

D59-15

RADIATION PROCESSING OF MEATS

**A Time-Temperature Relationship
for Heat-Enzyme Inactivation of
Radiation-Sterilized Beef and Pork**

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Irradiated raw meat products are not stable upon extended storage at ambient temperatures, because of residual enzyme activity, particularly proteolytic enzyme activity. Control of enzyme activity is possible by the use of heat, and this study defines the parameters of time and temperature—from 140° to 170° F.—required for inactivation of proteolytic enzymes in 5-megarad irradiated beef and pork meats. Data indicate that the irradiation causes no significant sensitization of the enzymes to heat at the temperatures used.

THE ATTAINMENT OF A RAW product which may be stored for extended periods of time without refrigeration was an original objective for the use of ionizing radiation in meat processing. Studies have indicated, however, that such irradiated raw meat products are not stable upon extended storage at non-refrigerated temperatures (1, 2, 4). Heat is at the present time the only practical method known for control of proteolytic enzyme activity in a meat product.

To design processing procedures for radiation-stabilized meat products, the relationship of time and temperature which would be required to inactivate tissue proteolytic enzymes in radiation-stabilized meats was studied. The proteolytic enzymes were chosen for this testing, because their activity correlated with the deterioration of radiation-stabilized meats on storage. Tissue proteolytic enzymes are relatively heat-stable, while most of the other enzymes present in the system are inactivated. Catalase was inactivated in samples which still had proteolytic enzyme activity.

Initially, the proteolytic enzyme was measured by use of a standard hemoglobin substrate and the Folin-Ciocalteu reagent. This approach was abandoned, because the assay was not sensitive enough at low levels of enzyme activity. Any data so obtained would require checking for application to muscle enzymes *in situ*.

The data herein reported have been obtained on muscle proteolytic enzyme *in situ*. Thin slices of meat in a flexible packaging material were used to minimize the heat transfer effects in the water bath. Incubated storage increased the sensitivity of the assay. Irradiation was used pre- or postheating in order to determine whether the enzymes were sensitized to heat inactivation by the irradiation. An increase in the sensitivity of *Clostridium botulinum* to heat after irradiation has been reported (3).

An irradiation dose of 5 megarad was used in these studies because investigations in other parts of the program have indicated that a dose in this range would be necessary to achieve a sterilizing radiation process with the same factor of microbiological safety as is used in thermal processing.

Experimental

Beef. Ten pounds of choice grade, frozen beef chuck were sliced into sections approximately 2 mm. thick, and circular pieces of meat 2 inches in diameter were cut from the lean portion of these sections. Each of these circular portions of meat was placed in a 2.5-inch packet made of a polyethylene-coated polyester film. (Scotchpak, Minnesota Mining & Mfg. Co., was chosen because of its resistance to changes upon irradiation and low gas transfer rate.) The packets were air evacuated and heat

sealed. The meat was kept frozen during both the cutting and packaging procedures. The packets were arranged by weight in sets of seven. The weights between sets varied from 2.1 to 5.3 grams, but the maximum variation within each set was 200 mg. The sets of lower weight were used at higher inactivation temperatures—where exposure time was short—in order to minimize the heat penetration time.

Temperatures of 140°, 150°, 155°, 160°, 165°, and 170° F. were used where the heat treatment was prior to irradiation and temperatures of 140° and 160° F. where the heat treatment was post-irradiation. At each of these temperatures, six heating intervals were investigated. The intervals were chosen so that three would be shorter than the ones requiring complete inactivation (as determined from preliminary experiments). For each interval at a given temperature there were seven packets; three were maintained frozen for controls and four were incubated for 6 weeks at 100° F. The incubated samples were held in tightly sealed jars in order to avoid loss of moisture during the storage period.

The temperature-controlled water bath used was of large volume. All of the samples for each specific temperature were thawed and immersed together. The packets at each heating interval were then removed and cooled rapidly in ice water. For irradiation, the packets were frozen, canned, and shipped in frozen

Table I. Decrease of Amino-Nitrogen Production in 5-Megarad Irradiated Beef and Pork after Heat-Enzyme Inactivation Treatment and 6 Weeks' Incubation at 100° F.

		Bath Temperature											
		140° F.		150° F.		155° F.		160° F.		165° F.		170° F.	
Time, min.	Amino N ^a		Time, min.	Amino N ^a , HI	Time, min.	Amino N ^a , HI	Time, min.	Amino N ^a		Time, sec.	Amino N ^a , HI	Time, sec.	Amino N ^a , HI
	HI ^b	IH						HI	IH				
BEEF													
0	135	109	0	143	0	167	0	159	127	0	125	0	162
8	101	86	4	46	1	103	1/2	114	64	10	118	5	165
16	72	65	6	33	3	47	1	67	45	20	99	10	136
20	62	59	8	28	6	36	2	27	27	40	40	20	34
24	53	53	10	29	10	39	4	18	22	60	26	30	26
32	54	49	16	25	15	35	6	15	19	90	27	60	24
PORK													
0	222	192	0	214	0	248	0	248	204	0	220	0	220
8	170	168	4	122	1/2	196	1/2	196	120	5	214	5	214
16	140	140	6	74	1	156	1	156	78	10	190	10	190
20	126	120	8	60	2	68	2	68	38	20	154	20	154
24	116	112	10	52	4	32	4	32	26	30	128	30	128
32	98	106	16	44	6	22	6	22	16	60	122	60	122

^a Amino nitrogen expressed as μ mole of tyrosine per gram of meat. Values are net—i.e., averaged amino-nitrogen content of counterpart samples kept frozen (average $80 \pm 12 \mu$ moles for pork and $37 \pm 6 \mu$ moles for beef) has been subtracted from the average of the four incubated replicates.

^b HI = heat treated preirradiation. IH = heat treated postirradiation.

condition to the gamma-radiation facility.

After the samples had been removed from storage, packets were placed in a boiling water bath for 10 minutes so that all samples were brought for analyses to a similarly cooked (denatured) condition. The meat from each packet was macerated in successive 1-ml. aliquots of 5% trichloroacetic acid (TCA) until a volume of 6 ml. was obtained. These extracts were allowed to stand in water baths at 55° to 60° C. for 30 minutes before filtering in order to increase the coagulation and precipitation of the trichloroacetic acid-insoluble materials. One milliliter of the filtrate was diluted to 200 ml. with distilled water, and the resulting solution was assayed for amino nitrogen using the ninhydrin method of Moore and Stein as modified by Rosen (5). The absorbance of the developed blue color was read on a Bausch and Lomb Spectronic 20 colorimeter at 570 m μ . The equivalent amount of amino nitrogen was read from a standard curve which used tyrosine as the standard amino acid. This value was converted to micromoles of tyrosine per gram of meat used.

For interpretation of results, the replicate determinations at each time interval were averaged for the incubated and the frozen samples, and the standard deviation was calculated. The average percentage error (standard deviation divided by the average value) for all determinations was $\pm 12\%$.

Pork. In general, the procedure with pork was similar to that used with beef. The exceptions are noted as follows:

Fresh pork loin was trimmed of surface fat, ground twice through a $3/16$ -inch plate, and then thoroughly mixed. Five-gram aliquots were flattened to approximately a 2-mm. thickness in the plastic

film packets, and the packets were air-evacuated and heat-sealed.

Temperatures of 140°, 150°, 160°, 165°, and 170° F. were used when the heat treatment was prior to irradiation, and temperatures of 140° and 160° F. when the heat treatment was postirradiation.

A 1 to 400 dilution of the trichloroacetic acid filtrate was used.

Results and Discussion

The experimental values for the amino nitrogen content of beef and pork are tabulated in Table I. The total proteolytic activity appears to be greater in pork than in beef. Whether this is due to a higher concentration of enzymes or to higher specific activities is not yet known.

The net increase in the amino-nitrogen content—incubated sample value minus frozen sample value—in samples that should have been completely inactivated by the enzyme is somewhat puzzling. This may be due to a naturally occurring hydrolysis of the pH 5.7 protein material at the 37° C. temperature used. The amounts found, however, appear to be high, particularly in half of the pork samples. Further investigations relating to the significance of the amino-nitrogen content to flavor acceptance of irradiated meats, and to the importance of particular enzymes or enzyme systems to the flavor of stored irradiated raw meats are indicated to be necessary.

A graph of time *vs.* amino-nitrogen content (expressed as micromoles of tyrosine per gram of meat) was plotted for each temperature studied. The graphs were all very similar in construction. A representative graph is shown in Figure 1. Inactivation was considered to be complete when no change in the amino-nitrogen content occurred with increasing

Table II. Time-Temperature Relationship for Inactivating Proteolytic Enzymes of Irradiated Meat

Temp., ° F.	Beef		Pork	
	HI ^a	IH Time, Minutes	HI Minutes	IH
140	23	23	30	27
150	6.0		8.4	
155	3.5			
160	1.5	1.6	2.4	2.2
165	0.65			
170	0.28		0.38	

^a HI = heat and treatment preirradiation. IH = heat treatment postirradiation.

heat exposure intervals, and appears on the graph as a plateau.

The point of intersection of a tangent—plotted at the last point before inactivation—and the plateau was used in order to arrive at a single time of inactivation for each temperature. At temperatures of 165° and 170° F. the heat exposure intervals were short enough and the inactivation was rapid enough so that the heat penetration time became significant. This is indicated by the smallness of the amino-nitrogen change in the first time interval. The time for enzyme inactivation, therefore, was corrected for heat penetration at these two temperatures as is indicated in Figure 1. The time for inactivation at each temperature is shown in Table II.

As little difference in thermal inactivation could be observed for samples heat-treated pre- or postirradiation, irradiation at the 5-megarad level caused no significant sensitization of the enzymes to thermal inactivation.

When the time of inactivation was plotted against temperature, the resulting curve appeared to be that of a log func-

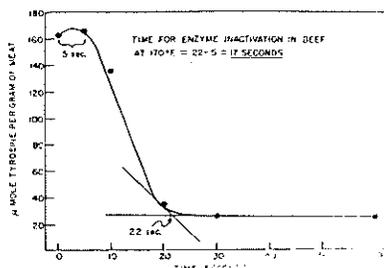


Figure 1. Enzyme inactivation at 170° F. vs. time in beef

Measured by the amino-N content of sample irradiated with a 5-megarad dose and stored at 100° F. for 6 weeks

tion. Plotting time on a log scale vs. temperature gave straight lines. This is in agreement with the generally accepted theory that the heat inactivation of enzyme systems follows a first order reaction curve.

The regression lines for the inactivation of beef and of pork are shown in Figure 2. The regression functions ($y = \log$ seconds) are as follows:

$$y_{\text{beef}} = 11.893 - 0.0623 x \text{ (std. error of estimate} = 0.0165 \text{ and correlation coefficient} = 0.9971)$$

$$y_{\text{pork}} = 11.664 - 0.0600 x \text{ (std. error of estimate} = 0.0224 \text{ and correlation coefficient} = 0.9953)$$

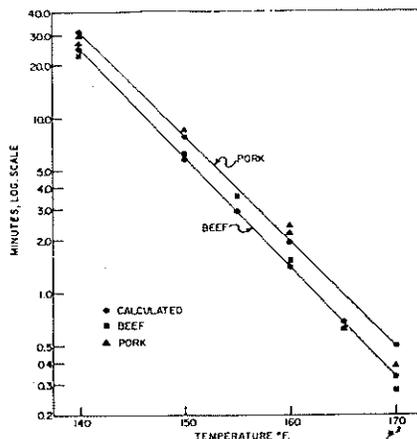


Figure 2. Time vs. temperature plot of regression lines for enzyme inactivation in irradiated beef and pork

This figure, then, shows the relationship of time and temperature which would be required to inactivate enzymes in radiation-sterilized beef and pork products. The difficulty of controlling any heat process, in order to achieve an internal temperature for the specified length of time should compensate for any errors which have been made because of assumptions concerning the point of inactivation at each temperature.

Whether this same time-to-temperature relationship is true of tissue proteolytic enzymes of organ meats, and of other species of animals, and what time-to-temperature ranges for complete inactivation of enzymes will produce the most acceptable product in terms of flavor, odor, color, texture, and storage stability is still to be determined.

Acknowledgment

The assistance of Robert Baldwin in performing analyses of the pork samples is gratefully acknowledged.

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Received for review April 2, 1959. Accepted August 5, 1959. Division of Agricultural and Food Chemistry, 134th Meeting, ACS, Chicago, Ill. Paper No. 954 in series approved for publication. The views or conclusions are those of the authors. Mention of company products does not constitute endorsement of the department over others not named.