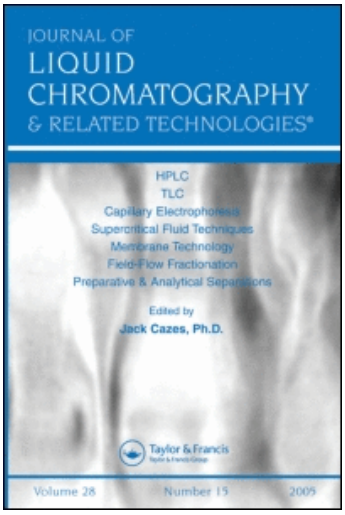

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Characterization of Branching-Characteristics of Starch-Glucans by Means of Combined Application of Complexation, Enzymatically Catalyzed Modification, and Liquid-Chromatography

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CHARACTERIZATION OF BRANCHING-CHARACTERISTICS OF STARCH-GLUCANS BY MEANS OF COMBINED APPLICATION OF COMPLEXATION, ENZYMATICALLY CATALYZED MODIFICATION, AND LIQUID-CHROMATOGRAPHY

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ABSTRACT

Molecular characteristics, especially the branching-structure, of glucans from starch granules isolated from tubers of potato species Ukomo are determined. Granule-fraction with different mean-diameters are provided by sedimentation as inital separation step for further investigations. Then aqueous/DMSO dissolved starch glucans were separated into a non-branched/long-chain-branched and a short-chain-branched fraction by precipitation with n-butanol and methanol, respectively. By means of preparative SEC glucan pools of these fractions were collected and as well analyzed by means of methylation/GC-MS as checked for their individual potential to complex with polyiodide anions. Additionally equivaalents of these fractions were debranched by the catalytic action of pullulanase to determine the constituting glucan chain distribution by means of analytical SEC. Results of branching characteristics in terms of average number of monomers between two adjacent branching positions (bp→bp), average

percentage of branching (b%) and E_{640}/E_{525}(nb/lcb / scb)-ratio from different techniques are in good agreement and yield significantly different values for glucans from large and small starch-granules.

INTRODUCTION

Multiple and superimposed heterogeneity is a basic quality of polymers[1], especially of biopolymers like polysaccharides[2,3]. Properties such as monomer composition, molecular weight (degree of polymerization) and branching-characteristics are examples for molecular properties which are controlling macroscopic material qualities. For this reason there is a strong effort to determine appropriate parameters at the molecular level to get reliable selection- and processing-critera[4,5]. Strategies to gain such characteristic parameters include purification of the material of interest without artefacts and the application of separation-techniques combined with suitable methods for identification and quantification[6,7,8]. Due to the fact that polymers show a range of distributions, separation-techniques are a most proper tool to increase the quality of information because they enable access onto the range and shape of distributions, whereas only average values can be achieved from bulk-characterizations[9,10]. But also the actually applied mode of separation has to be selected solemnly, stongly depends on the parameter of interest, and either is ΔH- or ΔS-controlled considering that any separation is attained by energy-differences in terms of delta Free Enthalpy (ΔG) which includes as well Enthalpy-(ΔH) as Entropy-($T\Delta S$) contributions. Generally, entropy-controlled ($\Delta H \rightarrow 0$) separation techniques enable the determination of size-/shape-distributions, whereas enthalpy-controlled ($\Delta H >> T\Delta S$) techniques, even refered as non-exclusion techniques, are applied to achieve distributions according to different chemical potentials ($\Delta\mu$)[11].

A key-subject in the analysis of biopolymers is the dependence of material-characteristics on the history of particle-genesis, on specific catalytic mechanisms

and on the influence of global (environmental) conditions[12,13]. The actual studies deal with this topic by investigating molecular characteristics, especially the branching-structure, of different fractions of potato-starch which will be determined by means of combined application of several preparative and analytical separation-steps, modification-procedures, complexation/precipitation-techniques and extended data analysis. Therefore starch granules from tubers of potato species Ukomo got fractionated yielding two initial pools of particles with different mean-diameter. Both granule-fractions were dissolved in aqueous DMSO and splitted into a non-branched/long-chain-branched and a short-chain-branched glucan fraction by precipitation with n-butanol and methanol, respectively. Preparative SEC of each of these four glucan-species provided glucan-pools which were enzymatically debranched and yielded the constituting glucan-chain distributions. Analysis of these hydrolization-products was performed by means of analytical SEC and included transformation of mass distribution into number distribution of molecular weights as an essential procedure.

MATERIALS AND METHODS

Starch

Starch granules were purified from ripe tubers of potato species Ukomo. The tubers were washed, peeled, smashed and suspended in pure water. The slurry was sieved to separate the starch components from fibers and then got centrifuged to remove water soluble components with the supernatant.

Sedimentation

For the pooling of starch granules according to their size the aqueous starch-slurry was applied at the top of a water-filled column (ID=63 mm, l=340 mm). Separation was achieved by different sedimentation times (t_s) of large (t_s: short) and small (t_s: long) particles. Fractions of decreasing particle size

were collected, a specimen of large granules (Fr.I: t_s=2-5 min) and another one of small granules (Fr.II: t_s>30 min) were utilized for further investigations.

Dissolution

Starch particles from different pools were dissolved in 90% aqueous DMSO with a concentration of 1% (wt/v). Dissolution was completed by stirring over night at 70°C. Clear solutions were obtained either immediately or after centrifugation (3000 rpm, 15 min). Analysis of the non-dissolved residues proved to be non-starchy materials. Dissolution of starch in DMSO, different to aqueous sodium hydroxid, represented an essential purification step because accompanying proteins and lipids were insoluble. DMSO dissolved starch samples could be stored for several weeks without significant aging-effects such as degradation, aggregation or retrogradation.

Precipitation

Fractionation of native starch into non-branched/long-chain-branched (nb/lcb) and short-chain -branched (scb) components was achieved by complexation of aqueous DMSO dissolved starch with n-butanol: nb/lcb glucans were precipitated, whereas the scb components remained in solution[14]. 450 ml of an aqueous 0.15% (wt./v) NaCl-solution was added to 100 ml of the 1% (wt./v) DMSO-starch-solution and mixed 10:1 with n-butanol. This solution was stored 2 days at 4°C and the resulting precipitate was centrifuged, washed with methanol, dried in vacuum and yielded a dry white powder. The scb-starch fraction which remained in the supernatant after the n-butanol complexation can be precipitated by adding an excess of methanol, i.e. by mixing 1:4 with methanol and storing over night at 4°C. This precipitate was centrifuged, washed two times with methanol and dried in vacuum. As well the nb/lcb-glucan-fraction as the scb-fraction easily can be redissolved in 90% aqueous DMSO for further investigations.

Staining:

Total carbohydrates in the eluate from the semi-preparative SEC-system have been determined by mixing 1 ml of the sample solution with 2 ml anthron reagent (200 mg cristalline anthron p.A. dissolved in 100 ml H_2SO_4 96 % p.A.) and heating the mixture for 10 min in a boiling water bath. After cooling the solution and degassing it by means of ultrasound, absorption at 540 nm has been measured and the concentration of total carbohydrates is read from a calibration curve obtained from D-glucose[15,16].

For the determination of nb/lcb- and scb-glucan-complexes with polyiodide anions 125 mg freshly sublimated iodine was dissolved in the presence of 400 mg potassium iodide in 1000 ml demineralized water and diluted 1:1 with 0.1 M acidic acid to ensure a final pH between 4.5 and 5 when mixed with the alkaline eluate from size-exclusion chromatography. λ_{max} for the iodine-glucan-complex is known to depend on both, the degree of polymerization (dp) and the branching structure of the glucan chains: at wavelengths above 600 nm nb/lcb-glucans give strong absorption (the typical blue amylose colour), below 600 nm the scb-glucans are absorbing (the typical violett amylopectin colour)[17,18]. Best results were obtained by vis-detection at 640 nm (nb/lcb) and 525 nm (scb), respectively. The reliability of the detection of scb-glucan-iodine-complexes at 525 nm is supported by the corresponding maxima in ORD/CD-spectra for α1-4-glucan-chains with dp-values smaller than 40[19,20].

Vis-absorption was detected by means of a Spectrophotometer 550, Perkin-Elmer/UK.

Methylation/GC-MS-analysis

Methylation/GC-MS-analysis was applied to the starch samples for the determination of branching characteristics[21]. Therefore aqueous/DMSO-dissolved starch was mixed with solid sodium-hydroxide and methyljodid. After shaking with chloroform, the organic phase got dried and the procedure performed a second time to ensure total methylation. For GC-MS-analysis the glucans were

hydrolized with trifluoroacidic acid, reduced with Na BH_4 and acetylated with acidic acid anhydrid.

Such treatment yields reaction products from starch-glucans with different retention-times at GC-MS-analysis, depending on the kind of monomer: glucose from the non-reducing end → 1,5-di-O-Ac 2,3,4,6-tetra-O-Me-D-sorbit, α1-4-linked glucose within the polymer-chain → 1,4,5-tri-O-Ac 2,3,6-tri-O-Me-D-sorbit and α1-4 linked glucose with α1-6-linked branches → 1,4,5,6-tetra-O-Ac 2,3-di-O-Me-D-sorbit[22].

Enzymatically catalized debranching

For the enzymatically supported branching analysis the starch-samples were dissolved in DMSO and diluted 1:2 with aqueous buffer and mixed with aqueous pullulanase-solution (purified NOVO Promozym 200 L, Novo, DK; EC 3.2.1.41), an alternative approach to the application of isoamylase[23,24]. Debranching action was performed for 48 h at 37°C with additional incubation of pullulanase after 24 h to ensure total hydrolysis of α1-6-glycosidic linkages.

Size-exclusion Chromatography

For **preparative SEC** a series of two columns S-1000 Superfine (l=850 mm, ID=16 mm, $M_{excl}=10^8$ g mol^{-1}) and S-200 Superfine (l=940 mm, ID=16 mm, $M_{excl}=10^5$ g mol^{-1}) with 0.005 N Na OH and a flow rate of 1.6 m min^{-1} were utilized.

For **semipreparative/analytical SEC of native glucan** samples a Sephacryl-system consisting of a S-1000 Superfine column (l=1290 mm, ID=16 mm, $M_{excl}=10^8$ g mol^{-1}), a S-400 Superfine column (l=900 mm, ID=16 mm, $M_{excl}=10^6$ g mol^{-1}) and a S-200 Superfine column (l=940 mm, ID=16 mm, $M_{excl}=10^5$ g mol^{-1}) were utilized in series with 0.005 N Na OH as eluent at a flow rate of 0.75 ml min^{-1}.

For **analytical SEC of debranched glucan-chains** a system of Superose 6 TM (Pharmacia, Uppsala/S) (l=290 mm, ID=10 mm, $M_{excl}=5.10^6$ g mol^{-1}),

Fractogel (Merck, Darmstadt/FRG) HW50 S (l=290 mm, ID=10 mm, M_{excl}=20 000 g mol^{-1}) and HW40 S (l=290 mm,ID=10 mm,M_{excl}=7000 g mol^{-1}) were utilized with 0.05 M KCl as eluent at a flow rate of 0.6 ml min^{-1}.

Mass of eluted glucans was detected by a differential refractometer R503, Waters/UK. Fractions for iodine and anthron staining were collected with a fraction collector Ultrorac type 7000, LKB/UK.

Branching Analysis

A well working technique to check the branching characteristics of glucans is their enzymatic debranching, that means selective hydrolysis of α1-6-glycosidic linkages combined with succeeding SEC-separation and analysis of the resulting glucan-chain distribution. Detection of SEC-separated glucan chains by means of a differential refractive index detector initially yields the mass-distribution (w(M)) which has to be transformed into number distribution (n(M)) for branching analysis in terms of percentage of branching positions within a glucan (b%) and an average number of monomers between two adjacent branching points (bp→bp). Transformation of mass distribution (w(M): eq.(1a)) into number distribution (n(M): eq.(1b)) can be achieved according eq.(1a), eq.(1b) and eq.(2)[25], where N(M) is defined as the number fraction, W(M) as the weight fraction of molecules in the molecular weight range dM.

(1a) $$n(M) = \frac{d\,N(M)}{dM}$$

(1b) $$w(M) = \frac{d\,W(M)}{dM}$$

(2) $$n(M) = \frac{w(M)}{M}$$

In the analysis of the n(M)-functions up to three peaks in the differential number distribution (Mp_I, Mp_II, Mp_III) were taken as representative glucan-chain

populations. Contributions for the different populations were estimated corresponding to their approximate percentage of area (n%_I, n%_II, n%_III) in the n(M)-function. An average number of monomers between two adjacent branching points (bp→bp) can be calculated according eq.(3) with i=I, II, III:

$$bp \rightarrow bp = \frac{\sum_i dp_{(Mp)_i} \cdot n\%_i}{100} \quad (3)$$

Introducing an norm-glucan with a reference degree of polymerization of $ref_{dp}=1000$, an average number of branching points within such a glucan with ref_{dp} (av_b) can be calculated according eq.(4):

$$av\_b = \frac{ref_{dp}}{bp \rightarrow bp} \quad (4)$$

Application of eq.(5) yields an average percentage of branching (b%) for a glucan with $dp=ref_{dp}$ yielding an average percentage of monomers with branching positions:

$$b\% = \frac{av\_b \cdot 100}{ref_{dp}} \quad (5)$$

RESULTS AND DISCUSSION

Starch-glucans from tubers of potato species Ukomo were investigated for their branching-chararacteristics by applying a range of separation-, identification- and quantification-methods (Table 1). The main topic of interest was the question whether granules with different biological history contain glucans with different branching-structure, and if so, can these dissimilarities be specified. For starch, in a first approach, different biological history was set identical with different

TABLE 1

Applied Techniques and Methods for the Characterization of Starch-glucans from Tubers of Potato Species Ukomo.

Method	**Glucan -Fractions** **applied analytical techniques** **glucan-component**			
sedimentation	Fraction I: Fr.I		Fraction II: Fr.II	
preparative SEC: iodine-staining anthron-staining	**Iodine-staining:** E_{640} nb/lcb-quantification E_{525} scb-quantification E_{640}/E_{525} starch composition **Anthron-Chromogen:** E_{550} total carbohydrates		**Iodine-staining:** E_{640} nb/lcb-quantification E_{525} scb-quantification E_{640}/E_{525} starch composition **Anthron-Chromogen:** E_{550} total carbohydrates	
precipitation	**n-butanol:** Fr.Ibut	**methanol:** Fr.Imet	**n-butanol:** Fr.IIbut	**methanol:** Fr.IImet
preparative SEC-pools: enzymatically catalized debranching	Fr.Ibut_a	Fr.Imet_a	Fr.IIbut_a	Fr.IImet_a
	Fr.Ibut_b	Fr.Imet_b	Fr.IIbut_b	Fr.IImet_b
	Fr.Ibut_c	Fr.Imet_c	Fr.IIbut_c	Fr.IImet_c
analysis	**de-branching:** pullulanase catalyzed hydrolysis of α1-6-glycosidic linkages **iodine-staining:** E_{640} non-branched/long-chain branched glucan-quantification E_{525} short-chain branched (scb) glucan quantification E_{640}/E_{525} starch composition: (nb/lcb / scb)-ratio **anthron-chromogen:** E_{550} total carbohydrates after acid-catalized total-hydrolysis **analytical SEC:** molecular weight distribution: weight w(M) → number n(M) **branching analysis:** average number of branching points within $dp=ref_{dp}$: av_b percentage of branching points within $dp=ref_{dp}$: b% average number of monomers between adjacent bp: bp→bp			

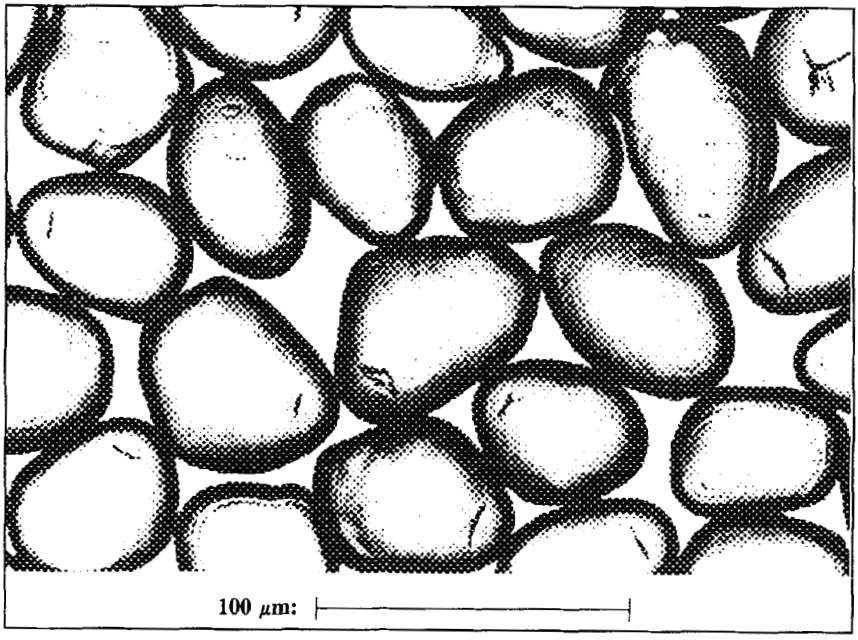

FIGURE 1 Starch granules fraction I (Fr.I) from sedimentation of aqueous starch slurry from tubers of potato species Ukomo. Particle diameters range between 45-70 μm.

granule-sizes[26,27] and thus, the starch particles initially were pooled according to increasing sedimentation times, i.e. with decreasing particle mean-diameters. Two of these granule-fractions, Fr.I: d=45-70 μm (Fig.1) and Fr.II: d=20-40 μm (Fig.2), were utilzed for further investigations. As well Fr.I as Fr.II were dissolved in aqueous 90% DMSO and got separated on the semi-preparative/analytical SEC-system. The amount of carbohydrates in each of the fractions was determined by means of vis-detection at 550 nm as the amount of anthron-coupled-glucose, previously produced by acidic hydrolysis of the glucans. Vis-detection at 640 nm (E_{640}) and 525 nm (E_{525}) for each of the fractions from SEC-separation enabled the determination of chromatograms for non-branched/long-chain-branched (nb/lcb) and short-chain-branched (scb) polyiodide-complexed glucans. Consequently, these E_{640}- and E_{525}-chromatograms enabled the

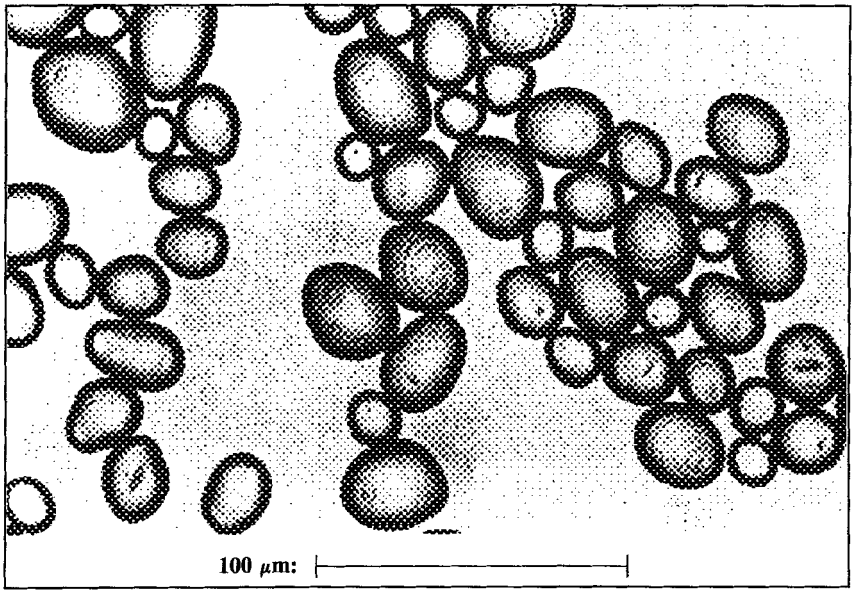

FIGURE 2 Starch granules fraction II (Fr.II) from sedimentation of aqueous starch slurry from tubers of potato species Ukomo. Particle diameters range between 20-40 μm.

computation of qualitative starch composition factors over the range of eluted fractions as the ratio of E_{640}/E_{525} (nb/lcb / scb). For both fractions (Fr.I: Fig.3; Fr.II: Fig.4) glucans at low-retention volumes show E_{640}/E_{525}-values close to and below 1.0, indicating a main-amount of scb-glucans, whereas high-retention-volume-glucans show E_{640}/E_{525}-values in the range of 2.0, an evidence for mainly nb/lcb-glucans. The midrange domain of eluted glucans with E_{640}/E_{525}-values rising from 1.0 up to 2.0 obviously indicates the transition from nb/lcb-majority to scb-majority of the eluted glucans. At this state of analysis no significant differences between starch from granules of Fr.I and Fr.II could be identified.

In a next step of differentiating the starch-glucans, the aqueous DMSO dissolved fractions Fr.I and Fr.II were mixed with n-butanol to precipitate the non-branched/long-chain branched components. The short-chain-branched glucans

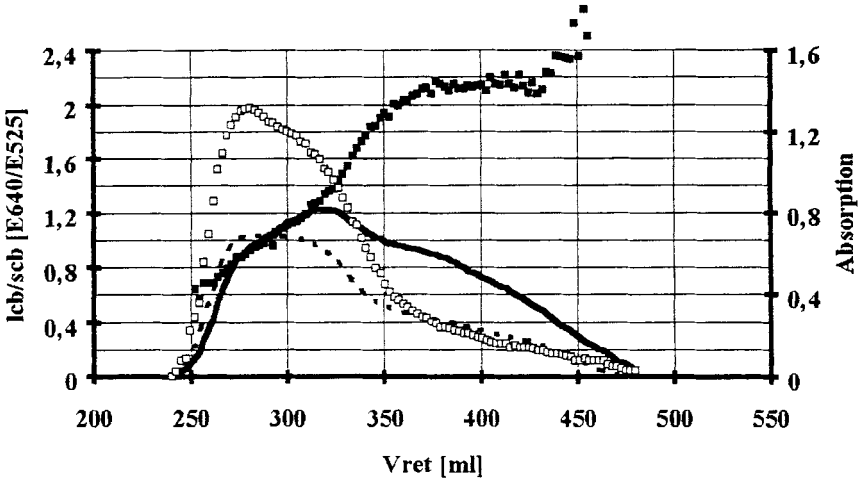

FIGURE 3 Large starch granules (Fr.I): dissolved in aqueous 90% DMSO and separated on semi-preparative/analytical SEC; fractions complexed with polyiodide anions: detection of non-branched/long-chain-branched (nb/lcb) glucans at 640 nm (————), detection of short-chain-branched glucans at 525 nm (— — —); starch composition ratio E_{640}/E_{525} (nb/lcb / scb)-ratio (■■■); total carbohydrates by detection at 550 nm of anthron-coupled glucose after acidic hydrolysis of glucans (□□□).

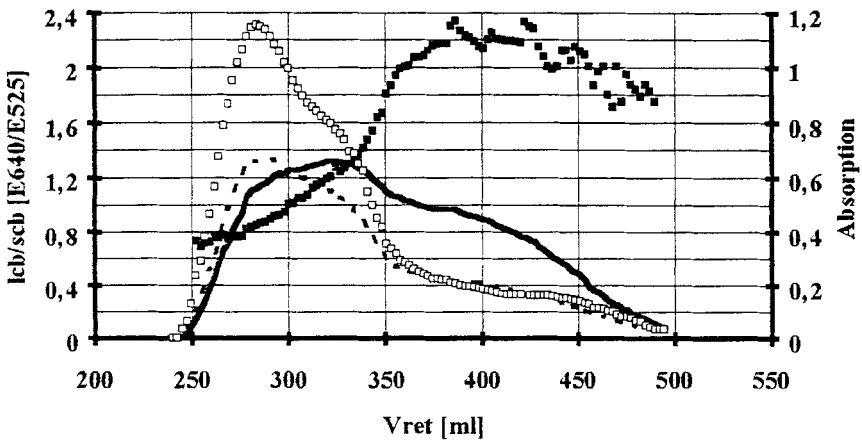

FIGURE 4 Small starch granules (Fr.II): dissolved in aqueous 90% DMSO and separated on semi-preparative/analytical SEC; fractions complexed with polyiodide anions: detection of non-branched/long-chain-branched (nb/lcb) glucans at 640 nm (————), detection of short-chain-branched glucans at 525 nm (— — —); starch composition ratio E_{640}/E_{525} (nb/lcb / scb)-ratio (■■■); total carbohydrates by detection at 550 nm of anthron-coupled glucose after acidic hydrolysis of glucans (□□□).

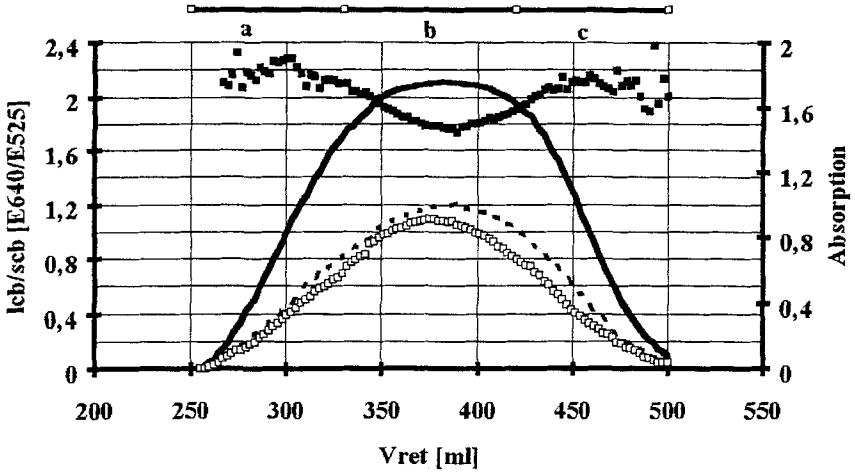

FIGURE 5 Large starch granules (Fr.I): n-butanol precipitated fraction from aqueous DMSO dissolved starch expected to represent a non-branched/long-chain-branched glucan fraction; separation on semi-preparative/analytical SEC; fractions complexed with polyiodide anions: detection of non-branched/long-chain-branched (nb/lcb) glucans at 640 nm (————), detection of short-chain-branched glucans at 525 nm (— — —); starch composition ratio E_{640}/E_{525} (nb/lcb / scb)-ratio (■■■); total carbohydrates by detection at 550 nm of anthron-coupled glucose after acidic hydrolysis of glucans (□□□); ranges of components which were pooled (Fr.Ibut_a, Fr.Ibut_b, Fr.Ibut_c) for enzymatically catalyzed debranching.

in the supernatant subsequently got precipitated by an excess of methanol. The four fractions (Fr.Ibut, Fr.Imet, Fr.IIbut, Fr.IImet) were washed, dried, redissolved in aqueous DMSO and again analyzed on the semi-preparative SEC-system. The starch composition factor computed as the E_{640}/E_{525}-ratio with values between 1.8 and 2.2 for the n-butnol-precipitates indicated as well for the large granules (Fr.Ibut: Fig.5) as for the small granules (Fr.IIbut: Fig.7) a majority of nb/lcb-glucans. The same procedure yields for the methanol-precipitates (Fr.Imet: Fig.6, Fr.IImet: Fig.8) E_{640}/E_{525}-values close to 0.8, indicating an expected majority of short-chain-branched glucans. Again, no significant difference between the nb/lcb-glucans of Fr.I and Fr.II could be identified at this state of analysis. The scb-glucans slightly differ in the tendency of their E_{640}/E_{525}-values

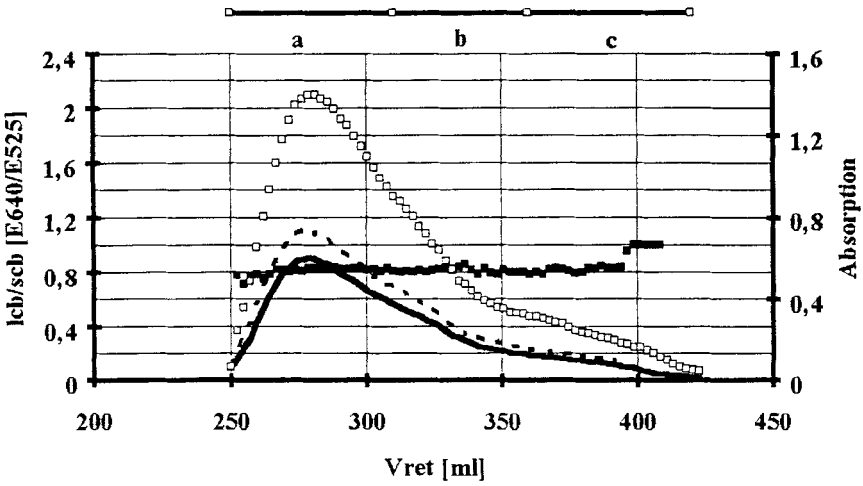

FIGURE 6 Large starch granules (Fr.I): methanol precipitate from the supernatant of the previous n-butanol precipitatation of aqueous DMSO dissolved starch expected to represent a short-chain-branched glucan fraction; separation on semi-preparative/analytical SEC; fractions complexed with polyiodide anions: detection of non-branched/long-chain-branched (nb/lcb) glucans at 640 nm (————), detection of short-chain-branched glucans at 525 nm (— — — —); starch composition ratio E_{640}/E_{525} (nb/lcb / scb)-ratio (■■■); total carbohydrates by detection at 550 nm of anthron-coupled glucose after acidic hydrolysis of glucans (□□□); ranges of components which were pooled (Fr.Imet_a, Fr.Imet_b, Fr.Imet_c) for enzymatically catalyzed debranching.

in the midrange domain of elution: scb-glucans from large granules (Fr.I: Fig.6) keep values close to 0.8, whereas scb-glucans from small granules (Fr.II: Fig.8) tend toward even lower values.

In a next step each of the four glucan-fractions were fractionated on the preparative SEC-system into glucan-pools at low- (a), midrange- (b) and high- (c) retention-volumes (fractions indicated in Fig.5-8, according Table 1). The glucans in each of these pools got debranched by catalytic action of pullulanase and the reaction-products were analyzed by means of analytical SEC and extended data analysis. As separation of the debranched glucans on the analytical SEC-system initally yields the mass-modulated molecular weight distribution (w(M)) application of eq.(2) to the w(M)-distributions is required to transform the w(M)- into

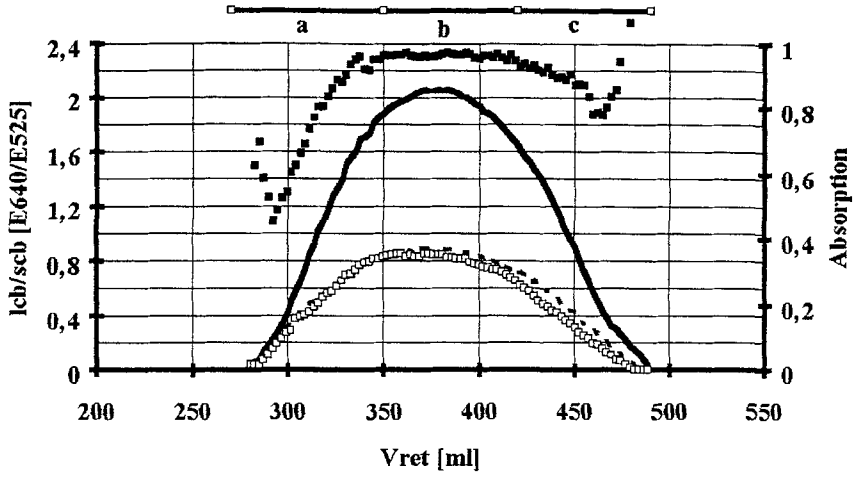

FIGURE 7 Small starch granules (Fr.II): n-butanol precipitated fraction from aqueous DMSO dissolved starch expected to represent a non-branched/long-chain-branched glucan fraction; separation on semi-preparative/analytical SEC; fractions complexed with polyiodide anions: detection of non-branched/long-chain-branched (nb/lcb) glucans at 640 nm (————), detection of short-chain-branched glucans at 525 nm (— — —); starch composition ratio E_{640}/E_{525} (nb/lcb / scb)-ratio (■■■); total carbohydrates by detection at 550 nm of anthron-coupled glucose after acidic hydrolysis of glucans (□□□); ranges of components which were pooled (Fr.IIbut_a, Fr.IIbut_b, Fr.IIbut_c) for enzymatically catalyzed debranching.

n(M)-distributions to give weight to the glucan-chains by their number and not by their mass. For each of the analyzed debranched glucan-pools both distributions (n(M) and w(M)) are illustrated in the indicated Figures 9-12. Peaks in the differential molecular weight distributions (Mp_x) were classified to be representative for a glucan-chain-population with specified degree of polymerization $dp_{(Mp)}$. The contribution of each of these populations was estimated from the percentage of area in the n(M)-function. By means of such $dp_{(Mp)}$- and n%-data average values of branching characteristics were calculated according to eqs.(3)-(5).

At this state of analysis the non-branched/long-chain-branched glucans from large (Fr.I) and small (Fr.II) granules showed significantly different branching characteristics. The average number of monomers between two adjacent

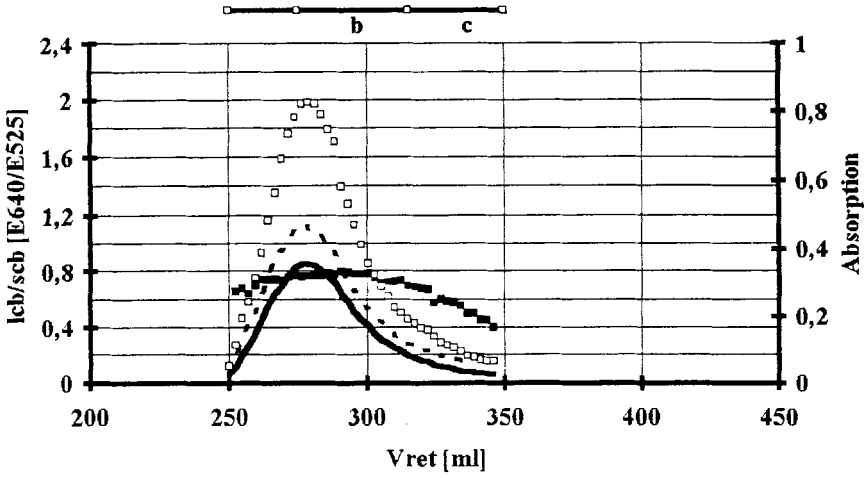

FIGURE 8 Small starch granules (Fr.II): methanol precipitate from the supernatant of the previous n-butanol precipitatation of aqueous DMSO dissolved starch expected to represent a short-chain-branched glucan fraction; separation on semi-preparative/analytical SEC; fractions complexed with polyiodide anions: detection of non-branched/long-chain-branched (nb/lcb) glucans at 640 nm (————), detection of short-chain-branched glucans at 525 nm (— — —); starch composition ratio E_{640}/E_{525} (nb/lcb / scb)-ratio (■■■); total carbohydrates by detection at 550 nm of anthron-coupled glucose after acidic hydrolysis of glucans (□□□); ranges of components which were pooled (Fr.IImet_b, Fr.IImet_c) for enzymatically catalyzed debranching.

branching positions results with bp→bp=94.5 for the initial (Fr.Ibut_a: Fig.9a, Tab.2a) and bp→bp=172.5 for the midrange (Fr.Ibut_b: Fig.9b, Tab.2a) preparative SEC-fraction of large granules. This average distance is significantly larger than that for nb/lcb-glucans from small granules: bp→bp=19 for the inital fraction of preparative SEC (Fr.IIbut_a: Fig.11a, Tab.3a), more indicating scb-glucans than a nb/lcb-fraction; bp→bp=52 for the midrange fraction (Fr.IIbut_b: Fig.11b, Tab.3a) and bp→bp=37 for the final fraction of preparative SEC (Fr.IIbut_c: Fig.11c, Tab.3a). Obvious dissimilarities between glucans from large and small granules, already documented by different average values for the parameters of branching characteristics, even more explicitely are illustrated by the absence of extremely high-dp glucan-chains in nb/lcb-glucan-fractions of small granules (large granules :Figs.9a-b, small granules: Figs.11a-c).

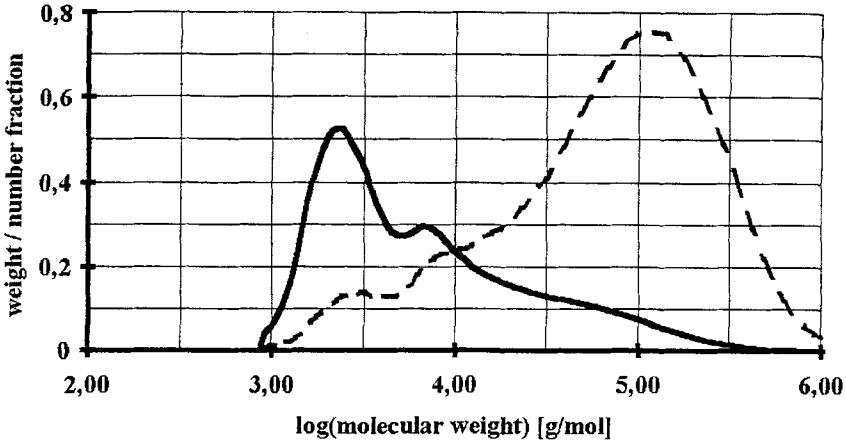

FIGURE 9a Large starch granules (Fr.I): n-butanol precipitated nb/lcb-glucan fraction; pool Fr.Ibut_a from preparative SEC, debranched by catalytic action of pullulanase; (- - -) mass distribution from DRI-detection of analytical SEC of debranched glucans; (———) number distribution according eq.(1). Results of branching analysis in Tab.2a.

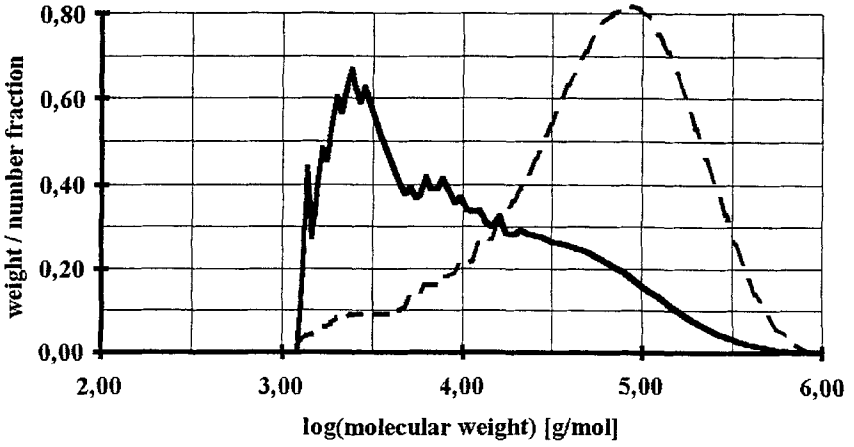

FIGURE 9b Large starch granules (Fr.I): n-butanol precipitated nb/lcb-glucan fraction; pool Fr.Ibut_b from preparative SEC, debranched by catalytic action of pullulanase; (- - -) mass distribution from DRI-detection of analytical SEC of debranched glucans; (———) number distribution according eq.(1). Results of branching analysis in Tab.2a.

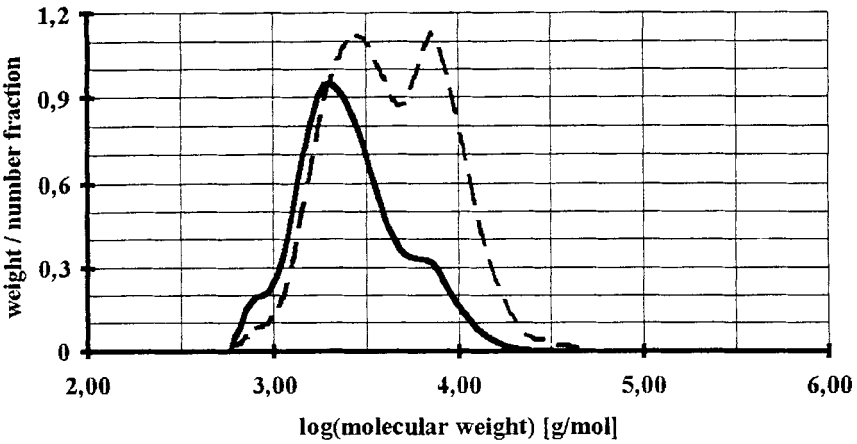

FIGURE 10 Large starch granules (Fr.I): methanol precipitate from supernatant of previous n-butanol precipitatation of aqueous DMSO dissolved starch expected to be a scb-glucan fraction; identical for pools Fr.Imet_a, Fr.Imet_b, Fr.Imet_c from preparative SEC, debranched by catalytic action of pullulanase; (- - -) mass distribution from DRI-detection of analytical SEC of debranched glucans; (———) number distribution according eq.(1). Results of branching analysis in Tab.2b.

Table 2a

Branching Analysis of Large Starch-granules (Fr.I) from Tubers of Potato Species Ukomo. (abbreviations in the text; illustrations for number and mass distributions of glucan chains: Figure 9a (Fr.Ibut_a) and Figure 9b (Fr.Ibut_b))

glucan-fraction	Mp [g mol^{-1}]	$dp_{(Mp)}$	n_%	branching characteristics		
				av_b	bp→bp	b%
Fr.Ibut_a: Mp_I	2 500	15	~ 70	**10.58**	**94.5**	**1.06**
Fr.Ibut_a: Mp_II	7 400	45	~ 20			
Fr.Ibut_a: Mp_III	122 000	750	~ 10			
Fr.Ibut_b: Mp_I	2 500	15	~ 45	**5.80**	**172.5**	**0.58**
Fr.Ibut_b: Mp_II	7 400	45	~ 35			
Fr.Ibut_b: Mp_III	122 000	750	~ 20			

Table 2b

Branching Analysis of Large Starch-granules (Fr.I) from Tubers of Potato Species Ukomo. (abbreviations in the text; illustrations for number and mass distributions of glucan chains: Figure 10 (identical for Fr.Imet_a, Fr.Imet_b, Fr.Imet_c))

glucan-fraction	Mp [g mol^{-1}]	$dp_{(Mp)}$	n_%	branching characteristics		
				av_b	bp→bp	b%
Fr.Imet_a: Mp_I	2 000	12	~ 80			
Fr.Imet_a: Mp_II	7 400	45	~ 20	**53.76**	**18.6**	**5.38**

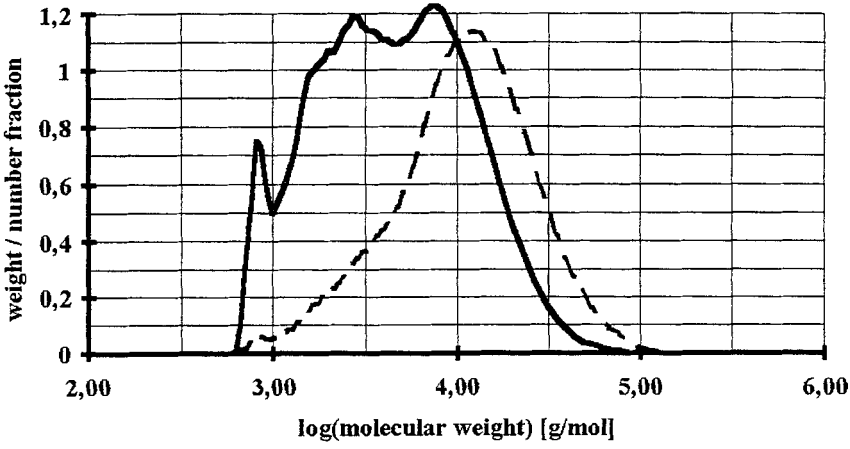

FIGURE 11a Small starch granules (Fr.II): n-butanol precipitated nb/lcb-glucan fraction; pool Fr.IIbut_a from preparative SEC, debranched by catalytic action of pullulanase; (- - -) mass distribution from DRI-detection of analytical SEC of debranched glucans; (———) number distribution according eq.(1). Results of branching analysis in Tab.3a.

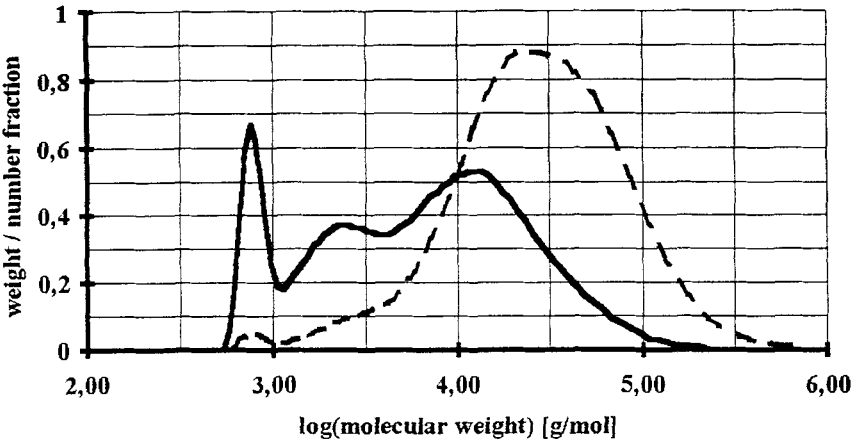

FIGURE 11b Small starch granules (Fr.II): n-butanol precipitated nb/lcb-glucan fraction; pool Fr.IIbut_b from preparative SEC, debranched by catalytic action of pullulanase; (- - -) mass distribution from DRI-detection of analytical SEC of debranched glucans; (———) number distribution according eq.(1). Results of branching analysis in Tab.3a.

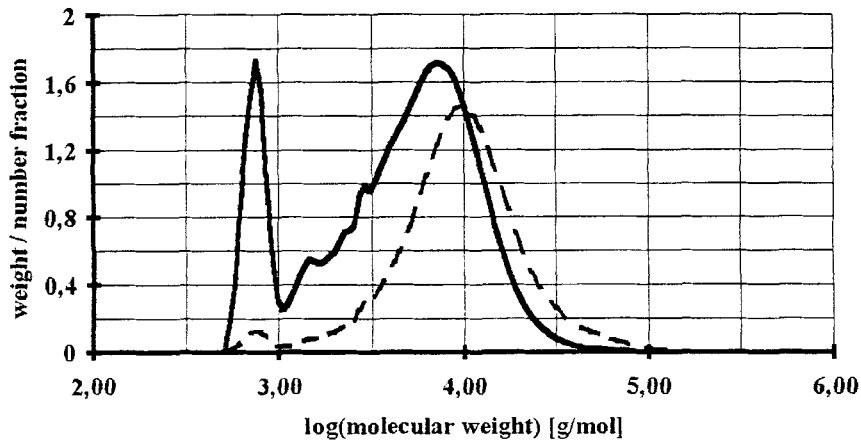

FIGURE 11c Small starch granules (Fr.II): n-butanol precipitated nb/lcb-glucan fraction; pool Fr.IIbut_c from preparative SEC, debranched by catalytic action of pullulanase; (- - -) mass distribution from DRI-detection of analytical SEC of debranched glucans; (———) number distribution according eq.(1). Results of branching analysis in Tab.3a.

Table 3a

Branching Analysis of Large Starch-granules (Fr.II) from Tubers of Potato Species Ukomo. (abbreviations in the text; illustrations for number and mass distributions of glucan chains: Figure 11a (Fr.IIbut_a), Figure 11b (Fr.IIbut_b) and Figure 11c (Fr.IIbut_c))

glucan-fraction	Mp [g mol^{-1}]	$dp_{(Mp)}$	n_%	branching characteristics		
				av_b	bp→bp	b%
Fr.IIbut_a: Mp_I	800	5	~ 5	**52.63**	**19.0**	**5.26**
Fr.IIbut_a: Mp_II	2 500	15	~ 80			
Fr.IIbut_a: Mp_III	7 400	45	~ 15			
Fr.IIbut_b: Mp_I	800	5	~ 20	**19.23**	**52.0**	**1.92**
Fr.IIbut_b: Mp_II	2 500	15	~ 20			
Fr.IIbut_b: Mp_III	13 000	80	~ 60			
Fr.IIbut_c: Mp_I	800	5	~ 20	**27.03**	**37.0**	**2.70**
Fr.IIbut_c: Mp_II	7 400	45	~ 80			

Table 3b

Branching Analysis of Large Starch-granules (Fr.II) from Tubers of Potato Species Ukomo. (abbreviations in the text; illustrations for number and mass distributions of glucan chains: Figure 12a (Fr.IImet_b), Figure 12b (Fr.IImet_c))

glucan-fraction	Mp [g mol^{-1}]	$dp_{(Mp)}$	n_%	branching characteristics		
				av_b	bp→bp	b%
Fr.IImet_b: Mp_I	2 500	15	~ 85	**49.38**	**20.3**	**4.94**
Fr.IImet_b: Mp_II	8 200	50	~ 15			
Fr.IImet_c: Mp_I	800	5	~ 20	**83.33**	**12.0**	**8.33**
Fr.IImet_c: Mp_II	1 600	10	~ 70			
Fr.IImet_c: Mp_III	6 500	40	~ 10			

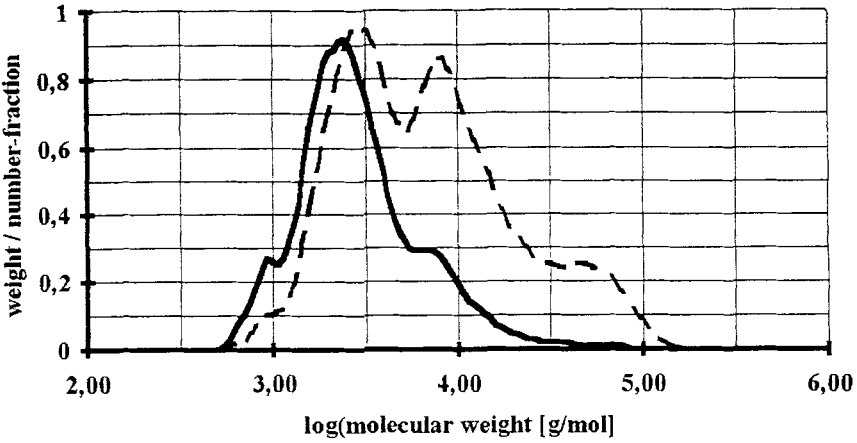

FIGURE 12a Small starch granules (Fr.II): methanol precipitate from supernatant of previous n-butanol precipitatation of aqueous DMSO dissolved starch expected to be a scb-glucan fraction; pool Fr.IImet_b from preparative SEC, debranched by catalytic action of pullulanase; (- - -) mass distribution from DRI-detection of analytical SEC of debranched glucans; (———) number distribution according eq.(1). Results of branching analysis in Tab.3b.

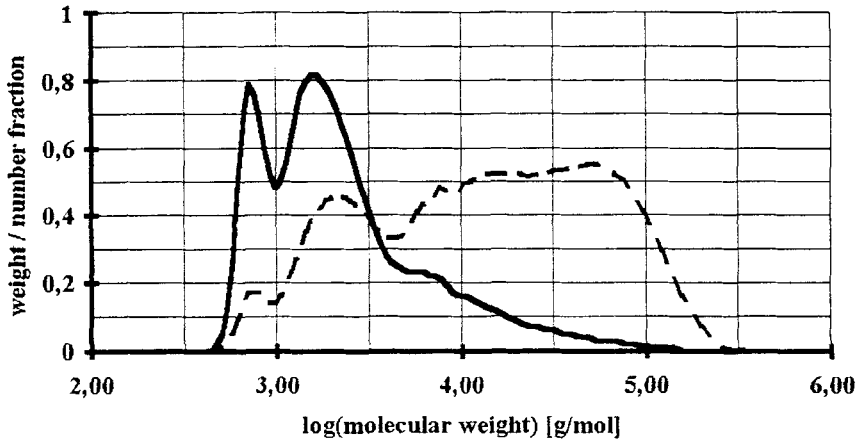

FIGURE 12b Small starch granules (Fr.II): methanol precipitate from supernatant of previous n-butanol precipitatation of aqueous DMSO dissolved starch expected to be a scb-glucan fraction; pool Fr.IImet_c from preparative SEC, debranched by catalytic action of pullulanase; (- - -) mass distribution from DRI-detection of analytical SEC of debranched glucans; (———) number distribution according eq.(1). Results of branching analysis in Tab.3b.

The short-chain-branched glucans of large granules are quite homogeneous in their branching characteristics over the total range of elution on the preparative SEC-system: an expected short average-distance between two branching positions was found with bp→bp=18.6 monomers for all debranched glucan pools (Fr.IImet_a, _b, _c: Fig.10, Tab.2b). A similar value of bp→bp-value of 20.3 monomers was found for the midrange fraction (Fr.IImet_b: Fig.12a, Tab.3b), but a significantly higher branched structure for the final fraction of preparative SEC (Fr.IImet_c: Fig.12b, Tab.3b) with bp→bp=12.0 monomers for the scb-glucans from small granules.

For a cross-check of these results methylation/GC-MS-analysis was applied to determine average branching characteristics. By means of this technique an average number of monomers between two branching points was determined as the ratio of the integrals of 1,4,5-tri-O-Ac 2,3,6-tri-O-Me-D-sorbit deriving from α1-4-linked glucose and 1,4,5,6-tetra-O-Ac 2,3-di-O-Me-D-sorbit deriving from α1-4 linked glucose with α1-6-linked branches. Obtained results can be judged to be reliable for scb-glucans, those for the nb/lcb-glucans better should be taken as trend, because the huge excess of α1-4-linked glucose over α1-4-linked glucose with α1-6-branching positions introducef high deviations in the calculated ratios. But even with this restriction a quite good agreement of GC-MS-results and results from analytical SEC was found (Tab.4).

Summarizing, differences in the branching-structure of glucans from starch granules with different biological history were identified and could be quantified as the lack of extremely high-dp glucan chains in the constituting glucan-chain distribution of starch from small granules. Obvious dissimilarities in mass- and number-distributions between glucans from large and small granules were found again, diminished but still significant, as different values of average distance between two branching points (bp→bp) and average percentage of branching (b%). In general, the majority of glucans from small granules show a higer average degree of branching than glucans from large granules, a fact that most likely is

Table 4

Branching Characteristics of Glucan Fractions from Starch-granules of Tubers of Potato Species Ukomo according to Table 1.

glucan-fraction	branching characteristics				
	SEC: av_b	SEC: b%	SEC bp→bp	GC-MS: bp→bp	iodine E_{640}/E_{525}
Fr.Ibut_a	10.58	1.06	94.5	70	2.2
Fr.Ibut_b	5.80	0.58	172.5	100	1.9
Fr.Imet_a	53.76	5.38	18.6	24	0.8
Fr.Imet_b	53.76	5.38	18.6	26	0.8
Fr.Imet_c	53.76	5.38	18.6	n.d.	0.8
Fr.IIbut_a	52.63	5.26	19.0	60	1.6
Fr.IIbut_b	19.23	1.92	52.0	119	2.2
Fr.IIbut_c	27.03	2.70	37.0	n.d.	2.0
Fr.IImet_b	49.38	4.94	20.3	18	0.8
Fr.IImet_c	83.33	8.33	12.0	n.d.	0.6

due to changing activities of (1-4)-α-D-glucan-branching 6-glycosyltransferase (EC 2.4.1.18) during biogenesis of starch in the investigated potato tubers.

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