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MODIFICATION OF BRANCHING PATTERN OF POTATO MALTODEXTRIN WITH Q-ENZYME

Abstract

Pure branching enzyme (Q-enzyme [(1→4)-α-D-glucan: (1→4)-α-D-glucan 6-glucosyltransferase, EC 2.4.1.18]) could be isolated from a crude potato tubers extract by means of a sequence of LC-techniques (HIC and IEC); Q-enzyme accepts native and synthetic non-branched (amylose-type) glucans as substrates and increased the percentage of branching by a combined hydrolytic and transfer-activity. Q-enzyme accepts technical-grade potato-maltodextrin as substrate: the resulting branching patterns depend strongly on the incubation temperature. Determined molecular-level characteristics clearly show the influence of modified branching pattern on glucan-coil dimensions, conformation and interactive properties. In particular, modification of interactive characteristics on the molecular level is strongly suspected to control macroscopic/technological qualities of starch-based materials such as gelation potential or freeze/thaw-stability.

Introduction

In the recent years an enhanced variability of starches on the molecular level was developed by new and improved breeding-techniques of starch containing plants, gene technology and enzymatically catalyzed modification of starches. Application of hydrolases for instance, produces a wide range of different starch hydrolizates with characteristics primarily controlled by the amount of applied enzymatic activity. Activity of transferases, such as Q-enzyme, is not tested yet, because no pure enzyme was available up to now.

A first step to improve understanding of the background of technological properties of starch containing goods is the development of analytical strategies to obtain reliable information about molecular-level characteristics of starch. Therefore, laboratory-made glucans of specific and varying molecular characteristics with respect to

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branching pattern, molecule dimension, conformation and interactive potential need to be established to investigate correlations between the molecular and the macroscopic/technological level.

A first kind of laboratory-made glucans are synthetic amyloses, non-branched α(1→4)-linked glucans, which are studied now for years already. *In vitro* synthesis of amylose by means of phosphorylase was introduced by Pfannemüller who investigated functionality of non-branched starch-glucans [1-3]. Sshe isolated highly active phosphorylase from potato and stabilized it to maintain constant substrate turnover during synthesis. Such Phosphorylase-catalyzed syntheses with glucose-1-phosphate as substrate and maltooligomers of dp≥3 as starters provide quite uniform polymers with a degree of polymerization which simply is controlled by the concentration of the starter-oligomers.

But non-branched glucans are just one kind out of a wide range of starch glucans if the branching pattern is considered as criterion for discrimination. Thus, for an appropriate correlation of molecular glucan-characteristics with macroscopic level starch-properties, additionally branched 'amylopectin'-type glucans with α(1→4) and more or less α(1→6) linked branches need to be investigated.

For the modification of amylose-type, purely α(1→4)-linked non-branched, glucans into amylopectin-type, short-chain branched glucans, branching-enzyme Q-enzyme [(1→4)-α-D-glucan: (1→4)-α-D-glucan 6-glucosyltransferase, EC 2.4.1.18] needs to be isolated from plants and stabilized after purification and during application [4-6]. Complexity is even increases as Q-enzyme owns a twofold activity: hydrolytic and transfer activity. As a result, depending on reaction conditions and amylose-characteristcs, a wide range of differently branched glucans may be achieved by the application of Q-enzyme. The scheme of transfer-activity of Q-enzyme is illustrated in Fig. 1 with non-branched glucan (nb-Glc) as substrate for the formation of short-chain branched glucans (scb-Glc).

$$\text{nb-Glc}_n + \text{nb-Glc}_m \longrightarrow [\alpha(1\rightarrow 4)_n\ \alpha(1\rightarrow 6)_n]\text{-scb-Glc}_{n+m}$$

Fig. 1. Scheme of modification of non-branched glucans (nb-Glc) into short-chain branched glucans (scb-Glc) by the transfer-activity of Q-enzyme.

Isolation and purification of Q-enzyme from potato and application of the stabilized branching enzyme [(1→4)-α-D-glucan: (1→4)-α-D-glucan 6-glucosyltransferase, EC 2.4.1.18] on technical-grade potato maltodextrin as substrate will be presented and discussed. Additionally, the consequences of modification of the

maltodextrin branching pattern on the physico-chemical characteristics will be illustrated.

Experimental

Material

Potato tubers, *vs.* Fambo, which were utilized to extract Q-enzyme, were grown in Großenzersdorf, Lower Austria/Austria at controlled conditions. Technical-grade potato maltodextrin (C-Pur 1906), which was used as substrate for the purified Q-enzyme, was provided by Cerestar/Belgium.

Preparation of the crude Q-enzyme extract from potato tubers

500 g freshly harvested potatoes were washed, peeled, cut in small pieces and homogenized after mixing with 20 mL of 4°C Cleland-buffer pH 7.3 [50 mM Tris (2-amino-2-(hydroxymethyl)-1,3-propandiol), 2.5 mM DTT (1,4-dithiothreitol), 5 mM EDTA (etylendiamintetraaceticacid), set to pH 7.3 by means of citric acid]. Particles exceeding the millimeter range were removed from the cooled (4°C) suspension by filtration, starch and starch-accompanying materials by centrifugation (20 min, 4°C, 30000 g). The clear filtrate was mixed with ammonium sulfate to yield a solution percentage of 20% and was stored over night for protein precipitation. The resulting precipitate was removed from the solution by centrifugation (20 min, 4°C, 30000 g). Then, ammonium sulfate concentration in the supernatant was increased to 50% and left once again over night at 4°C. This second precipitate again was removed from the solution by centrifugation (20 min, 4°C, 30 000 g), resuspended in a small volume of 0.01 M Cleland buffer and stored at -80°C under N_2 for final purification.

Purification of the crude Q-enzyme extract by means of Hydrophobic Interaction Chromatography (HIC)

15 mL of the -80°C/N_2-stored crude protein extract is applied to the Fractogel TSK Butyl-650 (M) HIC-system (Merck/FRG; 70x50 mm) which was equilibrated with Cleland-buffer dissolved 30% ammonium sulfate. Elution of different protein fractions at a flow rate of 4 mL/min was achieved by a step-gradient of decreasing ammoniumsulfate concentration: 30% → 20% → 15% → 10% → 0%. The 10%-fraction was collected and proteins precipitated over night by increasing ammonium sulfate concentration to 50%. The precipitate was separated from the supernatant by centrifugation (30 min, 4°C, 13000 g) and resuspended in a small volume of 0.01 M Cleland-buffer. The obtained enzyme could be stored without significant loss of activity for several days at 4°C.

Purification of an HIC-fraction of the crude Q-enzyme extract by means of Ion-Exchange Chromatography (IEC)

Before the proteins of the HIC-10%-eluted pool were applied on the ion-exchange matrix, the solution was de-salted by means of Centriprep-vials (Amicon, No.4306, cut-off 30 kDa) by two-times adding 0.01 M Cleland-buffer. Then, the obtained 15 mL of de-salted protein-solution was applied to a DEAE (diethylamionoethyl-cellulose)-matrix (Merck/FRG; Fractogel EMD DEAE 650 (S); 150x26 mm) and eluted with 0.01M Tris buffer and a NaCl step-gradient: 0.00 M → 0.30 M → 0.35 M. The major amount of Q-enzyme is eluted at 0.35 M NaCl conditions and proofed to be free of any kind of side-activities at electrophoretic tests.

PAGE of branching- and hydrolytic-enzyme: activity-staining [7]

For verification of branching activity of the obtained enzyme and to distinguish branching activity from purely hydrolytic activities, PAGE (polyacryl gel electrophoresis) with activity-staining was performed with a 12% PA-gel and 1% starch-containing gel on a Mini-Protean II (Biorad/FRG; P_{max}: 20 W, I_{max}: 70 mA, U_{max}: const. 200 V, gel thickness: 1mm); collector-gel: 4% PA ; collector-gel-buffer: 0.5M Tris with citrate acid at pH 6.8; separation-buffer: Tris/glycin pH= 8.3 (3.0g Tris and 14.4g glycin in 2 L Deionat). For activity staining after separation the gels were carefully washed with pure water and then equilibrated over night with incubation-buffer (50 mM Tris, 2 mM ascorbic acid set with citric acid to pH 7.5). The surface-cleaned equilibrated gel then is put for 30 min into an iodine-solution (0.1 g iodine, 1.5 g KJ per 1 L Deionat) for staining of reaction products.

Photometric test of Q-enzyme activity with iodine staining

1-2 mg of long-chain branched (lcb) starch glucan (Sigma S-4501) is dissolved in 1 mL Cleland buffer and mixed with an aliquot of enzyme-solution and stored for 48 hours at room temperature. Enzymatic activity is determined for test- and blind-mixtures by photometric scanning of the maxima of formed iodine/starch-complexes. The photometrically investigated solutions contain: 500 µL test-solution, 2 mL Deionat, 200 µL iodine solution (0.1 g iodine, 1.5 g KJ for 1 L Deionat).

Incubation of Q-enzyme with aqueous dissolved potato maltodextrins

A 40mg/mL solution of potato maltodextrin in 0.01M Cleland-buffer pH 7.3 was obtained by slightly raising temperature. 25 mL of this solution was diluted with buffer (blank) or Q-enzyme solution to yield a volume of 50 mL. A small amount of NaN_3 was added to the glucan-solution to prevent microbial growth; oxidation of Q-enzyme is prevented by N_2-atmosphere and reductive conditions in the solution. The batches were kept at two reaction-temperatures: 4°C and 20°C. After 5 days of incubation the

samples were analyzed with respect to the absorption spectra of glucan/iodine-complex and provided for further and more detailed destructive and non-destructive investigations.

Destructive analysis of Q-enzyme modified maltodextrin: controlled debranching with pullulanase and isoamylase combined with LC-fragment analysis

3 mL of each enzyme/substrate-solution was set to pH 3.7 with acetic acid/acetate-buffer, mixed with 10 µL isoamylase-suspension (Hayashibara Biochem. Lab./Japan, Lot No. 30600) and kept at 50°C for 24 hours. Then the solution was set to pH 5.5 with 0.1 M NaOH and mixed with 5 µL Pullulanase-suspension (Hayashibara Biochem. Lab./Japan, Lot No. 002232). After 6 hours at 37°C once again 5 µL of Pullulanase-suspension was added. After 24 hours at 37°C the solution was short-time boiled to denaturate proteins completely and then diluted 1:4 with Deionat for LC-analyses of obtained glucan-fractions.

50 µL of the completely debranched glucan fractions, representing the constituting glucan-chain length distribution, were applied to a Carbopack PA 100 (Dionex, 4x200 mm) and eluted at a flow rate of 0.8 mL/min with a continuos gradient starting from H_2O/1 M NaOH $\rightarrow$ 1 M NaOH/1 M NaAc. Detection of eluted carbohydrates was done with an electrochemical detector.

Non-destructive analysis of Q-enzyme modified starch-glucans: SEC-DRI/LALLS

Absolute molecular weight of the glucan-chain distributions was determined by means of size-exclusion chromatography combined with dual detection of scattering intensity (low angle laser light scattering device) and mass (differential refractive index detector (SEC-DRI/LALLS). 200 µL of each sample solution was separated on a series of SEC-columns (TSK/Japan; PW6000, 5000G, 4000G, 3000G: 300+300+300+300 x 7.5 mm) at a flow rate of 0.8 mL/min with 0.1 M aqueous NaCl as eluent. Individual SEC-separated fractions were detected with respect to their scattering intensity at a scattering angle of 5° (TSP/USA; KMX-6; λ=632 nm) and with respect to their mass (Wyatt/USA; Optilab 903, interferometric differential refractometer λ=630 nm). Data acquisition was performed with software package CODAwin, data processing and documentation with software package CPCwin (both: A.H group /Austria).

Results and discussion

For successful *in vitro* modification of branching patterns of starch polymers, branching enzyme (Q-enzyme) needs to be applied in an active form and in sufficient amounts. Q-enzyme, isolated from amyloplasts of storage cells of green plants, is an

oxidation-sensitive SH-enzyme and thus, requires a permanent reductive medium to stand the purification procedure.

Isolation and purification of Q-enzyme from potato

Pure and active branching enzyme (Q-enzyme) was isolated by a sequence of liquid chromatographic techniques from potato tubers and by fractionated precipitation with ammonium sulfate from the crude-extract obtained from the initial homogenate. To prevent oxidation, reducing agents such as sodiumdithionit or 1,4-dithiothreitol, were applied at each single step of purification.

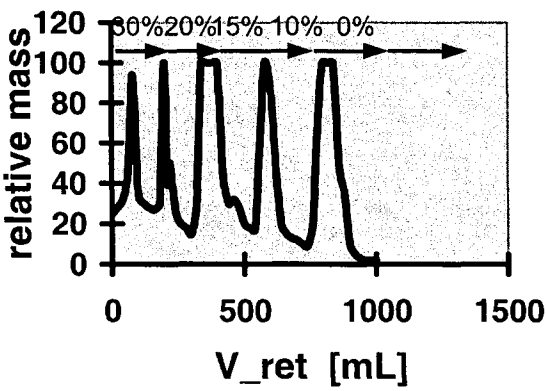

Fig. 2. Hydrophobic Interaction Chromatography (HIC) of crude enzyme extract from potato tubers with varying $(NH_4)_2\ SO_4$ content as step-gradient 30–0%; in the eluent: Clelandpuffer, pH 7.4; (pre-fractionated by $(NH_4)_2\ SO_4$-precipitation); Phosphorylase was identified in the 15% $(NH_4)_2\ SO_4$ pool; amylases were identified in the 0% $(NH_4)_2\ SO_4$ pool; Q-enzyme was eluted at 10% $(NH_4)_2\ SO_4$;

By means of hydrophobic interaction chromatography (HIC) phosphorylase, amylases, R-enzyme and Q-enzyme could be separated and eluted at different ammoniumsulfate molarities (Fig. 2). The PAGE-test of the different ammoniumsulfate-pools results in low amounts of hydrolytic side-activities for the Q-enzyme-pool (10% ammoniumsulfate). After purification of this pool with ion exchange chromatography (IEC), pure Q-enzyme, free of phosphorylase, amylases and R-enzymes, in appropriate amounts for application in modification reactions of glucan branching patterns could be achieved.

Branching activity of isolated and purified Q-enzyme was controlled by means of gel-electrophoresis (PAGE) combined with activity-staining to distinguish between amylases, R-enzyme and Q-enzyme.

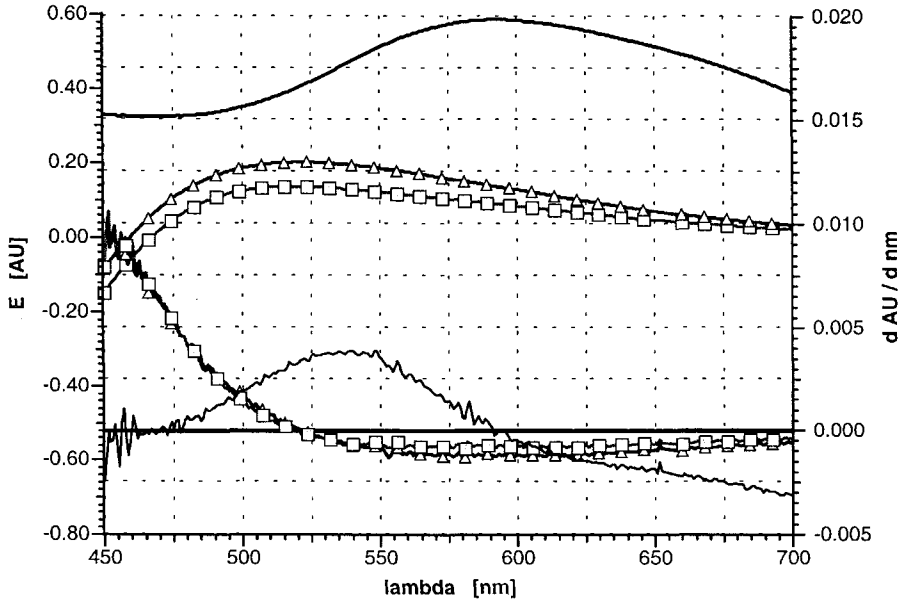

Fig. 3. VIS spectrum of long-chain branched (lcb) starch glucan, Sigma S–4501 (———), Q-enzyme modified starch glucan at 20°C (—□—), Q-enzyme modified starch glucan at 4°C (—Δ—); absorbance maxima: initial starch glucan: 590 nm, Q-enzyme modified at 20°C: 520 nm, Q-enzyme modified at 4°C: approx. 520 nm.

The gel-incorporated starch/iodine-complex comes up with a blue color, whereas at the Q-enzyme-position the gel-incorporated starch-complex turns red on a blue background, as it is a substrate for the Q-enzyme and becomes more branched than the initial sample. For purely hydrolytic enzymes, such as for amylases, either no color will found as the incorporated starch gets degraded and the glucan oligomers will be lost by diffusion, or a bright blue color comes up, such as for R-enzyme which hydrolizes branching positions. As a matter of fact, activity staining provides no quantitative information about enzyme Q-enzyme activity and about the formed products, however, it is a sensitive tool to identify presence/absence of pure Q-enzyme in protein fractions.

To investigate the activity of branching enzyme, long-chain branched (lcb) starch glucans ('amylose') was applied as substrates for the Q-enzyme. Fig. 3 shows a significant shift of the maximum to lower wavelengths in the absorption spectra of the glucan/iodine-complexes due to Q-enzyme activity. However, quantification of Q-enzyme activity due to the magnitude of the absorption maximum of glucan/iodine-complexes might be erroneous as the reducing conditions may interfere.

Modification of potato maltodextrin with Q-enzyme

Q-enzyme hydrolyzes α(1→4)-glycosidically linked glucans and transfers the resulting fragments inter- and/or intramolecular by formation of α(1→6)-glycosidic branching positions. For successful transfer the acceptor-glucan needs a non-branched α(1→4)-segments of at least dp 20-30. Degree of polymerization (dp) of hydrolytically formed glucans strongly depends on reaction temperature, however, the minimum for transfer is dp 6 [6, 8, 9].

Q-enzyme was applied to modify an water-soluble technical-grade potato maltodextrin with a high percentage of low-dp short-chain branched glucans (C-Pur 1906, Cerestar/B). In a first attempt Q-enzyme was incubated to aqueous 20 mg/mL potato starch maltodextrin solutions for 5 days at 20°C and at 4°C. Investigations of the formed products were focused on two major questions:

- is maltodextrin accepted as substrate: if yes, how will Q-enzyme modify these primarily low-dp short-chain branched glucans?
- if there is a modification of the branching pattern, will there be a correlated significant modification of physico-chemical characteristics?

As a first and qualitative indicator for the maltodextrin to be accepted as a substrate by the Q-enzyme, a shift of the maximum of glucan/iodine-absorption spectrum was observed (Fig. 4): the magnitude of the shift obviously depends on the reaction-temperature. The absorption maximum of the glucan/iodine-complex shifted from 540 nm to 520 nm at 20°C and close to 460 nm at 4°C.

Simultaneously, SEC-elution-profiles proof, that at both incubation temperatures molecular composition of potato maltodextrin was significantly modified by the Q-enzyme (Fig. 5). High-dp glucans of the initial maltodextrin were eliminated by the hydrolitic activity of Q-enzyme (V_{ret} 30 ... 36 mL), whereas compact low-dp glucan-coils were formed. Q-enzyme-activity is higher at 4°C than at 20°C.

To obtain molecular weight (degree of polymerization) distributions, molecular weight averages and additional molecular characteristics, SEC combined with dual-detection of scattering intensity and fraction masses was applied [10-13] (Fig. 6). Excluding the extremely high-dp 5% of observed components which most probably are due to aggregation phenomena, for both reaction temperatures average degree of polymerization decreased significantly due to the Q-enzyme action: $dp_w = 54$ for 4°C and $dp_w = 89$ for 20°C.

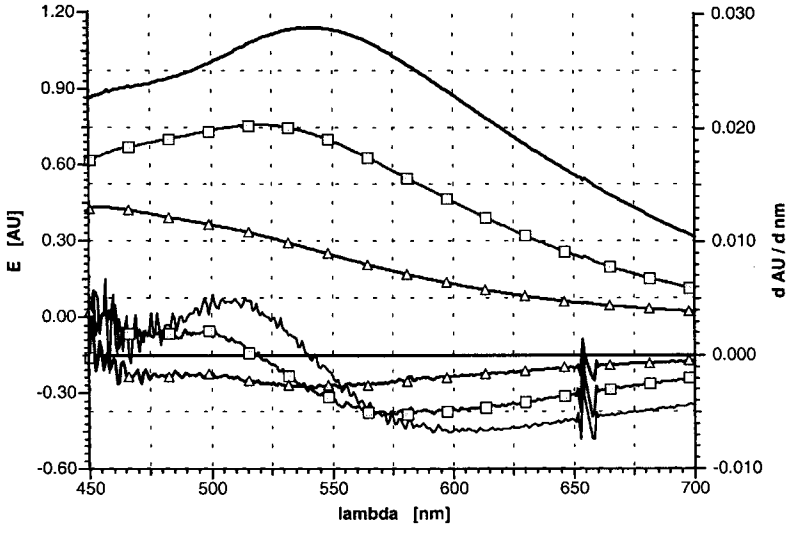

Fig. 4. VIS spectrum of potato maltodextrin (———), Q-enzyme modified potato maltodextrin at 20°C (—□—), Q-enzyme modified potato maltodextrin at 4°C (—Δ—); the first derivative of these spectra illustrate a shift of the absorption maximum (zero-intercept) and a broadening of absorbance in the wavelength-range below 550 nm; absorbance maxima: potato maltodextrin: 540 nm, Q-enzyme modified at 20°C: 520 nm, Q-enzyme modified at 4°C: approx. 460 nm.

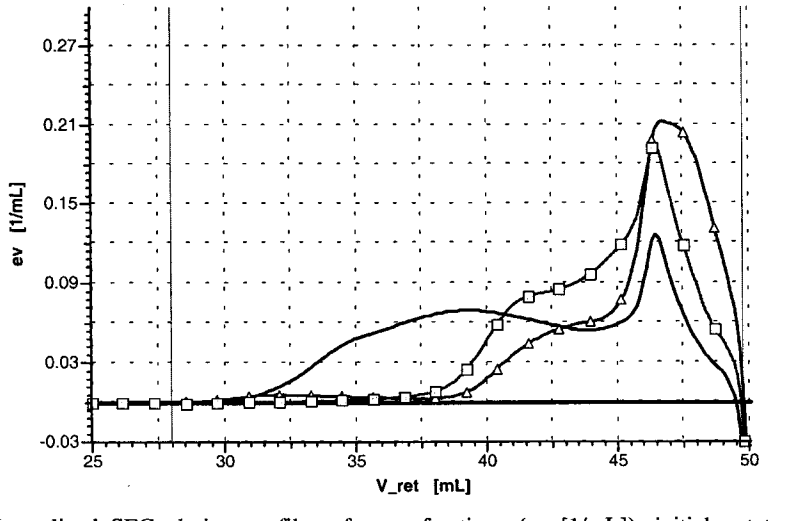

Fig. 5. Normalized SEC elution profiles of mass fractions (ev [1/mL]): initial potato maltodextrin (———); Q-enzyme modified potato maltodextrin at 20°C (—□—); Q-enzyme modified potato maltodextrin at 4°C (—Δ—).

For detailed investigations on Q-enzyme caused modifications in branching characteristics, the modified maltodextrins were selectively debranched with isoamylase and pullulanase. The obtained glucan-fragments then were analyzed by means of HPAEC-PAD (high performance anionic exchange chromatography – pulsed amperometric detection: DIONEX-system) and by analytical SEC. Component-composition of maltodextrins before and after debranching differ significantly and proof the increase of short-chain branching by Q-enzyme (Fig. 7). Hydrolysis by amylases can be excluded as the increase of low-dp glucan-chains is more pronounced at 4°C than at 20°C which is just the opposite of temperature dependence of amylase-activity. Results of average molecular weights and degree of polymerization, obtained from analytical SEC, are listed in Tab.1.

Table 1

Weight and number average molecular weight (M_w, M_n), weight and number average degree of polymerisation (dp_w, dp_n) and polydispersity (M_w/M_n) of initial - and Q-enzyme modified maltodextrin after debranching

	initial potato maltodextrin	potato maltodextrin Q-enzyme modified at 20°C	potato maltodextrin Q-enzyme modified 4°C
M_w [g/M]	7566	3045	1873
M_n [g/M]	2891	1382	1034
dp_w [Glc]	46	19	12
dp_n [Glc]	18	9	6
M_w/M_n	2.6	2.2	1.8

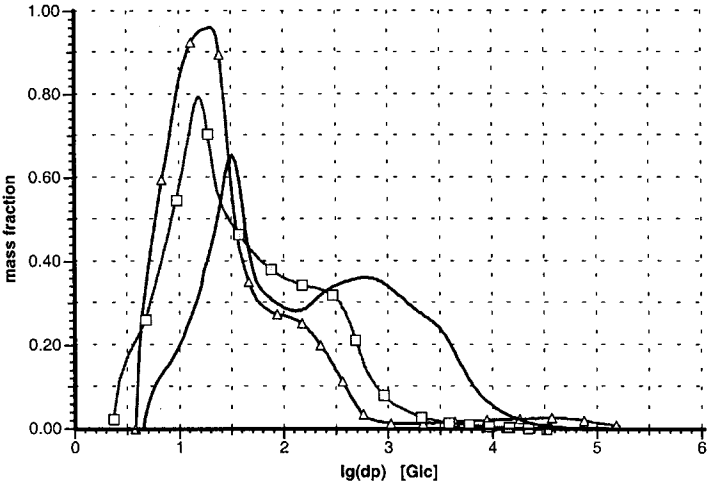

Fig. 6. Normalized differential degree of polymerization distribution: mass fractions (m_dpD_d, area = 1.0); initial potato maltodextrin (———);Q-enzyme modified potato maltodextrin at 20°C (—□—); Q-enzyme modified potato maltodextrin at 4°C (—Δ—).

Mass fractions of degree of polymerization distribution indicate that initially high-dp glucans have been transformed preferably into midrange-dp glucans by the Q-enzyme. Simultaneously, packing density and scb-characteristics of these midrange-dp glucan coils increased.

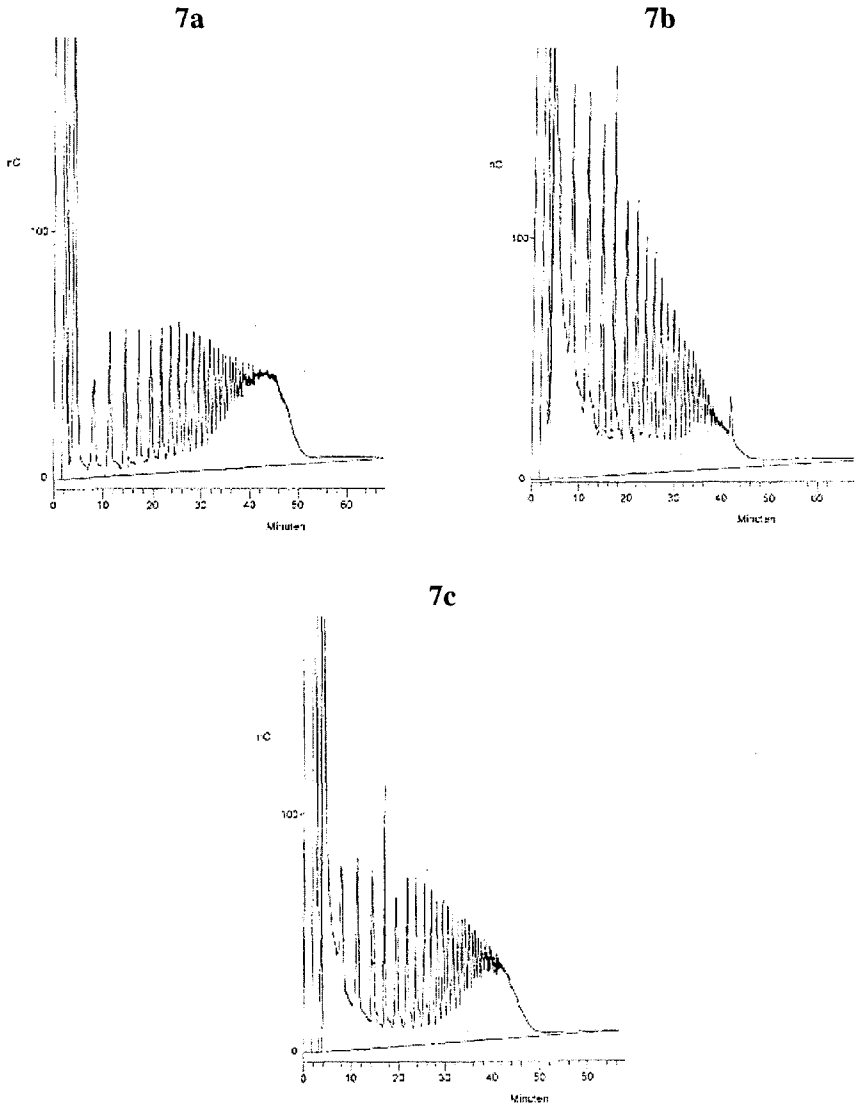

Fig. 7. HPAEC-PAD analysis of debranched potato maltodextrin; glucan chain length distribution of initial potato maltodextrin (a), Q-enzyme modified potato maltodextrin at 20°C (b) and Q-enzyme modified maltodextrin at 4°C (c).

Dionex- and SEC-profiles clearly show the differences in the activity of Q-enzyme at 20°C and at 4°C:
at both temperatures Q-enzyme eliminates high-dp components more or less completely by transforming them into short-chain branched glucans;
transferase activity obviously is more pronounced at 4°C than at 20°C; packing density of glucan-coils is higher at 4°C than at 20°C which strongly indicates more pronounced scb-characteristics for glucans formed at 4°C compared to those formed at 20°C;

Detailed physico-chemical analysis of initial and Q-enzyme modified potato maltodextrins then was achieved from analysis of SEC-DRI/LALLS-data [14]. Distribution profiles of intrinsic viscosity, Staudinger/Mark/Houwink-constants K (dissolution status of glucan coils) and a (coil conformation) were calculated (Fig. 8-10). Average values and occupied ranges of these parameters are listed in Tab. 2.

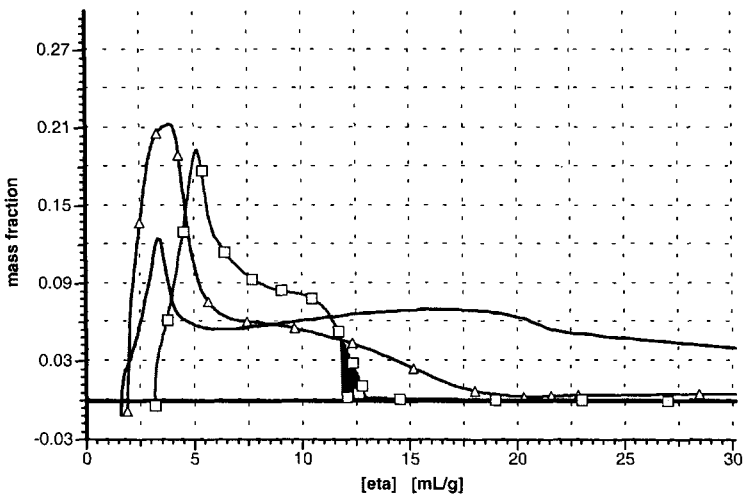

Fig. 8. Intrinsic viscosity distribution (m_IVD_d); initial potato maltodextrin (———);Q-enzyme modified potato maltodextrin at 20°C (—□—); Q-enzyme modified maltodextrin at 4°C (—Δ—).

A complex but nevertheless significant structure-sensitive parameter on the molecular level, intrinsic viscosity in terms of occupied volume per mass-unit of individual maltodextrin-components, can be achieved from absolute molecular weight data (SEC-DRI/LALLS) and universal SEC-calibration. Obviously Q-enzyme-activity stabilizes the glucan-coils by introducing branches which causes reduction of intermolecular polymer/polymer-interaction. Intrinsic viscosity monitors this modification quite sensitively and decreases significantly after Q-enzyme action. Of course, reduced occupied volumina partially are caused by reduced geometric coil dimensions but also

by reduced 'interaction-radii' of the modified glucans. Intrinsic viscosity according the power law $[\eta]=K.M^a$ can be splitted into contributions correlated with molecular dimensions (M: molecular weight), polymer-coil conformation (*a*) and contributions correlated with interactive polymer-characteristics.

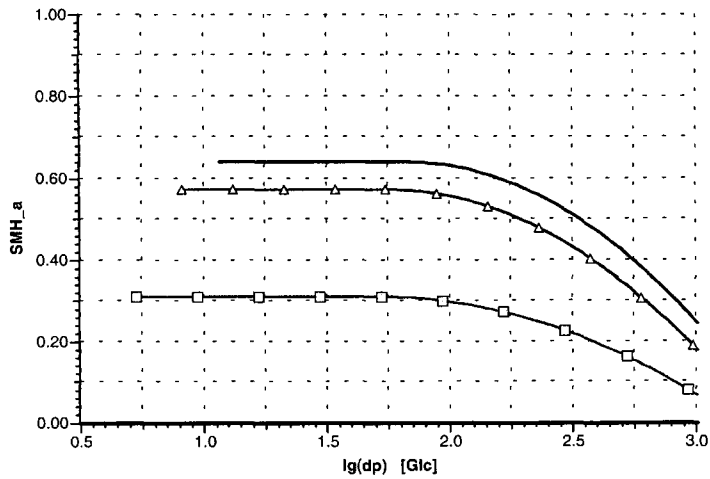

Fig. 9. Dependence of Staudinger/Mark/Houwink (SMH) exponent *a* of power low $[\eta]=K.M^a$ on degree of polymerization of potato maltodextrin (initial), Q-enzyme modified at 20°C and Q-enzyme modified at 4°C.

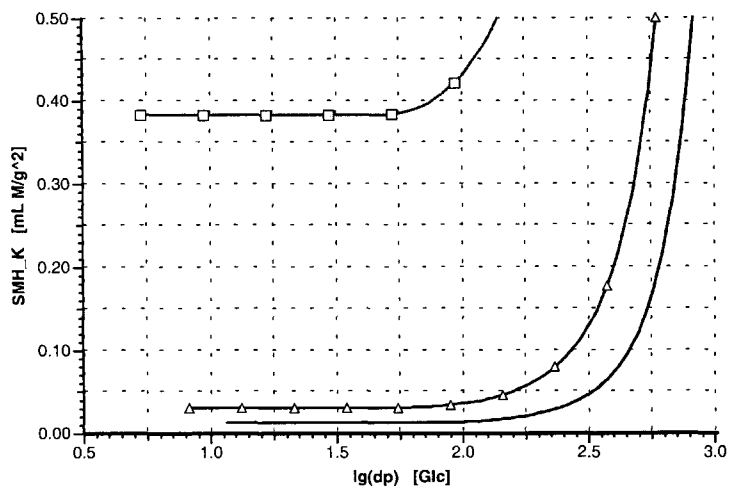

Fig. 10. Dependence of Staudinger/Mark/Houwink (SMH) factor *K* of power low $[\eta]=K.M^a$ on degree of polymerization of potato maltodextrin (initial), Q-enzyme modified at 20°C and Q-enzyme modified at 4°C.

Different *K*-values for Q-enzyme modified maltodextrins at 4°C and at 20°C strongly indicate different dissolution-states for the glucans: more pronounced polymer/solvent interaction for the glucans formed at 20°C than for those formed at 4°C. Although at both investigated reaction temperatures – 4°C and 20°C – glucan-coil dimensions decreased, a significant difference for products formed at 4°C and at 20°C was found: at 20°C the glucan-coils were even more compact than those formed at 4°C. Obviously and maybe primarily, Q-enzyme modifies interactive glucan-characteristics and therefore is suspected to be a key-tool to control interaction-correlated macroscopic/technological material properties such as gelation-potential, freeze/thaw-stability, etc. on molecular level.

Table 2

Molecular characteristics of initial potato maltodextrin, Q-enzyme modified at 20°C potato maltodextrin and Q-enzyme modified at 4°C potato maltodextrin

Characteristics	Initial potato MD	Q-enzyme modified at 20°C	Q-enzyme modified at 4°C
molecule dimension glucan coil radius [nm]	1 .. 45 10.1	1 .. 38 7.7	1 .. 36 4.0
molecule conformation SMH *a*	0.64	0.56	0.32
molecular interactive potential SMH *K* [mL M g^{-2}]	0.01	0.03	0.38
occupied molecule volume due to dimension, conformation and interactive potential $[\eta] = K.M^a$ [mL g^{-1}]	2 .. 120 23	2 .. 90 17	3 .. 31 8

Thus, some first answers to the initial questions concerning Q-enzyme activity were obtained:

Q-enzyme accepts short-chain branched and primarily low-dp starch glucans as substrates and modifies the branching pattern such, that primarily the interactive potential of the glucan-coils is modified. Extent of modification, i.e. intensity of scb-branching and actual packing density of glucan-coils, strongly depends on external conditions, such as reaction-temperature.

Modification of interactive characteristics on the molecular level is strongly suspected to control macroscopic/technological qualities of starch-based materials – at least such qualities, which obviously are correlated with interactive characteristics such as gelation potential or freeze/thaw-stability.

Summarizing, controlled modification of branching pattern of starch-glucans with Q-enzyme is equal to controlled modification of interactive starch-glucan properties on the molecular level with consequences on macroscopic/technological material qualities.

Thus, Q-enzyme seems to be a promising tool to improve processing-efficiency e.g. by 'tailoring' the raw material before it is transferred to specific traditional technological processing.

Acknowledgement

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MODYFIKOWANIE SPOSOBU ROZGAŁĘZIEŃ W ZIEMNIACZANEJ MALTODEKSTRYNIE ZA POMOCĄ ENZYMU Q

Streszczenie

Czysty enzym rozgałęziający (enzym Q [(1→4)-α-D-glukan: (1→4)-α-D-glukano 6-glukosylotransferaza, EC 2.4.1.180] został wyizolowany z ekstraktu z surowych bulw ziemniaczanych za pomocą kolejnych rozdziałów chromatograficznych (HIC i IEC). Enzym Q wykorzystuje jako substraty natywne i syntetyczne nierozgęzione glukany typu amylozowego i zwiększa procentową zawartość rozgałęzień przez połączoną aktywność hydrolityczną i transferową. Jako substrat enzym Q wykorzystuje też techniczną maltodekstrynę ziemniaczaną. Tworzenie rozgałęzień bardzo zależy od temperatury inkubacji. Znaleziona charakterystyka na poziomie molekularnym wyraźnie wskazuje na zależność sposobu rozgałęziania od rozmiaru zwoju w glukanie, konformacji i jego możliwości oddziaływań z otoczeniem. Szczególnie ten ostatni czynnik w wyraźnym stopniu wpływa na mnakroskopowe i technologiczne właściwości materiału skrobiowego, np. na zdolność do kleikowania i odporność na niskie temperatury.