EFFECTS OF CHEMICALS UPON ELECTRON SPIN RESONANCE (ESR) SIGNALS IN LETTUCE SEEDS

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ABSTRACT

ESR spectrometric study of chemically treated lettuce seeds has been carried out. Solutions of $HgCl_2$, Na_2S, KOH, KCN, KBr, KF, KIO_3, KIO_4, KCl, KI, and K_2S, as well as water and petroleum ether have been used. Germination power and respiratory activity of the same seeds have also been investigated. Boiled seeds and gamma irradiated seeds have also been studied. KI was found to be the most effective agent. Independence upon cation and pH was found. The effect of KI and KCN was found to be reversible. No correlation between respiratory activity and the number of free radicals seems to exist.

Seed coat is affected first and later on the embryo is also induced by the penetrating solution.

Free radicals have been implicated in biological oxidation-reduction processes (3, 9, 13), the action of ionizing, ultra-violet and visible radiation on biological systems (4, 14) and chemical carcinogenesis (8). Investigations of free radicals in living tissues were first undertaken by Commoner et al. (1, 2). Their results support the hypothesis of Michealis (9) which says that these free radicals are necessary intermediates in biological oxidation-reduction process. Water (13) has also postulated that free radicals take part in most enzyme reactions.

The purpose of these experiments is to contribute some more data to the not-well known knowledge of free radicals in seeds.

METHODS AND EXPERIMENTAL RESULTS

1. a). Lettuce seeds var. New York were soaked in different solutions for 6 hours. The treated seeds were then dried in dessicators for two nights. After this treatment the seeds were ready for Electron Spin Resonance spectrometric study of magnetic centers. Most of the chemicals used in these experiments are known as metabolic inhibitors (5, 7), which in their solid state do not give signals. The relative number of unpaired electrons was calculated from the product of the height times the square of the width of the derivative curve. The absolute number was calculated from pitch standards. The result suggests that the ESR signal in lettuce seeds can be modified by chemicals (fig. 1). The most effective agents are KCN, K_2S

and KI. These salts reduced the relative number of spins to about 10% of that of the untreated seeds. It seems that the relative number of spins is not dependent of pH. Two molar of KBr and 2 M of KF solutions, which have different pH, gave the same effect. On the other hand, 5 M solution of K_2S and 5 M solution of KOH, both have the same pH, changed the amount of free radicals differently.

b). When the treated seeds were washed in water for a couple of hours after the measurements and were dried again, the relative number of spins showed an increase and reached almost the original level (fig. 2 and fig. 3). This fact suggests that the effect of 5 M KCN and 2 M KI is reversible.

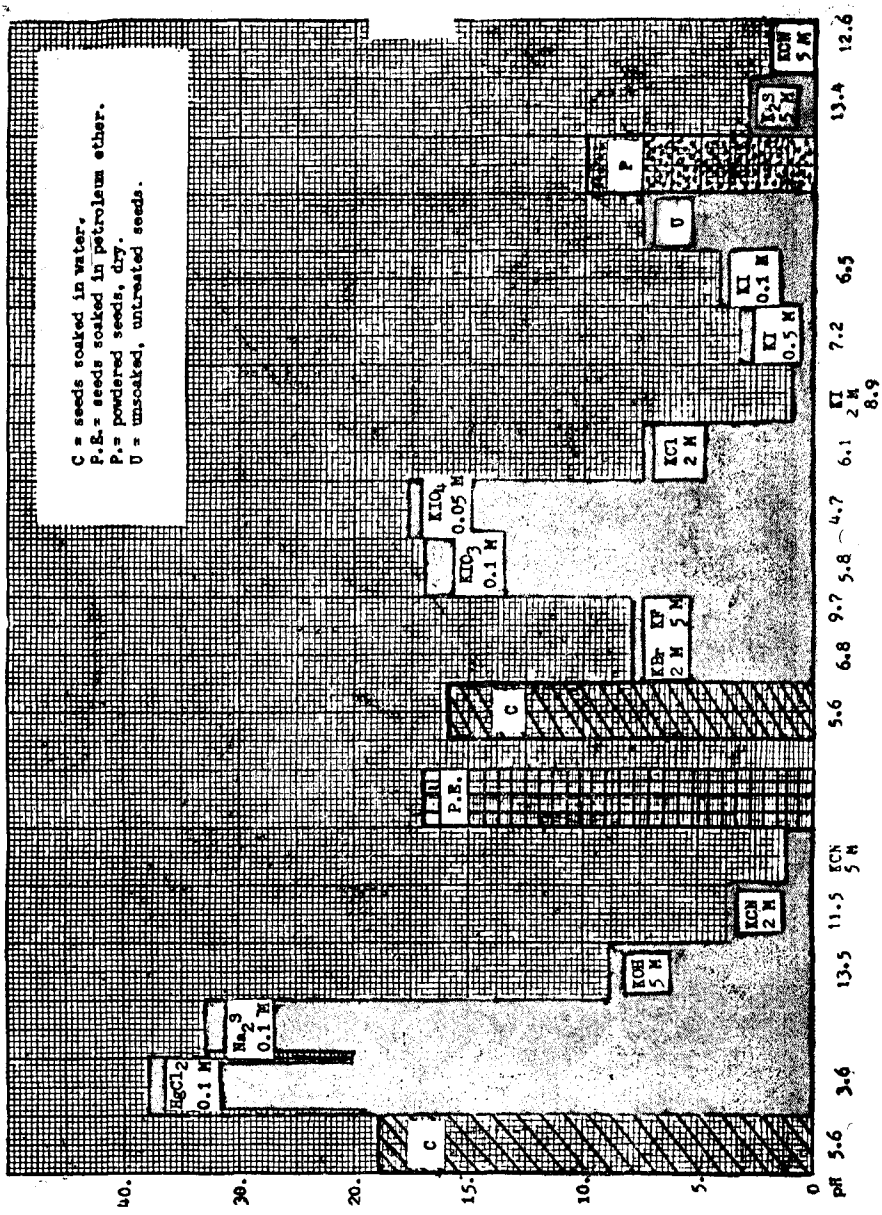
2. In another experiments the seeds were first boiled before the treatments. Boiled seeds treated with KCN solution of low concentration increased the number of spins, but the number decreased again when solutions of higher concentration were used. The opposite was true when KOH solutions were used. KI and the control seem have no effect (Table 1).

3. In the next experiment the seeds were soaked for an hour with 1 to 15 minutes interval, to see when the KI solution is the most effective. The result is shown in fig. 4. Approximately the same reduction of the relative number of spins (about 1/5 of that of the control) were detected in 1 minute as well as in 30 minute soaking times.

Table 1. Relative number of spins in lettuce seed, boiled for 10 minutes in water and then soaked for 6 hours in solutions

Unboiled				Boiled		
hw^2	spin/cm	spin/seed	sample	spin/seed	spin/cm	hw^2
28.4	3×10^{15}	—	standard	—	3×10^{15}	28.4
6.9	—	9.1×10^{12}	control	7.7×10^{12}	—	5.8
6.6	—	8.7×10^{12}	0.05 M KCN	15.1×10^{12}	—	11.4
2.9	—	3.8×10^{12}	0.1 M KCN	14.9×10^{12}	—	11.2
1.8	—	2.4×10^{12}	0.2 M KCN	11.8×10^{12}	—	8.9
1.5	—	2.0×10^{12}	0.5 M KOH	1.6×10^{12}	—	1.2
22.3	—	29.3×10^{12}	2 M KOH	0.7×10^{12}	—	0.5
44.6	3×10^{15}	—	standard	—	3×10^{15}	44.6
8.7	—	7.3×10^{12}	control	6.8×10^{12}	—	8.1
1.3	—	1.1×10^{12}	2 M KI	1.9×10^{12}	—	2.3

Fig. 1. Relative number of spins after 6 hours soaking in solutions of different pH
Relative number of spins per seed.



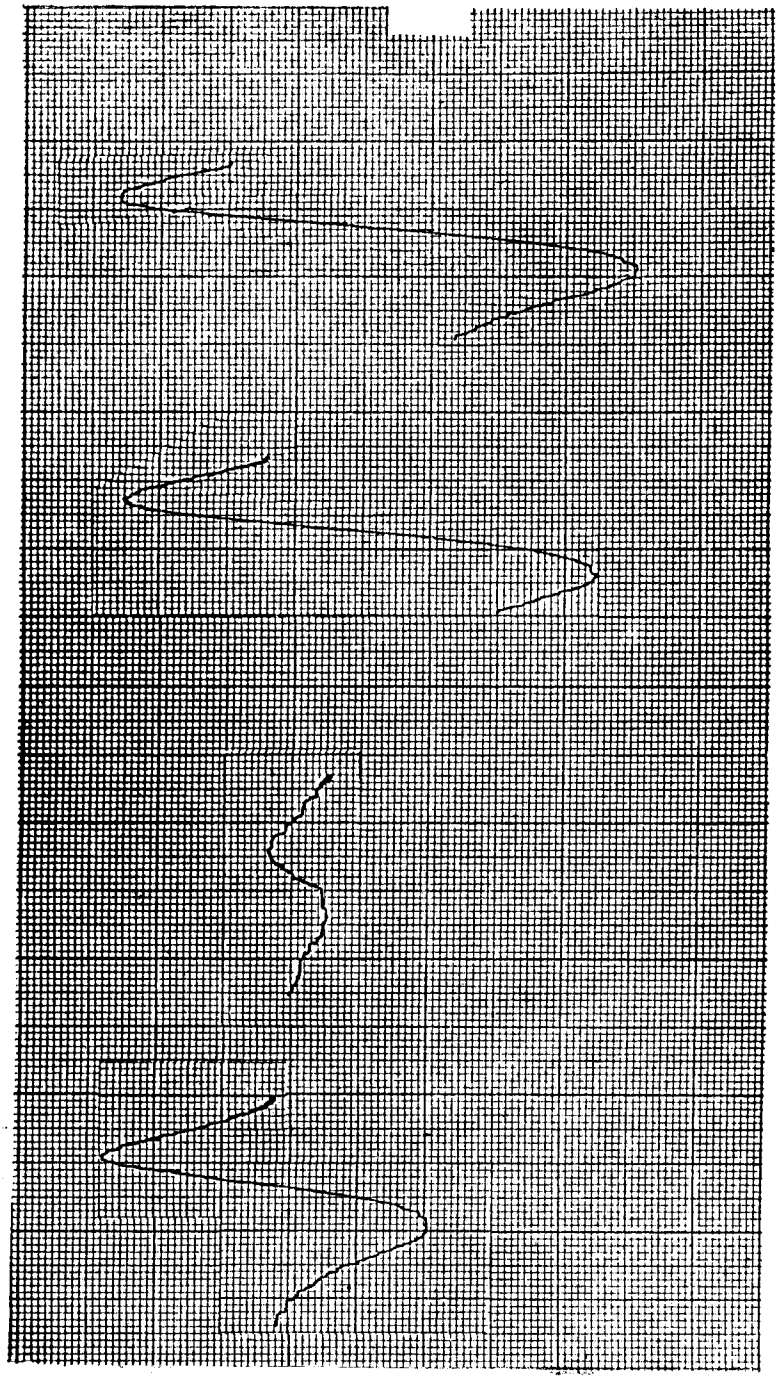


Fig. 2. The reversibility of free radicals in lettuce seeds, treated with KCN and then washed with water.

Fig. 3. The reversibility of free radicals in lettuce seeds, treated with KI and then washed with water.

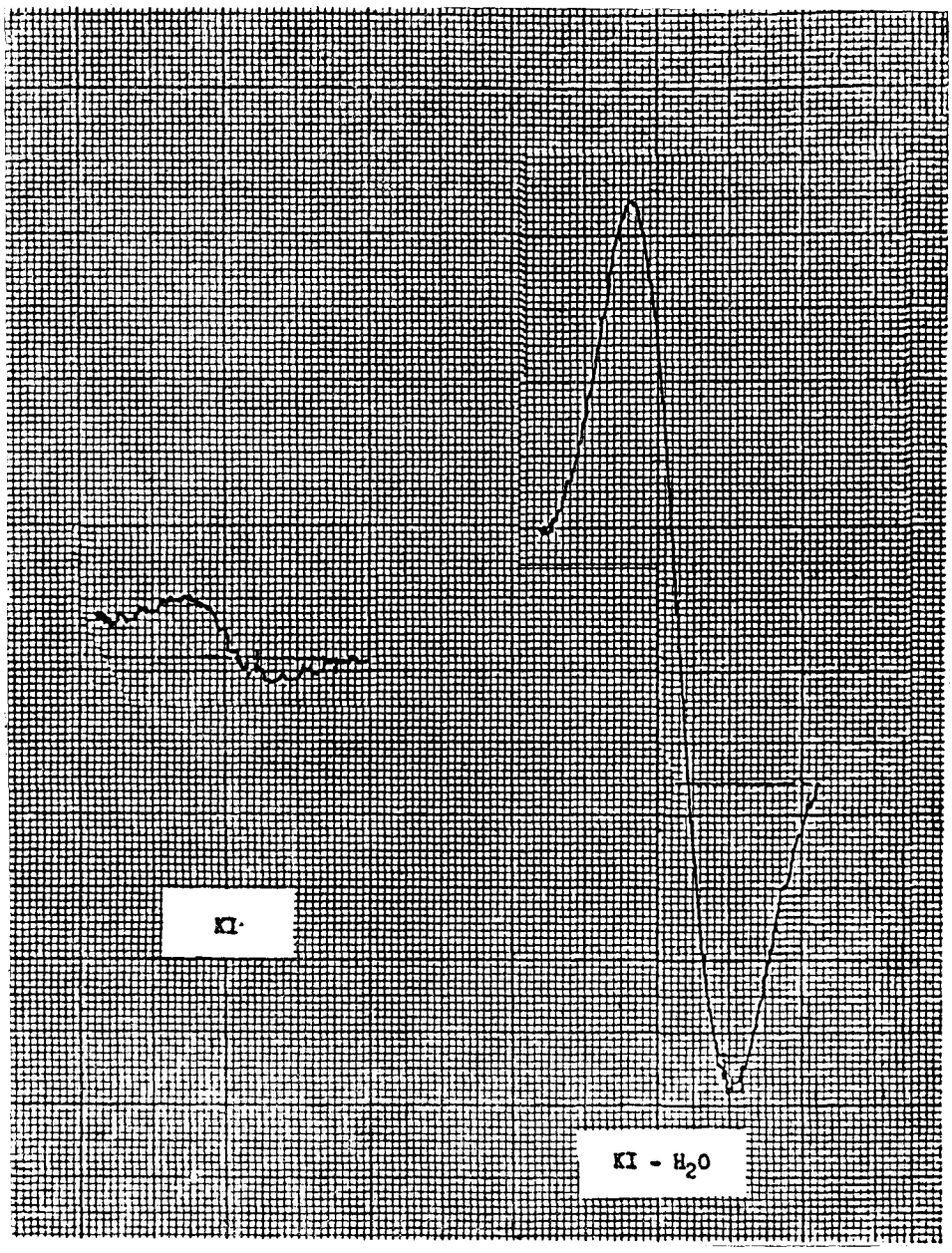
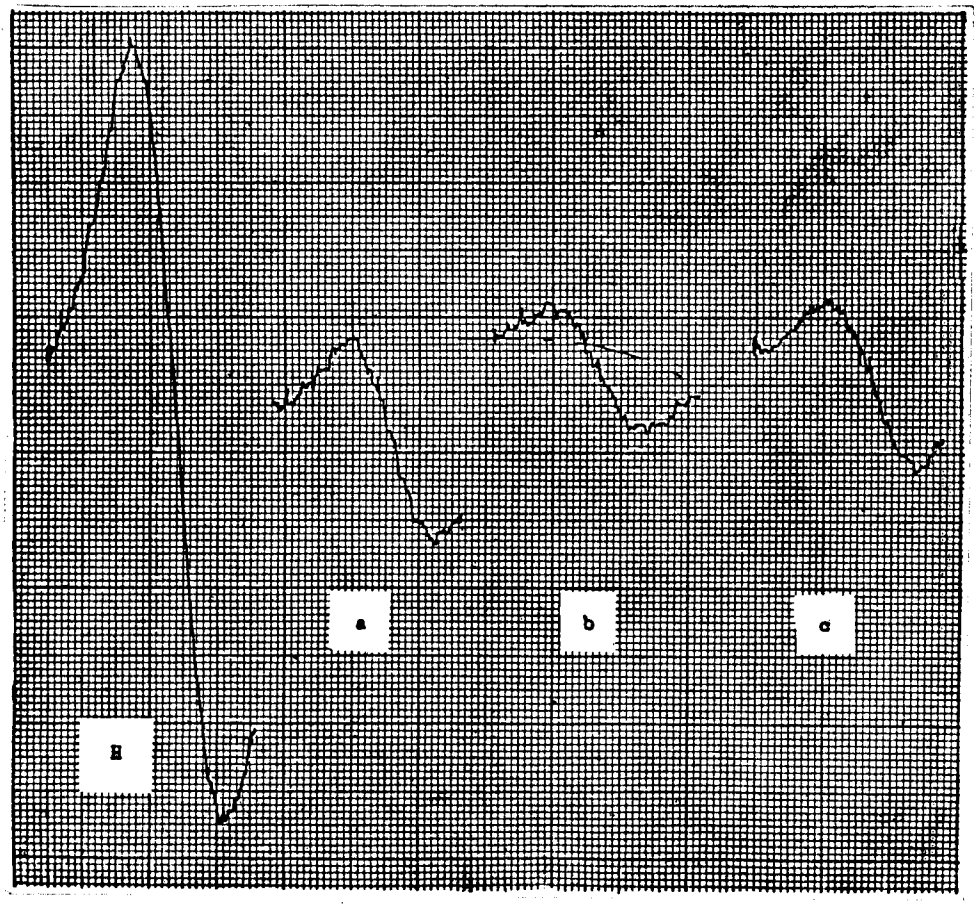


Fig. 4. The effect of KI on the relative number of free radicals of the seeds after the seeds have been soaked for different times. a = 1 minute, b = 30 min., c = 5 min. H = soaked in water.



4. The short-time effectiveness of KI arose a question that KI might change mostly the outer part of the seeds only. It is therefore interesting to investigate the relative numbers of free radicals in the seed coat, the embryo and the endosperm. These three parts were separated after boiling the seeds, taking advantage of the fact that boiling has no effect on the number of free radicals. The free radicals in the whole seeds ($hw^2 = 38.1$) were found to be divided 59.3% in the seed coat ($hw^2 = 22.6$), 24.8% in the embryo ($hw^2 = 9.4$), and 11.6% in the endosperm ($hw^2 = 4.4$).

5. a). The effect of the chemical substances on the respiration of lettuce seeds was also studied. The method of Warburg (12) was used. The seeds were soaked in the flasks for 2 hours before the manometers were read. The solutions were added after the second reading. According to the expectation (5.7), the chemicals were found to inhibit the respiration (see table 2). No respiration was detected in the boiled seeds. These results and the above mentioned ones suggest that the relative number of free radicals (fig. 1) is not necessarily dependent of the respiratory activity. $HgCl_2$ is known to be as effective respiratory inhibitor as KCN, but seeds treated with 0,1 M $HgCl_2$ showed relative number of spins 30 times greater than that of seeds treated with 5 M KCN. KBr, KCl, KI and KCN solutions of the same molarity (2 M) showed the same effect on the respiration, but their effects on ESR signals were not the same. Whether or not the respiratory activity depends on the free radicals, is not known.

b). After the ESR measurements, the same seeds were put in the Warburg flasks and their respiration was measured again. The results are summarized in table 3. The results suggest that the respiration of the chemically inhibited lettuce seeds could be detected and was raised after the seeds were washed in water. This fact seems to be in accordance with and in relation to the results shown in fig. 2 and fig. 3.

6. The ESR-investigated seeds were than sown on filter papers placed in petri dishes. Water was added and the effect of the chemicals on germination was followed during 4 days after the seeds had been sown.

Table 2. Oxygen consumption in lettuce seeds (in μ1/100 seeds/30 minutes).

(All chemicals were added at 3½ hour).

Time after moistening (hours)	Control	2M KCN	5M KCN	0.1M $HgCl_2$	Control	2M KBr	2M KCl	2M KI
3 to 3½	31.73	30.96	32.65	35.93	21.10	21.70	21.52	19.24
3½ to 4	33.51	15.41	15.18	29.83	22.13	9.19	9.58	7.79
4 to 4½	32.14	9.68	5.28	15.54	20.75	3.30	6.46	4.38
4½ to 5	32.35	7.76	2.40	8.88	22.76	0.6	1.94	0.96
5 to 5½	31.70	3.46	0.87	4.43	22.43	—	0.47	—
5½ to 6	31.67	0.68	—	1.06	23.68	—	—	—

Table 3. The "recovery" of the respiratory activity in treated lettuce seeds after having been washed with water (O_2 uptake in μ1/100 seeds/30 minutes).

Time after beginning (hours)	Control (wet)	Control (redried)	5M KCN (redried)	Control (redried)	2M KBr (redried)	2M KCl (redried)	2M KI (redried)
1½ to 2	29.90	32.20	8.90	32.15	10.31	7.11	12.89
2 to 2½	34.20	26.08	12.95	29.46	15.47	16.59	15.93
2½ to 3	32.80	27.50	14.40	32.20	20.63	21.37	17.87
3 to 3½	29.90	25.30	11.10	25.59	20.76	18.95	17.79
3½ to 4	31.40	34.28	16.40	34.22	17.61	21.31	15.89
4 to 4½	32.60	31.60	13.90	30.99	17.63	19.00	23.87

Solutions of 10^{-3} M NaN_3, 0.1 M $HgCl_2$ and 2 M KF were found to be the most effective. The seeds lost their germination power after having been treated in these solutions. Solutions of 2 M KCN, 2 M KBr, 2 M KCl and 2 M KI were found to have no effect on germination. Normal growth was detected in all cases but those of KI treatment. Solutions of 0,1 M to 2 M KI suppressed root hair development and chlorophyl production, and the leaf blades grew in a horizontal plane. These facts are in agreement with the results quoted by Hochster and Quastel (5).

7. The great effectiveness of KI was also found in the case of gamma-irradiated seeds (see table 4). The relative number of spins of KI treated gamma-irradiated seeds is almost the same as that of the un-irradiated seeds soaked in water, and about 1/3 of that of the irradiated seeds soaked in water (control).

Table 4. Relative number of free radicals in irradiated lettuce seeds, and then treated with chemicals. The gamma irradiation dose is 10 Mrads.

Sample	hw^2	spin/cm	spin/seed	note
Standard	30.0	3×10^{15}	—	
Irrad., unwet.	195.0	—	2.4×10^{14}	1 day after irradiation
Irrad., in H_2O	169.5	—	2.1×10^{14}	soaked for 3 hours
Irrad., in 2M KCN	92.1	—	1.1×10^{14}	„ „
Irrad., in 2M K_2S	115.6	—	1.4×10^{14}	„ „
Unirrad., in H_2O	10.1	—	1.3×10^{13}	soaked for 6 hours,
Irrad., in H_2O	23.8	—	2.9×10^{13}	12 days after irradiation
Irrad., in 2M KI	7.6	—	1.0×10^{13}	

None of these gamma-irradiated seeds showed any indication of respiration nor germination. This fact supports the conclusion that there is no correlation between the relative number of free radicals and the respiration or germination.

DISCUSSION AND CONCLUSION

The experiments showed that the ESR signals in lettuce seeds can be modified by chemical substances. KI showed the greatest effectiveness. Seeds which were soaked for 1 minute had almost the same relative number of free radicals as those which were soaked for 1 hour. Fig. 1 also showed that the changes in free radicals amount are not due to the cation, K^+, and that the change is independent of pH. The type of signals suggests that the magnetic centers are free radicals rather than transition metal cations or F centers. Even though the signal gave a single peak, it cannot be assumed that only one species of free radicals is present. The result of petroleum ether-soaked seeds suggest that the relevant molecules are not lipids.

The effect of KCN and KI was found to be reversible (fig. 2 and fig 3). The reversibility was also shown in the respiration experiments (Table 2 and table 3). It is hard to say however, whether or not the free radicals are necessary intermediates in a biological oxidation — reduction system (1, 2, 9, 13). The boiled seeds which have almost the same number of free radicals as the unboiled seeds, and the gamma-irradiated seeds which contain 10 to 20 times as many free radicals as the un-irradiated seeds (Table 4), did not show respiratory activity. These seeds also lost their germination power. It may be concluded, therefore, that the relative, number of free radicals in lettuce seeds is not necessarily dependent of the respiratory activity. Whether or not the respiratory activity depends on the amount of free radicals, is not known.

Since the boiled seeds had almost the same relative number of free radicals as the unboiled seeds (table 1), is it hard to conclude whether or not the free radical content is associated with the protein component (1, 2, 9, 13).

The short-time effectiveness of KI and the different free radical contents in the different parts of the KI-treated seeds, lead to a conclusion that KI changes the free radicals amount in the seed coat first, and later on it penetrates and induces the embryo. Further investigation in this problem is suggested.

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