

## Determination of Starch Solubilized by Means of Irradiation

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Solubilization of starch by irradiation can be utilized for analytical purposes. Water, chlorophyll, sugars, and other alcohol-soluble compounds must be removed before irradiation by means of extraction of the material with hot ethanol. After irradiation, starch is extracted with water and may be determined colorimetrically as the iodine complex or as glucose after hydrolysis. Since the method involves only simple and well-defined procedures, it seems well suited for rapid analysis of great numbers of samples. For application of the method a suitable radiation source is required, *e. g.* a (50—) 100—1 000 curie  $^{60}\text{Co}$  gamma-source or a van de Graaff accelerator.

In routine investigations of plants and plant materials, the analysis of starch has always been an intriguing problem. The quantitative determination of this inexactly defined compound is laborious and time-consuming, especially owing to difficulties in extracting and solubilizing the compound in a reproducible manner. It has previously been found that irradiation makes starch more easily soluble in water, chiefly due to the induction of carboxyl groups<sup>1</sup>. This investigation is primarily concerned with the question, whether irradiation can facilitate a rapid and reproducible quantitative determination of starch.

The methods in use for the determination of starch involve the extraction and dissolution by means of HCl,  $\text{HClO}_4$ ,  $\text{CaCl}_2$ , or alkalis. These chemicals are either so reactive that the structure of starch is changed, or, as in the case of  $\text{CaCl}_2$ , their dissolving capacity is rather small. Since the chemicals often also dissolve contaminating compounds, great difficulties arise which hinder subsequent procedure.

For the determination of starch several methods have been used. Determination of glucose after acid hydrolysis gives correct values only if the starch has first been isolated. The determination of glucose after hydrolysis by means of enzymes is also circumstantial, depending on, *i. a.*, variations in the amylose/

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amylopectin ratio. Between starches of different origin, differences in the proportions of these starch components also prevent the general use of a colorimetric determination of the starch-iodine complex. Since amylose and amylopectin exhibit different spectra with iodine, and perhaps also for other less well-defined reasons, the iodine complexes of starches of different origin exhibit different spectra and intensities of extinction<sup>2,3</sup>. Similarly, as in the case of enzyme hydrolysis, such factors may be corrected for, if the method is standardized by means of starch from the plant material under investigation<sup>4</sup>. Apart from the genetically controlled variation of the composition of starch, there is also a variation within the plant during its vegetative development<sup>5</sup>. The latter variation is rather small ( $\pm 2\%$ ) and has but an insignificant influence on the colorimetric determination of the iodine complex.

Polarometric methods<sup>6</sup> give reliable values only when fairly large amounts of starch are determined.

In summarizing this short review of methods for the determination of starch, it can be stated that the quantitative extraction and purification followed by acid hydrolysis permits the most exact determination<sup>7</sup>. This method is laborious, however, and not suited to routine determinations.

#### PROCEDURES

From the studies by Ehrenberg, Jaarma, and Zimmer<sup>1</sup> on the effects of roentgen- and gamma-radiations on starches it can be concluded that different physico-chemical changes are induced in the material leading to a greater solubility in water of the latter. This effect seems partly to be due to a disintegration of the molecules but especially to the introduction of acidic -COOH groups, which can be titrated and which, in neutral milieu, occur as the negatively charged base groups, -COO<sup>-</sup>. (Such changes are already obtained when monosaccharides are irradiated<sup>8</sup>; the true nature of the groups in the case of polysaccharides has not yet been determined.)

Two criteria must be fulfilled to permit the use of these radiation-induced changes for analytical purposes:

(1) Irradiation should not interfere with opportunities for determining starch colorimetrically (or, in tests aiming at a calibration, as glucose after hydrolysis);

(2) Irradiation of the plant material should permit the extraction and dissolution of starch.

Chemical changes which might be of importance to the first criterion were studied by irradiating starches of different origin (potato, rice, arrow root, maize) with doses  $10^5$ — $10^7$  rad of  $\gamma$ -radiation from a 60 curie <sup>60</sup>Co-source. Before irradiation, the starches were desiccated with acetone to a water content of 5 %.

Throughout the investigation, 0.1 % solutions of starch were prepared by dissolving 250 mg in 150 ml boiling water, and, after another 15 min of boiling and cooling, diluting to the mark of a 250 ml volumetric flask. For the colorimetric investigation of the iodine complex, 2 ml of the 0.1 % solution (*i. e.*, 2 mg starch) were introduced in a 100 ml volumetric flask, followed by 18 ml water, 2 ml 2 N acetic acid, 1 ml 10 % KI solution, and,

Table 1. Influence of gamma radiation on the properties of potato starch; cf. text.

Radiation dose Mrad	Extinction at $\lambda = 590 \text{ m}\mu$	Reducing sugar, % of starch	Specific viscosity	pH of 0.1 % solution
0	0.295	< 1	0.520	6.92
0.12	0.295	< 1	0.220	6.90
0.19	0.295	< 1	0.220	6.90
0.50	0.295	< 1	0.166	6.72
0.86	0.295	< 1	0.154	6.70
1.6	0.295	< 1	0.135	6.60
10	0.281	10	0.070	5.60

finally, 10 ml 0.01 N  $\text{KIO}_3$ . Water was then added to the mark. The extinction of the greenish solution was determined in a Beckman spectrophotometer at wave-lengths 500–650  $\text{m}\mu$ .

The radiation effects on the starch were also characterized by determinations of reducing sugars (5 ml samples of the 0.1 % solution were determined according to the method of Somogyi<sup>9</sup>), specific viscosities, and pH of the solutions.

The data demonstrate that up to doses of  $1\text{--}2 \times 10^6$  rad, no changes occur which could be suspected to appreciably affect the analytical properties of the starches. In Table 1 this is demonstrated for potato starch; the other starches behaved identically in all respects. Higher doses, around  $10 \times 10^6$  rad, are required to change the extinction. At the same time great amounts of reducing groups are introduced, and the viscosity indicates a considerable breakdown of the molecules<sup>1</sup>. The decreased extinction has to be ascribed, to a great extent, to a displacement towards red of the colour of the iodine complex (Fig. 1 and earlier publication<sup>1</sup>).

For the elucidation of factors pertinent for the second criterion it was important to include materials widely different in composition in general and in their starch content. Tissues storing starch (potato tubers and barley seeds), as well as vegetative parts of plants with a low starch content (leaves of potato and maple, *Acer platanoides*) were investigated. In the following, the pilot experiments with potato tubers and maple leaves will be described.

Roughly disintegrated parts of the material were dehydrated in boiling ethanol. This treatment extracts chlorophyll and other alcohol-soluble substances (simple sugars, glycosides, alkaloids, fats, and waxes). The enzymes become inactivated, which is of extreme importance in the determination of starch in green, vegetative parts of plants,

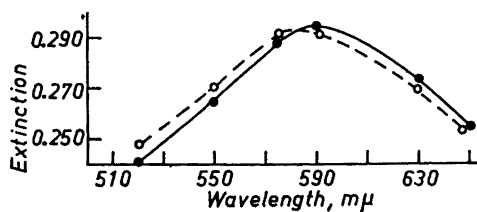


Fig. 1. Extinction curves of the iodine complex of potato starch (2 mg/100 ml). ● un-irradiated and irradiated with doses up to  $1.6 \times 10^6$  rad; ○ irradiated with  $10 \times 10^6$  rad.

Table 2. Starch content in the materials studied. Classic methods.

Material	1	2	3	4	5
	Residue after alcohol extraction %	Reducing sugars in residue (1) %	Pentosanes in residue (1) %	(2-3) × 0.9 %	Starch according to Pucher %
Maple leaves I	9.1	16.4	8.7	6.9	6.7
» » II	10.3	22.3	10.9	10.7	10.3
Potato tubers	24.8	66.4	2.1	57.9	55.3

where the (low) starch content undergoes rapid changes. Two extractions were performed at a final alcohol concentration of 75 %, and a third one at 80 % alcohol.

The dry material was ground in a Wiley mill (sieve 60). Parts of the samples were then very finely ground in a ball mill and sifted through silk-fabric.

The ball mills were prepared from thick-walled 100 ml cream bottles, each with 80–100 ball bearings 0.4–0.6 cm in diameter. In each bottle 0.5–0.8 g plant material was ground by placing the rubber-stoppered bottles in a shaking-machine. Gentle shaking (about 50 times per min) for 4–5 h was sufficient to disintegrate the material beyond a limit where no cells could be identified in the microscope.

Table 3. Colorimetrically determined starch in water extract of residue (column 1 in Table 2) after alcohol extraction. a = grinding in Wiley mill; b = grinding in ball mill (see text). Values related to residue after alcohol extraction and to values determined according to the method of Pucher *et al.*

Material	Radiation dose Mrad	Starch found			
		a: Wiley mill		b: Ball mill	
		% of extraction residue	% of value acc. to Pucher	% of extraction residue	% of value acc. to Pucher
Maple leaves I	0	1.70	25	2.00	30
	0.12	2.10	31.5	5.80	86
	0.5	2.35	35	6.60	98
	1.6	2.70	40	6.65	99
Maple leaves II	0	2.68	26	3.50	34
	0.13	2.99	29	3.92	38
	0.21	3.81	37	6.50	63
	0.52	3.90	38	10.1	98
	0.93	4.45	43	9.95	96
	1.7	5.35	52	10.4	101
Potato tubers	0	40.4	73	43.6	79
	0.12	46.1	83	47.5	86
	0.5	51.5	93	55.7	101
	1.6	52.5	95	54.8	99

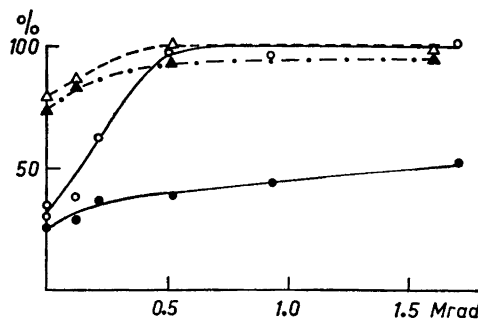


Fig. 2. Colorimetrically determined starch as a function of radiation dose. 100 % = starch content determined according to Pucher *et al.* ● Maple leaves: Wiley mill; ○ Maple leaves: ball mill; ▲ Potato tubers: Wiley mill; △ Potato tubers: ball mill.

200–500 mg material, depending on the starch content, is weighed, suspended in 25 ml water and then poured quantitatively into 150 ml of boiling water. The boiling is continued under reflux for 30 min. The addition of a few drops of *n*-octanol prevents foaming. After cooling, the solution is diluted to 250 ml, and after clearing by centrifugation, the iodine compound is determined colorimetrically at 590  $\mu$  and in accordance with the method given above. The starch concentration is calculated from a calibration curve, which is prepared utilizing isolated starch of the type to be determined<sup>10</sup>. (Since in some cases, *e. g.*, maple leaves, absolutely pure starch could not be easily prepared, the calibration curve was based on the amount of glucose determined after hydrolysis.)

In order to compare the data from irradiated materials with exact values for their starch contents, the latter were determined according to Pucher *et al.*<sup>7</sup>. In addition, a generally applied laboratory method was used, involving determination of total reducing sugars after 2.5 h hydrolysis in 0.7 N HCl, followed by subtraction of pentosanes, determined according to Tollens and Kröber<sup>11</sup>. The difference, multiplied by 0.9, was regarded as starch.

When the starch content had to be related to total dry or fresh weight, the loss of original material by the alcohol extraction was determined in a special sample. (1 g of material was weighed into a Soxhlet extraction thimble, extracted with alcohol as described above, dried and weighed again.)

In Tables 2–3 data are presented for three materials which were subjected to thorough study, *viz.* maple leaves I (harvested about June 1st), maple leaves II (harvested a month later), and potato tubers. In principle the other materials behaved identically. Table 2 gives the weight of the material after extraction with alcohol, as per cent of original fresh weight (column 1), as well as the starch values, as percentages of the extraction residue, determined as the difference between total reducing sugars and pentosanes, or according to Pucher. These two methods give acceptably agreeing values for the starch contents.

For the same materials, the results of the colorimetric analyses after irradiation are presented in Table 3. The influences of the radiation dose and of the grinding method are clearly demonstrated, as illustrated by the summarizing graph, Fig. 2. The following conclusions can be drawn from the data:

- (1) that with both methods of grinding, irradiation increases the possibility of extracting starch with water;
- (2) that effective extraction is reached, however, only in the finely ground samples (ball mill), *i.e.*, where all cells were destroyed;
- (3) that the dose required to make the starch fully soluble and extractable, amounts to about 500 000 rad. At this and higher doses good agreement was obtained with the data given in Table 1.
- (4) It was further demonstrated that irradiation of thermally dried samples, which had not been extracted by means of alcohol, did not lead to corresponding solubilization of the starch, not even at higher doses ( $> 10^6$  rad).

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