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Postmortem Changes of Sterile Fish Muscle Inoculated with a Proteolytic *Pseudomonas* sp.

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무균 어육에 단백질 분해세균 접종후의 저장중의 변화

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사후 저장기간 중 어육내에 진행되는 단백질 분해에 어육 자체효소와 세균활동 중 어느 쪽이 기여하는 바 큰가를 분별하기 위하여 금방 죽인 볼낙류(*Sebastodes*)의 어육을 무균방법으로 잘라내어 4 OZ 유리용기에 넣고 플라스틱으로 된 나사뚜껑으로 밀봉한 후 감마선 조사하지 않는 군(무균육과 무균육에 담백질 분해성 *Pseudomonas* 를 접종한 것으로 세분)과 0.5과 2.0 *Mrad* 선량 조사한 군(무균육, 조사 전에 접종시킨 어육, 그리고 조사후에 접종시킨 어육으로 세분)으로 나누어 0°C 에서의 저장 중의 어육의 총균수, pH, 관능 상태, 그리고 0.5*M* KCl 및 70% ethanol 가용성 총질소와 Amino-N 을 검측함으로써 어육 단백질 분해 과정을 조사하였다.

0.5 *Mrad* 감마선량은 무균 어육에 접종된 10^6 per *gm* Muscle *Pseudomonas* sp.를 완전 사멸시키는데 충분하였으므로 완전한 무균상태와 처음부터 단백질 분해성 세균활동이 활발한 상태에 놓여 있는 어육 내에 진행되는 단백질 분해과정을 비교할 수 있었다.

기름지지 않는 어류에 속하는 볼낙 어육의 저장 중에 일어나는 pH 의 상승, 관능상태의 저락, 그리고 단백질 분해에서 오는 총질소와 Amino-N 의 축적은 모두 세균의 번식활동과 직접 관련되어 있으며, 완전 무균 어육의 담백질 분해는 전혀 검측되지 안했으므로 통상 저장중에 일어나는 어육 담백질 분해는 전부가 세균활동에 기인됨이 확실해졌다.

감마선 조사 직후 단백질 분해성 세균을 접종시킨 어육을 조사치 않고 접종시킨 어육에 비교할 때, 전자의 경우 세균번식이 현저히 장해 받았으며, 이에 따라 pH 상승과 총질소 및 Amino-N 축적도 상대적으로 부진하였다. 이 방사선을 조사받은 어육에 접종된 단백질 분해성 세균활동의 장해는 조사선량에 비례함이 밝혀졌다.

관능상태로 판단할 때 0.5 *Mrad* 선을 조사받은 어육은 조사받지 않은 것보다 선도유지기간이 2배이상으로 연장되었다. 그러나 2.0 *Mrad* 선량 조사받은 어육의 선도유지는 오히려 조사받지 않은 것보다 못하였다. 이 고선량 조사로 인한 식품의 물리적 및 화학적 변화의 해독성은 접종시킨 세균 번식 장해현상으로 나타났으며, 이 현상은 조사식품의 안전성 검측을 위한 하나의 도구로 사용될 가능성을 말해준다.

1. Introduction

Our present-day knowledge on the relative contribution of muscle enzymes and microbial action to the postmortem breakdown of fish muscle proteins is mostly derived from comparative storage studies of sterile and spoiling fish muscle. Obtaining truly sterile muscle samples with minimum disruption of fish muscle system, however, has been the chief difficulty (Partmann, 1954).

In interpreting the data obtained from samples treated with toluene (Reed *et al.*, 1929; Beatty and Collins, 1939; Partmann, 1954), mixtures of toluene and thymol (Kamarove, 1933), or antibiotics (de Silva and Hughes, 1660) in order to suppress bacterial growth, the sterility of such samples must be taken into consideration, since such methods tend to be selectively bactericidal rather than sterilizing. The techniques involving dissection of sterile block of muscle (Partmann, 1954; Nickerson and Proctor, 1935; Hodgkiss and Jones, 1955; Shewan and Jones, 1957) or irradiation of whole fish or fish muscle to remove the surface microflora (Nickerson, *et al.*, 1950; Proctor, *et al.*, 1950) are most promising.

In recent years, a number of investigators have reported on autolytic proteolysis based on free amino acid changes occurring in fish muscle (de Silva and Hughes, 1960; Jones, 1955; Siebert and Schimitt, 1965; Shewan and Jones, 1957; Bramstedt, 1962). Their results invariably indicate that, in white muscle of fish, protein breakdown by muscle proteinases is quite negligible as compared to bacterial action during the practical storage period of fish. There is evidence, however, that a significant proteolysis independent of bacterial action occurs in fatty fish like mackerel (Nickerson *et al.*, 1950). In enzyme systems isolated from the muscle of a number of species of fish the enzymes from dark muscle are invariably more active than those from white muscle (Saito and Sameshima, 1958 a and b; Fujii *et al.*, 1951; and Groninger, 1964).

Even in the fatty fish muscle, however, the autolysis alone can proceed only so far in the absence of exogenous factors such as bacterial and digestive proteinases and contribution of endogenous enzymes of fish muscle to the post-mortem protein degradation under the normal storage condition is believed to be very slight (Jones, 1962).

The advent of irradiation preservation methods has provided a useful tool to study the relative importance of fish muscle proteinases as contrasted to those of exogenous origin. *Pseudomonas* sp. are frequently associated with the spoilage of fresh meats and fishery products(Bellamy *et al.*, 1955; Shewan, 1962) and are often strongly proteolytic (Vanderzant, 1957; Shewan, 1962; Peterson and Gunderson, 1960; and Hurley *et al.*, 1963). It is known that the *Pseudomonas* sp. are particularly radio-sensitive (shewan and Liston, 1958) and are completely inactivated by low doses (about 10^5 rad) of radiation (Niven, 1958). Since enzymes apparently are more radio-resistant than microorganisms (Sui, 1957; Raymann and Byrne, 1957; and Niven, 1958), it is possible that the spoilage of irradiation pasteurized products could continue even after the initial viable microbial population is greatly reduced.

To determine whether prolonged shelf life of fish fillets is feasible, it is necessary to establish

whether muscle proteinases (cathepsin) could be a principal cause of protein degradation durir non-frozen storage of a non-fatty fish.

2. Material and Methods

Rock fish (*Sebastodes caurinus* and *S. auriculatus*) were caught by otter trawl, transporte alive and kept in a circulating seawater aquarium until used. These species were chosen f this study because they are non-fatty round fish and could provide the large segments of steri muscle required for the study of chemical and microbiological changes during storage. The li fish, one at a time, were stunned by a blow on the head and the dorsal muscle was excise aseptically in a glove box. The aseptically excised muscle of 10–15 gm was placed in dr sterile, four oz widemouth bottles provided with plastic screw-caps. The exact weight of tl muscle sample (± 0.05 gm) was determined by weighing the bottles before and after tl transfer of samples.

The samples were divided into two unirradiated and three irradiated groups. The two unirr diated groups consisted of sterile muscle (Group I, unirradiated sterile muscle) and steri muscle inoculated with a proteolytic strain of *Pseudomonas* sp. to give 10^6 cells per gm musc (Group II, unirradiated inoculated muscle). The three irradiated groups were: Group II irradiated sterile muscle; Group IV, sterile muscle inoculated as in Group II before irradiatio and Group V, sterile muscle inoculated as in Group II, but after irradiation. The irradiate groups were exposed to either 0.5 or 2.0 Mrad of gamma radiation at 1°C. All samples we then stored at 0°C for the experimental period.

The proteolytic strain of *Pseudomonas* sp. used in these studies was isolated from cod durii ice storage (Liston and Kasemsarn, 1962). The celle free growth medium obtained from tl culture of this organism will digest actively (1.4 μM leucine N/hr/mg/N) proteins prepar from rockfish muscle (Chung, 1963). The inoculum for these present studies was subcultur twice on seawater MacLeod agar slants incubated at 20°C for 14hr. The bacterial cells in tl stationary phase of growth were transferred from the slants with a sterile cotton swab to 500 ml Erlenmeyer flask containing 150 ml of clear fish broth. This broth medium was prepar by boiling rockfish fillets in five volumes of tap water for one hr and then filtering through large, coarse filter paper. To this extract was added 0.05% yeast extract, 0.1% glucose and 5% NaCl. The pH of the medium was adjusted to 7.4 with 0.1*N* NaOH and sterilized f 15 min at 15 psi. The inoculated flask, containing 12 small glass beads to aid in preparing tl suspension, was shaken on a rotatory shaker at a low speed for 14 hr in a 20°C room ensure good growth. The culture suspension was spread on the surface of the muscle sampl (1 *ml* per 10 *gm* flesh), using a pipet. This volume was sufficient to wet the entire surface the muscle samples giving the bacterial density of 10^6 cells per gm muscle.

At intervals of 0, 1, 5, 10, 20, 40, and 60 days, one bottle was withdrawn from each grou and organoleptic characteristics were noted and then the muscle homogenized with 20 times i own volume of chilled 0.5*M* KCl solution in a sterile Waring blender for 2min at low spee Aliquots of the homogenate were used for determining the viable cell density, pH, and f

estimating the amino and total nitrogen soluble in 0.5 *M* KCl and 70% ethanol.

The viable count was estimated by the standard pour-plate technique, using phosphate buffer (pH 7.2) as a diluent and seawater MacLeod agar (pH 7.4) of MacLeod *et al.* (1954) as medium. The number of colonies were counted after 5 days of aerobic incubation at 20°C and the results expressed as logarithmic number of bacteria per gm muscle. The pH of the homogenate was measured electrometrically.

Fifty *ml* of the homogenate was centrifuged (9,750×g) for 30min at 0°C. Aliquots of the supernatant were used for estimating amino and total nitrogen content. These were the nitrogen contents of 0.5 *M* KCl soluble fraction (referred to as nitrogen soluble in 0.5 *M* KCl). Aliquots of the remaining portion of the supernatant were mixed with 95% ethanol to give 70% in ethanol. Precipitates were removed by filtering through Whatman #50 paper. The filtrate was used for estimating again amino and total nitrogen (referred to as nitrogen soluble in 70% ethanol).

The amino nitrogen was determined by the method of Moore and Stein (1954) and the total nitrogen by the micro-Kjeldahl method (A.O.A.C., 1955). All chemical analyses of the 0.5 *M* KCl soluble fraction were made on the day of sampling, whereas the 70% ethanol soluble fraction was stored at −17°C for later determination. All analyses were run in duplicate with proper controls and results expressed as micromole nitrogen per gm muscle.

3. Results

Total viable count: The changes in total viable count in all five sample groups are shown in Table 1. The unirradiated sterile muscle (Group I) remained sterile for 20 days, whereas the irradiated sterile muscle (Group III) and the muscle inoculated with a proteolytic strain of *Pseudomonas* sp. before irradiation (Group IV) remained virtually free of bacteria throughout the storage period. After 40 days the count in these latter sample groups was between 101 to 103 cells per gm muscle. These low counts at the end of storage, particularly in the 0.5 Mrad irradiated samples, were probably due to contamination. This indicates that 0.5 Mrad of gamma radiation is sufficient to inactivate the *Pseudomonas* sp. inoculated on the surface of the muscle samples to the density of 10^6 cells per gm muscle.

In the unirradiated inoculated muscle (Group II)and the samples inoculated after irradiation (Group V), the number of bacterium increased from 10^6 to $10^9 \sim 10^{10}$ cells per gm muscle by the 10th day. By the 40th day, the counts were declining. It was noted, however, not only the early phase of bacterial growth in the irradiated samples was significantly inhibited, but also the growth during subsequent storage period remained below the level of that in the unirradiated inoculated group (Group II). Furthermore, bacterial growth on the irradiated fish muscle appeared to reflect radiation dose in that the number of bacterium increased more rapidly in the unirradiated inoculated muscle (Group II), less rapidly in the 0.5 Mrad irradiated muscle, and least rapidly in the 2.0 Mrad irradiated muscle (Table 1).

pH: As shown in Table 2, the initial pH of all sample groups was 6. 80. In the samples which remained virtually sterile during the storage (Group III and IV), the pH decreased very slightly

on the 1st day, followed by a slight increase above 6.80, then decreased again below 6.80 at the end of the storage. The overall pH changes in these sample groups throughout the storage period was very slight. On the other hand the pH of the unirradiated muscle (Group Ⅰ) started to increase by the 40th day, reaching a value of 7.25 on the 60th day. This late increase in pH should be explainable by the increase in the total viable count (Table 1).

Table I. Changes of Total Viable Count of Rockfish Muscle at 0°C

(Log. No. bacteria/gm muscle)

Sample Group	Irradiation (Mrad)	Storage (day)						
		0	1	5	10	20	40	60
Ⅰ	0	0	0	0	0	2.1	4.6	4.3
Ⅱ	0	6.5	7.2	9.7	10.0	10.6	10.3	9.8
Ⅲ	0.5	0	0	0	0	0	0	1.5
〃	2.0	0	0	0	0	0	0	0
Ⅳ	0.5	0	0	0	0	0	2.5	3.5
〃	2.0	0	0	0	0	0	0	3.0
Ⅴ	0.5	6.5	5.4	8.2	9.4	9.8	9.8	9.7
〃	2.0	6.4	4.6	7.5	8.7	9.7	9.4	9.1

Group Ⅰ. Unirradiated sterile muscle; Group Ⅱ. Unirradiated sterile muscle inoculated with *Pseudomonas;* Group Ⅲ. Sterile muscle irradiated at 0.5 and 2.0 Mrad; Group Ⅳ. Sterile muscle inoculated before irradiation at 0.5 and 2.0 Mrad; Group Ⅴ. Sterile muscle inoculated after irradiation at 0.5 and 2.0 Mrad.

The pH of the unirradiated inoculated group (Group Ⅱ) and the muscle inoculated after irradiation (Group Ⅴ) increased with the storage, closely paralleling the increase in total viable count. Although the difference in pH values of these groups were slight, the unirradiated inoculated muscle had the highest pH values, and 0.5 Mrad irradiated muscle inoculated after irradiation had the next highest while the same sample groups irradiated at 2.0 Mrad had the lowest pH at each sampling interval.

Table 2. Changes of pH of Rockfish Muscle at 0°C

Sample Group	Irradiation (Mrad)	Storage (day)						
		0	1	5	10	20	40	60
Ⅰ	0	6.80	6.80	6.80	6.80	6.80	7.10	7.25
Ⅱ	0	6.80	6.80	6.80	7.10	7.40	7.60	8.50
Ⅲ	0.5	6.80	6.80	6.80	6.80	6.90	6.78	6.68
〃	2.0	6.80	6.75	6.80	6.85	6.90	6.82	6.70
Ⅳ	0.5	6.80	6.73	6.73	6.85	6.85	6.84	6.70
〃	2.0	6.80	6.73	6.80	6.80	6.90	6.79	6.70
Ⅴ	0.5	6.80	6.80	6.80	6.90	6.95	7.30	8.30
	2.0	6.80	6.80	6.86	6.87	6.90	7.20	8.20

Group Ⅰ. Unirradiated sterile muscle; Group Ⅱ. Unirradiated sterile muscle inoculated with *Pseudomonas;* Group Ⅲ. Sterile muscle irradiated at 0.5 and 2.0 Mrad; Group Ⅳ. Sterile muscle inoculated before irradiation at 0.5 and 2.0 Mrad; Group Ⅴ. Sterile muscle inoculated after irradiation at 0.5 and 2.0 Mrad.

Organoleptic characteristics: At each sampling interval, the general appearance and odor of each sample and its homogenate were recorded. In general, all samples irradiated at 0.5 Mrad

remained sea-fresh until after the 10th day, whereas the same stage of freshness for the unirradiated sterile samples was lost by the 5th day. The samples irradiated at 2.0 Mrad had a characteristic odor which can best be described as that of a spoiling smoked fish. In addition to this, these latter samples had tiny brown spots on the surface, probably due to irradiation burn, which became more or less uniformly brown (smoked fish appearance) by the 20th day, and by the 40th day became definitely sour and rancid. The 0.5 Mrad irradiated samples were also smoked fish-like in appearance and odor by the 40th day, but to a lesser degree, and the unirradiated sterile samples, which probably were contaminated during late storage, had developed a fluorescent yellow appearance at the end of storage. However, none of the samples inoculated before irradiation or the unirradiated sterile samples developed any putrefactive odor. The absence of any significant change in pH and negligible bacterial action in the samples irradiated at 2.0 Mrad indicated that the deterioration in organoleptic quality (appearance and odor) was due to some undetermined cause, such as oxidative rancidity and non-enzymatic browning.

Deterioration of organoleptic quality of the unirradiated inoculated muscle (Group Ⅱ) and the muscle inoculated after irradiation became evident as the bacterial activity increased. By the 5th day the former were distinctively putrefactive, whereas the latter were only slightly putrefactive. As the deterioration progressed, the bacterial growth on the surface of samples accompanied a softening of muscle tissue which progressed until the 60th day, when more than half of the unirradiated inoculated muscle samples (Group Ⅱ) were liquefied, whereas less than one-third of the surface of the samples inoculated after irradiation (Group Ⅴ) was liquefied.

Amino nitrogen: As shown in Table 3, the 0 day values of amino nitrogen content soluble in 0.5 *M* KCl ranged between 81.54 and 87.23 μ Mole leucine equivalent nitrogen per gm fish muscle. The changes during the storage in general closely paralleled the increase of bacterial numbers in each sample group. In Group Ⅲ and Ⅳ samples, which remained virtually sterile throughout the storage period, the amino nitrogen content declined with the storage in both 0.5 and 2.0 Mrad irradiated samples, but the overall decrease was very slight, ranging from 9.38 to 20.23 μ Mole per gm muscle. The unirradiated sterile muscle (Group Ⅰ) underwent a

Table 3. Changes of Amino Nitrogen in 0.5 *M* KCl Soluble Fraction

(μ Mole/gm musele)

Sample Group	Irradiation (Mrad)	Storage (day)						
		0	1	5	10	20	40	60
Ⅰ	0	81.54	76.69	76.23	83.92	77.85	79.85	76.75
Ⅱ	0	81.77	83.38	115.38	271.43	464.40	834.61	912.00
Ⅲ	0.5	82.38	78.85	68.85	79.49	62.38	66.85	62.15
〃	2.0	83.61	80.15	78.84	76.08	74.69	74.61	72.77
Ⅳ	0.5	87.23	76.77	74.30	68.15	64.53	75.92	77.85
〃	2.0	85.38	75.46	70.46	70.85	71.31	72.56	71.38
Ⅴ	0.5	83.41	75.62	76.54	135.69	208.38	378.00	803.07
〃	2.0	84.46	78.85	74.62	161.85	173.38	248.08	567.69

Group Ⅰ. Unirradiated sterile muscle; Group Ⅱ. Unirradiated sterile muscle inoculated with *Pseudomonas;* Group Ⅲ. Sterile muscle irradiated at 0.5 and 2.0 Mrad; Group Ⅳ. Sterile muscle inoculated before irradiation at 0.5 and 2.0 Mrad; Group Ⅴ. Sterile muscle inoculated after irradiation at 0.5 and 2.0 Mrad.

similar pattern of changes except that the value was slightly above the 0 day value on the 10th day probably due to the bacterial action.

In Group II and V samples, in which the proteolytic strain of *Pseudomonas* sp. was actively growing, the amino nitrogen values steadily increased with the storage, resulting in an overall increase of 7 to 11 fold (Table 3). It is significant to note that the increase was the greatest in the unirradiated muscle (Group II), the next greatest in the 0.5 Mrad irradiated and the least in the 2.0 Mrad irradiated muscle samples of Group V.

The amino nitrogen in 70% ethanol soluble fraction of 0 day samples ranged from 59.82 to 63.31 μ Mole per gm muscle (Table 4) and, when compared to the values of 0.5 *M* KCl

Table 4. Changes of Amino Nitrogen in 70% Ethanol Soluble Fraction

(μ Mole/gm muscle)

Sample Group	Irradiation (Mrad)	Storage (day)						
		0	1	5	10	20	40	60
I	0	61.00	56.34	55.26	59.93	52.40	56.24	58.65
II	0	63.31	63.08	107.99	221.80	363.47	546.90	686.75
III	0.5	60.93	55.32	50.81	56.81	49.36	49.21	51.70
〃	2.0	59.82	55.16	51.97	50.79	46.79	48.11	50.97
IV	0.5	61.20	56.70	52.18	49.84	46.61	50.26	48.38
〃	2.0	60.98	55.20	51.17	49.20	47.56	48.96	48.25
V	0.5	62.44	58.81	55.53	127.17	157.68	269.46	635.93
〃	2.0	61.43	57.46	54.36	122.77	135.58	219.62	471.16

Group I. Unirradiated sterile muscle; Group II. Unirradiated sterile muscle inoculated with *Pseudomonas;* Group III. Sterile muscle irradiated at 0.5 and 2.0 Mrad; Group IV. Sterile muscle inoculated before irradiation at 0.5 and 2.0 Mrad; Group V. Sterile muscle inoculated after irradiation at 0.5 and 2.0 Mrad.

soluble fraction, it was 21.72 to 23.93 micromoles less. This difference was due to the removal of proteins soluble in 0.5 *M* KCl by precipitating in 70% ethanol.

Table 5. Changes of Total Nitrogen in 0.5 *M* KCl Soluble Fraction

(μ Mole/gm/muscle)

Sample Group	Irradiation (Mrad)	Storage (day)						
		0	1	5	10	20	40	60
I	0	1,017.89	976.10	957.22	986.88	869.82	841.51	937.00
II	0	1,179.91	1,143.51	1,295.62	1,810.63	2,010.63	2,279.80	2,144.99
III	0.5	1,004.41	932.95	949.13	947.62	748.25	723.26	667.36
〃	2.0	1,087.76	966.66	981.57	818.36	740.49	716.10	647.14
IV	0.5	1,024.63	935.65	920.96	779.26	705.78	672.72	714.32
〃	2.0	1,094.50	965.03	924.63	721.29	678.14	632.21	622.87
V	0.5	1,144.86	959.92	1,094.74	1,344.16	1,540.99	2,042.52	2,082.97
〃	2.0	1,193.15	1,045.50	1,004.41	1,228.63	1,290.23	1,871.77	3,082.97

Group I. Unirradiated sterile muscle; Group II. Unirradiated sterile muscle inoculated with *Pseudomonas;* Group III. Sterile muscle irradiated at 0.5 and 2.0 Mrad; Group IV. Sterile muscle inoculated before irradiation at 0.5 and 2.0 Mrad; Group V. Sterile muscle inoculated after irradiation at 0.5 and 2.0 Mrad.

The changes of the amino nitrogen in 70% ethanol soluble fraction during the storage followed the identical pattern that was observed in 0.5 *M* KCl soluble fraction.

Total nitrogen: The total nitrogen soluble in 0.5 *M* KCl on 0 day ranged from 1,004.41 to 1,193.15 μ Mole per gm muscle (Table 5). As it was with the amino nitrogen, the total nitrogen values of the irradiated sterile muscle (Group Ⅲ) and the muscle inoculated after irradiation declined gradually during the storage, whereas the values of Group II and V samples increased steadily, closely paralleling the bacterial growth. Again increase was the greatest in the unirradiated, inoculated muscle (Group Ⅱ), the next greatest in the 0.5 Mrad irradiated, inoculated and the least in the 2.0 Mrad irradiated, inoculated muscle of Group V.

The total nitrogen soluble in 70% ethanol was between 360.42 and 377.24 μ Mole per gm muscle (Table 6) and, as compared to the values of 0.5 *M* KCl soluble fraction, the 70%

Table 6. Changes of Total Nitrogen in 70% Ethanol Soluble Fraction

(μ Mole/gm muscle)

Sample Group	Irradiation (Mard)	Storage (day)						
		0	1	5	10	20	40	60
Ⅰ	0	372.31	350.11	348.86	363.53	338.83	328.81	344.01
Ⅱ	0	373.12	365.12	470.43	906.77	1,421.74	1,786.71	1,812.65
Ⅲ	0.5	360.42	342.81	349.15	333.75	325.09	316.26	306.06
〃	2.0	362.12	352.90	333.42	312.18	322.66	305.89	284.98
Ⅳ	0.5	362.00	350.23	338.25	308.25	305.59	298.31	354.01
〃	2.0	369.74	345.43	326.02	305.79	298.12	291.88	288.24
Ⅴ	0.5	370.59	350.52	360.26	470.57	627.95	818.96	1,506.38
〃	2.0	377.24	367.74	363.19	450.79	596.74	786.61	1,279.26

Group Ⅰ. Unirradiated sterile muscle; Group Ⅱ. Unirradiated sterile muscle inoculated with *Pseudomonas;* Group Ⅲ. Sterile muscle irradiated at 0.5 and 2.0 Mrad; Group Ⅳ. Sterile muscle inoculated before irradiation at 0.5 and 2.0 Mrad; Group Ⅴ. Sterile muscle inoculated after irradiation at 0.5 and 2.0 Mrad.

ethanol soluble fraction contained approximately one-third the value contained in the 0.5 *M* KCl soluble fraction. Again this difference in the total nitrogen content between the 0.5 *M* KCl and 70% ethanol soluble fraction was due to the removal of soluble proteins by precipitating in 70% ethanol. Two distinct patterns of changes during the storage were observed as before, one for groups (Group Ⅰ, Ⅲ, and Ⅳ) in which bacterial action was either absent or very slight and another for groups (Group Ⅱ and Ⅴ), in which the inoculated proteolytic strain of *Pseudomonas* sp. was actively growing.

4. Discussion

As aforementioned, the dose of 0.5 Mrad was sufficient to inactivate the inoculated *Pseudomonas* sp. in Group Ⅳ and therefore the microbiological and chemical changes underwent a pattern identical to that of the sterile sample groups (Group Ⅰ and Ⅲ). The samples of Group Ⅱ and Ⅴ, in which the inoculated bacterium was actively growing, represented the spoiling fish muscle. One of the objectives of present investigation was to obtain a sharp distinction between endogenous enzymes and bacterial activities for their relative contribution to post-mortem breakdown of fish

muscle proteins. For this the aseptically excised fish muscle were inoculated with a proteolytic strain of *Pseudomonas* sp. to the level of 10^6 cells per gm muscle and as sterile muscle the aseptically excised muscle were irradiated at 0.5 or 2.0 Mrad in insure sterility. Thus the pattern of proteolysis occuring in intact muscle completely independent of bacterial action and that in the muscle under the overwhelming influence of a proteolytic bacterial action could de compared.

When the changes in pH, viable cells, and amino and total nitrogen values of the samples inoculated after irradiation and the unirradiated, inoculated samples are compared to those of the unirradiated sterile, irradiated sterile, and samples inoculated before irradiation, in which bacterial action was either absent or negligible, it is clear that the relative activity of bacteria is directly correlated to the changes observed and that virtually no change can be attributed to endogenous tissue enzymes when samples free from bacterial action are stored at 0°C as long as 60 days.

The growth of the inoculated *Pseudomonas* sp. in the irradiated fish muscle (Group V) was severely retarded on the 1st day and the viable cell counts remained below those in the unirradiated inoculated muscle (Group II) throughout the storage (Table 1). Although the dependency of inhibitory effect upon radiation dose applied during the early storage period could not be clearly

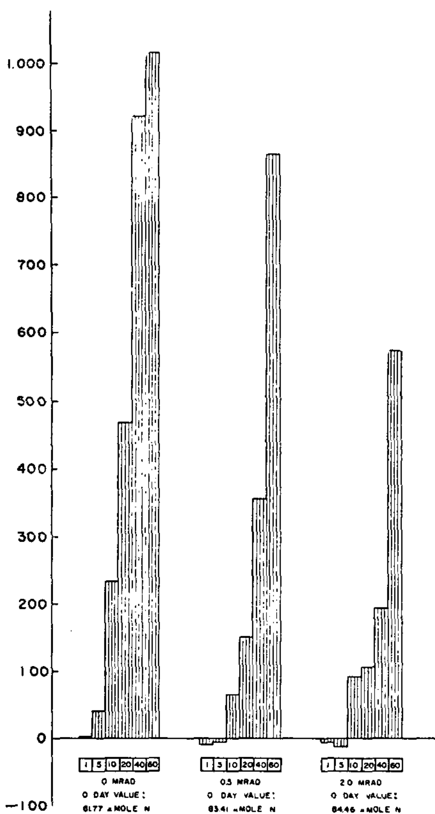

Fig. 1. Amino nitrogen in 05. M KCl soluble fraction of rockfish muscle inoculated with *Pseudomonas* (percent increase over 0 day value).

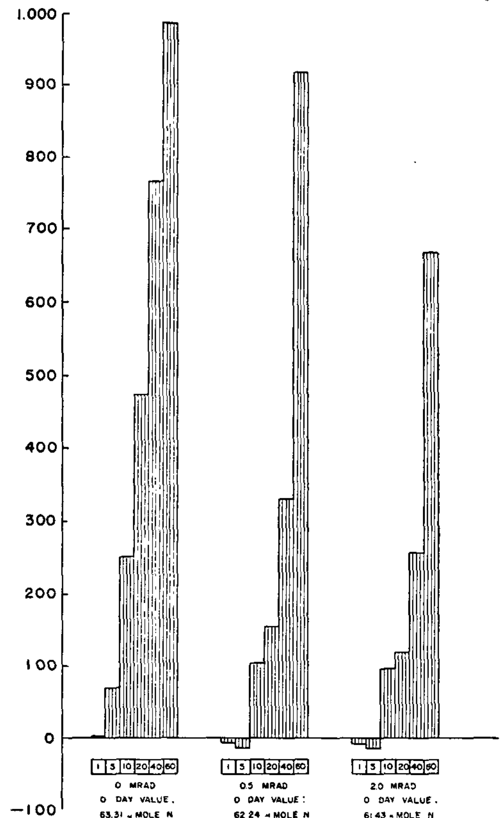

Fig. 2. Amino nitrogen in 70% ethanol soluble fraction of rockfish muscle inoculated with *Pseudomonas* (percent increase over 0 day value).

established due to insufficient sampling between 0 and 5th day, the inhibition was more pronounced in the 2.0 Mrad irradiated group than in the 0.5 Mrad irradiated throughout the storage (Table 1).

Pugsley *et al.* (1935) noted that the growth of *E. coli* and *Sarcina lutea* was retarded when inoculated onto nutrient agar previously exposed to X-rays. The inhibitory effect dissipated completely as the time between the exposure and inoculation was extended to 349 min. Solberg and Nickerson (1963) also observed a similar finding with *Staphylococcus aureus* (ATCC 9664) when inoculated onto the 0.6 Mrad irradiated chicken muscle.

The inhibitory effect of irradiated fish muscle upon the growth of the *Pseudomonas* sp. was also reflected on the accumulation of amino and total nitrogen content in the 0, 0.5, and 2.0 Mrad irradiated muscle of Group II and V. It is apparent from the per cent increase at each storage interval of amino nitrogen in 0.5 *M* KCl as well as in 70% ethanol soluble fraction over respective 0 day value as shown in Figs. 1 and 2 that the growth of the proteolytic strain of *Pseudomonas* sp. inoculated in the unirradiated fish muscle continued with no apparent lag (Table 1) and this unhindered metabolic activity of *Pseudomonas* sp. was reflected in the steady accumulation of aimo nitrogen content. In the irradiated muscle, however, the inhibition of growth of the inoculated *Pseudomonas* sp. resulted in the corresponding decrease of amino nitrogen content below 0 day value until the 5th day and a significant increase was delayed until the 10th day. At each storage interval the per cent increase over 0 day value was the highest in the unirradiated muscle (Group II), the next highest in the 0.5 Mrad irradiated and the least in the 2.0 Mrad irradiated muscle of Group V (Figs. 1 and 2).

The inhibitory effect of irradiated fish muscle upon the accumulation of total nitrogen content in both 0.5 *M* KCl and 7o% ethanol soluble fractions was also the same as with the amino nitrogen, thus establishing the dose depency of growth and metabolic activities of the *Pseudomonas* sp. when inoculated in irradiated fish muscle.

This dose dependency was also apparent from the extent of liquefraction of inoculated muscle samples; by the end of storage approximately one-third of muscle inoculated after irradiation (Group V) was liquefied, whereas more than half of the unirradiated inoculated muscle was liquefied.

The results on the inhibitory effect of irradiated fish muscle on bacterial growth and metabolic activities suggest an interesting possibility of measuring the extent of damage done to food as a result of irradiation from the growth response of a well defind strain of bacterium highly sensitive to irradiated foods.

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