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Carbohydrate Polymers 47 (2002) 181–189

Carbohydrate Polymers

www.elsevier.com/locate/carbpol

Study of a preparative-scale process for the production of amylose

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Received 21 December 1999; revised 24 November 2000; accepted 21 December 2000

Abstract

A method for the preparative-scale production of amylose was studied. Wrinkled pea starch was chosen as the starting material because the amylose content of this starch is especially high. The main steps in the process were production of a molecularly dispersed starch solution by pressure cooking the starch and debranching the amylopectin with the industrial enzyme, Promozym®, from Novo. The resulting product contained the amylose and oligosaccharides from debranched amylopectin. The amylose was complexed with butanol-1 and the complex was separated from the solution by centrifuging, while the oligosaccharides remained in solution. Butanol-1 was removed by reflux cooking of the sediment in water. Then the amylose was precipitated with ethanol and dried. The temperature for the production of the starch solution was optimized so that the starch was molecularly dispersed but not appreciably degraded. The industrial debranching enzyme, Promozym®, contains also a small amount of glycosidic activity, Consequently, the quantity of enzyme had to be adjusted to ensure that degradation of the amylose was not excessive. Two amylose samples with different molar mass distributions were prepared on a kilogram scale.

Keywords: Starch; Amylose; Pullulanase; Debranching; Molecular weight; Molecular weight distribution; HPSEC–MALLS

1. Introduction

Materials from renewable resources such as starch have shown promising development and received increasing interest in recent years. The importance of starch for such materials has been described in several papers (Doane, 1994; Lloyd & Kirst, 1963; Röper & Koch, 1990;), but relatively little is known about the influence of the amylose/amylopectin ratio on the mechanical properties of these materials (Lourdin, Valle & Colonna, 1995). To evaluate the potential of starch for the use in material production, an investigation of the pure amylose and amylopectin components was needed (Fink et al., 1998). Amylopectin is easily available from waxy maize starch, but pure amylose is not available on the market in sufficiently large amounts. The aim of this work is to develop an approach which could be used for the production of amylose on a preparative scale.

The fractionation of starch into the two polysaccharides, amylose and amylopectin, can be carried out by a number of methods because of their different structural behaviour. The degree of branching and the molecular weight distribution of amylose and amylopectin cause differences in solubility and diffusion behaviour, hydrodynamic properties and complexing reactions. Preparative methods of amylose separation, such as extraction of amylose which has diffused from the starch granule (Adkins & Greenwood, 1966), complexing of amylose and precipitation (Adkins & Greenwood, 1969; Ceh, Stropnik & Leskovar, 1985; Banks, Greenwood & Muir, 1991), or the combination of both (Mua & Jackson, 1995), primary precipitation of amylopectin (Matheson, 1990; Matheson & Welsh, 1988; Stepanenko & Avakyan, 1974), preparative ultracentrifugation (Montgomery, Sexson & Senti, 1961), gel permeation chromatography (GPC) (Ebermann & Schwarz, 1975; Kennedy, Rivera, Lloyd & Warner, 1992;Radosavljevic, 1991; Taki, Suzuki, Taki, Hisamatsu & Yamada, 1988) and debranching of amylopectin by enzymes (Ong, Jumel, Tokarczuk, Blanshard & Harding, 1994) have been described. In many cases, a combination of different methods proved most effective.

Only small amounts of the pure components could be produced by ultracentrifugation and size exclusion chromatography (SEC). Three methods are suitable for the production of amylose on a kilogram-scale: separation of amylose diffused from the granule, complexing of amylose after complete destruction of the starch granular structure by different dissolution procedures, or preparation of amylose after debranching of amylopectin. These methods were investigated recently for the separation of potato amylose (Haberer, 1999). The results were evaluated by characterizing

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0144-8617/02/$ - see front matter
PII: S0144-8617(01)00164-3

the molecular weight distribution of the fractions produced by SEC method with multiangle laser light scattering (SEC-MALLS) and by the iodine reaction. Diffusion amylose is mainly the low-molecular-weight fraction of amylose and contains a certain amount of amylopectin, depending on the leaching temperature (Haberer, 1999; Roger & Colonna, 1993). Further cleaning steps were required to obtain pure diffusion amylose. Complexing of amylose, after different dissolution procedures for the starch, initially yields an amylose fraction with about 10% amylopectin. Two or more further dissolution and complexing steps were necessary to obtain pure amylose (Haberer, 1999).

The most promising method was isolation of amylose after enzymatic debranching of the amylopectin. Debranching enzymes catalyze the hydrolysis of α-1,6-glycosidic bonds in amylopectin and/or glycogen. With regard to their specificity, they are divided into two groups: pullulanases and isoamylases. Pullulanase is able to split off α-1,6-glycosidic bonds with at least two or more α-1,4-glycosidic-bonded glucose units in the chain. Isoamylase is not able to split off very short side chains with two or three glucose units. The preparation of very pure debranching enzymes is only possible on a very small scale, as these preparations were used only for analytical purposes. The technical pullulanase enzyme, Promozym®, contains also a low amount of glycosidic activity. As a result of the action of this enzyme, not only does debranching of amylopectin take place but also a certain degradation of amylose. To ensure the optimum debranching effect of the enzyme, the starch polysaccharide must be in a molecularly dispersed state of solution.

This paper will describe a process for the preparation of amylose from starch using the debranching method on amylopectin, followed by butanol-1 complexing of the amylose. The production of amylose comprises the following main steps:

Wrinkled pea starch was chosen as the starting material because of its high amylose content. The molecularly dispersed solution was obtained by pressure cooking at the optimum temperature under a nitrogen atmosphere.
The amylopectin was debranched using the industrial pullulanase, Promozym®. Estimation of the optimum enzyme addition and reaction conditions for the Promozym® were therefore necessary steps.
After the debranching reaction, the solution contains amylopectin side chains and some oligomers in addition to the amylose.
The amylose was separated from the reaction mixture by complexing with butanol-1.
After removal of the butanol, the amylose was precipitated with ethanol and dried.

Investigations into the reproducibility of the whole process from preparation of the starch solution and debranching through to isolation were accompanied by analysis of the composition of the solutions and isolated fractions.

2. Materials and methods

2.1. Material

Wrinkled pea starch was purchased from Amylose KG Stauderer (Germany). The amylose content of the wrinkled pea starch sample was 75% as determined by iodine titration (Richter, Augustat & Schierbaum, 1969).

The enzyme, Promozym® 200L, was supplied by Novo Nordisk A/S (Denmark) with a debranching activity of 200 U ml^{-1}.

2.2. Analytical methods

The weight average molecular weight M_w, number average molecular weight M_n, and molecular weight distribution of the starch samples after pressure cooking, after debranching and of the purified amylose samples were determined by HPSEC–MALLS. The HPSEC system consisted of a 600MS pump module, 717 autoinjector, column compartment, RI-detector 410, and MALLS detector Dawn-F-DSP laser photometer (Wyatt Technology, Santa Barbara) fitted with a S2 flow cell and an Ar-ion laser operating at $\lambda_0 =$ 488 nm and equipped with 18 detectors at angles ranging from 7.5 to 157°. The columns used were Waters Styragel HMW 7, HMW 6E and HT 3 with dimensions of 300×7.8 mm. Elution of the samples was carried out with dimethyl sulfoxide (DMSO) containing 0.09 m $NaNO_3$ at a flow rate of 0.5 ml min^{-1} and a temperature of 60°C. 100 μl of a 0.5% solution were injected. The MALLS detector was serially connected with the refractive index detector (DRI). During a sample run on the HPSEC–MALLS system, the data from the DRI and MALLS detector were collected and processed using ASTRA software 4.70.07 to give the molecular weight M_i at each retention volume. The basis for the absolute characterization of macromolecules by HPSEC-light-scattering experiments was described by Wyatt (1993)).

Amylose content was established according to Richter et al. (1969). Iodine spectroscopy has been described elsewhere (Schierbaum, Radosta, Richter, Kettlitz & Gernat, 1991). The maximum absorption of the polysaccharide–iodine complex was estimated at two iodine concentrations, (λ_{max1} at a low (1×10^{-4} N) and λ_{max2} at a high (4×10^{-4} N) iodine concentration.

2.3. Preparative methods

Because starches are not molecularly soluble in boiling water, heating has to be carried out under autoclaving conditions while stirring.

2.3.1. Laboratory-scale investigations

These investigations were carried out in a *Roth* laboratory autoclave with a maximum capacity of about 200 ml. The procedure for dissolving starches in this autoclave has been described elsewhere (Aberle, Burchard, Vorwerg &

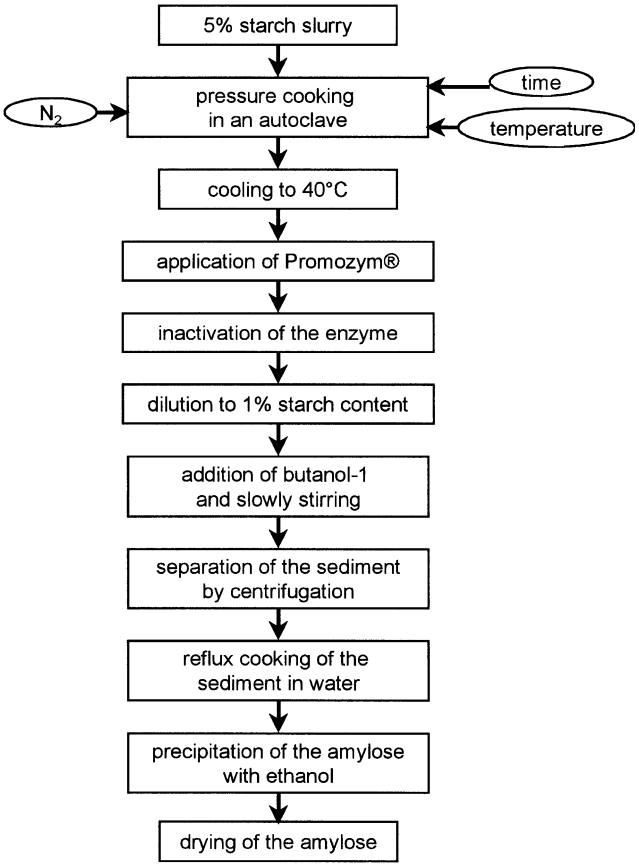

Fig. 1. Diagrammatic representation of the method for amylose preparation.

Radosta, 1994). After pressure cooking, the solutions were quenched by immersing the autoclave in cold water. Part of the solution was diluted with DMSO for GPC analysis. To investigate the debranching action of Promozym®, the wrinkled pea starch solution was transferred from the autoclave to a temperature-controlled, stirred tank, which was held at 40°C. The pH was adjusted to 6 and the required amount of debranching enzyme (12.5–150 U Promozym®/g starch) was added. The debranching reaction was carried out under gentle stirring for 15 min. The enzyme was deactivated by cooking at 100°C for 10 min. Again a part of the solution was diluted with DMSO for GPC analysis.

2.3.2. Pilot-scale investigations

For pilot-scale investigations, an autoclave with a calculated maximum volume capacity of 15 l and an anchor agitator was used. The reactor had an oil recirculation heating system with a heating power of 9 kW. This heating system is the limiting factor for the temperature–time program used. Cooling of the reactor was controlled by the same driving unit that controlled the heating. A special cooling system, flushed with tap water, was used to cool part of the circulating heating medium. For the preparation of the pea starch solution eleven litres of distilled water were preheated in the reactor to 40°C. 600 g of the starch (d.m.) with 1 l cold water were suspended in the preheated water while stirring (80 min^{-1}). The reactor was closed and flushed with nitrogen up to a pressure of 6 bar. Then the temperature was raised to 150°C over about 30 min while stirring the suspension. The reactor pressure, after reaching the required temperature, was 9.5 bar. Pressure cooking was done for 20 min at 150°C. After that, the reactor was cooled down to 95°C as quickly as possible. Usually, it took 20 min for this cooling procedure. The reactor pressure was reduced to normal pressure and the reactor was opened. The starch solution was homogenized with a Turrax (IKA Ultra-Turrax T50, 10 000 min^{-1}) for 2 min. At this point, a sample was taken to investigate the molecular weight distribution of the starch. The reactor was then cooled down to 40°C over 50–60 min. For debranching the amylopectin, the required amount of pullulanase (100 and 200 U Promozym®/g starch) was added. After 15 min, the enzyme was inactivated by heating to 100°C. A sample was taken to investigate the molecular weight distribution of the debranched starch sample. The hot solution was diluted with warm water to 1% starch content and cooled down to 70°C. Butanol-1 (42 g l^{-1}) was added to the solution and it was stirred gently for 1 h. Then the solution was cooled down to 40°C over 4 h and again stirred (25 min^{-1}) overnight. The nearly clear supernatant was decanted from the bottom product and

Table 1
Molecular weight, recovery rate, and iodine absorption maximum of pea starch solution after disintegration of the starch granular structure in the mini autoclave. Conditions: 5% dry matter, 20 min, nitrogen atmosphere, 9.5 bar

Temperature (°C)	M_w (10^6 g mol^{-1})	Recovery (%)	Iodine absorption	
			λ_{max1} (nm)	λ_{max2} (nm)
140	15.7	75	629	625
150	15.9	85	626	625
155	16.3	88	625	619
155	17.5	89	625	620
155	18.5	89	625	619
160	17.3	92	619	619

the amylose–butanol complex was separated from the moist sediment by centrifugation (Heraeus-Cryofuge 6000) at 4200 min^{-1} for 20 min. The amylose–butanol-1 complex was homogenized with water and transferred, together with 6 l water, into a round-bottom flask with stirrer, reflux cooler and drip funnel. The solution was heated to boiling for some minutes to destroy the amylose–butanol complex. After that, the solution was cooled down to 70°C and at this temperature the amylose was precipitated with 9 l ethanol over 2 h. The sedimented amylose was suction filtered on a sintered glass frit, suspended in ethanol and heated to boiling to dehydrate the amylose. The cooled suspension was again filtered on a sintered glass frit and the amylose was dried in a vacuum oven (12 mbar, 50°C, 60 h). A sample of the amylose so-produced was dissolved in DMSO for GPC analysis.

Fig. 1 summarizes the main steps of the amylose preparation process.

3. Results and discussion

To find the optimum dissolving conditions for wrinkled pea starch and for the debranching procedure, laboratory-scale investigations were carried out with an agitated mini autoclave (Roth).

3.1. Preparation of a molecularly dispersed starch solution

Under certain conditions, dissolving starches in DMSO yields a molecularly dispersed solution. The average molecular weight determined for wrinkled pea starches in DMSO was about $15(\pm 3) \times 10^6$ g mol^{-1}. To obtain a molecularly dispersed solution of pea starch in water, this molecular weight range has to be achieved by pressure cooking in an autoclave. From preliminary studies, the optimum pressure cooking time was established as 20 min. The higher the concentration of starch in the solution, the higher would be the amylose yield. The highest concentration that gave a homogeneous starch solution was 5% dry matter. At higher concentrations, aggregation of starch occurred so quickly that heterogeneous solutions were produced. The influence of the pressure cooking temperature on the molecular weight distribution of the wrinkled pea starch is shown in Table 1 and Fig. 2. All the temperatures between 140 and 160°C yielded molecular weights in the range expected for wrinkled pea starch (for 155°C three parallel measurements were undertaken). On the basis of recovery rates from the HPSEC, it could be assumed that the starch granular structure was not dissolved fully at a temperature of 140°C. The solution may have contained particles or aggregates that were too large to be separated by HPSEC. A small

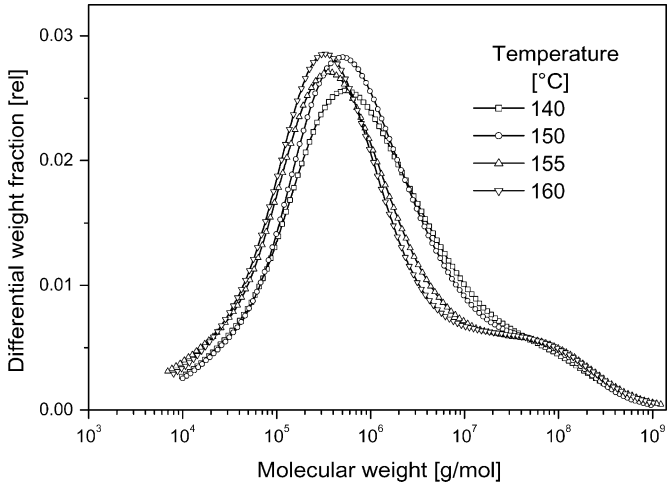

Fig. 2. Molecular weight distribution of wrinkled pea starch dissolved at different autoclaving temperatures.

Table 2
Weight average molecular weights of wrinkled pea starch samples after debranching with Promozym®

Enzyme dose (U g^{-1} starch)	M_w (10^6 g mol^{-1})
12.5	1.940
25	1.350
50	0.930
100	0.274
150	0.180

part of the amylopectin may be retained in the membrane filter prior to HPSEC or on the SEC column. This could also be concluded from the molecular weight distributions in Fig. 1, where the samples dissolved at higher temperatures contain a somewhat higher amount of amylopectin in the molecular weight range between 4×10^7 and 10^9 g mol^{-1}. The higher content of amylopectin could also explain the increasing M_w values at higher autoclaving temperatures. The recovery rate rose with increasing autoclave temperature, indicating a better solution state of the polysaccharides. The absorption maximum λ_{max} of the iodine–amylose complex, however, was shifted to lower wavelengths. The lower the wavelength, the shorter the amylose chains that react with iodine (Schierbaum et al., 1991). The decrease in the maximum absorption wavelength with increasing solution temperature and the shift of the amylose peak to lower molecular weight indicated that degradation of the amylose was beginning. For this reason, a temperature of 150°C was chosen for autoclave preparation of wrinkled pea starch solutions in the pilot-scale process.

3.2. Investigations into debranching

Five different amounts of the enzyme, Promozym®, were added to the wrinkled pea starch solution to investigate the debranching action. The results of these experiments are summarized in Table 2 and shown in Fig. 3.

As mentioned earlier, not only does debranching take place but also a certain degradation of the amylose. Fig. 3 shows the chromatograms and the absolute calibration curves of the samples after the action of different amounts of the enzyme. It can be concluded that with 12.5 and 25 U Promozym® /g starch, the debranching action was not complete. A certain amount of amylopectin was preserved in the samples and the M_w were higher than 1×10^6 g mol^{-1}. The absolute calibration curves showed two distinct slopes over elution time in the chromatogram region. The lower slope in the first part of the chromatogram was characteristic of residual amylopectin and the somewhat higher slope in the second part was due more to the amylose. With 50 U Promozym®/g starch, the amylopectin fraction in the solution was further diminished and the slope of the calibration curve was more strongly influenced by the amylose fraction. The cleaved amylopectin chains and the oligosaccharides could be observed as a shoulder at the low-molecular-weight end of the chromatogram. The last peak of the chromatogram was related to the amount of enzyme added. With 100 U Promozym®/g starch, the calibration curve was linear and the debranched sample contained only amylose and the cleaved side chains of the amylopectin. The highest enzyme addition of 150 U g^{-1} starch delivered a slightly degraded amylose and again the amylopectin side chains. Because the absolute calibration curve of this sample was superimposed on the sample with 100 U g^{-1} starch, the shift of the concentration signal of the amylose peak to higher elution volumes could be seen to represent a lower molecular weight.

A further increase in enzyme addition reduced the M_w of the amylose but was also associated with difficulties in the procedure. The short-chain amylose tended to aggregate, even during the debranching procedure, and was precipitated from the solution. The aggregates were very stable and were not completely dissolved, even by pressure cooking. As a result, the amylose yield was considerably reduced at the higher enzyme additions.

The chromatograms of the debranched pea starches (Fig. 3) showed an interesting phenomenon. The peak maximum of the amylose concentration signal was unexpectedly shifted to lower elution volumes, corresponding to higher

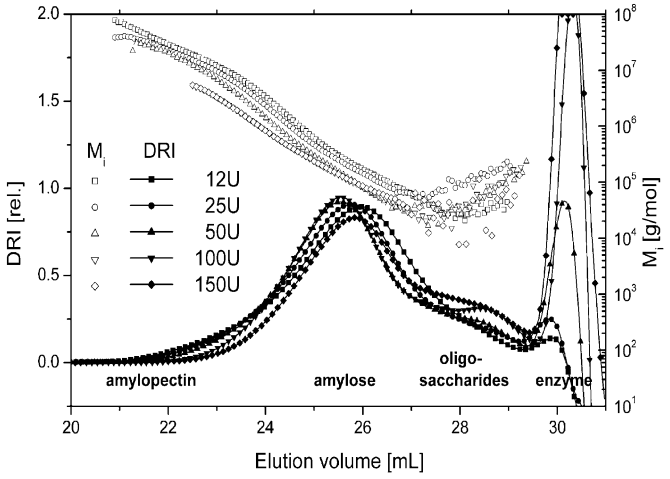

Fig. 3. HPSEC chromatograms and absolute calibration curves for wrinkled pea starch solutions debranched with different amounts of Promozym®.

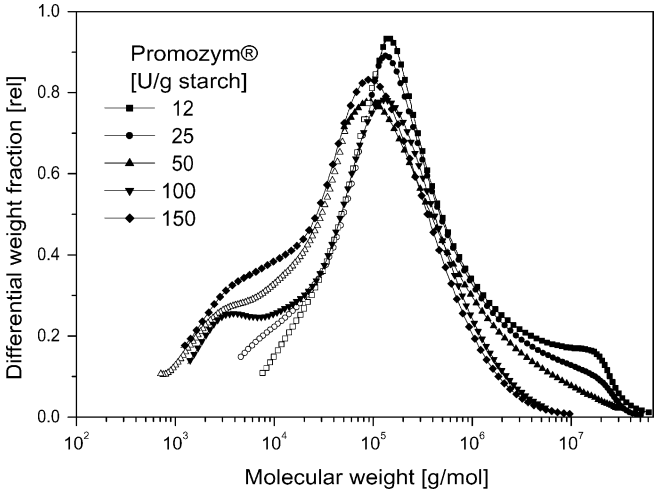

Fig. 4. Molecular weight distribution of wrinkled pea starch solutions debranched with different amounts of Promozym®. The filled data points were calculated with the fitted calibration curve and the open data points come from linear extrapolation to the low-molecular-weight range.

hydrodynamic volumes, with increasing enzyme addition up to 100 U g^{-1} starch. This would imply that the hydrodynamic volume of the amylose was increased in the absence of large amounts of amylopectin. On the other hand, the absolute calibration curves were moved slightly towards lower molecular weights with increasing enzyme addition. Consequently, the resulting M_w were decreased and the molecular weight distributions (calculated from the concentration signal and the absolute calibration curve) were displaced towards lower M_i values with increasing enzyme addition. To calculate the molecular weight distributions, the absolute calibration curves for the samples debranched with 12.5 to 50 U Promozym®/g starch were fitted fifth degree polynomial and the last part was linearly extrapolated. The calibration curves for 100 and 150 U were linearly fitted. Fig. 4 shows that the molecular weight distribution curve and especially the peak maximum of the amylose peak was relocated to lower M_i values with higher enzyme addition.

3.3. Pilot-plant production

3.3.1. Amylose with a molecular weight of about 2×10^5 g mol^{-1}

The analytical results for 17 different batches of amylose

Table 3
M_w of wrinkled pea starch after pressure cooking, after debranching reaction (100 U Promozym®/g starch), and of the amylose after butanol-1 complexing (n.d.: not determined)

Batch number	M_w after pressure cooking (10^6 g mol^{-1})	M_w after debranching (10^5 g mol^{-1})	M_w after complexing (10^5 g mol^{-1})
18	9.779	2.245	1.939
19	11.010	2.587	1.932
20	11.060	n.d.	2.396
21	11.400	3.280	1.684
22	9.892	2.039	2.031
23	13.570	2.631	2.151
24	10.790	2.273	1.910
25	8.269	3.154	2.016
26	11.330	2.657	2.190
27	14.100	2.633	2.010
28	15.050	2.780	1.960
29	11.890	2.689	2.502
30	13.940	2.739	2.416
31	10.240	3.124	2.157
32	14.750	3.022	2.105
33	18.230	2.631	2.351
34	12.390	2.524	2.045
Average	12.217 ± 2.379	2.672 ± 0.304	2.106 ± 0.207
Mixture of batch 21–34, calculated			2.109 ± 0.205
Mixture of batch 21–34, measured			1.970

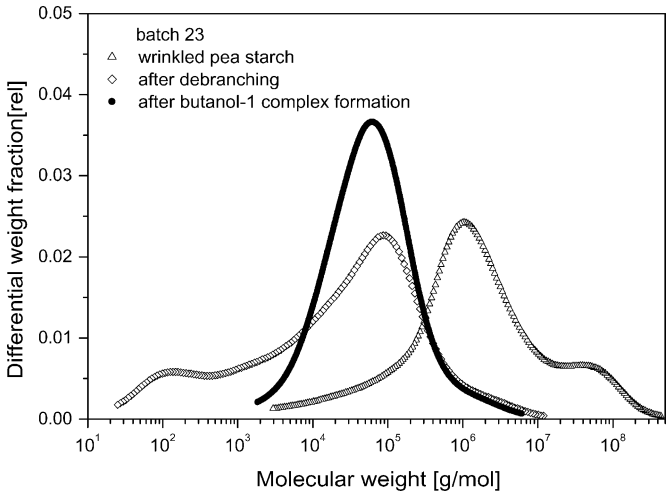

Fig. 5. Molecular weight distribution of wrinkled pea starch solution, debranched wrinkled pea starch and amylose after butanol-1 complexing for batch 23.

produced on a pilot-plant scale and debranched with 100 U Promozym®/g starch are summarized in Table 3. The reproducibility of the batch production of wrinkled pea starch solutions was relatively good. The average M_w was $12.22 \times 10^6(\pm 19.5\%)$ g mol^{-1}. This value was somewhat lower than that for dissolving in the Roth autoclave. The reason may be that the heating procedure took a longer time in the larger autoclave and the mechanical agitation was more intensive so that disintegration of the starch and especially the amylopectin was improved. After debranching the amylopectin fraction, the M_w was reduced to $2.67 \times 10^5(\pm 11.4\%)$ g mol^{-1}. As a consequence of the debranching reaction, the product contained the amylopectin side chains in an amount up to 20% d.m. and some lower oligosaccharides due to the amylolytic activity of the Promozym®. The molecular weight distribution of the debranched sample was, therefore, very broad. These short chains could modify the mechanical properties of amylose products to a considerable degree. To prevent this influence, the amylopectin side chains and other oligosaccharides contained were separated from the mixture by complexing the amylose with butanol-1. The M_w of the pure amylose was determined as $2.11 \times 10^5(\pm 9.9\%)$ g mol^{-1}. The removal of the short chains from the mixture should result in a higher M_w for the separated amylose but this was not the case. The value found for the amylose, after complex formation, was somewhat lower than the M_w of the sample with the oligosaccharides. The reason for this slight diminution could be the loss of a small amount of high-molecular-weight amylose during reprocessing of the amylose–butanol-1 complex.

Table 4
M_w of wrinkled pea starch after pressure cooking, and of the amylose after butanol-1-complexing (200 U Promozym®/g starch)

Batch number	M_w after complexing (10^5 g mol^{-1})
38	0.572
39	0.589
40	0.548
41	0.556
42	0.555
43	0.528
44	0.554
Mixture of batch 38–44, calculated	0.557 ± 0.020
Mixture of batch 38–44, measured	0.580

The recovery rate from the HPSEC columns was between 95 and 100% for the wrinkled pea starch samples after debranching and also for the amylose samples after butanol-1 complex formation. A sub-fractionation of the samples during chromatography could be excluded therefore.

The ASTRA program makes it possible to calculate the M_n values of the molecular weight distribution and the polydispersity index, P, as the quotient of M_w/M_n. The prerequisite for this calculation is that every slice of the chromatogram contains only one species of molecule. This prerequisite is not perfectly fulfilled in the SEC separation therefore only the range of the calculated polydispersities should be considered. Absolute polydispersity values estimated by a separate method could be different from these calculated values. Nevertheless, the calculated polydispersities impart information about the width of the molecular weight distribution. The polydispersity of the wrinkled pea starch solutions after pressure cooking were calculated as $35(\pm 20\%)$. After the debranching reaction, polydispersity was increased by about one order of magnitude to about $300(\pm 20\%)$. The butanol-1 complexing reduced the polydispersity of the amylose produced to $8.5(\pm 11\%)$ and to $5.0(\pm 11\%)$ in the case of the lower molecular weight amylose (see Section 3.3.2). Modification of the width of the molecular weight distribution as a result of the different processing steps is shown in Fig. 5, using the example of batch 23.

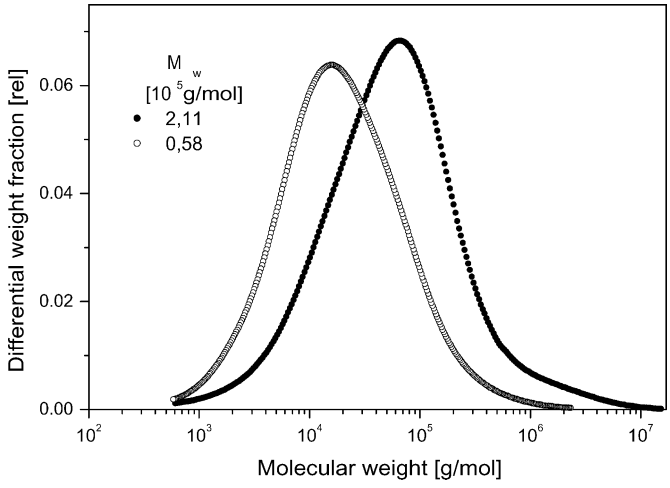

Fig. 6. Comparison of the molecular weight distribution of two amylose samples.

The amylose yield in relation to the starch mass was 51% and in relation to the amylose content 68%. The amylose content of the high-molecular-weight amylose produced was 98(±2)% as determined by iodine-binding capacity.

3.3.2. Amylose with a molecular weight of about 6×10^4 g mol^{-1}

The lower molecular weight of the amylose was achieved by increasing the enzyme addition to 200 U per g starch and extending the hydrolysis time to 20 min. The relatively short-chain amylose showed a retrogradation tendency even during hydrolysis. For this reason, the solution was heated to 150°C after hydrolysis to obtain a filterable solution after cooling to 98°C. The amylose was also reprocessed with butanol-1 to obtain a narrower molecular weight distribution. The results for seven batches of this low-molecular-weight amylose are summarized in Table 4. In this case only the M_w of the amylose after butanol-1 complexing was recorded because the product after debranching contained too many aggregates as a result of retrogradation; thus, SEC-MALLS analysis was not possible. The M_w of the mixture of seven batches was 5.8×10^5 g mol^{-1}. The molecular weight distribution of the mixture of the seven batches of this amylose sample in comparison with the mixture of 14 batches of the higher-molecular-weight amylose sample is shown in Fig. 6.

The yield of the low-molecular-weight amylose was about 40% in relation to the wrinkled pea starch and about 55% in relation to the amylose. The determination of iodine-binding capacity was not possible because the low-molecular-weight amylose retrograded too fast in the analysis solution.

4. Conclusions

A process for the preparative-scale production of amylose by debranching the amylopectin and complexing the amylose with butanol-1 from the solution mixture was investigated. Wrinkled pea starch was chosen as the starting material because of the high amylose content but it is possible to take any other starch raw material as a basis for this process.

Optimum dissolution of the wrinkled pea starch was reached at a temperature of 150°C over a time of 20 min under a nitrogen atmosphere with a pressure of 9.5 bar. The required units of Promozym® amounted to 200 U g^{-1} starch with a reaction time for debranching the amylopectin of 20 min. The inactivation of Promozym® was carried out by heating the reaction solution to 100°C. The amylose was separated by complexing with butanol-1 and centrifuging at 4200 min^{-1} for 20 min. The amylose–butanol complex was dissolved in water and amylose was precipitated with ethanol, separated and dried.

The reproducibility of the process was very good. The M_w of the separated amylose could be varied by using different amounts of Promozym®. Two amylose samples with different M_w of 2.1×10^5 and 5.8×10^4 g mol^{-1} and different molecular weight distributions were produced on a kilogram scale. The highest M_w would be possible with pure pullulanase, but this would generally increase the costs of the process.

Acknowledgements

This work was supported financially by the German Federal Ministry for Agriculture (No. 93 NR 136-F)

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