AN EXPONENTIAL MODEL EQUATION FOR THIAMIN LOSS IN IRRADIATED GROUND PORK AS A FUNCTION OF DOSE AND TEMPERATURE OF IRRADIATION

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Abstract—The effect of low dose γ-irradiation on the thiamin content of ground pork was studied in the range of 0–14 kGy at 2°C and at radiation doses from 0.5 to 7 kGy at temperatures −20, 10, 0, 10 and 20°C. The detailed study at 2°C showed that loss of thiamin was exponential down to 0 kGy. An exponential expression was derived for the effect of radiation dose and temperature of irradiation on thiamin loss, and compared with a previously derived general linear expression. Both models were accurate depictions of the data, but the exponential expression showed a significant decrease in the rate of loss between 0 and −10°C. This is the range over which water in meat freezes, the decrease being due to the immobolization of reactive radiolytic products of water in ice crystals.

INTRODUCTION

The use of low dose irradiation (0.3–1.0 kGy) of pork to kill trichinae was approved in 1985 by the Food and Drug Administration, and in 1986 by the Food Safety Inspection Service of the Department of Agriculture (1986). The effect of low-dose irradiation on the vitamins of meat is not well-defined, for most of the work done to date has been carried out at sterilizing doses of irradiation (tens of kiloGrays), and usually at very low or unreported temperatures (Alexander *et al.*, 1956; Josephson *et al.*, 1978: Kennedy and Ley, 1971; Thomas and Calloway, 1957; Thomas *et al.*, 1981). Groninger *et al.* (1956) observed no thiamin losses at low doses up to 3.0 kGy, implying that there is a threshhold value below which thiamin is not destroyed. The effect of temperature on thiamin loss in minced beef was studied by Wilson (1959) who found that at temperatures below freezing, where most of the water in the meat is immobilized as ice, the rate of thiamin loss was reduced with respect to the loss that occurred in the unfrozen state. Since low dose irradiation is to be carried out under commerical conditions with temperatures varying about the freezing point of meat, a predictive equation is needed to fit the data with as high a degree of accuracy and precision as possible. This study was initiated to determine the mechanism and significance of the loss of thiamin in pork at low doses of γ-radiation at temperatures ranging about freezing, with and without cooking, and to develop a predictive model for the data, based on previous observations.

EXPERIMENTAL

Sample preparation for irradiation

Ground pork was used in order to have as homogeneous a substrate as possible. Pork loins were purchased from a local meat wholesaler from carcases of the previous day's slaughter, and were placed in a glove bag flushed with nitrogen. Lean meat from the eye was trimmed of fat and ground twice in a meat grinder through a 3/16″ (5 mm) plate, and thoroughly mixed. *Ca* 20 g portions were then placed into low oxygen/water permeable bags (IKD ALLVAK no. 13, International Kenfield Distribution Co., Rosemont, IL), pressed out into squares about 6 cm on a side, 0.5 cm thick, removed from the glove bag and heat-sealed under vacuum. The details for the preparation and irradiation of pork chops whose thiamin data were also analyzed exponentially, are given by Fox *et al.* (1989).

Irradiation

All meat samples were irradiated in a cesium 137 source operating at 0.125 kGy/min. Dose rates were determined by the ferrous-cupric sulfate dosimetry method of Jarrett and Halliday (1979). Pouches containing the ground pork samples were arranged in a wire basket so that all samples received equal radiation. For experiments with long radiation periods, two sets of samples were placed in the basket, the basket was removed from the source after one of the shorter periods had elapsed, the appropriate samples were removed and the basket was returned to the source to complete the irradiation. The temperature was controlled by placing a thermocouple in the radiation chamber, which controlled a valve in a nitrogen line to the chamber. When the temperature rose, the valve opened and admitted the cold gaseous phase of liquid nitrogen to cool the chamber. Control of the chamber was within ± 1.5°C, varying equally and regularly about the target dose. The internal temperature variation of the samples was measured by inserting a thermocouple into a 3/8 in. thick pork chop and measuring the temperature during several cycles of the chamber variation. The variation was less than the noise, which itself was equivalent to less than 0.01°C.

Radiation protocols

The first experiment was a test of radiation doses ranging from 0.25 to 14 kGy at 0°C. The second experiment used a protocol (cf. Table 1) based on a central composite design for the determination of a second order response surface (John, 1971). Samples were held in a refrigerator at 0 ± 0.5°C overnight before irradiation, and sample preparation was initiated immediately after irradiation.

Heating/cooking

One half of the samples of the second ground pork study were heated at 82.5°C by submerging the pouches in a temperature controlled water bath for 10 min followed by immersion in cold water. The cooked samples had some drip loss, but the drip was mixed with the solid portion of the samples and the whole analyzed. The pork chops were cooked on an electric grill until an internal temperature of 76°C was reached (Fox *et al.*, 1989). The moisture contents of the raw and cooked pork chops were 72.3 ± 0.2% and 52.1 ± 1.4%, respectively, for a cooking loss of 37.3 ± 1.4%. There was no statistically significant variation of any of these values with radiation dose or temperature of irradiation.

Sample preparation for thiamin determination

Technicon procedure 479–77A (1977) was used, with one modification. The weighed meat samples were placed into 250 ml tall form beakers containing 50 ml 0.1 N HCl, instead of the volumetric flasks specified by Technicon. This made it easier to disperse the samples into the acid, handle them during autoclaving, adjust the pH and add the enzyme after autoclaving. To disperse the comminuted samples in the acid solutions, a length of nylon rod 5 cm by 1.7 mm diameter (as used in grass whips or weed whackers) was mounted in a snug fit hole drilled on a diameter at one end of a 1/4 in. stainless steel rod. The rod was mounted in the chuck of a variable speed laboratory stirrer. The sample was dropped into the acid solution, the whip immersed and the motor speed brought up to *ca* 800 rpm, just fast enough to disperse the particles without splashing. From 10 to 20 s were sufficient to thoroughly disperse the samples. The samples were then autoclaved for 0.5 h at 15 psig (pounds/in^2 gauge or 1.054 kg/cm^2 above atmospheric), cooled and adjusted to pH 4.3 with sodium acetate. Then 1 ml of 5% α-amylase (Sigma A-0273) was added and the samples incubated overnight at 3°C. After incubating, the samples were quantitatively transferred to 100 ml volumetric flasks and brought to volume with pH 4.3 acetate/metaphosphoric buffer. The solutions were clarified by filtration through Whatman No. 2V filter paper.

Thiamin determination

The concentration of thiamin was determined using a Technicon Autoanalyzer according to the manufacturer's instructions (Technicon, 1977). In this procedure, thiamin is determined fluorometrically as the thiochrome resulting from oxidation of the vitamin with alkaline potassium ferricyanide. The thiochrome is extracted into isobutyl alcohol in order to eliminate interference from other fluorescing compounds.

Data management

The data were converted to μg vitamin/g wet meat in a Lotus 123 worksheet (1986) and transferred to a SAS worksheet (SAS, 1985). Two equations were used to fit the data. The first was a linear equation developed for remaining concentration:

$$[T]_{t,d} = a_0 + a_1 \cdot d + a_2 \cdot t + a_3 \cdot d \cdot t + a_4 \cdot d^2 + a_5 \cdot t^2; \quad (1)$$

in which the factors are: $[T]$, μg thiamin/g pork; t, temperature in °C; d, dose in kGy; and a_0, the initial concentration. The calculations on the linear model were made using the regression procedures of John (1971).

The second equation was developed from previous observations concerning the dose and temperature dependence of the thiamin loss. From the observation that the loss of thiamin is exponential with dose (Thomas *et al.*, 1981), the following equation was written:

$$[T]_d = a_0 \cdot e^{-kd}; \quad (2)$$

where $[T]_d$ is the concentration after dose d, a_0 is the initial concentration and k is the dose constant. The dose dependence of the reaction was a function of the temperature, so the following expression was written:

$$k = a_2 \cdot t + a_1; \quad (3)$$

where a_2 represents the dependence of k on the temperature and a_1 is a factor which allows for the possiblity that the temperature curve might not pass through the origin. Another factor is needed to account for the reduction in the dose dependence which takes place during freezing. The mathematical term which will effect such a shift is:

$$a_3\left[\frac{e^{(t-c)}}{1 + e^{(t-c)}}\right]; \quad (4)$$

where c is a temperature in the center of the range of interest, in this case the midpoint of the range over which meat freezes, about -5°C. At negative values of the exponent $(t - c)$ the fraction in the parentheses tends towards zero, at positive values it goes to one. In going from temperatures below -5°C to temperature above, the value of the entire expression goes from 0 to a_3, the magnitude of the difference between the dose dependence above and below the range of the shift. Combining equations (2, 3 and 4) and inserting the appropriate temperature, the following expression is obtained:

$$[T]_{t,d} = a_0 \cdot e^{-d}\left\{a_2 \cdot t + a_3\left[\frac{e^{(t+5)}}{1+e^{(t+5)}}\right] + a_1\right\}. \quad (5)$$

An iterative nonlinear regression program (SAS, 1985) was used to fit this exponential model to the data. For both calculations, the zero temperature/zero dose value was included for each temperature of irradiation.

RESULTS

Dose

The result of the study of the loss of thiamin over a broad range of doses is shown in Fig. 1. The loss of thiamin was an exponential function of the dose over the entire range studied, and the exponential curve passed through the initial value. The equation for the regression is:

$$\ln[T] = 2.072 - 0.112\,\text{kGy} \quad R^2 = 0.992.$$

Temperature/dose

Table 1 lists the values for the dose/temperature study and Table 2 lists factors for the remaining thiamin concentration for both the linear and exponential model equations. The data from a study of the thiamin content of pork chops (Fox *et al.*, 1989) was also fitted with the exponential expression and is shown in Table 2. For all factors, a negative coefficient indicates loss of vitamin and a positive coefficient an increase. As will be noted from the R^2 values, both model equations for thiamin are highly significant, both increasing temperature and dose resulting in increasing losses of vitamin. In the exponential equation the offset term coefficient (a_3) is highly significant, that is, there is a discontinuity in the data for the effect of temperature on the dose dependence in the range from -10 to 0°C. The difference in the calculated curves for the two equations, especially with respect to the temperature dependence, is seen in Fig. 2.

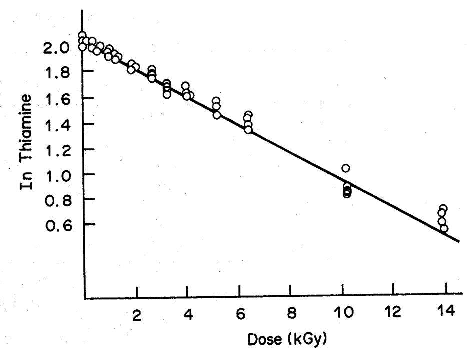

Fig. 1. Effect of irradiation of thiamin over a broad range of doses at 2°C.

DISCUSSION

Exponential loss of thiamin

The data of this study do not show the 3 kGy threshold value reported by Groninger *et al.*, (1956) as the exponential curve passed through the initial concentration of thiamin. The lowest dose reported

Table 1. Micrograms thiamin per gram of irradiated ground pork

	Dose in kiloGray					
Temperature (°C)	0	0.50	1.75	3.50	5.25	7.0
Raw						
−20		8.60 (0.10)[1]		7.58 (0.21)		
−10			7.82		7.10 (0.45)	
0>	8.40[2] (0.16)	7.85 (0.18)		5.51[2] (0.48)		4.15 (0.11)
10			6.28 (0.28)		4.05 (0.02)	
20		7.15 (0.12)		4.58 (0.11)		
Cooked						
−20		6.73 (0.18)		6.12 (0.35)		
−10			6.24 (0.05)		6.04	
0>	7.38[2] (0.28)	6.98 (0.12)		4.44[2] (0.46)		3.36 (0.03)
10			5.37 (0.05)		3.51 (0.14)	
20		6.33 (0.33)		3.79 (0.01)		

[1]Numbers in parentheses are standard deviations.
[2]All values are averages of duplicates except for these values for which $n = 5$.

Table 2. Factors of the two model equations for μg thiamin/g meat in irradiated ground pork and pork chops

Linear model equation

$$[T] = a_0 + a_1 \cdot d^1 + a_2 \cdot t^1 + a_3 \cdot d \cdot t + a_4 \cdot d^2 + a_5 \cdot t^2$$

Thiamin	R^2	a_0	a_1	a_2	a_3	a_4	a_5
In ground pork							
raw	0.950	8.30	−0.85	−0.0054	−0.0240	0.0443[2]	0.003[3]
cooked	0.942	7.31	−0.95	−0.00042	−0.0177	0.066	0.0001[3]
In pork chops							
raw	0.920	11.03	−1.29	−0.0077	−0.0233	0.0411[3]	0.009[3]
cooked	0.912	12.16	−2.30	−0.0279	−0.0283[4]	0.166	0.00022[3]

Exponential model equation
(in logarithmic form)

$$\ln[T] = \ln a_0 + a_1 \cdot d + a_2 \cdot d \cdot t + a_3 \cdot d\left[\frac{e^{(t+5)}}{1+e^{(t+5)}}\right]$$

	R^2	a_0	a_1	a_2	a_3
In ground pork					
raw	0.958	2.10	−0.061	−0.00286	−0.0437
cooked	0.937	1.94	−0.070	−0.00234	−0.0452
In pork chops					
raw	0.949	2.42	−0.101	−0.00179	−0.053
cooked	0.938	2.48	−0.149	−0.00313	−0.0459

[1]d is dose in kGy, t is temperature in °C. All values in the table are highly significant ($Pr > F = 0.0001$) except for: [2]significant at the $Pr > F = 0.0023$ level; [3]not significant; [4]significant at the $Pr > F = 0.019$ level.

by Thomas *et al.* (1981) was 1 Mrad (10 kGy), yet the curves they obtained also extrapolated through the initial value at zero time. They reported a value of 76% destruction at 1 Mrad (10 kGy), 5°C, which, if translated into a first order dose constant, gives a value of 0.135 kGy^{-1} comparable to the values of 0.122 and 0.105 kGy^{-1} for the raw ground pork experiments (multiple dose and dose/temperature experiments, respectively). Similar exponential dependences have been reported for the thermal degradation of thiamin, (Proctor and Goldblith, 1952), X-ray degradation of cocarboxylase (thiamin pyrophosphate), (Mulley and Stumbo, 1975), electron radiation degradation of both histidine and pepsin (Ebert and Swallow, 1957) ascorbate, (Proctor *et al.*, 1952) and biotin (Watanabe *et al.*, 1976) and in fact is common to almost all food component degradations (Thompson, 1982). During the process of irradiation, the hydrolytic radicals are produced at a constant rate, and if produced in excess over the compounds with which they are reacting, will remain at constant concentration. Under these conditions, the reaction rate becomes dependent on the concentration of the compound reacting with the radical, that is, the reaction is first order with respect to the reacting compound.

Comparison of the two model equations

Dose. Figure 2 shows the three dimensional curves (response surfaces) for raw ground pork, derived from the linear and exponential expressions. The regression line for the linear expression (2B) shows a concave upwards curvature with dose, the net result of the negative dose term (a_1) and the positive dose^2 term (a_4). The dose^2 term becomes comparatively larger with dose than the negative value of the dose term, causing an upwards curvature. The curvature reflects the exponential character of the effect of radiation dose, which in the log plot appears as a straight line. Both equations fit the dose curve with a high degree of significance $Pr > F \leqslant 0.0001$ (probability that the effect was due to random variation was less than 0.0001).

Temperature

The linear expression does not show any curvature with temperature. Since the exponential equation shows the temperature dependence curve to be sigmoid, a single inflection curve represented by a two term (t and t^2) expression would not show the double curvature. The temperature^2 term coefficient (a_5) was

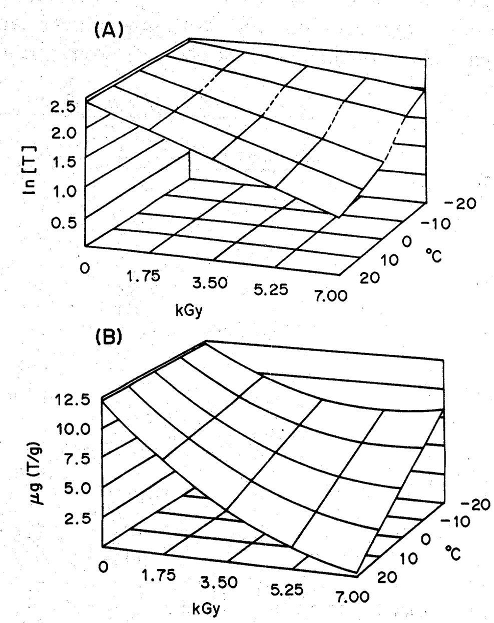

Fig. 2. Exponential (A) and linear (B) three dimensional plots (response surfaces) for the loss of thiamin as a function of dose and temperature of irradiation. [T] in μgT/g.

not significant—statistically or mathematically—for either ground pork or pork chops, raw or cooked (Fox *et al.*, 1989). In the exponential equation, however, the offset term coefficient (a_3), representing the discontinuity through the freezing range, was highly significant for both ground pork and pork chops. Furthermore, it was of the same magnitude as the other dose terms. For example, in raw ground pork the sum of the coefficients a_1, a_2 and a_3 goes from -0.032/kGy at -10°C to -0.105/kGy at 0°C, which difference is the result of a decrease of 0.029/kGy due to the temperature term (a_2) and 0.044/kGy due to the offset term (a_3). In raw pork chops, the sum goes from -0.083 to -0.154, due to decreases of 0.018/kGy for a_2 and 0.054/kGy for a_3. From the results it is evident that the exponential expression is the preferred model for the temperature dependence.

The a_3 term in the exponential equation has the form of an associative phenomenon. The decreased rate of vitamin destruction in frozen meats has been attributed to immobilization in the ice crystals of the radical compounds formed from the irradiation of water. Since the relative number of molecules in the frozen and liquid states is a function of the temperature of the meat, the a_3 term may be thought of as representing the equilibrium of water molecules between the two states. The term therefore is a measure of the decrease in availability of radicals for the reaction, further modified by a factor allowing for the increased concentration of the reactants due to exclusion of the latter from the ice crystals.

The cross product term coefficient, a_2, is also highly significant and is a measure of the effect of temperature on loss of thiamin due to irradiation. The factor introduces about a 3% increase in the dose response per 10°C for both ground pork and pork chops. The derivation makes no provision for a difference in the slope in the frozen and unfrozen states, but if the mechanism of the reaction of the radicals produced from the irradiation of water with thiamin were to be the same above and below freezing, the slopes would be the same. If water were to continue to crystallize out of solution below -10°C, the temperature curve in the frozen meat would be expected to have a slightly higher slope than the curve in the unfrozen meat.

Effect of cooking

The effect of cooking was significant ($Pr > F \leqslant 0.0001$) for the thiamin content of ground pork (a_0). The expressed liquid from the ground pork was mixed with the solid material before thiamin determination, so that the difference represented thermal loss due to cooking. It will be noted in Table 2 that the concentration of thiamin/g of meat in the cooked pork chops was 10% higher than in the uncooked chops. Since the chops lost about 37% of their weight during cooking, the loss of thiamin was about 30% $[(11.03 - \text{loss})/0.63 = 12.16, \quad \% = \text{loss} \times 100/11.03]$. This loss may have been due to thermal degradation or to drip loss (Thomas and Calloway, 1957; El-Bedewy *et al.*, 1978) since only the meat of the chop was analyzed. The only other effect of cooking was to cause an increase in the loss due to radiation in the cooked chops as compared with the raw (factor a_1 in both expressions, $Pr > F \leqslant 0.0001$). Regardless of these differences, the sigmoid shape of the temperature dependence curve was retained after cooking.

CONCLUSION

The data for thiamin loss may be fitted with either an exponential or a linear model expression to a high degree of precision. In view of the initial broad dose range experiment and previous results in the literature, the exponential model is more in keeping with the chemical basis for the loss. In addition, the exponential expression shows an offset in the temperature curve over the range in which meat freezes, which the linear expression does not show. For greater accuracy and precision the exponential form is preferred.

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