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Process Development of Safe and Ready-to-eat Raw Oyster Meat by Irradiation Technology

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***Abstract*—**White scar oyster (*Crassostrea belcheri*) is often eaten raw and being the leading vehicle for foodborne disease, especially *Salmonella* Weltevreden which exposed the prominent and most resistant to radiation. Gamma irradiation at a low dose of 1 kGy was enough to eliminate *S*. Weltevreden contaminated in oyster meat at a level up to 5 log CFU/g while it still retain the raw characteristics and equivalent sensory quality as the non-irradiated one. Process development of ready-to-eat chilled oyster meat was conducted by shucking the meat, individually packed in plastic bags, subjected to 1 kGy gamma radiation at chilled condition and then stored in 4oC refrigerated temperature. Microbiological determination showed the absence of *S*. Weltevreden (5 log CFU/g initial inoculated) along the whole storage time of 30 days. Sensory evaluation indicated the decreasing in sensory scores along storage time which determining the product shelf life to be 18 days compared to 15 days of non-irradiated one. The most advantage of developed process was to provide the safe raw oyster to consumers and in addition sensory quality retained and 3-day extension shelf life also exist.

***Keywords*—**decontamination, food safety, irradiation, oyster, *Salmonella* Weltevreden

I. Introduction

OYSTERS are of the greatest importance with regard to food safety because consumers prefer to eat them in the raw state, in which their unique flavor and nutritive value are preserved. However, consuming fresh oyster has often been associated with food borne disease outbreaks in many countries as it was considered important vehicles for pathogenic bacteria [7,10]. The technologies normally used to reduce the pathogen from food usually governing heat treatment in which practically must turn the products into cooked form. In order to decontaminate the oyster, concerns regarding preserve the raw condition was of the major consideration. The preservation methods, such as cold treatments [13], high temperature [6], vacuum packaging [4], electrolyzed water [14], high-pressure treatments [3] have been widely introduced. Ionizing radiation is one of the best emerging preservation technologies to improve microbiological of food [5, 8, 11]. The main purpose of irradiation fresh seafood is to control pathogens during storage and hence extending shelf life. Irradiations with gamma rays is receiving special attention from researchers because it reduces the numbers of both pathogenic and spoilage microorganism, not only rendering food safer but also increasing its shelf life.

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However the dose of ionizing radiation required to reduce the microbial population to safe level can cause undesirable changes in sensory qualities. The combination of irradiation with other preservation treatment such as storage at low temperatures can possibly solve the problem.

Among the pathogens commonly found in oyster including *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio vulnificus* and *Salmonella* spp., *Salmonella* Weltevreden was proved to be the most resistant specie to radiation [15]. It was reported that the radiation decimal reduction dose (D_{10}) of *Salmonella* Weltevreden was about twice of those of *Vibrio parahaemolyticus* and *Vibrio vulnificus* in inoculated white-scar oyster (*Crassostrea belcheri)* homogenate. It meant that *Vibrio* spp. was also be destroyed as *Salmonella* spp. which was considered as target pathogen was destroyed by irradiation process.

The aim of this study was to develop process of chilled ready-to-eat raw oyster meat for safety consumption using gamma irradiation. According to the concerns regarding the difficulty to shuck the oyster meat from its shell when consumer would like to consume the fresh oyster at home brought to the form of individual pack of ready-to-eat shucked oyster meat being investigated in this study. Based on previous investigation [15], *Salmonella* Weltevreden was used as target pathogen in oyster meat in this study. Sensory evaluation was used to determine the shelf life of the products.

II. Materials and Mthods

A. Sample preparation

1. Oysters

Oysters (*Crassostrea belcheri*) were obtained from Bandon Bay, the Gulf of Thailand in the Surat Thani Province and were transported to the laboratory within 24 h after harvest under ambient temperature.. It was then washed and cleaned using a brush to remove dirt deposited on the outside of the shell, taking care not to damage the joint between the valves. Oysters were aseptically shucked from their shells.

2. Microorganisms

Salmonella Weltevreden DMST 33380 (isolated from shellfish by Department of Medical Sciences, Ministry of Public Health of Thailand) were used in this study.

3. Preparation of the inoculum and inoculated samples

S. Weltevreden was kept in tryptic soy broth (TSB, Merck) contained 15% glycerol at -20 °C. To prepare the inocula, *S.* Weltevreden was grown in a TSB and incubate to log phase for 6 h at 35°C The activated cell cultures were centrifuged (6000 rpm for 30 min at 4°C) in a

refrigerated centrifuge (Hettich Model Universal 32R, Germany) and the culture were washed with sterile saline (0.85% NaCl). The pellet was finally suspended in sterile saline (0.85% NaCl) to a cell density of approximately 10^8 CFU/ml and kept as the stock inoculum.

Each shucked oyster meat was inoculated with 1 ml *S.* Weltevreden stock inoculum (10^6 CFU/g) by manually aseptic injection using a sterile syringe. The injection was done at 90° angle into oyster meat at 4 different points (0.25 ml/point) to a final concentration of approximately 10^5 CFU/g. Inoculation method was modified the method described by Lee and Bigelow [9]. The inoculation level was determined prior to the experiment. Each inoculated oyster meat was packed in low density polyethylene bag (approx.15 g: bag) and sealed. The oyster samples were then kept in refrigerated condition of 4 ±2°C. The samples either irradiated or control (non-irradiated) used for sensory evaluation were not inoculated for the safety concern of panelists.

Individual oyster packs were packed in polystyrene foam box (22 oysters / box) together with dry ice to keep cool temperature. The packing pattern was prior studied to provide the regularly distribution of cool temperature inside the box, special caution taken to prevent the oyster meat to be frozen.

B. Irradiation process

Cobalt-60 irradiator (JS-8900, Canada) with the source strength of ~255,500 Ci. was used to irradiate the samples at Thai Irradiation Center, Office of Atoms for Peace, Ministry of Science and Technology Bangkok, Thailand**.** The absorbed doses, measured by nylon thin film FWT-60-00 dosimetry (USA), at room temperature

Predetermination of optimum irradiation dose

The samples were subjected to radiation dose of 1, 2, 3 kGy. while the control samples was the non-exposed one (0 kGy). Survival of *S.* Weltevreden in each samples were determined after irradiated and the minimum radiation dose at which no survivals detected was selected as effective dose for this process.

Irradiation of oyster samples

Oyster samples were subjected to gamma radiation at the minimum absorbed dose selected from 2.1.1. After irradiation samples were kept in refrigerated condition as soon as possible.

C. Determination of product quality and shelf life

All samples were stored in chilling condition at 4±2°C. For quality determination, samples were sampling for microbiological and sensory analysis on day 0, 3, 6, 9, 12, 15, 18, 21, 24, 27 and 30 post-irradiation storage.

Microbiological analysis

Inoculated samples were examined for survival of *S.* Weltevreden according AOAC [2] and counting the visible colonies by surface plating onto a Xylose lysine desoxycholate agar (Merck) by macerating 25 g sample was aseptically homogenized for 2 min in a sterile stomacher bag containing 225 ml of sterile saline (0.85% NaCl) After serial dilution, a 100 µl aliquot from an appropriate dilution was plated onto a Xylose lysine desoxycholate agar. Plates were incubated at 35°C for 24 h and the colony forming unit (CFU) per gram was counted at 25-250 CFU per plate. After incubation period the plates were counted and 10 colonies from each plate were tested to confirm *Salmonella* spp. according AOAC [2]. Total viable bacterial count was also determined according to the procedure of AOAC [2].

D. Sensory evaluation

Non-inoculated oysters were used for sensory evaluation, both irradiation and control samples for panelist safety concern. A 6-person trained panels were used to evaluate color, odor, texture and acceptability of both raw and cooked irradiated (1.0 kGy) and non-irradiated (control) samples. Flavor attribute was additionally evaluated in cooked sample. Cooked samples were prepared by washing the raw oysters meat, wrap in foil, and steaming for 3 min. A scoring-test was used to evaluate previous mentioned attributes, scores 1-5 was ranked for magnitude of differences for each attribute. For overall acceptability attribute, score 5 represented extremely accepted, score 1 represented extremely unaccepted and score at 3 was adopted as the lowest limit for acceptance. Minimum storage time before any of the attribute scores reach under 3 was set as shelf life.

E. Statistical Analysis

Data of sensory evaluation was performed in randomized complete block design and compared means using t-test. All the statistical analyses were conducted at the 0.05 level of significance. All experiments were performed in twice.

III. Results and Discussion

A. Predetermination of optimum irradiation dose

The present study investigated the effect of gamma irradiation on *S.* Weltevreden after irradiation treatment of chilled oyster meat samples. From the previous study, Thupila *et al.* [15] reported the radiation decimal reduction dose or D_{10}-value of 0.33 for *S.* Weltevreden in oyster meat homogenate, thus low dose range of 1-3 kGy were examined as expected to cover the achievement of approximately 4-log kill. Table I shows the survival of total plate counts and *S.* Weltevreden in irradiated samples after irradiated for 0, 1, 2, 3 kGy. It was clearly shown that in order to eliminate 4.22 log cfu/g initial contamination of *S.* Weltevreden in chilled oyster meat, only 1 kGy as target dose (or 1.37 actual dose in this study) was sufficient for total elimination. However not all the microorganisms were destroyed by irradiation, as 0.87 log cfu/g still survived at this dosage level. It's not necessary and may not possible to destroy all the microbial load in food since some was resistant to radiation. Those survivals may not account as harmful health hazard since the most radiation-resistant pathogen commonly found in oyster was investigated in this study.

TABLE I
SURVIVAL OF TOTAL PLATE COUNT (TPC) AND *SALMONELLA* WELTEVREDEN CFU/G IN INOCULATED OYSTER MEAT AFTER IRRADIATED WITH DIFFERENT DOSES

Intended doses (kGy)	Actual absorbed dose range (kGy)	TPC (log CFU/g)	*Salmonella* Weltevreden (log CFU/g)

0	0	4.78±0.04	4.22±0.02
1	1.37-2.13	0.87±0.12	0.00±0.00
2	2.70-4.20	0.15±0.21	0.00±0.00
3	3.62-5.61	0.09±0.12	0.00±0.00

TPC= Total Plate Count

The appearance of the oyster after irradiation was still present in raw state without any adverse effects upon visual inspection. Therefore irradiation may be considered as a non-thermal technology or so call cold sterilization [5, 8] to preserve oyster against the harmful pathogens, and could be offered as an alternative technology to provide products of chilled ready-to-eat oyster meat in the commercial market. In this study, using target 1 kGy radiation dose of raw oyster was established for the irradiation process.

B. Quality of irradiated oyster during chilled storage

1. Microbiological quality

Survival of Total Plate Count (TPC) and *Salmonella* Weltevreden in inoculated oyster meats after subjected to1 kGy irradiation along the post-irradiate storage of 30 days at 4±2°C were compared to non-irradiated samples as presented in Fig 1. Initial total plate count and *S.* Weltevreden were 5.3 and 5.2 log cfu/g respectively. Irradiate at 1 kGy dose (1.25 kGy for actual absorbed dose) reduced about 1.5-2 log cfu/g the amount of TPC compared to the non-irradiated one along the storage time. Noticeable result demonstrated none of viable cells *S.* Weltevreden was detected after irradiated and along the storage time whereas the non-irradiated one still contain approximately 4 log cfu/g until the end of storage. The results obtained in this study indicated that the irradiation process established at 1 kGy radiation dose could effectively decontaminate oyster meat for the purpose of food safety.

2. Sensory quality

Fig. 2 presented the sensory scores evaluated on irradiated and non-irradiated raw and cooked oysters meat during 30-day chilling storage by trained panelists. Evaluation of oyster samples was conducted on both raw and cooked forms. Even though normally oyster was consumed in raw form but in order to differentiate the quality attributes that might arising from the irradiation process, the cooked samples should also conducted since it may gave different detectable sensory attributes from the raw ones.

The result showed that the sensory scores of all attributes gradually decreased along the storage time in both irradiated and non-irradiated one either evaluated on raw or cooked form. After irradiate (day 0), all attributes of irradiated oyster still obtained the highest scores of 5 which indicated that the sensory qualities was equivalent to the fresh one. Development of off-odor or irradiated odor or flavor such as rotten egg, bloody, cook meat, burnt, sulfur, metallic, alcohol, acetic acid, liver-like, etc. could be found in many kinds of irradiated foods reported by several researchers [1, 12, 16]. They also indicated that the off-odor developed immediately after irradiation and disappeared or was reduced, during storage. The off-odor may also be due to the rancidity of fat which affected the flavor and brought about lower score for irradiated samples than that received by control samples [16]. Apparently, no obvious off-odors or flavors were detected in this study and in addition, the sensory scores obtained from irradiated samples were slightly higher than the control samples and had longer storage acceptable time of 21days versus 15 days for control. The sensory attributes including color, texture, and overall acceptability were not accepted after 18 days of storage for irradiated oyster while 15 days for control samples which consequently determined the shelf life of chilled oyster meat.

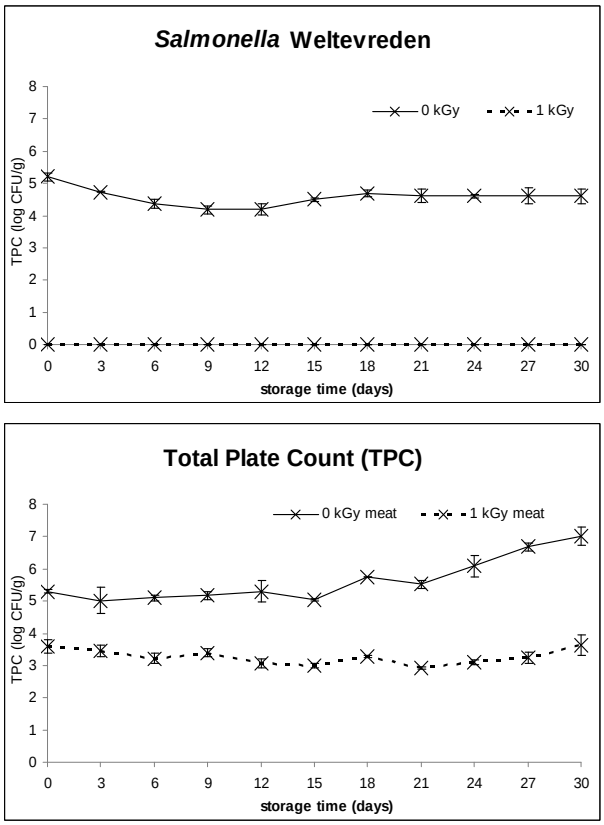

Fig. 1 Survival of Total Plate Count (TPC) and *Salmonella* Weltevreden CFU/g in 1 kGy irradiated and non-irradiated oyster meats during storage at 4°C

These indicated that not only the sensory acceptability of irradiated oyster was not less than the non-irradiated one, but also 3 days of chilled oyster shelf life was extended. Even though it was not considered long shelf life extension but the safety for consumption aspect was realized to be the advantage of this process. The result was in agree with several studies reported that doses of 1.0-2.0 kGy have been found to extend the shelf life of fish without changing their sensory attributes [16, 17].

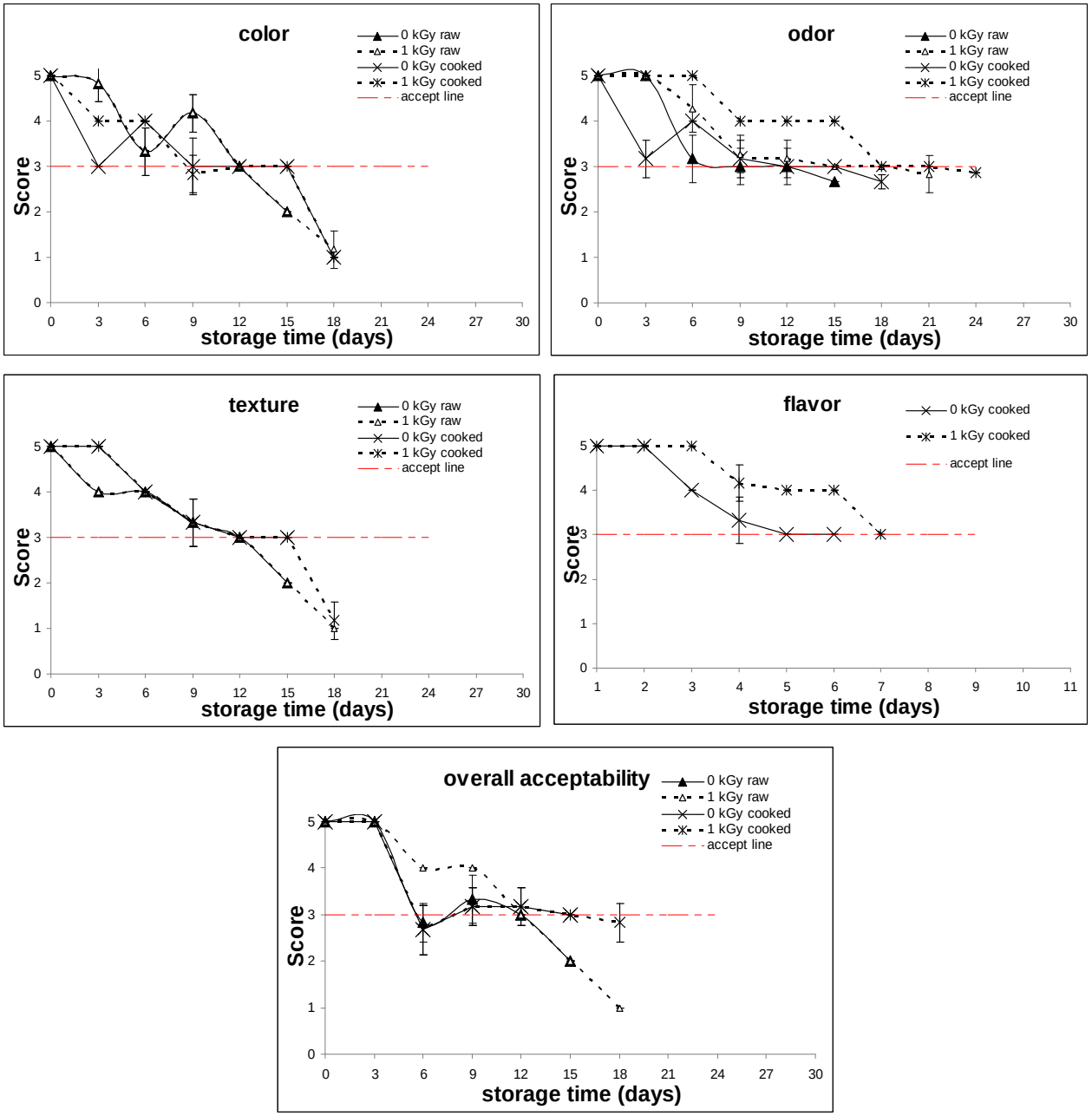

Fig. 2 Sensory quality scores of irradiated and non-irradiated oyster meat during chilling storage

IV. Conclusion

Process development of ready-to-eat chilled oyster meat was conducted by shucking the meat, individually packed in plastic bags, subjected to 1 kGy gamma radiation at chilled condition and subsequently stored at 4±2°C. The present study indicated that radiation dose of 1.0 kGy can totally eliminate 5 log cfu/g initial inoculated *Salmonella* Weltevreden after irradiation and along the whole storage time of 30 days. This process provided products with equivalent sensory quality as the non-irradiated one and also 3-day of shelf life extension.

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