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The Influence of Ionizing Radiation on the Proline Content in Potato Tubers

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Proline contents in common Swedish potato varieties have been determined by paperchromatography before and after irradiating the tubers. The results of the investigation indicate that there is a correlation between proline content and sprouting. It is also shown that there is an influence of γ-rays on the proline metabolism. The reason for the accumulation of proline in irradiated tubers is discussed.

The object of this study was to investigate the changes in proline concentration in potato tubers induced by ionizing radiation (γ-rays from ^{60}Co) and to ascertain whether the amino acid proline could be connected with the sprouting of the tubers.

An investigation on the amino acid content in γ-irradiated and in non-irradiated tubers,[1] respectively, which was started in 1958 at this Institute, indicated *inter alia* that the amount of proline in the tubers varied greatly. In freshly harvested tubers only traces of proline could be found while after storing for several months there was a considerable amount of this amino acid in the tubers. In potato tubers which had just begun to sprout there was more proline in the apical end than in the other parts of the tuber. This fact indicates that the proline metabolism directly or indirectly might be connected with the mechanism of sprouting.

Recent research on potatoes [2–6] has shown that not only changes in cultivation but also in storage conditions influence the tubers both physiologically and chemically. Among these investigations, Werner and Levertons work [3] on the influence of external factors on the ascorbic acid content in potato tubers should be mentioned; Herrmann and Raths [4,5] have also emphasized the influence of the manuring and the storing conditions on the chemical composition of the tubers, on the yield etc. Fischnich *et al.*[6] have even observed great changes in the content of free amino acids in the tubers by cultivating them at different amounts of nitrogen, phosphorus (P_2O_5), and potassium (K_2O) in the artificial manuring. By halving the amount of nitrogen, the other factors remaining unchanged, they found that the proline content was decreased by

50 %, while a double amount of all three components, the nitrogen, phosphorus, and potassium, increased the proline content by 55 %.

With respect to these facts, it is a matter of course that the prerequisite for a reliable analysis of proline content in potato tubers is that all of these influencing variables are taken in consideration.

Thus in the present study all samples of the tubers used for the investigation (with one exception) were cultivated under known and controlled conditions. The tubers were thus stored in a cold-room at constant temperature (+ 4°C).

MATERIALS AND METHODS

Potato tubers of three common Swedish varieties, Bintje, King Edward, and Early Puritan, were used for the examinations. The γ-doses (^{60}Co), 15 and 100 kilorad, were given at a dose rate of 100 r/sec. For the low "stimulating" dosage, a small ^{60}Co-source (about 1 Curie giving 1.6 rhm) was used. In this case the doses were 28 and 76 rad, dose rate 1.6 rh, and 144 and 288 rad, dose rate 6 rh. For each sample of irradiated tubers, the corresponding non-irradiated control tubers were stored at the same temperature during the treatment.

The analyses of proline were performed on press-juices of the tubers. The juice was prepared as described elsewhere.[7] The whole procedure was achieved in a cold-room at + 3—4°C. The press-juice was kept at 0°C; in less than 10 min after the centrifugation it was spotted on chromatogram papers (Whatman No. 1). The solvent used for separation was butanol-acetic acid-water (4:1:1). The chromatograms were run three times at 16—17°C with ascending technique. According to this method the proline was well separated from the other amino acids present. The spots were developed with Folin reagent.[8] The elution was performed with water instead of methanol and the absorption was measured at 504 mμ. Special care was taken to spray the spots equally and also to avoid high background colours. For instance, phenolic vapours cause extremely blue coloured backgrounds giving quite wrong results. To obtain reliable results, the proof and the known sample, respectively, should not differ more than 15 %. The method is time-consuming since it is necessary first to encircle the concentrations before the conclusive chromatographing

Table 1. The content of proline in the apical end of the tubers of the Bintje variety, harvested in September and irradiated on October 19, dose 15 kilorad.

Analyzed in	μg proline/ml press-juice		Dry matter in the	
	Irradiated	Control	juice %	tuber %
September	Traces		4.80	22.5
»		Traces	4.78	21.9
October	16		4.78	22.0
»		16	4.79	22.5
January	160		4.80	24.3
»		152	4.82	23.0
April	304		4.89	23.4
»		364	4.57	22.5
May	287		4.75	22.0
»		300	4.55	21.0
June	298		4.80	23.8
»		285	4.53	20.5
July	302		4.63	24.0
»		240	4.41	21.6
October	280		4.95	23.8
»		210	3.97	19.7

Table 2. Proline content in different parts of tubers of the Bintje variety, irradiated on October 19. Analyses performed 7 and 9 months after irradiation.

Time after irradiation, months	Part of the tuber	μg proline/ml press-juice		Dry matter in press-juice %	
		Irradiated	Control	Irradiated	Control
7	Apical end	304	364	4.89	4.57
	Middle	290	310	3.95	3.90
	Opposite end	300	330	3.81	3.75
	Sprouts		1300		6.10
9	Apical end	298	285	4.80	4.53
	Middle	206	204	3.90	3.93
	Opposite end	278	212	4.10	4.00
	Sprouts		1410		6.80

can be performed, but instead there is no need for any expensive apparatus. The smallest amount of proline to be determined quantitatively in this way is 3 μg; qualitatively the lower limit is 0.3 μg. By immersing the chromatograms in isatin-reagent [9] it is possible to detect 0.1—0.2 μg proline.

Each analysis of proline was accompanied by an investigation of the dry matter in the press-juices and for each treatment of the tubers an investigation of their dry matter was also performed.

RESULTS AND DISCUSSION

In the present study it is shown (Table 1) that there is a striking increase in the content of free proline during the storage of the potato tubers. In freshly harvested tubers there are only traces of proline, then the content increases until sprouting commences, reaching its maximum value and then decreasing with continued development of sprouts. The proline concentration in the sprouts is up to seven times higher than the concentration in the tubers (Table 2). These results are in accordance with those reported by Breyhan *et al.*[10] Only concerning the influence of storage temperature is there some discrepancy. The authors mentioned above found only traces of proline in tubers stored for about a year at temperatures above + 1°C. In the present study, however, it is shown that there is a considerable content of proline in tubers stored for more than a year at + 4°C, as recorded in Table 1. In tubers irradiated immediately after harvest with a dose of 15 kilorad — which totally inhibited the sprouting — the changes in proline content were smaller than in the untreated control tubers. As is also seen from Table 2, the proline is unequally distributed in different parts of the tuber. The concentration is highest in the apical end and lowest in the middle of the tuber.

In tubers irradiated in February with the high dose 100 kilorad the amount of proline, found in the apical end by analyzing immediately after irradiation, was unchanged in June (328 μg/ml press-juice). This indicates that the irradiation induces a disturbance in the proline metabolism. Such a disturbance is to be seen to some degree already at "sprout-inhibiting" dose in the investigations on the King Edward variety. The results are given in Table 3. This sample of tubers was a commercial product, seed potatoes bought in April 1965. The

Table 3. Proline content in tubers of the King Edward variety irradiated April 22 with a dose of 15 kilorad.

Date of proline determination	μg proline/ml press-juice		Dry matter in press-juice, %	
	Irradiated	Control	Irradiated	Control
April 22	151	150	5.30	5.36
July 7	312	260	4.70	4.50

conditions of cultivation and storage before procuring are unknown. These tubers had no visible sprouts on arrival April 22. They were stored for 24 h at + 4°C before irradiation. No changes in proline content could be observed immediately after the irradiation. Repeated analyses after 11 weeks' storage at + 4°C showed, however, an increased proline concentration; the increase was 74 % in the untreated and 108 % in the irradiated tubers. The sprouts of the controls were now well developed and contained 750 μg proline per ml press-juice. According to Breyhan *et al.*[11] there is simply a transport of proline in the tubers and from the tubers into the sprouts. These authors have even observed an increase in proline content caused by sprout-stimulating agents such as gibberelline and rindite.[10]

Consequently an increased amount of proline in tubers irradiated with "stimulating" doses should be demonstrable (for such doses see page 324). Tubers of the three earlier named varieties were given doses of 28—288 rad. Preliminary examinations indicated a pronounced stimulation of the sprouting in this dose range. No change in proline content however could be observed under the influence of such low doses of γ-rays.

The results of the present investigation clearly indicate that there is a correlation between proline content and sprouting; γ-rays also exert an influence on the proline metabolism. The higher doses have a more striking effect than

Table 4. Proline contents in irradiated and non-irradiated tubers of the King Edward variety as determined in press-juices and calculated for whole tubers and sprouts. I_0 = tubers irradiated with the dose 15 kilorad; I_3 = irradiated tubers 3 months later; C_0 = control tubers before sprouting; C_3 = control tubers well sprouted 3 months later.

Proline content, μg/ml press-juice before sprouting		after sprouting		Control tuber Weight g	Water content %	Sprouts from one tuber Weight g	Water content %	μg proline/ml press-juice from sprouts
I_0	C_0	I_3	C_3					
151	150	312	261	77	75	4.8	81.9	750

Calculation: Proline $I_3 - C_3$ = 51 μg/ml press-juice; water content in the tuber: 57.8 ml; decrease of proline in the control tuber, 51 × 57.8 = 2948 μg; water content in the sprouts: 3.9 ml; total proline content in the sprouts from one tuber: 2925 μg.

the lower doses. This is valid even for many of the other amino acids present in the tubers. Whether it is a question of an inhibition of enzymes concerned with transaminating reactions, with decarboxylation, or with oxidative deamination, or whether the irradiation strikes a weak point in the protein molecule so that an abnormal splitting of peptide bonds occurs are problems still waiting to be solved.

If the hypothesis of Breyhan *et al.*[10] that proline participates in the synthesis of chlorophyll, is correct, the reason for the proline accumulation by irradiation could be a blocking of one or some of these reactions.

As shown in the calculation in Table 4, the difference in proline content between irradiated and untreated tubers is equal to the amount found in the sprouts of the control tubers. Thus it is also possible to explain these results as a simple inhibition of the transport of the proline from the tubers into the sprouts.

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