 1992; Jane et al., 1999).

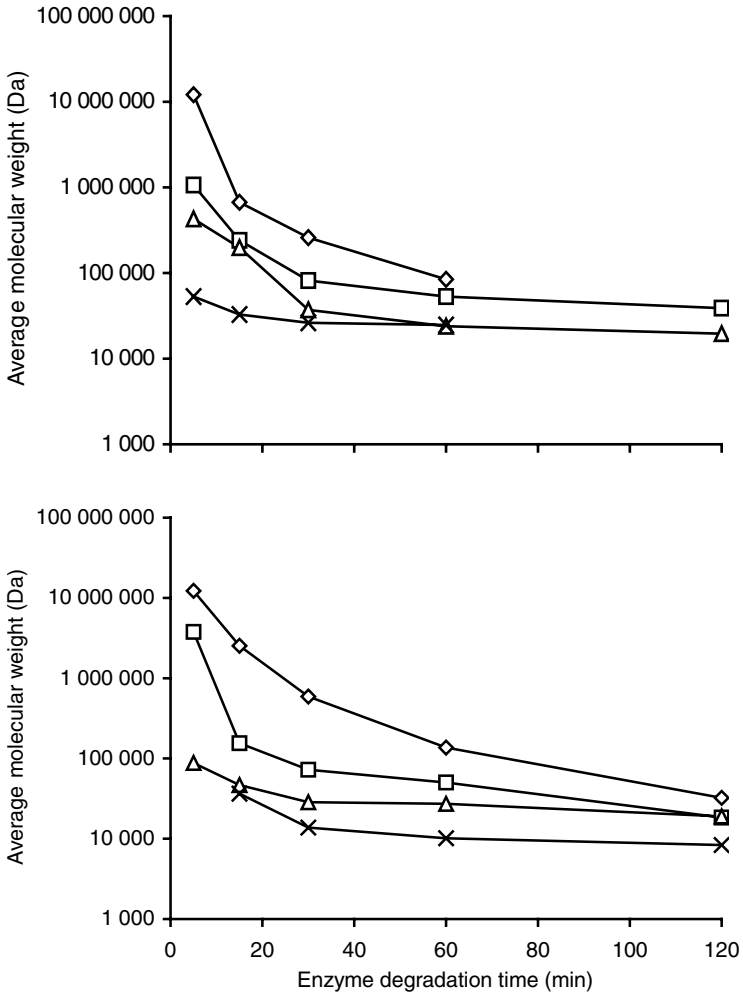

Fig. 3. Molecular weights of Potato (upper) and Barley (lower) amylopectin fractions obtained from enzyme degradation with α-amylase for different periods of time and subsequent sequential ethanol precipitation at 20 (◇), 40 (□), 60 (△)and 80 % (×) (w/v). Missing values were not determined because of the limited amount of material.

3.2. Partial hydrolysis of amylopectin with α-amylase

The modification of amylopectins from potato and barley was performed by enzymatic hydrolysis with α-amylase from *B amyloliquifaciens*. Prior to the enzyme treatment the amylopectin was gelatinised and treated mechanically in order to reduce the viscosity. Different concentrations of potato and barley amylopectin (0.5 g and 1 g/100 ml, respectively) were used, based on results from pre-experiments. The difference in treatment of the two types of amylopectins was based on the difference in apparent gelatinisation behaviour and also by measuring the viscosity of gelatinised and mechanically treated amylopectin suspensions (results not shown). No change in viscosity of the gelatinised amylopectins was seen if the mechanical treatment was performed for periods of time longer than 10 s. Heitmann, Wenzig and Mersmann (1997) studied the effect of stirring at high shear rates and reported a mechanical disruption of potato starch granules. They degraded the starch granules in three steps (3 × 1 min at 20,000 rpm) and after the last treatment the high molecular mass fraction almost disappeared, leaving a more low molecular weight peak of partly soluble starch that stayed mostly unaffected during further treatment. In the present study the mechanical treatment procedure was found to be detrimental for the repeatability of the enzymatic hydrolysis. This could be due to the fact that it was difficult to produce a completely dissolved sample by gelatinisation at the conditions used. During gelatinisation, starch granules will undergo swelling and partial solubilisation. Excessive shearing treatment of the gelatinised starch may break and disintegrate the granular structure (Colonna, Leloup &Buleon, 1992).

The mechanical treatment was followed by incubation with α-amylase for different periods of time at 30°C. The enzymatic hydrolysis was followed by sequential ethanol precipitation in order to fractionate the enzyme-treated material into different molecular weight fractions. The yield of these fractions of potato and barley amylopectin was measured after both the selected enzyme hydrolysis times and ethanol concentrations (Fig. 2). The total yield

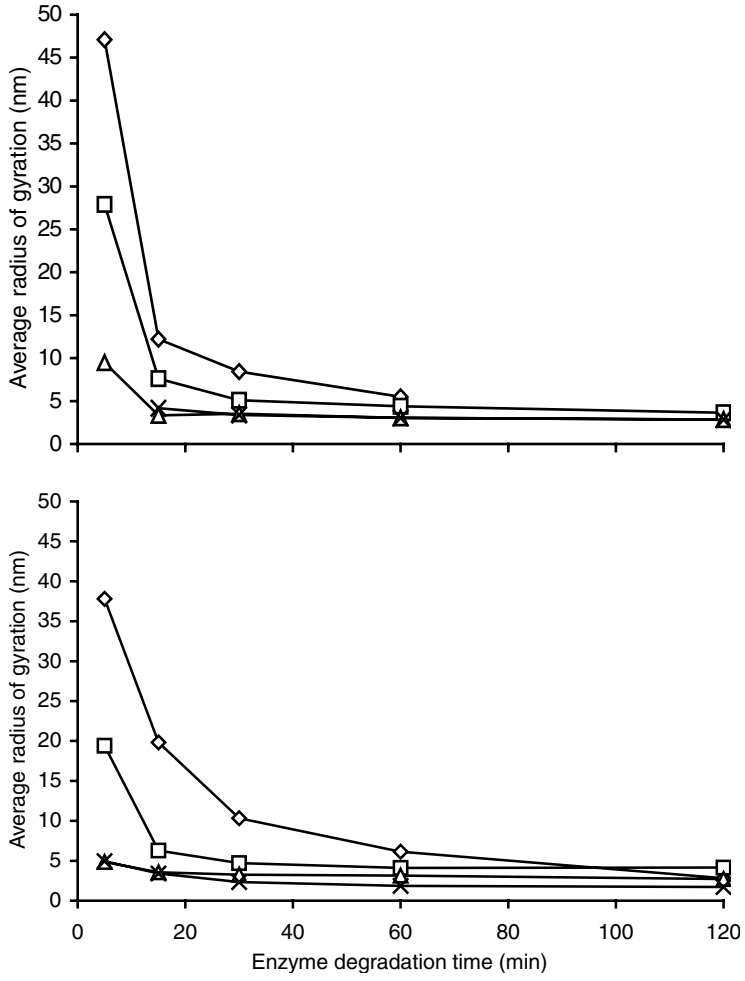

Fig. 4. Radius of gyration of Potato (upper) and Barley (lower) amylopectin fractions obtained from enzyme degradation with α-amylase for different periods of time and subsequent sequential ethanol precipitation at 20 (◇), 40 (□), 60 (△) and 80 % (×) (w/v). Missing values were not determined because of the limited amount of material.

varied between 45 and 91% and the highest yield was achieved with the shortest enzyme treatment time. These results clearly showed that some of the material was of low molecular weight and consequently did not precipitate with 80% ethanol. The results also showed that the yield of amylopectin treated for 5 min with α-amylase decreased from the lowest to the highest ethanol concentration. As the ethanol concentration increased the fraction giving the highest yield was shifted with increasing enzyme treatment time. The primary target was to reproducibly achieve fractions of enzyme-treated amylopectin with narrow molecular weight distributions at relatively high yields. As mentioned, the viscosity and homogeneity of the starch solution were found to be the two very important factors influencing the repeatability of the enzyme treatment. Marchal, van de Laar, Goetheer, Scimmelpennink, Bergsma, Beeftink and Tramper (1999) used amylopectin potato starch and α-amylases from different sources (including *B amyloliquifaciens*) and their study showed that different temperatures (50,70 and 90°C) during hydrolysis influenced the initial overall molecular weight distribution with the higher temperatures giving a more homogenous distribution. Their study also showed that at the same DE (dextrose equivalents, which is directly related to the number average molecular weight) the oligosaccharide composition depended on the hydrolysis. In the present study it was also confirmed that by using mechanical treatment, the course of enzymatic hydrolysis could be altered, suggesting that the enzyme gained access to a larger portion of the substrate. Previous studies report the susceptibility of gelatinised starch and of less organised amorphous regions of starch granules to be more susceptible to enzyme attack (Gallant, Bouchet & Baldwin, 1997; Krishnakumari & Thayumanavan, 1995; MacGregor, 1993). At a macroscopic level, the enzymatic attack seems to be controlled by the structure of starch granules and an extensive disruption of this organisation makes the enzyme gain more access to the granule interior. This is probably accompanied with a more even hydrolysis producing more homogeneous molecular mass fractions with different structures. Processed starch is thermodynamically an unstable structure. Dynamic processes such as retrogradation decrease the rate of hydrolysis by α-amylase (Fredriksson,

Table 1
Polydispersity index ($\bar{M}_w/\bar{M}_n$) of potato and barley amylopectin fractions obtained from enzyme degradation with α-amylase and subsequent sequential ethanol precipitation. Missing values were not determined because of the limited amount of material in those fractions

Enzyme degradation time (min)	Polydispersity index ($\bar{M}_w/\bar{M}_n$)			
	Ethanol concentration			
	20%	40%	60%	80%
Potato				
5	1.67	–	–	–
15	2.58	2.84	1.28	1.95
30	1.81	1.42	1.60	1.31
60	1.22	1.25	1.30	1.24
120	–	1.15	1.21	1.15
Barley				
5	1.68	5.62	2.40	–
15	3.12	1.98	1.82	2.14
30	2.54	1.41	1.38	1.62
60	1.47	1.26	1.32	1.49
120	1.38	1.28	1.18	1.31

Björck, Andersson, Liljeberg, Silverio, Eliasson & Åman, 2000). This dynamic behaviour of amylopectin may also have influenced the outcome of the results in the present study.

3.3. $\bar{M}_w$ and $\langle\bar{s}\rangle_w$ of amylopectin fractions

The amylopectin fractions were analysed for their molecular size distribution in order to determine the impact of enzyme treatment and ethanol precipitation. Generally the fractions obtained in this study showed a pattern of both decreasing molecular weight and radius of gyration $\langle\bar{s}\rangle_w$ with increasing enzymatic degradation time (Figs. 3 and 4). The different lines in the graphs represent the different ethanol concentrations, revealing that the precipitation process yielded amylopectin fractions with the lowest ethanol concentration giving the highest values of both $\bar{M}_w$ and $\langle\bar{s}\rangle_w$. The advantage of using MALLS together with viscometry is the possibility to use two independent calculations to determine $\bar{M}_w$ and $\langle\bar{s}\rangle_w$. MALLS together with RI and the ASTRA software was used to calculate $\bar{M}_w$. $\langle\bar{s}\rangle_w$ was calculated with the TriSEC software using the signal from the viscometer, RI and the 90° angle in the light scattering detector. The approach using viscometry gives more accurate values for $\langle\bar{s}\rangle_w$ when it is close to the theoretical limit of MALLS. For barley the 20 % ethanol treatment gave $\bar{M}_w$ ranging from 3×10^3 to $12\ 250\times10^3$ g/mol and a $\langle\bar{s}\rangle_w$ between 2.8 and 37.8 nm. The 60 % ethanol concentration gave $\bar{M}_w$ and $\langle\bar{s}\rangle_w$ between $19.0\times10^3$and 87.8×10^3 g/mol and 2.8 and 4.8 nm, respectively. The potato amylopectin followed the same pattern but the absolute values were somewhat different, for 20 % ethanol the values for $\bar{M}_w$ were 84.23×10^3–$12\ 090\times10^3$ g/mol and for $\langle\bar{s}\rangle_w$ 5.5–47.1 nm and for 60% ethanol $\bar{M}_w$ were 19.63×10^3–428.7×10^3 g/mol and $\langle\bar{s}\rangle_w$ 2.8–9.5 nm. The material precipitating with 20% ethanol showed the largest decrease in $\bar{M}_w$ over enzyme treatment time. This may be explained by the fact that these fractions contain all material over a certain $\bar{M}_w$ and thus cover a wider range of molecular weights. This explanation is however not valid for the fraction precipitating at higher ethanol concentrations, since their ranges are limited by the sequential ethanol precipitation procedure. This indicates that the enzyme treatment affected the precipitation behaviour of the fractions. $\bar{M}_w$ values were obtained from RI-MALLS (refractive index-multi angle laser light scattering) and $\langle\bar{s}\rangle_w$ from RI-RALLS-viscometry (right angle (90°) laser light scattering). The use of both techniques was based on limitations of MALLS in determining $\langle\bar{s}\rangle_w$ of very small molecules and RI-RALLS-viscometry in determining $\bar{M}_w$ of high molecular weight samples. When calculating the radius of gyration, these values seem to be relatively low compared to the molecular masses obtained. The polydispersity ($\bar{M}_w/\bar{M}_n$) of the different fractions generally decreased with enzyme treatment time but not with the ethanol concentration (Table 1). Aberle, Burchard, Vorwerg and Radosta (1994) found molar masses of 601×10^3 up to $1\ 101\times10^3$ g/mol and a radius of 220 nm for amylopectins from different sources. However White (1999) reported $\bar{M}_w = \sim10\ 000\times10^3$ g/mol, $\langle\bar{s}\rangle_w = 57$ nm and a $(\bar{M}_w/\bar{M}_n) = \sim2.3$ for native potato amylopectin, which agrees with the results obtained in the present study. Discrepancies in measuring molecular mass and size of amylopectin in solution could be due to the fact that the enzymatic hydrolysis allows inter- and intramolecular aggregations to occur, giving an overestimation of molecular weights. In a molecular modelling study it was found that certain internal lengths lead to parallel double helices, influencing the degree of local crystallinity (O'Sullivan & Perez, 1999). This computer simulation may also suggest the occurrence of aggregates at certain unit chain lengths in the amylopectin structure. With regards to the results obtained for $\bar{M}_w$ and

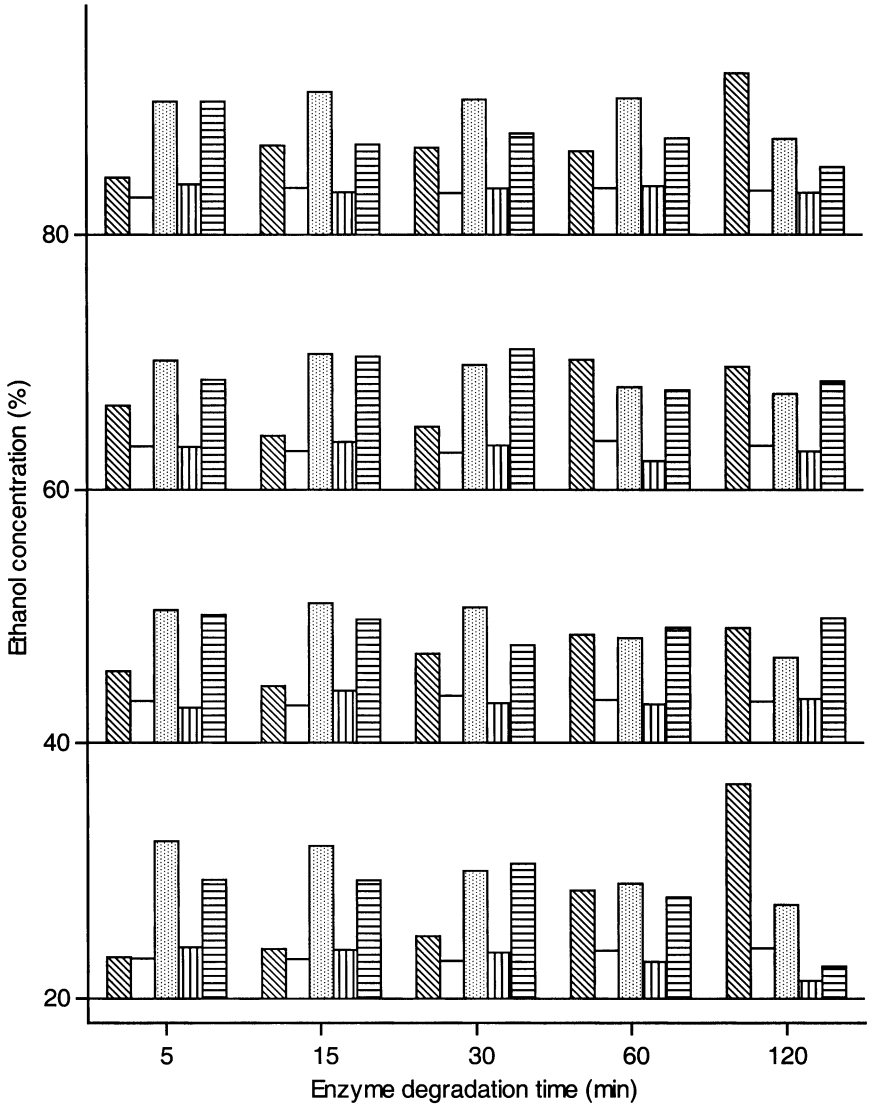

Fig. 5. The chain length distribution of Potato amylopectin fractions analysed after enzymatic hydrolysis with α-amylase and subsequent sequential ethanol precipitation divided into classes of DP 2–8 (▨), 9–11 (□), 12–20 (▩), 21–25 (▥) and >26 (▤).

$\langle \bar{s} \rangle_w$, consideration also has to be given to the shear sensitivity of very high molecular weight amylopectins as they are separated on size exclusion columns. Values of molecular weight and radius of gyrations determined online in a size exclusion chromatography system have been reported to be significantly lower than those determined with an offline (batch mode) MALLS detector, implying shear degradation (Klavons, Dintzis & Millard, 1997; You et al., 1999). However, the explanation could also be the domination of the light scattering signal by high molecular weight aggregates when measuring in batch mode. The result in that case would be a shift of the $\bar{M}_w$ towards a higher value than the real average. A different explanation for differences found in studies concerning determination of size and mass could be the fact that some amylopectin molecules may not elute through the size-exclusion columns at all.

3.4. Structural features of amylopectin fractions

Debranching of the amylopectin and analysis of the unit chains on an anion exchange column revealed information about the changes in structure caused by the α-amylase degradation process and the subsequent ethanol precipitation. PCA analysis was applied to the unit chain length distribution profiles determined by HPAEC-PAD for all individual fractions i.e. the different enzyme degradation times and ethanol concentrations. The relationship between the variables were visualised by the loadings of the two first principal components, and the results showed that the variables were clustered into five groups. Variables that belonged to the same group were internally correlated and were therefore considered as one parameter in the following interpretation. This procedure provided a systematic way to divide the unit chains into specific groups. It was found that

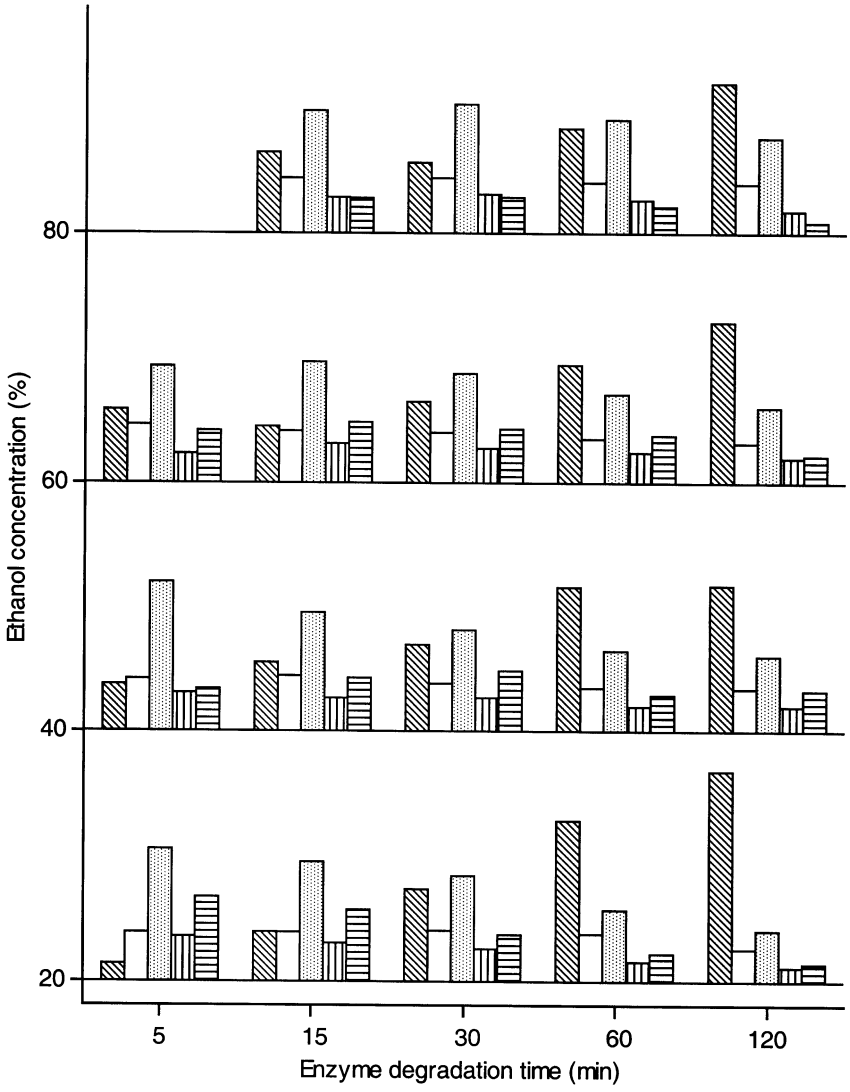

Fig. 6. The chain length distribution of Barley amylopectin fractions analysed after enzymatic hydrolysis with α-amylase and subsequent sequential ethanol precipitation divided into classes of DP 2–8 (▨), 9–11 (□), 12–20 (▩), 21–25 (▥) and >26 (▤).Missing values were not determined because of the limited amount of material in these fractions.

the different unit chains were clustered into the ranges, DP = 2–8, 9–11, 12–20, 21–25 and >26. These ranges were thus set by the way the enzymatic attack affects the structure of the fractions. The results were recalculated as the sum of the individual unit chains within each range (Figs. 5 and 6). Generally with increased enzyme degradation time, a change in the proportions of the different ranges was found. The amylopectin fractions had different amounts of unit chains in specific ranges and the outcome appeared to be independent of the starting material. For both potato and barley, the clearest change was seen with increased enzyme degradation time giving a shift in the structure towards a higher proportion of the unit chain lengths in the ranges DP 2–8 and a decreasing proportion of DP 12–20. The unit chains between 9–11 and 21–25 seemed to stay mostly unchanged with increasing enzyme degradation time. The proportion of unit chains above DP 26 generally decreased for the barley amylopectin while this trend was absents for the potato amylopectin. Comparing the results in the direction of increasing ethanol concentration, the amylopectin fractions showed no clear systematic change in proportions for each time of hydrolysis. As seen in Figs. 3–6, the fractions obtained by ethanol precipitation had similar unit chain length distribution although their molecular weight and size differed. Precipitation by a specific ethanol concentration was expected to give similar fractions but in this case the results showed a variation in molecular weight. Thus the results obtained suggested that the fractionation was structure dependent. Marchal et al. (1999) studied the effect of different temperatures on enzymatic hydrolysis of amylopectin from different sources and found that at the same dextrose equivalent value the

oligosaccharide composition was dependent on the treatment temperature. In that investigation they used α-amylase from different microbial sources, including *B amyloliquifaciens*. This onset of different activities of the enzyme at different temperatures propose that the hydrolysis was carried out in a non-random fashion, as suggested in earlier discussions about the mechanism (Colonna et al., 1992; Hizukuri, 1996).

Application of mild enzymatic treatment to starch produces maltodextrins with low dextrose equivalents (DE). One way to produce these starch hydrolysis products (SHPs) is via α-amylase action on starch slurries before and during gelatinisation. An other possible way to achieve such SHP:s is by mechanical degradation with or without the combination of enzymatic hydrolysis. The SHPs have the capability to form thermo-reversible gels, and possess a similar mouth-feel to fat (Woods & Swinton, 1997). Modification of amylopectin structure may also lead to specific changes in the physico-chemical properties of starch. Previously, the expression of the glycogen branching enzyme was used to increase the degree of branching in an amylose-free potato mutant (Kortstee, Suurs, Vermeesch, Keetels, Jacobsen & Visser, 1998). This led to a higher proportion of shorter chains in the amylopectin, which showed a lower peak viscosity and a tendency to form weaker gels. Another possible strategy for achieving structurally different amylopectin fractions is the one used in our study.

4. Conclusions

Fractions with relatively narrow molecular weight distributions at high yields were obtained as a result of gradual enzymatic degradation of potato and barley amylopectin followed by sequential ethanol precipitation. When α-amylase cuts the α-(1 → 4)-glycosidic bonds in the inner and outer chains of amylopectin it gives products that vary in molecular weight and unit chain lengths. Generally the fractions obtained showed a pattern of both decreasing molecular weight and radius of gyration with increasing time of enzymatic hydrolysis. The unit chain length distribution varied with the time of hydrolysis and the sequential ethanol precipitation process was structure dependent. These results clearly indicate that mechanical degradation, enzymatic hydrolysis and ethanol precipitation of amylopectin starches from potato and barley may result in fractions with unique molecular characteristics. Such products may be of interest in different future applications. In order to reveal their usefulness the physical behaviour is studied in ongoing research.

Acknowledgements

Professor Ron Bhatty from the University of Saskatchewan, Saskatoon, Canada is greatly acknowledged for providing the barley grain. We wish to thank Lyckeby Stärkelsen for providing the potato starch and preparing the barley starch samples. We also thank Camilla Olsson at SIK (Swedish Institute for Food and Biotechnology) for providing us with viscosity data on mechanically treated starch.

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