Effect of gamma irradiation on sprout inhibition and its physiological mechanism of Chestnuts

Y. UCHIYAMA

Okayama Agricultural Experiment Station, Okayama City

Summary

From the results of this experiment on the effects of gamma irradiation on the sprout inhibition and its physiological mechanism purporting the extension of storage life of chestnuts, the following summary can be made.

Chestnuts harvested in October were irradiated with 1×10^4 , 5×10^4 roentgen of Co 60 gamma rays, then stored in moist sawdust at room temperature. On February, over 50 percent of nuts which had been received in low doses roentgen showed visible growth of roots, but in the untreated nuts only 30 percent. After that, the roots of untreated nuts had continued vigorous growth, whereas the rooting of the treated nuts with the lower dose were gradually prevented, but not completely stunted. On the other hand, high doses completely inhibited the rooting and sprouting. Most of treated with the nuts with the higher doses were still salable in April. More rotting were found in the treated nuts higher doses than those with the lower doses. Such unfavorable effects tended to increase in the nuts with higher under the elongated storage. From

these observations concerning results with beneficial effect of root and sprout inhibition, it is supposed that the range of 3.5×10^4 5×10^4 roentgen is the most desirable level of treatments.

Total ascorbic acid contents were slightly diminished in parallel with increasing dose levels and elongation of storage times. Sugar contents showed that the conversion of starch to sugars progressed slightly by the irradiation. Soluble astringent contents did not show any change by the irradiation.

The effects of irradiation on the respiratory activity were obviously recognized, but it seemed that the changes of respiration had not so much direct correlations with mechanism of root and sprout inhibition.

The amylase activities decreased immediately after irradiation but rapid and significant increasing followed just after. No important variations were found in either polyphenoloxidase or peroxidase, though showed a tendency to decrease with small inactivation by irradiation. The correlation between the rotting and the oxidase activity was not clear.

Introduction

Gamma irradiation has recently been reported to be an effective sprouting inhibitor for potatoes and onions^(4,10). SPARROW and CHRISTENSEN⁽¹²⁾ reported that the sprouting of potatoes was greatly reduced with a dose of 5,000 rep and completely inhibited at 20,000 rep or higher. Brownell and coworkers⁽²⁾ indicated that doses from 10,000 to 15,000 rep were minimum that were necessary for complete sprout inhibition of potatoes. On the other hand^(7,8,11), it was also observed that the tubers which received comparatively high doses of gamma irradiation increased in the percentage of the loss resulted from rotting during the storage. Generally, the irradiation dose of approximately 10,000 roentgen seems to be preferred by many investigators. Those statements were based on experiment with a number of different vegetables, not including chestnuts.

The only report on the application of gamma irradiation for sprout inhibiting of chestnuts is that of T. IWATA and K. OGATA⁽⁹⁾. They conducted some observations on IMAKITA and GINYOSE varieties immediately after treatment with 0, 15,000, 30,000 and 60,000 roentgen, and after various intervals during the storage. They reported that almost complete inhibiting effects were obtained with all the doses used regardless of varieties, and that no significant

Received for publication August 2, 1966

differences were indicated in the chemical constituents between the irradiated and the un-irradiated chestnuts.

Preliminary experiments at this station gave the same indications with these effects among the higher doses, whereas the increased effects on the sprouting and rooting were observed in the early growing stage of chestnut at the lower doses, though storage temperature and irradiation doses were somewhat different from those on the work of IWATA and OGATA.

This work was carried out at this station for a period of six months to determine whether this method has any potential usefulness for the storage of chestnuts. Besides the effects on sprouting and rotting has been also investigated, including determination of chemical constituents and activities of some enzymes such as amylases, polyphenoloxidase and peroxidase.

Materials and Methods

The nuts of *Castanea crenata* cultivar, Tsukuba were harvested in the orchard located at Osa, Okayama Prefecture in October and stored in moist sawdust at room temperature.

A 1,800-curie Co 60 with a dose rate of about 41 roentgen/min. to a 225 sq. cm surface was adapted for continuous operation during off-clinical hours at the faculty of medicine, Okayama university. In each dose 60 nuts were arranged in the tray and set in frame to be kept in fixed positions. The top of the upper layer of nuts was 75 cm below the radiation source. Doses were 10,000, 20,000, 35,000 and 50,000 roentgen, with respective dwell times in the irradiator of 4, 8, 15 and 20 hours. After the desired dose levels had been obtained, the portions of the treated and untreated samples were taken to the laboratory for analysis, and the remaining samples were stored in moist sawdust at room temperature.

Nuts were examined at regular intervals for observable changes in the appearance of rooting, sprouting and rotting. The occurred symptoms were expressed as percent.

Ascorbic acid was determined by a modification of the 2,4-dinitrophenylhydrazine procedure as described by T. TAKAHASHI and K. KONO⁽¹⁵⁾. Aliquots of extracted solution with meta phosphoric acid was used for direct determination of dehydro ascorbic acid (DA). Another aliquot was used for determination of total ascorbic acid (TA). Reduced ascorbic acid (AA) was measured by the difference between DA and TA. Results were show in terms of DA, TA and AA.

Reducing and total sugar contents were determined by Somogyi's method with some modifications.

Soluble tannin as astringent materials was determined by the titration method of potassium permanganate. Aliquots of extracted solution with water were used for its determination.

Carbon dioxide evolution was measured as follows: nuts were placed in desicator, and the carbon dioxide given off from nuts was collected in 25 ml of 2 N potassium hydroxide solution placed at the bottom of desicator, and was filled up to 250 ml with water adding 10 ml of 25 percent barium chloride solution, then titrated with 0.2 N hydrochloric acid solution.

Estimation of amylase activity was as follows: the peeled nuts were carefully cut and chopped with stainless knife, then 5 g of tissues were weighed and homogenized at high speed for 4 min. in 20 ml of cold phosphate buffer solution (pH 5.5), and filtered after centrifugation at 4,000 rpm for 5 min. Appropriate filtrate from the homogenates were immediately assayed

for the activity of amylase by the method of OSTWALD viscosimeter, that is, 2 ml, of filtrate (as crude enzyme solution) was added to 10 ml of 1 percent starch solution previously prepared to pH 5.5 with phosphate buffer solution and held at 37.5°C. When the mixture was kept at 37.5°C, viscosity changes were measured at the given intervals in the reaction of 0, 5, 10, 15, 20 and 30 min. Exactly 30 min. after, the mixture was quickly alkalified by the addition of Somogyi's reagent, and liberated reducing sugar was determined from the residual iodoine which was titrated with standard thiosulfate solution. Reducing power of amylase was expressed in term of monomolecular reaction rate constant K , which was calculated by the use of the following equation :

$$K=1/t \cdot \log a/a-x$$

where KMonomolecular reaction rate constant
 t Reaction time (30 min.)
 a Amount of starch, which hypothesized as 75 percent of a given substrate (mg)
 x Liberated reducing sugar as maltose (mg)

On the other hand, the percentage of degradation of substrate was calculated basing on the result of viscosity. Such resulting value was represented by the following equation :

$$D=\eta_{sp}-\eta'_{sp}/\eta_{sp}-1 \times 100$$

where DPercent of degradation of substrate
 η_{sp} Relative viscosity at zero hour
 η'_{sp} Relative viscosity at each definite time

Estimation of peroxidase activity was as follows: 10 g of peeled and chopped tissues were weighed, and homogenized in 10 ml of cold phosphate buffer solution (pH 6.0), then centrifuged and filtered as well as for the assay of amylase. The filtered extract was frozen immediately and stored at -15°C until analysis was made. The filtrate was assayed for enzyme activity by method of Warburg manometry⁽¹⁾ at 20°C. The samples were removed from frozen storage and 0.1 ml of defrosted solution, as crude peroxidase solution, was placed in the side arm. The substrate (contained 0.2 ml of 5 percent pyrogallol, 0.5 ml of phosphate buffer (pH 6.0) and 1.5 ml of 0.01 M hydrogen peroxide solution) was placed in the main compartment. The gas phase in the manometer was air, and they were equilibrated at 20°C for 10~20 min., then the content of side arm was mixed with the solution of the substrate in the main compartment. Reading was taken at 3, 5, 6, 10 and 30 min. after mixing and recorded. The results of measurement of the enzyme activities were expressed as carbon dioxide ml/g/hr.

Estimation of polyphenoloxidase activity⁽¹⁾ was as follows: for the assay, the enzyme solution was prepared as well as peroxidase, but the provided phosphate buffer solution to extract was pH 7.3. The rate of oxygen uptake on the enzyme activity was measured with Warburg manometer. The side arm contained 1 ml of enzyme solution. The main compartment contained 2 ml of 0.03 M phosphate buffer solution, which was previously prepared to pH 7.3 and was 10 mg of catechol added. A strip of whatman No. 40 filter paper and 0.2 ml of 2N potassium hydroxide solution were placed in the center well of each flask. The gas phase in the manometer was air. After equilibrated at 25°C for a time the measurement was carried out. Reading was taken as well as peroxidase and results were expressed as O_2^{μ} l/g/hr.

Results and Discussion

Rooting, Sprouting and Rotting

The sprouting of chestnut begins with the break up of its husk at the upper end, then the root come out. When the root grows to a considerable length (about 20~30 mm), the sprout appears. The results on rooting, sprouting and rotting of chestnuts after irradiation are summerized in Table 1.

No significant effects on the quality of nuts up to the December 14 were observed. On January 15, untreated and treated nuts with 1×10^4 and 2×10^4 roentgen slightly rooted. On February 15, over 50 percent of treated nuts with the lower doses (at the doses of 1×10^4 and 2×10^4 roentgen) showed visible length of roots, but only 30 percent of the untreated nuts. Such effect of irradiation (in case of lower doses) which appear to be stimulative at early stage of rooting may suggest that this lower doses would activate the physiological mechanisms of the chestnuts, and as a result, the nutritious condition was effectively utilized causing the rapid growth. The increased amylase activity was recognized in the irradiated nuts, especially lower irradiated nuts (this fact will be discussed in later section). Therefore, it may be considered that there exist a relationship between the promoting effect of sprouting and the increased amylase activity. However, the accelerated growth was only temporal, and did not last long. During the storage, the growth of rooting was gradually prevented and fairly stunted. On the other hand, vigorous rooting in the untreated nuts was continued. On

Table 1. Effects of gamma irradiation on rooting, sprouting and rotting of chestnuts (Tsukuba).

Days after irradiation	Doses ($\times 10^4$ roentgen)	Rooting %	Sprouting %	Root length mm	Rotting %	Sound %
1 15/11	0	0	0	0	0	100
	1	0	0	0	0	100
	2	0	0	0	0	100
	3.5	0	0	0	0	100
	5	0	0	0	0	100
63 15/1	0	20	0	4	0	80
	1	30	0	2	0	70
	2	20	0	2	0	80
	3.5	0	0	0	0	100
	5	0	0	0	0	100
95 15/2	0	30	0	7	0	70
	1	60	0	5	0	40
	2	55	0	4	0	45
	3.5	0	0	0	0	100
	5	0	0	0	0	100
120 15/3	0	100	60	62	0	0
	1	90	20	28	0	10
	2	90	0	15	3	10
	3.5	0	0	0	3	97
	5	0	0	0	3	97
120 5/4	0	100	100	105	3	0
	1	100	90	41	3	0
	2	93	10	20	6	0
	3.5	2	0	2	8	90
	5	0	0	0	10	90

March 15, the untreated and treated nuts with the lower doses almost rooted, while most of the treated nuts with the higher doses were still salable. From these results, the lower limit of irradiation for inhibition of rooting seemed to be about 3.5×10^4 roentgen. More rotting was found in the treated nuts with the higher doses than those treated with the lower doses. Such unfavorable effects on the treated nuts increased as the storage time elongated, moreover the affected nuts were putrefied with fungal developments at last. It has been often reported that rot incidence in the potato tubers occurred when they received relatively high dose of gamma irradiation. IWATA and OGATA⁽⁹⁾ indicated that the gamma rays-treated chestnuts also seemed to become susceptible to storage rotting. From these observations, concerning results with the beneficial effect of rooting inhibition it is supposed that from 3.5×10^4 to 5×10^4 roentgen is the most desirable level of treatment.

Chemical Components

Analytical results are shown in Table 2.

Ascorbic acid: Total ascorbic acid (TA) contents in the treated nuts were slightly lower than the untreated, which the concentration diminishing with increasing dose levels. A slight but consistent decrease in ascorbic acid during the storage were observed as in many other fruits^(5,13).

Sugar: Sugar contents are also included in Table 2. There is the suggestion that the conversion of starch to sugar progressed slightly in the lots given each roentgen. BROWNELL⁽³⁾ reported that the initial decreases and the following rapid increases in sugar contents of potatoes were observed after irradiation. In this work, there were only slight increases in

Table 2. Effects of gamma irradiation on chemical components of chestnuts.

Days after irradiation	Doses ($\times 10^4$ roentgen)	Ascorbic TA	acid DA	mg % AA	Total sugar %	Reducing sugar %	Tannin as astringents % (all nuts)
1 15/11	0	18.14	9.17	8.97	6.26	1.17	0.36
	1	17.58	9.12	8.46	7.23	1.20	0.41
	2	16.95	9.38	7.57	7.85	1.22	0.40
	3.5	16.69	9.28	7.41	8.09	1.32	0.39
	5	16.54	9.47	7.07	8.23	1.47	0.36
63 15/1	0	16.61	10.93	5.68	9.10	1.41	0.32
	1	16.39	10.87	5.52	8.86	1.27	0.38
	2	16.00	10.98	5.02	8.74	1.33	0.46
	3.5	15.94	11.00	4.94	8.59	1.22	0.35
	5	15.33	11.10	4.23	7.26	1.10	0.35
93 15/2	0	16.84	11.41	5.43	8.72	1.55	0.43
	1	16.09	11.64	4.45	8.46	2.09	0.40
	2	15.93	11.57	4.36	8.87	1.66	0.38
	3.5	15.77	11.98	3.79	8.68	1.23	0.46
	5	15.84	11.71	4.13	8.38	1.29	0.43
120 15/3	0	15.96	11.70	4.26	6.45	1.31	0.34
	1	16.08	12.17	3.91	7.55	1.09	0.33
	2	15.73	12.40	3.33	7.24	1.10	0.36
	3.5	15.55	12.50	3.05	8.75	1.65	0.38
	5	15.73	12.67	3.06	7.32	1.30	0.46
140 5/4	0	15.21	12.34	2.87	6.14	1.21	0.41
	1	15.17	12.14	3.03	6.66	1.08	0.44
	2	15.03	12.81	2.22	7.24	1.03	0.44
	3.5	15.06	12.74	2.32	7.10	1.27	0.45
	5	14.73	12.61	2.12	7.33	1.27	0.48

sugar contents among the treated nuts, however, not any decreases until about 60 days after irradiation. The respiration of nuts markedly increased either immediately or shortly after irradiation (this fact will be discussed in later section). However, the behavior of sugar which seems to be utilized as the source of energy for the respiration, and as a result, causing the decrease of sugar content is not clear from this results.

Soluble tannin as astringent contents: There were no significant effects of irradiation on the soluble astringent contents as shown in Table 2.

Respiration

The effects of irradiation on the carbon dioxide evolution of chestnuts are shown in Fig. 1.

Fig. 1. Effects of gamma irradiation on the carbon dioxide evolution.

As indicated in Fig. 1, nuts irradiated had a marked increase in carbon dioxide evolution immediately after the treatment. The stimulative effect of irradiation were increased with increasing doses, that is, the carbon dioxide evolution of nuts which had been treated at 5×10^4 , 3.5×10^4 , 2×10^4 and 1×10^4 roentgen was about each 3.2, 2.3, 2.0 and 1.5 times greater than that of the untreated. On the day succeeding the treatment, however, these increased curves rapidly lowered as low as that of the untreated about two weeks after irradiation. In April, untreated and treated nuts with the lower doses showed again the considerable increasing curves, but nuts with the higher doses were slight in change. The effects of irradiation on the respiratory activity which seems to be physiologically important behaviour of chestnuts were obviously recognizable, but it seemed that the changes of respiration had not so much correlation with

the mechanism of root inhibition directly. The untreated group and the lower irradiated nuts reactivated their respiration approximately after 60 days and followed by sprouting. On the other hand, the higher irradiated nuts did not any sprout in spite of their increased respiration during storage.

Enzyme Activities

Amylase: The effects of irradiation on amylase activities in the given nuts were measured by fall of viscosity or by estimating some new reducing groups formed during the course of the reaction. Table 3 gives the degradation rate of starch by the viscosity. Table 4 gives the variances in the values of amylase activities by reducing power. The discrepancy between the changes in viscosity and in reducing power undergoing hydrolysis by amylase seems to indicate the pattern of enzyme action to the glycosidic linkage in the starch macromolecule. The results given in Table 3 indicates that the major degradation occurred very early in the reaction. As indicated in Table 3 the values of viscosity changes are very sensitive indication of even traces of amylase activities. Fig. 2 shows a comparison of the rate of degradation on

Table 3. Effects of gamma irradiation on amylase activities : Rate of degradation of starch (on the occasion of viscosity).

Doses ($\times 10^4$ roentgen)	Time of reaction min.	Days after irradiation (Date)				
		1 (15/11)	63 (15/1)	93 (15/2)	120 (15/3)	140 (5/4)
0	5	32.939	34.433	45.489	48.625	49.000
	30	75.740	79.175	87.594	93.359	31.571
1	5	37.014	65.055	68.085	69.750	57.053
	30	50.096	92.913	85.461	39.750	74.526
2	5	39.510	69.231	74.436	75.748	53.468
	30	62.610	91.346	94.737	92.027	61.850
3.5	5	15.931	64.235	61.495	55.432	45.416
	30	55.278	86.007	34.890	97.342	69.252
5	5	14.384	63.165	58.941	53.748	41.679
	30	33.790	88.722	80.588	92.027	48.560

Table 4. Effects of gamma irradiation on amylase activities : Monomolecular reaction rate constant (on the occasion of reducing power)

Doses ($\times 10^4$ roentgen)	Days after irradiation (Date)				
	1 (15/11)	63 (15/1)	93 (15/2)	120 (15/3)	140 (15/4)
0	0.00664	0.00382	0.00392	0.00657	0.00657
1	0.00453	0.00392	0.00532	0.00798	0.00839
2	0.00417	0.00575	0.00564	0.00726	0.00867
3.5	0.00449	0.00938	0.00564	0.00275	0.00746
5	0.00216	0.00765	0.00795	0.00327	0.00891

Fig. 2. Effects of gamma irradiation on amylase activities (on the occasion of viscosity).

treated nuts, especially in the lower irradiated. The change of reducing and viscosity by amylase in the untreated nuts were observed with the values that seems to proportionate to each other.

There appear to be important variations in the amylase activities (Fig. 2) with irradiation. The activities decreased immediately after irradiation, but rapid and significant increasing followed just after. In December, the treated nuts with the higher doses showed again the

the occasion of viscosity changes at 5 min. in the reaction. The values of reducing power presented in Table 4 shows the narrower range of values compared to those show in Table 3 and Fig. 2. Therefore, this differences in the values of hydrolysis of starch which basing on these two changes in reducing power and viscosity seems to suggest that the manner of hydrolysis by amylase goes through changes rapidly from macromolecule to relatively larger molecule and then follows slowly and gradually to the smaller molecule (reducing groups: maltose). Such manner in the amylase reaction were recognized in the

Fig. 3. Effects of gamma irradiation on the peroxidase activities.

Fig. 4. Effects of gamma irradiation on the polyphenoloxidase activities.

considerable decreases in the activity, but the treated with the lower doses in March. The activity of the untreated nuts continued gradually to increase in during storage. From above results, gamma irradiation on amylase proved to cause inactivation by its direct action immediately after treatment and by indirect action during the storage. However, it is not clear whether increased tendency observed during the storage is caused by the stimulative effect of gamma radiation or by some formation of substrates which become more reactionable to enzyme actions, or both. As protein^(2,14) is known to be very sensitive to irradiation, most of enzymes are also oxidized to be inhibited their activities by irradiation. Many investigators^(6,14) have reported in recent years concerning the inactivation of enzymes by irradiation which could decrease by the addition of various compound to the enzyme solution. It is assumed that some chemical compounds which should be protected to amylase activities were contained in this nuts, though such effects of protection were temporary.

Oxidase: Polyphenoloxidase is known to catalyze the aerobic oxidation of various naturally occurring *o*-dihydroxyphenols to the corresponding *o*-quinones. Peroxidase, furthermore, can catalyze the oxidation of certain polyphenols in the presence of organic peroxide (or *o*-quinone) to form dark colored polymers. The author of the present study had originally expected a significant correlation between blackening or rotting of chestnuts and oxidase activity, moreover these reactions may be also correlative with mechanism of nuts sprouting, but could not find a distinct relations between them. Fig. 3 and Fig. 4 shows the oxidase activities. The curves shown in these Figs. indicate that the oxidase enzyme conditions were not greatly affected by the irradiation under the given doses. Polyphenoloxidase and peroxidase activities tended to decrease slightly with small inactivation by the irradiation.

Aknowledgment

The author wish to express his sincer thanks to Mr. Y. HAYASHI, Head of the Station, and Mr. H. YUNOMURA, for their advices. Thanks are also due to Dr. M. YAMAMOTO, Prof. of Okayama University, for the use of Co 60 gamma irradiation facility.

Literatures Cited

1. AKABORI, S. 1956. Research methods of enzyme (in Japanese). Asakura Book Co. 2: 337—359.
2. BARRON, E. S. G., S. R. DICKMAN., T. P. Singer., and J. A. MUNTZ., 1954. Biological effects of external X and gamma radiation. Mc-Graw Hill Book Co.
3. BROWNELL, L. E., C. H. BURN., and P. G. GUSTAFSON, 1956. Storage properties of gamma irradiated potatoes. Food Tech. 10: 33.
4. BROWNELL, L. E., L. C. ANDERSON., H. J., COMBERG, L. L. KEMPE., J. J. MARTIN., J. V. NEHEMIAS., and R. A. WOLF., 1954. Utilization of the goss fission products. Uni. Mich., Eng. Res. Inst. Prog. Rpt. No. 7: 64, 84. 102.
5. CLAUDIA, E. W., A. T. DORIS. and C. M. DUDLEY, 1963. Ascorbic Acid Retention and color of strawberries as related to low-level irradiation and storage time. Food Tech. 17: 77.
6. DALE, W. M. 1956. Radiation Biology. Mc-Graw Hill Book Co.
7. DALLIN, S. L. and R. L. SAWYER., 1959. Effect of gamma and fast electron irradiation on storage qualities of onions. Proc. Amer. Soc. Hort. Sci. 73: 390—397.
8. ———. 1959. Effect of sprout inhibitions levels of gamma irradiation on the quality of onions. Pro. Amer. Soc. Hort. Sci. 73: 398—406.
9. IWATA, T. and K. OGATA, 1961. Studies in the storage of chestnuts treated gamma radiation. Bull. Inst. Chem. Res., Kyoto Uni. 39: 112.
10. OGATA, K., T. IWATA., and K. CHACHIN., 1959. The effect of gamma radiation on sprout prevention and its physiological mechanism in the potato tubers and the onion bulb. Bull. Inst. Chem. Res., Kyoto Univ. 37: 425.
11. SCHREIBER, J. S. and M. E. HIGHLAND., 1953. A study of the bio chemistry of irradiated potatoes stored under commercial conditions. Food Res. 23: 464—472.
12. SPARROW, A. H. and E. CHRISTENSEN., 1954. Improved storage of potato tubers after exposure to ^{60}Co 60 gammas. Nucleonics. 12: 16.
13. SALUNKHE, D. K., R. K. GERBER., and L. H. POLLARD., 1959. Physiological and chmical effects of gamma radiation on certain fruits, vegetables and their products. Pro. Amer. Soc. Hor. Sci. 74: 423.
14. TANAKA, S., H. HATANO., S. GANNO, and T. OKAMOUO, 1959. Actions of gamma radiation on enzymes and enzyme systems. Bull. Inst. Chem. Res., Kyoto Univ. 37: 374.
15. TAKAHASHI, T. and K. KONO, 1954. Vitamin (in Japanese). Shibata Book Co.

クリの発芽抑制と生理機構に及ぼすガンマー線の影響

内 山 善 雄

(岡山県立農業試験場)

摘 要

クリ(品種:筑波)の貯蔵期間を延長するため、収穫後のクリに $\text{Co } 60\text{-}\gamma$ 線を照射し、線量と発芽抑制、貯蔵性の関係を検討した。

なお、2, 3の化学成分、酵素活性の消長をあわせて検討した。

その結果は次のとおりである。

1. 低線量(10,000~20,000r)は一時的に発芽現象を促進したが続いて生育する根に生理障害を与えた。
2. 高線量(35,000~50,000r)は完全に発芽抑制した。貯蔵6か月後(11月~4月)なお健全であった。

3. 貯蔵末期に腐敗、黒変果が線量に比例して多発した。

4. 照射はアミラーゼ活性に特異的な影響を与え、またオキシダーゼ、(パーオキシダーゼ、ポリフェノールオキシダーゼ)にわずかな活性低下を及ぼした。

5. 呼吸は照射により急激に上昇したが、日数の経過とともに次第に正常にかえる傾向を示した。

6. 照射はアスコルビン酸と糖の含量にわずかな変化を及ぼしたが、タンニン含量には影響を与えなかつた。