Reports

Proteolytic Enzyme Activity in Irradiation-Sterilized Meat

The recorded formation of tyrosine crystals in the storage of irradiation-sterilized raw meat (*1*) is indicative that a general proteolysis occurs in irradiated meat samples (tyrosine is the least soluble of the amino acids). The principal proteolytic enzymes present in beef muscle have been identified as cathepsins (*2*). It has been reported that an irradiation dose of 1.6 million rep inactivates only 50 percent of the proteinase activity in samples of beef muscle (*3*).

Additional pertinent information is provided by data taken from three different investigations in the Radiation Preservation of Foods Project at the Quartermaster Food and Container Institute for the Armed Forces (*4*).

Extensive crystal formation gave a very unappetizing appearance to all samples of irradiated pork tenderloin that had been stored for 3 months at 100°F. Samples stored at 72°F had an increased free amino acid content in the fluids that were squeezed from the meat, but enzyme activity had not produced sufficient concentration of tyrosine to form crystals. Variables in the study were an initial freezing or wet-ice pack before irradiation, an irradiation dose of 2 or 3 million rep, and storage at either 72° or 100°F.

Table 1 shows the results obtained from paper-chromatographic, amino-acid analyses of residue fluids after steam-distillation of various samples of ground beef. The preirradiation heat treatment was based on conditions determined to be sufficient for inactivating catalase by heat and consisted of heating the meat in a steam retort and holding it for 10 minutes at an internal temperature of 160°F. The results show that proteolysis has been inhibited in the meat in which the enzymes were heat-inactivated.

One milliliter of 1-percent solutions of ascorbic acid or of cysteine (known cathepsin activators) and 1 ml of copper sulfate or of hydroquinone (cathepsin inhibitors) were added to 200-g samples of ground beef prior to irradiation at 3, 6, or 12 million rep. Some samples were stored at 76°F, and the rest at 100°F.

No tyrosine crystal formation was evident in sample cans that were opened after 3 months' storage. After 7 months' storage at 100°F, however, crystals were found in the 3-million-rep-dose cans containing the added cathepsin activators. No crystals were observed in the cans used to test the other variables. These results may be interpreted as follows:

1) The rate of enzyme activity is accelerated at the higher storage temperatures.

2) The inhibition of enzyme activity at greater doses of irradiation may be due to destruction of the enzyme activators. More likely, however, this work confirms the report (*3*) that proteinases exhibit greater resistance than bacteria to inactivation or destruction by irradiation. The effect is opposite to that encountered in heat sterilization of foods where the amount of heat necessary to inactivate enzymes is less than that required to destroy microorganisms.

3) A supplementary confirmation is made of cathepsins as the principal proteolytic enzymes present in beef muscle.

The data cited show that prolonged storage and storage at elevated temperatures will destroy meat structure and probably develop a bitter taste in it (most L-amino acids are bitter). The necessity for inactivation of the proteolytic enzymes is indicated, therefore, if irradiation-sterilized meat is to become an acceptable food product.

Table 1. Semiquantitative paper-chromatographic, amino-acid analyses of residue fluids from steam distillations of equal weights of fresh and irradiated samples of ground beef. The presence of free amino acids is indicated by +.

Treatment	Presence of free amino acids	
	Before storage	After storage
Fresh	+	
Irradiated (2×10^6 rep)	+	++++*
Irradiated (3×10^6 rep)	+	++++*
Preirradiation heat-treated (3×10^6 rep)	+	+†

* Stored 3 months at 76°F; † stored 5 months at 76°F.

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References and Notes

1. G. B. Pratt and O. F. Ecklund, *Food Technol.* 10, No. 10, 496 (1956).
2. A. K. Balls, *Ice and Cold Storage* 41, No. 4, 85 (1938).
3. D. M. Doty and J. P. Wachter, *J. Agr. and Food Chem.* 3, 61 (1955).
4. This report is paper No. 681 in a series of papers approved for publication. The views or conclusions are ours and are not to be construed as necessarily reflecting the views or endorsement of the Department of Defense. A fuller account of the several studies on which this report is based is in preparation.

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Effect of Ionizing Radiation on Rust Reaction in Plants

Studies on the nature of resistance of plants to rust diseases have long been hampered by the fact that the rust fungi are obligate parasites. One approach to the problem is the alteration of the disease reaction by manipulation of environment (*1*) or by chemical treatment (*2*) of the host plant, followed by appropriate physiological or biochemical analyses of the change induced. Recently, ionizing radiation (*3*) has been investigated as a possible therapeutic agent and as a tool for studying the vascular wilt disease of tomato, which is caused by the heterotrophic fungus, *Fusarium oxysporum* f. *lycopersici.* This report (*4*) records the effects of chronic gamma- and acute x-ray treatments on the host-parasite interaction in several rust diseases: flax rust (*Melampsora lini,* race 1); wheat stem rust (*Puccinia graminis tritici,* races 15B and 111); oat stem rust (*P. g. avenae,* race 7A) and crown rust of oats (*P. coronata avenae,* race 202).

For chronic gamma treatments, seedlings grown to the first- or second-leaf stage in plastic pots were exposed to radiation from a 9.4-c Co^{60} source in the greenhouse. Dose, dose rate, stage of growth, and time of inoculation were the main variables. For x-ray treatments, the seedlings were grown in a mixture of loose soil and peat, removed, washed and enclosed in plastic film for irradiation. Plants were inoculated immediately after irradiation and transplanted to soil or grown in liquid nutriculture. Lead shielding and x-rays were used for partial-plant exposures.