Functional properties of genetically modified potato starches

Bente Wischmann[1], Andreas Blennow[2], Finn Madsen[3], and Ole Bandsholm[4]

[1] FBEG, BioCentrum, DTU, Technical University of Denmark, DK-2800 Kgs. Lyngby, Denmark. [2] Department of Plant Biology, Royal Veterinary and Agricultural University, 40 Thorvaldsensvej , DK-1871 Frb.C, Denmark. [3] Danisco Cultor, 38 Edwin Rahrs Vej, DK-8220 Brabrand, Denmark. [4] KMC, Herningvej 60, DK-7330 Brande, Denmark

ABSTRACT

Some results from a large collaborative study of a series of 30 different starches derived from genetically modified potato plants are presented. The analysis utilises both physical/chemical and functional properties and some conclusions are drawn focusing on the amylopectin chain length and content of phosphate in the starch samples.

INTRODUCTION

Native starch is modified genetically, physically or chemically to obtain a wide range of useful functional properties in food products[1]. The functional properties are mainly determined by the structure of the starch. The covalently bound phosphate in potato starches provides these starches with unique functional properties. Furthermore it is known that an important parameter, which characterises the physical/chemical properties of starch is the degree of branching and chain length of amylopectin[2].

As a part of a concerted action a series of genetically modified potato starches was analysed in order to look for starches with new or altered functionality, but also to achieve knowledge about the correlation between structural, physical/chemical and functional properties. This study included 30 different starches, which were obtained by transformation of potato plants with either starch branching enzyme (BE) or E.coli glycogen branching enzyme (G).

The analysis may be divided into chemical, rheological, textural and thermal analysis. The chemical analysis included: phosphate content, amylose %, protein, ash, pH, dry weight and amylopectin chain length. The functional properties were characterised both by thermal and rheological analysis. The rheological analysis included: rapid visco analysis (RVA), dynamic oscillation measurements using a Stress Tech rheometer. Also freeze/thaw stability and retrogradation measured by pulse-NMR, and texture profile analysis (TPA) using a texture analysis instrument was performed. The thermal properties of the starches were analysed by differential scanning calorimetry (DSC).

To be able to handle and overview the data a multivariate statistical approach has been taken. The data is treated by focusing on the degree of phosphorylation of the starches and its relation to starch structure and functional properties.

MATERIALS AND METHODS

Starch material

Genetic modification of the potato plants was carried out to alter the functional properties of the starches. One series of potatoes (B1 – B19) corresponds to starches derived from potatoes in which potato starch branching enzyme one (BE1) is reduced by the antisense RNA technology. The other series (G20 to G33) is starch samples derived from potato plants in which the

E.coli glycogen synthase has been inserted in order to get it expressed in the plants.

Chemical analysis

All starch samples were characterised by a series of standard industrial quality parameters including pH, dry weight, and content of ash and total protein. Determination of amylose was done as described in Bay-Smidt et al.[2]. Starch bound phosphate was determined as released Glc-6-P after acid hydrolysis following the method of Bay-Smith et al.[3].

Thermal analysis

The thermal properties of the starch samples were analysed by differential scanning calorimetry (DSC) using a Seiko DSC220 run from 25 °C to 100°C at 5 °C/min. All Starch samples were analysed in slurries of 2 mg sample and 8 µl 10 mM NaCl in triplicates.

Rapid visco analysis

The pasting properties of starches were analysed using a Rapid Visco Analyser (RVA) model 4 (Newport Scientific, Warriewood, Australia). The starch concentration was 4% starch on dry weight basis in slurries of 10 mM NaCl.

Rheological testing

Rheological testing was performed using a Stress Tech controlled stress rheometer (Rheologica AB, Lund Sweden). The measurement geometry used was a 4 cm plate-plate. All measurements were carried out in triplicates at 25 °C on 5% starch gels in 10 mM NaCl. Oscillatory measurements were carried out within the linear viscoelastic region, over a frequency range of 0.1Hz to 7.0Hz. The relative gel strength was calculated as the slope of log G' versus log frequency. All measurements were done in triplicates.

Table 1. The different parameters used to examine all transgenic potato starch samples.

Variable group	Variable	Description
Chemistry	P	Phosphate content (nmol/mg starch)
	Amyl	Amylose (%)
	DW	Dry weight (%)
	Ash	Ash (%)
	Prot	Protein (%)
	pH	PH
HPAEC-PAD	6-60	Amylopectin chain length (DP6 to DP60)
TPA	Cohes	Cohesiveness
	Adhes	Adhesiveness
	Spring	Springiness
	Gum	Gumminess
	Chew	Chewiness
P-NMR	Retr1	Retrogradation*)
	Retr3	Retrogradation*)
	Retr7	Retrogradation*)
	f/t1	1 freeze/thaw cycle**)
	f/t2	2 freeze/thaw cycles**)
	f/t3	3 freeze/thaw cycles**)
RVA	Peak1	Peak viscosity
	Through1	Lowest visc. after peak1
	Breakdown	Difference between Peak 1 and Through 1
	Final visc	Final viscosity
	Setback	Difference between Through 1 and final viscosity
	Peak time	Time at which peak viscosity is reached
	Pasting temp	Width of viscosity peak
DSC	To	Onset temp. (°C)
	Tm	Peak temp. (°C)
	Tc	Completion temp.(°C)
	ΔH	Enthalpy change (mJ/mg)
Oscillation	G'	Storage modulus (Pa)
	Slope G'	Slope of G' values around 1.3Hz
	G''	Loss modulus (Pa)

*)Retrogradation was measured at 5°C after storage of 1, 3 and 7 days.

**)Freeze/thaw stability was measured after 1, 2 and 3 cycles of –18°C to +20°C.

Texture profile analysis

A texture analyser TA-XT2 was used for the texture profile analysis (TPA). The double compression was carried out in TPA

dishes (50 mm in diameter, 13 mm in height) with a cylindrical probe (TA 5 bob, ½''), which was pressed 66% into the 5% starch gels (based on dry weight) in 10 mM NaCl at a speed of 1 mm/sec. All measurements were carried out in duplicates.

Amylopectin chain length

The chain length distribution of debranched amylopectin was carried out as described by Blennow et al.[4].

Pulse-nuclear magnetic resonance (P-NMR)

A low field pulsed NMR spectrometer (Bruker Minispec NMS 120) operating at 20MHz – 0.47 Tesla was used to determine the freeze/thaw stability of the starch samples measured at 20 °C after 1, 2 or 3 cycles of freeze/thaw. Degree of retrogradation was measured at 5 °C at day 1, day 3 and day 7. Measurements were carried out in triplicates or with four repetitions, respectively. As in the other analysis samples were 5% starch on dry weight basis in 10 nm NaCl.

DATA TREATMENT

In order to get an overall view of the results a multivariate statistical approach was taken by use of Unscrambler 7.6 (Camo A/S, Tronheim, Norway). Both a principal component (PCA) and a principal least square analysis (PLS) was carried out.

RESULTS AND DISCUSSION

As listed in Table 1 a range of different analysis was performed in order to characterise the different transgenic starches looking for starches with new functional properties. The results presented here will focus on some of the results and concentrate on the correlation between phosphate content of the starch samples and amylopectin chain length.

Table 2. Phosphate content of selected starch samples. Mean values (three determinations) of the five highest (B samples) and five lowest (G samples)

Starch sample	Phosphate [nmol G6P/mg]
B2	18.1
B4	17.9
B5	15.1
B6	15.3
B10	14.3
G21	6.7
G23	6.9
G24	7.0
G28	6.8
G29	6.4
Control	9.6

Amylopectin chain length

The starches are derived from two different transformations. The starches in the series named B are expected to have fewer branches in the amylopectin molecules due to the lowering or elimination of one of the enzymes responsible for starch branching (*starch branching enzyme 1*, SBE1). These starches may therefore contain relatively longer amylopectin chains. The other starch series named G, stems from a transformation in which an enzyme (*glycogen branching enzyme* from E.coli), with branching activity has been inserted. These starches are therefore expected to contain relatively more branches and therefore shorter chains in the amylopectin molecules.

By HPAEC-PAD the branch pattern of the debranched amylopectin molecules was determined of all the starches. In Fig. 1 the chain length distributions are shown for all the starches. For clarity the mean values of the five highest and the five lowest phosphorylated starches and the control starch is shown in the lower panel. It is seen that the starches from the G-series contain more chains of degree of polymerisation

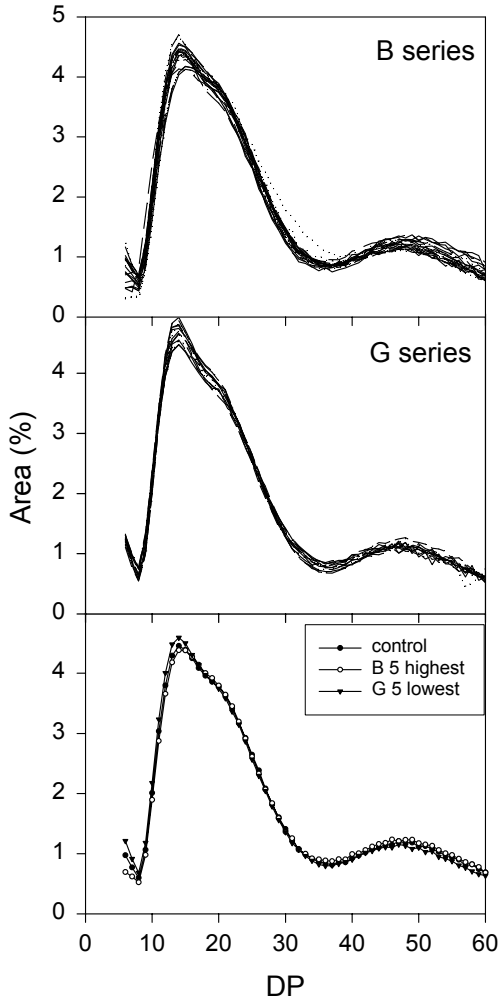

Figure 1. Amylopectin chain length distributions of transgenic potatoes B1-B19 (B series) and G20-G33 (G series). Bottom: Mean values of the five highest and the five lowest phosphorylated starches and control starch.

(DP) around 13-15 compared to the B-series starches (Fig. 1, lower panel).

Chemical analysis show a small but significant difference between the phosphate content in the starches both among and between the two starch series B and G. Table 2 show the phosphate content in the five highest and the five lowest starches as well as in the control, which is not genetically modified.

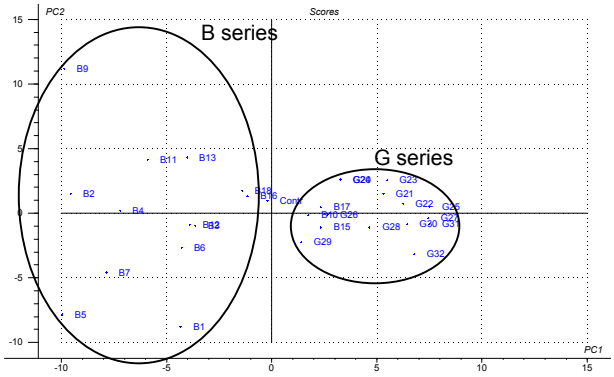

Figure 3. PCA score plot of all samples. Circles indicate the relative distribution of the two series of transformants.

Principle component analysis

To obtain an overview of the data a principal component analyses (PCA) was performed including all samples and analysis. The resulting score plot is shown in Fig. 3. The first principle component (PC1) describes the difference between the two series of transformants, B and G. Circles Fig. 3 indicate these groups. Especially differences in starches in the B series seem to be explained by PC2. Note that the control starch has negligible influence on the plot. PC1 and PC2 explain 38% and 15% of the variance, respectively.

The corresponding loading plot is shown in Fig. 4. The PC1 distinguishes between some of the TPA results (i.e. Adhes, Spring and Chew) together with one RVA result: Breakdown on one side (to the right of the plot) opposed to most of the chemical data and the rest of the RVA data on the other side (i.e. to the left of the plot). The degree of polymerization (DP) of debranched amylopectin is seen as the DP numbers 6 to 60. Circles in Fig. 4 indicate three groupings of DP, which is: shortest chains (I), medium length chains (II) and longest chains (III). PC1 describes the difference between DP group-I and DP groups -II and -III. The positioning of the phosphate content is indicated. It is seen that phosphate content is closely correlated to the longest DP chains. This confirms earlier results that 'the longer chains in starch the higher phosphate

content'[4]. The scores and loading plot confirms that the starches in the G series contain shortest amylopectin chains and lowest content of phosphate. On the other side of the plots the starches in the B series contain the larger amylopectin chains and the B starches with the highest amount of phosphate are closely correlated to the phosphate.

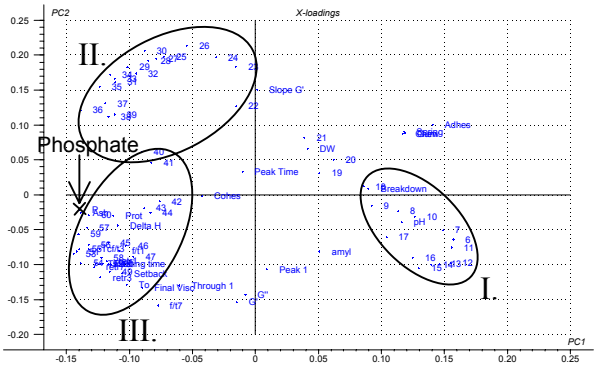

Figure 4. PCA loading plot of all variables. Circles indicate the relative position of amylopectin chain lengths: the shortest chains (I), medium length chains (II) and largest chains (III). The positioning of the phosphate content in the starch is also indicated.

Partial least squares regression analysis

Focusing on the structure – functional relationship a partial least squares regression analysis (PLS) was made in order to relate the chain length to the physical/chemical and functional parameters. In Fig. 5 and 6 the resulting PLS is seen. To the left of the score plot Fig. 5 the starch series B1 to B7 is situated. Opposed to this and following the PC1 the starch series G20 to G32 is found. In the top of the plot the rest of the B starch series (B8 to B19) is found. This distribution agrees well with the observation that amylopectin longer chains (DP > 20) are situated in the upper left field of the X-loading weights and Y-loadings plot Fig.6 and is opposed to the very short chains situated in the lower right field (Fig.6).

The slope of G' draw samples of the B-series (B8 to B19) along the PC2, and thereby contributes to the separation of the B-series starches into two groups.

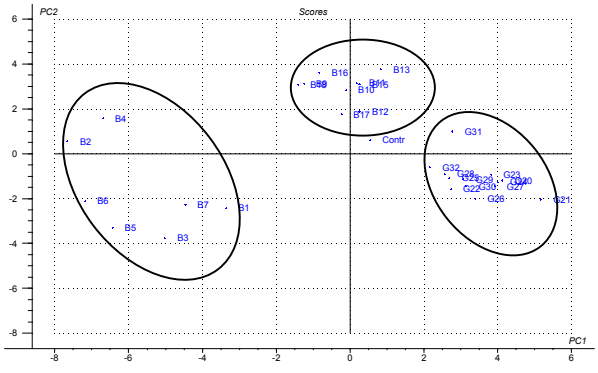

Figure 5. PLS scores plot showing the relation between starch samples and physical/chemical and functional parameters (se Table 1 for details of the parameters). Circles indicate three groupings of the starches.

Starches with relatively short amylopectin chains contain less phosphate; form gels that are adhesive, chewy and gummy and these starches also show high values of breakdown determined by the RVA. The

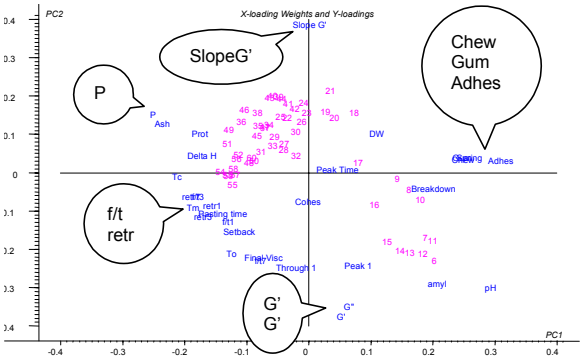

Figure 6. PLS X-loading weights and Y-loading weights for the model between structural characteristics (amylopectin chain length) and functional parameters Important parameters are indicated by callouts. (Se Table 1 for details of these parameters).

gels from B1 to B7 starches show low freeze/thaw stability as well as high degree of retrogradation compared to the G series of the starches. They are rather strong gels as indicated by the close relation to G' and G'' and to the reverse of slopeG', which is situated in the lower and upper part of the plot, respectively.

CONCLUSION

Taking a multivariate statistical approach it was possible to get an overview of the many data and draw some conclusions. The two different genetic modifications were clearly differentiated and the B transformant series could furthermore be divided into two subgroups. The positive correlation between amylopectin chain length and parameters such as phosphate content was confirmed. Focusing on the structure - functional relationship it was also possible to relate the amylopectin chain length to some of the gel properties of the starches as f. ex. G', low freeze stability and high degree of retrogradation.

ACKNOWLEDGMENTS

The work presented here is financed through a centre contract: '*Centre for development of new starches for use in food, (1999-2003)'*.

REFERENCES

1. Ellis, R., Cochrane, M., Dale, M., Duffus, C., Lynn, A., Morrison, I., Prentice, R., Swanston, J., and Tiller, S.J. (1998), "Starch production and industrial use", *J. Sci. Food Agric.,* **77**, 289-311.

2. Bay-Smidt, A.M., Blennow, A., Bojko, M., and Møller, B.L. (1999), "The effect of phosphate and amylopectin molecular size on peak viscosity of starch pastes", *Ann.Trans.Nordic Rheol.Soc*. **7**, 31-38

3. Bay-Smidt, A.M., Wischmann, B., Olsen, C.E., and Nielsen, T.H. (1994), "Starch bound phosphate in potato as studied by a simple method for determination of organic phosphate and 31P-NMR", *Starch*, **46**, 167-172

4. Blennow, A. Bay-Smidt, A.M., Wischmann, B, Olsen, C.E. and Møller,B.L. (1998), "The degree of starch phosphorylation is related to the chain length distribution of the neutral and the phosphorylated chains of amylopectin", *Carb.Res.*, **307**, 45-54