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Existence of pathogenic bacteria (*Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, and *Vibrio parahaemolyticus*) in Pado traditional food from West Sumatera

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ABSTRACT

Pado made by the author that when made deliberately inoculated or not inoculated with *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus cereus* do not contain all three of these microbes when it is finished and ready to be consumed. *Pado* purchased on the market (Bukittinggi, Maninjau and Lubuk cone) also does not contain such microbes. All *Pado* (self-made and purchased in the market) does not contain *Vibrio parahaemolyticus*. Microbes are not found either in the fraction of a fish (FF), and the fraction of not fish (FNF) of *Pado*

Key Words: *Pado*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus* dan *Vibrio parahaemolyticus*

INTRODUCTION

Pado is a traditional food made from Picung seed (*Pangium edule* reinw, as showed figure 1), coconut pulp and raw fish. The materials are mixed and stored, covered, for 3-5 days before being consumed or sold. The area where they found *Pado* maker is Tiku, Lubuk Basung, Maninjau, and Balingka Matur, Agam district in West Sumatra province. Based on the experience of the manufacturer and merchants *Pado*, traditional food can be kept relatively long. It also fits with the observation of the writer, *Pado* purchased in a market that has been stored for more than 2 months at room temperature can still be consumed.

There are two versions of how making *Pado*. The first is a dry version, where the Picung seeds that have been dried and then pulverized and mixed with dried coconut pulp as well. This mixture was used to cover a layer of fish are layered in containers such as plastic boxes, basin or bamboo baskets. The composition of the material in containers ranging from basic wadalah is Picung mixture and coconut pulp - fish - Picung mixture and coconut pulp, and so on [1]. *Pado* is referred to as a dry.

The second is a wet version. Dried Picung seeds in the form of rough pieces soaked with water and then mixed with coconut pulp. This mixture was used to cover the layers of the same fish as the manufacture of dry *Pado*. *Pado* is referred to as wet.

At this time, wet *Pado* was mostly made as preferred by consumers. *Pado*'s maker also prefer to make wet. With the same amount of raw materials will be obtained more results for the manufacture of wet *Pado* added some water. In terms of durability store, *Pado* can also be stored as long as dry *Pado* at room temperature. At this time, the term generally refers *Pado* was related which wet *Pado*. This is because, the manufacture of dry is rarely found.



Figure 1. Picung (*Pangium edule reinw*) (a) Picung Plant ; (b) Picung fruit

Pado is used as one of the ingredients in the manufacture of *Pado* 'sambal' flavor. Besides *Pado*, the same material as the manufacture of *sambal* generally, ie chili, onion, garlic, tomatoes, salt, lemon and tomato. All the ingredients are mixed, then saute until cooked. *Pado* additions will give a distinctive smell and taste *Pado* and color condiment somewhat blackish. Sometimes *Pado* added after all the sautéed ingredients. Although made from raw fish, sometimes *Pado* eaten directly, mixture Picung seed and coconut pulp, and raw fish. Who consume *Pado* believes that safe to be consumed without cooking or warming process before hand. So far, there are no reports about the health problems caused by consuming raw *Pado*.



Figure 2. Picung (*Pangium edule reinw*) fruit and dried Picung seeds

Potentially pathogenic microbes as contaminants during manufacture *Pado*. All material is processed without passing through heating process. The water used is raw water. The fish used are raw fish. Microbial contaminants potentially as a pathogen or toxin can contaminate these products, such as *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, and *Vibrio parahaemolyticus*. *Escherichia coli* is commonly found in fish so that the numbers are most likely (APM) for these microbes are limited to 2 by 01-2729-1-2006 SNI [2].

S. aureus including microbes most likely to contaminate raw food in the processing lot of contact with hands and human skin. Skin, mouth and nose man is the network it used to contain these microbes. Up to 30% of the normal population are carriers of *S. aureus* [3]. These microbes are classified as resistant to food situation is slightly acidic (pH 4-4.5), osmotic pressure and high salt levels (Food Standards Australia New Zealand, 2013). Food processing raw when frequent contact with human skin (eg hands were not given gloves) has a great opportunity contaminated with microbes.

B. cereus is a bacteria that usually contaminate food and can develop if the food is not stored in cold temperatures. *B. cereus* is a microbe commonly found in food and the spores are resistant to normal heating temperature for cooking food. The resulting toxin causes symptoms of vomiting and diarrhea (Food Standards Australia New Zealand, 2013). *Vibrio parahaemolyticus* is a bacterium that is commonly found on the sea and become a microbe most frequently reported cause of food poisoning outbreaks [4].

Various research, finding out that Picung seeds can inhibit or slow the growth of microbes. Husni, Samah, and Apriliza, (2007); and Heruwati, Widyasari and Policy (2007) using the seed powder and salt [5; 6]. This mixture can preserve fresh fish between 4 to 6 days. Yusra, Irawati, and Yogi (2008) uses a mixture of shredded fresh Picung fruit and salt to preserve fish bloating [7]. Somewhat different way done by Sukarti (2011) by using a pericarp (fruit) paste of fresh seeds to preserve carp Majalaya [8]. Yusra *et al* did not report that on the 6th day, mackerel by Picung

pericarp (fruit) mixture and salt, the bacteria population is lower than the control [7]. While Sukarti (2011) reported that the shelf life of carp Majalaya by Picung pericarp (fruit) pasta be up to 5 days [8].

This study aims to analyze the presence of some microbes as a potential pathogen in Pado, namely *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, and *Vibrio parahaemolyticus*. Pado which investigated is sold in the market and commonly consumed by people; and Pado homemade inoculated and not inoculated with *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus cereus*.

EXPERIMENTAL SECTION

Pado materials studied are homemade; and Pado bought at the market Bukittinggi, Maninjau and Lubuk Basung, West Sumatera. Pado sold in Bukittinggi, Maninjau, Lubuk Basung and also used in this study. Pado made with the usual way by the makers of Pado in Agam District. Dry Picung seeds was pounded with chilli grinding stone bruise or rupture but not until smooth. 1 kg of dried Picung is immersed in 2 liters of water for ± 5 minutes. Into this marinade added coconut pulp 1 kg.

The mixture was stirred and knead until well blended and colored black. After that, this mixture (here in after referred to as fractions instead of fish, or FBI-Not Fish Fractions) divided into two equal lots and one dab called FBI 1 and FBI 2, which placed in plastic LDPE (low density polyethilen) bag, into ± 1 cm thick layer. FBI layer arranged above a layer of mackerel (4-5 mice) that have been weeded out, then on top of a layer of fish is placed again one layer of the fraction is not-fish (FBI). This carried on until all the fish fraction (FI) and Not-Fish Fraction (FBI), was used up, and the top layer is the Not-Fish Fraction (FBI) as showed figure 3.

After that, the top of the bag tied up with rubber bands. Furthermore, this bag pierced with a pin tightly and evenly across its surface. After that, the radially bag tied with a rubber band attached-connect. Binding start from the top center of the bag that had been tied before, continue down the side toward the base and continue to the other side, then back again to to the bottom and the top center of the bag. This binding resumed again as before on the bag that has not been bound. Distance bond on the side of ± 1 cm. This binding results in blackish brown liquid will come out through a wall of a punctured bag.



Figure 3. Pado Fraction (a) Fish Fraction and (b) Not-Fish Fraction and (c) Fish Fraction and Not-Fish Fraction

This method is usually done by Pado craftsmen. Pado would made if the amount is not much. If Pado made quite a lot, the container used was a large plastic bag that is enclosed in a plastic bag. After the sack is closed by tying its upper end, a sack over his walls pierced with nails, then sack weighted with stones (weighing approximately 10-15 kg). Override this will cause the liquid will drip through a wall of a punctured bag.

While the FBI 2 inoculated beforehand with a suspension of *S. aureus*, *B. cereus* and *E. coli*, each of 100 μ L. Mackerel to be mixed with the FBI-2 also inoculated with the bacteria in the same amount. The next process is done the same as the previous Pado manufacture (using FBI 1). Wet Pado in the bag tight bound is placed on the support that the punch and discharge accommodated. Pado left in this condition for 4 days, then just do the quantitative analysis against *E. coli*, *S. aureus*, *B. cereus* and *V. Parahaemolyticus*.

The suspension is prepared by inoculating 1 needle tip ose of bacteria from agar slant (aged 7-10 days stored at temperatures C°5-7) into a flask containing 25 ml of liquid medium nutrient broth (NB), then incubated at 32 -34 °C to wobble 50 rpm for 24 hours. In addition to self-made Pado, also used the example Pado derived from market Bukittinggi, Maninjau and Lubuk Basung in Agam regency of West Sumatra. Currently in the three areas that are still traded Pado.

Observations made qualitatively against *E. coli*, *S. aureus*, *B. cereus* and *V. parahaemolyticus* conducted by the Regional Technical Implementation Unit Health Laboratory Provincial Health Office of West Sumatra in Padang.

RESULTS AND DISCUSSION

Tests showed that *Pado* created by the author do not contain *S aureus*, *E coli* *B cereus* and *V parahaemolyticus*. *Pado* that when making deliberately inoculated with *S aureus*, *E coli*, *B cereus* also does not contain all three of these bacteria 4 days later (the normal time to finalize *Pado's* ready to eat) in both the fish fraction (FI), and the fraction is not a fish (FBI) as showed in table 1.

Table 1. *S aureus*, *E coli*, *S aureus* and *V parahaemolyticus* in fish fraction (FI) and the fraction is not a fish (FBI) on *Pado* made by the author

<i>Pado</i> Fraction	<i>S. aureus</i>	<i>B. cereus</i>	<i>E. coli</i>	<i>V. parahaemolyticus</i>
<i>Pado</i> 1 ^{*)}				
Fish Fraction	(-)	(-)	(-)	(-)
Not-Fish Fraction	(-)	(-)	(-)	(-)
<i>Pado</i> 2 ^{**)}				
Fish Fraction	(-)	(-)	(-)	(-)
Not-Fish Fraction	(-)	(-)	(-)	(-)

*) Pathogenic bacteria inoculated on the FBI and FI at the time of manufacture

**) Pathogenic bacteria are not inoculated at the FBI and FI at the time of manufacture

Tests on *Pado* purchased in Bukittinggi, Maninjau, Lubuk Basung and also indicates the absence of *S aureus*, *E coli* *B cereus* and *V parahaemolyticus*, both on Not-Fish fractions (FBI) and the fraction of the fish (FI) as showed in table 2.

Table 2. *S aureus*, *E coli*, *S aureus* and *V parahaemolyticus* in fish fraction (FI) and the fraction is not a fish (FBI) on *Pado* purchased in some areas in Agam District

Region	<i>S. aureus</i>	<i>B. cereus</i>	<i>E. coli</i>	<i>V. parahaemolyticus</i>
Bukittinggi				
• Fish Fraction	(-)	(-)	(-)	(-)
• Not-Fish Fraction	(-)	(-)	(-)	(-)
Maninjau				
• Fish Fraction	(-)	(-)	(-)	(-)
• Not Fish Fraction	(-)	(-)	(-)	(-)
Lubuk Basung				
• Fish Fraction	(-)	(-)	(-)	(-)
• Not-Fish Fraction	(-)	(-)	(-)	(-)

Thus, taking *Pado* in a raw state or who are not getting enough heat in its processing will not endanger the health of the danger comes from *S aureus*, *E coli* *B cereus* and *V parahaemolyticus* mentioned above.

CONCLUSION

Pado made his own, and *Pado* purchased in some areas (Bukittinggi, Maninjau and Lubuk Basung) not containing *S aureus*, *B cereus* *E coli* and *V parahaemolyticus* good at fractions of fish (FI) and the fraction is not a fish (FBI) of *Pado*. Negative results were also founded at *Pado*, when making inoculated with *S aureus*, *B cereus* and *E. coli*. The negative result is believed to be related to their anti-microbial activity on *pado* which derived from its constituent materials.

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