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Comparison of Chemical Changes in Potato Tubers Induced by γ-Irradiation and by Chemical Treatment

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The contents of the free amino acids, γ-aminobutyric acid and glutamic acid, in potato tubers have been examined by paper chromatography. The changes in these two compounds induced in the tubers by γ-irradiation and by treatment with the sprout-inhibitor CIPC (isopropyl-*N*-(3-chlorophenyl) carbamate) as compared with untreated tubers has been investigated. In parallel, the influence of both treatments on the saccharides, the total nitrogen, inorganic, acid labile, and total phosphorus has been analysed. A comparison of effects caused by γ-rays given with low-dose rate and with high-dose rate has been performed. The sprout-inhibiting effect of all three treatments has also been compared.

It has long been known that ionizing radiation can be used to inhibit sprouting of potato tubers. Nearly two decades have passed since the first reports by Sparrow and Christensen.[1,2] Wide-spread interest has been shown in investigating the chemical effects induced in the tubers after exposure to radiation. Most apparent is an increase of the sucrose concentration which, in some cases, persists for several months after the treatment. A number of papers have been published to this effect, *e.g.* Refs. 3—7.

Much less interest has been devoted to the changes, induced by irradiation, in the concentrations of some of the free amino acids in the tubers. As early as 1958 Herrmann and Raths,[8] however, reported radiation induced changes in the levels of γ-aminobutyric acid (GABA) and glutamic acid (GA), α-alanine, and valine. The effects at different doses caused changes in different directions. From the results obtained, the authors concluded that the increase of GABA and decrease of GA was not caused by simple decarboxylation of GA. This interpretation is supported by investigations performed by the present author (to be published), where the interaction between GA and GABA in the cell-sap from potato tubers was observed to be influenced by addition of isolated potato-mitochondria, but also by addition of mannitol only.

Fujimaki *et al.*[9] recently investigated the effect of γ-irradiation on the content of amino acids of potatoes and found some induced changes, particularly in the level of GA 15 days after exposure. The re-investigation after 105 days' storage of the tubers, however, indicated that the differences in amino acid concentrations had been equalized. As reported earlier,[10] the present author has observed the changes of GABA and GA levels, induced after exposure of the tubers to γ-rays, to persist at least 180 days after a treatment with 14 kilorad. Therefore it is difficult to agree with the opinion presented by the authors quoted. It should also be observed that GABA, although one of the larger components of white potato tissue in the western hemisphere, is not to be found in the Japanese potato variety Dansyaku. It might be that the varieties investigated do not react in the same way. Parameters such as variety, dose-rate used, storage temperatures for the tubers, before and after the treatment, and of course cultivation conditions have great influence on the chemical composition of the tubers.[6,11] The importance of such factors as environment and cultivation has also been emphasized by investigating untreated tubers.[12]

There are very few reports in the literature concerning changes in the chemical composition induced in potato tubers after treatment with chemical sprout-inhibitors. About 10 years ago Schwimmer and Weston[13] examined the chlorophyll formation in potato tubers as influenced by γ-irradiation and by chemicals. In 1966 Baijal and van Vliet[14] investigated the influence of CIPC-treatment on the chemical composition in different parts of the potato tuber during storage and found no evidence of an influence of the sprout-inhibitor on the distribution pattern.

The object of the present work was to investigate whether CIPC-treatment induced similar changes in the contents of the free amino acids, GABA and GA, in the tubers which have been observed after exposure of the tubers to γ-rays[8-10] The aim was further to search for possible changes in the saccharide levels. If the sucrose accumulation in potato tubers treated by irradiation can be connected with the sprout-inhibition, sucrose could be expected to accumulate also in chemically sprout-inhibited tubers. In addition, it was intended to determine the content of soluble nitrogen and of different phosphorus-containing compounds.

MATERIALS AND METHODS

The potato variety Bintje has been used for the main part of this investigation. Complementary analyses were performed with tubers of the variety Magnum Bonum. The tubers were stored at +5°C, except those which were investigated with respect to the influence of changed temperatures on the chemical composition.

Irradiation treatment. The tubers were exposed to γ-rays from a Hot-Pot ^{60}Curie source containing about 2 500 Curie. The dose given at room temperature was 10–14 kilorad and the dose-rate 180 rad/sec, referred to below as high dose-rate. The same dose was given at a low dose-rate, 40–80 rad/h with aid of a small ^{60}Co source containing about 0.5 Curie. The temperature at this latter exposure was about +10 to +15°C.

CIPC-treatment. The main part of the tubers (Bintje) was treated by powdering with a commercial sprout inhibitor, containing 1 % CIPC. 2 g powder per kg tubers was used. A minor part of the tubers was treated by soaking them in a thick suspension for 5 sec during rigorous stirring. The amount of the preparation necessary for reaching the same result as by powdering, 2 g per kg tubers, had been tested in advance by soaking small lots in suspensions of different strengths, drying them rapidly in a stream of cold air and weighing them. The treatment by soaking was performed in order to obtain a more homogeneous distribution of the CIPC. To ascertain that the soaking as such had no influence on the chemical composition, tubers soaked only in water were also examined. In addition a small lot of tubers of the variety Magnum Bonum was treated by soaking in CIPC in the manner described above. The treatments were performed in January before any sign of sprouting was visible (Bintje) and in April when sprouting had commenced (Magnum Bonum).

Preparation of extract. The tubers were rapidly peeled, 200 g peeled tuber very rapidly cut into pieces and homogenized in a Waring blendor for 30 sec, and 20 g of the potato mash was pressed through double gauze in a special press giving a pressure of 4000 kg/cm^2. The cell-sap was centrifuged once at 800 *g* to get rid of starch and cell-debris and thereafter once at 10 000 *g*. 20 g fresh-weight of potato tuber tissue gave about 19—20 ml centrifuged cell-sap. The temperature throughout the procedure was maintained at 0—2°C.

Analyses. The amino acid determinations were performed by paper chromatography using a special method described earlier.[10] The saccharides were determined spectrophotometrically: total sugars by using an anthrone method,[15] the sum of ketoses according to Roe,[16] and the sucrose by the method of Rorem *et al.*[17]

Phosphate-determinations. The inorganic phosphate and the acid labile phosphate (after 7 min hydrolysis at 100°C) were determined according to Martin and Doty [18] as modified by Ernster *et al.*[19] The total phosphorus was determined using the same method and also by a modified Fiske-Subbarow method.[20,21] The content of soluble nitrogen was determined according to the Kjeldahl micro method.

Control of sprouting was performed every second week for each treatment.

RESULTS AND DISCUSSION

The results obtained indicate that it is possible to induce an irreversible sprout-inhibition after exposure of potato tubers to a dose of 10—14 kilorad, given at a dose rate as low as 40—80 rad/h. It is evident that the chemical changes induced in the composition of the tubers after treatment with the same dose given at a high dose rate (180 rad/sec) and the low dose rate mentioned above differ very little. The time (10 days) necessary for reaching the dose 10—14 kilorad at the low dose rate used, explains the result obtained, *i.e.* that the changes in saccharide concentrations are present already on the day when irradiation ceases. It is known that the sucrose content particularly is influenced already at the dose-level 4000 rad. The slowly accumulated dose permits the changes to start several days before the desired dose is reached. It is, however, of practical as well as of theoretical value to know that this low dose rate can be used for sprout-inhibition. Of course such a small irradiation source as that used for the investigation reported here, could not be used in practice for larger lots of potatoes. As mentioned under Materials and Methods, the source used here now contains about 0.5 Curie.

As shown in Table 1, minor changes in both amino acid concentrations and saccharide levels in potato tubers treated with CIPC as compared with untreated tubers have been observed. Particularly after the commence of the sprouting, there is a tendency in the same direction as in irradiated tubers *viz.* a decrease of the glutamic acid and an increase of γ-aminobutyric acid. These changes are more pronounced in the soaked tubers than in the powdered ones. It must be pointed out that the distribution of CIPC among the tubers treated by powdering is far from homogenous. The purpose of soaking with CIPC-solution was, in fact, to obtain an equal distribution in all the tubers treated.

Table 1. Contents of free amino acids in potato tubers treated with γ-rays, 10–14 kilorad, dose rate 180 rad/sec. (I) or with CIPC: P=powdered; S=soaked; W=soaked in water; U=untreated. Details of treatments given in the text. Amino acids expressed as μmoles/g fresh weight of the potato tissue.

Interval between treatment and analysis days	Treatment	GABA μmoles	GA μmoles	N/ml cell-sap mg
0 [a]	I	5.70	5.30	2.90
	P	4.37	6.26	2.75
	S	6.31	5.65	2.97
	W	4.47	5.31	3.05
	U	5.66	5.35	2.82
60 [b]	I	7.86	1.46	3.40
	P	5.15	5.94	2.78
	S	5.63	6.77	3.10
	W	6.12	7.04	3.08
	U	5.44	5.37	3.15
120	I	7.46	1.30	3.50
	P	6.50	4.73	2.85
	S	9.32	2.65	2.98
	W	5.70	6.20	3.00
	U	5.45	5.30	3.05
150	I	6.60	1.21	3.45
	P	6.70	4.55	2.80
	U	5.15	5.24	3.08

[a] Preparation started within one hour after the treatment.
[b] Sprouting commences.

Table 2. Saccharide concentrations in CIPC-treated potato tubers (Bintje). Values given in mg sugar/ml cell-sap. For abbreviations see Table 1.

Interval between treatment and analyses	Sucrose				Sum of ketoses				Total sugars			
	P	S	W	U	P	S	W	U	P	S	W	U
days												
0	2.6	2.4	2.2	2.5	2.7	2.8	2.8	2.8	6.8	8.0	6.8	7.1
10	2.9			2.5	4.2			3.8	11.6			10.6
15	2.7			2.8	3.8			3.8	10.9			10.5
20	3.0	3.1	2.3	2.7	3.8	4.0	3.5	3.1	9.3	9.6	9.0	9.0
30		3.1	2.7			4.5	3.5			10.5	10.5	
60 [a]	2.8	3.7	2.6	2.6	5.1	6.3	4.9	4.9	13.5	16.5	13.3	13.3
70		4.5	2.6			7.6	5.5			16.8	13.4	
100		3.8	3.1			5.8	6.6			14.2	14.1	
120	2.8			3.0	4.8			4.9	10.5			11.0

[a] Sprouting commenced.

The changes in saccharide concentrations, which were also recorded after CIPC-treatment, were slowly equalized during storage. Here too, the most pronounced changes were observed in the soaked tubers at the time for sprout development (Table 2).

An increased level of sugars was observed also in tubers of the Magnum Bonum variety 45 days after the treatment with CIPC (by soaking).

The concentrations of saccharides in the potato tubers before and after exposure to γ-irradiation are given in Table 3. As mentioned above, the increase

Table 3. Concentrations of saccharides in potato tubers before and after exposure to γ-irradiation. Dose = 10 – 14 kilorad; I_H dose rate 180 rad/sec. I_L 40 – 80 rad/h. U = untreated tubers.

Interval between treatment and analysis days	Potato treatment	mg/ml cell-sap sucrose	total ketoses	total sugar
0 [a]	I_H	2.1	3.4	9.0
	I_L	6.0	7.4	16.0
	I_L [b]	9.8	8.9	23.8
	U	2.2	3.5	9.1
5	I_H	14.4	11.3	25.0
	I_L	6.8	8.2	21.3
	U	2.5	2.8	7.1
60	I_H	8.9	7.7	17.9
	I_L	8.2	7.8	17.7
	U	3.3	6.6	14.1
120	I_H	3.7	4.5	11.9
	I_L	3.6	4.6	12.0
	U	2.3	5.4	11.4
180	I_H	2.4	5.3	12.5
	U	2.6	8.1	15.9

[a] Preparation started within one hour after the treatment.
[b] The irradiation repeated 20 days later to verify the high starting values obtained.

of the sugar concentrations in the tubers irradiated at low dose-rate exists already when the treatment is finished. In the tubers irradiated with the same dose but at high dose-rate there is a minor decrease of the sugar concentrations immediately after the exposure. The accumulation of sugars is, however, apparent already 24 h after the treatment, and the concentrations subsequently increase to reach their maximum 5 – 7 days later. Different lots retain the high sugar levels for different times. Usually the sucrose concentration persists at a higher level for a longer time than the concentrations of the other sugars. After 3 – 4 months the differences between irradiated and untreated tubers is, however, equalized. When investigating the sugar concentrations six months after the irradiation-exposure, provided that this was performed before the tubers were in "sprout-temper" (German: "Keimungsstimmung"), a small decrease of the sugar concentrations was observed as compared with those in the untreated tubers.[1]

The concentrations of phosphate in the cell-sap from potato tubers after the different treatments are recorded in Table 4. Although not given in the

Table 4. Concentrations of phosphate in cell-sap from potato tubers treated with γ-rays and CIPC. Symbols for treatments as in Table 1. Values given as μg P/ml cell-sap. Pi = inorganic phosphate, Pa = acid labile phosphate, and P = total phosphate.

Potato variety	Interval between treatment and analysis days	Treatment	P μg	Pi+Pa μg	P μg	Pa μg
Bintje	0 [a]	I	220	250	299	30
		P	199	220	264	21
		S	201	242	280	41
		W	190	232	275	42
		U	200	225	270	25
»	120	I	183	240	380	57
		P	175	208	270	33
		S	146	192	254	46
		W	180	210	285	30
		U	190	230	300	40
»	180	I	171	243	485	72
		U	149	219	293	70
»	1	I [b]	130	163	260	33
Magnum Bonum	1	I [b]	232	306	394	74
		U	188	302	368	114
»	45	S	165	268	315	103

[a] Preparation started within one hour after treatment.
[b] Irradiation performed after eight months' storage of the tubers.

table, a small decrease of total soluble phosphate 1—5 days after the irradiation of the tubers (Bintje) at the high dose-rate was observed. A determination performed two weeks after the exposure revealed that the changes had already been equalized. After prolonged storage an increased concentration of the total soluble phosphate was again observed, as is to be seen in Table 4. The γ-irradiation exposure was repeated after eight months' storage of the tubers and the same investigations were performed. Again a lower concentration of phosphates was found in the cell-sap from the irradiated tubers than in the sap from the untreated tubers of the Bintje variety, whereas the opposite was true for tubers of the Magnum Bonum variety. In these tubers, however, a decrease of phosphates after the CIPC-treatment was observed. As also shown in Table 4 no significant differences in phosphates were found between CIPC-treated and untreated tubers of the Bintje variety.

The values of extractable nitrogen, given in Table 1, were somewhat higher in the irradiated tubers 60—150 days after exposure than in the tubers from the other treatments and in the untreated tubers. These differences were, however, not significant.

The sprout-inhibition caused by irradiation at both dose levels and by CIPC-treatment was 100 %. It is evident from this investigation, that the changes in chemical composition of the tubers caused by irradiation-exposure are much more pronounced than the changes caused by the chemical treatment. In both cases the changes are reversible.

Concerning the amino acids in the tubers it has also been observed that GABA is much more susceptible to changes in the storing temperatures than is GA. It should be borne in mind that all comparisons performed between different treatments and the chemical changes caused by them have to be done on tubers stored at the same temperature and humidity. It is evident that though smaller variations in reactions to treatments do occur in tubers of different varieties, the chemical changes in the main are equivalent.

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