The effect of phosphate and amylopectin molecular size on peak viscosity of starch pastes

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ABSTRACT

Potato starch contains covalently bound phosphate. The aim of this study was to get more detailed knowledge of the role of the starch bound phosphate in determining rheological properties. In order to eliminate interfering parameters, starches with varying levels of phosphate were produced within the same cultivar and separated into four granule size classes resulting in 20 starch samples with a 3-fold difference in phosphorylation level. In all samples phosphate content, amylose content, chain length distribution of debranched amylopectin were analysed and the viscosimetric properties were determined using a rapid visco-analyser (RVA). Unexpectedly, no correlation was found between phosphate content and peak viscosity. Size exclusion chromatography of the starches revealed great variations in amylopectin molecular size which correlated to viscosimetric properties.

INTRODUCTION

Native as well as modified starches are widely used to control the stability, consistency and texture of many composite food products, ranging from sauces, soups, dressings and spreads to frozen or chilled products such as drinks, ice creams and desserts. The rheological and gelling properties of the starches in question are responsible for the quality of the various products.

A large range of native starches contain covalently bound phosphate[1]. Compared to other commercial starches, potato starch contains high amounts of covalently bound phosphate which means that in average one out of every 350 glucosidic residues is phosphorylated[1].

A significant positive correlation is generally found between peak viscosity of a starch paste and phosphorylation level in starches from different potato cultivars[2,3]. The phosphorylation level, however, cannot fully explain the variation in peak viscosity.

In addition, the presence of starch bound phosphate increases swelling capability and results in clear gels[4].

Phosphate groups are mainly located in the longer unit chains (30-100 glucose residues) of amylopectin[1,5], and our recent results show that a major part of starch phosphate is located in the amorphous part of the granule. Gelatinization of a starch granule is initiated by an endothermic absorption of water mainly in the amorphous growth rings resulting in swelling of this region and liberation of amylose[6]. Subsequently, the crystalline regions are disrupted.

Natural variations in amylose content do not affect peak viscosity[3]. The amylose free potato starch, however, displays a somewhat lower viscosity than wildtype starch[7].

In order to obtain deeper insight into the role of starch molecular structure in controlling viscosimetric properties, we

have analysed starches with regard to starch paste viscosity using a rapid visco-analyser, and phosphate content, amylose content, chain length distribution of amylopectin and the distribution of amylose and amylopectin by size exclusion chromatography (SEC).

MATERIALS AND METHODS

Plant material:

Starches from *Solanum tuberosum*, cultivar `Dianella´ with altering degree of phosphorylation were obtained by growing the plants in pots containing 10 L vermiculite (medium grade) in a growth chamber, day/night temp.: 20-21°C/12°C, where plants received 0 mg phosphate (P)*L^{-1}, 3 mg P*L^{-1}, and 12 mg P*L^{-1}, respectively, (see Table 1) and optimum levels of all other nutrients. Each plant received 1L of nutrient solution per week and tap water if necessary from the time of emergence. Tubers from the first growth season were harvested and a part of them were used for starch isolation (see below). The remaining part was used as seed tubers in the following growth season as follows: Tubers from plants that received 0 mg P*L^{-1} and 3 mg P*L^{-1}, respectively, were laid under the same conditions as described above and the plants received 0 mg P*L^{-1}. Dianella 2 and 87-BDN-56 were grown in the field.

Table 1. Experimental design for production of starches with altering phosphorylation level.

Sample	Phosphate nutrition mg P*L^{-1}
A	0
B	3
C	12
D (seed tubers from experiment A)	0
E (seed tubers from experiment B)	0

Isolation of starch

Double ion-exchanged water was used in all purification steps. Tubers of the cultivar Dianella grown in climate chamber were washed and passed through a juice centrifuge (Moulinex) and the starch suspension was centrifuged for 15 min., 1500 rpm, 4°C. The starch was passed through a 140 µm mesh with addition of water, washed twice in water. The second wash was followed by vacuum filtration and then recovered. The starches were dried in a ventilated chamber over night at 28°C. Before subjected to analysis of viscosimetric properties, the starches were carefully grounded with a mortar and pestle to obtain single grain preparations.

Starches from Dianella2 and 87-BDN-56 were isolated as described in Bay-Smidt et al.,(1994)[8]

Gajutzu starch was a kind gift from Kazuo Yamamoto, Hokuren Federation Agricultural cooperatives, Japan.

Separation of potato starch granules into four granule size classes.

Separation of starch granules from the native starches A,B,C,D, and E of the cultivar Dianella into 4 granule size classes was accomplished by wet-sieving through nylon filters fitted in a non-pressurized AMICON stirring cell. Filtration was carried out in ice-cold double ion exchanged water under vigorous stirring. In the first step, cell wall remains and starch granules larger than 70 µm were retained on the filter. The procedure was repeated with each run-through with 50 µm, 31 µm and 20 µm filters. The fraction containing granules below 20 µm was allowed to settle overnight before the water was sucked off and the granules were dried at 28°C in a ventilated chamber. Completion of each step was determined by light microscopy with a measuring ocular mounted on the microscope. Throughout the filtration procedure, granules were stained with iodine to assess their intactness. No sign of breakdown was observed.

Determination of starch bound phosphate

Starch bound phosphorus were determined as Glc-6-P released after acid hydrolysis of starch as described in Bay-Smidt et al., (1994)[8].

Determination of amylose content

Starch (100 mg) was dissolved in 50 ml 1M NaOH under vigorous stirring over night followed by addition of 5 N HCl (0.33ml. per ml starch solution). The sample was thoroughly mixed. To 50 µl sample was added 1000 µl iodine solution (0.26 g I_2, 2.6 g KI in 10 ml H_2O diluted 1000 times) and the absorption at 550 nm and 620 nm was measured on a Shimadzu UV-160 A spectrophotometer. A standard curve (5-40 % amylose) was made by mixing pure amylose and pure amylopectin (Sigma Chemical Company).

Chain length distribution of debranched amylopectin

The determined was carried out as described in Blennow et al., (1998)[5]

Starch paste viscosity analysis

The pasting properties of starches were analysed using a Rapid ViscoAnalyser (RVA) model 4 (Newport Scientific, Warriewood, Australia). The viscosity of the sample is sensed by using a precision motor to continuously rotate a plastic paddle in the sample at constant speed and controlled temperature regime. Current through the motor is electronically measured and digitised for computer storage. The starch concentration was 2 g on a dry weight basis, corrected to 14% moisture, in 25 ml double ion-exchanged water. The moisture content was calculated from the weight loss after freeze drying the sample for 24 h. In the RVA, the starch suspension was held at 50°C for 1 min and subsequently heated to 95°C at 12.3°C/min. Holding time at 95°C was 2.5 min. Subsequently, the sample was cooled to 50°C at a cooling rate of 12.3°C/min, where it was kept for 2 min. The rotation speed of the paddle was 160 rpm and cooling was performed with tapwater.

Size exclusion chromatography (SEC)

10 mg of starch was transferred to a glass tube containing 40 µl 2 M NaOH. After 15 min of incubation at room temperature the tube was transferred to a boiling water bath. The starch gel was carefully disrupted by dilution to 5 ml with double ion-exchanged water and pipetting for 4 min. 200 µl of the sample was immediately applied to a TSKW 75 column (26mm*300mm) and eluted with 10 mM NaOH at a flow rate of 0.75 ml/min at 50°C. Refractive index was measured by a Differential refractometer (Waters 410, Millipore).

Determination of λ-max

λ-max of the iodine/starch complex was measured in SEC fractions by mixing 900µl of sample with 100 µl Lugol's solution and measure the absorbance spectra from 700 to 400 nm using a Shimadzu UV-160A spectrophotometer.

RESULTS

Chemical characterization of the starch samples

Potato starches with a 3-fold difference in the level of starch bound phosphate (Table 2) were obtained by growing the cultivar Dianella under optimum or restricted phosphate supply, as indicated in table 1 and by separation of the starches into four different granule size classes. A strong positive correlation between the level of starch bound phosphate and amylose content was found in all granule size classes and in the native starches of Dianella (Table 2). This relationship is not found among potato cultivars (data not shown). The phosphate content was highest in the small granules and the amylose content was highest in the large granules.

Analysis of chain length distribution of debranched amylopectin showed no significant difference between potato starch

samples from the cultivar Dianella (data not shown). A positive relationship between phosphorylation level and chain length distribution in starches from different species and from different potato cultivars has been demonstrated earlier.[5]

Table 2. Phosphate and amylose content of starch samples.

	Phosphate content (nmol Glc-6-P/mg starch)	Amylose %
Dianella 2	17.0	n.d.
87-BDN-56	25.5	n.d.
gajutzu	58.8	n.d.
Dianella native starches		
A	8.6 ± 0.2	25.9
B	8.4 ± 0.2	26.4
C	13.5 ± 0.3	28.0
D	6.5 ± 0.2	23.9
E	5.7 ± 0.3	24.0
avg. ± SD.	8.6 ± 3.0	25.6 ± 1.7
0-20 µm		
A	8.3 ± 0.4	26.1
B	8.5 ± 0.3	25.8
C	14.3 ± 0.7	27.8
D	7.2 ± 0.3	23.2
E	6.6 ± 0.3	23.1
avg. ± SD	9.0 ± 3.1	25.2 ± 2.0
20-31 µm		
A	6.6 ± 0.2	27.8
B	6.4 ± 0.7	27.5
C	11.1 ± 0.1	29.7
D	5.1 ± 0.1	25.6
E	4.6 ± 0.3	26.1
avg. ± SD.	6.8 ± 2.6	27.3 ± 1.6
31-50 µm		
A	4.5 ± 1.1	27.6
B	6.5 ± 1.0	28.6
C	8.6 ± 0.1	29.9
D	4.1 ± 0.3	26.2
E	4.0 ± 0.1	26.2
avg ± SD	5. 5 ± 2.0	27.7 ± 1.6
50-70 µm		
A	5.0 ± 0.1	29.4
B	5.9 ± 0.2	29.8
C	8.0 ± 0.2	29.8
D	4.0 ± 0.1	27.1
E	3.9 ±0.1	27.1
avg. ± SD	5.4 ± 1.7	28.6 ± 1.4

Table 3. Correlation coefficients (r) in linear regression analysis of amylose content and phosphorylation level (table 2). The level of significance (P) in Students t-test of the hypothesis H_0: b=O, (b = slope of regression line) is calculated when r > 0.7 and visual evaluation of a graphic presentation of the data supported the statistical evaluation.

	Dianella native starches	<20 µm	20-31 µm	31-50µm	50-70 µm
r	0.95	0.86	0.95	0.96	0.81
P	0.005	0.025	0.005	0.001	0.05

SEC

Separation of starch into amylopectin subfractions and amylose was achieved by SEC. As determined by its iodine complex, amylopectin elutes between fraction 30 and fraction 53 and amylose elutes approximately from fraction 54-65. A low molecular weight amylopectin co-elutes with the amylose fraction as observed by separation of pure amylopectin (waxy) starch.

Viscosimetric analysis

Granule size had no significant influence on peak viscosity (Table 4). Neither the amylose content nor the phosphate content showed any correlation to peak viscosity, except in granule size class 31-50 µm, where negative correlations were found (Table 4).

The potato cultivar Dianella 2 had higher peak viscosity but lower phosphate content than the cultivar 87 BDN-56 (Table 2, Table 4) The extremely high phosphorylated gajutzu starch had lower viscosity than the two potato cultivars (Table 4, Fig. 5).

Fig.1. shows the profiles of SEC and RVA for starches with granule size 20-31µm. Generally, these starches had low amounts of early eluting (low MW) amylopectin. The samples B and D with the

highest amounts of early eluting amylopectin clearly showed the highest peak viscosity, and a linear relationship was found between these parameters (Table 5). Starch sample C 31-50 μm (Fig. 2) had the highest phosphorylation level in this series but the lowest peak viscosity. In analogy with the results for the series 20-31 μm, a very low amount of early eluting amylopectin is found in samples with low viscosity (Table 4).

Table 4. Peak viscosity and % of fraction 32-37 of total starch separated by SEC

	Peak viscosity (RVU)	% frac.32-37 from SEC
Dianella 2	558	37.3
87-BDN-56	246	26.6
gajutzu	205	29.9
Dianella native starches		
A	290	39.0
B	365	41.2
C	330	30.2
D	332	43.7
E	27	45.3
0-20 μm		
A	157	27.5
B	283	44.0
C	267	34.5
D	209	42.2
E	181	42.5
20-31 μm		
A	86	9.0
B	235	24.8
C	87	3.3
D	152	23.5
E	76	7.5
31-50 μm		
A	145	27.0
B	165	23.0
C	93	8.4
D	234	36.0
E	199	30.2
50-70 μm		
A	180	22.5
B	121	14.6
C	n.d.	0
D	246	27.4
E	156	22.1

Table 5. Correlation coefficients (r) in linear regression analysis of peak viscosity versus phosphate content, amylose content and percentage of fraction 32-37 in SEC-analysis. P-level is calculated as described under Table 3 and written under the r-value when calculated.

	Dianella native starches	<20 μm	20-31 μm	31-50 μm	50-70 μm
Phosphate content vs peak viscosity	0.0	0.6	-0.2	-0.8 0.025	-0.2
amylose content vs peak viscosity	0.3	0.5	0.1	-0.9 0.005	0.0
% frac. 32-37 vs peak viscosity	0.0	0.4	0.9 0.01	0.9 0.0005	0.9 0.0005

Fig. 3A and Fig. 3B show a linear correlation between peak viscosity and the fraction of early eluting amylopectin (Fig. 3B). No such correlation was found between phosphorylation level and peak viscosity.

The five native starches of Dianella (Fig. 2) had very similar SEC and RVA profiles, except sample C which had a lower amount of early eluting amylopectin. In this series, no correlation was found between peak viscosity and fractions 32-37 from SEC (Table 5).

Dianella 2 had a higher amount of early eluting amylopectin than 87-BDN56 and gajutzu starch (Table 4, Fig. 5). This correlates well with the RVA data, in that the highest peak viscosity was found in Dianella 2.

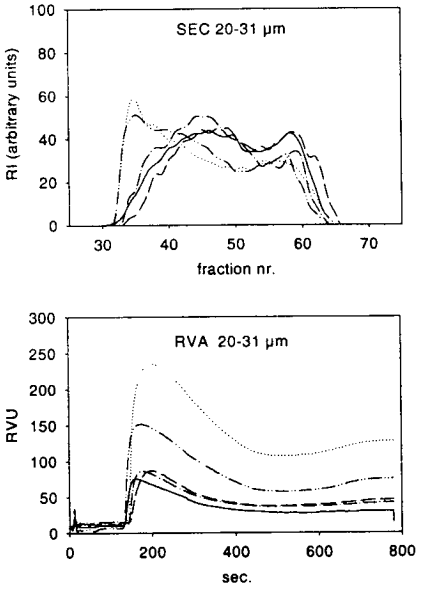
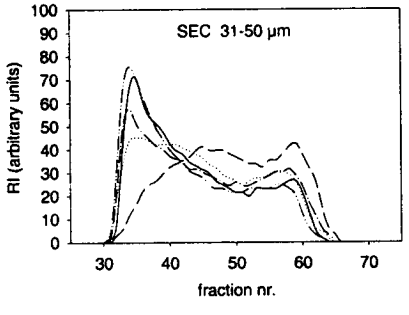


Figure 1. SEC and RVA analysis of starches from potato cv. "Dianella" with granule size 20-31 µm. A 20-31µm (_._), B 20-31µm (.....), C 20-31 µm (-----), D 20-31 µm(_.._), E 20-31 µm(____)



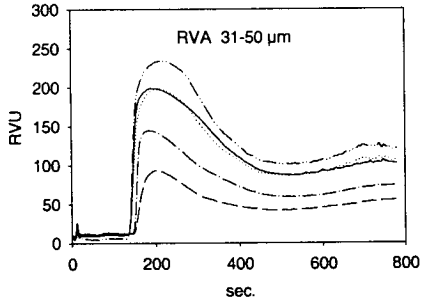


Figure 2. SEC and RVA analysis of starches from potato cv. "Dianella" with granule size 31-50 µm. A 31-50 µm (_._), B 31-50 µm(.....), C 31-50 µm (-----), D 31-50 µm(_.._), E 31-50 µm(____)

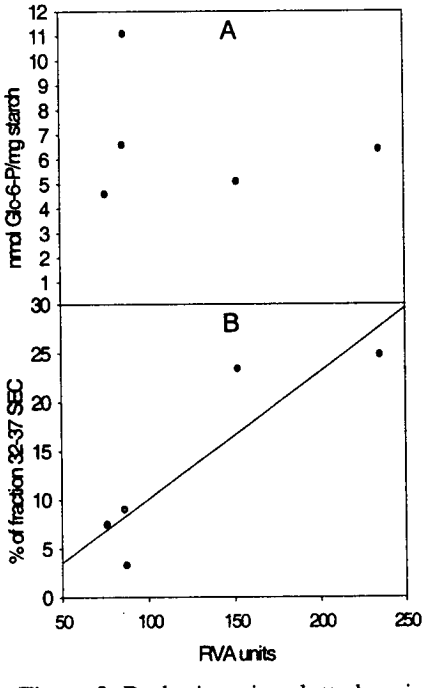


Figure 3. Peak viscosity plotted against phosphorylation level (A) and peak viscosity plotted against % of fraction 32-37 in SEC (B) in granule size 20-31 µm

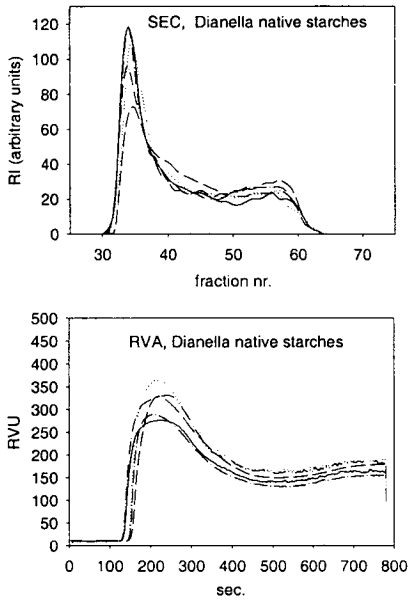


Figure 4. SEC and RVA analysis of starches from potato cv. "Dianella", grown under various phosphate supply.
A (_._), B(.....), C(-----), D(_.._), E(___)

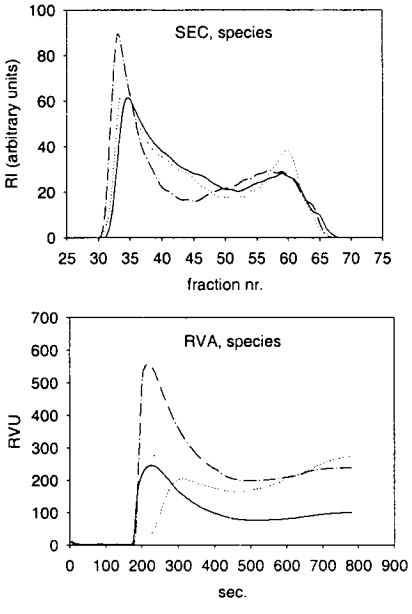


Figure 5. SEC and RVA analysis of starches from potato cv.Dianella (_._) and potato cv. 87-BDN-56(___) and gajutzu starch (.....)

DISCUSSION

The correlation coefficient between phosphorylation level and peak viscosity of potato starch samples is reported to be in the range from 0.6 [3] - 0.8 [2].

In this study, we did not find a positive correlation between these parameters. Analysis of the distribution of starch components by size exclusion chromatography showed large differences in the distribution profiles which could be related to the viscosimetric properties. A low percentage of early eluting amylopectin correlated well with a low peak viscosity. According to Bello-Perez et al. (1998)[5], the early eluting amylopectin in SEC analysis represent true high molecular mass amylopectin. By multiple angle laser light scattering, we are currently analysing whether this is also found in our samples. The molecular mass of potato starch amylopectin is between 10^7 -10^9 Da[10]. In the

series of starches from the potato cultivar Dianella with varying levels of starch bound phosphate (native Dianella starches), no correlation between peak viscosity and percentage of early eluting amylopectin was found (r = 0.4). However, looking closer into the profiles in this series it appeared that sample C which had the highest amount of starch bound phosphate had a peak viscosity similar to sample D which was less phosphorylated. This can be explained by the fact that sample C had a very low amount of early eluting amylopectin as compared to the other samples in this series. The same arguments holds true when two potato starches and gajutzu starch are compared. From these data we conclude that the positive effect on peak viscosity of starch bound phosphate is enhanced by high amounts of early eluting amylopectin and diminshed if the percentage of early eluting amylopectin is low. This result is of great importance for the tailoring of starches with specific properties.

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