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Rapid estimation of the amylose/amylopectin ratio in small amounts of tuber and leaf tissue of the potato

J. H. M. HOVENKAMP-HERMELINK[1], J. N. DE VRIES[1], P. ADAMSE[1], E. JACOBSEN[1], B. WITHOLT[2] and W. J. FEENSTRA[1]

[1] Department of Genetics, University of Groningen, Center of Biological Sciences, Kerklaan 30, 9751 NN Haren, the Netherlands
[2] Department of Biochemistry, University of Groningen, Nijenborgh 16, 9747 AG Groningen, the Netherlands

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Summary

Small amounts of potato tuber and leaf tissues are extracted in perchloric acid. After staining with I_2-KI solution absorbancies at 618 and 550 nm are measured. The amylose/amylopectin ratio can be estimated from the ratio of the absorbancies by using a formula or a graph in which the specific absorptions of the two compounds are introduced. Weighing of samples is not required. The method can be used for the estimation of the starch composition of minitubers and is especially suitable for the detection of mutants with an altered starch composition.

Introduction

Different methods are described in the literature for the determination of the relative amounts of amylose and amylopectin in starch. Estimation of the amylose and amylopectin fractions is possible by means of amperometric or potentiometric titration with iodine or by a colorimetric procedure (Banks et al., 1971; Bates et al., 1943; Knutson, 1986; Richter et al., 1968; Williams et al., 1970). However, these procedures are not suited for the analysis of small tissue samples of which the starch content is unknown. A method which is applicable to microgram amounts of starch has recently been described by Lustinec et al. (1983). However, this method is laborious and time consuming and, therefore, not suited for the screening of large numbers of samples, such as is required for the isolation of mutants.

Here another procedure for starch analysis in plant tissues is described. It is faster and more convenient than the methods mentioned above and is appropriate for analysis of small quantities of starch. The method combines extraction of starch in perchloric acid (Lustinec et al., 1983) with determination of the absorption at two wavelengths after staining with I_2-KI solution. Amylose and amylopectin show different absorption maxima under these conditions. Since each compound also shows considerable absorption at the wavelength of maximal absorption of the other, a correction must be applied in calculating the relative amounts of the two starch components. For a comparable situation, the ratio of the amounts of chlorophylls a and b, Bruinsma (1963) showed that this ratio can be calculated from the ratio of the absorbancies at the two peak values.

In this paper we describe this procedure and use it to determine the percentage of

amylose in starch from tubers and leaves of normally and in vitro grown potato plants. The results obtained with this procedure compared favourably with those obtained with an amperometric titration.

Material and methods

Four different genotypes of *Solanum tuberosum* were used: cv. Astarte (2n = 4x = 48), the monoploid clone H7322 (2n = 1x = 12), doubled H7322 and the interdiploids HH578 and HH260 (2n = 2x = 24). The plants were grown in manured leaf-soil in a temperature-controlled glasshouse (19/17 °C, day/night); H7322 was also grown in vitro on a Murashige and Skoog medium, supplemented with 1 % sucrose and 0.8 % agar (Roest and Bokelmann, 1976), at 21 °C, 14 h light intensity 3200 lux. Induction of minitubers on stem explants was according to the procedure of Hovenkamp-Hermelink et al. (1988).

Stock solutions of starch components were made by dissolving 25 mg of amylose or amylopectin, extracted from potato (Sigma Chemical Co. Ltd) in 10 ml of 45 % (w/v) aqueous $HClO_4$ and adding H_2O to a final volume of 100 ml. Stock solutions were mixed to obtain starch solutions with 10, 20 and 60 % amylose. Stock solutions and mixtures were diluted with water to obtain final starch concentrations of 6.25, 3.13 and 1.56 mg/100 ml.

For starch extraction from tubers 10–50 mg of tissue (fresh weight) was coarsely minced and immersed in 0.5 ml of 45 % $HClO_4$. After 4 minutes 8 ml of H_2O were added; after mixing the tuber debris settled to the bottom of the test tube. One hundred to 200 mg of leaf material was needed to extract a sufficient amount of starch. The material was collected in late afternoon, cut into small pieces and likewise immersed in 0.5 ml 45 % $HClO_4$ for 4 minutes, under gentle squeezing with a glass rod. The suspension obtained was diluted with 8 ml of H_2O and centrifuged for 60 s at 650 g.

I_2-KI staining was achieved by mixing 4 ml of starch solution or extract and 5 ml of a diluted (1:2, v/v) Lugol's solution (Merck, 2 g KI + 1 g I_2 in 300 ml H_2O). Absorbancies were measured immediately after mixing in a Beckman spectrophotometer model DU-5, equipped with a program for measuring at two wavelengths and recording the ratio of the absorbancies after correcting for the blanks which consisted of a mixture of 4 ml of appropriately diluted $HClO_4$ and 5 ml of diluted Lugol's solution. Spectra were determined in a Beckman spectrophotometer model 24.

Starch composition in tubers of cv. Astarte was also analysed by determining the iodine-complex-binding capacity with the amperometric titration test (Williams et al., 1970), after extraction of isolated starch in sodium hydroxide (Varns & Sowokinos, 1974).

Results

Absorption spectra of amylose and amylopectin showed maxima at 618 and 550 nm, respectively. We determined the absorbancies at these wavelengths of solutions of pure amylose and amylopectin at three concentrations in order to test whether there is a linear relation between concentration and absorption at the I_2-KI concentration used (Fig. 1). Since the absorptions were linear over the concentration range used the absorption coefficients (a) could be determined from Fig. 1. The values obtained (in OD

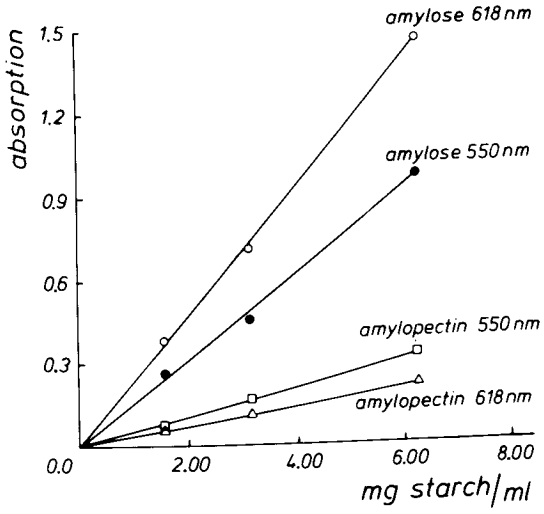

Fig. 1. Absorption values of amylose and amylopectin solutions of various concentrations.

units·ml·mg^{-1}) were (am = amylose, ap = amylopectin): a(am 618) = 23.4; a(am 550) = 15.5; a(ap 618) = 3.5; a(ap 550) = 5.1. Assuming additivity of absorption values, the ratio (R) of the absorbancies at 618 and 550 nm of mixtures of amylose and amylopectin can be calculated according to the formula:

$$R = \frac{P \times G \times a(\text{am } 618) + (1 - P) \times G \times a(\text{ap } 618)}{P \times G \times a(\text{am } 550) + (1 - P) \times G \times a(\text{ap } 550)} = \frac{P \times 23.4 + (1 - P) \times 3.5}{P \times 15.5 + (1 - P) \times 5.1}$$

in which G = starch concentration and P = fraction amylose. It is clear that R is independent of G and varies between 0.69 for $P = 0$ and 1.51 for $P = 1$. The curve giving the relation between R and P is given in Fig. 2. When R is known the fraction amylose

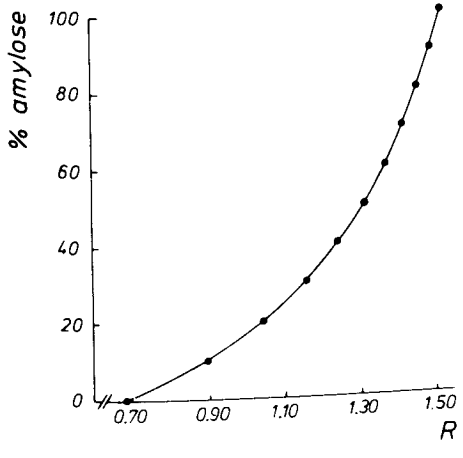

Fig. 2. Calculated relation between the percentage of amylose and the ratio of the absorbancies at 618 and 550 nm (R) of amylose/amylopectin mixtures in solution.

Table 1. Comparison of prepared and calculated amylose contents of artificial amylose/amylopectin mixtures.

% Amylose as prepared	Starch concentration (mg/100 ml)				% Amylose as calculated from average *R*-value
	1.563	3.125	6.25		
		*R**		average	
10	0.904	0.870	0.927	0.900	10.3
20	1.018	1.077	1.108	1.068	22.1
60	1.345	1.400	1.415	1.387	65.3

* Absorption at 618 nm/absorption at 550 nm.

can be read directly in the graph. Alternatively, P can be calculated using the formula $P = (3.5-5.1\ R)/(10.4\ R-19.9)$ which is obtained from the formula given above for R by extracting P.

As a test for the reliability of the method the R-values of mixtures of amylose and amylopectin containing 10, 20 and 60 % amylose, respectively, were determined and the ensuing amylose percentages were calculated (Table 1). The calculated compositions showed slightly higher amylose percentages than those which were present according to the preparation of the mixtures.

Amylose percentages in starch extracts from tubers and leaves were determined (Table 2). In soil-grown tubers of four different genotypes the amylose percentages varied between 21.5 and 23.2 and appeared to be highest in cv. Astarte. A comparable percentage (21.5) is observed in minitubers of H7322 induced in vitro. The percentages of amylose in starch from leaves of H7322 and cv. Astarte were strikingly lower and did not exceed 15 %.

For comparison, the amylose percentage of purified starch isolated from tubers of cv. Astarte was determined amperometrically and spectrophotometrically. The spectrophotometric determination was performed with starch solutions prepared with

Table 2. Amylose % in tubers and leaves of different clones.

Clone	Tissue	Number of samples	Average of *R*	Standard deviation of *R*	Amylose %
H7322	minituber *	20	1.06	0.026	21.5
	leaf	4	0.96	0.019	14.1
Doubled H7322	tuber	5	1.06	0.015	21.5
HH578	tuber	7	1.07	0.007	22.3
HH260	tuber	7	1.06	0.012	21.5
Astarte	tuber	10	1.08	0.018	23.2
	leaf	6	0.89	0.021	9.8

* Grown in vitro.

perchloric acid or with sodiumhydroxide. The results were 23.2 and 23.5 %, respectively, which is not very different from the amperometrically determined value of 21.2 %.

Discussion

Our results show that by extracting starch in perchloric acid and measuring the absorption at two wavelengths, after staining the solution with I_2-KI, the percentage of amylose in the sample can be determined semi-quantitatively in a simple way. Neither the weight of the sample nor the concentration of the starch in the extract is required for the analysis. The procedure was applied to potato starch but might be used for other starches as well, provided that amylose and amylopectin from such starches are available in pure form.

Potato starch normally contains about 20 % amylose. Amperometric titration of amylose in soil-grown tubers of cv. Astarte revealed an amylose content of 21.2 %, whereas our spectrophotometric analysis yielded 23.2 %. The difference was not due to the extraction procedure: the use of sodium hydroxide as the solvent instead of perchloric acid yielded 23.5 %. The difference could be due to differences between the structures of amylose and amylopectin in native starch and those of the purified components. However, amylose percentages determined for artificial mixtures were slightly higher than expected and, thus, the actual amylose percentage in tubers of cv. Astarte could be slightly lower than the value which was determined spectrophotometrically. The important advantage of the method is that it can be carried out routinely in a short period of time, allowing the analysis of 100 samples per day, and requiring only a few milligrams of tissue per sample. This renders it applicable to the analysis of minitubers, grown in vitro on stem segments of a monoploid clone. The amylose percentage in such tubers proved not to differ from that in tubers grown in soil on plants of the same genotype, or on the diploidized form. Two other diploid genotypes gave comparable values. All of these values were slightly lower than that of tubers of cv. Astarte, which is a typical starch potato.

The method is certainly useful for the detection of relative differences, as is the case in a search for mutants. In this respect starches containing low or zero amounts of amylose, comparable to waxy starch of maize and also starches with a grossly increased amount of amylose are of interest. The method is especially valuable for the detection of the latter type of aberrations because visual screening is not possible here. Such a screening can only be carried out for amylose-free mutants, which stain reddish-brown with iodine.

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