Storage Characteristics of Irradiated Pacific Oysters, *Crassostrea gigas*

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감마선에 쬔 참굴의 저장성

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산란기 직전의 참굴(*Crassostrea gigas*)을 통상 방법으로 개각하여 0.3 및 2.0 Mrad의 감마선에 각기 쬔후 0°C의 온도하에 저장기간중 번식하는 총 세균수와 pH 변화를 측정하고 부패상을 관찰함으로써 방사선을 쬔 참굴의 저장성을 조사하였다.

자외선을 쬔 무균해수에 개각하지 않고 정화시킨 굴과 보통 해수에 수용하였던 굴을 비교하여 정화 처리에 의한 세균 제거의 효율을 조사하였으나 4—5일의 장기간 처리에도 불구하고 별다른 세균제거 결과를 볼 수 없었다. 이것은 굴이 보유하고 있는 본래의 총 세균수가 적기 때문이라고 추측된다.

감마선을 쬐지 않은 굴은 저장 15일째에 부패했음이 완연했으나 0.3 Mrad의 감마선을 쬔 굴은 35일후까지도 시큼할뿐 변로 변질되지 않았다. 2.0 Mrad의 감마선을 쪼인 굴은 관능상의 선도 유지에 있어서 0.3 Mrad로 쬔 굴보다 훨씬 나빴고 off-odor가 심했다.

굴 저장중의 pH 변화로 선도를 판정함은 그 신빙성이 극히 희박하였고 선도의 저하와 함께 pH가 내려간다는 일반적인 인식과는 달리 어류부패의 경우와 같이 저장 초기에는 약간 하강하나 부패의 진행과 함께 상승함이 확인되었다.

1. Introduction

Since oysters are mostly consumed fresh and oysters grown in waters contaminated with fecal materials have been responsible for the outbreaks of typhoid in the past, much attention has been given to oysters as carriers of infectious enterobacteria. During the storage of shucked oysters at refrigerated temperatures, however, the population of the enterobacteria declines gradually (Hoff, *et al.*, 1967), and is completely overgrown by psychlophiles that are normally associated with oysters. Fieger and Novak (1961) in a literature review state that the early stage of spoilage of oysters is due to the activities of *Serratia, Pseudomonas, Proteus, Clostridium, Bacillus, Aerobacter,* and *Escherichia* and later in the course of spoilage streptococci, lactobacilli and yeasts become predominating microflora. Thus the pattern of microbial spoilage of oysters is different from that of fish in that the decomposition of oysters is of fermentative nature as evidenced by progressive decrease in pH and development of souring in meats (Gardner and

Watts, 1956).

Radiation pasteurization may be useful in altering the pattern of microbial spoilage of oysters. Radiation of seafood products, such as cod, haddock, shrimps, and crabmeats, will extend their shelf life by reduction in the number of bacteria. However, there has been only limited application of this technique to the preservation of oysters. Southern oysters (*Crassostrea virginica*) (Gardner and Watts, 1957) were irradiated at doses of 6.3×10^4 to 6.3×10^5 rep (ca 0.06 to 0.6 Mrad) and 8.3×10^5 to 3.5×10^6 rep (ca 0.7 to 2.9 Mrad) and during storage at 5°C and room temperature pH measurement and organoleptic judgements on both irradiated and unirradiated oysters were made. Doses less than 0.6 Mrad failed to retard the spoilage as indicated by pH change and organoleptic judgement of fresh uncooked oysters, while doses above 0.7 Mrad caused an off-odor described as "grassy". All oysters, irradiated at doses as high as 2.9 Mrad or unirradiated, became sour and pH decreased progressively during the storage, suggesting the endogenous enzymes, rather than bacterial action, as the cause of these changes. On the other hand, the Louisiana State University research team recently reports that doses up to 0.3 Mrad do not impart any off-odor on oysters (Novak, *et al.*, 1964). They suggest 0.3 Mrad as the most suitable pasteurizing dose for the oysters.

Since the studies to date have been limited, it was decided to irradiate Pacific oysters and determine the storage characteristics at ice temperature (0°C).

2. Material and Method

The Pacific oysters (*C. gigas*) of 7–10 cm in length were purchased from an oysterman in the latter part of April. The oysters were almost ready to spawn, therefore they were at their best quality as far as the fatness was concerned. A portion of the oysters was treated for 96 to 120 hours in a depuration unit at the Shellfish Sanitation Laboratory at Purdy, Washington, in which the oysters were fed with constantly flowing seawater disinfected with ultraviolet radiation. Kelly (1961) reports that the unit lowers the coliform content of the oysters artificially contaminated with fecal coliforms to 2.5% of the initial level in 8 hours during the summer months when the oysters were fed with seawater disinfected with an ultraviolet radiation.

While oysters were being depurated for 96–120 hours the other portion of oysters remained in oysterbeds undisturbed until all the oysters, depurated and non-depurated, were taken out of water to be transported to the Processing Laboratory at the College of Fisheries for this study.

The depurated oysters were shucked into a sterile glass container as aspectically as possibile. The non-depurated oysters were handled by ordinary commercial methods, but taking great care not to introduce a massive contamination of oysters with bacteria during handling. All Shucked oysters were unwashed before they were packed into sterile, dry 4-oz wide-mouth bottles. The oyster liquor was drained out prior to packing in order for the liquor to be packed as little as possible with oyster meats. Three to five oysters were required to fill each bottle. Each bottle was closed tightly with a plastic screw cap. Exact weight of each sample was determined by weighing the bottle before and after the transfer of oysters.

Both the depurated and non-depurated oyster samples were exposed to gamma radiation of 0.3

and 2.0 Mrad at 1°C while the unirradiated samples were kept at 0°C. All samples were then stored at 0°C. At intervals of 0, 1, 2, 4, 7, 10, 15, 20, and 35 days, two bottle from each group were withdrawn and the oyster meats homogenized with two times the volume of precooled, sterile, distilled water for two minutes at high speed, using the Osterizer blendor.

Using the homogenates, aerobic bacterial count and pH measurement were made. The standard pour-plate technique was employed for the bacterial count, using nutrient agar (Difco) with 0.5% NaCl as medium and phosphate buffer (pH 7.2) as diluent. The colonies on plates were counted after five days of incubation at 20°C and expressed as number of bacteria per gm oyster meat. The pH was measured electrometrically.

3. Results and Discussion

The number of bacteria present in the depurated oysters as determined 6 hours after they were taken out of water was 560 per gm oyster and it was not significantly different from the comparable number present in the non-depurated oysters (Table I). This is favorably compared with the bacteriological criteria established for commercially shucked Eastern oysters at the market level in the Cooperative Program for the Certification of Interstate Shellfish Shippers during 1959 and 1960, which define the oysters having a plate count of not exceeding 500,000 per ml of sample as "Satisfactory" (Kelly, 1960). Presnell, *et al.*, (1963) report that samples of Pacific oysters taken from a processing plant have a total plate count (at 35°C) of nearly 10^4 per gm oyster meat on 0 day. This almost 100-fold greater number of bacteria present in the commercially shucked oysters as compared to the results of this study indicates the importance of good sanitary conditions in and around the processing facilities and well enforced sanitary practice by handling personnel.

The absence of any significant difference in the bacterial count between the depurated and non-depurated oysters persisted throughout 35 days of storage at 0°C. As shown in Table 1, for unirradiated samples the bacterial count started to increase on the fourth day and it continued to increase during the remaining period of storge. The level of bacterial population reached 10^7 on the 20th day and was in the stationary phase on the 35th day. All unirradiated oysters became sour, stale and then slightly putrid by 15th day.

Although Kelly (1961) reports that 97.5% purification is achieved in 8 hours during summer months when Pacific oysters are allowed to cleanse themselves in a depuration unit at Purdy, Washington, no quantitative deta on the purification of non-coliforms hardbored by oysters are available. If the almost identical level of bacterial present in the depurated and the non-depurated oysters should indicate a negligible degree of contamination of the oysters with fecal coliforms in oyster beds, the data shown in Table 1 indicate then that depuration of Pacific oysters for 96 to 120 hours, using the same facilities described by Kelly (1961), apparently did not bring about the removal of what are presumed to be the microflora normally associated with the oysters.

The microflora of the oysters of both depurated and non-depurated was inactivated at dose as low as 0.3 Mrad and the samples remained free from bacteria (as determined to a number less than 33 per gm oyster) until the 7th day. In both groups of the irradiated oysters, the count

on the 10th day ranged 10^3 per gm oyster and on the 35th day it reached 10^6 for the 0.3 Mrad irradiated and 10^1 to 10^3 for the 2.0 Mrad irradiated oysters.

The pH of ground oysters ranged from 6.22 to 6.58 on 0 day and the effect of treatments, in respect to depuration and irradiation at 0.3 and 2.0 Mrad, on the pH value was not noticeable. For the unirradiated oysters, however, pH increased on the 4th day slightly above the initial value, then decreased below the value on the 7th day (Table). Thereafter, the pH remained more or less unchanged until the value increased above the 0 day level on the 35th day. During the period between the 20th and 35th day, there was no significant increase in number of bacteria; therefore, if the increase in pH reflects the bacterial activities, it is presumed that the type of microflora developing in oysters must have changed from primarily acid producing bacteria to putrifying types.

The pH of the 0.3 Mrad irradiated samples also decreased on the 7th day, but, unlike the unirradiated samples, the value remained more or less unchanged during remaining period of storage. The 2.0 Mrad irradiated samples, however, underwent essentially no change in pH throughout 35 days of storage. The bacterial count of the 2.0 Mrad irradiated samples of both depurated and non-depurated groups was negligible until 10th day, and during the period between 15th and 35th day, the level of bacterial population in the samples ranged from 0 to 10^3 (Table 1). This level of bacteria is not sufficient to contribute to the changes. Therefore, when bacterial activities were absent or negligible in oysters, the initial pH value remained unchanged throughout 35 days of storage at 0°C. This is contrary to the finding by Gardner and Watts (1957) that in Southern oysters irradiated at doses as high as 2.9 Mrad, the pH decreased progressively and souring developed during storage at 5°C and room temperature. Since the dose of 2.9 Mrad would completely inactivate the microflora present in oysters, they concluded these changes were caused by endogenous enzymes of oysters that survived the does of 2.9 Mrad gamma radiation. Their study, however, did not include microbiological investigation.

The possible usefulness of pH measurement as an objective quality index of commercially shucked oysters has been suggested by Hunter and Linden (1925) on Atlantic coast oysters (*C. verginica*); Baldwin, *et al.*, (1941) and Pottinger (1948 and 1951) on Eastern oysters(*C.virginica*); Pickur (1947) on Pacific oysters (*C. gigas*); and Gardner and Watts (1956) on Southern oysters (*C.virginica*). However, due to the variations with species, locality, and season, the establishment of quality criteria of oysters singularly based on pH measurement would be of only limited value. As shown in Table 2 in the absence or negligible influence of bacterial activity, pH of oysters during 35 days of storage at 0°C was not significant, therefore, pH as main criteria for determining the quality of irradiated oysters can not be applied.

4. Conclusions

Following conclusions may be drawn from this study:

1. Depuration of oysters apparently did not lower the bacterial population harbored by oysters. This may be due to either the initial bacterial load of the oysters (10^2 per gm oyster) was too low to be effective or non-coliforms are not removed from oysters by the depuration process

Table 1. Bacterial Count of Irradiated and Unirradiated Pacific Oysters During Storage at 0°C (No/gm oyster meat)

Irradiated at (Mrad)		Storage (day)								
		0	1	2	4	7	10	15	20	35
Depurated oysters	0	*5.6×10^2	1.3×10^3	3.9×10^3	2.8×10^3	2.8×10^4	2.7×10^5	1.1×10^6	1.5×10^7	3.8×10^7
	0.3	**0	0	0	0	0	5.5×10^3	2.3×10^3	4.4×10^5	8.2×10^5
	2.0	0	0	0	0	0	0	8.0×10^2	1.2×10^3	1.3×10^3
Non-depurated oysters	0	7.8×10^2	5.2×10^2	7.3×10^2	2.0×10^3	4.8×10^4	4.5×10^5	2.5×10^6	2.0×10^7	3.1×10^7
	0.3	0	0	0	0	0	4.0×10^3	6.5×10^2	6.8×10^3	3.9×10^5
	2.0	0	0	0	0	0	0	0	1.4×10^3	5.0×10^1

*Arithmetic average of 4 plate counts representing 6-10 individual oysters.
**Zero count taken as 33 per gm oyster meat.

Table 2. pH of Irradiated and Unirradiated Pacific Oysters During Storage at 0°C

Irradiated at (Mrad)		Storage (day)								
		0	1	2	4	7	10	15	20	35
Depurated oysters	0	6.30	6.33	6.30	6.46	5.98	5.95	5.98	6.00	6.48
	0.3	6.22	6.29	6.24	6.46	5.98	5.95	6.02	6.05	5.96
	2.0	6.45	6.34	6.33	6.57	6.29	6.22	6.41	6.30	6.20
Non-depurated oysters	0	6.40	6.35	6.30	6.57	6.15	5.92	6.01	6.03	6.25
	0.3	6.52	6.39	6.35	6.50	6.04	6.23	6.06	6.01	6.94
	2.0	6.58	6.40	6.38	6.60	6.21	6.26	6.28	6.20	6.24

even after a prolonged treatment. Or, on the contrary to the effectiveness of this depuration process reported by Kelly (1961), the conditions were such that the physiological activities of oysters were hindered while they were being subjected to seawater disinfected with ultraviolet radiation. The conditions affecting water pumping activity of oysters during the depuration process have to be investigated.

2. Dose of 0.3 Mrad rendered the oysters free from bacteria. The quality of the 0.3 Mrad irradiated oysters during 35 days of storage at 0°C was superior to the unirradiated oysters. The unirradiated samples became sour, stale, and then putrid by 15th day, while the 0.3 Mrad irradiated samples were only slightly sour on the 35th day.

3. The initial pH value of the 2.0 Mrad irradiated samples did not undergo any significant changes throughout 35 days of storage and this is due to the absence or negligible influence of bacterial activities in the samples. The 0.3 Mrad irradiated samples, however, had pH value comparable to that of 7 day old unirradiated samples.

4. pH measurement as an objective quality index appears to have only a limited usefulness for irradiated oysters.

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