[Reprinted from Food Research, 1957, Vol. 22, No. 5, Pages 455–461]

THE EFFECT OF RADIATION PROCESSING UPON THE *IN VITRO* DIGESTIBILITY AND NUTRITIONAL QUALITY OF PROTEINS

A. LEONARD SHEFFNER, RICHARD ADACHI, AND HARRY SPECTOR
Quartermaster Food and Container Institute for the Armed Forces, Chicago, Illinois

(Manuscript received March 9, 1957)

The potential use of gamma radiations for the preservation of food makes it important to determine the effect of this type of treatment upon the nutritional quality of food proteins. In this respect it has been shown that although moderate doses of ionizing radiations produce significant destruction of individual amino acids in pure solution *(3, 4, 7, 13)*, proteins must be irradiated at several times the usual sterilization dose range of 0.5 to 6.0 million rep before nutritionally significant losses of amino acids are observed *(1, 8)*. However, viscosity and solubility studies indicated that irradiation produces definite increases in cross linking as well as degradation of the treated proteins *(1)*. Since these structural changes can modify the susceptibility of the treated proteins to digestion it became of interest to study the *in vitro* digestibility of irradiated proteins and to estimate the effects of digestibility changes upon nutritional value. Recently, the relationship between the pattern of amino acids released by digestive enzymes and the biological value of food proteins was studied in this laboratory. The pattern of amino acids released *in vitro* by pepsin revealed differences between proteins which were not apparent from their total essential amino acid content nor from the patterns existing when the pepsin digests were further digested with trypsin and erepsin. As a consequence of these results, an amino acid index was devised which took into account the availability of amino acids during *in vitro* pepsin digestion. The new index combined the pattern of essential amino acids released by *in vitro* pepsin digestion with the amino acid pattern of the remainder of the protein to produce an integrated index—the Pepsin Digest-Residue (PDR) Amino Acid Index. The PDR index was closely correlated with the net protein utilization value (biological value x digestibility) of a variety of proteins *(12)*. Subsequent application of the new procedure to heat-processed protein foods indicated that the PDR index also accurately predicted changes in the net utilization of the treated proteins *(11)*.

The purpose of the present study was (a) to determine the patterns of amino acids released by *in vitro* enzymatic digestion of representative food proteins subjected to ionizing radiation and (b) to compare the nutritional quality of the irradiated and heat processed proteins as measured by their PDR index values.

METHODS AND MATERIALS

Procedures for preparation of the enzymatic digests, microbiological assay for amino acids, and calculation of the PDR index have been previously described *(12)*.

Evaporated milk was prepared at the Pet Milk Company, New Glarus, Wis. by the usual production procedure. In this procedure the raw milk is heated for 10 minutes at 210° F. and then condensed in a double-effect evaporator. For the first effect the milk

is treated at 180° F. for 2½ minutes; in the second effect the milk is further treated at 125° F. for 2½ minutes. Following canning of the unsterilized condensed milk, samples for control purposes and for subsequent irradiation were removed from the conveyor belt. Remaining samples were heat-sterilized at 242° F. for 14 minutes. All canned condensed milk samples were kept chilled until analyzed. Freezing of milk samples was avoided to minimize structural changes in the proteins.

Beef [a] used in this study was obtained from the *longissimus dorsi* muscles of premium grade steers. Meat was trimmed, 20% fat by weight was added, and ground through a 1 inch by 1½ inch plate and then a ¼ inch plate. Pork meat was obtained from the *longissimus dorsi* muscles of matched pork loins. After being trimmed to 20% trimmable fat, the pork meat was ground in the same manner as the beef. Samples were canned under 20 inches vacuum in No. 2½ cans. Heat processed samples were autoclaved at 240° F. for 161 minutes (FO = 6.95). Unsterilized samples prepared for irradiation were stored frozen.

Turkeys used in this study were all of the same brood and fed in the same flock. Meat was removed in large pieces and excess fat removed. Both dark and light meat and skin were cut into strips, ground, and mixed. Samples were vacuum packed in No. 2½ cans. Heat processing was performed at 240° F. for 114 minutes (FO = 11.6). Unsterilized samples prepared for irradiation were kept frozen. All meat samples were stored at −20° F. after processing until analyzed.

Irradiation sterilization was done by gamma irradiation from spent fuel rods at the Argonne National Laboratory.[b] All samples were given radiation doses of 2 million rep as determined by ferrous dosimetry. Milk samples were held at approximately 35° F. and meat samples were kept frozen in solid CO_2 packs until placed in the irradiation canal. Since the longest period of irradiation was approximately 2 hours, the meat samples were probably frozen throughout the treatment. Previous tests had indicated that aqueous solutions in No. 2 cans remain frozen for 3 hours in the canal.

RESULTS

Irradiation with 2 x 10^6 rep gamma rays produced no significant destruction of amino acids in milk, turkey, or beef protein. In pork there was a 16% loss of cystine following irradiation, but no other destruction was observed. Following heat processing, no loss of amino acids was found in any of the protein foods studied.

Rate of release of amino acids during pepsin digestion was approximately the same for both irradiated and non-irradiated turkey meat (Table 1). In the case of irradiated evaporated milk the liberation of cystine was reduced 29%, but the remaining amino acids were unaffected. Similarly, with irradiated pork, only phenylalanine was not as readily released by pepsin, and for beef only phenylalanine and tryptophan were reduced. On the other hand, there was a decided decrease in the release by pepsin of all the amino acids measured in the heat-processed meat samples. For heat-processed evaporated milk, only the rate of release of cystine was reduced.

Incubation of the 4 protein foods with pepsin and pancreatin in succession also indicated that irradiation with 2 x 10^6 rep did not produce marked changes in the susceptibility of the proteins to enzymatic digestion (Table 2). In evaporated milk the release of all the amino acids studied except cystine was not affected by irradiation; the liberation of cystine was 57% less in the irradiated milk digests than in the unprocessed samples. In the pepsin plus pancreatin digests of irradiated beef protein, only the quantity of histidine was reduced; and for turkey and pork no differences were noted between the irradiated and unprocessed samples.

Contrary to the limited effect of irradiation, heat processing markedly decreased the susceptibility of the 3 meat samples to the combined action of pepsin and pancreatin. However, in the case of evaporated milk similar to the results obtained with irradiation,

[a] Beef, pork, and turkey samples were prepared in the Animal Products Branch, Quartermaster Food and Container Institute for the Armed Forces under the direction of Mr. R. Buscemi.

[b] The samples were irradiated under the direction of Miss H. Gladys Swope, in charge of the Gamma Radiation Facility, Argonne National Laboratories.

TABLE 1

The concentrations of amino acids in pepsin digests of food proteins subjected to irradiation and thermal processing

	Evaporated Milk			Turkey			Pork			Beef		
	Con.[1]	Irr.[2] *mg/g*	Therm.[3]	Con.	Irr. *mg/g*	Therm.	Con.	Irr. *mg/g*	Therm.	Con.	Irr. *mg/g*	Therm.
Methionine	1.2	1.0	1.2	6.9	6.8	4.8	10.0	10.0	6.6	10.0	9.3	7.0
Cystine	0.17	0.12	0.15	1.04	0.99	0.52	1.4	1.1	0.44	1.1	1.1	0.32
Phenylalanine	5.7	5.2	6.2	8.9	8.1	4.8	10.0	9.1	4.7	11.6	9.5	5.1
Tyrosine	5.5	5.4	5.9	5.5	5.4	3.1	5.6	5.4	3.0	6.5	5.7	3.1
Histidine	0.26	0.22	0.26	1.7	1.6	1.2	1.6	1.5	1.1	2.6	2.3	2.1
Lysine	0.60	0.70	0.74	2.8	2.6	2.1	3.2	2.4	1.8	3.4	3.1	1.9
Tryptophan	4.5	4.2	4.7	4.2	3.6	1.5	5.2	4.6	2.0	7.0	5.3	2.1
Leucine	39.0	37.0	41.7	46.9	47.5	30.4	48.5	46.1	27.2	62.2	59.0	38.2
Isoleucine	8.9	7.8	7.8	34.3	35.0	31.5	25.4	24.8	14.0	29.2	30.1	14.8
Valine	2.5	2.4	2.7	10.6	10.8	6.8	11.5	10.6	6.9	12.4	11.4	7.0
Threonine	13.8	13.4	14.4	31.0	28.0	21.2	29.0	26.9	16.5	27.1	27.4	17.5

[1] Unprocessed controls.
[2] Irradiated with 2 x 10^6 rep.
[3] Thermal processed (see Methods).

TABLE 2

The concentrations of amino acids in pepsin plus pancreatin digests of food proteins subjected to irradiation and thermal processing

	Evaporated Milk			Turkey			Pork			Beef		
	Con.[1]	Irr.[2] *mg/g*	Therm.[3]	Con.	Irr. *mg/g*	Therm.	Con.	Irr. *mg/g*	Therm.	Con.	Irr. *mg/g*	Therm.
Methionine	12.8	11.8	12.6	19.7	19.4	16.2	22.5	23.7	17.4	21.0	20.4	17.8
Cystine	1.4	0.6	0.9	4.3	4.6	2.8	6.5	5.9	3.3	6.6	5.9	2.8
Phenylalanine	22.6	22.5	23.9	29.5	28.1	23.2	30.8	34.4	21.9	28.3	29.8	23.4
Tyrosine	27.5	25.0	28.7	28.9	28.5	23.9	29.7	28.0	21.9	27.6	28.4	23.5
Histidine	14.2	13.2	12.2	18.0	16.6	14.4	14.5	14.4	10.4	19.3	14.4	16.2
Lysine	61.6	60.3	57.5	82.6	76.8	68.6	83.9	81.8	63.3	81.2	82.0	68.2
Tryptophan	10.8	9.1	10.4	10.0	9.7	8.0	10.8	12.6	8.1	10.6	10.1	8.1
Leucine	81.5	74.6	80.5	69.4	72.1	59.2	71.0	70.8	55.5	83.4	69.2	76.3
Isoleucine	37.5	37.0	39.8	52.4	48.9	43.8	38.6	40.4	32.2	42.8	40.7	41.0
Valine	22.7	23.0	21.6	37.7	38.0	30.5	41.0	40.4	30.6	40.6	40.6	31.6
Threonine	34.7	31.8	35.4	45.8	41.1	38.5	41.7	52.0	36.0	42.7	42.8	37.2

[1] Unprocessed controls.
[2] Irradiated with 2 x 10^6 rep.
[3] Thermal processed (see Methods).

the liberation of only cystine was reduced following heat processing. It should be noted that the heat processing time for evaporated milk is much shorter than that necessary for meat samples.

PDR amino acid indexes of net protein utilization (biological value x digestibility) for the 4 proteins studied in this series of experiments are presented in Table 3. In each

TABLE 3

The PDR index of net protein utilization of foods subjected to thermal and radiation sterilization

Protein	PDR Index[1]		
	Unprocessed	Thermal Processed	Irradiated
Beef.....	86	85	87
Pork.....	88	80	89
Turkey.....	74	71	74
Evap. milk.....	75	75	74

[1] Standard errors; beef 0.76, pork 1.29, turkey 0.92, and milk 0.83.

case, irradiation with 2×10^6 rep produced no significant change in nutritional quality as measured by the PDR index. Heat processing produced an apparent but not significant (5% level) decrease in the PDR value of turkey and a very significant lowering in the PDR index of pork protein.

DISCUSSION

The biological value of evaporated milk has been reported to be significantly lowered by irradiation with 3×10^6 rep gamma rays *(5)*. No change in digestibility was observed. These results were interpreted as suggesting that some essential amino acid was partially destroyed or bound so as not to be available at an optimum rate for most efficient utilization. In subsequent reports from the same laboratory *(6, 9)* it was shown that cystine and methionine were destroyed to the extent of 43.7% and 29.4% respectively by 3×10^6 rep. They also found that the effects of irradiation upon the biological value of the milk proteins could be compensated for by supplementation of the irradiated milk with 0.3% cystine. Nevertheless, the data reported in the present study indicated that irradiation with 2×10^6 rep does not significantly destroy amino acids in evaporated milk. Also, with the exception of cystine there was no significant change in the enzymatic availability of amino acids in the irradiated milk proteins. Diminished enzymatic liberation of cystine by itself would not be expected to have nutritional significance since the concentration of cystine in milk proteins is very low, especially in comparison with that of methionine. However, calculation of the PDR index of irradiated milk using methionine and cystine values which reflect the destruction reported by Rama Rao and Johnson *(9)* produces a decrease of 4.5% in the PDR index which is similar to the 5.0% decrease in biological value found by Metta and Johnson in their later experiments *(6)*. Results thus suggest that gamma irradiation with up to 2×10^6 rep does not cause significant destruction or change in availability of amino acids in the proteins of evaporated milk; however, with larger doses some destruction of cystine and methionine may occur which depresses the nutritional value of the irradiated milk.

In the case of turkey, pork, or beef, irradiation with 2 x 10^6 rep caused only minor alterations in the concentration or enzymatic availability of amino acids. The PDR indices of these protein foods were therefore not significantly changed. In this respect, Metta and Johnson *(5)* reported no change in the biological value of beef following irradiation with 3 x 10^6 rep and Calloway *et al. (2)* obtained similar results with turkey irradiated up to 6 x 10^6 rep. Also, Rosner *(10)* found no amino acid destruction or change in the pepsin digestibility of beef irradiated up to 6 x 10^6 rep.

Heat-treated proteins of turkey, pork, and beef did not show evidence of amino acid destruction nor of changes in the pattern of amino acids released by digestive enzymes. However, the heat treatment did cause a significant decrease in the quantity of amino acids released by *in vitro* incubation with pepsin. It is evident from previous work *(11)* that diminished susceptibility to *in vitro* pepsin digestion usually results in a decrease in the nutritional quality of a protein. The observed decrease in pepsin digestibility of heated pork and turkey, were thus reflected in lower PDR index values, although only for pork was the change statistically significant. However, whereas heated beef protein was less susceptible to pepsin digestion, the PDR index value reported here and the biological value as obtained by the Mitchell method of nitrogen balance *(5)* were not decreased. Inspection of the data for calculation of the PDR index indicated that the heated beef had a higher essential amino acid content per unit of nitrogen than did the untreated beef. This phenomenon, which may be due to volatilization of non-essential nitrogenous compounds, is possibly one reason that heat-processed beef protein appears to retain completely its nutritional value on a nitrogen basis, in spite of the fact that the protein is considerably less susceptible to enzymatic digestion. A more important reason is related to the fact that following treatment with pepsin the amino acid pattern in the undigested fraction of heated beef proteins is superior (relative to the standard egg protein) to the pattern of the same residual fraction of raw beef protein. This improvement in the amino acid pattern of protein not digested by pepsin but subsequently digested by intestinal enzymes probably compensates for the effect of decreased pepsin susceptibility of the heat processed beef proteins.

SUMMARY

Representative protein foods were subjected to 2 x 10^6 rep gamma rays from spent fuel rods and to thermal processing.

Irradiation produced no significant destruction of essential amino acids in milk, turkey, or beef protein. In pork, no destruction of amino acids was observed except for a small loss of cystine. Following heat processing no loss of amino acids was found in any of the protein foods studied.

Irradiation resulted in substantially no change in the enzymatic availability of amino acids, whereas thermal processing caused significant decreases in the digestibility of the proteins studied.

No significant changes in the protein quality, as measured by the PDR index, occurred from irradiation. Heat processing produced a small but not significant decrease in the PDR index of turkey and a very significant lowering in the PDR index of pork protein.

LITERATURE CITED

1. Bellamy, W. D., Clark, L. B., Mondenhall, R. M., and Schwartz, P. Study of chemical changes produced in irradiated proteins and amino acids. *Prog. Rept.* (final), U. S. Army Quartermaster Corps Contract No. DA19-129-qm-129, 30 April (1956).
2. Calloway, D. H., Cole, E. R., and Spector, H. The nutritive value of turkey preserved by ionizing radiation. I. Growth-promoting quality and biological value. *J. Am. Diet. Assoc.,* (In press, 1957).
3. Drake, M. P., Giffee, J. W., Johnson, D. A., and Koenig, V. Effects of ionizing radiation on proteins. I. Effect of gamma radiation on the amino acid content of insulin. *J. Am. Chem. Soc.,* (In press, 1957).
4. Maxwell, C. R., Peterson, D. C., and Sharpless, N. E. The effect of ionizing radiation on amino acids. I. The effect of x-rays on aqueous solutions of glycine. *Rad. Research,* 1, 530 (1954).
5. Metta, V. C., and Johnson, B. C. The effect of radiation sterilization on the nutritive value of foods. *J. Nutrition,* 59, 479 (1956).
6. Metta, V. C., and Johnson, B. C. On the nutrient value of the major nutrients of irradiated foods. *Prog. Rept.,* U. S. Army Office of Surgeon General Contract No. DA-49-007-MD-544, March 28–July 5 (1956).
7. Proctor, B. E., Lockhart, E. E., and Goldblith, S. A. Fundamental physical and biochemical changes which occur as a result of radiation treatment resulting in modification of flavor, color, and texture. *Prog. Rept.,* U. S. Army Quartermaster Contract No. DA19-129-qm-522, 1 April to 31 Oct. (1954).
8. Proctor, B. E., and Bhatia, D. S. Effects of high voltage cathods rays on amino acids in fish muscle. *Food Technol.,* 4, 357 (1950).
9. Rama Mao, P. B., and Johnson, B. C. On the nutrient value of the major nutrients of irradiated foods. *Prog. Rept.,* U. S. Army Office of Surgeon General Contract No. DA-49-007-MD-544, July 6-September 26 (1956).
10. Rosner, L. *Prog. Rept.,* U. S. Army Quartermaster Corps Contract No. DA19-129-qm-618, Oct. 4 (1956).
11. Sheffner, A. L., Adachi, R., and Spector, H. Measurement of the net utilization of heat processed proteins by means of the pepsin digest-residue (PDR) amino acid index. *J. Nutrition,* 60, 507 (1956).
12. Sheffner, A. L., Eckfeldt, G. A., and Spector, H. The pepsin digest-residue (PDR) amino acid index of net protein utilization. *J. Nutrition,* 60, 105 (1956).
13. Stein, G., and Weiss, J. Chemical action of ionizing radiations on aqueous solutions. IV. The action of x-rays on some amino acids. *J. Chem. Soc.,* 3256 (1949).