

Effect of Radiation and Conventional Processing on the Thiamin Content of Pork

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ABSTRACT

A study was undertaken to compare the effect of ionizing radiation and thermal processing on the thiamin content of pork. Ground pork was either thermally processed in cans or, after enzyme-inactivation, irradiated in pouches with electrons or gamma rays. Thiamin in treated and untreated samples was measured by the thiochrome method. The results show that thiamin retention in pork decreases as the irradiation dose increases, but that retention increases as the temperature of irradiation decreases. Moreover, irradiation with gamma rays (low dose-rate) leads to lower retention than irradiation with electrons (high dose-rate). Results also show that conventional thermal processing causes loss of thiamin comparable to or greater than irradiation at effective processing conditions for sterilization.

INTRODUCTION

DURING the past several years, considerable research has been performed by our laboratories on irradiating food as a means of achieving long term preservation. All methods to preserve food reduce the nutritive value to some extent, and further loss in nutrient content could be encountered as a result of storage and of heating in preparation for consumption. Irradiated foods, however, can be kept for months without spoilage in the absence of refrigeration. This present work was undertaken to provide nutrient data to support petitions to Food and Drug Administration for clearance of foods so treated.

Previous studies by our laboratories (Thomas and Callo-way, 1961) have shown that irradiation causes some destruction of thiamin and pyridoxine, but relatively little destruction of riboflavin and niacin. At the same time, comparisons were made between irradiation and conventional processing methods indicating that vitamin losses were of the same magnitude (Thomas and Josephson, 1970). From this point on, all studies were conducted with pork, and analyses made only for thiamin because it is easy to determine and its concentration is high in pork.

In the past we have reported that pork irradiated with electrons retains more thiamin than when irradiated with gamma rays (Thomas et al. 1975). This study was designed to address this phenomenon, as well as to make additional comparisons with thermal processing.

MATERIALS & METHODS

THE PROCESSING of the pork was essentially as published by Shults et al. (1976). The raw material for this study was fresh (3-5 days post-slaughter), boneless pork hams, with the skin and external fat removed. The raw ham muscles were cut into 50-500g chunks, ground through a 1.25 cm blade, and vacuum mixed in a mechanical mixer together with 0.75% NaCl, 0.3% sodium tripolyphosphate (TPP), and 3% crushed ice. After mixing, the product was stuffed into fibrous, regenerated cellulose casings (102 mm in diameter) to make 12 rolls; the stuffed product was then stored in a refrig-

erator at 2-5°C overnight (15 hr) prior to enzyme-inactivation.

The enzyme-inactivation was accomplished in a steam-heated cookhouse, utilizing the cooking cycle shown in Table 1. This process brings the internal temperature of the rolls to the range 75-77°C, at which point, the steam is discontinued to reduce the dry bulb temperature to 74°C when an additional heat treatment of 3 to 4½ hr is given to allow each roll to reach an 88% yield. During this time, the internal temperature of the rolls remained 75-77°C. The rolls are then removed from the cookhouse and chilled to 2-5°C by refrigerating overnight.

The final steps include cutting and packaging. By removing the end pieces, the chilled pork rolls were trimmed to 45 cm lengths and then sliced into 13 mm sections. For the irradiation, representative samples of each roll were vacuum packaged in flexible pouches 11.5 x 17.8 cm in size, as described by Killoran et al. (1979).

For thermal processing, the product was vacuum packed in 404 x 309 (10.6 cm diameter x 9.0 cm high) metal cans and processed at retort temperatures of 116°C (240°F) for 170 min at 25 psi or 121°C (250°F) for 120 min at 30 psi to the lethality value of $F_0 = 6.0$ using the method of Cohen (1974).

Irradiation was performed over a range of doses by exposure to electrons from a 10 Mev Linac (linear accelerator) or gamma rays from ⁶⁰Co or ¹³⁷Cs sources. Jarrett and Halliday (1979) have described the dosimetry techniques used for both the Linac and the megacurie ⁶⁰Co source. The dose spread in the electron and gamma ray irradiated samples was about 15%. The experimental plan is given in Table 2.

All samples were equilibrated to a selected starting temperature prior to irradiation and maintained within ±5°C of that temperature despite the irradiation process. For gamma ray irradiation, the sample temperature was controlled by continuous cooling. For electron irradiation, the temperature increase was minimized by continuous cooling and by interrupting the irradiation after specific doses for a period of time adequate to reequilibrate the samples.

All samples were analyzed at least in duplicate for moisture and fat contents as well as thiamin. For the thermally sterilized product, six replicates were analyzed and their means compared. For the irradiation sterilization at specific temperatures at least two samples were analyzed for each increment of temperature and dose, and the data graphically analyzed. Moisture and fat determinations were made following the procedures described in *Official Methods of Analysis* (1975). Thiamin content was measured by the thiochrome method published in *Methods of Vitamin Assay* (1966) and all values were corrected to a moisture and fat-free basis.

RESULTS

THE DATA OBTAINED show that thermal sterilization significantly reduces thiamin and that irradiation sterilization leads to a loss of thiamin dependent on phase, temperature, dose, and dose-rate.

Table 1—Conditions for enzyme-inactivation of pork

Time hr	Temperature °C	
	Dry bulb	Wet bulb
2	66.0	48.9
1	76.7	57.2
2.5	90.0	71.1
3-4.5	74.0	57.2

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Thermal sterilization

As shown previously, the destructive effect of heat on thiamin is significant and depends on the temperature and processing time. For samples processed to a F_0 value of 6.0, the retention is only $12 \pm 2\%$ and $9 \pm 2\%$ at 116°C and 121°C , respectively (Table 3).

Irradiation sterilization

As results in Table 3 show, the effect of irradiation on thiamin is such that the retention decreases with dose, increases with decreasing temperature, and differs depending on whether gamma rays or high energy electrons are used.

Dose response. For both gamma ray and electron irradiation, the retention of thiamin decreases regularly with increasing dose. All samples, which initially contained about 0.9 mg of thiamin per 100g pork, were irradiated at dose increments that would bring about a reduction in content adequate for analysis, but would not lead to complete loss. The results for samples irradiated in the frozen state are plotted as the logarithm of the fraction of thiamin retained against dose (Fig. 1). Results for the unfrozen sample have been similarly analyzed but not shown here. The conform-

ity to an exponential dependence of retention on dose is particularly good for the electron irradiated samples and adequate for the gamma ray irradiated samples. Such plots are convenient and allow one to use the slope, which corresponds to m in the expression

$$2.3 \log_{10} [T] / [T]_0 = mD$$

(where T represents thiamin and D represents dose), for comparing differences in the response. The very limited scatter of the points about the lines reflects the close control of dose and temperature, the uniformity of the samples, and the reproducibility in the analyses ($\sim \pm 2\%$).

Phase and temperature effects. The results clearly indicate the beneficial effect on thiamin retention of freezing the samples and lowering the temperature of the frozen samples. To depict this influence, the quantity "fraction lost per Mrad," which is

$$(1 - e^{-mD}),$$

was calculated (using m from the graphical analyses and unity for D) and plotted against reciprocal temperature (Fig. 2). This figure shows that the loss in the gamma ray irradiated unfrozen sample is clearly above the others, and that the losses in gamma ray and electron irradiated samples in the frozen state follow a quasi-Arrhenius temperature dependence, the lines for both being practically parallel.

This representation of the data is convenient for comparing the effect of freezing on thiamin retention. If the line for the gamma ray irradiated frozen samples is extrapolated to the freezing point of pork, taken as -2°C , the fraction lost per Mrad would be about 0.5. If it is assumed that the fraction lost (0.76) in the unfrozen sample at $+5^\circ\text{C}$ would be the same at -2°C , then about 50% more thiamin is lost in going from the frozen to the unfrozen state.

The figure is also useful for comparing the influence of temperature on thiamin loss in frozen samples, but it must be put into perspective. First, no data were specifically obtained for temperatures below -45°C . Considerable evidence exists to indicate that at still lower temperatures the fraction lost per Mrad might become constant. Secondly,

Table 2—Experimental design

Treatment	Temp °C	Dose Mrad						
Thermal, $F_0 = 6.0$	116							
	121							
Gamma rays (^{137}Cs)	5	0	0.25	0.50	0.75	1.0	2.0	
	-15	0	1.0	2.0	3.0	4.0	5.0	
	-30	0	1.2	2.4	3.6	4.8	6.0	
	-45	0	1.5	3.0	4.5	6.0	7.5	
Electrons (LINAC)	-30	0	1.2	2.4	3.6	4.8	6.0	
	-45	-	1.5	3.0	4.5	6.0	7.5	

Table 3—Effect of processing on thiamin in pork

Treatment	Temp °C	Dose Mrad	Retention %
Thermal, $F_0 = 6.0$	116		12
	121		9
Gamma rays (^{137}Cs)	-45	1.5	72
		3.0	50
		4.5	40
		6.0	35
		7.5	27
Electrons (LINAC)	-20	1.2	82
		2.4	68
		3.6	57
	-45	1.5	83
		3.0	75
		4.5	66
		6.0	58
	7.5	52	
	9.0	50	

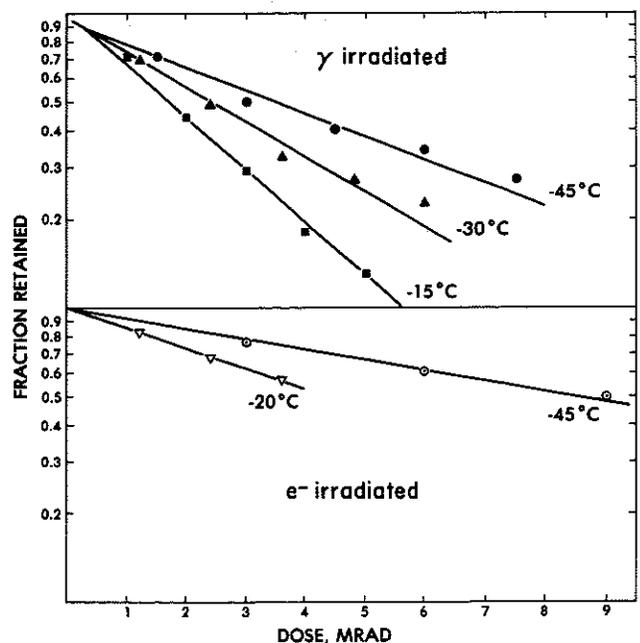


Fig. 1—Fraction of thiamin retained as a function of dose. Each point is an average of two samples. Initial temperatures are given; temperatures throughout irradiation rose less than 5°C . All samples had an initial concentration of thiamin of about 0.9 mg/100g pork.

only two points are shown for the electron irradiated samples. However, two preliminary experiments were conducted at -37°C and -25°C that are consistent with the basic findings shown here. Thirdly, this plot is not valid for determining an "activation energy," except when m in the above expression is small. A plot of m vs $1/T$ is valid and has been made, but is not shown.

Dose-rate effect. The differences in thiamin loss between gamma ray and electron beam irradiation, which are reflected in the less steep slopes of Figure 1 and the correspondingly lower fractional losses of Figure 2, are shown to be consequences of a dose-rate effect. Relevant results were obtained for samples containing initially about 0.9 mg/100g pork irradiated at a fixed temperature to a fixed dose using the linear accelerator. Since it is possible to vary the peak current (by approximately a factor of 50) in the short pulses of the electron beam, three different dose-rates were used. These results are given in Table 4.

To show the comparison more graphically, and to suggest what other experiments may have to be done, the electron irradiation results and the relevant gamma ray irradiation result are plotted in Figure 3. The thiamin loss after 6 Mrads of irradiation is shown, in effect, as a function of dose-rate, which is proportional to beam current. The dashed line represents the continuous gamma ray irradiation, which corresponds to a very low dose-rate of about 10^{-6} of the usual dose-rate associated with the 600 ma electron beam. The dotted line is assumed to correspond with the increased loss that would be encountered at dose-rates lower than those actually used. The solid curve was drawn through the data points on the basis of upper and lower limits.

DISCUSSION

THE LOSS OF THIAMIN upon irradiation in the frozen state and the dependence of this loss on dose, temperature, and dose-rate can be understood by considering the nature of the pork system, the basic radiation phenomena, and specific radiation chemical mechanisms.

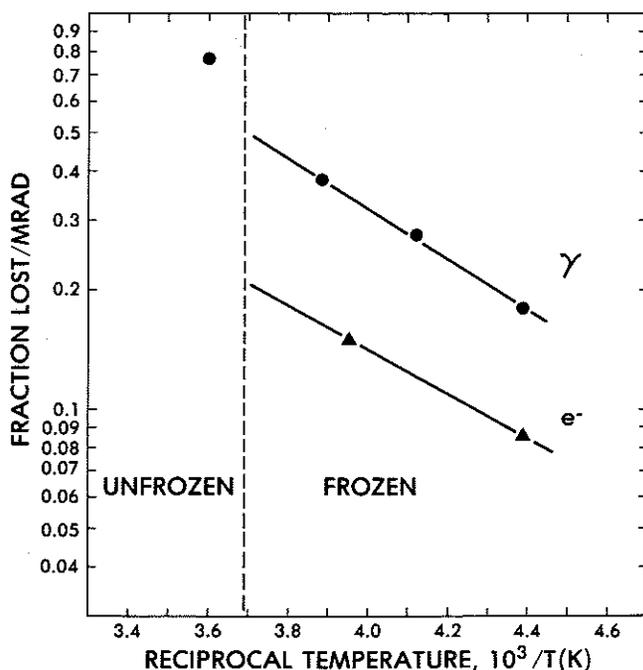


Fig. 2—Fraction of thiamin lost per Mrad of absorbed dose as a function of reciprocal temperature. Vertical dotted line corresponds to the transition point of 271°K (-2°C).

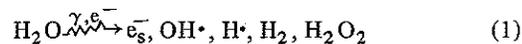
Frozen pork system

In terms of the radiolysis, the frozen pork can be considered as having three independent phases: the aqueous phase; the hydrated myofibrillar protein phase; and the lipid phase. The frozen aqueous phase is primarily polycrystalline and contains vitamins, soluble proteins (myoglobin, serum albumin), small peptides, free amino acids, and added salts. Thiamin, either free or bound to specific proteins (*Methods of Vitamin Assay*, 1966), will be considered as occurring primarily in this phase. Analysis of thiamin in an aqueous extract from a compressed cooked sample, in fact, accounted for over 90% of the total for that sample.

Basic radiolytic phenomena

The exposure of an aqueous phase to high energy irradiation leads to the formation of ions, excited molecules, and free radicals from the water that are initially distributed non-uniformly in regions called "spurs." Most of these reactive entities recombine in the spurs, while some diffuse away reacting with neighboring solutes, depending on the temperature and/or the viscosity of the system. Ultimately, a small fraction of these initial entities becomes uniformly distributed, particularly the smaller and more mobile entities.

For water, both in the liquid and frozen states, the radiolysis is described by reaction (1):



The yield of each entity (which is given by the G-value, the number of molecules formed per 100 ev of absorbed energy) depends on the phase and temperature. In ice, $G(e_s^-)$ is ~ 0.3 at -5°C and decreases rapidly with decreasing temperature

Table 4—Effect of dose-rate on thiamin in pork

Temp $^{\circ}\text{C}$	Dose Mrad	Current ma	Retention %
-45	6.0	600	60
-45	6.0	150	63
-45	6.0	24	52

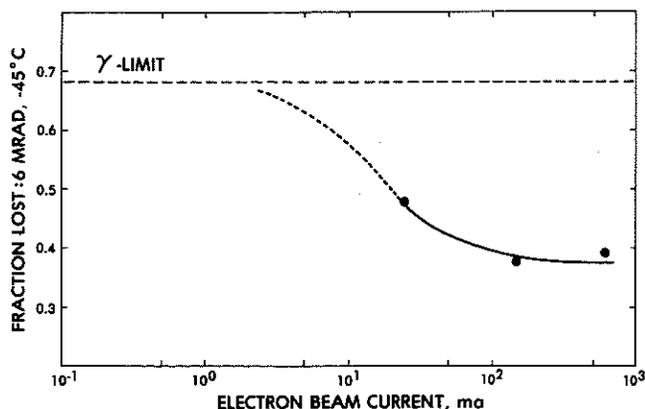


Fig. 3—Fraction of thiamin lost at -45°C for a 6 Mrad dose as a function of dose-rate. The abscissa is given in milliamperes (ma) of peak electron beam current during the $\sim 5 \mu\text{sec}$ pulses from the LINAC. Dose-rate is proportional to beam current. The solid line is drawn through the points corresponding to the indicated peak currents. Each point represents the average of two samples. The dashed line represents the results for gamma ray irradiation, which corresponds to a low dose-rate limit. The dotted line is drawn hypothetically to connect the lower and upper dose-rate limits.

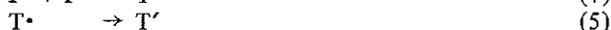
(Taub and Eiben, 1968); $G(H\cdot)$ is ~ 0.7 ; and $G(OH\cdot)$ is ~ 1.0 . Both e_s^- and $H\cdot$ are mobile in ice at -45°C . Reactions of these radicals, which have been extensively studied in liquid water, could take place to a limited extent with solutes in the ice, depending on temperature and solute concentration.

Two possible sequences of reactions could occur in the aqueous phase of the pork that would be relevant to the effects observed. One involves a competition by the soluble proteins and the thiamin for the primary water radicals; the other involves reaction between secondary radicals formed and the thiamin. These are designated below as Scheme I (reactions 2–5) and Scheme II (reactions 3, 6, 7, 5), in which only $H\cdot$ as representative of other water radicals is shown; P and T represents proteins and thiamin, respectively; $P\cdot$ and $T\cdot$ represent the radicals derived from them; and T' represents a new product resulting from the decay of $T\cdot$.

Scheme I



Scheme II



Note that in Scheme I some $H\cdot$ reacts with T, while in Scheme II all $H\cdot$ reacts with P and some of the resulting $P\cdot$ reacts with T. A similar scheme could be written for e_s^- and for $OH\cdot$.

Evidence has been presented for the reactions of e_s^- and $OH\cdot$ with solutes in frozen aqueous systems (Taub et al., 1978) and for the presence and decay of protein radicals in frozen systems of amino acids, peptides, and sarcoplasmic and myofibrillar proteins (Taub et al., 1979a; Shieh et al., 1980). Such evidence would indicate that reaction 3 could compete against reaction 2 at high concentrations of P, but that reaction 4 could only account for a small fraction of the decay of $H\cdot$, since the concentration of T is low. Such evidence also indicates that the molecular dimensions of $P\cdot$ and the temperature determine the likelihood of $P\cdot$ decay in these systems. Small radicals, such as dipeptide radicals, decay at about -60°C , while large myosin radicals decay at -10°C . No direct evidence, however, is available to show that $P\cdot$ would react with T.

Dose-rate effect

Three conditions have to be met for dose-rate to influence the loss of thiamin in the frozen pork. First, the free radicals reacting with thiamin must be short-lived, short enough to decay to a significant extent between successive pulses of the linear accelerator. Second, these radicals must be uniformly distributed to conform to simple laws of kinetics. And third, they must have concurrent pathways

for reaction conforming at least to second- and first-order processes. In effect, the radicals must be formed in a high, uniform concentration and rapidly decay in a mixed order process after each pulse. Otherwise, they would continue to increase in concentration until a steady-state level is achieved, as happens upon continuous gamma ray irradiation. The basis for these and related considerations have been discussed previously by Taub et al. (1979b).

Direct evidence, using electron spin resonance (Halliday et al., 1979), has been obtained recently indicating that the radicals formed from either the drippings from cooked pork or the juices expressed from this meat decay rapidly at $>-60^\circ\text{C}$. This evidence is consistent with other data obtained from experiments on frozen water and frozen solutions of myoglobin. Consequently, the first condition is met.

No directly applicable evidence is available relating to the uniform distribution of these radicals. However, the highly mobile e_s^- reacts in crystalline ice at -14°C by a second-order process, consistent with a uniform distribution (Taub and Eiben, 1968). The same can be assumed for $H\cdot$. Other radicals, such as those from the soluble protein, should be nearly uniformly distributed. In this case, the solutes probably retain almost their original (before freezing) distribution and they give rise to radicals upon reaction with water radicals that have dispersed or are constrained in spurs close to them. Consequently, the second condition is assumed to be met at or above -45°C .

No direct evidence is available relating to the mixed order decay of those radicals capable of reacting with thiamin. It is reasonable, however, to expect $H\cdot$ to decay both by reaction with another $H\cdot$ or with P or T. The former is a true bimolecular process; the latter is a pseudo-first-order process, since the concentration of P and T are high compared to $H\cdot$. A similar situation could prevail in which $P\cdot$ reacts with another $P\cdot$ in competition with reacting with T. Consequently, the third condition is hypothesized to account for the observations.

The results shown in Figure 3 can be explained on the basis of these conditions being met in either Scheme I or Scheme II. At the very low dose-rates corresponding to gamma ray irradiation, the low steady-state concentration of $H\cdot$ (Scheme I) favors reactions (3) and (4) over reaction (2), and more thiamin is lost. Similarly, the low steady-state concentration of $P\cdot$ (Scheme II) favors reaction (7) over reaction (6). As the dose-rate is increased, such as in the electron beam irradiation using 24 ma current, the instantaneous concentration of $H\cdot$ (or $P\cdot$) after each pulse is high enough for some bimolecular combination reaction to occur, diverting radicals away from thiamin and decreasing its loss. At the still higher dose-rates corresponding to the electron irradiation at peak currents of 150 and 600 ma, a considerable portion of the radicals decay bimolecularly and therefore more are diverted from thiamin. [It is possible that a high dose-rate limit is reached whereby only reaction (2) [or reaction (6)] occurs and the thiamin is lost solely due to direct effects.]

Further proof of this interpretation must await additional experiments at dose-rates equivalent to peak currents below 24 ma and at higher initial thiamin concentrations. If these schemes are applicable, then (provided the data are expressed in terms of number of moles lost) the S-shaped curve in Figure 3 would shift to the right at a higher thiamin concentration, since reaction (4) [or reaction (7)] becomes more important at any fixed dose-rate.

Temperature effect

The increase in thiamin loss as the temperature increases from -45°C to -15°C can be understood in terms of a higher yield of a primary radical and/or a difference in

Table 5—Thiamin retention in beef and chicken after processing^a

Process	Product	
	Beef	Chicken
Thermal	21%	22%
Gamma irradiated	23%	27%
Electron irradiated	44%	66%

^a Data adapted from Letterman Army Institute for Research, McGown et al. (1979a, 1979b).

activation energies of the reactions relating to the competition between thiamin and other entities for the relevant radical.

If e_s^- contributes to the thiamin loss through reactions analogous to (3) and (4) in Scheme I or just (3) in Scheme II, then the increased loss reflects the increase in $G(e_s^-)$ over this temperature range. Since the G-value for thiamin loss at -15°C in low dose-rate irradiation is about 0.01 and $G(e_s^-)$ at this temperature in ice is 0.16, only a small fraction of the solvated electrons either reacts with the thiamin or forms protein radicals that subsequently react with the thiamin.

If the increased thiamin loss is exclusively or additionally due to activation energy differences, then reactions (3) and (4) Scheme I or reactions (6) and (7) in Scheme II would most likely be involved. In both schemes, the reaction involving thiamin would have to have the higher activation. However, it is likely that $\text{H}\cdot$ reacts with thiamin in (4) by a low activation "addition" process, while $\text{H}\cdot$ reacts with proteins in part by a high activation "abstraction" process. The effect therefore would be opposite to that observed. On the other hand, it is likely that $\text{P}\cdot$ reacts with thiamin in (7) by a high activation abstraction process compared to the low activation radical combination process given by (6). The effect in this case would be as observed.

In any case, the temperature effects are the same for both gamma ray and electron irradiation, implying that the same entities and the same mechanisms are involved.

Dose effect

The loss in thiamin according to Schemes I and II should increase with dose, but the exact functional dependence for all the different conditions cannot be predicted from the data currently available. For the loss in the frozen system at low dose-rate, the exponential dependence can be explained on the basis of Scheme I. Under these conditions, $\text{H}\cdot$ reaches a low steady-state concentration and the loss of thiamin can be expressed as follows:

$$-d[\text{T}]/dD = ck_4[\text{T}]/(k_3[\text{P}] + k_4[\text{T}]),$$

where c is a constant and k_3 and k_4 are appropriate rate constants. Since the concentration of thiamin is so low compared to the protein, it is valid to eliminate $k_4[\text{T}]$ from the denominator on the right-hand side and to consider $[\text{P}]$ as constant, giving

$$-d[\text{T}]/[\text{T}] = (ck_4/k_3)dD,$$

which leads to the exponential loss expression. This relation might not hold for systems with much higher thiamin concentrations.

Comparison with other meats

These results for pork irradiated under strictly controlled conditions are consistent with results for thiamin loss in beef and chicken irradiated on a production basis for animal feeding studies. Samples of this beef and chicken were used by the Letterman Army Institute for Research to investigate, and ultimately to demonstrate the absence of, any anti-thiamin properties. Their analyses of the irradiated and thermally processed samples are shown in Table

5. Note that the electron irradiated products retain 2–2.5 times as much thiamin as the gamma ray irradiated products.

Comparison of irradiated and thermally processed pork

Comparison of pork products having similar shelf-stability shows that irradiation processing leads to equivalent or greater thiamin retention than thermal processing. In both cases, sterilized products are being considered. The thermal processing corresponds to a $F_0 = 6$ at 121°C ; the irradiation processing would be done with a dose at 4.5 Mrad at -45°C . In this report, the former leads to a range of 10–20% thiamin retention (Tables 3 and 5). The latter, if done with gamma rays (low dose-rate), leads to about 42% retention; but if done with electrons (high dose-rate), leads to a 69% retention (Tables 3 and 4).

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