การใช้รังสีกำจัดเชื้อ *Escherichia coli* O157:H7 ในกุ้งต้มแช่เยือกแข็ง

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บทคัดย่อ

การศึกษานี้มีวัตถุประสงค์เพื่อศึกษาผลของรังสีจากอิเล็กตรอนต่อการทำลายเชื้อ *Escherichia coli* 0157:H7 ในกุ้งต้มแช่เยือกแข็ง ทำการศึกษาโดยทำให้กุ้งต้มแช่เยือกแข็งมีการปนเปื้อนเชื้อ *E. coli* O157:H7 จำนวน 5สายพันธุ์ที่ระดับ 1.7 และ 2.747 log CFU/g แล้วนำไปฉายรังสีจากอิเล็กตรอนที่ปริมาณรังสี 0, 1, 2 และ 3 กิโลเกรย์ในสภาพแช่เยือกแข็ง (-20 °C) พบว่า ตรวจไม่พบเชื้อ *E. coli* 0157:H7 (<10 CFU/g) ในตัวอย่างกุ้งต้มแช่เยือกแข็งที่มีปริมาณเชื้อ 2.747 log CFU/g แล้วฉายรังสีที่ปริมาณรังสี 2 และ 3 กิโลเกรย์ แต่ตรวจพบเชื้อ *E. coli* 0157:H7 จำนวน10 CFU/g ในตัวอย่าง 1 ซ้ำจากตัวอย่าง 3 ซ้ำในตัวอย่างที่ปนเปื้อนเชื้อในระดับสูง 2.747 log CFU/g ซึ่งฉายรังสีที่ปริมาณ 1 กิโลเกรย์ อย่างไรก็ตาม ตรวจไม่พบเชื้อ *E. coli* 0157:H7 ในตัวอย่างกุ้งที่มีการปนเปื้อนเชื้อที่ระดับ 1.7 log CFU/g ซึ่งฉายรังสีขนาด 1, 2 และ 3 กิโลเกรย์ ดังนั้นการใช้รังสีขนาด 2-3 กิโลเกรย์ จึงสามารถควบคุมเชื้อนี้ได้ และการฉายรังสีจึงเป็นวิธีการหนึ่งที่สามารถใช้กำจัดเชื้อ *E. coli* 0157:H7ได้

Effects of Irradiation on *Escherichia coli* O157:H7 of Peeled and Cooked Frozen Shrimp

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ABSTRACT

Peeled and cooked frozen shrimp imported from Thailand were inoculated with a five-strain mixture of *Escherichia coli* 0157:H7 and frozen., the contamination levels of *E. coli* O157:H7 were 1.7 and 2.747 log CFU/g The frozen samples (-20 °C) were exposed to irradiation treatment by using electron beam accelerator with doses 0, 1, 2 and 3 kGy. The irradiated sample was investigated for *E. coli* 0157:H7.

Survivors (<10 CFU/g) were not detected in samples exposed to 2 and 3 kGy of irradiation at the level of contamination at 2.747 log CFU/g. In one of three replicate of contamination at 2.747 log CFU/g, 10 CFU/g was found in sample that were irradiated at 1 kGy. However, no survivors (<10 CFU/g) were found in samples exposed to 1, 2 and 3 kGy at the level of contamination at 1.7 log CFU/g. Irradiation at dose 2-3 kGy could controlled this pathogen. Therefore, irradiation is one of the effective methods to control *E. coli* 0157:H7.

INTRODUCTION

The export of frozen shrimp have risen from 20,000 metric tons in 1983 to 121,000 metric tons in 1991 and became 148,861 metric tons in 1993 at a value of 37,841 million baht from export (Department of Business Economic, 1993). The problem of export are the quality and safety of food products. Late restrictions have been enforced concerning the quality of frozen marine products exported from Thailand to some European countries, Japan and USA. Precooked shrimp are more hazard than uncooked products, because they are commonly eaten, without further cooking, in salads, cocktails, etc.

E. coli O157:H7 has been shown to be the causative agent of several foodborne outbreaks of haemorrhagic colitis and haemolytic uraemic syndrome leading to death in many cases. These bacteria were first isolated from outbreaks in Oregon and Michigan (1982), where contaminated hamburgers seemed to be the spreaders of the agent[1]. Other outbreak have been traced back to the consumption of contaminated ground beef. But also raw milk and vegetables have been implicated as vehicles of *E. coli* O157:H7[2]. Several studies , investigation of food of animal origin for this serotype of *E. coli* becomes more and more necessary in the microbiological quality control of foods. In 1996, isolates of *E. coli* O157:H7 caused numerous foodborne outbreaks, including a serious outbreak in Japan. Now the import products to Japan is very concerned on *E. coli* O157:H7.

E. coli O157:H7 organisms are gram-negative rods which belong to the family Enterobacteriaceae. Gastrointestinal infections caused by *E. coli* O157:H7 have led to public health problems in various countries[3].

One treatment of product which could effectively solve the problem of the presence of food poisoning pathogens is that of irradiation of packaged product prior to export. Irradiation can decrease the microbial load of foods and also eliminate food poisoning pathogens on meat products without changing their nutritive and sensory qualities.[4-6]

The purpose of this investigate was to eliminate pathogens (*E. coli* O157:H7) in peeled and cooked frozen shrimp by using irradiation. The objective of this study was to determine the effects of electron beam irradiation on *E. coli* O157:H7 of peeled and cooked frozen shrimp.

MATERIALS AND METHODS

1. Preparation of Inoculated Shrimps

Pure culture of 5 strains of *E. coli* O157:H7 from Iowa State University were stocked and maintained on tryptic soy agar slants at 4°C. Each strains of *E. coli* O157:H7 from stock slants was cultured in 10 ml of tryptic soy broth (TSB) and incubated for 18-20 h at 37 °C. Then, each loop of 5 strain was inoculated in the same 10 ml of TSB and incubated for 18 h at 37 °C. Following incubation, the number of a viable five-strain mixture of *E. coli* O157:H7 per 25 g of inoculum were estimated by the direct surface plate count procedure. Decimal dilution of bacteria were prepared in 0.1% peptone water until $1:10^8$, and counted by using surface spreaded plate of tryptic soy agar (TSA) at 37 °C for 18-20 h. Each developed colony was assumed to have grown as one viable unit.

Peeled and cooked frozen shrimp (product imported from Thailand) was obtained from a commercial supermarket and stored at -20 °C until used. The peeled and cooked frozen shrimp of each replicate were determined for *E. coli* O157:H7 to be sure that test products were free from *E. coli* O157:H7. The following method was used for preparation of inoculated test products. 0.1 ml of suspension of the peptone water diluent with the *E. coli* O157:H7 in different quantity was added to 25 g of each test products which was contained in a sterile polyethylene bag (Fig1). The inoculated samples were packed and heat sealed , then frozen at -20 °C. The designs level of *E. coli* O157:H7 were approximately 10^2 and 10^3 CFU/g .

The inoculated samples were irradiated with a Linear Electron Accelerator at Linear Accelerator Facility at Iowa State University. The samples were irradiated at temperature -20 °C at doses 0, 1, 2 and 3 kGy (energy level 10 MeV and power level 8 kw). Immediately after irradiation treatment, irradiated samples were returned to frozen -20 °C storage. All processes were repeated 3 times.

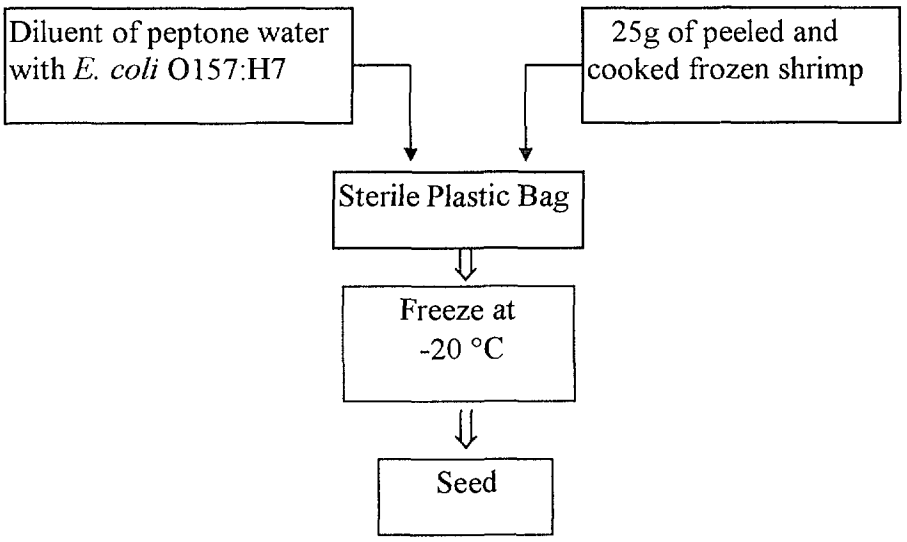

Fig.1 Preparation of Inoculated Shrimps

2. Method for *E. coli* O157:H7 Detection

The 25 g of samples were homogenized for 2 min with 225 ml 0.1 %peptone water in a stomacher. The homogenized samples were prepared serial tenfold dilutions in peptone water diluent in accordance with anticipated level of contamination then applied 0.1 or 1 ml of each dilution in duplicate for the surface spreaded plate on MacConkey Sorbitol Agar (MSA), incubated at 37 $^{\circ}$C for 18-20 h. Typical colonies of *E. coli* O157:H7 (sorbitol negative) were counted and randomly selected colonies were confirmed as *E. coli* O157:H7 by gram strain, biochemical test: indole and 4-methyl- umbelliferone glucuronide (MUG) and serological test by using *E. coli* O157 latex agglutination assay (Oxoid) and Reveal for *E. coli* O157:H7:Neogen). The detection method was modified from Hitchins et al.,[2] (Fig.2). The detection limit of this method was less than 1 log CFU/g.

3. Statistical Analysis

Microbiological data were converted to logarithms of the number of Colony Forming Unit / g ($\log_{10}$ CFU/g). Average data and standard deviation were calculated from three replications. The results were mostly investigated the relationship between the number of *E. coli* O157:H7 in samples and elimination of *E. coli* O157:H7 in each doses of irradiation by using analysis of variance (ANOVA) procedure with the computer software Excel version 5.0.

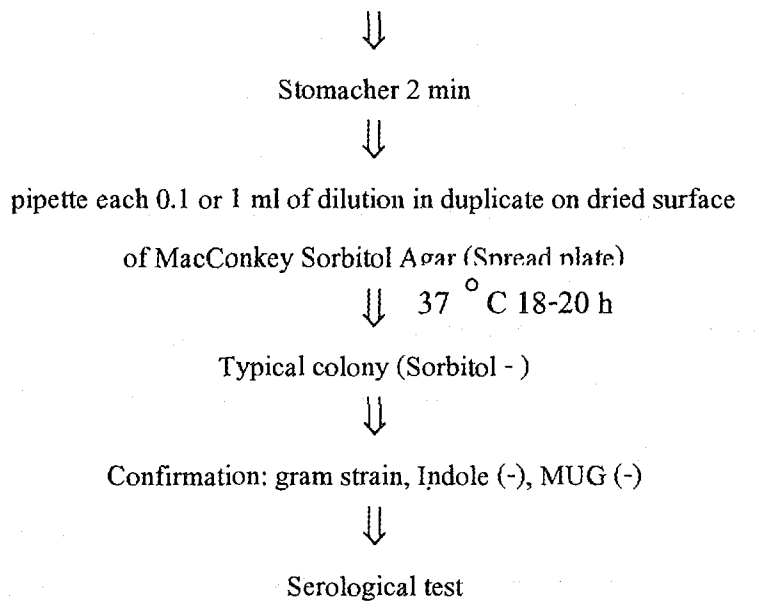

Fig. 2 Flow diagram of methods for detecting *Escherichia coli* O157:H7

RESULTS AND DISCUSSION

Table 1 shows the actual doses of electron beam irradiation for peeled and cooked frozen shrimp. There were no significance differences in each replicate for each dose.

The population of *E. coli* O157:H7 in inoculated shrimp was decreased for 1.6 log cycle after frozen, but all of samples still had this food-borne pathogen as shown in table 2 and 3. Doyle and Schoeni[7] also showed that *E. coli* O157:H7 can survive well in ground beef during frozen storage for up to 9 months.

Irradiation effectively reduced the number of *E. coli* O157:H7 in peeled and cooked frozen shrimp. There was a significant difference in the number of survivor of *E. coli* O157:H7 in non-irradiated and irradiated samples. Survivors (<10 CFU/g) were not detected in samples exposed to 2 and 3 kGy of irradiation at the level of contamination at 2.747 log CFU/g. In one of three replicate of contamination at 2.747 log CFU/g, 10 CFU/g was found in sample that were irradiated at 1 kGy. However, no survivors (<10 CFU/g) were found in samples exposed to 1, 2 and 3 kGy at the level of contamination at 1.7 log CFU/g.

From this high level of contamination, D_{10} reduction of *E. coli* O157:H7 by irradiation was <0.57 kGy. Irradiation at 2.0 kGy was effective in reducing this food borne pathogen in this product. Thayer and Boyd[8] reported that D_{10} value of *E. coli* O157:H7 at -5 °C was 0.44 kGy and they found that *E. coli* O157:H7 greater resistance to the effects of irradiation at temperature below freezing. Clavero, M.R.S. et al.[9] also reported that *E. coli* O157:H7 in raw ground beef had a significantly ($P<0.05$) D_{10} value when irradiated at -15 to-17 °C than when irradiated at 3 to 5 °C. Fu[10] also showed that *E. coli* O157:H7 in streaks were reduced at least 1 log by 0.6 kGy electron beam irradiation at 25 °C and *E. coli* O157:H7 in ground beef were 2 log cell reduction by 0.8 kGy irradiation at 25 °C.

Therefore, application of irradiation at dose 2-3 kGy could complete inactivated this organism with a high probability. Irradiation is one of the effective methods to control *E. coli* 0157:H7.

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Table 1 Desired and actual doses of irradiation for peeled and cooked frozen shrimp.

Sample	Actual dose for peeled and cooked frozen shrimp		
	1 kGy	2 kGy	3 kGy
Replicate 1	1.094±0.087	2.127±0.075	2.951±0.078
Replicate 2	1.030±0.060	2.075±0.163	3.256±0.355
Replicate 3	1.102±0.049	2.130±0.052	3.086±0.131
Average ± SD	1.076±0.063	2.111±0.088	3.098±0.220

Table 2 Effect of irradiation on *E. coli* O157:H7 of peeled and cooked frozen shrimp which inoculated at low level of contamination.

Sample	*E. coli* O157:H7 (log CFU/g)				
	Before frozen	After frozen, irradiated at doses			
		0 kGy	1 kGy	2 kGy	3 kGy
R1	3.114	1.000	< 1	< 1	< 1
R2	3.312	2.255	< 1	< 1	< 1
R3	3.477	1.845	< 1	< 1	< 1
Ave ± SD	3.301±0.182	1.7 ± 0.64	< 1	< 1	<1

Note: Detection limit is 1 log CFU/g.

Table 3 Effect of irradiation on *E. coli* O157:H7 of peeled and cooked frozen shrimp which inoculated at high level of contamination.

Sample	*E. coli* O157:H7 (log CFU/g)				
	Before frozen	After frozen, irradiated at doses			
		0 kGy	1 kGy	2 kGy	3 kGy
R1	4.176	2.371	1	< 1	< 1
R2	4.217	2.653	< 1	< 1	< 1
R3	4.267	3.217	< 1	< 1	< 1
Ave ± SD	4.22±0.045	2.747± 0.431	< 1	< 1	<1

Note: Detection limit is 1 log CFU/g.

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