Bull. Korean Fish. Soc. 7(4); 1974 韓水誌7(4); 1974

Microbiological and chemical changes in the Pacific oysters, *Crassostrea gigas* exposed to gamma radiation during ice storage

Jong Rak CHUNG *

감마선 조사된 참굴의 빙장 기간중의 세균학 및 화학적 변화

정 종 락*

산란직전의 남해산 참굴(*Crassostrea gigas*)을 산채로 구입하여 통상조건하에서 탈각하여 얻은 굴육질을 polyethylene laminated aluminium pouch (Al+PE : 0.03+0.03*mm*) 속에 밀봉하여 살균선량(0.2Mrad) 및 멸균선량(2.0Mrad)의 감마선으로 조사하고, 한편, 100ppm의 염소수에 굴육질을 2분간 침지한 후 탈수하여 같은 방법으로 포장하여 35일간의 빙장기간중 세균학적 및 화학적 변화를 대조 굴(조사치 않고 염소수 처리하지 않은)의 경우와 비교하였다.

굴육질의 시초의 총균수는 *g*당 700-800에 달했고, 0.2 Mrad의 감마선 조사는 빙장 10일째의 저장 기간중의 총 균수를 검출할 수 있는 선이하로 감소시켰으며 2.0 Mrad 조사는 20일째 까지 거의 무균상태를 가져왔다.

감마선 조사는 또한 세균활동을 표시하는 대사물질인 TMA와 TCA soluble nitrogen의 축적을 현저히 억제시켰으며 빙장후기부터 번식하기 시작하여 총균수가 *g*당 100,000을 초과케 된 35일째까지도 대조굴의 동일한 총균수 시기에 비하여 이 대사물의 축적억제가 현저했음은 감마선 조사의 효과가 총균수의 양적인 감소뿐만 아니라 동시에 TMA를 생산하고 단백질을 분해할 능력이 있는 세균 제거 내지는 번식의 억제라는 선택적인 양면을 가지고 있음을 보여 준다.

염소수 처리한 굴육질의 경우 총균수의 양적인 감소효과는 현저하지 않했는데도 불구하고 감마선 조사 경우처럼 세균대사물질 축적의 현저한 억제를 가져 왔음은 주목할만 하다.

굴육질의 glycogen 함량은 사후 저장 기간중 쉽사리 감소되지 않했으며 pH 측정으로 본 생굴의 신선도와의 관련성은 희박하며 조사와 염소수 처리굴의 경우 더욱 그러했다.

INTRODUCTION

Oysters have been proposed as one of the marine species that can be irradiated at sub-sterilization levels without imparting changes in odor and flavor (Shewan, 1959). Gardner and Watts (1957) reported that radiation at doses of less than 0.6 Mrad failed to retard the spoilage of fresh Southern oysters(*Crassostea virginica*) and that an adverse odor developed which was described as "grassy" when the irradiation exposure was greater than 0.7 Mrad. On the oter hand, Novak *et al* (1954)

*한국 과학기술 연구소, Korea Institute of Science and Technology

1. This work was supported in part by the International Atomic Energy Agency (IAEA), Vienna, Austria

2. Present address: Korea Advanced Institute of Science P.O. Box 150, Cheongyangni, Seoul, Korea

have reported that Gulf oysters (*C. virginica*) irradiated at 0.3 Mrad had a longer storage life when compared to those irradiated at 0.2 Mrad, although the doses above 0.2 Mrad imparted a slight adverse change in organoleptic quality. In their further study with the same species, it was suggested the dose of 0.2 Mrad to be the maximum and the oysters irradiated at the dose were still acceptable after 21 days of storage in ice while the unirradiated became unacceptable within 7 days of the storage (Novak *et al*, 1966).

The present study was initiated to obtain information on microbiological and chemical changes which would be found when Pacific oysters (C. *gigas*) were subjected to ionizing radiation required for pasteurization, i.e. 0.2 Mrad as recommended by Novak *et al* (1966) or for sterilization, i.e. 2.0 Mradas recommended by Hannan(1955).

MATERIALS AND METHODS

Pacific oysters (*Crassostrea gigas*) were purchased from local fish dealers in the early May. The oysters packed in straw sacks holding approximately 60*kg* are freighted by train during overnight from growing areas along the south coast of Korea. The conditions of handlings during and after the freighting were such that all oysters were delivered in live conditions to the processing laboratory of the Korea Institute of Science and Technology (KIST).

Preparation of oysters.

Oysters were shucked into stainless steel containers under as sanitary conditions as possible. After shucking, the free liquor was drained and the oyster meats were packed, unwashed into polyethylene laminated alminium pouch (Al+PE, 0.03+0.03*mm*). Each sample pouch contained 70-80 g(4-6 oyster meats) and was heat sealed with a minimum of air space. The weight(+0.05*g*) of each sample was determined by weighing each pouch before and after filling with the oyster meats. A portion of the shucked oyster meats were treated by immersion in a sodium hypochlorite solu tion of 100 ppm available chlorine for 2 min at 20℃ before they were packed into the Al+PE pouch.

Treatment and storage.

The samples were divided into four groups; those irradiated at 0.2 and 2.0 Mrad, the unirradiated but treated in chlorine solution and the unirradiated control. The sample packages were well iced in an insulated wooden box and transported to the Korea Atomic Energy Research Institute where a shipboard irradiator was installed for general use. The irradiator is of the Brookheaven National Laboratory Mark II type of approximately 22,000 Ci strength of Cobalt-60 and Slavin *et al*(1966) reported in details on the description and the operational procedure of a similar type of shipboard irradiator.

No effort was made to keep the samples at ice temperature during the time of irradiation. The unirradiated samples were kept in ice while other samples were being irradiated. All samples, irradiated and unirradiated, were transported back to the processing laboratory of KIST and stored under well iced conditions. At intervals of 0, 1, 2, 4, 7, 15, 20 and 35 days, two sample pouchs from each group were withdrawn and prepared for microbiological and chemical evaluation.

Total viable counts.

The oyster meats were transferred to chilled, sterilized, 2-oz glass blender jars and blended for 2 min at high speed with an equal weight of pre-chilled, sterile distilled water, using an Osterizer

blender. Serial decimal dilutions of the homogenate in phosphate buffer (pH 7.2) were plated into tryptone glucose yeast extract agar (Scholz *et al*, 1962) containing 0.5% NaCl. The colonies were counted after 5 days of incubation at 20℃ and expressed in log number bacteria per *g* oyster meat.

Dry matter and pH.

Dry matter was determined by drying the 2-*ml* homogenate samles in a circulating air oven at 105℃ until a constant weight was reached (approximately 30 min of drying time). The results of all chemical analyses were calculated on the dry matter basis.

Ten *ml* of the homogenate was combined with an equal volume of distilled water and the pH determined, using a Beckman Zeromatic pH meter.

Glycogen.

One *ml* of the homogenate was digested with 2 *ml* of 30% KOH in a boiling water bath and the glycogen precipitated with ethanol, using the method of Good *et al.* (1933), as modified by Dubois *et al.* (1956).

TCA soluble amino nitrogen.

Ten *ml* of the homogenate was mixed with an equal volume of 10% trichloroacetic acid (TCA) to give a final TCA concentration of 5%, heated in a 45℃ water bath for 30 min, and then filtered through Whatman No. 1 filter paper. The leucine equivalent amino nitrogen in the TCA filtrate was determined to follow proteolysis in the oyster meats photometrically, using the ninhydrin method of Moore and Stein (1954).

Total volatile bases.

Total volatile bases (TVB) were determined by the microdiffusion method of Conway and Byrne (1933), as modified for fish by Beatty and Gibbons (1939).

Results and Discussion

Total viable counts.

The total viable bacterial count of the unirradiated control oysters on 0 day ranged from 700 to 800 per *g* oystermeat. After a lag period, the count started to increase after 2*nd* day of ice storage and exponentially by the 4th day, approaching 10^7 per *g* oyster meat by the 20th day (Fig. 1). The counts remained more or less unchanged thereafter until the 35th day.

No bacteria could be detected in any of the samples subjected to the ionizing radiation during the early storage period. For the oysters irradiated at 0.2 Mrad, the counts of a little over 10^2 per *g* started to be detected by the 10th dayand increased to the level of 10^5 per *g* by the 35th day. The bacterial growth in the oysters irradiated at 2.0 Mrad, on the other hand, was retarded until the 20th day and the counts increased to level of only 10^3 per *g* by the and of the storage period.

For the unirradiated, chlorine dipped oysters, the counts were less than those in the unirradiated control at each sampling interval, but followed an identical pattern of the changes observed in the unirradiated throughout the storage period.

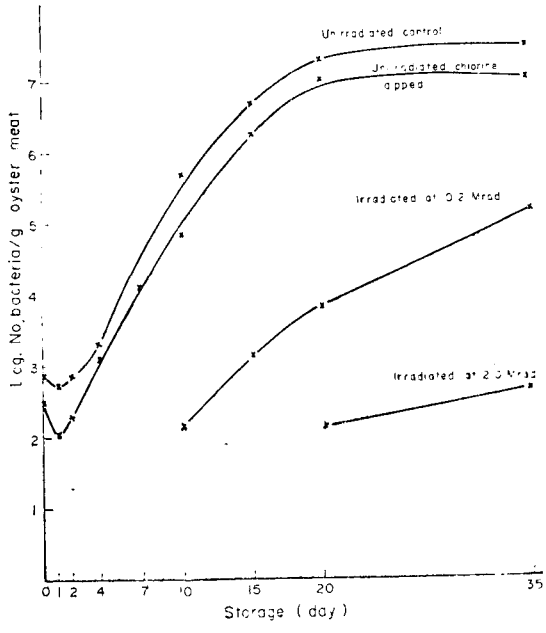

Fig. 1. Changes in total bacterial counts of Pacific oyster meats during ice storage.

pH

The initial pH values of Pacific oysters were between 6.2 and 6.5 and the values decreased slightly during 35 days of ice storage, but the overall decrease was less than 0.5 pH unit. The pHu values of the unirradiated control, after progressive decrease during the early storage period, started to rise by the 20th day and increased above the initial values by the 35th day, due probably to the increase in bacterial activities, while those of the irradiated and unirradiated, chlorine-dipped samples continued to decrease (Table 1).

Piskur (1947) suggested pH changes could serve as quality index for commercially shucked Pacific oysters held in storage at 32-34° F, since in their studies the pH values fell from 6.4 on 0 day (fresh) to 6.1 on the 6th day (fair), to 5.9 on the 13th day(stale), and to 5.5 on the 30th day (sour or putrid). Similar results have been reported for the Atlantic oysters (*C. virginica*) (Hunter and Linden, 1932; Baldwin *et al.*, 1941; Pottinger, 1948 and 1951), and for the Southern oyster (*C. virginica*) by Gardner and Watts (1956). Gardner and Watts (1956) also report that the spoilage of oysters is a fermentative one, as evidenced dy a progressive decrease in pH and development of souring in meats during 20 days of storage at 5℃.

Table 1. Changes in Pacific oyster meats during ice storage-pH

	storage (day)								
treatment	0	1	2	4	7	10	15	20	35
unirradiated control	6.30	6.33	6.30	6.46	5.98	5.95	5.98	6.00	6.48
irradiated at 0.2 Mrad	6.22	6.29	6.24	6.46	5.98	5.93	6.02	6.05	5.96
irradiated at 2.0 Mrad	6.45	6.34	6.33	6.47	6.29	6.22	6.41	6.30	6.20
unirradiated, chlorine-dipped	6.32	6.30	6.30	6.35	5.95	5.92	6.05	6.02	5.98

In present study, the pH value measurements were not accompanied with organoleptic assessment of the samples. However, it is apparant that the pH changes alone can not serve as a quality index for the Pacific oysters, especially for the irradiated samples although, it is reasonable to conclude that the rise of pH values of oyster meats towards the end of storage period was due primaily to the increased bacterial activities. On the other hand, the pH values in the unirradiated, chlorine-dipped samples continued to decrease through the late storage period although bacterial counts in the samples were as comparably high as those in the unirradiated control.

Glycogen, The glycogen content of all sample groups regardless of the treatments remained more or less unchanged throguhout 35 days of storage, although there were some variations. The glycogen content of the oysters averaged 35.2% on a dry basis, about 5.9% on a wet weight basis

Table 2. Changes in Pacific oyster meats during ice storage: Glycogen (% dry tissue)

treatment	storage (day) 0	1	2	4	7	10	15	20	35
unirradiated control	35.5	35.0	35.1	35.5	34.9	36.1	35.2	36.1	35.1
irradiated at 0.2 Mrad	35.2	35.6	36.0	35.7	36.1	35.7	35.6	36.3	34.0
irradiated at 2.0 Mrad	35.1	36.5	35.9	35.6	34.1	35.8	35.5	36.2	35.9
unirradiated, chlorine-dipped	35.0	36.1	36.3	35.9	35.3	37.5	36.4	36.5	35.6

(Table 2). This compares favorably with 5.96% for Adriatic oysters (*Ostrea edulis*) during the month of February, when the glycogen content reaches its peak (Krvariv, 1953). The glycogen content of Gulf oysters(*C. virginica*) has been reported to be 4.28% in May (Fieger *et al.*, 1958).

Takagi and Simidu (1963) reported there occured a rapid decline in glycogen contents of shucked oyster (*Ostrea laperousei* Schrench) from 2.97% on 0 day to 0.54% by the 8*th* day of storage at 2~3℃. The rusults found in the present study are quite contradictory to their results obtained from the *Ostrea* species, indicating that glycogen is a storage product not easily utilized to meet the metabolic requirements of the shucked oyster, nor rapidly degradated by the actions of spoilage bacteria. Liuzzo *et al* (1969) also reported that there occured no significant changes in the glycogen contents of the Gulf oysters (*C. virginica*) both irradiated (at 0.2 or 0.4 Mrad) or unirradiated, during 20 days of ice storage.

Total volatile bases and TCA soluble amino nitrogen.

The total bacterial counts (Fig. 1), TVB (Fig. 2), and TCA soluble nitrogen (Table 3) reflect clearly the effects of irradiation upon the spoilage pattern of oysters during ice storage. In the chlorine-dipped oysters, the total viable population increased in the same manner as in the unirradiated oysters, while accumulation of TVB(Fig. 2) and TCA soluble nitrogen (Table 1) was suppressed. This indicates the alteration of the spoilage pattern found in oysters under normal conditions as a result of the chlorine treatment.

The TVB on 0 day ranged from 30.6 to 40.3μ moles per g dry matter, and the values of all the sample groups increased progressively. However, the rate of increase in the unirradiated oysters accelerated on the 15th day, reaching 384.6 to 397.8μ moles by the 35th day, a 900 to 1,150% increase over the 0 day values (Fig. 2). The 15th day coincided with the period when the total viable population reached the late exponential phase

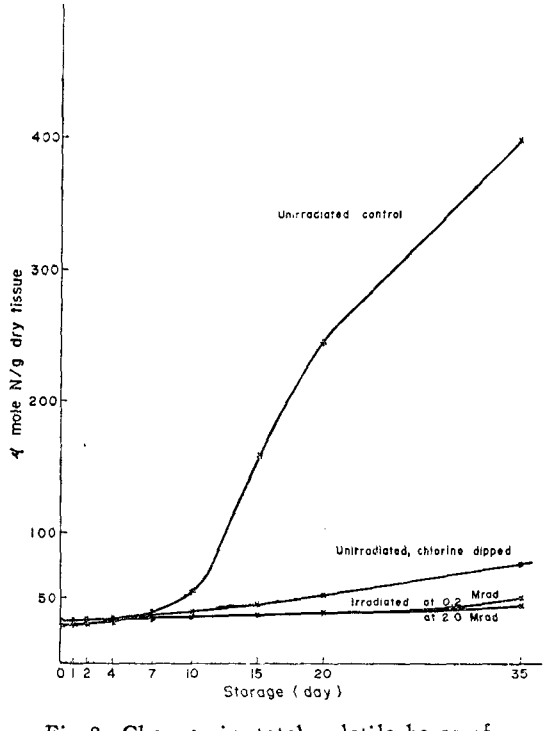

Fig. 2. Changes in total volatile bases of Pacific oyster meats during ice storage.

of growth, In the irradiated samples, on the other hand, the overall increase ranged only from 27 to 54%. About one-half of the small increase found during the storage of irradiated oysters could be accounted for on the basis of deamination of nucleotides by endogenous enzymes, as reported by Guardia and Dollar (1970).

Table 3. Changes in Pacific oyster meats during ice storage: TCA soluble nitrogen. (μmoles leucine N per g dry tissue)

	storage (day)								
treatment	0	1	2	4	7	10	15	20	35
unirradiated control	1.3	1.2	1.1	1.2	1.1	1.0	1.4	2.8	5.8
irradiated at 0.2 Mrad	1.2	1.2	1.1	1.1	1.0	1.0	1.0	0.9	0.9
irradiated at 2.0 Mrad	1.2	1.2	1.2	1.2	1.1	1.0	0.9	0.9	0.9
unirradiated, chlorinedipped	1.3	1.2	1.1	1.0	1.0	1.0	1.0	1.0	0.9

The TCA soluble amino nitrogen on 0 day ranged from 1.2 to 1.3 μ moles leucine *N* per *g* dry matter and, except for the unirradiated groups, decreased gradually during the storage period (Table 3). The amino nitorgen of the unirradiated groups began to increase on the 15th day, and by the overall increase was nearly 440% over the 0 day values. It is again important to note that, as it with the TVB accumulation, accumulation of the TCA soluble nitrogen in the chlorinedipped sample group was negligible despite the high level of bacterial population developed during the ice storage. The absence of proteolytic activity in the chlorine-dipped sample group may be due most probably to possible inactivation or suppression effect of chlorine dipping treatment upon proteolytic microorganisms initially present in the oyster meats. Of course this must be substantiated from studies designed to show the proteolytic capability of isolates of microflora developing in the chlorine-dipped oyster samples.

CONCLUSIONS

Treatment of Pacific oysters with 0.2 Mrad of gamma radiation reduced the number of total bacterial counts below detectable limits during the first 10 days of post-irradiation storage in ice, while those irradiated at 2.0 Mrad remained virtually sterile up to the 20th day. Reduction of the total bacterial counts due to the radiation treatment appeared to be not only quantitative but also qualitative as evidenced by a severe suppression of TVB and TCA soluable nitrogen content throughout the storage period despite the high dacterial level developed during the latter half of the storage peiiod in the samples irradiated at 0.2 Mrad.

The treating the oyster meats in chlorine solution (100 ppm of available chlorine) brought about an effect similar to the radiation treatment as far as the suppression of TVB and TCA soluble nitrogen is concerned, although the total bacterial counts increased with storage in same manner as the unirradiated control.

TVB appeard to be an important spoilage indicating substance of the unirradiated oyster meats undergoing a normal spoilage during ice storage. Because of a sample-to-sample variation as well as the insignificant overall change, pH measurement can not be a good spoilage indication for both the irradiated and unirradiated oyster meats.

Oyster glycogen was not affected by gamma radiation or by spoilage during 35 days of ice storage.

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