Nutritive Composition of Irradiated Gulf Oysters Stored in Ice

Joseph A. Liuzzo, Stephen C. Lagarde, and Arthur F. Novak

Radiation preservation of Gulf oysters has been reported to be feasible in past studies on the basis of organoleptic, chemical, and bacteriological tests when doses of 0.2 Mrad were employed. In this study, freshly shucked oysters were divided into three treatments: nonirradiated, irradiated at 0.2 and 0.4 Mrad with Co^{60}, and iced-stored for 0, 5, 10, 15, and 20 days. At these intervals, the oysters were analyzed for moisture, ash, glycogen, crude protein, nonprotein nitrogen, true protein, crude fat, and soluble sugars. Crude protein was the only nutrient significantly affected by irradiation. When this fraction was converted to true protein, the significance of the decrease was lost. Ash content was significantly decreased as storage time increased. Although not significant, glycogen decreased and soluble sugars increased during storage. Because none of the major nutrients was greatly affected, the feasibility of radiation preservation of oysters is strengthened.

The success of radiation preservation of foods is dependent, in part, upon the stability of the nutrients during or after irradiation. Considerable research has been reported on the effects of radiation on proteins, vitamins, and fats in various foodstuffs (Ambe and Tappel, 1961; Brooke and Steinberg, 1964; Liuzzo *et al.*, 1966; Nawar and Dubravcic, 1966; Proctor *et al.*, 1950; Proctor and Goldblith, 1951; Reber and Bert, 1967; Sheffner *et al.*, 1957; Schweigert and Doty, 1958). However, less research has been reported on the effects on food carbohydrates (Cloutier *et al.*, 1959; Dollar *et al.*, 1964; Schweigert and Doty, 1958). Most work with radiation effects on carbohydrates has been conducted with sugar solutions and extracts rather than with carbohydrates in their native state and environment.

Results found in the literature are contradictory concerning actual effects of radiation on the nutritive composition of foods. These contradictions are due to variations in dose levels employed, radiation environment, condition of the food prior to radiation, and storage conditions maintained after treatment.

Radiation preservation of Gulf oysters has been reported to be feasible from the standpoint of organoleptic, chemical, and bacteriological tests (Novak *et al.*, 1966). The work herein reported was conducted to ascertain if the moisture, ash, glycogen, crude protein, nonprotein nitrogen (NPN), true protein, crude fat, and soluble sugar contents remained stable during irradiation of Gulf oysters at the dose level (0.2 Mrad) recommended by Novak *et al.* (1966).

EXPERIMENTAL METHODS

Oysters used in these studies were collected from beds near New Orleans, La., and transported to a nearby commercial packing house in a refrigerated truck maintained at 4° C. They were stored in a refrigerated room overnight at 4° C. and shucked by professional shuckers early the next morning. The facilities of this commercial packing plant enabled oysters to be packed according to present FDA regulations (Food and Drug Administration, 1967).

After the oysters were packed in 1-pint glass jars, they were transported to the Louisiana State University Nuclear Science Center and divided into three treatments (nonirradiated and irradiated at 0.2 and 0.4 Mrad). Samples to be irradiated (350 grams, each treatment) were placed inside a 53 × 28 cm. diving bell and lowered to the bottom of a 6-meter well filled with water. The samples remained in close proximity to a 36,000-curie source of Co^{60} until the desired dose level of gamma radiation had been administered. The temperature of the oysters during irradiation was 28° C. The dose level employed was 2000 rads per minute. Therefore, the samples remained in the irradiator for 1 hour and 40 minutes to obtain a dose of 0.2 Mrad and twice this time for the 0.4-Mrad dose. The 0.4-Mrad level was used as a positive control.

The nonirradiated and irradiated oysters were stored in ice and samples were withdrawn for analyses at 0, 5, 10, 15, and 20 days of storage. Determinations were made from duplicate samplings for moisture, ash, glycogen, crude protein, NPN, true protein, crude fat, and soluble sugars. Two collections of oysters, procured several months apart, were analyzed to compensate for seasonal variations in nutrient composition.

Moisture and Ash Determinations. Fifty grams of each sample was homogenized for 3 minutes with a Waring Blendor. Aliquots of this homogenate were placed in previously ignited and weighed crucibles. The samples were dried for 24 hours at 80° C. under 20 inches of vacuum, and per cent moisture was determined. The dried samples were ashed at 550° C.

Glycogen and Soluble Sugar Determinations. Excess oyster liquor from 50 grams of each sample was removed with blotting paper and the samples were homogenized for 3 minutes. Aliquots of 10 grams were homogenized with 10 volumes of 80% ethanol and allowed to settle for 10 to 14 hours. The homogenate was centrifuged at 2000 r.p.m. for 15 minutes. The supernatant (I) contained the soluble sugars and the precipitate the glycogen. The latter was homogenized with 100 ml. of 20% trichloroacetic acid (TCA) and centrifuged for 15 minutes at 2000 r.p.m. To the supernatant, which contained the glycogen, was added 4 volumes of 95% ethanol. The resultant precipitate (II) was centrifuged and used for glycogen analysis.

Ten-milliliter aliquots of the initial supernatant (I) containing the soluble sugars were evaporated to dryness on a steam bath. The residue (III) was employed for the sugar determinations. The phenol-sulfuric acid test (Montgomery, 1961) was used to analyze precipitate (II) for glycogen and residue (III) for soluble sugars.

Protein Determination. To obtain the true protein content of the samples, both total nitrogen and nonprotein nitrogen (NPN) were determined (total nitrogen minus NPN equals protein nitrogen). Duplicate 10-gram samples from each lot were placed in Kjeldahl flasks, to which were added 10 grams of

Department of Food Science and Technology, Louisiana State University, Baton Rouge, La. 70803

Table I. Effect of Radiation and Iced Storage on Per Cent Moisture of Oysters[a]

Storage Time, Days	Irradiation Dosage, Mrad 0	0.2	0.4	Av.
0	88.10	85.38	86.82	86.77
5	88.41	88.88	88.88	88.72
10	88.19	89.58	88.81	88.86
15	88.27	87.92	89.21	88.47
20	87.59	88.27	87.66	87.84
Av.	88.11	88.01	88.28	88.13

[a] Averages of 2 replications.

Table II. Effect of Radiation and Iced Storage on Per Cent Ash of Oysters[a]

Storage Time, Days	Irradiation Dosage, Mrad 0	0.2	0.4	Av.
0	1.11	1.33	0.93	1.23
5	0.77	0.53	0.62	0.64[b]
10	0.76	0.65	1.09	0.83[b]
15	0.79	0.64	0.81	0.75[b]
20	0.71	0.70	0.67	0.69[b]
Av.	0.83	0.77	0.82	0.81

[a] Averages of 2 replications.
[b] % ash significantly lower than at 0 days of storage ($P < 0.05$).

Table III. Effect of Radiation and Iced Storage on Glycogen Content of Oysters[a]

Storage Time, Days	Irradiation Dosage, Mrad 0	0.2	0.4	Av.
0	16.13	15.03	9.03	13.40
5	9.52	13.44	8.43	10.46
10	10.23	11.76	9.29	10.43
15	9.40	11.04	20.83	13.76
20	10.65	10.97	8.68	10.10
Av.	11.19	12.45	11.25	11.63

[a] Glycogen values expressed in mg./g. of oyster. Values given are averages of 2 replications.

Table IV. Effect of Radiation and Iced Storage on Soluble Sugar Content of Oysters[a]

Storage Time, Days	Irradiation Dosage, Mrad 0	0.2	0.4	Av.
0	19.39	14.42	15.61	16.47
5	15.73	17.47	17.57	16.92
10	21.07	16.01	16.71	17.93
15	20.65	19.53	16.99	19.05
20	20.23	23.44	20.86	21.51
Av.	19.41	18.17	17.55	18.38

[a] Carbohydrate values expressed in mg./g. of oyster. Values given are averages of 3 replications.

Table V. Effect of Radiation and Iced Storage on Per Cent Crude Protein of Oysters[a]

Storage Time, Days	Irradiation Dosage, Mrad 0	0.2	0.4	Av.
0	7.45	7.11	7.39	7.32
5	7.27	6.97	6.97	7.07
10	7.41	6.97	7.15	7.18
15	7.30	7.18	6.64	7.04
20	7.37	6.93	7.17	7.16
Av.	7.37	7.03[b]	7.07[b]	7.16

[a] Averages of 2 replications.
[b] % crude protein significantly lower than at 0.0 Mrad ($P < 0.05$).

Table VI. Effect of Radiation and Iced Storage on Per Cent Nonprotein Nitrogen of Oysters[a]

Storage Time, Days	Irradiation Dosage, Mrad 0	0.2	0.4	Av.
0	0.222	0.218	0.244	0.228
5	0.361	0.209	0.241	0.270
10	0.186	0.183	0.209	0.193
15	0.256	0.278	0.254	0.263
20	0.246	0.186	0.167	0.200
Av.	0.254	0.215	0.223	0.231

[a] Averages of 2 replications.

Table VII. Effect of Radiation and Iced Storage on Per Cent True Protein of Oysters[a]

Storage Time, Days	Irradiation Dosage, Mrad 0	0.2	0.4	Av.
0	5.88	5.75	5.81	5.81
5	5.69	5.63	5.63	5.65
10	5.88	5.63	5.75	5.75
15	5.75	5.81	5.25	5.60
20	5.81	5.56	5.81	5.73
Av.	5.80	5.56	5.65	5.64

[a] Obtained by subtracting averages of 2 replications of NPN from averages of 2 replications of total nitrogen.

Table VIII. Effect of Radiation and Iced Storage on Per Cent Crude Fat of Oysters[a]

Storage Time, Days	Irradiation Dosage, Mrad 0	0.2	0.4	Av.
0	2.48	2.24	2.10	2.17
5	2.73	2.40	2.79	2.64
10	2.18	3.11	2.83	2.71
15	2.76	2.89	2.84	2.83
20	2.28	2.56	2.24	2.36
Av.	2.49	2.64	2.56	2.56

[a] Averages of 2 replications.

K_2SO_4 and 0.3 gram of $CuSO_4$. Forty milliliters of concentrated H_2SO_4 was added to each flask and total nitrogen was determined by the Kjeldahl-Gunning procedure (Association of Official Agricultural Chemists, 1965).

To separate the protein nitrogen from the NPN, approximately 50 grams of each sample was homogenized for 3 minutes with 2 volumes of 5% TCA. The homogenate was centrifuged for 15 minutes and the supernatant, containing NPN, was separated from the precipitated protein by means of a suction filter. Duplicates, using 0.5 ml. of each supernatant, were placed in micro-Kjeldahl flasks and digested according to Koch and Hanke (1953). The digested samples were further treated and determined by methods described by Hawk *et al.* (1954).

Crude Fat Determination. Approximately 50 grams of oyster samples were homogenized for 3 minutes. Homogenate duplicates of 10 grams each were placed in Soxhlet thimbles and fat was determined by the SI-MO-FAT method of Davis *et al.* (1966).

An analysis of variance was conducted for each set of data obtained, using the methods of Snedecor (1962).

RESULTS AND DISCUSSION

The average nutrient contents of the oysters are shown in Tables I through VIII. These tables reflect any changes in concentration resulting from irradiation and iced storage.

The only nutrient significantly affected by irradiation was

crude protein (Table V). However, when these values were converted to true protein, the significance was lost (Table VII). The decreases observed in NPN after irradiation (Table VI) were not large enough to be credited with the significant decreases in crude protein. Since these differences just entered the fiducial limits of the 5% probability level, the minute factors which caused them were probably negated when the crude protein fraction was divided into NPN and true protein.

Soluble sugar concentrations were also decreased by irradiation of the oysters; however, they were not significant (Table IV). Higher doses of ionizing radiation lower the polysaccharide content and subsequently raise the soluble sugar concentration (Saini, 1968). However, if relatively high amounts of water are present, the soluble sugars formed are subject to oxidation (Long and Lirot, 1957). Since oysters contain a large amount of water, it is possible that the slight decreases observed in soluble sugars are due to radiolysis.

The most critical reduction of nutrient composition observed with storage was in the ash content (Table II). It is possible that appreciable amounts of minerals leached into the liquid medium from the oyster tissue during storage time. Although not significant, glycogen levels decreased with storage time (Table III). This was probably due to hydrolytic mechanisms which were active even during iced storage. This reduction can be correlated with an increase in soluble sugars as storage time increased. These increases approached significance at 15 and 20 days. All other nutrients remained relatively stable during storage.

The results of this investigation strengthen the conclusions of Novak *et al.* (1966), who proposed that Gulf oysters can be successfully preserved for extended periods with gamma irradiation at levels of 0.2 Mrad and subsequent iced storage.

LITERATURE CITED

Ambe, K. S., Tappel, A. L., *J. Food Sci.* **26,** 448 (1961).
Association of Official Agricultural Chemists, "Official and Tentative Methods of Analysis," 10th ed., 1965.
Brooke, R. O., Steinberg, M. A., *Food Technol.* **18,** 1056 (1964).
Cloutier, J. A. R., Cox, C. E., Manson, J. W., Clay, M. G., Johnson, L. E., *Food Res.* **24,** 659 (1959).
Davis, C. E., Ocherman, H. W., Cahill, V. R., *Food Technol.* **20,** 1475 (1966).
Dollar, A. M., Liston, J., Matches, J. A., Shabyj, P. A., Annual Progress Report, U. S. Atomic Energy Commission, Contract AT-(54-1)-1544/1730 (1964).
Food and Drug Administration, "Definitions and Standards for Shellfish," Title 21, Part 36, Section 36.10, 1967.
Hawk, P. B., Oser, B. L., Summerson, W. H., "Practical Physiological Chemistry," 13th ed., pp. 544–7, 603–4, 878–80, Blakiston, New York, Toronto, 1954.
Koch, F. C., Hanke, M. E., "Practical Methods in Biochemistry," 5th ed., p. 497f, Williams and Wilkins, Baltimore, 1953.
Liuzzo, J. A., Barone, W. B., Novak, A. F., *Federation Proc.* **25,** 722 (1966).
Long, L., Jr., Lirot, S. J., U. S. Army Research and Development Series, No. **9,** 126 (1957).
Montgomery, R., *Biochem. Biophys. Acta* **48,** 591 (1961).
Nawar, W. W., Dubravcic, M., Proceedings of Sixth Annual Food Irradiation Contractor's Meeting, U. S. Atomic Energy Commission, Washington, D. C., 1966.
Novak, A. F., Liuzzo, J. A., Grodner, R. M., Lovell, R. T., *Food Technol.* **20,** 201 (1966).
Proctor, B. E., Goldblith, S. A., *Food Technol.* **5,** 376 (1951).
Proctor, B. E., Nickerson, J. T. R., Goldblith, S. A., *Refrig. Eng.* **58,** 357 (1950).
Reber, E. F., Bert, M. H., Proceedings of Seventh Annual Food Irradiation Contractor's Meeting, U. S. Atomic Energy Commission, Washington, D. C., 1967.
Saini, V., *J. Food Sci.* **33,** 136 (1968).
Schweigert, B. S., Doty, D. M., Research Dept. S-507, U. S. Army Q. M., Food and Container Institute, Chicago, 1958.
Sheffner, A. L., Adachi, R., Spector, H., *Food Res.* **22,** 455 (1957).
Snedecor, G. W., "Statistical Methods," 5th ed., Iowa State College Press, Ames, Iowa, 1962.

Received for review June 12, 1968. Accepted April 11, 1969. Work supported in part by a research grant under Contract AT-(40-1)-2951, U. S. Atomic Energy Commission.