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The application of a high voltage electric field (HVEF) to reduce *Escherichia coli* and *Salmonella thyphimurium* bacteria in red snapper (*Lutjanus* sp.) fillets

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Abstract. *Escherichia coli* and *Salmonella thyphimurium* are commonly found pathogenic bacteria in red snapper fillets that must be reduced. (HVEF) is non-thermal method used to reduce bacteria. The aim of this study was to evaluate the ability of HVEF in relation to the reduction of *Escherichia coli* and *Salmonella thyphimurium* in red snapper fillets. Cut-shaped filets (4 x 2 x 1.5 cm) contaminated with *Escherichia coli* and *Salmonella thyphimurium* (10^4 cfu) were the main sample. The HVEF treatment was performed at different voltages (10 $kVcm^{-1}$ and 16 $kVcm^{-1}$) for 30 seconds in a cross-section of stainless steel. The effectiveness of HVEF was evaluated by the changes in the *Escherichia coli* (MPN/g) counted and in the *Salmonella thyphimurium* (qualitative +/-). *Escherichia coli* was reduced by 32.67 MPN/g (10 $kVcm^{-1}$) and 109.27 MPN/g (16 $kVcm^{-1}$) ($P<0.05$). The presence of *Salmonella typhimurium* in the fillets was negative (10^{-1} dilution) after being treated with 10 $kVcm^{-1}$ and 16 $kVcm^{-1}$, while the control was positive (10^{-1} dilution). The HVEF treatment did not significantly affect the changes in the texture value, centrifugal loss, and water content ($P>0.05$). The results of the research were informed by the development of the preservation technology of fish fillets using the HVEF method in the fish processing industry, because it reduced *Escherichia coli* and *Salmonella thyphimurium*.

1. Introduction

Red snappers are one of the resources that can be processed in the form of fillets [1]. Red snapper fillets are export products that have a value of 100,000/kg IDR which indicates high economic value [2]. Red snapper fillets are easily deteriorated due to enzymatic and microbiological activities [3]. The total number of bacteria in red snapper fillet products is one of the important factors involved in product acceptance by importing countries. Fillet products that have total number of bacteria that is not in accordance with the specifications of fresh fillet products will be rejecting by the importing country [4].

Data on the cases of rejection of Indonesian fishery products in 2010 - 2012 reached 1,203 cases. In 2012, there were at least 118 cases out of 419 cases of rejection of fishery products due to bacterial contamination [4]. According to [5], in 2013, there were 90 cases of rejection of fishery products caused by bacterial contamination. According to [6], *Escherichia coli* and *Salmonella* are bacterial contaminations found in fishery products. The reduction of *Escherichia coli* and *Salmonella* needs to be done in order to reduce the risk of rejects [4].

A High Voltage Electric Field (HVEF) is a non-thermal process conducted by utilizing electricity in a relatively short amount of time in order to maintain the shelf life of food products by reducing the bacteria [7]. The application of HVEF can reduce bacterial activity through the electroporation process [8]. HVEF applications on food products have been widely used to reduce bacterial contamination in food products, but it is limited to liquid products only (milk) [9]. Limited information regarding the potential of HVEF as a processing technique for fillet products in reducing the number of *Escherichia coli* and *Salmonella thyphimurium* was the background of this study. The purpose of this study was to determine the effect of HVEF on the reduction of *Escherichia coli* and *Salmonella thyphimurium* in red snapper fillets.

2. Materials and methods

2.1.Materials

The frozen red snapper fillet was obtained from PT. Kelola Mina Laut, Gresik, East Java. The fillet was vacuum packed using polyethylene and transported to the laboratory. The fillet was cut into 4 x 2 x 1.5 cm chunks. The sample was stored in a laboratory freezer (-18^{o}C) before being processed. *Escherichia coli* ATCC 8739 and *Salmonella thyphimurium* ATCC 14028 in glycerol was obtained from the Testing Unit of Faculty of Pharmacy, Universitas Airlangga.

2.2. Bacterial isolate preparation

Escherichia coli and *Salmonella typhimurium* on glycerol media were cultured in tryptone soya broth (TSB) and incubated at 37° C for 24 hours. The bacterial isolates in TSB were cultured on tryptone soya agar (TSA), incubated at 37° C for 24 hours [10].

2.3. Bacterial contamination in red snapper fillet

The isolates of *Escherichia coli* ATCC 8739 and *Salmonella thyphimurium* ATCC 14028 on tryptone soya agar (TSA) were diluted in a 0.9% NaCl solution to obtain a bacterial dilution of 1 x 10^4 cfu. The solution for each bacterium was poured into a 6 x 11 cm plastic bag containing a sample, soaked for ± 10 minutes [5].

2.4. High voltage electric field treatment

The sample was placed between the electrodes connected to a high voltage generator 50 kV capacity (Sulis, Indonesia). The sample was induced by an electric field of 10 kVcm^{-1} and 16 kVcm^{-1} for 30 seconds with one sample as the control [11]. The electric strength formula was as follows (Eq (1)):

$$E_{electric\ strength}\ (kVcm^{-1}) = \frac{\text{voltage (kV)}}{\text{gap (cm)}} \quad (1)$$

2.5. Enumeration of Escherichia coli

The enumeration of *Escherichia coli* in the fillet was done using the Most Possible Number (MPN) method. The sample was crushed and diluted in 9 ml of 0.9% NaCl by as much as one gram in order to get the mother solution (10^0 dilutions). The mother solution was diluted to a 10^{-3} dilution. One milliliter of each dilution solution was dissolved in lactose broth (LB) supplemented with Durham tubes upside down by three series, incubated at 37° C for 48 hours [12]. The medium in the Durham tubes containing air were cultured on eosin methylene blue (EMB) agar, incubated at 37° C for 48 hours [13]. The sample series was positive for *Escherichia coli* compared to the MPN index [14].

2.6. Existence of Salmonella typhimurium

The sample was crushed and diluted in 9 ml of 0.9% NaCl by as much as one gram in order to obtain the mother solution (10^0 dilutions). The mother solution was diluted again to 10^{-2} dilution. The sample solution with a dilution of 10^0, 10^{-1}, and 10^{-2} was cultured in salmonella-shigella agar (SSA), incubated at 37° C for 24 hours. The positive media containing *Salmonella thyphimurium* was characterized by the presence of black colonies [15].

2.7. Texture (Hardness) analyze

We tested the texture (hardness) using the texture analyzer (Brookfield, USA). The sample was pressed using a measuring needle 0.5 cm in diameter with a pressure speed of 10 mm/s. The texture values were detected automatically in grams (g) [11].

2.8. Centrifugal loss test

The fillet was cut into a thickness of 0.5 cm and 1 cm long, before being weighed (W_1) and coated with filter paper to fit into a 1.5 ml micro tube. Centrifugation (Micro CL 21R, England) was carried out for 10 minutes at 10°C, at a speed of 10,000 rpm. The sample was removed from the filter paper and weighed (W_2). The centrifugal loss formula was as follows [16] (Eq (2)):

$$Centrifugal\ loss\ (\%) = \frac{W1-W2}{W1}\ x\ 100\% \quad (2)$$

2.9. Water content test (AOAC, 2005)

The sample was crushed and put into a dry Porcelain dish as much as ± 5 grams (W_1). The sample was treated at 105°C for 12 hours (Memmert, Germany). The dry sample was weighed (W_2). The water content formula was as follows (Eq (3)):

$$Water\ content\ (\%) = \frac{W1-W2}{W1}\ x\ 100\ \% \quad (3)$$

2.10. Data analyze

The Data Most Possible Number (APM) changes of the *Escherichia coli*, texture, centrifugal loss, and water content was analyzed using ANOVA (Analysis of Variance) and continued with Duncan's Multiple Range Test (DMRT). The data on the changes in relation to the existence of *Salmonella thyphimurium* in red snapper fillets was analyzed descriptively.

3. Results and discussion

3.1. Changes in MPN Escherichia coli

The analysis of the changes in MPN Escherichia coli can be seen in Table 1.

Table 1. Results of the Analysis of Changes in Escherichia coli MPN in the Red Snapper Fillets.

Treatment	No *Escherichia coli* added (MPN/g)	Added *Escherichia coli* (MPN/g) (B)	After treatment (HVEF) (MPN/g) (C)	Change of MPN *Escherichia coli* (MPN/g) (C-B)	SNI 01-2696.1 2006
0 $kVcm^{-1}$	0.00 ±0.00	80.07 ±112.68	99.33 ±121.97	19.27^a ±9.30	Max<2
10 $kVcm^{-1}$	3.13 ±5.43	41.33 ±30.00	8.67 ±12.50	-32.67^a ±18.00	APM/g
16 $kVcm^{-1}$	0.00 ±0.00	124.33 ±33.71	15.07 ±19.86	-109.27^b ±41.00	

Note: Different letters in the same column indicate significant differences (P<0.05) (n = 3)

The changes in the APM Escherichia coli in red snapper fillets were treated with a high voltage electric field (HVEF) and showed significant differences in each treatment (P<0.05) (Table 1). The HVEF 16 $kVcm^{-1}$ treatment showed the highest change in APM Escherichia coli (-109.27 ± 41.00) compared to KLTT 10 kVcm-1 (-32.67 ± 18.00) and the control (19.27 ±9.30). The results were

in line with [17], in which the HVEF treatment (17 $kVcm^{-1}$ - 30 $kVcm^{-1}$) applied to tangerine juice products can reduce the number of Escherichia coli in each treatment. The results were also supported by [18], who found that the HVEF treatment (16 $kVcm^{-1}$) in dairy products can reduce the number of Escherichia coli to negative. Bacterial reduction in liquid food tends to be more effective, as the ion content spread in liquid food products can distribute electricity more than solid food [19].

Electric field induction in Escherichia coli cells can cause damage to the cell walls and cell membranes which results in leaks in *Escherichia coli* cells (lysis) [17]. The treatment of HVEF against bacteria can induce pore formation (electroporation) in cells [20]. Electroporation causes osmotic pressure changes [21], external material enters the cell (swelling) and this causes cell lysis due to internal cell pressure [22].

3.2. Existence of Salmonella typhimurium

The results on the existence of *Salmonella thyphimurium* in red snapper fillets can be seen in Table 2.

Table 2. Results of the existence of *Salmonella typhimurium* in Red Snapper Fillets.

Treatment	N	No *Salmonella thyphimurium* added			Result	Added *Salmonella thyphimurium*			Result	After treatment (HVEF)			Result	SNI 01-2696.1 2006
		10^0	10^{-1}	10^{-2}		10^0	10^{-1}	10^{-2}		10^0	10^{-1}	10^{-2}		
0 $kVcm^{-1}$	1	-	-	-		+	+	+		+	+	-		Max negative
	2	-	-	-	-	+	+	-	+	+	+	-	+	
	3	-	-	-		+	+	+		+	+	-		
10 $kVcm^{-1}$	1	-	-	-		+	+	+		+	-	-		
	2	-	-	-	-	+	-	-	+	+	-	-	+	
	3	-	-	-		+	+	+		-	-	-		
16 $kVcm^{-1}$	1	-	-	-		+	+	+		+	-	-		
	2	-	-	-	-	+	+	-	+	+	-	-	+	
	3	-	-	-		+	+	+		+	-	-		

Note: (+) positive*Salmonella thyphimurium*
(-) negative *Salmonella thyphimurium*

The existence of *Salmonella thyphimurium* in red snapper fillets after being treated with HVEF (10 $kVcm^{-1}$ and 16 $kVcm^{-1}$) tends to decrease at the 10^{-1} dilution (negative) compared to the control (positive) (Table 2). The results were in line with [23], in that the results showed a decrease in the number of *Salmonella thyphimurium* on an agar medium treated with HVEF. The results were also supported by [24], in that the results showed that an increase in the voltage electric field on the egg yolk can reduce the number of *Salmonella enteritidis*.

Salmonella thyphimurium cell damage is caused by bacterial cell pore formation (electroporation) [8]. *Salmonella thyphimurium* cell electroporation was formed due to changes in the potential differences in the cell wall and cell membrane components [20]. The external material enters the cell due to differences in the osmotic pressure in the cell and the cell environment [25]. Cell lysis is due to the amount of material that enters the cell [22].

3.3. Texture (Hardness), centrifugal loss, and water content

The analysis of the texture (hardness), centrifugal loss, and water content can be seen in Table 3.

Table 3. Analysis of the Texture (Hardness), Centrifugal Loss, and Water Content in Red Snapper Fillets.

Parameter	Treatment		
	0 $kVcm^{-1}$	10 $kVcm^{-1}$	16 $kVcm^{-1}$
Texture (*Hardness*) (g)	213.40^{a} ±30.02	194.47^{a} ±38.71	263.23^{a} ±36.49
Centrifugal Loss (%)	18.93^{a} ±1.70	17.90^{a} ±4.16	15.00^{a} ±2.86
Water Content (%)	76.12^{a} ±1.92	77.17^{a} ±1.15	78.05^{a} ±2.54

Note: Same letter in the same row indicate no significant difference (P>0.05) (n = 3)

The results of the analysis showed that the HVEF treatment had no significant effect on hardness, centrifugal loss, and water content in the red snapper fillets (P>0.05). The results were in line with [26], in that the HVEF treatment (18.6 $kVcm^{-1}$) did not affect the protein structure of the salmon. The results were also supported by [27], in that the HVEF treatment did not affect the beef weight loss. The texture (hardness) of the red snapper fillets was affected by the condition of the myofibril protein [28], as well as by centrifugal loss and water content [11]. The effect of the HVEF was not significant in relation to texture (hardness), centrifugal loss and water content. This can be caused by the inability of the electric field to degrade the red snapper fillet protein [27].

4. Conclusion

High voltage electric field (HVEF) applications can reduce *Escherichia coli* and *Salmonella thyphimurium* in red snapper fillets. The research results can be useful information for the fish processing industry to increase the preservation of fish fillets using HVEF technology.

5. Reference

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