EFFECT OF GAMMA RAY IRRADIATION AND FRYING ON THE THIAMINE CONTENT OF BACON

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ABSTRACT

The effects of ionizing irradiation and frying on the thiamine and riboflavin content of bacon were determined. Significant destruction of thiamine but not riboflavin occurred during both the irradiation and frying. The destruction of thiamine was found to be directly related to the dose of ionizing radiation or the degree of cooking. Frying before irradiation, reduction of water content by lyophilization, and irradiation at −40°C rather than 2°C produced significantly greater retention of thiamine. Frying the bacon following irradiation produced radiation dose-related, nonadditive, increased destruction of thiamine. The protective effect of frying before irradiation was not directly linked with the reduction of moisture by frying.

INTRODUCTION

It is important to determine whether interactions occur between the processes of cooking and irradiation and if so to determine whether the order of processing affects the final result. It is well known that vitamin losses do result from both irradiation and cooking. Vitamin degradation by ionizing radiation has been studied since at least 1919 (Sugiura and Benedict 1919). Following World War II the Atoms for Peace program led to studies of the use of ionizing radiation to preserve foods and included several studies of its effects on vitamins (Alexander *et al.* 1956; Day *et al.* 1957; Ebert and Swallow 1957; Groninger *et al.* 1956; Groninger and Tappel 1957; Richardson *et al.* 1958; and Ziporin *et al.*

1957). Ziporin *et al.* (1957) reported that bacon which had received a radiation dose of 55.8 kGy at 24°C at a dose rate of 27.9 kGy per hour lost 93% of its thiamine. Groninger *et al.* (1956) reported that pork lost 88% of its thiamine following a radiation dose of 27.9 kGy in the presence of oxygen or nitrogen, but riboflavin and niacin were relatively stable to irradiation in both beef and pork. Ziporin *et al.* (1957) associated thiamine retention in irradiated powdered milk to its lack of moisture. Richardson *et al.* (1958) reported considerable loss of thiamine and some of riboflavin, pyridoxine, pantothenic acid, and folic acid when the vitamins were mixed with aqueous casein and irradiated but not when the vitamins were mixed with dry casein and irradiated.

Wilson (1959) discovered that radiation induced destruction of thiamine in minced beef could be eliminated by irradiation at a temperature of −75°C and that a nitrogen atmosphere provided little protection for the thiamine. A decrease in temperature from +20°C to 0°C made little difference in thiamine retention. A 10 kGy radiation dose destroyed 58%, 24%, 12%, and 0% of the thiamine in minced beef at 0°C, −10°C, −20°C, and −75°C, respectively (Wilson 1959). The protective effects of low temperatures for vitamins during irradiation were confirmed by other workers (Diehl 1979; Thomas *et al.* 1981).

Diehl (1969, 1975) reported that synergistic effects occurred among irradiation, heating, and storage and the loss of thiamine in wheat flour, crushed oats, and dried whole egg. Much greater losses of thiamine occurred in irradiated products during cooking and/or storage than in the non-irradiated products. Thomas and Calloway (1957) reported that the retention of thiamine in irradiated turkey decreased with the intensity of the radiation dose.

The purpose of the present study was to determine the effects of ionizing radiation and its possible interaction with moisture content and/or frying on the thiamine and riboflavin content of bacon. A nitrite level of 40 ppm was selected for processing because we wished to evaluate the effects of ionizing radiation on reduced nitrite bacon. Two experimental studies were performed. The effects of ionizing radiation on the thiamine and riboflavin content of raw and fried bacon and on the thiamine content of bacon irradiated prior to frying was investigated in the first experiment. The interaction between the moisture contents of raw, lyophilized, and fried bacon and radiation dose was investigated in the second experiment. The dose range covered in these experiments was limited to those that might be employed for preservation of the product.

MATERIALS AND METHODS

Bacon Processing

Six fresh skinless pork bellies were injected to 111% of raw weight with a curing solution containing 15% NaCl, 7.5% sucrose, 3% sodium tripolyphosphate, 0.55% sodium ascorbate, and 0.04% sodium nitrite. Following an overnight brine drainage period at 1.1°C, the bellies were processed in a gas-heated, wood-smoke supplied smokehouse to an internal temperature of 54°C and an approximate belly yield of 100% (raw belly weight). The processing objective was a smoked bacon product containing 1.5% NaCl, 0.75% sucrose, 0.3% sodium tripoly-phosphate, 40 ppm $NaNO_2$, and 550 ppm sodium ascorbate where irradiation would be substituted for nitrite to control *Clostridium botulinum*.

The smoked bacon bellies were chilled to 1.1°C and then sliced from brisket to flank end into ca. 2.8 mm thick slices. The slices were vacuum packaged in groups of eight consecutive slices in barrier pouches ("IKD-ALLVAK" #13 International Kenfield Distribution Co., Rosemont, Illinois)[2] and identified according to within-belly source. Composite samples were prepared which included eight-slice packages from each of five subsections within each of the six individual bellies.

Raw Bacon Samples

Bacon samples assigned to the raw irradiation treatments were briefly firmed at −27°C, removed from the sample pouches, and combined into one lot of bacon slices. These were subsequently cut and mixed in a Haegger-Alpina bowl cutter (Model #PB-5-890-II; Koch Supplies, Kansas City, MO) using techniques described by Pettinati *et al.* (1983). The cut and mixed samples were vacuum packed in 30–50g portions in the "IKD-ALLVAK" #13 pouches before irradiation treatment. Three 150g samples were frozen and retained for proximate analysis.

Freeze-Dried Bacon Samples

Raw cut bacon prepared as described above was subdivided into four identical samples for lyophilization treatments (raw control, low moisture, medium moisture, and high moisture). The condenser temperature during lyophilization was −40°C, and the vacuum pressure was ca. five microns.

The moisture contents were reduced from that of the nonlyophilized bacon (40.6 ± 0.12%), to high moisture (35.2 ± 0.19%), medium moisture (23.5 ± 0.20%), and low moisture (2.2 ± 0.03%). After lyophilization the bacon was recut and mixed in a Hobart bowl cutter (Troy, Ohio) using the techniques described by Pettinati *et al.* (1983). The lyophilized samples were then vacuum packaged as described above. Three 150g samples of each of the lyophilized bacons were frozen and retained for proximate analysis.

Fried Bacon

Raw bacon slices were weighed and then fried in preheated electric skillets at 177°C for 1.5 min, 3.0 min, or 3.5 min per side to produce slightly fried, medium, and well done "crisp bacon." The yields were 60.3, 33.2, and 27.4% of the raw product. The fried bacon was then cut, mixed, and vacuum packaged as described above. Three 150g samples of each of the fried bacons were frozen and retained for proximate analysis. All samples were stored at 1°C prior to radiation treatment.

Radiation Treatment

The vacuum packaged samples were irradiated in a self-contained 147,000 Ci cesium-137 radiation source at a dose rate of 0.1 kGy per min at 2°C in crushed ice and water or at −40°C in the gas phase from liquid nitrogen. Following the irradiation treatment all samples were stored frozen until analyzed.

The dosimetry and dose distribution for this radiation source was described by Shieh *et al.* (1985).

Analytical Methods

Moisture, protein, and fat contents were determined at least in duplicate, as indicated in Table 1, according to the appropriate AOAC (1984) methods respectively 24.003, 24.027, and 24.005. Duplicate 2.53g samples were analyzed by the thiochrome and fluorometric methods, respectively, for thiamine in the range 0.003–0.15 μg/ml and riboflavin in the range ≥0.1 μg/g using semi-automated procedures (AOAC 1984; Anon. 1977). The samples of meat were dispersed in 0.1 N HC1 and autoclaved for 30 min at 15 psi to extract the vitamins as described in the Technicon Autoanalyzer manual. All procedures are described in the instrument manual (Anon. 1977). No evidence was found that substances were produced by the irradiation treatment which interfered with the analysis of either thiamine or riboflavin. In order to allow comparison of results between samples that would have different levels of moisture and/or fat, the results for thiamine and riboflavin were reported on the basis of protein. All samples were analyzed in duplicate. The total number of samples analyzed are indicated in the appropriate table.

Statistical Methods

Statistical analysis was performed by the application of the general linear model procedure of the Statistical Analysis System (SAS) (SAS INSTITUTE, INC. 1985). Analyses included the use of analysis of variance to investigate the effects of dose, temperature, and type of preparation on the amounts of thiamine and riboflavin. In addition, regression techniques (Draper and Smith 1981) were used to build response surface models for thiamine as a function of moisture and dose.

RESULTS AND DISCUSSION

Proximate Analyses

Analytical results for moisture, fat, and protein content of the bacon products are summarized in Table 1. Because the bacon described as "irradiated-fried" was fried after the radiation treatments, it was necessary to determine proximate analyses for each treatment. All values for thiamine and riboflavin are reported as mg/100g protein because both the moisture and the crude fat levels were dependent on the treatment (e.g., none, frying or lyophilizing). Irradiation "per se" did not produce any changes in moisture, fat, and protein content of the samples (Table 1).

Effect of Radiation Dose on Thiamine Content

The analytical results for thiamine and riboflavin for each treatment, radiation dose, and irradiation temperature are presented in Table 2. As expected from other studies (Thomas *et al.* 1981), less loss of thiamine occurred when the bacon was irradiated at −40°C rather than at 2°C. Gamma irradiation of raw bacon at 2°C resulted in an exponential loss of thiamine in the product, which agrees with the conclusions of Thomas *et al.* (1981). When the irradiation of raw bacon was conducted at −40°C, thiamine degradation was more nearly linear than exponential. It is presumed that an exponential rate would have been observed if the study had included greater radiation doses at −40°C. Since the diffusion of both the solvated electron and hydroxyl radicals, which are known to be involved in the radiation destruction of thiamine, are greatly impeded in ice (Taub *et al.* 1979) the greatly reduced destruction of thiamine at −40°C was expected.

Effect of Frying and Then Irradiating on Thiamine Content

Interpretation of the data presented in Table 2 indicated that frying degraded approximately 48% of the thiamine. Irradiation of the bacon after frying degraded additional thiamine but at a much reduced rate. After frying, a gamma radiation dose of 30 kGy degraded approximately 36% of the residual thiamine whereas approximately 91% of the thiamine content in raw bacon was destroyed by the same gamma radiation dose. Thomas *et al.* (1981) compared rates of thiamine destruction at different temperatures by plotting the $\log_{10}$ of the fraction of thiamine retained $[T]/[T_o]$ versus the radiation dose. This assumes that the retention on dose is logarithmic and could therefore be described by the equation

TABLE 1.
MOISTURE, FAT AND PROTEIN CONTENT OF BACON PRODUCTS

Product	Radiation Dose kGy	Irradiation Temp. (°C)[a]	N[b]	Moisture $\bar{x} \pm$ S.D.[b]	N	Crude Fat $\bar{x} \pm$ S.D.	N	Protein $\bar{x} \pm$ S.D.
				g/100 g wet wt				
				------ Experiment 1 ------				
Raw	all[c]	2	12	32.8 ± 1.45	9	54.9 ± 3.72	20	9.64 ± 0.47
		-40		32.8 ± 1.45		54.9 ± 3.72		9.64 ± 0.47
Fried-irradiated	all	2	6	12.8 ± 0.15	9	47.2 ± 2.28	11	27.3 ± 2.30
		-40		12.8 ± 0.15		47.2 ± 2.28		27.3 ± 2.30
Irradiated-fried	0	2	3	13.7 ± 0.31		ND	2	29.6 ± 0.76
		-40		13.7 ± 0.31		ND		29.6 ± 0.76
	7.50	2	3	8.67 ± 0.10		ND	4	25.8 ± 2.10
		-40	3	14.0 ± 0.10		ND	2	27.5 ± 5.04
	15.0	2	3	8.29 ± 0.25		ND	2	21.5 ± 0.34
		-40	3	11.6 ± 0.24		ND	2	32.2 ± 0.11
	30.0	2	3	8.34 ± 0.13		ND	3	23.6 ± 1.92
		-40	3	10.6 ± 0.19		ND	4	30.5 ± 3.45

TABLE 1. continued

				------ Experiment 2 ------				
Raw	all	2	3	40.6 ± 0.12	3	44.8 ± 0.30	2	11.3 ± 0.05
Lyophilized	all	2	3	2.17 ± 0.03	2	73.8 ± 0.12	2	18.0 ± 0.07
Lyophilized	all	2	3	23.5 ± 0.20	2	56.9 ± 0.28	2	14.7 ± 0.23
Lyophilized	all	2	3	35.2 ± 0.19	2	48.9 ± 0.38	2	12.6 ± 0.08
Fried	all	2	3	7.88 ± 0.11	2	39.6 ± 0.08	2	41.5 ± 1.21
	all	2	3	17.4 ± 0.27	2	38.9 ± 0.99	2	35.4 ± 0.12
	all	2	3	28.5 ± 0.34	2	48.7 ± 0.01	2	20.8 ± 0.13

[a] Temperature during irradiation treatment

[b] N = number of samples analyzed; $\bar{x}$ ± S.D. = mean ± standard deviation

[c] The term all refers to the averaging of values for all radiation doses (0, 7, 5, 15.0 and 30.0 kGy) because the irradiation treatment should not alter the proximate analysis of sealed samples. In experiment one, three samples were analyzed for each dose. In experiment two three samples were analyzed for each composite sample before irradiation treatment.

TABLE 2.
THIAMINE AND RIBOFLAVIN IN RAW, FRIED, FRIED-IRRADIATED, AND IRRADIATED-FRIED BACON AT FOUR RADIATION DOSES AND TWO IRRADIATION TEMPERATURES.; EXPERIMENT 1

Treatment	Radiation Dose (kGy)	N[a]	Thiamine mg/100g protein Irradiation Temp. 2°C $\bar{x} \pm$ S.D.[b]	Thiamine mg/100g protein Irradiation Temp. -40°C $\bar{x} \pm$ S.D.	N[a]	Riboflavin mg/100g protein Irradiation Temp. 2°C $\bar{x} \pm$ S.D.	Riboflavin mg/100g protein Irradiation Temp -40° C $\bar{x} \pm$ S.D.
None (Raw)	0	4	4.42 ± 0.26	4.54 ± 0.20	2	1.33 ± 0.04	1.32 ± 0.03
	7.5	4	1.77 ± 0.04	3.84 ± 0.27	2	1.32 ± 0.01	1.33 ± 0.04
	15.0	4	0.95 ± 0.14	3.09 ± 0.14	2	1.25 ± 0.04	1.32 ± 0.00
	30.0	4	0.40 ± 0.12	1.70 ± 1.18	2	1.26 ± 0.01	1.31 ± 0.01
Fried-irradiated	0	4	2.32 ± 0.10	2.18 ± 0.16	2	1.16 ± 0.01	1.13 ± 0.02
	7.5	4	2.02 ± 0.09	1.94 ± 0.08	2	1.20 ± 0.01	1.14 ± 0.00
	15.0	4	1.78 ± 0.16	1.73 ± 0.08	2	1.14 ± 0.04	1.13 ± 0.01
	30.0	4	1.49 ± 0.24	1.48 ± 0.08	2	1.14 ± 0.01	1.15 ± 0.00
Irradiated-fried	0	2	2.28 ± 0.03	2.28 ± 0.03			
	7.5	2	0.76 ± 0.05	1.73 ± 0.02			
	15.0	2	0.40 ± 0.04	1.34 ± 0.11			
	30.0	2	0.07 ± 0.01	0.16 ± 0.04			

[a] N = number of samples

[b] $\bar{x} \pm$ S.D. = mean ± standard deviation

$\frac{\ln[T]}{[T_o]}$ = mD where [T] represents the thiamine concentration after radiation dose D and [T_o] represents the initial thiamine concentration and m the slope. Further, since all such retention lines pass through 1.0 at a zero dose, the slope m can be determined from the equation $m = \frac{\{\text{sum } \ln [T]/[T_o]\}}{(\text{kGy})}$ (Snedecor and Cochran 1980). If the slope for the degradation of thiamine in raw bacon by irradiation at 2°C is computed by this method, a value of −0.102 is obtained. The equivalent slope for a straight line plotted through the retention values after various radiation doses at 2°C for bacon which had been fried prior to irradiation was −0.017 and that for raw bacon irradiated in the frozen state at −40°C was −0.029. Whereas the lower rate of thiamine degradation by ionizing radiation in the frozen bacon can be attributed to inhibited diffusion of the solvated electrons and hydroxyl radicals, the lower rate of degradation in bacon that was fried prior to irradiation may be linked to the effects of heating on protein structure, protein and/or phosphate binding of the vitamin, and/or the water content of the bacon. Irradiating the fried bacon in the frozen state did not markedly alter the rate of degradation (−0.015) compared to the rate when irradiated at 2°C (−0.017).

Janitz and Grodyka-Zapytowska (1981) found bound thiamine to be more sensitive to thermal destruction than free thiamine, and that pork that had been refrigerated for 7 days had a higher proportion of free than of bound thiamine. Mulley *et al.* (1975) reported that under identical heating conditions cocarboxylase was destroyed more rapidly than thiamine hydrochloride. The observed lower rates of thiamine degradation by ionizing radiation in bacon which has been fried may be due at least in part to thermal destruction of bound cocarboxylase. This may indicate that protein bound cocarboxylase is more sensitive to ionizing radiation, but this was not tested in this study.

Effect of Irradiating and Then Frying on Thiamine Content

When bacon was fried after irradiation at either 2°C or −40°C additional thiamine destruction occurred (Table 2). If the thiamine destruction in the raw product by gamma irradiation at 2°C was as reported above, then the effect of frying after irradiation can be determined by subtracting the irradiated-fried values from the corresponding irradiated-raw values. As noted above, approximately 48% of the initial thiamine content [(4.42 - 2.30) ÷ 4.42] × 100 of nonirradiated bacon was destroyed by frying. Thus, at a dose of 7.5 kGy, the retention of thiamine in raw bacon was 1.77 mg/100g. Subtracting the value (0.76 mg/100g) that was obtained by irradiating bacon to an absorbed dose of 7.5 kGy before frying gives an estimate of 1.01 mg/100g as the amount of thiamine that was destroyed by frying the irradiated bacon. This estimate indicates that 57% of the residual thiamine following irradiation was destroyed by frying

[(1.01 ÷ 1.77) × 100]. Similar values can be calculated for radiation doses of 15 and 30 kGy giving values of 58 and 82%, respectively, for the residual thiamine destroyed by frying. The results obtained with bacon irradiated at −40°C were very similar to those at 2°C in that 55, 57, and 91% of the residual thiamine was destroyed by frying after radiation doses ot 7.5, 15.0, and 30.0 kGy, respectively.

Another way to assess the effect of frying following irradiation would be to multiply the retention values for thiamine in the irradiated raw bacon by the fraction retained after frying nonirradiated bacon (0.52). Thus, the predicted values would be: (4.42 mg/100g) × 0.52 = 2.30 mg/100g or 48%, at 0 kGy; (1.77 mg/100g) × 0.52 = 0.92 mg/100g or 21%; at 7.5 kGy; (0.95 mg/100g) × 0.52 = 0.49 or 11%, at 15 kGy and (0.40 mg/100g) × 0.52 = 0.21 or 4.7% retention, at 30 kGy. The actual retention values were 52%, 17%, 9%, and 1.6% at doses of 0, 7.5, 15, and 30 kGy; so a 3% greater loss was observed than was predicted. Comparison of the slopes for the linear regression of the natural logarithms of the retention values on dose were −0.102 for the predicted values and −0.126 for the actual irradiated and then fried sample values. All three methods for evaluating the effect of frying on the thiamine content indicate that greater thermal destruction of the vitamin occurred when the bacon was irradiated prior to frying. Because increased destruction was 7% for 0 to 7.5 kGy, 2% for 7.5 to 15 kGy, and 34% for 15 to 30 kGy it was only moderately dose related. For this reason and because of the low number of replicate samples in the study, additional work will be required to test the hypothesis that thermal destruction interacts synergistically with the effects of ionizing radiation.

Effect of Treatments on Riboflavin Content

The effects of radiation on riboflavin in raw and fried bacon at 2° and −40°C were determined and are reported in Table 2. Statistically, a very small decrease in riboflavin was identified in raw bacon irradiated at 2°C but not at −40°C. Frying destroyed approximately 13% of the initial riboflavin content but did not increase the sensitivity of the riboflavin to radiation. The amount of radiation-induced destruction of riboflavin observed in this experiment does not allow any estimate to be made of the rate of destruction. It is presumed that such destruction would have occurred at much higher radiation doses. One can conclude from this study that riboflavin associated with bacon is much more resistant to the effects of ionizing radiation than is thiamine. Thus, at radiation doses (15 kGy), sufficient to eliminate *Clostridium botulinum* in bacon (Rowley *et al.* 1983), riboflavin remained virtually unaffected. Other workers have reported that riboflavin was apparently more stable to radiation than was thiamine in foods (Kennedy and Ley 1971; Thomas and Calloway 1957; Tobback 1977). Tobback (1977) speculated that the resistance of riboflavin to the effects of radiation would be expected since it is bound to proteins which generally would protect prosthetic groups from radiation.

Interaction of Water With Radiation Effects

The observation in Experiment One that thiamine in fried bacon was apparently much less sensitive to subsequent effects of ionizing radiation led us to question why this occurred. First, it should be considered that this lack of sensitivity could have practical significance since prefried bacon, which is used by restaurants, might have an extended shelf life if irradiated. Bacon irradiated after frying would have significantly greater retention of thiamine than bacon irradiated before frying and would, therefore, have greater nutritional and commercial value. The decreased radiation sensitivity of thiamine in the fried product could be related to any of several changes brought about by the process of frying, such as thermal denaturation of proteins, loss of fat, loss of moisture, thermal effects on protein binding of the vitamin, drip loss of the vitamin (a less stable form ?), and thermal alteration of the vitamin. Of the above, both moisture and fat loss are clearly demonstrable by examination of the proximate analysis values in Table 1. Further, because of these losses the protein content of the fried product is much greater than that of the raw bacon. The linkage of vitamin and protein contents was recognized in the experimental design. Since all vitamin data are reported in terms of mg vitamin/100 g protein, it is not possible to separate the protein interaction from vitamin retention in this study. Nor would it be meaningful in this case since cooking drastically alters the ratio of protein to vitamin content as described above. In order to differentiate between the effects of cooking (frying) and those of water content, an experiment was designed in which the thiamine contents of limp, crisp, and dark-brown fried bacon and raw bacon lyophilized to three moisture levels were compared to the thiamine content of raw bacon following treatment with radiation. The results are reported in Fig. 1 and 2.

The degradation of thiamine in the fried samples did not follow the same pattern as that in the raw irradiated bacon. For all three fried products, the retention slopes (Table 3) were much less than for raw irradiated bacon; the regression of thiamine content with radiation dose became more linear as the cooking level was increased. As was expected, the greater the extent of cooking the greater the degree of thiamine degradation due to the cooking process in nonirradiated bacon (Table 3, Fig. 1). When the effects of frying the raw bacon (nonirradiated values) are subtracted from the totals of the thiamine degradations at each radiation dose (Table 3), it is apparent that the net vitamin losses due exclusively to the gamma irradiation of the raw and fried bacon were directly proportional to each bacon's moisture content when irradiated.

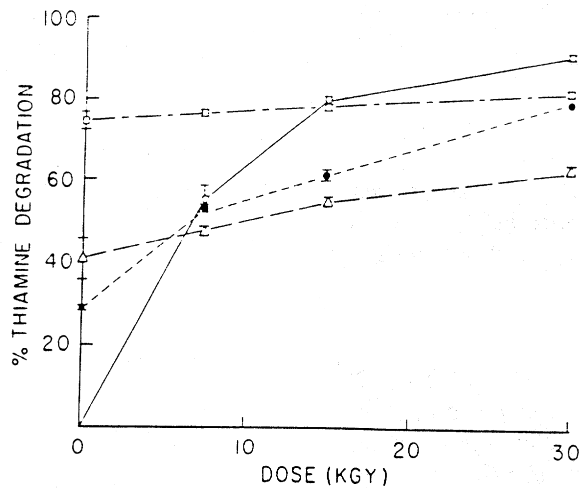

FIG. 1. % THIAMINE DEGRADATION CAUSED BY GAMMA RADIATION OR COOKING OF RAW AND THREE DIFFERENT LEVELS OF FRIED BACON

The bars indicate two standard deviations (S.D.). Where bars are not shown the S.D. were too small to be plotted.

(0 - raw bacon 40.6% H_2O; ● - slightly fried bacon 28.5% h_2O; Δ - medium fried bacon 17.4% H_2O; □ - well done "crisp" bacon 7.8% H_2O)

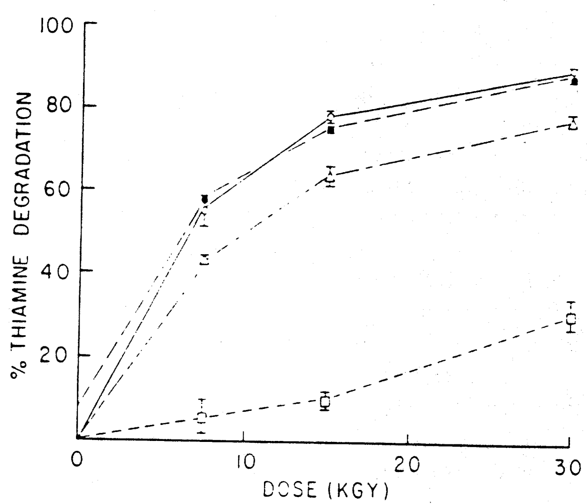

FIG. 2. % THIAMINE DEGRADATION CAUSED BY GAMMA RADIATION OF RAW BACON LYOPHILIZED TO VARIOUS FINAL MOISTURE LEVELS

(0 - raw bacon 40.6% H_2O; ● - lyophilized bacon 35.2% H_2O; Δ - lyophilized bacon 23.5% H_2O; □ - lyophilized bacon 2.2% H_2O)

TABLE 3.
EFFECTS OF LYOPHILIZATION OR FRYING OF BACON PRIOR TO IRRADIATION ON RETENTION OF THIAMINE.

Treatment	Radiation Dose (kGy)	Thiamine mg/100g protein $\bar{x} \pm$ S.D.[a]	Ratio $[T]/[T_o]$	Retention Slope m $m = \Sigma \ln \frac{(T)/(T_o)}{(kGy)} / N$
Raw ($40.6\%\ H_2O$)	0	3.14 ± 0.02	1.0	-0.095
	7.5	1.41 ± 0.12	0.449	
	15.0	0.67 ± 0.03	0.213	
	30.0	0.32 ± 0.01	0.102	
Fried (limp) ($28.5\%\ H_2O$)	0	2.23 ± 0.02	1.0	-0.044
	7.5	1.51 ± 0.03	0.677	
	15.0	1.23 ± 0.04	0.552	
	30.0	0.69 ± 0.03	0.309	
Fried (medium) ($17.4\%\ H_2O$	0	1.84 ± 0.15	1.0	-0.016
	7.5	1.64 ± 0.01	0.891	
	15.0	1.43 ± 0.01	0.777	
	30.0	1.19 ± 0.05	0.647	
Fried (crisp) ($7.77\%\ H_2O$)	0	0.78 ± 0.07	1.0	-0.008
	7.5	0.75 ± 0.00	0.962	
	15.0	0.68 ± 0.00	0.872	
	30.0	0.60 ± 0.02	0.769	
Lyophilized ($35.2\%\ H_2O$)	0	2.92 ± 0.07	1.0	-0.088
	7.5	1.31 ± 0.02	0.449	
	15.0	0.77 ± 0.00	0.264	
	30.0	0.36 ± 0.01	0.123	
Lyophilized ($23.5\%\ H_2O$)	0	2.94 ± 0.08	1.0	-0.059
	7.5	1.78 ± 0.00	0.605	
	15.0	1.14 ± 0.08	0.388	
	30.0	0.69 ± 0.05	0.235	
Lyophilized ($2.2\%\ H_2O$)	0	3.15 ± 0.03	1.0	-0.008
	7.5	2.96 ± 0.12	0.940	
	15.0	2.82 ± 0.07	0.895	
	30.0	2.52 ± 0.12	0.800	

[a] $\bar{x} \pm$ S.D. = mean ± standard deviation

The patterns of thiamine degradation in lyophilized bacon (Fig. 2, Table 3) were similar to those observed for raw bacon except for the pattern of thiamine degradation for bacon in which the water content had been reduced to 2.2% H_2O. The latter bacon may have been subjected to some thermal damage during the lengthy lyophilization process required to remove that much moisture from the product, and as a result some of the thiamine may have been affected in the same manner as in the fried samples. It should be noted, however, that no thiamine degradation was observed in the nonirradiated sample of the 2.2% moisture bacon and, though the pattern was convex rather than concave, these products were clearly more resistant to radiation damage.

The analyses reported above led to the tentative conclusion that the reduction in water content by the frying process was at least partially responsible for the radiation protective effect. If, however, reduction of water was the sole reason for the radiation protective effect of frying, then one would expect the radiation-related destruction of thiamine to occur in a pattern similar to that observed for lyophilized bacon (Fig. 2). One could form the hypothesis that the response of thiamine to a given radiation dose, if such a response is totally related to the moisture content, should be predictable from knowledge of the radiation dose and moisture content of the irradiated sample. To test this hypothesis, the following least squares linear regression equations were developed from the study with lyophilized bacon:

7.5 kGy $y = 2.963 - 0.043\,x;\ R^2 = 0.972$

15 kGy $y = 2.802 - 0.057\,x;\ R^2 = 0.973$

30 kGy $y = 2.470 - 0.059\,x;\ R^2 = 0.961$

where: y = mg thiamine/100g protein

x = % water

R^2 = coefficient of determination

These equations were then used to predict expected responses of thiamine at the moisture levels found in the fried bacon products. In each case the predicted value exceeded the observed thiamine value in the fried and irradiated product. When the loss of thiamine due to frying the nonirradiated product was added to the observed value for the fried-irradiated product, that value always exceeded the predicted value for a nonfried product containing an equivalent amount of moisture. Thus, the hypothesis was rejected because moisture content alone could not account for the observed results with irradiated fried bacon.

The analysis of variance (ANOVA) confirmed the existence of a significant ($P < 0.0001$) three-way interaction among dose, temperature, and type of preparation for thiamine. The ANOVA revealed a significant ($P < 0.0001$) effect due to the experimental bacon treatment for riboflavin. Subsequent orthogonal polynomial contrasts revealed that only in the raw bacon irradiated at 2°C was there a statistically significant ($P < 0.01$) regression of riboflavin content with radiation dose. The linear regression equation was $Y = 1.325 - 0.0257x$; $R^2 = 0.404$ where Y = mg riboflavin/100g protein; and x = radiation dose kGy.

Orthogonal polynomial contrasts of the results for thiamine indicated significant ($P < 0.0001$) responses to the radiation dose at either 2°C or −40°C in all three treatments (raw, fried-irradiated, and irradiated-fried bacon). The ANOVA confirmed the existence of significant effects ($P < 0.0001$) in the responses of thiamine to radiation due to the raw, the fried-irradiated, and the irradiated-fried bacon products. The effects of irradiation, frying, their interaction, and the interaction of these three terms with the temperature of irradiation were contrasted. Two-way tables of frying and irradiation levels at each dosage and type of frying treatment for each irradiation temperature were compiled and used to estimate A, B, and AB in the model:

$$Y_{ij} = M + A_i B_j + AB_{ij}$$

where: M = average effect,
A_i = frying effect $i = 1,2$,
B_j = irradiation effect $j = 1,2$,
with jth irradiation level,
and Y_{ij} = thiamine value for the
ith frying level and
jth irradiation level.

By collating the results of the contrasts with the two-way tables and the estimates for A, B, and AB, it is evident that there are significant interactions between frying and irradiation. These interactions were temperature dependent and gave significantly smaller interactions at −40°C than at 2°C. The frying effects were greater when the bacon was fried after, rather than before, irradiation.

The group comparisons obtained by ANOVA for Experiment Two, which concerned the effects of the level of frying or moisture on the response of thiamine to radiation, were used to fit second order response surface models for fried and lyophilized study results. The following are the response surface equations and their associated coefficients of determination:

$$\text{Frying } Y = 0.02214 + 0.1374 \text{ x moisture} - 0.3480 \text{ x dose} - 0.02459 \text{ x moisture x dose} - 0.1817 \text{ x moisture}^2 + 0.001678 \text{ x dose}^2;\ 100\ R^2 = 86.64$$

$$\text{Lyophilized } Y = 3.681 - 0.06485 \text{ x moisture} - 1.192 \text{ x dose} - 0.01682 \text{ x moisture x dose} + 0.001119 \text{ x moisture}^2 + 0.003154 \text{ x dose}^2;\ 100\ R^2 = 95.24$$

where Y = mg thiamine/100g protein
moisture = % moisture
and dose in kGy

The response surfaces generated by these equations are presented in Fig. 3 and 4. It is apparent by comparing the surfaces that the protective effect of frying before irradiation involves more than just the removal of water from the samples.

It was presumed from the start of the experiment that a relationship would exist between vitamin and protein contents of the samples. Indeed both the processes of lyophilization and frying increased the relative contents of protein in the samples before irradiation, and in both cases the samples with the greatest relative content of protein provided the greatest protection to thiamine. The obvious difference between the raw, lyophilized, and fried samples, other than moisture content, is that thermal denaturation of protein and thermal destruction of thiamine had occurred in the fried samples. It was mentioned earlier that thermal destruction of protein bound thiamine (cocarboxylase) may have taken place and that the residual thiamine was also more resistant to the effects of radiation. The differences between the response surfaces generated in Fig. 3 and 4 may be due to differences in the interaction of radiation-induced free radicals with the denatured proteins. The denatured proteins may have more reactive moities and thus more effectively protect the vitamin from radiation damage.

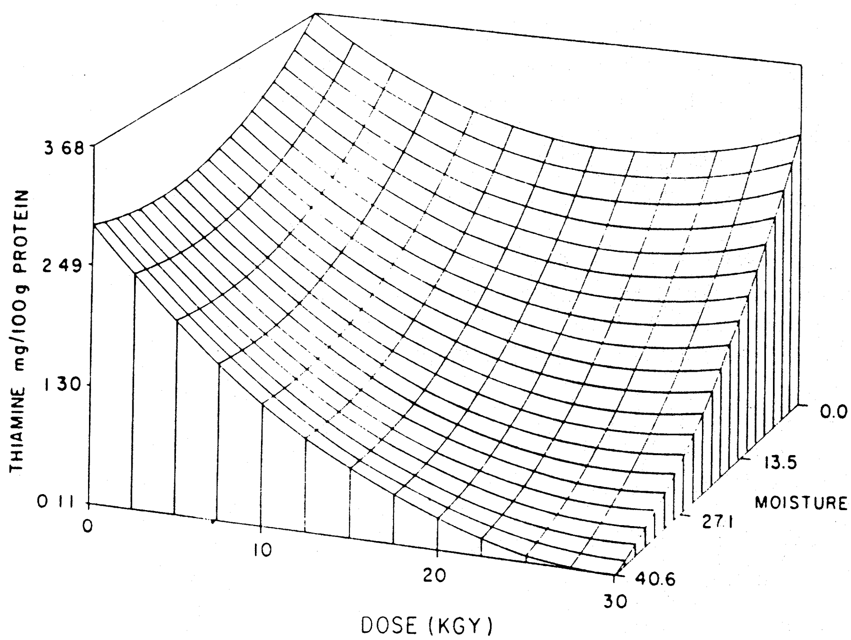

FIG. 3. RESPONSE SURFACE FOR EFFECT OF MOISTURE LEVEL ON DESTRUCTION OF THIAMINE IN LYOPHILIZED BACON BY GAMMA RADIATION

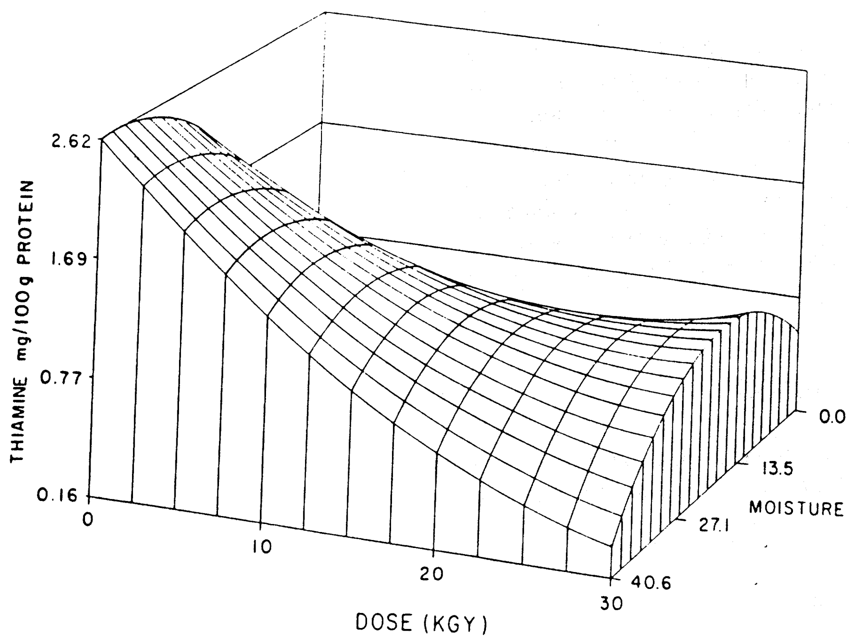

FIG. 4. RESPONSE SURFACE FOR EFFECT OF FRYING AND FINAL MOISTURE LEVEL ON DESTRUCTION OF THIAMINE IN BACON BY GAMMA RADIATION

This study has confirmed that low-temperature irradiation of bacon results in greater retention of thiamine, similar to that reported by Wilson (1959), and Thomas *et al.* (1981) for minced beef and ground pork, respectively. This study has also identified a protective effect for thiamine when bacon is cooked before irradiation. That protective effect was not identical to that produced by removal of water by lyophilization from the products prior to irradiation. The observation by Thomas and Calloway (1957) that postirradiation cooking of turkey produced a synergistic effect on thiamine was tentatively confirmed in this study with bacon. When irradiated bacon was fried, a greater amount of thiamine was lost than would be predicted if the loss were simply additive. The association of radiation resistance of vitamins with the amount of water by Ziporin *et al.* (1957) and Richardson *et al.* (1958) was demonstrated to be true for bacon in the case of thiamine. The sensitivity of fat to oxidation by both radiation and heating is well documented. Possible subsequent reactions of oxidized fat with thiamine or riboflavin are unknown, and for that reason the analysis was included. However, no obvious conclusions could be drawn from the results. The analysis for riboflavin was not performed on the irradiated-fried samples because no significant changes were observed in either the raw-irradiated or the fried-irradiated bacon samples. Riboflavin in bacon was found to be almost completely insensitive to gamma radiation at either 2°C or −40°C, which contrasts with a report by Thomas and Josephson (1970) that the relative sensitivities of vitamins in pork were in the order riboflavin (78% retention) and thiamine (85% retention). Approximately 48% of the thiamine was destroyed by moderate frying. A radiation dose of 15kGy, which is adequate to maintain microbiological safety of reduced nitrite bacon (Rowley *et al.* 1983), destroyed 78.5% or 31.9% of the thiamine in raw bacon at irradiation temperatures of 2°C or −40°C, respectively. Bacon that received the 14 kGy irradiation dose at 2°C prior to frying lost a much greater proportion (91.0%) of its thiamine content.

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