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RADIATION STERILIZATION OF FOODS

Biological Value of Gamma Irradiated Corn Protein and Wheat Gluten

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Effect of gamma-ray irradiation and of heat-cooking on the nutritive value of corn protein and wheat gluten was studied. Irradiation did not produce off-flavors or odors in corn or wheat gluten; the lysine in corn or wheat gluten and the tryptophan in corn were not destroyed. Radiation-sterilized corn and wheat aluten were completely acceptable for the growing rat during a 20-day feeding period. Irradiation, like heat-cooking, did not affect the digestibility of corn protein or the digestibility of wheat gluten. The biological value of corn protein and of wheat gluten were also not affected. However, irradiation of corn at 9.30 million rad lowered its digestibility by 5% but did not lower its biological value.

 $\mathbf{B}^{ extsf{efore}}$ any new food processing method can be put into commercial use, foods so processed must be proven satisfactory with respect to the effect of the processing on wholesomeness (lack of toxicity), acceptability (palatability), and nutritive value of both the micro- and macro-nutrients. Irradiation sterilization has little or no deleterious effect on the nutritive value of some animal and legume proteins for the growing rat (3, 4). The protein sources tested were milk, beef, peas, and Lima beans, all of which are deficient in sulfur amino acids. While irradiation did not affect the digestibility of the biological value of the beef and Lima bean proteins, it lowered the biological value of milk and pea proteins by about eight percentage points. Cystine and methionine seemed to be particularly sensitive to ionizing radiation (2). A nutritional study of two typical cereal proteins, corn and wheat, in which the limiting amino acids are generally lysine and/or tryptophan, was undertaken. The changes due to gamma irradiation and conventional heat cooking, respectively, in the digestibility and the biological value of corn and wheat gluten are reported.

Experimental

Illinois high protein corn was selected for studying the effect of irradiation on the nutritive value of a protein deficient in tryptophan-and lysine. Even though high protein corn has been reported to be lower in biological value than low protein corn, owing to a significant increase in the zein proportion of the kernel (9), the former was used, because when only 50% of the corn is included in the diet it provides 10%protein, and thus its high protein content makes it convenient for formulating a balanced ration. Tryptophan-and lysine-continue to remain the limiting amino acids in the high protein corn.

The corn was finely ground and the corn and wheat gluten-which was obtained from a commercial sourcewere suspended in water, in each case, at a 35% concentration, canned in No. 2 cans, and frozen. Some of these canned samples were gamma-ray irradiated at the desired dose. Wheat gluten was irradiated at 2.79 million rad, while corn samples were treated at 2.79 million rad and 9.30 million rad, respectively. (One rad is defined as 100 ergs of radiant energy absorbed per gram of material irradiated.) A portion of nonprocessed suspended corn and wheat gluten, respectively, was cooked in an autoclave for 4 minutes at 15 pounds pressure. To obtain nutritional data on these samples owing to cafeteria methods of feed processing, all samples of corn and wheat gluten,

including the controls, were then dried in vacuum at room temperature and finely reground. Proximate analysis on the nonprocessed, heat, and irradiation processed corn and wheat gluten samples indicate (Table I) that irradiation did not result in significant loss of nitrogen in corn or wheat gluten.

The corn and wheat gluten samples were analyzed microbiologically for lysine using L. mesenteroides-P-60 (1) after hydrolysis with 2.5N hydrochloric acid at 15 pounds pressure for 6 hours. The corn samples were also analyzed microbiologically for tryptophan (5)after hydrolysis with 6N barium hydroxide; Streptococcus fecalis R. was the organism employed; and light transmittance was read at 650 m μ in a Coleman spectrophotometer.

The biological value method of Mitchell (6, 7) with certain modifications was used to measure the nutritive value of the corn protein and the wheat gluten. The vacuum-dried samples of corn and wheat gluten-whether nonprocessed, heat-processed, or irradiation-processed-were incorporated into balanced diets to provide 10% protein $(N \times 6.25)$, on a moisture-free basis (Table II).

The modification of the Mitchell method consisted of using two instead of three feeding periods. The first experimental period of 20 days was

followed by a standardization period of 14 days. Growing rats received equal and adequate amounts of the test diets during the experimental period and similar amounts of a 4% whole egg protein diet—otherwise similar to the test diets—during the standardization period. The rats were on constant food intake during the last 14 days of the first as well as the second feeding period. Individual collections of urine and feces were made during the last 7 days in either period. There were 10 rats per treatment. Ferric oxide was used as the feces marker.

Results and Discussion

When the irradiation sterilized cans containing corn were opened, no significant off-flavors or odors were noticed. Even irradiation at 9.3 million rad did not result in any detectable off-flavors in the case of corn. Rats avidly consumed the diets containing irradiated corn. No food refusals were noticed in any group during the 20-day feeding period; irradiated corn was completely acceptable to the growing rat during this short-term study. The food intake of each rat in each group was 8.0 grams a day during the last 14 days of the experimental period; the rats grew at the rate of approximately a gram a day, and there was no difference between groups.

Using the method of analysis of variance, no difference was found in the biological values of the protein of the nonprocessed (48%) and the processed (heat-cooked, 2.8 or 9.3 million rad irradiated) corn. Similarly, no differences were noticed in the digestibility of the proteins of the nonprocessed (91%), heat-processed (92%), and 2.8 million rad irradiated (93%) corn. However, the digestibility of the 9.3 million rad irradiated corn protein was significantly lower (p < 0.01) than the nonprocessed corn-the difference being 4.8 \pm 0.71—or the heat-cooked corn the difference being 6.1 \pm 0.91—or the 2.8 million rad irradiated corn-the difference being 6.6 ± 0.98 . Nitrogen metabolism values of 91% for the digestibility and of 48% for the biological value for the Illinois high protein corn are in good agreement with the reported values (9).

Irradiation at 2.8 million rad, which is effective for food sterilization (10), did not result in any nutritional damage to the proteins of corn. The lysine and tryptophan contents of the corn protein were not affected by irradiation. The nonprocessed corn protein (N \times 6.25) had 1.96% lysine and 0.60% tryptophan. Irradiation at 2.8 million rad or even 9.3 million rad—which dosage is far in excess of the dose required for food sterilization—did not result in any significant loss in either of these amino acids. The biological value of the 2.8 million

Table I. Chemical Analysis of Seed Corn and Wheat Gluten

	Corn, %				Wheat Gluten, %		
	Non- processed (control)	Heat cooked	Irradi- ated (2.8 million rad)	Irradi- ated (9.3 million rad)	Non- processed (control)	Heat cooked	Irradi- ated (2.8 million rad)
On Fresh Basis							
$\begin{array}{c} \text{Moisture} \\ \text{Crude protein} \\ (N \times 6.25) \end{array}$	7.72 21.3	3.65 21.7	4.31 21.3	4.44 21.2	6.59 77.1	3.51 79.9	3.32 80.30
Ether extract Crude fiber Heat of com- bustion (cal.)	4.80 2.31 430	3.67 2.29 446	4.57 2.20 445	4.42 2.21 448	0.81 0 526	0.41 0 547	0.77 0 548
		On	MOISTURE-	FREE BASIS			
Crude protein $(N \times 6.25)$	23.1	22.6	22.3	22.2	82.5	82.8	83.0
Ether extract Crude fiber Heat of com- bustion (cal.)	5.20 2.50 469	3.81 2.38 463	4.77 2.30 465	4.63 2.31 469	0.87 0 563	0.43 0 566	0.80 0 567

Table II. Composition and Proximate Analyses (Per Cent) of Corn and Wheat Gluten Diets Per Cent

	Control Corn Diet	Cooked Corn Diet	2.8 × 10 [₫] Rad Corn Diet	9.3 × 10 ⁸ Rad Corn Diet	Control Wheat Gluten Diet	Cooked Wheat Gluten Diet	2.8 × 10 Rad Wheat Gluten Diet
Corn, uncooked	46.9						· · · · ·
cooked		44.3	11111				
2.8×10^6 rad		• • • • •	46.9				· · · · ·
9.3×10^6 rad	· · · · ·			47.1	• • • • •	• • • • •	
Wheat gluten, control					13.0		
cooked						12.6	
$2.8 imes10^{6} m rad$							12.4
Starch	15.4	16.8	15.4	15.1	52.0	52.5	52.5
Sucrose	20.0	20.0	20.0	20.0	10.0	10.0	10.0
Cerelose		5.0	5.0	5.0	5.0 8.0	5.0 8.0	5.0
Lard Corn oil	$5.0 \\ 0.2$	5.0	0.2	0.3	2.0	2.0	$\frac{8.0}{2.0}$
Cod liver oil	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Wheat germ oil	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Vit. cerelose					1.0	1.0	1.0
(concn.)							
Vit. cerelose	5.0	5.0	5.0	5.0	· ; · <u>-</u> ·	· · · · · ·	· · · · · ·
Mineral mix 446	4.5	4.5	4.5	4.5	4.5 0.5	4.5 0.5	4.5 0.5
NaCl Wood flock	1.0	1.0	1.0	1.0	2.0	2.0	2.0
WOOD HOCK	1.0	<u> </u>	1.0	1.0			
Total	100	100	100	100	100	100	100
		Сн	emical An	ALYSIS			
Moisture	6.8	8.1	4.2	4.0	6.5	6.1	6.5
Total nitrogen	1.591	1.624	1.579	1.559	1.652	1.672	1.675
Protein (N \times 6.25		10.2	9.9	9.7	10.3	10.5	10.5
Ether extract	9.6	10.2	9.9	9.6	12.2	12.0	12.4
Gross energy (cal.)	427	455	438	428	547	526	548
							<u> </u>

rad irradiated corn was 47%, and that of the 9.3 million rad irradiated corn was 46%, as compared with the value of 48% for the nonprocessed corn and 46% for heat-cooked corn; there is no statistical difference among these values. Thus, irradiation or heat cooking does not affect the availability of the absorbed nitrogen. However, irradiation of corn at 9.3 million rad resulted in lowering the digestibility of the corn protein by about 5% (p < 0.01). Even though a damage of five percentage points is not serious, this is interesting as irradiation at 9.3 million rad did not alter the biological value—i.e., the utilization of the absorbed nitrogen—of the corn.

0 0 1 1 0

The irradiation-processed wheat gluten looked similar to the nonprocessed wheat gluten and did not have a different odor.

Rats in all treatments consumed completely the diets offered to them during the entire feeding period. They received their respective diets at the rate of 9 grams during the last 14 days of the feeding period and grew at the rate of 0.55 grams a day. There was no difference in growth among groups.

The summary of the nitrogen metabolism data is given in Table III. Anal-

Table III. **Results of Nitrogen Studies with Growing Rats**

	nin oroning have					
Processing	Apparent Digesti- bility, %	True Digesti- bility, %	Bio- Iogical Value, %			
	Corn					
Control Cooked, 4 minutes, 15 pounds	83 . 4 83 . 1	91.1 92.4	48.0 45.8			
Irradiated, 2.8×10^{6} rad	84.3	92.9	47.1			
Irradiated, 9.3 \times 10 ⁶ rad	76.4	86.3	46.3			
	WHEAT (Gluten				
Control Cooked, 4 minutes, 15 pounds	91.8 91.6	98.7 98.5	41.6 41.4			
Irradiated, 2.8 × 10 ⁶ rad	91.4	99.1	41.7			

ysis of variance showed no difference in digestibility (99%) or biological value (42%) in wheat gluten owing to processing-heat or irradiation. The limiting amino acid in wheat gluten is lysine. Heat processing of proteins has been reported to bring about reduced availability of lysine probably owing to the formation of a new peptide linkage

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between ϵ -amino group of lysine and a free carboxyl group of other amino acids. There was apparently no such chemical change due to irradiation as no reduction in the biological value was observed when wheat gluten in water suspension was autoclaved for 4 minutes at 15 pounds pressure or was irradiated at 2.8 million rad. The nitrogen metabolism data on wheat gluten are in excellent agreement with the reported values (8). The lysine content of wheat gluten (2.2%) did not change owing to heat cooking or irradiation.

Thus, lysine in corn or wheat gluten is not damaged due to irradiation sterilization. There was no change in the digestibility or the biological values of the proteins of the corn and wheat gluten when processed at 2.8 million rad gamma irradiation.

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Comparison of Solubility Characteristics of Selected Seed Proteins

N A RESEARCH program on the chem-I N A RESEARCH program on the ical composition of seeds from plants not now cultivated as economic crops in the United States (18), information was desired permitting selection of seed species containing protein constituents extractable in high yield under mild conditions. It was also desirable to classify and characterize protein systems under study, at least on an empirical basis. To this end, meals from a number of seed species were extracted with a series of solvents and the percentage of extractable nitrogen was determined.

The suitability of proteins for given uses is difficult to define in terms of fundamental structure, composition, and properties in the same fashion that amino acid content is related to nutritive value. Present commercial uses for protein

have generally been developed empirically (12). Practically all current industrial applications for proteins, such as production of fibers, sizes, adhesives, ingredients of coatings, emulsifiers, and food additives depend upon bringing proteinaceous material into solution. The material is usually derived in high yield from some high protein source. Hence a knowledge of solubility would appear to be an important factor in selection of vegetable proteins for possible industrial applications. Some of these applications would also depend on use of the mildest possible extraction conditions.

Selection of Materials. Factors considered in selecting seed species for these extractions were: high protein and/or oil content, general prominence or promise of the botanical family to which the spe-

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cies belongs, and desirability of varying widely the spectrum of plant families included. Emphasis was placed on little-investigated seed species; certain extensively studied ones, such as wheat, corn, soybeans, and flax, were included for comparison.

Method. To compare solubility characteristics of seed protein constituents, the following solvents were selected: 0.01M sodium hydroxide (pH 11.7 to 11.9); 0.5M disodium phosphate (pH 8.9); 0.5M sodium chloride; 70% ethyl alcohol, and water. These selections were more or less arbitrary and changes in concentration of solutions used as extractants would doubtless have produced different results. As a measure of nonprotein nitrogen the trichloroacetic acid extraction procedure of Becker,