

## INHIBITION OF *Escherichia coli* O157:H7 CONTAMINATION ON CHICKEN MEAT BY NATURAL VINEGAR PREPARED FROM BANANA PEEL AND COCONUT WATER

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### ABSTRAK

Penelitian terkait penggunaan vinegar sebagai pengawet alami untuk menginaktivasi pertumbuhan mikroba pada daging. Vinegar yang digunakan berasal dari kulit pisang dan air kelapa, dan dilakukan pengujian terhadap kemampuannya menghambat *Escherichia coli* O157:H7. Daging ayam diberi perlakuan larutan vinegar (setara dengan 1% asam asetat), larutan asam asetat (1%), dan larutan asam laktat (2%), kontrol (air destilasi). Sampel direndam dalam larutan asam perlakuan selama 1 menit (1,125:1 w/v; daging ayam : perlakuan larutan). Sampel yang sudah diperlakukan diinokulasi dengan *E. coli* O157:H7 di atas permukaan daging ayam, kemudian didiamkan selama 20 menit untuk absorpsi bakteri ke dalam daging. Pengamatan dilakukan pada hari ke 0,3, 6, 9 dan 12 pada penyimpanan suhu rendah, dan jam ke 0, 6, 12, 18, 24 pada penyimpanan suhu ruang. Hasil menunjukkan bahwa vinegar kulit pisang dan vinegar air kelapa efektif sebagai pengawet alami. Kesimpulannya, vinegar berpotensi menghambat pertumbuhan *E. coli* O157:H7 pada daging ayam sampai 12 jam pada penyimpanan suhu ruang dan 9 hari pada suhu dingin.

*Kata kunci* : vinegar kulit pisang, vinegar air kelapa, *Escherichia coli* O157:H7, daging ayam

### ABSTRACT

The use of vinegar as a natural preservative to inactivate microbial growth in meat was investigated. Vinegar was prepared from banana peel and coconut water, and the efficacy against *Escherichia coli* O157:H7 was tested. Chicken meat were treated with vinegar solution (equals to 1% acetic acid), acetic acid solutions (1%), and lactic acid solutions (2%), control (distilled water). Samples were soaked with acid solution treatment for 1 minute (1.125:1 w/v; chicken meat: solution treatment). Treated samples were inoculated with *E. coli* O157:H7 on the surface of the chicken meat, then left for 20 minutes for absorption of bacteria into the meat. Observation was done at 0, 3, 6, 9, and 12 days at low temperature storage, and at 0, 6, 12, 18, 24 hours at room temperature storage. The results showed that banana peel vinegar and coconut water vinegar were effective as natural preservative. In conclusion, the vinegar were potentially inhibited *E. coli* O157:H7 growth at chicken meat until 12 hours at room temperature storage and 9 days at cold temperatures.

*Keywords* : Banana peel vinegar, coconut water vinegar, *Escherichia coli* O157:H7, poultry meat

## INTRODUCTION

Vinegar is widely used as an antimicrobial due to its ability to lower the pH and cause instability in the bacterial cell membrane. Vinegar is known as a producer of acetic acid as the primary metabolite, tartaric acid, and citric acid (Global Agrisystem, 2011).

Acetic acid is generally considered safe for use (Generally Recognized as Safe/GRAS category). This regulations were found in FSIS-USDA as an acidifier (which estimated has an antimicrobial effect) on meat products (USDA, 1995; USDA, 1996). Several studies have been done to apply organic acid which is GRAS category at a certain concentration (Dorsa *et al*, 1997; Diez *et al*, 2008; Jamilah *et al*, 2008; Okolocha and Ellerbroek, 2005; Zhou *et al*, 2010). Organic acid is commonly used in food preservation. Few studies showed inhibition properties of acetic acid on *E.coli* O157: H7 in carcass surface tissue (Bell *et al*, 1986; Corner *et al*, 1997; Cutter *et al*, 1997; Kochevar *et al*, 1997).

Vinegar is a product made from fermented material that contain sugar or polysaccharide (Kwartiningsih and Mulyati, 2005), and easily prepared by fermentation process. Natural vinegar, a GRAS organic acid have been developed from many fruits and grains such as apple, wine, malt, sugar, glucose (Kwartiningsih and Mulyati, 2005), rice (Chang and Fang, 2007), grape (Sengun and Karapinar, 2004) and coconut water (Fardiaz and Nuraeni, 1996). Agricultural wastes such as banana peel, coconut water, cocoa pulp, and molasses were potentially used to vinegar production, because of sugar or polysaccharide containing in this material by using slight modification process such as aging for banana peels. The superiority of acetic acid from fermentation process like vinegar is better flavor than that was produced from chemical industry, easily prepared, and inexpensive.

The application of rice vinegar as an antimicrobia have been done on iceberg lettuce (Chang and Fang, 2007), grape vinegar mixture with lemon juice to carrots (Sengun and Karapinar, 2004). Studies on application of vinegar was prepared from agricultural waste to preserve fresh meat has not been found in published literature. In the other side, fresh meat often contaminated with pathogenic bacteria such as *Escherichia coli* O157:H7, *Salmonella* spp., *Listeria monocytogenes*, *Clostridium botulinum*,

*Campylobacter*, *Clostridium perfringens*, *Staphylococcus aureus*, *Aeromonas hydrophyla* and *Bacillus cereus* (Huffman, 2002). In Indonesia, poultry especially chicken is a source of animal protein that is very popular in the community. However, the supply of chicken meat/postharvest processing done by the slaughterer/butcher primarily small to medium-scale enterprises is still lacking to keep the sanitation and hygiene, so that cases of food poisoning are still common (foodborne illness). This study was design to study inhibition of *Escherichia coli* O157:H7 in chicken meat by natural vinegar prepared from banana peel and coconut water. Observation at this study only was done according to *E.coli* O157:H7 in chicken meat.

## MATERIAL AND METHODS

### Sample Preparation

Chicken carcass was taken immediately after slaughtered from Jambu Raya Slaughtering House . The meat was cut into cubical shapes, with approximately weight 75 g and three replications. Samples were packed with polyethylene plastic bag and put into the ice box that containing ice gel. The temperature in the ice box  $\pm 4^{\circ}\text{C}$ . The samples were then transported to the laboratory for observation.

### Bacterial Culture

Bacteria test used was *Escherichia coli* O157:H7, a pathogenic bacteria that commonly found in meat. That bacteria strain was obtained from Indonesian Research Center for Veterinary Science (Bogor, Indonesia). The isolate was inoculated in peptone water (0.1%) and the cell concentration was adjusted to  $10^6$  CFU/ml of population of bacteria.

### Preparation of Vinegar

Vinegar was prepared using procedures developed by Radiyati and Darmajana (2003), Kwartiningsih and Mulyati (2005) and Global Agrisystem (2011) with slight modifications. Aging of banana peels was need to convert carbohydrate to simple sugar, also addition of alfa amylase and glucoamylase enzyme at the certain stage of preparation banana peel vinegar.

Agricultural waste (banana peel and coconut water) were boiled. Ingredien (sugar cane 200g/kg, ammonium sulfate 0.33 g/L, and ammonium phosphate 0.05 g/L) were then added to the solution. The pH of solution was

maintained between 3-4.5. *Saccharomyces cereviceae* (15%) was put into the jar, then tight packed and stored at room temperature. After 4 days anaerob fermentation, *Acetobacter aceti* (10%) was added to the bottle. Aerob fermentation was then done for acetic acid formation from alcohol.

#### Acid Treatment

Samples of chicken meat were randomly divided into fivegroups, each sample was 75 g weight. The treatment used including banana peel vinegar (equal to 1% acetic acid), coconut water vinegar (equal to 1% acetic acid), acetic acid solution (1%)(MERCK), lactic acid solution (2%) (MERCK), and control (distilled water). Samples were soaked with acid solution treatment for 1 minute (1.125:1 w/v; chicken meat: solution treatment). Treated samples were inoculated with 10<sup>6</sup>CFU/mL of *E. coli* O157:H7 on the surface of the poultry meat (Dorsa *et al.*, 1996). The samples were left for 20 min for absorption of bacteria into the meat. The samples were then packed with sterile polyethylene plastic bag. All samples were stored at low temperature (5-7°C) and room temperature (27-30°C). Observation was done at 0, 3, 6, 9, and 12 days at low temperature storage, and at 0, 6, 12, 18, 24 hours at room temperature storage.

#### Bacterial Enumeration

Treated samples were serially 10-fold diluted with 9 ml sterile buffered peptone water

(OXOID). Diluted samples were spread plated on to Eosin Methylene Blue (EMB) media (OXOID), as selective media to enumeration of *E.coli* O157:H7. Media treated were then incubated at 37°C for 24 h before observation.

#### Active Component

Active component of vinegar was done by using Gas Chromatography (GC). Samples of banana peel and coconut water vinegar were extracted with SPME (DVB/CAR/PDMS) at temperature 65°C 30minute, than inject to GC.

#### Experimental Design and Data Analysis

Design of this study was complete randomized design. Experiment was performed in triplicate. The data were analyzed statistically by using Statistical Analysis System (SAS)version 9.1. Significant differences between population of pathogen (TPC) were determined using Duncan’s multiple range test, with 5% and 1% level of significance.

### RESULTS AND DISCUSSION

Table 1 shows the results of the observations that have been carried out for 24 hours. The result showed that all samples of poultry meat treated with acid was lower TPC than control. Application of banana peels vinegar and coconut water vinegar can reduce *E. coli* O157:H7 approximately 1.3 log CFU/mL and 1.4 log CFU/mL, respectively.

Table 1. Effect of Soaking Treatment of Vinegar to Poultry Meat to *E.coli* O157:H7 (log CFU/mL) Population at Room Temperature (27-30°C)

Treatment	Hour				
	0	6	12	18	24
Control (distilled water)	5.3007 <sup>a</sup>	6.5863 <sup>a</sup>	7.5910 <sup>a</sup>	8.6812 <sup>a</sup>	9.8282 <sup>a</sup>
Banana peels vinegar (contain 1% acetic acid)	4.6531 <sup>c</sup>	5.4719 <sup>c</sup>	5.9150 <sup>b</sup>	6.4719 <sup>d</sup>	6.9187 <sup>d</sup>
Coconut water vinegar (contain 1% acetic acid)	4.7015 <sup>b</sup>	5.6428 <sup>b</sup>	5.8975 <sup>b</sup>	6.4912 <sup>d</sup>	7.9242 <sup>b</sup>
Acetic acid (1%)	3.8450 <sup>c</sup>	4.8279 <sup>d</sup>	5.4464 <sup>d</sup>	6.8303 <sup>b</sup>	7.7480 <sup>c</sup>
Lactic acid (2%)	4.5046 <sup>d</sup>	4.7897 <sup>d</sup>	5.5245 c	6.6425 <sup>c</sup>	7.8624 <sup>b</sup>

Mean values in each column with different superscript indicate significant effect between interaction of treatment at 1% Duncan test

The observation at hour 6 to 24 also showed similar trend. Capability of banana peels vinegar and coconut water vinegar to inhibit *E. Coli* O157:H7 at poultry meat showed no significantly different. The previous study (Rhee *et al.*, 2003) indicated that vinegar had the ability and effectiveness to inhibit the growth of *E.coli* O157:H7. The vinegar was applied to fresh fruit and vegetables. The use of organic acids (acetic acid and lactic acid) can reduce the population of *E.coli* O157: 7, but its ability decreased after 18 hours. Soaking treatment using distilled water (control) at 0 hour reduced the population of *E. coli* O157:H7, and increased in line with the duration of storage. Banana peels vinegar and coconut water vinegar more capable to inhibit *E. coli* O157:H7 growth at low temperature. Statistical analysis showed a significant difference (Table 2). Table 2 shows the results of the observations that have been made for 12 days. Population of *E.coli* O157:H7 decreased 1.2 log CFU/ml till 3 days. Reduction decreased after 6 and 9 days of storage. Application of vinegar was

not effective after 9 days of storage. The use of organic acids (acetic acid and lactic acid) can reduce the population of *E.coli* O157:H7 up to 12 days of storage.

Banana peel vinegar and coconut water vinegar were produced from agricultural waste. Both of these vinegar having nearly the same physical characteristic, ie. color and odor. As seen from the chromatogram (Figure 1) that acetic acid was one of organic acid component of banana peel vinegar (retention time 25,8) and coconut water vinegar (26,18).

Organic acid are considered to affect microbial activity by two primary mechanism : by cytoplasmic acidification with uncoupling of energy production and regulation, and by accumulation of the dissociated acid anion to toxic levels (Mani-Lopez *et al.*, 2012). Besides organic acid, vinegar also contains phenolic compounds. Phenolic compounds are known to have membrane-active properties against microorganisms causing leakage of cell constituents (Johnston *et al.*, 2003). Permeability

Table 2. Effect of Soaking Treatment to *E.coli* O157:H7 (log CFU/mL) Population at Low Temperature (0-4°C)

Treatment	Day				
	0	3	6	9	12
Control (distilled water)	5.3007 <sup>a</sup>	5.9682 <sup>a</sup>	6.0086 <sup>a</sup>	6.6301 <sup>a</sup>	6.7304 <sup>a</sup>
Banana peels vinegar (contain 1% acetic acid)	4.6531 <sup>c</sup>	4.7481 <sup>d</sup>	5.7672 <sup>b</sup>	5.9154 <sup>b</sup>	6.4719 <sup>b</sup>
Coconut water vinegar (contain 1% acetic acid)	4.7015 <sup>b</sup>	4.7780 <sup>c</sup>	5.5088 <sup>c</sup>	5.6625 <sup>d</sup>	6.3977 <sup>c</sup>
Acetic acid (1%)	3.8450 <sup>e</sup>	4.7584 <sup>cd</sup>	4.8521 <sup>d</sup>	5.3007 <sup>e</sup>	5.9474 <sup>d</sup>
Lactic acid (2%)	4.5046 <sup>d</sup>	4.9067 <sup>b</sup>	5.3274 <sup>e</sup>	5.7369 <sup>c</sup>	5.9188 <sup>d</sup>

Mean values in each column with different superscript indicate significant effect between interaction of treatment at 1% Duncan test

Table 3. Organic Acid Component Vinegar

Vinegar	Components
Banana peel vinegar	Propanoic acid, Ethyl Acetate, n-Propyl acetate, acetic acid
Coconut water vinegar	Ethanol, acetic acid, benzoic acid

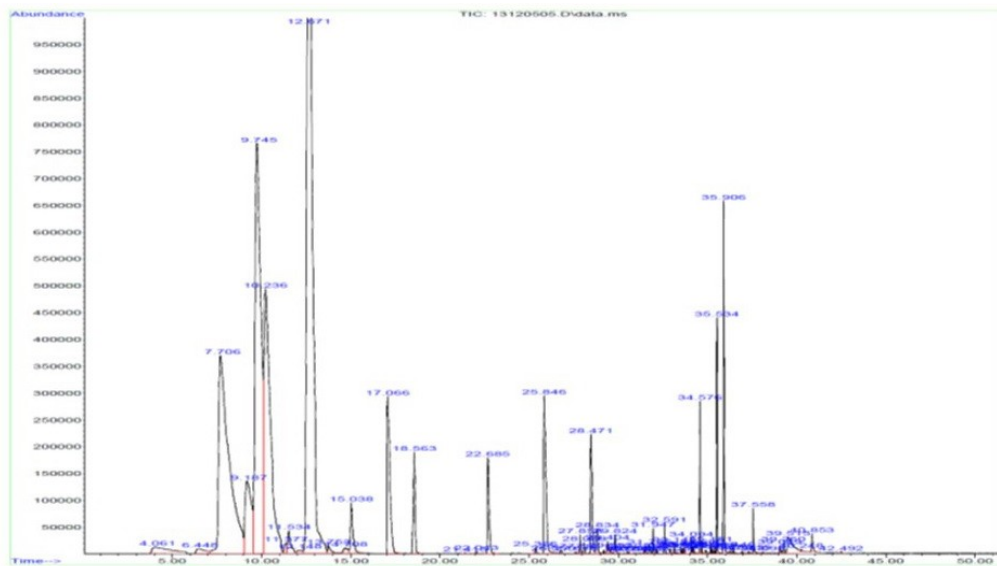


Figure 1. The Chromatogram of Banana Peel Vinegar

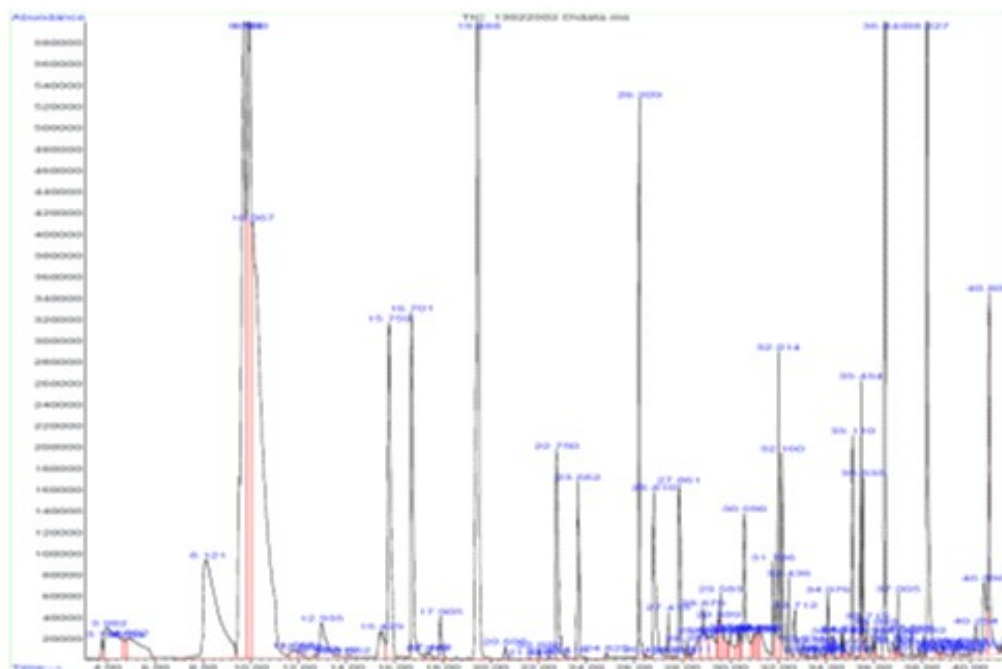


Figure 2. The Chromatogram of Coconut Water Vinegar

of the membrane of phenolic compounds is not as fast as organic acids, but after successfully damaging the membrane, its ability to inhibit longer. This is because the phenolic compounds damage microbial cell by altering the permeability of the cytoplasmic membrane, causing leakage of

intracellular materials. These compounds also denature proteins and inactivate proteins such as enzymes. The cell wall of Gram negative bacteria (*E.coli*) are thinner so the phenolic compound easier to attack the protein found in the cell wall of bacteria *E.coli*. Protein in bacteria is one of the

constituent components of the cell wall and plasma membrane, where the protein on the cell wall of the damaged or denatured bacteria cell wall be easily penetrated by a chemical substance which causes impaired metabolism of bacteria (Mailoa *et al.*, 2014).

Table 3 shows the organic acid component vinegar. Acid is known as one of component phenolic derived from plant source. Phenolic compound found in vinegar include *gallic acid*, *4-Hydroxybenzaldehyde*, *catechin*, *vanillic acid*, *caffeic acid*, *syringic acid*, *vanillin*, *syringaldehyde*, *p-coumaric acid*, *m-coumaric acid*, *anisaldehyde*, *epicatechin*, *sinapic acid*, *Salicylaldehyde*, *scopoletin*, *veratraldehyde* and *o-coumaric acid*. Phenol compounds can also denature enzymes that responsible for spore germination or affect the amino acids involved in the germination process. Flavonoids have a broad spectrum of antimicrobial activity with reduced immunity in the target organism (Naidu, 2000). Phenolic compound also can form hydrogen bonds with the protein contained in bacteria *E. coli*, if the hydrogen bonds formed between phenolic compound with protein will be denatured proteins possibility that bacterial metabolism becomes impaired (Mailoa *et al.*, 2014).

## CONCLUSION

Banana peel vinegar and coconut water vinegar were effective as natural preservative to chicken meat. These vinegar potentially inhibited the growth of *E. coli* O157:H7 on chicken meat until 12 hours at room temperature storage and cold temperatures for 9 days. Vinegar used in this study were better inhibit pathogenic bacteria than acetic acid and lactic acid.

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