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《Original》

Radurization of Packaged English Sole Fillets

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Abstract

English sole (*Paronphrys vetulus*) fillets packaged in polymylar bags were irradiated at 500 Krad, using a Cobalt-60 irradiator and the accumulation of spoilage indices substances and bacterial growth in the irradiated and unirradiated samples were measured during 36 days of storage at 0–2°C. A casein agar plate technique was developed for a direct enumeration of proteolytic bacterial population, thus enabling the determination of relative proportion of proteolytic bacteria in the total microflora at each storage interval.

Irradiation at 500 Krad resulted in a ten fold reduction of microflora and throughout the storage period the level of microflora lagged behind that of the unirradiated, by as much as one thousand fold. This was accompanied by a remarkable suppression of TVB and TMA accumulation in the irradiated, never reaching a spoliage level. Proteolytic bacterial population also was reduced to below one per cent of the total viable count and remained so throughout the storage period, while proreolytic bacteria in the unirradiated increased proportionately with the storage, comprising 85.5% of the total microflora by the twenty-second day. This selective removal of proteolytic bacteria must account for the reduced rate of proteolysis occurred in the irradiated during the storage.

요 약

English sole(가재미종) 어편을 Polymylar bag에 밀봉하여 500 Krad의 감마선에 조사한 후 36일간의 0—2°C 저장기간중 조사한 것과 조사하지 않은 어편내에 축적되는 부패표시 물질과 총 균수를 측정하였다. 단백질 분해균을 직접 계수하기 위하여 Casein agar Plate법을 만들어 저장기간중의 총균수에 대한 단백질 분해균의 비율을 측정하는데 사용하였다.

500 Krad 선량 조사결과 어편의 총 균수는 10분의 1로 감축되었고 저장중 조사하지 않은 어편의 총균수 보다 훨씬 적어 1000분의 1선까지 내려갔다. 이에 따라 TVB와 TMA 축적도 억제되어 부패선을 넘지 못했으며 단백질 분해균 역시 총균수의 1% 이하선으로 감축되었으며 저장 기간중 이 선을 넘지 못했다. 반면에 조사하지 않은 어편엔 총 균수에 대한 이들의 비율은 저장기간중 점차 상승하여 22일째 까지 총균수의 85•5%를 차지 하였다. 방사선 조사로 인한 이 단백질 분해균의 선택적인 제거는 그 어편의 단백질 분해 부진 현상을 초래했다.

Introduction

Immediately after death of a fish, a series of deteriorative changes in flavor, odor and appearance start to occur, causing the fish to proceed from an initially fresh condition to staleness and then eventual putrifaction. These deteriroative changes, termed spoilage, are brought about in fish, as in other foods, by autolysis, chemical reaction, and microbial activities. All evidence indicates that the latter contributes most to the spoilage process, producing striking and undesirable changes. Thus our present-day knowledge of fish spoilage is derived mostly from studies designed to correlate the accumulation of various spoilage incides substances with bacterial growth.

Radiation treatment of fish brings about an interuption of the normal process of spoilage as a result of selective reduction of radiation sensitive bacteria, the degree of reduction being dependant upon the dose of radiation absorbed by fish. It is well established that at a dose high enough to achieve sterilization, there occur adverse changes in odor or flavor. rendering food products unacceptable or even harmful for human consumption (Hannan. 1955; Niven, 1958; and Coleby and Shewan 1665). Therefore the recent emphasis has been in the application of low dose radiation for the purpose of extending shelf-life of fresh foods (Miyauchi *et al.* 1965; and Schultz and Lee, 1966).

Research efforts up to date, however, have been concerned for the most part on methodology and post-irradiation storage characteristics in an attempt to determine the minimum dose range to achieve shelf-life extension of various food products and very little information is available concerning the fundamental aspects underlying the desired end-result of shelf-life extension.

This study was designed to investigate the spoilage pattern of English sole (*Parophrys vetulus*) fillets packaged in polymylar bags irradiated at 0.5 Mrad of gamma radiation during storage at 0–2°C with particular reference to the accumuation of nitorgenous end-products and to bacterial growth in both irradiated and uniraadiated smaples. The role of proteolytic bacteria in fish muscle deterироration was also investigated.

Materials and Methods

Preparation of Samples

English sole (*Parophrys vetulus*) caught by otter trawl were well iced with minimum handling immediately after capture aboard the vessel and brought to processing laboratory for this study. By this time fish had been in ice for no longer than 6 hours counting from the time of capture.

Fish were filleted in the same manner as commercial practice, and approximately 100 gm flesh was packed in polymylar bags and heat sealed. One portion of the smples was exposed to 500 Krad of gamma radiation at 0–2 C°, using a 20,000 Ci cobalt-60 irradiator, Mark II, while unirradiated fillets were stored at °C. Both irradiated and unirradiated fillets were then stored at 0–2°C and at intervals of 0, 4, 7, 11, 14, 19, 22, and 36 days, two samples from each group were processed for microbiological and chemical examinations.

Total viable count

Forty-five gm of flesh was homogenized in a sterile Waring blender with four times its volume of 0.1% sterile peptone water for one and one-half minute and the appropriate decimal dilutions were used for estimating the total viable number of bacteria by the standard pour plate method. Trypticase phytone yeast extract agar and 0.1% peptone water were used as medium and diluent respectively. Colonies were counted after incubating plates at 8°C for two weeks and the results expressed as logarithmic number of bacteria per gm fish flesh.

Proteolytic count

Two-tenths ml of the dilutions used in plating was placed on the surface of the casein agar and spread evenly with a bent-glass rod. The casein agar plate, consisting of underlaying bacto-agar and overlaying casein agar, was prepared as following.

One and one-half per cent bacto-agar was dis-

solved in tap water by steaming, pH adjusted to 7.2 with 1 N NaOH, then heat sterilized in French square bottles at 15 psi for 15 minutes. After cooling, 10 ml of the bacto-agar was poured onto petridishes and allowed to solidify.

For the overlaying casein agar, 15 gm bacto-agar dissolved in 800 ml mineral solution for *Pseudomonas*-type orgarnisms (Nimmo-Simth and Apleyard, 1956) containing 1 gm yeast extract by steaming and 80 ml each into French square bottle was heat sterilized as before. At the same time 20% purified casein (Difco) was prepared in 0.5% Na_2CO_3 solution, pH adjusted to the range of 6.5–7.2 and separately heat sterilized also. Twenty ml of the casein solution was added to French bottle containing 80 ml of the overlaying agar while it was very hot and, after mixing well, poured onto the underlaying agar plates (no more than 3 ml per plate so that the overlaying agar would not be thicker than 2 mm). The plates were dried, then wrapped air tight in plastic bags, and stored in refrigerator for prolonged use.

The casein agar plates spread with the decimal dilutions on the sureface were flooded with $HgCl_2$ solution after incubating them at 8°C for two weeks. The colonies of proteolytic bacteria were surrounded by a "clear zone." The proteolytic colonies were counted after 30 minutes of standing at room temperature and results expressed as per cent proteolytic bacteria of the total viable count. By this method it was possible to estimate directly the changes in the relative proportion of proteolytic bacteria in the microflora of fish fillets during storage.

Chemical Analysis

Fish tissue surplus from the microbiological examinations was frozen at -40°C for subsequent chemical analysis. Twenty gm of the frozen sample were homogenized in 2 oz wide-mouth jar, using an Osterizer homogenizer. The sample was homogenized first with 10 ml of distilled water for 30 seconds, then for 60 more seconds after adding 40 ml of 10% trichloroacetic acid.

The homogenates were filtered under reduced pressure through Whatman No. 1 paper and the filtrate used for determining total volatile bases (TVB), trimethylamine (TMA) and tryosine-N. Each result was expressed as mgN per 100 gm fish flesh.

TVB were estimated by microdiffusion method of Conway and Byrne (1933) as modified for fish by Beatty and Gibbons (1936).

TMA content was determined by the same method as TVB, but after the addition of the sample to the outer well, 1 ml of neutralized fromalin was added and the two were mixed well before adding saturated K_2CO_3. To prepare the neutralized formalin, 500 ml of formaldehyde (analytical grade) was treated with 5 gm of magnesium carbonate, filtered, and made to 1 liter with distilled water.

Tyrosine-N was determined by Lowry-Folin procedure (Lowry *et al.*, 1951) as modified by McDonald and Chen (1965). This method determines tyrosine as well as peptides, therefore is highly sensitive for measureing proteolysis. Tyrosine standard solution was prepared by the method of Greenberg (1955).

Results

The viable cells in the unirradiated fillets were a little over 10^2 per gm muscle on O day. The population started to increase exponentially on the 4th day, reaching a value of 10^8 by the 19th day; thereafter it entered into the stationary phase of growth. The changes of the viable population in the irradiated smaples generally followed a pattern similar to the above, but at each storage interval the viable cells in the irradiated smaples were significantly lower than those in the unirradiated by 10 to 1,000 fold (Figure 1).

The proteolytic population in the unirradiated samples also increased during storage, closely paralleling the increase of total viable population (Figure 1). The percentage of proteolytic population to the total flora was estimated by the proportionate number of proteolytic colonies on the casein agar plate of 25 to 200 colonies at each smpling interval. Approximately 31.3% of the total population was proteolytic on O day and the percentage increased steadily with the storage, after a decrease on the 4th day (Table 1).No proteolytic bacteria were

detected in the irradiated smaples throughout the storage. However, since detectability of proteolytic bacteria by this technique is limited to one per cent, a medium selective to proteolytic bacteria is necessary for a direct enumeration of proteolytic population less than one per cent of the total viable population. This must have been the case with the irradiated samples.

The direct enumeration technique, which involves spreading smaple diluent on the sureface of casein agar plates and, after growth, flooding the plates with $HgCl_2$ solution, then counting the colonies with surrounding "clear zone", offers several advantages over the conventional method: 1) The process of picking representative colonies off the poured plates, of testing of proteolytic capabilities of each isolate using a suitable substrate, and of enumerating proteolytic bacteria are eliminated; 2) Results are more direct than those obtained from the isolation technique; 3) this technique can substitute the total viable count in a mixed pure culture study involving proteolytic and non-proteolytic bacterium.

TVB content in O-day fish was 7.77 mg N per 100 gm muscle, and the content of the unirradiated samples increased steadily but at a slow rate during the first 11 days, then at a rapid rate thereafter, reaching a peak of 35.98 mg N per 100 gm muscle on the 19th day (Figure 2). The rapid increase started to occur while bacterial growth was well under the exponential phase (Figure 1 and 2). The TVB production in the irradiated smaples on the other hand never exceeded a level of 10mg N per 100 gm muscle until the 36th day. After an initial slow increase, as was observed in the unirradiated samples, but slightly below the level of the unirradiated smaples, perhaps due to a smaller degree of bacterial action, the TBV in the irradiated samples remained more or less unchanged until after the 28 th day (Figure 2.)

Interestingly enough, a singnificant increase of TVB started to occur on the 11th day in the unirradiated smaples, while viable population was still in the mid-phase of logarithmic growth, but such an increase started on the 28th day in the irradiated samples, corresponding to the late stationary phase. Furthermore, when the level of microflora in the two groups of samples at this point is compared, the microflora in the irradiated smaples is more than 10 times that in the unirradiated smaples (Figure 1 and 2).

TMA content in O-day fish was 0.47 mg N per 100 gm muscle, approximately one-sixteenth the content of the TVB. The increase during storage in the unirradiated smaples followed almost an identical pattern to that of TVB. reaching a mximum value of 25.51 mg N per 100 gm muscle. The TMA in the irradiated smaples, however, increased steadily from the initial value of 0.53 to 2.55 mg N per 100 gm muscle by the 36th ady, however, the overall increase was very slight (Figure 3).

The tryosine values of the unirradiated samples increased at a slow rate from the initial value of 8.20 to 12.25 mg per 100 gm muscle by the 7th day, and the increase was accelerated thereafter, reaching a maximum value of 30.05 on the 22nd day. The initial value of the irradiated smples was 9.75, and it increased rather rapidly, after a slow rate of increase during the first 14 days, reaching a value of 29.50 by the 36th day (Figure 4).

Proteolytic bacteria in unirradiated English sole fillets stored in polymylar bags at 0–2°C

Proteolytic bacteria	Storage (day) 0	4	7	11	14	19	22
Log No./gm tissue	2.11	2.36	3.56	5.74	7.57	8.30	8.30
Per cent of total count	31.3	17.6	58.3	76.0	80.0	81.8	85.5

Discussion

A dose of 500 Krad was chosen in this study because the dose is known to be in the borderline of the so-called thershold dose for flatfish like sole (Coleby and Shewan, 1965), that may be defined as the maximum permissible dose at which no significant adverse odor or flavor is imparted to the products.

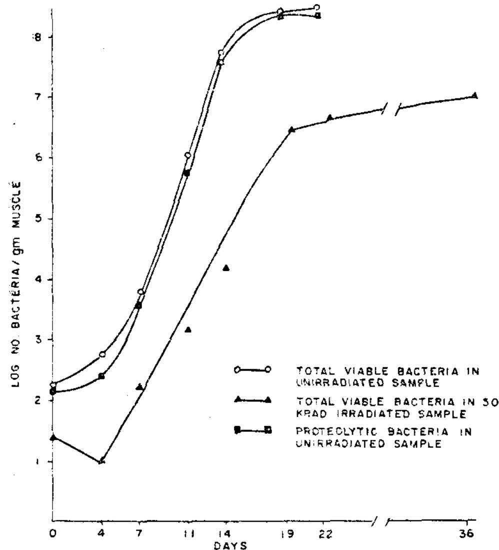

Figure 1. Bacteria in English sole fillets stored in polymylar bags at 0–2°C. (Each point represents four separate counts).

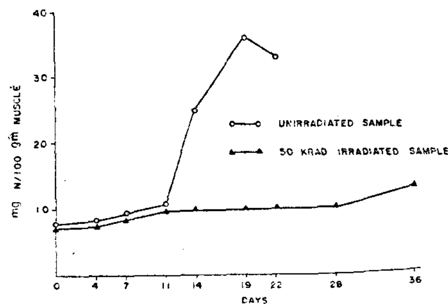

Figure 2. TVB in English sole fillets stored in polymylar bags at 0–2°C. (Each point represents a duplicate determination.)

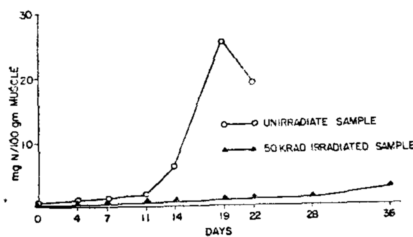

Figure 3. TMA in English sole fillets stored in polymylar bags at 0–2°C. (Each point represents a duplicate deternimation).

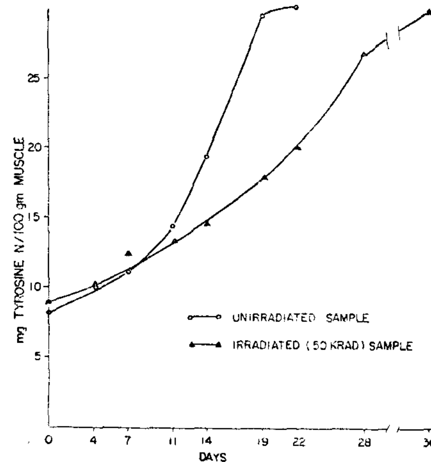

Figure 4. Tyrosine-N in English sole fillets stored in polymylar bags at 0–2°C. (Each point represents the four separate determinations).

The immediate effect of raduarization of English sole fillets was shown in ten fold reduction of microflora as compared to the unirradiated samples and the margin became wider, reaching as much as 1,000 fold difference, with storage period (Figure 1). This is commonly observed in irradiated foods and in turn serves the basis for explanining the shelf-life extension of such products.

Since fish microflora are mainly consisted of Gram-negative asporogenous rods (Shewan, 1961) and these organisms are readily inactivated by a low dose raiation (Niven, 1958), it is no coincidence that the possibility of shelf-.life extension by means of radurization is known to be particularly great with fishery products. (Shewan, 1959).

The reduction of microflora in the irradiated samples was accompanied by the suppression of TVB and TMA accumulation, never reaching a spoilage level throughout 36 days of storage (Figure 2 and 3), although the viable bacterial count exceeded a level of 10^7 cells per gm fish flesh (Figure 1). At this cell density, there were 20 mg TVB N per gm fish flesh in the unirradiated samples, but only 9.5 mg N in the irradiated samples. These substances serve as common spoilage indices in fishery products and are used for determining their freshness (Farber, 1965). The suppression of these spoilage indices

substances in spite of high terminal viable bacterial cell density of the iraadiated samples indicates that the bacterial population producing TVB and TMA was selectively removed at the radurization dose of radiation.

Results on the direct proteolytic bacterial count revealed that proteolytic bacterial population in the irradiated samples was reduced to below one per cent of the total viable count and remained so throughout the storage period, while proteolytic bacteria in the unirradiated samples increased proportionately with the storage from 31.3% on O day to 85.5% on the twenty-second day (Table). This striking removal of proteolytic bacteria as a result of radurization is reflected in the reduced rate of proteolysis occured during the storage of the irradiated samples.

Deducing from the data, it is clear that radurization selectively inactivates major portion of fish microflora that cause the accumulation of TVB and TMA in normal fish spoilage and it is tempting to conclude that the microflora affected most by radiation treatment of fish represent in effect the proteolytic bacteria.

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