Radiat. Phys. Chem. Vol. 29, No. 3, pp. 215–218, 1987
Int. J. Radiat. Appl. Instrum. Part C
Printed in Great Britain.

0146-5724/87 $3.00 + 0.00

DETECTION OF GAMMA IRRADIATED PEPPER AND PAPAIN BY CHEMILUMINESCENCE

ABDUS SATTAR,[1] H. DELINCÉE[2] and J. F. DIEHL[2]

[1]Nuclear Institute for Food and Agriculture, Peshawar, Pakistan and [2]Federal Research Centre for Nutrition, Karlsruhe, F.R.G.

(Received 27 July 1986)

Abstract—Chemiluminescence (CL) measurements of black pepper and of papain using luminol and lucigenin reactions were studied. Effects of grinding, irradiation (5–20 kGy) and particle size (750–140 μm) on CL of pepper, and of irradiation (10–30 kGy) on CL of papain, were investigated. All the tested treatments affected the luminescence response in both the luminol and lucigenin reactions; however, the pattern of changes in each case, was inconsistent. Optimum pepper size for maximum luminescence was 560 μm, and optimum irradiation doses were >15 kGy for pepper and >20 kGy for papain. Chemiluminescence may possibly be used as an indicator or irradiation treatment for pepper and papain at a dose of 10 kGy or higher, but further research is needed to establish the reliability of this method.

INTRODUCTION

Radiation decontamination of spices, dry ingredients, herbs and enzyme preparations, has been carried out and found technically feasible, economically viable and a safe physical process. The studies on commercial enzyme preparations have shown that radiation doses of 10–30 kGy reduced the microbial load to negligible levels and 3–10 kGy can reduce the counts to $10^4\,g^{-1}$.[1] It has been concluded that the irradiation of any food commodity up to overall average dose of 10 kGy presents no toxicological hazard.[2] A number of countries have already cleared radiation processing of these dry food products, but in others the food laws prohibit the use of a radiation treatment. In order to ensure compliance with such food laws, identification procedures for gamma irradiated food materials are desirable. Chemiluminescence (CL) measurements have been found useful in radiation dosimetry,[3] and have recently been tested as an identification method for some irradiated foods.[4–10] However, information regarding chemiluminescence of pepper in relation to particle size, and of enzymes is not available. Chemiluminescence is observed when a chemical reaction yields an electronically excited product, which either luminesces or transfers its energy to another molecule, which then luminesces.[11,12] Reactions that produce CL are relatively uncommon. Well investigated are reactions based on cyclic acyl hydrazides (e.g. luminol) and acridine derivatives (e.g. lucigenin), and their mechanisms have been detailed earlier.[13–15] Reactions of spices with luminol have been tested[4–10] while comparable lucigenin reactions have been little reported.

The demand for dry ingredients and enzyme preparations of good microbiological quality is expected to increase in the future. It was, therefore, considered worthwhile to investigate in this study to what extent the CL-measurements of irradiated spices and commercial enzymes are suitable for a speedy and reliable identification of radiation processing.

MATERIALS AND METHODS

Materials

Black pepper was obtained from a food store, ground in a stainless steel grinder, sieved using 750, 560, 300, 200 and 140 μm screens, and kept in glass vials. The commercial enzyme preparation of papain from papaya latex was of Sigma-crude type 1 (product No. P3250). All other chemicals were of analytical grade, and the solutions were made with double-glass-distilled water.

Irradiation

Irradiation was done at room temperature at doses of 5, 10, 15 and 20 kGy for the peppers, and 10, 20 and 30 kGy for papain with 10 MeV-electrons. Nominal doses are average values ± 15%, the dose rate was 10^8 Gy/s during the pulse.

Luminol reaction

Light emission (chemiluminescence) was initiated by automatic dispension of 200 μl of luminol reagent (5-amino-2,3-dihydro-1,4-phthalazine dione) added to unirradiated and irradiated pepper and papain samples pre-weighed in polystyrene cuvettes. The composition of the reagent was as described first by Atari and Ettinger[16] and also used by Bögl and co-workers:[4–10]

125 mg	luminol	(0.7 mM)
2.5 mg	hemin	(3.8 μM)
1.25 g	Na_2CO_3	(11.8 mM)

The solution was adjusted with 1 M HCl to pH 10.0

and the volume made to 1000 ml with double distilled water. The sample weight for each measurement was 15 mg with at least 5 repeats in every case. All tests were done at room temperature and measurements recorded at 5 mV.

Lucigenin reaction

Again light emission was initiated by automatic dispension of 200 μl of lucigenin reagent (bis-*N*-methylacridinium nitrate) added to irradiated and unirradiated samples in polystyrene cuvettes.

The lucigenin solution was a combination of:

51 mg	lucigenin	(0.1 mM)
1.25 g	Na_2CO_3	(11.8 mM)

The solution was adjusted to pH 12.0 and the volume made to 1000 ml with water. The sample weight for every measurement in each case was 15 mg with at least 5 repeats. The measurements were recorded at 50 mV at room temperature conditions.

Chemiluminescence measurements

In order to measure the emitted light, a luminescence photometer of LKB Co. (model 1251) was used. The integral CL intensity was registered during the first 5 s after injecting luminol/lucigenin into the polystyrene cuvettes containing irradiated and unirradiated samples of the pepper and the papain as well as the maximum of the CL intensity (peak) also during the first 5 s. A time constant of 0.2 was used as a smoothing factor. Mostly, samples were measured within 6 h after the irradiation treatment.

RESULTS AND DISCUSSION

Initially, the influence of the grinding process on the formation of free radicals was considered to be of interest. The data regarding chemiluminescence of luminol and lucigenin reactions in relation to particle size (surface area) of ground pepper are presented in Table 1. As is evident from peak-max as well as integral values, luminescence of these unirradiated samples was detectable by both the luminol and lucigenin reactions. However, the intensity was higher with lucigenin than luminol. The luminescence response generally increased in peppers ground to pass through 750–300 μm sieves, whereas it inconsistently decreased when the sample particle size varied 200–140 μm. These results indicate that grinding, and/or the surface area of the samples play a role in causing chemiluminescence. Determination of the coefficient of variation (CV) of the data also revealed wide differences among the values. Table 2 shows the CL-intensity of luminol and lucigenin reactions as a function of irradiation dose applied to the peppers (750 μm). Again the intensity for the lucigenin reaction was much higher than the luminol reaction both in the form of peak-max and integral value. The CL-intensity was unexpectedly found to decrease with the 5.0 kGy treatment as compared to the unirradiated control in both the reactions used. A radiation dose up to 10.0 kGy did not result in any pronounced effect on luminol-CL, rather it further decreased the lucigenin-CL. However, at doses of 15.0–20.0 kGy, the CL-intensity was much higher than the unirradiated sample in both the luminol and lucigenin reactions. Comparison of CL-means of irradiation and grounding treatments showed marginal differences, which are inadequate for proving the suitability of this method for detection of irradiation treatment, especially at lower doses.

The effect of irradiation treatment (10 kGy) and the particle size of pepper (750–140 μm) on the CL-intensity employing luminol and lucigenin reactions, was also investigated, and the results are presented in Table 3. The data revealed an increased

Table 1. Effect of grinding with different sieves on the chemiluminescence intensity of pepper

	Luminol-CL		Lucigenin-CL	
Sieve-size (μm)	Peak-max (mV)	Integral-value (mV/5 s)	Peak-max (mV)	Integral-value (mV/5 s)
750	2.9 ± 0.9	5.3 ± 1.0	87 ± 7	275 ± 15
560	3.1 ± 0.8	5.5 ± 1.0	96 ± 6	301 ± 20
300	5.6 ± 1.0	5.4 ± 0.9	93 ± 8	258 ± 6
200	1.3 ± 0.2	3.4 ± 0.9	94 ± 9	233 ± 11
140	1.3 ± 0.2	4.3 ± 0.8	89 ± 6	255 ± 19
Mean	2.9	4.8	92	264
CV	61	19	4	10

Table 2. Effect of gamma irradiation doses on the chemiluminescence intensity of pepper (750 μm)

	Luminol-CL		Lucigenin-CL	
Irradiation dose (kGy)	Peak-max (mV)	Integral-value (mV/5 s)	Peak-max (mV)	Integral-value (mV/5 s)
Unirradiated	2.4 ± 1.0	6.9 ± 1.4	107 ± 19	266 ± 11
5.0	2.0 ± 0.7	6.9 ± 0.7	97 ± 11	231 ± 5
10.0	3.9 ± 1.1	7.4 ± 3	79 ± 10	255 ± 28
15.0	8.2 ± 3	11.1 ± 2	111 ± 12	385 ± 27
20.0	4.6 ± 0.4	9.9 ± 1.4	128 ± 20	428 ± 42
Mean	4.0	8.5	104	313
CV	31	21	14	7

Table 3. Effect of irradiation (10 kGy) and sieve size on the chemiluminescence intensity of pepper

	Luminol-CL		Lucigenin-CL	
Sieve-size (μm)	Peak-max (mV)	Integral-value (mV/5 s)	Peak-max (mV)	Integral-value (mV/5 s)
750-Unirradiated	4.7 ± 1.4	12.4 ± 0.6	106 ± 13	285 ± 38
-Irradiated	11 ± 3	20.4 ± 1.8	106 ± 9	328 ± 28
560-Unirradiated	24 ± 5	27 ± 6	137 ± 32	318 ± 56
-Irradiated	36 ± 9	43 ± 13	118 ± 23	308 ± 52
300-Unirradiated	9.7 ± 1.9	16 ± 2	95 ± 20	208 ± 41
-Irradiated	17 ± 6	22 ± 3	77 ± 7	184 ± 22
200-Unirradiated	5.5 ± 1.3	11 ± 3	92 ± 6	189 ± 18
-Irradiated	7.3 ± 3	13 ± 6	81 ± 2	210 ± 7
140-Unirradiated	3.5 ± 1.8	12 ± 6	83 ± 6	226 ± 10
-Irradiated	8.5 ± 3	22 ± 4	87 ± 10	283 ± 37
Mean	13	20	98	254
CV	81	48	19	22

influence of irradiation on luminol-CL in contrast to the generally irregular effect on lucigenin-CL. However, a profound effect on the CL of both lucigenin and luminol reactions occurred in relation to mesh size. Maximum luminescence intensity was found in the samples ground through a sieve of 560 μm in both the cases of luminol and lucigenin reactions. A decrease in CL with decreasing particle size was also observed for irradiated carbohydrates.[3] In order to make an overall estimate of dispersion of CL-values in relation to both the mesh size and irradiation, the coefficient of variation was measured. This revealed striking differences in the values. However, general occurrence of higher standard deviation damages the suitability of this method as an accurate and reproducible technique. This may also be the reason for the large deviations in intensity if one compares the results in Table 2 and 3.

In order to test the influence of irradiation treatment during different gaseous atmospheres on the CL-intensity of pepper, experiments were carried out using air, argon and vacuum. The results of these studies are presented in Table 4. It was found that an irradiation dose of 10 kGy increased the intensity in both the CL-reactions. Strangely enough, the CL-intensity with luminol was more in the samples irradiated under argon and vacuum than those under air, whereas a reverse trend was observed in the case of lucigenin-CL. However, these changes were not statistically significant because of large CV. Determination of CV is especially appropriate under conditions where there are extreme values or when it is desired to express variation as a percentage of the average around which the deviations are taken.

Further experiments were conducted to test the suitability of CL-measurements for identification of irradiated commercial enzymes, here papain. The data in Table 5 showed almost a regular increase in the luminol-CL, but an irregular increasing trend in the lucigenin-CL. Maximum luminol-CL was observed at 30 kGy, while with lucigenin-CL it was at 20 kGy. In this case the influence of sample-particle size on CL of papain was not studied as it might affect the activity and charge properties of papain.

Although CL-measurement for detection of irradiated enzymes have not been attempted by others,

Table 4. Effect of irradiation environment on the chemiluminescence intensity of pepper (750 μm). Radiation dose 10 kGy

	Luminol-CL		Lucigenin-CL	
Material	Peak-max (mV)	Integral-value (mV/5 s)	Peak-max (mV)	Integral-value (mV/5 s)
Pepper unirradiated	1.7 ± 0.5	4.6 ± 1.2	73 ± 16	163 ± 14
Pepper irradiated (air)	7.3 ± 3	6.3 ± 2	119 ± 7	303 ± 30
Pepper irradiated (argon)	12.6 ± 0.9	8.1 ± 1.1	96 ± 14	295 ± 33
Pepper irradiated (vacuum)	11.1 ± 2	8.3 ± 2	96 ± 14	244 ± 25
Mean	10.7	6.8	96	251
CV	14	25	13	10

Table 5. Effect of irradiation doses on the chemiluminescence intensity of papain

	Luminol-CL		Lucigenin-CL	
Irradiation dose (kGy)	Peak-max (mV)	Integral-value (mV/5 s)	Peak-max (mV)	Integral-value (mV/5 s)
Unirradiated	4 ± 2	10 ± 4	264 ± 54	606 ± 20
10.0	8 ± 3	16 ± 3	276 ± 42	600 ± 200
20.0	6 ± 2	16 ± 2	348 ± 24	908 ± 146
30.0	14 ± 6	21 ± 4	344 ± 34	830 ± 103
Mean	8	16	308	736
CV	40	21	12	16

application of this method for spices[4-10] and several other food-stuffs, has been tried.[5,6] These authors had concluded that the CL-method permits quick and reliable detection of treatment with ionizing rays at least with some spices. However, the findings of the present study on black pepper and papain showed generally inconsistent and irreproducible results obtained by this method. Other treatments, e.g. the grinding process and especially the particle size of the test sample were found to have a profound effect on CL-values. Occurrence of large standard deviations in many cases obviously shows a need for further research in the chemiluminescence measurements as an identification method for gamma irradiated food-stuffs. We wish to point out that all results obtained with black pepper in this study were derived from one sample of pepper. Our experience with samples of different origin shows even greater deviations and thus even greater uncertainties in the use of chemiluminescence as a method of identifying irradiated samples.

Acknowledgements—This research was carried out during the IAEA fellowship programme of Dr A. Sattar. We wish to thank Mr D. Ehlermann for general assistance during this research programme. The excellent technical help of Mrs S. Delincée and Mrs L. Zimmermann is gratefully acknowledged.

REFERENCES

1. J. Farkas, Radiation decontamination of dry food ingredients and processing aids. *IFFIT Report 49*, pp. 1–24. Wageningen, 1984.
2. WHO, Wholesomeness of irradiated food. Report of a Joint FAO/IAEA/WHO Expert Committee. *Technical Report Series 659*, Geneva, 1981.
3. K. V. Ettinger and K. J. Puite, *Int. J. Appl. Radiat. Isot.* 1982, **33**, 1115.
4. W. Bögl and L. Heide, *Fleischwirtschaft* 1984, **64**, 1
5. W. Bögl and L. Heide, in *Analytical Applications of Bioluminescence and Chemiluminescence* (Edited by L. J. Kricka), pp. 573–576. Academic Press, New York, 1984.
6. W. Bögl and L. Heide, *Radiat. Phys. Chem.* 1985, **25**, 173.
7. L. Heide and W. Bögl, *Z. Lebensm. Unters. Forsch.* 1985, **181**, 238.
8. L. Heide and W. Bögl, *Z. Anal. Chem.* 1985, **320**, 682.
9. L. Heide and W. Bögl, *GIT Fachz. Lab.* 1985, **9**, 833.
10. L. Heide, E. Stumpf, S. Albrich and W. Bögl, *Bundesgesundheitsblatt* 1985, **29**, 51.
11. E. H. White and M. M. Bursey, *J. Am. Chem. Soc.* 1964, **86**, 941.
12. R. C. Baugh, H. D. Colby and R. Miles, *Arch. Biochem. Biophys.* 1979, **192**, 344.
13. W. R. Seitz, *CRC Crit. Rev. Anal. Chem.* 1981, **13**, 1.
14. L. J. Kricka and G. H. G. Thorpe, *Analyst* 1983 **108**, 1274.
15. D. F. Roswell and E. H. White, *Meth. Enzymol.* 1978, **57**, 36.
16. N. A. Atari and K. V. Ettinger, *Nature* 1974, **247**, 193.