

Microbial quality of yellow seasoned “pindang” fish treated with turmeric and tamarind

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Abstract. The objective of this study was to determine the microbial quality of yellow seasoned pindang fish. The fish was treated using combination of turmeric and tamarind at different ratio. This research used Randomized Block Design with 2 (two) factors ie concentration of turmeric (0%, 2%, and 6%) and concentration of tamarind (0%, 3%, and 6%). Each treatment was replicated 3 times to obtain 27 experimental units. The parameters observed were total microbe, total fungi and some pathogenic bacteria. Some microbial data were analyzed using descriptive method, however, the number of *S. aureus* was analyzed at 5% significance level by using software co-Stat and if there was a real difference then tested further by test Honestly Significant Difference (HSD). The results showed that increasing the use of curcumin and tamarind tended to decrease the total number of microbial from treatment control 5.1×10^5 CFU/gram to $<1.0 \times 10^3$ CFU/gram. All the treatment produced yellow seasoned pindang fish with fungi $<1.0 \times 10^2$ CFU/gram. The products contain pathogenic bacteria *E. coli* < 3 MPN/ gram; *S. aureus* $<1.0 \times 10^3$ CFU/gram; *Salmonella* and *V. cholerae* were negative in 25 gram of sample. Based on microbial quality, it is recommended that the use of 3-6% of turmeric and 2-4% of tamarind are the best spices combination to produce safe consumption of yellow seasoned pindang fish.

Keywords: *curcumin, microbial, quality, seasoned, yellow*

1. Introduction

Fishing techniques is one of the fish processing efforts to extend the shelf life and increase the economic value of fish. Fish processing by boiling “pindang” ranks second after the salted fish. This process can be done by heating the fish in a salty condition for a certain time. The use of salt is generally low in concentrations less than 5% all. This causes the pindang fish in terms of the preferred flavor of the salted fish but has a short shelf life. According to [1] pindang fish can only survive for 3-4 days after the cover of the container is opened, even pindang fish processed in one of the centers of yellow seasoned pindang fish in Rumbuk village, East Lombok only able to survive for 1 day. This product is processed by utilizing turmeric, tamarind and salt. So far, the use of such spices has not been standardized, so that the quality of the products varies greatly between producers and the shelf life is short due to very limited sanitation. Pindang fish generally have a relatively high water activity, so it is very suitable for the growth of microorganisms. [2] showed that the water content of the yellow seasoned pindang fish ranged from 60.92 to 68-65%.



Traditionally processed yellow seasoned pindang fish are very susceptible to damage marked by mucus and growth of fungi. Fungi and bacteria are very common in pindang especially from Micrococcus group [3] According to the National Advisory Committee on Microbiological Criteria for Foods [4] and [5] there are several types of pathogenic microbial grown in fishery products such as *Bacillus cereus*, *Salmonella*, *Staphylococcus aureus*, *Vibrio parahaemolyticus*. In addition, *Escherichia coli* is often found as an indicator of poor sanitation. According to [14] to control or suppress the growth of microorganisms there are some hurdle techniques that can be done that are controlling intrinsic and extrinsic factor. Control of extrinsic factors, among others, by providing antimicrobial compounds both natural and synthetic.

Natural antimicrobials such as spices are very commonly used in traditional Asian cooking processing as in processing with the technique of boiling ('pemindangan' –in Bahasa). With a good technique of preservation by regulating the concentration of natural preservatives such as turmeric and tamarind is expected to suppress the growth of some pathogenic bacteria in yellow seasoned "pindang" fish. Turmeric contains various compounds such as curcumin and essential oil [6]. Turmeric is known as a natural dye also and has antibacterial effect. Some studies have shown that turmeric antibacterial effect can inhibit *Clostridium botulinum* (MIC 500 ug / mL) and bactericidal effect against Gram positive bacteria such as *Bacillus subtilis* and *Lactobacillus acidophilus* [7]. In addition to turmeric, tamarind is also known to have an inhibitory effect on a number of bacteria and fungi. [8] showed that the use of 1.5% salt and 3.5% tamarind is the best treatment to produce the best quality and the longest shelf life of dried lemuru fish. The purpose of this study was to determine the microbial quality (total microbial) and some potential pathogenic bacteria in yellow seasoned "pindang" fish processed by using combination of turmeric and tamarind.

2. Materials and Methods

2.1. Materials

The materials used in this research were fresh Cakalang fish and spices such as turmeric, tamarind and salt obtained from Kebon Roek market, Mataram, West Nusa Tenggara (NTB). Materials used for analysis consisted of butterfield's phosphate buffered, Plate Count Agar (PCA) (Merck., Germany), Potato Dextrose Agar (PDA) (Merck., Germany), Lauryl Tryptose Broth (LTB) (Oxoid Ltd., UK), EC Broth (Oxoid Ltd., UK), Eosin Methylen Blue Agar (EMBA) (Oxoid Ltd., UK), Lactose broth (Merck., Germany), Rappaport-Vassilidis (RV) (Oxoid Ltd., UK), Tetreathionate Broth (TTB), Hectoen Enteric (HE) (Oxoid Ltd., UK), Xylose Lysine Desoxycholate (XLD) Merck., Germany), Tripel Sugar Iron (TSI) (Oxoid Ltd., UK), Lysine Iron Agar (LIA) (Merck., Germany), Alkaline Peptone Water (APW), Thiosulfate-Citrate-Bile-Salt-Sucrose Agar (TCBS) (Oxoid Ltd., UK), Trypticase Soy Agar (TSA), NaCl, Baird Parker Agar (BPA) (Oxoid Ltd., UK), and egg yolk telurit (Oxoid Ltd., UK).

2.2. Methods

2.2.1. Processing of yellow seasoned pindang fish. The yellow seasoned pindang fish was made by modifying the processing of the yellow seasoned "pindang fish of Rumbuk's producer at east Lombok by following the process stages: sortation to obtain the fish with fresh condition, weeding to clean the fish from the stomach and gill contents, filleting to obtain slices meat of fish with size \pm 4-5 cm (width) x 7-8 cm (long) and 2-3 cm (height) with average fish weight \pm 70 gram. Next, fish fillet was washing and sieving, weighing and mixing with turmeric spices (0%, 3% and 6%) and tamarind (0%, 2% and 4%). Fish fillet used \pm 750 gram per unit of experiment with combination of treatment in processing as follows: % turmeric: % tamarind (p1 = 0: 0; p2 = 0: 2; p3 = 0: 4; p4= 3: 0; p5 = 3: 2; p6 = 3: 6; p7 = 6: 0; p8 = 6: 2; and p9 = 6: 4). Before the spice used added 2% salt and water as much as 275 mL. Further, cooking for 10 minutes at a temperature of 100°C was done.

2.2.2. Microbial Assay. Determination of total microbial count in yellow pindang was conducted by referring to [9] procedure with modification on the number of samples used. Fungi determination followed modified procedure by [10]. Determination of the amount of *E. coli* in yellow pindang was done with reference to Most Probable Number techniques 3 series tube procedure of [11] with modification on the number of samples used. The positive results were shown from the formation of colonies with black dots, with or without metallic greens (pure culture control of *E. coli* forms metallic green color). Determination of Salmonella in yellow pindang was done by referring to Indonesia National Standard [12] procedure with modification on the number of samples used. Determination of *V. cholerae* was done by referring to the procedure of [13] with modification to the number of samples used. Observed growth by seeing changes in the media. For further confirmation observations were made with gram staining. Determination of the amount of *S. aureus* in yellow pindang fish was done by referring to procedure [14] with modification on the number of samples used.

2.3. Statistical Analysis

The experimental design used in this study was Randomized Block Group with two factors: turmeric concentration (0%, 3% and 6%) and Tamarind (0%, 2% and 4%). Each treatment performed three replications so that 27 units of experiments were obtained. The data of observation parameter of *S. aureus* were analyzed by analysis of variance at 5% real level using Co-stat software and the real difference was tested using 5% of Honestly Different Test [15]. While the total microbial parameters, total fungi, and some pathogenic bacteria (*Escherichia coli*, *Salmonella*, *Vibrio cholerae*, *Staphylococcus aureus*) were analyzed using descriptive method.

3. Results and Discussions

Fish is known to be an excellent growing medium for many types of microorganisms [16] because it contain important nutrients such as water (60-84%), protein (18-30%), fat (0.1-0.2%) and carbohydrates (0-0.1%). Analysis of microbial quality in yellow fish was done to determine total microbial growth and growth of some common pathogenic microbes in fishery products such as *Escherichia coli*, *Salmonella*, *Staphylococcus aureus* and *Vibrio cholerae*. According to [13] that *Staphylococcus aureus*, *Salmonella thypimurium* and *Vibrio* belong to a class of pathogens found in fishery products that are important due to cause disease or poisoning for consumers.

3.1. Total Microbes

Based on [17], pindang fish products had maximum limit of total microbial content of 1.0×10^5 CFU / gram. Table 1 below shows total microbials in yellow seasoned pindang fish with turmeric and turmeric treatment.

Table 1. Total microbe of yellow pindang seasoning fish with turmeric and tamarind treatment.

Treatment	Total Microbe (CFU/g)		
	Tamarind (0%)	Tamarind (2%)	Tamarind (4%)
Turmeric (0%)	5.1×10^5	4.5×10^4	3.4×10^4
Turmeric (3%)	2.6×10^4	$<1.0 \times 10^3$	$<1.0 \times 10^3$
Turmeric (6%)	8.3×10^3	$<1.0 \times 10^3$	$<1.0 \times 10^3$

Table 1 shows that the use of turmeric and tamarind can suppress microbial growth in total. The higher the concentration of turmeric and the acid used, the lower the microbial content of the yellow seasoned pindang fish. The highest total microbial found in pindang fish without the use of turmeric and Tamarind that exceeds the standard of SNI that is equal to 5.0×10^5 CFU / gram, so the opportunity to cause a very short shelf life. While the use of turmeric and tamarind reduced the total amount of microbes to $<1.0 \times 10^3$ CFU / gram. The use of turmeric up to 6% without using tamarind

resulted in total microbial of 8.3×10^3 CFU / gram. The table also shows that the use of turmeric and tamarind together can decrease the total number of microbes up to $<1.0 \times 10^3$ CFU / gram. Results of research conducted by [18] in vitro, proved that the active compound in the turmeric rhizome is able to inhibit the growth of fungi, viruses and bacteria both gram positive and gram negative. In addition, according to [16] and [19], that bioactive components of tamarind such as tannins, flavonoids and saponins are known to have antibacterial effects through reactions with cell membranes, enzyme inactivation and inactivation of genetic material functions.

3.2. Total Fungi

Fungi are one of the most common types of microbes contaminating food products including fishery products. Table 2 shows that the mold content in yellow spiny pindang fish with all treatments $<1.0 \times 10^2$ CFU / gram.

Table 2. Total fungi of yellow pindang seasoning fish with turmeric and tamarind treatment.

Treatment	Total Fungi (CFU/g)		
	Tamarind (0%)	Tamarind (2%)	Tamarind (4%)
Turmeric (0%)	$<1.0 \times 10^2$	$<1.0 \times 10^2$	$<1.0 \times 10^2$
Turmeric (3%)	$<1.0 \times 10^2$	$<1.0 \times 10^2$	$<1.0 \times 10^2$
Turmeric (6%)	$<1.0 \times 10^2$	$<1.0 \times 10^2$	$<1.0 \times 10^2$

It is suspected that the absence of fungi, without and addition to the antimicrobial effects of turmeric and tamarind is also due to the cooking process used sufficiently to kill the fungi in general. It is known that the processing of yellow seasonings was done by cooking temperature 100°C for 10 minutes. The absence of fungi in fishery products in accordance with the requirements of fungi free (negative result) as indicated in Indonesia National Standard Quality Requirement for Pindang Indonesia National Standard 2717.1: 2009 [20]

3.3. Total *Escherichia coli*

Escherichia coli is known to have the optimum temperature for growth is 37°C (with a range of $10-40^\circ\text{C}$), optimum pH 7.0-7.5 (minimum 4.0 and maximum 8.5). *E. coli* is relatively heat sensitive and immediately destroyed by pasteurization temperature and by cooking. *E. coli* is known as an indicator bacterium whose existence is closely related to poor hygiene (indicator of sanitation) [22].

Table 3. Total *Escherichia coli* of yellow pindang seasoning fish with turmeric and tamarind treatment.

Treatment	Total <i>E.coli</i> (Colony/g)		
	Tamarind (0%)	Tamarind (2%)	Tamarind (4%)
Turmeric (0%)	< 3	< 3	< 3
Turmeric (3%)	< 3	< 3	< 3
Turmeric (6%)	< 3	< 3	< 3

Table 3 shows that the addition of turmeric and acid treatment produced yellow pindang seasoning fish with very low of total *E. coli*. The amount of *E. coli* obtained is in the safe position according to Indonesia National Standard 2717.1: 2009 [20], based on the Most Probable Number test method about <3 colony / gram. The low amount of *E.coli* in the yellow spicy pindang fish is suspected to be due to the antimicrobial effect of the tamarind by decreasing the internal pH of the cell, thus interfering with *E.coli* growth also due to the phenolic component of the antibacterial turmeric. It is also suspected that the processing and sanitation processes are appropriate. This is in line with the

opinion of [21] that generally the content of pathogenic microbes in fishery products will be low if the process fulfills the requirements of good processing.

3.4. Total number of *Salmonella*

Salmonella is a microorganism that can cause poisoning if ingested in food consumed. [9] and [16] emphasize that *Salmonella* is one type of pathogenic bacteria whose existence is very harmful to consumer health. In addition, Indonesian National Standard requires that bacteria not be found in the processed fishery products. The following table shows *Salmonella* content in yellow seasoned pindang fish that is processed with the use of turmeric and tamarind. Based on Table 4 it can be seen that the yellow pindang fish produced does not contain *Salmonella* in all treatments, meaning that no *Salmonella* contamination in the yellow seasoning pindang fish was produced. The Indonesia National Standard states that *Salmonella* should not be found (negative) for every 25 grams of material weight [20].

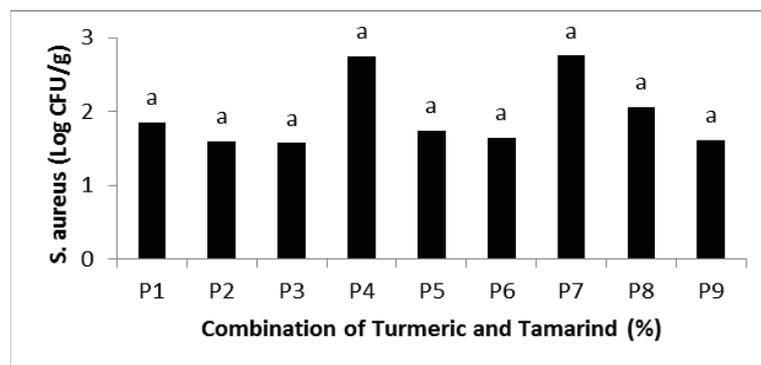
Table 4. Total *Salmonella* of yellow pindang seasoning fish with turmeric and tamarind treatment.

Treatment	Total <i>Salmonella</i>		
	Tamarind (0%)	Tamarind (2%)	Tamarind (4%)
Turmeric (0%)	-	-	-
Turmeric (3%)	-	-	-
Turmeric (6%)	-	-	-

Description: - = no growth found (Indonesia National Standard requirement for *Salmonella* : negative / 25 g)

3.5. Total number of *Staphylococcus aureus*

Staphylococcus aureus is a class of pathogenic microbes that cause disease through intoxication. This bacterium has the optimum temperature characteristics for the growth of 35°C-37°C, with a minimum temperature of 6.7°C and a maximum temperature of 45.5°C. These bacteria can grow at pH 4.0 to 9.8 with an optimum pH of 7.0-7.5 [9]. The following figure shows the amount of *S aureus* in the resulting of yellow pindang seasoning fish.



Description : % Turmeric : % Tamarind (p1= 0:0; p2= 0:2; p3 = 0:4; p4= 3:0; p5=3:2; p6=3:6; p7= 6:0; p8=6:2; and p9=6:4)

Figure 1. The content of *Staphylococcus aureus* on the Yellow Pindang Seasoning fish

Figure 1 shows that turmeric and tamarin combination treatments have no effect on the bacteria content of *S. aureus*, with a range <3 log CFU / gram or <of 1.0×10^3 CFU / gram. The maximum limit

of this pathogen content in fishery products according to Indonesian National Standard is 1.0×10^3 CFU / gram. From Figure 1 it appears that the yellow pindang produced contains *Staphylococcus aureus* below the maximum standard so that it can be said that the resulting product is safe from the pathogen. Although safe, the number of such microorganisms was higher than the content of other microbes tested. This is allegedly due to the growing ability of *S aureus* is much wider range of pH 4.0-9.8.

3.6. Total *Vibrio cholerae*

Vibrio cholerae is one of the pathogenic microbes that can cause cholera disease. The following table shows that no vibrio was found in yellow seasoned pindang fish either without or using a combination of turmeric and tamarind. Indonesian National Standard requires no *Vibrio* to be found in fishery products.

Table 5. Total *Vibrio cholerae* of Yellow Pindang Seasoning fish with turmeric and tamarind treatment.

Treatment	Total Fungi		
	Tamarind (0%)	Tamarind (2%)	Tamarind (4%)
Turmeric (0%)	-	-	-
Turmeric (3%)	-	-	-
Turmeric (6%)	-	-	-

Description: - = no growth found (SNI requirement: negative / 25 g)

3.7. pH value

The storage capacity of food products in general can be determined by the pH of the product. Figure 2 shows the changes in the pH value of the yellow spiny fish during storage. Figure 2 shows that the pH tends to be stable by the addition of turmeric and tamarind treatment with a pH range of 4.9 to 5.6. The addition of turmeric did not affect the pH value but the increased acid concentration decreased the pH of the yellow spice pindang. This is in line with [22] which says that the addition of acid with a certain portion can increase the acidity causing the decrease of pH of the material. Decreased pH is one of the factors that can inhibit microbial growth even though microbes have been clearly sensitive to low pH. According to [23] tamarind contains a number of organic acids such as malic acid, tartaric acid, citric acid, formic acid, succinic acid, and acetic acid.

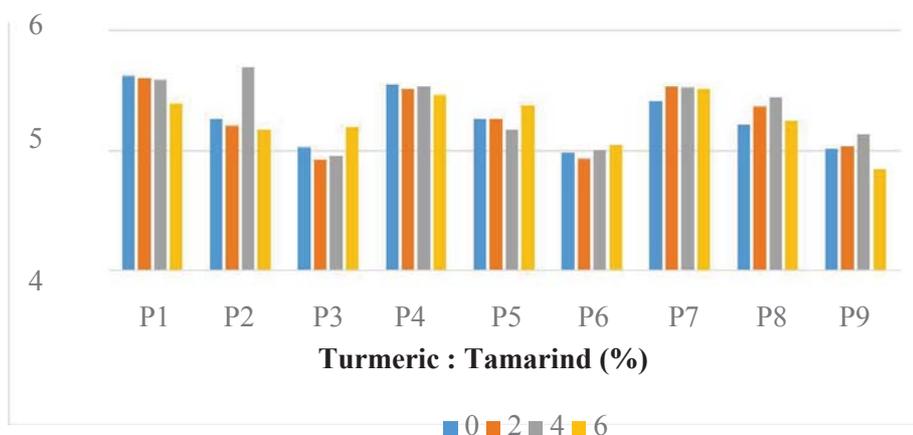


Figure 2. Changes in pH value during storage of yellow pindang seasoning fish.

Description : % Turmeric: % Tamarind (p1= 0:0; p2= 0:2; p3 = 0:4; p4= 3:0; p5=3:2; p6=3:6; p7= 6:0; p8=6:2; and p9=6:4)

4. Conclusions

The use of a combination of turmeric and tamarind can suppress the total growth of microbes. The higher the concentration of turmeric or tamarind, the lower the total microbes up to the safe limit according to SNI. Processing of yellow seasoned pindang fish without the use of turmeric and acid resulted in total microbes exceeding the Indonesia National Standard by the number of days to 0 by 5.0×10^5 CFU / gram. No fungi and pathogenic bacteria of *Salmonella* and *Vibrio cholerae* found in yellow seasoned pindang fish. In addition, pindang fish containing *E coli* and *S. aureus* are under the requirements of Indonesia National Standard 2717.1: 2009.

Acknowledgment

Thanks to the Higher Education Research and Technology Ministry who has funded this research activity through the MP3EI Skim Corridor Fisheries 2017.

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