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STUDY ON SOME CHEMICAL CHANGES IN IRRADIATED SPICES**

I. GROUND PAPRIKA

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Abstract. — To study chemical changes in irradiated spices an analytical procedure was developed using ground paprika as a model system. The effect of ionizing radiation on the colour and some paprika constituents has been investigated. In the low to moderate dose range (0—0.5 Mrad) as well as at the level of irradiation required for sterilization (1.5—2 Mrad) qualitative and quantitative changes were not observed. The only exception is the content of carbonyl compounds which increases with increasing radiation dose. Some other constituents, particularly carbohydrates, are resistant against radiation at doses even as high as 5 to 10 Mrad.

Radiation induced free radicals were followed by ESR technique. Further study of ESR-signals may yield valuable information in experiments on the demonstration of irradiation effects in ground paprika.

I. INTRODUCTION

The bacteriological aspect of spices is important since they are used as ingredients in many types of prepared food. Spices are very often the carriers of spoilage microorganisms and unless specially treated contain bacteria and moulds. The total cell count of the most highly contaminated spices may reach the 80—100 million/g level. In many cases these contaminations are responsible for the spoilage of

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products in the meat industry. Often these necessitates a heat treatment that ensures microbiological stability only at the cost of substantial reduction in the flavour and colour characteristics. Fumigation with ethylene oxide or propylene oxide is used in several countries to reduce the cell count in seasonings. However, chemical treatment is inseparable from the danger of chemical residues which may be persistent toxic compounds. Therefore, it seeems desirable to find physical methods for sterilization of spices.

Early work on the treatment of food with ionizing radiation concentrated mainly on food preservation. In the course of these studies it became apparent that irradiation may also enhance the technological and/or hygienic quality of food. Microbial contamination of some non- perishable (dry) food ingredients, e. g. spices, can be decreased considerably, thus increasing their hygienic quality and avoiding contamination, introduced by these ingredients, in the perishable (high-moisture, low-acid) food produced with their aid. To achieve practically total sterility in spices 1.5—2 Mrad is required, depending on the initial contamination.

The use of radiation makes it possible to sterilize spices in whole or broken form, in packages of any sizes produced of different materials which protect the product from a secondary infection. It seems, therefore, that gamma irradiation treatment would be useful in rendering spices free from bacteria, while not appreciably aggravating the problems associated with flavour and colour characteristics.

The industrial application of any radiation processing in food industry is usually preceded by detailed studies on the effect of ionizing radiation upon the chemical composition of food products. The chemical changes taking place in irradiated food and food constituents can be investigated in two ways. First, by evaluating the structure of the radiation-induced free radicals by the interpretation of the electron spin resonance spectra and secondary, by an exact analysis of radiolytic products. The study of the chemical mechanism is usually very difficult and complicated. Nevertheless, for application of radiation chemistry to practical problems it is necessary to analyse irradiated food.

The aim of our study was to follow radiation-induced changes in the chemical composition of spices. As model system we have used ground paprika, a spice from the morphological group of true spices. The chemical composition of paprika is very complex. It contains carbohydrates, proteins, water, lipids, essential oils (which mostly consists of carbonyls), vitamins and carotenoids. Some of these constituents, although present in very small amounts, are important for the use of paprika as a spice. For instance, carotenoides — the source of red colour in paprika, are present only in about 0,1%. Besides flavouring effects, the colour makes the use of paprika important in the food processing industry. In the meat industry and in particular in preparation of uncooked products such as saussages, colouring and loss of bloom is quite critical. Therefore, the use of paprika for enhancing and prolonging the colouring effects is quite widespread.

Despite numerous studies on the effect of ionizing radiation on ground paprika (1—5), changes in the chemical composition are still far from being definitely established. Besides, some of the detailed assays have been made only on volatile compounds produced. From the hygienic standpoint, particularly as regards human consumption, we are of course concerned with the non-volatile compounds remaining in the spice after irradiation. Therefore, the effect of gamma irradiation on some paprika constituents (carbohydrates, carbonyl compounds) as well as on the colour of ground paprika were investigated. Ultra-violet absorption of paprika extracts and formation and decay of free radicals in irradiated samples were also followed. In order to compare the results obtained for irradiated samples with some other methods of sterilization, samples of ground paprika heated at 100°C were also analyzed.

II. MATERIALS AND METHODS

Noble, sweet ground paprika (variety R-I, Kneževac) of 10% moisture content, available on the market was used in experiments.

Irradiation was carried out in a gamma ^{60}Co radiation source of 10 kC i nominal activity, at a dose rate of 1.3 Mrad h^{-1}. Samples were irradiated in glass ampoules at ambient temperature and in the presence of air. They were treated with doses ranging from 0.25 Mrad to 2 Mrad, and in some cases with doses higher than 2 Mrad.

The moisture content was determined in a drying oven at $95 \pm 2°C$ by drying to constant weight. The total pigment content, carbohydrates, carbonyl compounds and formaldehyde were determined spectrophotometricaly (6—9). Absorbancies were measured with a Varian 634 S spectrophotometer.

Free radicals were observed using electron spin resonance (ESR) method. The experiments were carried out with a Varian E-109 equipment with modulation of 100 kHz. ZnS with Mn^{2+} as an added impurity was used as internal standard.

III. RESULTS AND DISCUSSION

1. Influence of irradiation on the colour of ground paprika

The colour due to carotenoids is one of the most important criteria of the ground paprika quality. The principal carotenoids in paprika are: capsanthin, which constitutes about 40% of the total carotenoids present, beta-carotene (20%), and capsorubin (10%) (10). Carotenoids were extracted with benzene and determined according to Benedek method adapted for spectrophotometric technique (6). Absorbancies were measured at 477 nm where most important components of paprika colour show isoabsorption points. The total solvent-extractable colour was determined according to a beta-carotene standard from the formula:

$$B = \frac{A_m}{1783 \times b} \text{ where}$$

B — is the total pigment concentration in mg/g of paprika;

A_m — is the absorbancy measured in 100 ml of benzene extract;

1783 — is the percent absorptivity of beta-carotene standard;

b — is the quantity of paprika powder in g.

Experimental results obtained for the control sample, irradiated and heated ground paprika are given in Table 1.

Table 1. — The effect of gamma irradiation and heating time on the colour of ground paprika

irradiation dose or heating time*	B**)	% reduction
0	0.953	—
0.50 Mrad	0.948	0.5
1 Mrad	0.948	0.5
2 Mrad	0.869	9
6 Mrad	0.454	52
8 Mrad	0.252	74
1 hr	0.802	16
2 hr	0.701	26

*) Samples heated at 100°C;
**) Pigment content in mg/g of paprika.

No colour differences between the irradiated and unirradiated samples could be established visualy. The pigment content as extracted with benzene decreased to a comparatively small extent as an effect of irradiation at a comparatively high dose level (Table 1). This supports the findings of *Lukton and Mackinney* (11) on the resistance to irradiation of carotenoids in plant tissue. The consistency of the red colour, however, was strongly affected by high dose level, i. e. at 6 Mrad the measured decrease was 52%. Also, the visual comparison of irradiated and heated paprika indicated apparent differences between the samples. The paprika irradiated with doses up to 1 Mrad had better colour and appeared to be of much brighter red than the heated samples. Browning associated with the application of heat (12) in the production of „dark surface-colour" was not observed with the irradiated paprika.

2. Analysis of the aqueous extract

In the aqueous extract of ground paprika carbohydrates, carbonyl compounds and formaldehyde were determined. Prior to extraction paprika powder (20 to 100 mg) was homogenized by grinding with a little water. The sample was quantitatively transferred into a 100 ml volumetric flasc with distilled water, vigorously agitated and allowed to stand at least for 2 hr. For each spectrophotometric method used, the absorption spectrum was measured and the Beer's law validity (absorbancies vs. mg of paprika) was determined. Whenever possible pure compounds were used for comparison.

(a) Carbohydrates. Carbohydrates were determined with phenol reaction (7). Under the influence of the phenol reagent in the presence of concentrated sulfuric acid, an acidic hydrolysis of carbohydrates to simple sugars takes place, followed by their condensation with phenol and the formation of a coloured product. Glucose was used as a standard in carbohydrates determination.

In clear extracts, obtained by decantation or filtration, the content of sugars soluble in water was measured. The absorption spectrum has a maximum at 490 nm, characteristic of sugars with phenol reagent.

The phenol method was also applied to the mixture of paprika with water. If small quantities of paprika powder (about 5 mg) were well grinded and mixed with water, cloudy but almost homogeneous „solutions" are obtained. It seems reasonable to assume that in this way all carbohydrates present in paprika (soluble sugars plus polysaccharides) could be estimated. Characteristic yelow colour was developed and again the spectrum with the maximum at 490 nm was observed. It was checked with cellulose powder treated in the same way. Data obtained by analysing irradiated and heated ground paprika are presented in Table 2.

Table 2. — The effect of gamma irradiation and heating time on carbohydrates in ground paprika.

irradiation dose or heating time*)	Soluble sugar content**)	Total carbohydrate content**)
0	21.70	43.60
0.25 Mrad	20.65	40.65
0.5 Mrad	20.30	36.80
1 Mrad	20.70	35.00
1.6 Mrad	21.40	36.40
2 Mrad	20.80	36.20
2.5 Mrad	21.80	36.40
5.2 Mrad	20.50	35.80
8.4 Mrad	20.90	38.60
9.4 Mrad	20.00	40.80
1 hr	16.70	32.80
2 hr	15.10	32.00

*) samples heated at 100°C;

** content in per cent dry weight calculated as glucose.

No qualitative changes in absorption spectra of the samples irradiated with doses even as high as 5 to 10 Mrad were noticed. Also, the irradiation of ground paprika does not bring about any distinct changes in the concentration of sugars over the range of doses up to 10 Mrad (Table 2). At the same time a slight decrease in the total carbohydrate content was observed up to doses of about 8 Mrad, while for higher doses it began to increase. In samples heated at 100°C a decrease in both sugar and total carbohydrate content was obtained. Results obtained with ground paprika confirm the fact that carbohydrates are quite resistant against radiation when occuring in the natural system. They also show that heating affects the carbohydrate content in ground paprika more than irradiation.

(b) Carbonyl compounds. According to the study of carbonyl compounds in ground paprika (4), irradiation even as high as 5 Mrad did not cause qualitative changes in carbonyls. However, changes in quantity were not established. We tried, therefore, to measure carbonyls in ground paprika quantitatively. The steam distillation method, used to separate carbonyl compounds from carbohydrates (4), proved to be inconvenient for quantitative measurements. We applied, therefore, a modified 2,4-dinitrophenylhydrazone method (8), which could be carried out rapidly in the presence of carbohydrates without prior separation. The variety of carbonyl compounds present in paprika makes it difficult to select an appropriate compound as a standard. Because of that experimental results are expressed as $A^{1\%}_{1cm}$ which is the absorbancy of a 1% solution with an 1 cm optical path, calculated on the assumption of Beer's law $A^{1\%}_{1cm}$ — values were calculated from the formula:

$$A_m = A^{1\%}_{1cm} \times d \times b \text{ where}$$

A_m — is the absorbancy measured in 100 ml aqueous extract;

d — is the cell length;

b — is the per cent quantity by weight of the paprika powder.

The results of quantitative analysis of carbonyl compounds are presented in Fig. 1.

For doses below 2 Mrad there is first a rapid increase in the concentration of carbonyls untill the curve (Fig. 1) reaches its maximum, and for higher doses the concentration diminishes. The dependence of carbonyl content on dose indicates the existence of two competitive processes. One process is the formation of carbonyls, and the other which is competitive in relation to the first, leads to their conversion. The formation of carbonyls was observed in many irradiated food products and food constituents, raising the problem of possible toxicity. It was found, however, that the acute toxicity in mammals of α,β-unsaturated carbonyl compounds is fairly low (13). The saturated aldehydes (glycol aldehyde, glyceraldehyde and propion aldehyde) were found to be less cytotoxic than α, β — unsaturated carbonyls (croton aldehyde, ethylvinyl ketone) (13). Since many carbonyls are at the same time formaldehyde- releasing compounds, we measured the concentration of the latter with chromotropic acid method. As can be seen from the results obtained (see Table 3) the effect of irradiation on formaldehyde-releasing compounds content is negligible even for high doses.

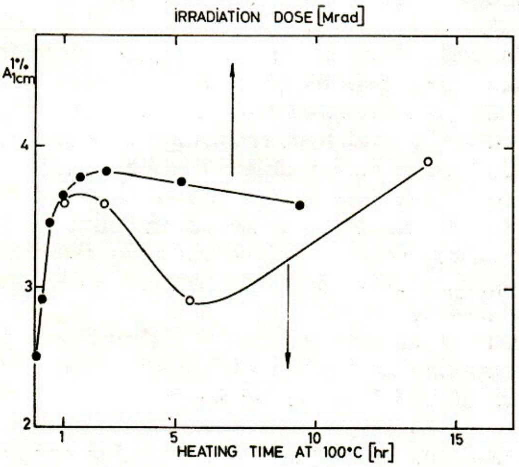

Fig. 1. — Carbonil formation in irradiated and heated ground paprika; o — irradiated; o — heated at 100°C.

Furthermore, qualitative investigation of carbonyls as performed using thin-layer chromatography (4) did not show any difference between the control and irradiated samples, i. e. no evidence was obtained that the qualitative composition of irradiated sample with regard to carbonyls is different from unirradiated ground paprika.

Related to the formation of carbonyl compounds are the organic peroxides, which are formed in the presence of oxygen but which hydrolyse to carbonyls in the presence of water (13). Since irradiation was carried out in the presence of air, but in closed ampoules, it might be that for higher doses oxygen is consumed and peroxides do not form. This, also, may be the reason for a decrease of carbonyls at higher doses. However, for a complex system as paprika it is not possible to make any definite conclusions about the chemical mechanism even for low absorbed doses.

Heating at 100°C acts first in the same way as irradiation, i. e. a rapid increase is observed, while for longer heating times carbonyl content decreases. However, here the decrease is more pronounced than for irradiation. For very long heating times (14 hr) an increase of about 60% as compared to control sample was measured. As we used heating only for comparison, the behaviour of carbonyl compounds in paprika treated with heat was not investigated in more details.

(c) Formaldehyde and formaldehyde-releasing compounds. Formaldehyde was determined by the chromotropic acid method (9). By the same procedure but through the use of a higher temperature, many formaldehyde-releasing compounds give a positive purple colour and thus can be determined. A formaldehyde-releasing compound is defined as any organic compound which is hydrolized or oxidized, under test conditions, to give formaldehyde. Therefore, aqueous extracts were analyzed according to two procedures, A and B, of chromotropic acid method. In procedure A, after adding the reagent, the samples were heated at 110°C for 1 hr, while in procedure B they were allowed to stand at normal temperature for 30 minutes. Formaldehyde was measured by procedure B. The difference between procedures A and B corresponds to the content of formaldehyde-releasing compounds. Formaldehyde-releasing compounds may, however, interfere with formaldehyde determination. Thus the content measured by procedure B does not represent the exact concentration of formaldehyde in ground paprika. As we, in the first place, studied the effect of irradiation on the contents measured by the chromotropic acid method, the determination of exact formaldehyde concentration was of less importance here.

The absorption spectrum of aqueous extract of unirradiated sample was compared to the spectrum of pure formaldehyde; they both have the maximum at 580 nm at which absorbancies were measured. Experimental results obtained for irradiated and heated ground paprika are given in Table 3. No difference between the absorption spectra of the control and irradiated samples (irradiated with 1,5 and 10 Mrad) could be notced.

In the whole dose range employed as well as in the samples heated at 100°C no apparent changes in CH_2O and CH_2O-releasing compounds content were noticed. It is important for practical application of radiation since formaldehyde is a toxic compound.

Table 3. — The effect of gamma irradiation and heating time on formaldehyde and formaldehyde-releasing compounds in ground paprika

irradiation dose or heating time[1])	content in per cent dry weight calculated as formaldehyde		
	A[2])	B[3])	(A—B)[4])
0.25 Mrad	0.59	0.27	0.33
0	0.61	0.26	0.33
0.5 Mrad	0.58	0.23	0.35
1 Mrad	0.57	0.25	0.32
1.6 Mrad	0.52	0.24	0.28
2 Mrad	0.52	0.22	0.30
2.5 Mrad	0.39	0.23	0.16
5.2 Mrad	0.37	0.24	0.13
9.4 Mrad	0.45	0.19	0.26
1 hr	0.56	0.17	0.39
2 hr	0.58	0.18	0.40

[1]) samples heated at 100°C; [2]) A = $CH_2O + CH_2O$-releasing compounds; [3]) B = CH_2O; [4]) (A—B) = CH_2O-releasing compounds.

3. Ultra violet absorption

Ultra violet absorption spectrum of paprika aqueous extract is presented in Fig. 2. It displays two absorption peaks: one at 196 nm

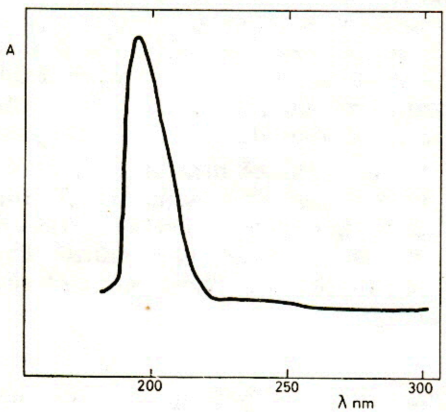

Fig. 2. — Absorption spectrum of the aqueous extract of ground paprika.

and another at 240 nm, which is characteristic of carbonyls (14). Since below 200 nm the error of the reading of the instrument is large, water absorbs in this region too, and the peak at 240 nm is of low intensity, the absorption of aqueous extract could not be used to fol-

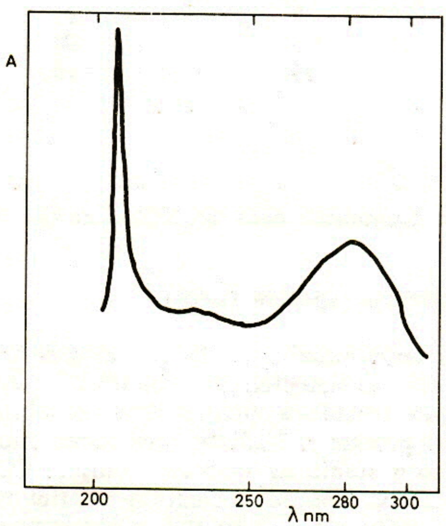

Fig. 3. — Absorption spectrum of the ground paprika with 6N CHl.

low irradiation in ground paprika. However, if the samples of paprika were heated in 6N HCl on a boiling water bath, a spectrum with well defined maximum at 280 nm was observed and is presented in Fig. 3.

The fact that the absorption at 280 nm was observed only in warm 6N HCl indicated that it is due to some hydrolysis products of ground paprika constituents (proteins and/or cellulose). Having in mind that under experimental conditions hydrolysis is far from being complete, we tried to use absorption at 280 nm to demonstrate the irradiation effects in ground paprika. Therefore, the percentage absorbancy was determined and reproducible results were obtained only for small quantities of paprika powder (1—5 mg) treated with 6N HCl. The results obtained for irradiated and heated samples are given in Table 4. No effect of irradiation or heating on the position of the absorption maximum and on the character of the ultra violet absorption was noticed. Unambigous changes of $A^{1\%}_{1cm}$ values measured in irradiated samples (Table 4) that might have served as a basis of detecting irradiation were also not observed. Very long heating time (14 hr) was employed, but, nevertheless, no apparent change could be obtained. This is an indication that substances absorbing in ultra violet are not sensitive to both irradiation and heating.

Table 4. — The effect of gamma irradiation and heating time on ultra violet absorption of ground paprika

Dose in Mrad	$A^{1\%}_{1\ cm}$	Time of heating*) in hours	$A^{1\%}_{1\ cm}$
0	103	0	103
0.25	100	1	100
0.5	100	2	97
1	98	5	96
1.6	99	14	95
2.5	98		
5.2	99		
9.4	96		

*) samples of paprika before treatment with 6N HC1 were heated at 100°C.

4. Electron spin resonance spectra (ESR)

Irradiation of solid material with ionizing radiation induces the formation of free radicals which can be detected in principle, by electron spin resonance. This technique, therefore, should yield an identification method for irradiated food products. Radiation-produced radicals are, in general, very reactive so a stabilization occurs only if they are trapped in an appropriate matrix. The identification of the radicals is in these cases often very diffucult due to the occurrence of large anysotropies of the spectroscopic splitting tensor and hyperfine coupling tensor. Since the most important aspect from our point of view is the stability of radiation induced radicals this complication is of less importance here.

The ESR spectra of ground paprika, irradiated with 2 Mrad, were taken immediately after irradiation. The spectra were not sufficiently fine-structured to allow an exact analysis of the radicals. It was, however, possible to distinguish three radical species which we called A, B and C. As the main component singlet A with the line width $\Delta H = 9 \pm 1G$ was obtained at higher microwave powers ($>$ 1 mW). It is presented in Fig. 4. At lower microwave powers ($>$ 1 mW) more pronounced was singlet B, given in Fig. 5, with the line width $\Delta H = 10 \pm 1G$

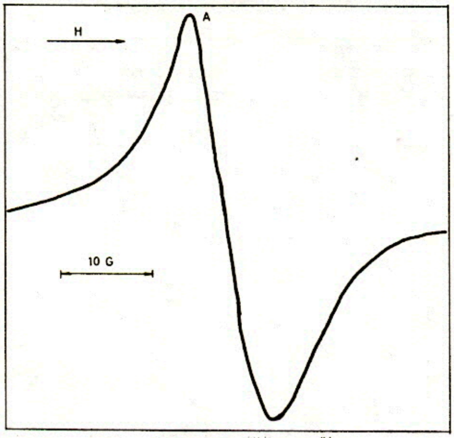

Fig. 4. — ESR-signal of irradiated ground paprika recorded at microwave power of 20 mW.

In both cases only the mean value of the spectroscopic factor g could be estimated: g(A) = 2.0042 ± 0.0005 and g (B) = 2.0041 ± ± 0.0005. Although the spectra A and B have similar ESR parameters, their behaviour with the increase of microwave power and stability at

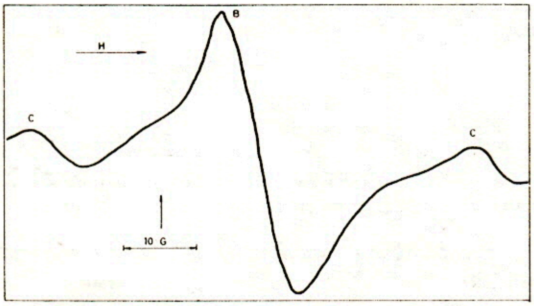

Fig. 5. — ESR-signal of irradiated ground paprika recorded at microwave power of 1 mW.

normal temperature are different. The signal A saturates at 50 mW, while the signal B is saturated already at 20 mW. The intensity (I), directly proportional to the concentration of the paramagnetic species, of the signal A was found to decrease to 70% in one day, and of the signal B to 90% in 10 days, of their initial intensities (I^0). The third signal C (Fig. 5) a symetrical doublet, could be easily seen at low microwave powers (< 10 mW). The spectrum C has a spectroscopic hyperfine splitting of outer lines a = 59.3 ± 1 G and the estimated g-factor g(C) = 2.0032 ± 0.0005. With increasing microwave power no saturation was noticed at all, which supports the assumption that the signal C is due to third radical species.

The concentration of free radicals was determined using DPPH (1,1—diphenyl 1-2 pycrilhydrazil) in KCl as a standard. Under exprimental conditions the absolute concentration of radical species was estimated to be 10^{15} spins per gram of ground paprika (± 40%).

Only the decay of the radical A was followed in more details. The decay observed immediately after irradiation seems to follow the first order kinetic and thus a straight line on a plot like the one in

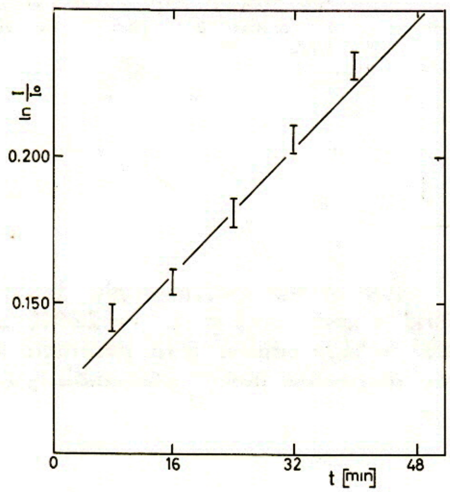

Fig. 6. — First-order decay of radical species A immediately after irradiation.

Fig. 6. The rate constant k = 1.1 x 10^{-3} s^{-1} and half-life time t $1/_2$ = = 10 hr 30 min.were obtained for this process. However, the total kinetic of radical A decay is very complex and is of mixed order.

It should be pointed out that the control sample of ground paprika contains free radicals too, caused by grinding. It was found that these free radicals do not disappear during a storage period as long as one year (15). Furthermore, the moisture content strongly affects the amount of free radicals formed during irradiation and their decay; in a space of high moisture content, radiation-induced free radicals decay rapidly (4).

IV. CONCLUSIONS

The summarized results show that at the level of irradiation required for complete sterilization, i. e. with doses up to 2 Mrad, any important and noticeable changes in the chemical composition of ground paprika were not observed. Even more, it was shown that some constituents, particularly carbohydrates, are resistant against radiation in the whole dose range employed (0.25 — 10 Mrad). In all cases the effect of heating was either more pronounced or the same as of irradiation. The only exception is the carbonyl content which increases with the increases of radiation dose. However, the same was observed in samples treated by heat. As it is generaly recognized, ordinary cooking and autooxidation of food and food constituents produces compounds similar to those produced by irradiation.

The fact that the colour of paprika did not change by relatively high absorbed doses is wery important for the practical use of radiation. Undesirable browning of paprika, produced by heat, was also not observed in irradiated samples.

The ESR-method might be suitable for detecting free radicals in irradiated ground paprika. Attention must be paid to the moisture content and to long lived radicals caused by grinding. Nevertheless, methodological studies of ESR spectra would be valuable as a possibility to develop a method of detection.

With regard to the possibility of using ionizing radiation for the sterilization of spices, chemical analyses as performed with ground paprika are, certainly, very useful. Since paprika was used as a model system, the same approach will be applied to other spices too. Studies on this subjects for other spices are in progress.

REFERENCES

1. *J. Farkas, I. Kiss, E. Andrášsy:* Food Irradiation (Proc. Symp. Karlsruhe, 1966), IAEA, Vienna, 1966, p. 601.
2. *J. Farkas, J. Beczner, J. Incze:* Radiation Preservation of Food (Proc. Sym. Bombay, 1972). IAEA, Vienna, 1973, p. 389.
3. *J. Kiss, J. Farkas, S. Ferenczi, B. Kálmán, J. Beczner:* Improvement of Food Quality by Irradiation (Proc. Panel, Vienna, 1973), IAEA, Vienna, 1974, p. 157.
4. *J. Beczner, J. Farkas, A. Waterich, B. Buda, J. Kiss:* Identification of Irradiated Foodstuffs (Proc. Colloq. Luxembourg, 1974), EUR 5126, 1975, p. 255.
5. *M. Vajdi:* M. Sc. thesis, of Manitoba, 1970.
6. *A. Laszlo:* Gordian, 73, 466 (1973).
7. *M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers, F. Smith:* Anal. Chem., *28,* 350 (1956).
8. *E. B. Sandres, J. Schubert:* Anal. Chem., *43,* 59 (1971).
9. *B. West, P. Sen:* Z. Anal. Chem., *153,* 177 (1956).
10. *J. Kanner, S. Harel, D. Pavletich, I. Ben-Gera:* J. Food. Technol., *12,* 59 (1977).
11. *A. Lukton, G. Mackinney:* Food Technol., *10,* 630 (1959).
12. *L. F. Lippert, M. O. Hall:* Hortiscience, *8,* 38 (1973).
13. *J. Schubert:* Improvement of Food Quality by Irradiation, (Proc. Panel, Vienna, 1973), IAEA, Vienna, 1974, p. 1.
14. *E. S. Stern, C. J. Timmons:* Electronic Absorption Spectroscopy in Organic Chemistry, Edward Arnold (publishers) LTD, London, 1970.
15. *P. M. T. Hansen, W. J. Harper, K. K. Sharma:* J. Food Science, *35,* 598 (1970).