

## Thiamin, Riboflavin and $\alpha$ -Tocopherol Retention in Processed and Stored Irradiated Pork

### ABSTRACT

Combination treatments for preservation of irradiated pork were investigated with respect to vitamin loss. Ground pork was prepared under nitrogen and packaged in anaerobic foil. The samples were enzyme denatured by heating before and after irradiation, then cooked and stored. Irradiation resulted in thiamin loss, but neither riboflavin nor  $\alpha$ -tocopherol was affected. Neither thiamin nor riboflavin was affected by heat denaturation, cooking or storage, but heating and cooking increased the measured  $\alpha$ -tocopherol. The lack of loss of the vitamins was attributed to the exclusion of oxygen.

Key Words: Pork, thiamin, riboflavin,  $\alpha$ -tocopherol, irradiation

### INTRODUCTION

IN ORDER TO REDUCE ENZYME DEGRADATION of muscle tissue during prolonged storage of sterilized irradiated meats (doses of 30 kiloGray and higher), they are heated to 70°C before irradiation to inactivate proteolytic enzymes by denaturation. Low temperature heating is also used in lightly processed products where combination treatments are useful for bacterial control. Radiation increases the heat sensitivity of *C. sporogenes* and *S. typhimurium* (Thayer et al., 1991) and heating before irradiation enhances the antimicrobial effects of irradiation (Farkas, 1990). An important question with respect to combined treatments using pasteurizing doses (2 to 3 kiloGray) and low temperature processing is the effects of such treatments on the radiation loss and storage stability of vitamins. Combined treatments may reduce or eliminate metabolic activity and prevent vitamin loss during irradiation and storage. The rate of loss of thiamin was inversely related to the titratable reducing capacity of animal tissues, yet there was a reduced rate of thiamin loss in meat (as compared with aqueous solutions) that was not entirely accountable for by the reducing capacity (Fox et al., 1993). Such a protective effect in raw tissues could result from the oxidase reduction of the hydroxyl radical as a competitive effect, or the subsequent enzymatic deactivation of a semi-stable partially oxidized thiamin or riboflavin, since both are electron transfer coenzyme precursors. Anaerobic glycolysis is stopped in meat due to lack of oxygen, but glycolytic enzymes could still produce oxidizable intermediates to affect storage stability of vitamins. Denaturing many of the proteins of muscle tissue would alter the availability of sulfhydryl groups which exert a strong protective effect on vitamins during irradiation.

The major emphasis on food irradiation is on pasteurizing doses of 1 to 10 kiloGray (kGy). This covers the initial phases of the loss of most radiation sensitive vitamins for which rates of loss have been reported (Fox et al., 1992, 1995). Most studies on irradiated, stored meats, including fish, have used higher doses, low temperatures or recorded only amounts of vitamins remaining (Diehl, 1979; Krylova, 1973; Hozova et al., 1986). Jenkins et al. (1989) reported on thiamin losses in raw pork irradiated at low doses, cooked and stored. Our objective was to study the three most sensitive vitamins,  $\alpha$ -tocopherol, thiamin and riboflavin, simultaneously for retention in a single substrate

that had been enzyme inactivated, cooked and stored. Enzymes were denatured by heating to 70°C, both before and after irradiation, all samples were cooked to 98°C and stored for 4 wk at 0°C.

### MATERIALS & METHODS

#### Substrate, processing conditions

Pork was studied because it has a high concentration of thiamin (~10  $\mu$ g/g lean tissue as compared to 1  $\mu$ g/g in other meats) and has a relatively low fat content (2% as compared with 4–5% fat in lean beef). Pork tenderloins (*Psoas major*), 3 to 5 days old, were used since that muscle tissue was quite uniform from one end of the muscle to the other and trimmed out cleanly with very little intracellular fat. They were purchased in anaerobic packs from a local supermarket and processed in a glove bag in an atmosphere of nitrogen to exclude oxygen as much as possible. The loins were trimmed of fat (Fig. 1), ground through a 4.76 mm plate and mixed thoroughly by hand for 10 min. About 20g portions of the ground pork were weighed, placed in plastic/foil bags (MIL-B-131H, Type 1, Class 1, O<sub>2</sub> transmission = 0.0006 cc/645 cm<sup>2</sup>/24 hr. Georgia Packaging, Inc., Columbus GA) and the bags closed by heat sealing. Only then were the samples removed from the nitrogen atmosphere. This generally anaerobic technique was employed to simulate whole meats where the interior of the product is in an anaerobic state. The bags were stored at 0°C 4–5 wk.

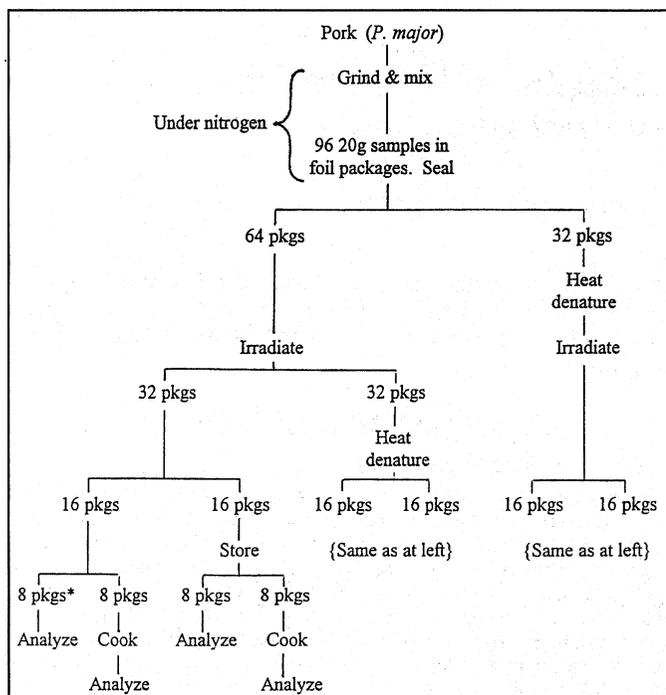
Sets of 32 packages were subjected to one of the following treatments: (1) irradiated raw, (2) heat denatured (enzyme inactivated) then irradiated, and (3) irradiated then heat denatured (Fig. 1). The raw, irradiated samples were controls in which any enzymatic interactions with the radiation process would produce different results from the other two sets. One-third of the samples (32) was heated to 70°C in a water bath, held at that temperature for 2 min and cooled. The temperature of the packages was determined by placing a small banjo thermistor in one of the foil packs with meat which was immersed with the other packages. The packages were agitated continuously and moved from place to place rapidly throughout the bath to ensure near uniform temperature distribution. All 96 samples were then irradiated in a <sup>137</sup>Cesium  $\gamma$ -ray source for total absorbed doses of 0.0, 0.50, 1.0, 1.50, 2.0, 3.0, 6.0 or 10.0 kGy; 12 packages for each dose consisting of 4 packages of each set (1, 2, 3). The packages were taped to the inside of an 8" (20.3 cm) Sonotube (a heavy cardboard tube used in building construction) which held the packages in a uniform circle around the periphery of the irradiation chamber at a uniform distance from the encircling <sup>137</sup>Cesium source rods. The tube also eliminated the "Compton effect" from metal walls of the chamber. After the irradiation process, 32 of the unheated samples were heated to 70°C as before (set 3, irradiated/denatured). The remaining samples were kept as raw/irradiated (set 1) and denatured/irradiated (set 2).

A radiation dose series of samples (0 to 10 kGy) of sets 1, 2, 3 were analyzed for thiamin, riboflavin and  $\alpha$ -tocopherol. Those irradiated to 0.0, 0.5, 1.0, 1.5, 2.0 and 3.0 kGy were analyzed for  $\alpha$ -tocopherol and those irradiated to 0.0, 1.0, 2.0, 3.0, 6.0 and 10.0 kGy were analyzed for thiamin and riboflavin.

Another dose series of sets 1, 2, 3 were cooked by immersing them in a boiling water bath. The samples were heated until the thermistor indicated they had attained equilibrium at 97.5  $\pm$  0.5°C, held in the bath an additional 5 min, after which they were analyzed for vitamins. The remaining sets were stored at 0°C for 4 wk and then analyzed as before, with and without cooking. The experiment was replicated twice.

#### Radiation source and temperature control

The self-contained <sup>137</sup>Cs gamma-radiation source (Lockheed-Georgia, Marietta, GA, Model LG 20000) had a strength of about 121,220 Ci



**Fig. 1—Flow sheet for sample preparation; heat denaturation, irradiation, cooking and storage.** \*Each set contains a full complement of doses.

(4.48 Pbc) and a dose rate of  $0.103 \text{ kGy min}^{-1}$ . The dose rate was established using National Physical Laboratory (Middlesex, United Kingdom) dosimeters and corrected on a weekly basis for decay of the isotope. Samples were maintained at  $5 \pm 0.5^\circ\text{C}$  during irradiation by thermostatically controlled injection of cold, gaseous nitrogen into the irradiation chamber. Sample temperature was monitored continuously during irradiation using calibrated thermocouples.

#### Vitamin determination

Samples for vitamin determination were prepared as described previously (Fox et al., 1993), except that the filtration step after centrifugation was eliminated. The extract (0.2 mL) was injected into a HPLC system without a column. The solution passed first through a fluorescence detector to determine the 530 nm fluorescence of riboflavin. The stream was then mixed with an equal volume of 0.2%  $\text{K}_3\text{Fe}(\text{CN})_6$  in 2% NaOH to oxidize thiamin to thiochrome and the fluorescence at 450 nm measured. For blanks the stream was mixed first with 2% NaOH, which destroyed the riboflavin, and then run through the two detectors.  $\alpha$ -Tocopherol was determined as described by Lakritz and Thayer (1992).

#### Statistical analysis

The GLM procedure of SAS (SAS Institute, Inc., 1987) was used for the ANOVA of the data. For the determination of the significance of differences between subsets of the data, the Bonferroni test was used. The significance of the data is presented in the form,  $p < 0.05$ .

## RESULTS

THE HEAT DENATURATION STEP resulted in a firm textured material which was relatively elastic, had a pale, grayish-white color indicative of heme pigment denaturation and no drip loss. Cooking hardened the sample, and there was some drip loss. The zero dose concentrations of vitamins in all samples showed no differences, even after all other treatments. The intercepts of the calculated regression curves did not vary significantly from the unirradiated samples' initial values (0 dose).

**Table 1—Loss of thiamin in irradiated pork as related to denaturation, cooking and storage**

Sample	Initial conc ( $\mu\text{g/g}$ ) Replicate		First order rate constant ( $\text{kGy}^{-1}$ ) Replicate	
	1	2	1	2
<b>0 time</b>				
<b>Uncooked<sup>a</sup></b>				
Raw/irrad	8.5	10.4	-0.09	-0.11
Denat/irrad	9.5	10.1	-0.14	-0.20
Irrad/denat	9.9	10.9	-0.16	-0.15
<b>Cooked</b>				
Raw/irrad	10.0	10.0	-0.15	-0.15
Denat/irrad	7.6	7.4	-0.14	-0.13
Irrad/denat	9.5	8.4	-0.10	-0.15
<b>Stored</b>				
<b>Uncooked</b>				
Raw/irrad	9.4	10.6	-0.13	-0.12
Denat/irrad	9.7	11.1	-0.19	-0.13
Irrad/denat	9.7	11.0	-0.15	-0.11
<b>Cooked</b>				
Raw/irrad	9.3	9.9	-0.14	-0.10
Denat/irrad	10.5	9.4	-0.14	-0.19
Irrad/denat	7.9	7.0	-0.08	-0.11
Average	9.49	Average	-0.134	
Std dev	1.12	Std dev	0.030	
C.V.	11.8	C.V.	22.3	

<sup>a</sup> For sequence of operations, see Fig. 1.

#### Thiamin

The initial thiamin concentrations and rate constants for all treatments were compared (Table 1). The average thiamin concentration was  $9.49 \pm 1.12 \mu\text{g/g}$  tissue and the average rate constant for the loss was  $-0.134 \pm 0.030 \text{ kGy}^{-1}$ . Respective coefficients of variation were 11.8 and 22.3%. The range of rate constants for  $1 \sigma$  was  $-0.104$  to  $-0.164$ , comparable to ranges previously reported (Fox et al. 1989, 1995). No readily observable trend was apparent, nor were there any differences between the two replicates, so both were combined for the ANOVA. The intercepts, dose and cooking effects were highly significant ( $p < 0.0001$ ). In order to determine the magnitude of the differences and where they occurred, the data were subjected to a test of the heterogeneity of the slopes and intercepts (Table 2) as a function of the heat/irradiation treatments, storage time and cooking.

#### Riboflavin

Initial concentrations and loss of riboflavin during irradiation (Table 3) showed riboflavin averaged  $1.23 \pm 0.493 \mu\text{g/g}$  tissue and the average rate constant for the loss was  $-0.0041 \pm 0.0291 \text{ kGy}^{-1}$  (C.V. = 714). This rate of loss was not significant and the data were no further analyzed.

#### $\alpha$ -Tocopherol

Analysis of the data showed that the two replicates had different concentrations of  $\alpha$ -tocopherol ( $p < 0.0001$ ), although neither the regression on dose nor the storage was significant ( $p > 0.05$ ). There were significant differences ( $p < 0.0001$ ) among the sets (raw/irradiated, denatured/irradiated and irradiated/denatured), cooking (uncooked vs cooked) and treatment/cooking (Table 4). In order to determine where the other differences arose, the raw data were divided into uncooked and cooked sets and subjected to a Bonferroni test (SAS Institute, Inc., 1987) (Table 4). In uncooked samples, sets 2 and 3 that had been heat denatured had higher  $\alpha$ -tocopherol concentrations than the raw samples (set 1). When the samples were cooked, the  $\alpha$ -tocopherol increased and the differences due to the treatment disappeared in all sets.

**Table 2**—Detailed ANOVA of initial concentration and rate constant for thiamin loss in pork subjected to various treatments storage and cooking

Sample	Treatment	Effect of storage			Effect of cooking				
		Intercept <sup>ab</sup>		Slope	Treatment	Intercept		Slope	
		µg thiamin/g		kGy <sup>-1</sup>		µg thiamin/g		kGy <sup>-1</sup>	
Raw/irrad	Uncooked	n.s.		O	-0.10 <sup>c</sup>	0 time	n.s.	U	-0.15 <sup>c</sup>
	Cooked	n.s.		S	-0.12	Stored	n.s.	C	-0.10
Denat/irrad	Uncooked	n.s.		O	-0.15			0 Time	U
	Cooked	n.s.		S	-0.12	Stored	C	8.1	-0.11
Irrad/denat	Uncooked	n.s.		n.s.	n.s.	0 Time	U	10.5	n.s.
	Cooked	n.s.		O	-0.11	Stored	C	8.9	n.s.
	Uncooked	n.s.		S	-0.16 <sup>c</sup>	0 time	n.s.	n.s.	n.s.
	Cooked	O	8.9		-0.17	Stored	U	10.9	n.s.
		S	6.8		-0.12		C	6.8	

<sup>a</sup> The concentration values are the intercept values from the first order equation for the thiamin loss. Each value in the pair, O: S, UC, is significantly different from the other.

n.s.=no significant difference, values not shown.

<sup>b</sup> Codes: O, zero time; S, stored; U, uncooked; C, cooked.

<sup>c</sup> Values which were outside  $\pm 1\sigma$  in Table 1.

**Table 3**—Concentration and rate constants for loss of riboflavin in irradiated pork that has been enzyme denatured, cooked and stored

Sample	Initial concentration µg/g		Rate constants in kGy <sup>-1</sup>	
	Replicate 1	Replicate 2	Replicate 1	Replicate 2
<b>0 time</b>				
Uncooked				
Raw/irrad	0.64	1.48	0.0014	0.012
Denat/irrad	0.91	2.73	-0.035	0.012
Irrad/denat	0.35	1.48	-0.007	0.012
Cooked				
Raw/irrad		1.25		0.017
Denat/irrad		1.43		-0.013
Irrad/denat		1.56		0.002
<b>Stored</b>				
Uncooked				
Raw/irrad	0.46	1.18	0.053	-0.030
Denat/irrad	1.41	1.42	-0.058	-0.019
Irrad/denat	0.65	1.56	0.073	-0.031
Cooked				
Raw/irrad	1.00	1.44	-0.013	-0.004
Denat/irrad	1.21	1.39	0.0018	-0.040
Irrad/denat	0.88	1.30	-0.016	0.0003
Average	1.23	Average	-0.0041	
Std dev	0.49	Std dev	0.029	
C.V.	40.2	C.V.	716.0	

**Table 4**—Effect of heat denaturation and cooking on concentration of  $\alpha$ -tocopherol in gamma irradiated pork

Process	Avg conc of $\alpha$ -tocopherol in ng/g meat		
	Raw/irrad	Denat/irrad	Irrad/denat
Uncooked	437	728 <sup>a</sup>	826
Cooked	781	783	868
Replicate no.	One	Two	
	966	526	
Process	Cooked	Uncooked	
	780	663	

<sup>a</sup> Underlined values in each row of the table not significantly different; all other are ( $p < 0.0001$ ). Significant differences between the means determined by the Bonferroni test.

## DISCUSSION

DIFFERENCES IN THIAMIN DATA (Table 2) were not great. Intercepts determined from the first order regression expressions, except one, were within the 95% ( $2\sigma$ ) confidence limits. One value in Table 1 ( $-0.204$  kGy<sup>-1</sup>) was outside the 95% confidence limits ( $2\sigma$ ,  $-0.074$  to  $-0.194$  kGy<sup>-1</sup>) but was not associated with significant differences in Table 2. No consistent trend correlated with any of the treatments, and the differences appeared to be randomly distributed. Coupled with the observation that the range of values was consistent with previously observed ranges, (Fox et al., 1989, 1992, 1995) leads to the conclusion that the observed differences are not of practical concern.

## Enzyme denaturation

The low temperature heating of the samples before or after irradiation had no effect on the rate of loss of thiamin or  $\alpha$ -tocopherol, or stability of riboflavin so there is no evidence of residual enzymatic activity affecting vitamin loss. The initial reaction of ionizing radiation with water to form the hydroxyl radical is primarily driven by the energy of the photon and is independent of other systems. The second step reaction of the hydroxyl radical with metabolites is oxidation, and most endogenous compounds that compete with the vitamins are in place and available for reaction, even the sulfhydryl groups of actomyosin. The samples had been prepared under nitrogen which precluded any further oxidative reactions that might have affected stability of the vitamins.

## Vitamin loss

The thiamin losses during irradiation in general confirmed previous reports (Jenkins et al., 1989; Hozova et al., 1986; Krylova, 1973; Fox et al. 1992, 1994) and the lack of significant loss of riboflavin also confirms previous observations (Hozova et al., 1986). Loss or gain in thiamin during enzyme denaturation, cooking and storage is questionable. Jenkins et al. (1989) reported thiamin losses of about 11% in cooked ground pork, but Fox et al. (1989) found increases of about 10% in measured thiamin and 50% in measured riboflavin in cooked pork chops and losses of 8% thiamin in cooked chicken breasts. In what must be regarded as a closed system, Kennedy (1965) found no loss of thiamin in eggs during thermal pasteurization. The effects of storage are even more diverse. Losses on storage have been reported for thiamin (Hozova et al., 1986; Diehl, 1969; Krylova, 1973; Sorman et al., 1986) yet no losses of thiamin on storage were reported from other studies (Jenkins et al. 1989; Mameesh et al. 1965; Syunyakova and Karpova, 1966; Brooke et al. 1964; Ang, 1986). The reported losses are generally a few percent, usually less than the standard deviation for the determination procedures. Our determination of thiamin showed a coefficient of variation of  $\pm 12\%$ , yet the increase on storage was only 3.5%. Precision in determination of vitamins in meat is unreliable because they occur in very low concentrations,  $\mu\text{M}$  or less. Also, the stability of thiamin in meat is dependent on a wide variety of factors (Fox et al., 1993, 1994) including interactions between thiamin and other vitamins and metabolic intermediates (Goldblith, 1955; Fox et al., 1992). A relatively short exposure to air could introduce enough oxygen to affect all the vitamins and their loss rate during irradiation, as well as lower the amounts of reducing compounds that may affect the rates of that loss (Kishore et al., 1978; Fox et al., 1992, 1993). The lack of significant loss of thiamin due to enzyme inactivation, cooking or storage may be attributed to the anaerobic conditions of our samples. The interior of whole meat cuts are

essentially anaerobic and bulk ground meats after standing a day or longer are anaerobic in the interior. The loins we studied were originally packaged in an oxygen impermeable film and showed only the faint purplish color of the reduced heme pigments with no "bloom" (pink color). Prior to the original packaging the loins must have been exposed to air, but only on the surface, so that by the time we obtained them the tissues were free of oxidizing substances, specifically oxygen lost to metabolic activity. During our preparation procedure the meat did not bloom, strong presumptive evidence that there was no oxygen present. Exposure of the ground meat to air resulted in the rapid development of pink color. Since ultimate destruction of vitamins requires oxidizing compounds, heat treatments or storage of the samples *per se* should not result in appreciable loss of thiamin or riboflavin.

### $\alpha$ -Tocopherol

There was about twice the amount of  $\alpha$ -tocopherol in the first replicate than there was in the second (Table 4). The relatively mild heat treatment used to inactivate the enzymes by thermal denaturation resulted in significant increases in the extractable vitamin, while subsequent cooking released more (Table 4). Note that the pooled  $\alpha$ -tocopherol value for the cooked samples was higher than that of the uncooked samples. The most likely explanation is that heating broke some of the ester links, releasing free  $\alpha$ -tocopherol which has 9 times the fluorescent intensity of esterified tocopherol. The amount of the latter produced would depend on a number of factors, including chain length. Heating may also have liberated tocopherols adsorbed on surface of proteins or cellular tissue. Either of these effects would result in a marked increase in measured fluorescence with no change in total tocopherol. Since these studies were conducted in nitrogen there was no loss of  $\alpha$ -tocopherol due to irradiation because, as Diehl (1979) has shown, oxygen is required.

### CONCLUSION

ENZYMATIC ACTIVITY had no effect on loss of vitamins in meat, whether or not irradiated, fresh or stored. Thiamin was lost only by radiation oxidation which occurred at the same rate for all treatments. Riboflavin showed no losses due to any treatments. The rigorous exclusion of oxygen, while it did not prevent the loss of thiamin on irradiation, may protect all of the vitamins due to heating, cooking and storage. While no destruction of  $\alpha$ -tocopherol was observed due to irradiation or storage, heating to low or high temperatures resulted in an apparent increase in the vitamin, probably due to freeing of esterified or bound tocopherol.

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Ms received 1/7/97; revised 5/15/97; accepted 5/31/97.