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Short communication

Characterisation of potato leaf starch with iodine-staining

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Abstract

The structure of starch from potato leaves collected early morning and late afternoon in the middle of July was studied. Starch was extracted from the leaves with dimethylsulphoxide and purified in several steps. Sepharose CL-2B elution profiles of the starches detected by iodine-staining showed that amylopectin from the leaf starches had broader size ranges than tuber starch amylopectin. Additionally, in leaf starch there was an increase during the day in the proportion of a fraction that had a similar size range to amylopectin from tuber starch. Amylopectins in both leaf and tuber starches were found to have similar average chain length, while amyloses showed different spectra with iodine-staining. Potato leaf and tuber starches seemed to be composed only of amylose and amylopectin with similar chain length distribution over their molecular weight ranges.

Keywords: Leaf starch; Tuber starch; Iodine-staining; Starch extraction; HELP

1. Introduction

Starch is a storage carbohydrate deposited as granules in chloroplasts of photosynthetic tissues or in amyloplasts of storage tissues. In storage organs, the synthesis of starch is continuous, whereas in leaves the starch is accumulated during the day and used at night to achieve a more or less constant supply of sucrose (Buléon, Colonna, Planchot, & Ball, 1998).

In most plants the starch is built up of 70–80% amylopectin and 20–30% amylose. Amylopectin is a branched polymer with a molecular weight of 10^7–10^8 Da and an average unit chain length between 18 and 25 glucose residues. Amylose has a molecular weight of 10^5–10^6 Da and an average chain length between 100 and 550 glucose residues (Nakamura, 1996).

The formation of colour by the interaction between starch and iodine is an important property for starch characterisation. Iodine forms a complex with α-1,4 linked glucans by insertion in the hydrophobic cavity of the linear glucan helices. This complex is generated if the chains are sufficiently long. After passing the point where colour formation occurs (DP 12 with a λ_{max} of 490 nm), the iodine colour changes through brown, red, purple and finally becomes blue when the chain length is about DP 45 (with a λ_{max} of 570 nm). Thereafter λ_{max} continues to rise, but no visible change in the hue of the colour takes place. A λ_{max} of 645 nm is not attained until DP 350–400 is reached (typical for potato amylose). After DP 400, λ_{max} is insensitive to chain length (Bailey & Whelan, 1961; Banks, Greenwood, & Khan, 1971). The λ_{max} value for amylose of Arabidopsis leaf starch is substantially lower than that reported for amylose from other species (usually greater than 600 nm), suggesting that either amylose from Arabidopsis leaves is more branched than that from other species, or that the analysed amylose fraction is contaminated with branched glucans (Zeeman et al., 2002). Iodine staining of starch granules from sunflower leaves showed two distinctive layers, a central region with a deep blue colour and a light purplish blue outer layer (Radwan & Stocking, 1957).

The present work complements a previous characterisation of potato leaf starch (Santacruz, Koch, Andersson, & Åman, 2004). In that work it was found that the starch content of potato leaves varied between 2.9 and 12.9% (dry matter basis) over night and day in the middle of July. Scanning electron microscopy of the isolated leaf starch samples showed that the granules had either an oval or

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a round shape and did not exceed 5 μm in size. The elution profile on Sepharose CL-6B of debranched leaf starch showed the presence of a material with a unit chain length distribution between that generally found for amylose and amylopectin. Amylopectin unit chains of low molecular size were present in a higher proportion in the afternoon leaf starch sample than in the morning leaf starch sample. Unit chains, mainly between DP 15 and 24 and above DP 35, increased in proportion during the synthesis over the day. In the present study, starch extracted from the morning and afternoon leaves were characterised by iodine-staining and compared with potato tuber starch.

2. Materials and methods

Potatoes (c.v. *Producent*) were grown south of Uppsala, Sweden. Leaves were collected in sunny conditions on July 12 (sunrise 3.52 and sunset 21.59 hours), 250 large leaves at 5:00 and 17:00 h. All leaves were transported in a cooler bag and then immediately stored at −20 °C. Normal potato tuber starch was obtained from Lyckeby Stärkelsen AB (Kristianstad, Sweden).

The starch extraction was essentially performed according to Santacruz et al. (2004). Enzymes were inactivated and chlorophyll and other low molecular weight compounds were extracted from disintegrated freeze-dried leaves with hot ethanol. Pectins and water extractable compounds were extracted with ethylenediamintetra acetic acid (EDTA) at room temperature. Starch was extracted from the residue with hot 90% dimethylsulphoxide (DMSO). DMSO of several extractions was mixed with water to obtain a 50% DMSO solution. The solution was filtered in a stirred ultra filtration cell (model 8400, Amicon Corp., Danvers, MA 01923, USA) with a membrane (YM3, diameter 76 mm, Amicon Corp., Danvers, MA 01923, USA) able to retain polymers with a molecular weight higher than 3000 Da. The filtrate was discarded and the retained fraction (approx 50 ml) was mixed with 99.5% ethanol and DMSO (1:9 v/v). The solution was left refrigerated overnight and thereafter centrifuged (1000*g* for 10 min). The supernatant was discarded and half of the pellet was mixed with 1 ml 1 M NaOH and heated gently in a boiling water bath (approx. 45 s). Water (9 ml) was added in steps during four hours. The starch solution was centrifuged (1000*g* for 5 min) and the supernatant applied on a Sepharose CL-2B (Amersham Biosciences, Uppsala, Sweden) column (70×1.6 cm) using 0.01 M NaOH as eluent. The flow rate was 0.4 ml/min and fractions of 2 ml were collected.

The elution profile was analysed by the phenol-sulphuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). Amylose and amylopectin were detected by iodine-staining (Morrison & Laignelet, 1983). Fractions obtained from Sepharose CL-2B were mixed with 0.4 ml of I_2–KI solution (2 mg I_2, 20 mg KI/ml). A spectrum of each fraction was measured between 500 and 800 nm (UV–VIS Spectrometer, Shimadzu, Kyoto, Japan) 15 min after addition of the I_2–KI agent. The spectra from each chromatographic run were compiled in a two-way data matrix with retention volume and spectral wavelength defining the vertical and horizontal dimensions, respectively. Heuristic evolving latent projections (HELP) were used to identify selective chromatographic regions and to resolve spectra and chromatograms of the pure constituents amylose and amylopectin (Kvalheim & Liang, 1992).

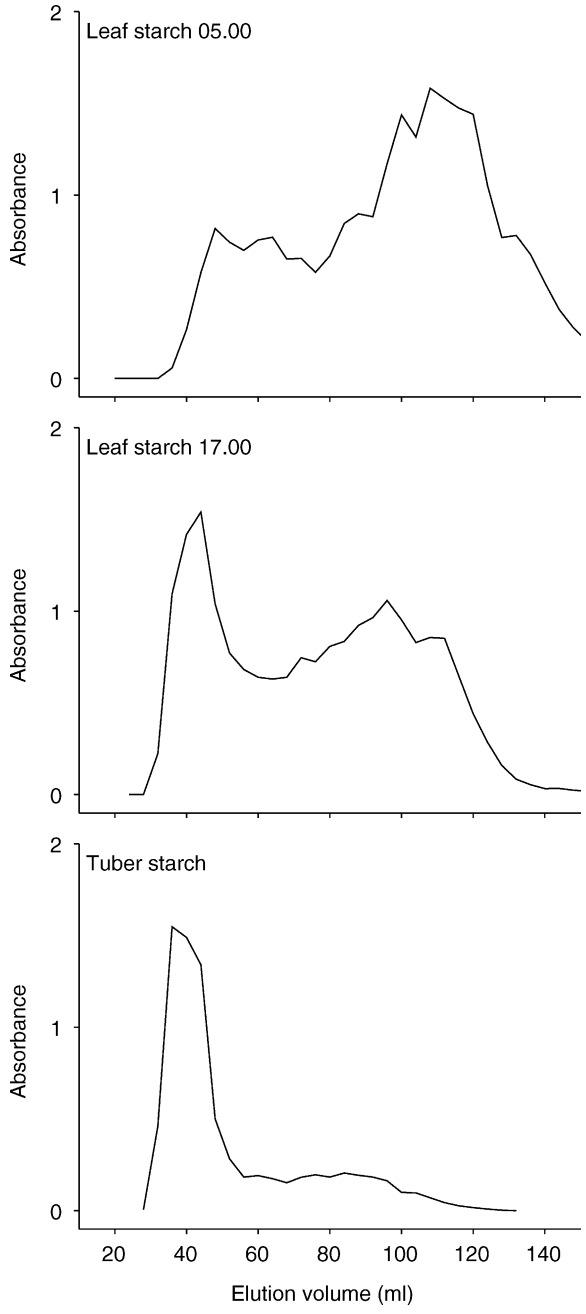

Fig. 1. Sepharose CL-2B elution profiles of potato leaf and tuber starches, detected by the phenol–sulphuric acid method.

3. Results and discussion

DMSO-extracted leaf starches were fractionated on Sepharose CL-2B and detected with phenol–sulphuric acid (Fig. 1). The chromatograms revealed a peak between 30 and around 60 ml elution volume, which corresponds to amylopectin, and a second peak that corresponds to amylose. The afternoon leaf starch sample had a higher relative proportion of amylopectin and less amylose than the morning sample, as reported earlier by Santacruz et al. (2004). The tuber starch had a higher relative amount of amylopectin than the leaf starches.

The fractions from the Sepharose column were also analysed by iodine-staining and the results reported as contour plots, where all the spectra are shown (Fig. 2). The amylose from tuber starch had a smaller elution volume compared to the volume for the leaf starch samples, suggesting a higher molecular weight.

The λ_{max} of each spectrum in Fig. 2 is shown in Fig. 3. The morning leaf starch sample showed a region between 40 and 60 ml elution volume with a λ_{max} of 550 nm, which corresponds to amylopectin, and a region above 100 ml with a λ_{max} higher than 600 nm, which corresponds to amylose. The afternoon sample showed similar regions although its λ_{max} corresponding to amylose was slightly lower than

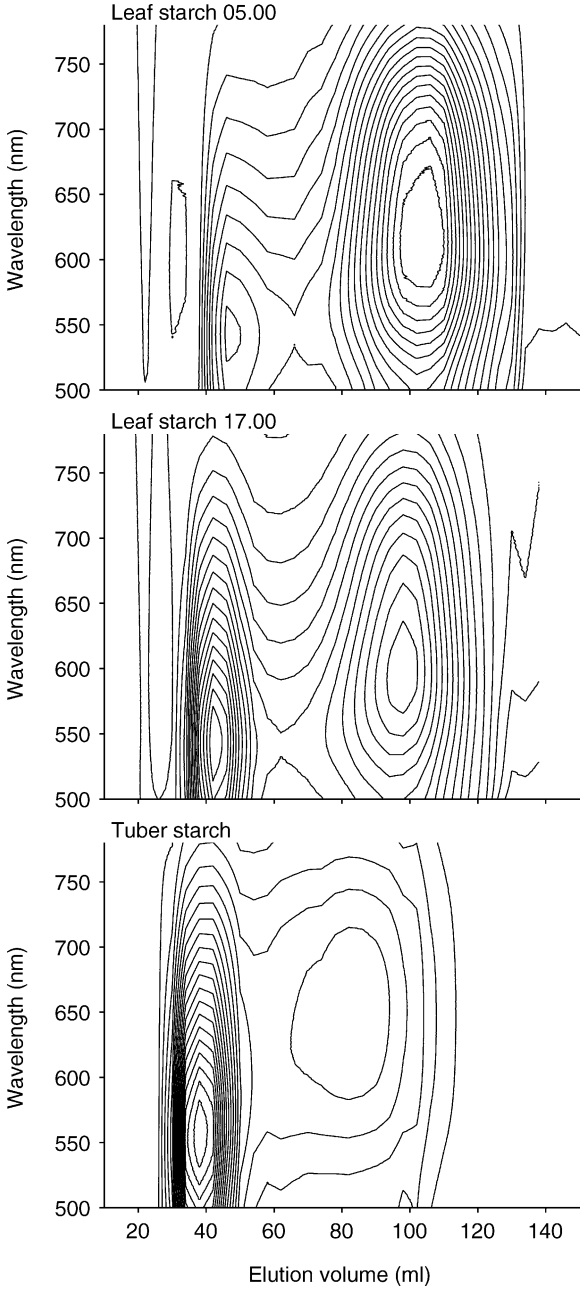

Fig. 2. Contour plots of potato leaf and tuber starches fractionated on Sepharose CL-2B and detected by iodine-staining.

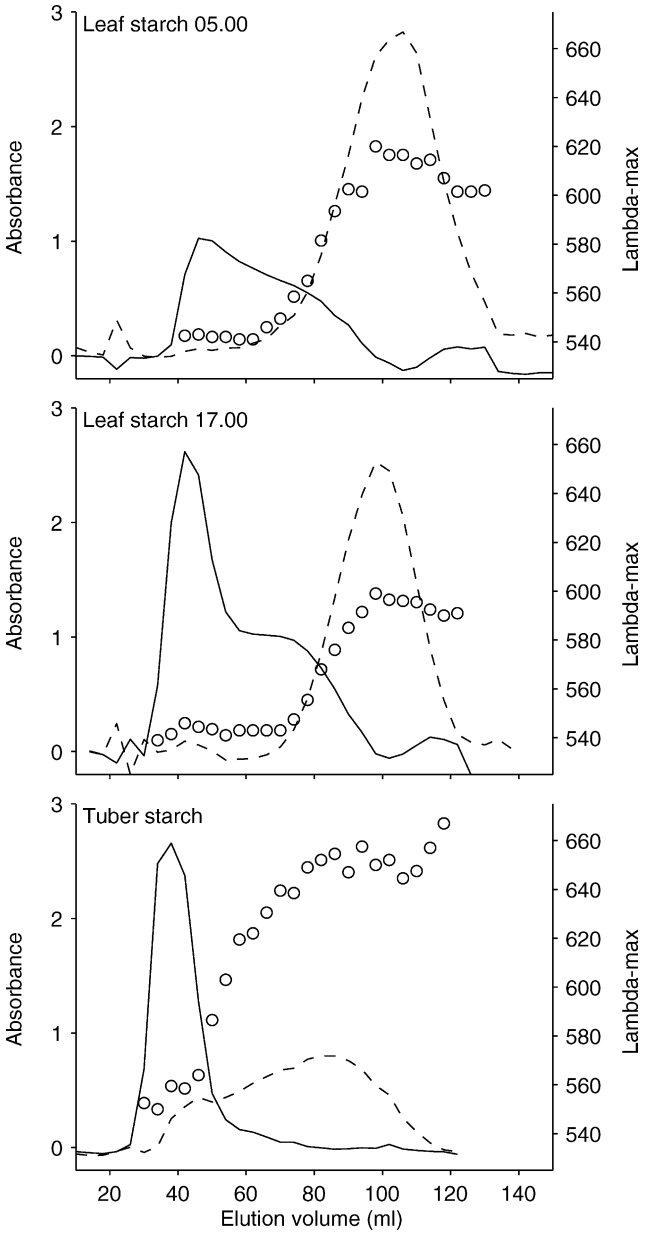

Fig. 3. Resolved chromatograms of amylopectin (—) and amylose (– – –) from potato leaf and tuber starches on Sepharose CL-2B. The λ_{max} of the fractions is shown with open circles.

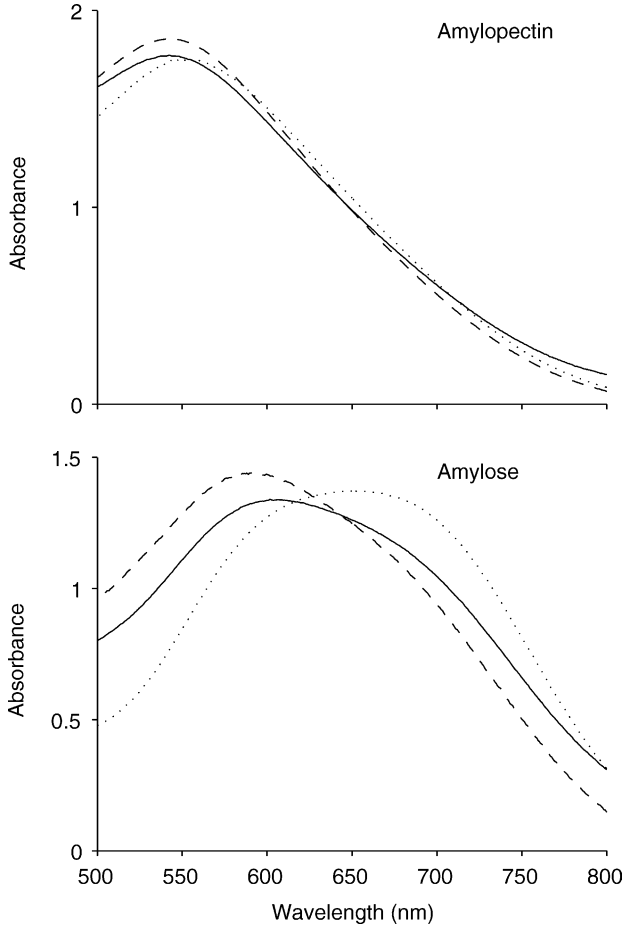

Fig. 4. Spectra of amylopectin and amylose from potato leaf and tuber starches stained with iodine. Leaf starch 05.00 (—), leaf starch 17.00 (– – –), tuber starch (···).

600 nm. Tuber starch showed a narrow region below 40 ml with a λ_{max} of approximately 550 nm, which corresponds to amylopectin, and one above 80 ml with a λ_{max} of 650 nm corresponding to amylose.

The resolved chromatograms of amylopectin and amylose were computed using the spectra extracted from their identified pure regions. It is noteworthy that all spectra in the overlapping regions could be accounted for by this method, indicating that the chain length distribution of both amylopectin and amylose was constant over their molecular size ranges (Fig. 3). Consequently, no third compound (intermediate material) is needed to explain the spectra of the mixtures. The tuber starch amylose showed a broader molecular size range than the leaf starch amylose, where the high molecular fraction was absent. The amylopectin, on the other hand, had broader size ranges in the leaf starches, but the fraction increasing in proportion during synthesis over the day had a size range similar to that of tuber starch amylopectin.

The leaf and tuber starch samples had amylopectins with similar iodine spectra, indicating similar average chain length (Fig. 4). The amylose spectra differed, showing that the amylose fraction from the afternoon sample had a higher proportion of short chains than that from the morning sample and the amylose fraction from tuber starch appeared to have a smaller proportion of short chains compared to the leaf starches.

The present work complements a previous characterisation of potato leaf starch (Santacruz et al., 2004). The results in the present study confirm that the afternoon leaf starch had a higher proportion of amylopectin than the morning leaf starch. Amylose from the afternoon sample had a higher proportion of short chains and a smaller proportion of long chains than the morning leaf starch sample. Amylopectin from both leaf starches and the tuber starch seemed to have similar average chain length. Both leaf and tuber starch seemed to be composed only of amylose and amylopectin, with constant chain length distribution over their molecular weight ranges. Consequently the material between the pure amylopectin and amylose is a mixture of the two and not an intermediate material.

References

Bailey, J., & Whelan, W. (1961). Physical properties of starch. I. Relationship between iodine stain and chain length. *The Journal of Biological Chemistry*, *236*, 969–973.

Banks, W., Greenwood, C., & Khan, K. (1971). The interaction of linear amylose oligomers with iodine. *Carbohydrate Research*, *17*, 25–33.

Buléon, A., Colonna, P., Planchot, V., & Ball, S. (1998). Starch granules: Structure and biosynthesis. *International Journal of Biological Macromolecules*, *23*, 85–112.

Dubois, M., Gilles, K., Hamilton, J., Rebers, P., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, *28*, 350–356.

Kvalheim, O., & Liang, Y. (1992). Heuristic evolving latent projections: Resolving two-way multicomponent data. 1. Selectivity, latent-projective graph, datascope, local rank, and unique resolution. *Analytical Chemistry*, *64*, 936–946.

Morrison, W., & Laignelet, B. (1983). An improved colorimetric procedure for determining apparent and total amylose in cereal and other starches. *Journal of Cereal Science*, *1*, 9–20.

Nakamura, Y. (1996). Some properties of starch debranching enzymes and their possible role in amylopectin biosynthesis. *Plant Science*, *121*, 1–18.

Radwan, M., & Stocking, C. (1957). The isolation and characterization of sunflower leaf starch. *American Journal of Botany*, *44*, 682–686.

Santacruz, S., Koch, K., Andersson, R., & Åman, P. (2004). Characterisation of potato leaf starch. *Journal of Agricultural and Food Chemistry*, *52*, 1985–1989.

Zeeman, S., Tiessen, A., Pilling, E., Kato, L., Donald, A., & Smith, A. (2002). Starch synthesis in arabidopsis. Granule synthesis, composition and structure. *Plant Physiology*, *129*, 516–529.