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IOP Conf. Series: Journal of Physics: Conf. Series **953** (2018) 012014 doi:10.1088/1742-6596/953/1/012014

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Abstract.

The aims of this studies were to prepare juice and raw condiment to be come semi-preserve pastes, and to do microbial assessments on the both pastes during storing in refrigerator and ambient temperatures. For both pastes in refrigerator, samples were taken at 0, 2, 4, 5, 6, 8, 10, 15, 20, 25, and 30 days, and in ambient temperature samples were taken at 0, 1, 2, 3, 4, and 6 days. Assessment were done for TPC, total coliform and *E. coli*, *Salmonella* sp, *Staphylococcus* sp., *Vibrio* sp., pH and water content.
The results shown that juice paste stored in refrigerator still good until 30 days (TPC $1{,}5x10^4$ CFU/g), and in ambient temperature still good until 6 days ($2x10^4$ CFU/g). Condiment paste stored in refrigerator still good until 30 days ($6.5x10^3$ CFU/g), and in ambient temperature still good until 6 days ($1.17x10^4$ CFU/g). However, recommended that condiment paste stored in ambient temperature only until 4 days ($7.3x10^3$CFU/g), while that juice paste until 5 days ($7.8x10^3$CFU/g). There were no pathogenic bacteria found in all samples.

1. Introduction

Sustainab6 fisheries required people to manage natural resources with the principle of z6 waste, however, by-products were still traditionally considered to be a low value or as a problem. Improved utilization of by-products has several reasons, including environmental, economic and the possibilities to produce more food from limited resources, been much focused in the last couple of decades [1]. There are five big *Katsuobushi* (*ikan kayu*) factories in Bitung and Amurang cities of North Sulawesi Indonesia, produced juice as a by-product. Every one to two days, each factory produced 10,000 to 15,000 liter of juice. This juice have a high potential to be processed for a natural condiment, but until now all nutritious juices were thrown away, and created environment problems. Condiment is frequently used in cooking in order to add flavor.
It is time to create a food security strategy by utilizing juice to obtain nutritious, healthy, and high-value food or condiments and can increase people's income. People's preferences for using flavor enhancers is very high although so far some of the flavorings on the market are still controversial. Nowadays the community's dependence on food flavoring products is very high, for example in China and Indonesia. Thus, it is necessary to produc9 natural flavoring as an alternative by utilizing juice that contain lots of glutamic acids. Glutamate is the purest taste of *umami*, while ingredient preparation and seasoning to enhance umami has been part of our21od culture for millennia. Discovery of glutamate is the key of the fifth taste after the four previous basic tastes i.e. sweet, sour, salty and bitter [2].
Possible sources of microbial contamination may come from poor quality of raw materials, time and temperature abuse during boiling and storing [3], insanitary equipment and utensil, poor hygienic practices of food handler's hand [4] and recontamination [5]. Preparation of food involved a lot of contact surface of utensils such as cutting board, knife, blenders, table of preparation, containers, surfaces of grillers, food handlers hand, skewer, spoon, etc. Various studies have reported that complete

elimination of pathogens from food processing environment and utensils are difficult, as many foodborne pathogens are known to be able to attach on food contact surfaces [6]. Examination of foods for microbial indicator organisms, such as Aerobic Plate Count, *Enterobacteriaceae* count, Vibrio count and *Staphylococcus* count has become normal practice to monitor food safety and quality control of food, besides evaluating the overall food sanitation system applied to the food operation. In addition, *S. aureus* count was used to determine the possibility of contamination come from food handlers. There is limited research has been reported on semi-preserved natural condiment paste.

Paste is a semi dried product, where prepared in mini plants in order to make easier the product to be transported from mini plat to the condiment factory. However, as a semi dried product, paste was very potential to be contaminated since in processing, packaging, transporting, and storing paste were handled manually. Aerobic plate counts on fish and fishery products generally do not relate to food safety hazards, but sometimes can be useful to indicate quality, shelf life and post heat-processing contamination. Coliform and *E. coli* are indicator used for evaluating quality or safety of row or processed food products such as sanitizing systems. Pathogens bacteria release toxins such as *Salmonella typhi, Vibrio parahaemolyticus*, and *Staphylococcus aureus*.

2. Material And Method

2.1. Material and Equipments

The material used in this research, was juices produced by "PT. Cellebes Mina Pratama" *Katsuobushi* factory at Bitung, North Sulawesi, Indonesia. Six microbiological parameters assessment using the Bergey's manual of determinative bacteriology [7] and two chemical parameter [8] were used. Eosin Methylene Blue Agar (Himedia) and Lactose Broth (Himedia) were used for the enumeration of coliforms and fecal coliforms; Bismuth Sulfite Agar (BSA) and Xylose Lysine Deoxycholate (XLD) agar were used for the detection of *Salmonella* spp. whereas Tiosulfate Citrate Bile Salt (TCBS) agar and Alkaline Pepton Water were used for the detection of *V. cholerae*. Mannitol Salt Agar were used for *Stapylococcus sp.* NaCl 0.9%, and buffer solution (pH 4, pH 7, and pH 10) were used for dillution. Equipment used were micro pippet (Dummo), erlemeyer, tube Hach and Durham, timer, thermocouple, incubator (YCO-N01), waterbath (Nesco), Oven, magnetic stirer (Vienna Type 206), laminary flow (Panasonic), microscope (Motic Type DMB01) and autoclave (Midnif), analytical balance, pH meter, vacuum packaging (Powerpack).

2.2. Paste Processing

Juice was taken from *Katsuobushi* factory "PT Celebes Mina Pratama" Bitung, filled into sterile plastic container and then placed in a cool box with ice. The juice was then transported to laboratory by car for 90 minutes. In laboratory, juice was mixed with corn flour and salt, and dried in a mixer evaporator at 60°C, for about one to two hours, to a semi-dried paste (Figure 1). The ratio juice to corn flour was 5:1 (v/w).

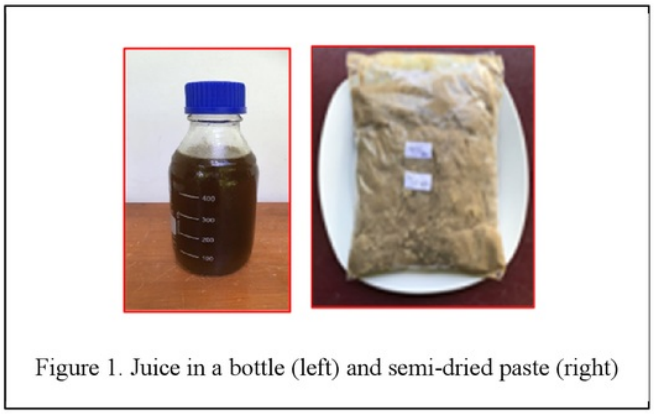

Figure 1. Juice in a bottle (left) and semi-dried paste (right)

Raw condiment was prepared, consist of tomato, onion, and mushroom, with ratio of 4:2:4. Each raw

seasoning was extracted, and then mixed together. The mixed extracts were then dried in mixer evaporator at the same way as juice paste.

2.3 *Treatment and parameter analysis*

The juice and raw condiment pastes, where then stored in refrigerator and ambient temperatures. For both pastes in refrigerator, samples were taken at 0, 2, 4, 5, 6, 8, 10, 15, 20, 25, and 30 days, and in ambient temperature samples were taken at 0, 1, 2, 3, 4, and 6 days. Assessment were done for TPC, total coliform and *E. coli*, *Salmonella* sp, *Staphylococcus* sp., *Vibrio* sp., water content (AOAC, 2005) and pH (AOAC, 2005).

2.3.1 Bacterial Isolation

Each Sample was weighed as much as 25 g, input into 225 ml of sterile 0.9% NaCl solution and shacked strongly, and then taken 1 ml suspension and input into 9 ml of sterile 0.9% NaCl solution, formed 10^{-2} dilution, and then homogenized by shaking the tube, and then taken 1 ml from 10^{-2} dilution input into 9 ml suspension formed a dilution rate of 10^{-3}. The procedure be continued in the same way for further dilution necessary.

Furthermore, from each dilution was taken 1 ml suspension and transferred into the NA medium for TPC test with a pour plate method, and BSA medium for *Salmonella* with scratch method, APW-TCBS medium for *Vibrio parahaemolyticus* test, and lactose broth-EMBA medium for Coliforms and *E. Coli*, and each of medium using the Most Probable Number (MPN) method. All samples were labelled indicated sample type and dilution level. Petri dish of media NA and BSA, Hack tube containing medium APW and lactose broth in a Hach tube with Durham, were kept in an incubator and incubated at 37 °C for 24 and 48 hours for observation.

3. Results and Discussion

In the present study, six microbiological and two chemical parameters i.e. total plate count, total Coliform, *Escherichia coli, Salmonella*, *Vibrio*, *Staphylococcus*, water content, and pH were carried out. This study provided information about microbiological present in juice and condiment pastes, both stored in refrigerator and ambient temperature.

3.1. Total Plate Count (TPC)

Total Plate Count data of juice and condiment pastes can be seen in Figures 2 and 3. Juice paste which were stored at room temperature shown that the lowest TPC was 10.6 x 10^2 (sample at day 0), and the highest was 10.8 x 10^3 (sample at day 6th), while those stored at refrigerator, the lowest was 5.4 x 10^2 (sample at day 0) and the highest was 9.9 x 10^3 (sample at day 25th). In both pastes stored in ambient temperature have TPC value higher than those in refrigerator. Indonesian National Standard (SNI) for fishery product allowed maximum TPC (30ºC, 72 hrs) is 5 x 10^5 CFU/g [9,10]. The results shown that juice paste stored in refrigerator still good until 30 days (TPC 1.5x10^4 CFU/g), and in ambient temperature still good until 6 days (2x10^4 CFU/g). Condiment paste stored in refrigerator still good until 30 days (6.5x10^3 CFU/g), and in ambient temperature still good until 6 days (1.17x10^4 CFU/g). However, recommended that condiment paste stored in ambient temperature only until 4 days (3.4x10^3CFU/g), while that juice paste until 5 days (7.8x10^3CFU/g).

Total Plate Count (TPC), or Aerobic Plate Count (APC), or mesophilic Plate Count (MPC) are indicators of the level of bacteria in a food or on a food contact surface. They are do no determine presence of specific pathogens, but may be an indication of poor sanitation practices in the facility. The total plate count is the enumeration of aerobic, mesophilic organism that grow in aerobic conditions under moderate Temperatures of 20-45ºC. This count includes all pathogens and non-pathogens and is used to determine the hygienic status of food produced. This microbiological growth medium is not a selective medium. Aerobic plate counts on fish and fishery products generally do not relate to food safety hazards, but sometimes can be useful to indicate quality, shelf life and post heat- processing contamination.

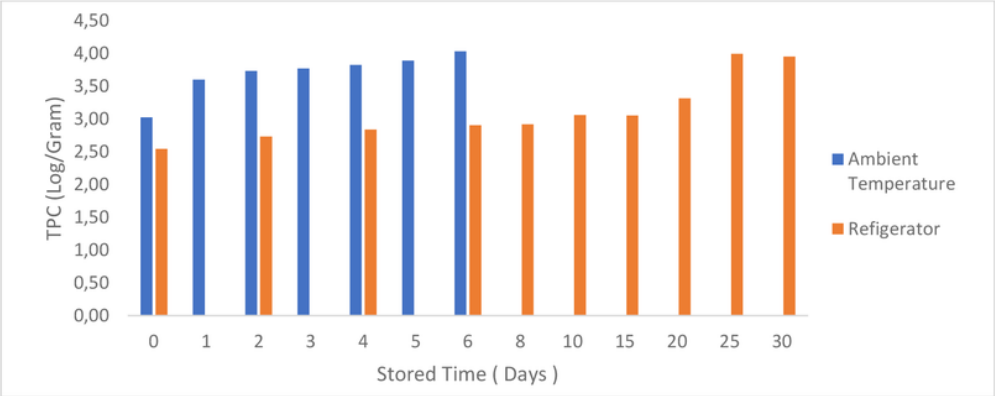

Figure 2. TPC values of Juice paste during stored in refrigerator and ambient temperature

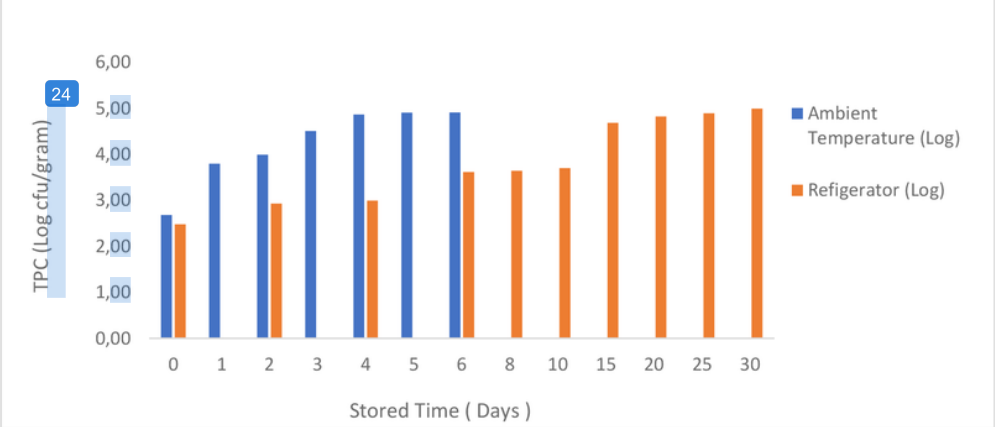

Figure 3. TPC values of condiment paste during stored in refrigerator and ambient temperature

3.2. Pathogens

There were no pathogens found in all samples, and it is means that no contamination hapenned from human, materials, or equipment used during processing. Indicator microorganisms are used for a variety of purposes in food systems including in evaluating quality or safety of raw or processed food products and validating effectiveness of microbial control measures such as sanitizing systems. Ideally, total coliform is a group of bacteria present all around us, most of which are not dangerous to human health. However, these bacteria are not naturally present in groundwater and are an indication that more harmful organisms might be present. Fecal Coliform and *E. coli* are subgroups within the total coliform group which primarily come from the feces of warm blooded animals. Presence of *E. coli* indicates that the water has been exposed to feces and an immediate risk to human health exists. The presence of *E. coli* in ready-to-eat foods is undesirable because it indicates poor hygienic conditions which have lead to contamination or inadequate heat treatment. Ideally *E. coli* should not be detected and as such a level of <3 per gram (the limit of the Most Probable Number test) has been given as the satisfactory criteria for this organism. Levels exceeding 100 per gram are unacceptable and indicate a level of contamination which may have introduced pathogens or that pathogens, if present in the food prior to processing, may have survived. The lower number of coliforms can be beneficial for pointing out the effectiveness of safety procedures during processing and handling [11]

Pathogens bacteria, such as *Vibrio parahaemolyticus* is relevant to seafoods only. High levels of *V. parahaemolyticus* gram) in cooked seafoods indicates that the food has been inadequately cooked or cross-contaminated after cooking with subsequent time/temperature abuse and should result in an investigation of the food handling controls used by the food expected because of natural contamination from the aquatic environment, however, levels from gram in raw seafoods would indicate inadequate

temperature controls since harvesting and should be considered as unsatisfactory [6]. The potentially hazardous level of *V. parahaemolyticus* relates to Kanagawa-positive strains. Levels of *V. parahaemolyticus* of ≥ in food borne illness (rel s to Kanagawa-positive strains). Salmonella is everywhere and is known as a zoonotic agent. The optimum growth temperature is 37.5 ºC and the pH value is 6-8, but at a temperature of 56 ºC in dry state it will die. There are many types of Salmonella causes of food borne disease, including *S. typhimurium*. More than 50,000 cases / year of food poisoning in the USA is caused by Salmonella. The amount of *Salmonella* that can cause Salmonellosis, i.e. between 107-109 c s / g.
According to [12], it is important to note that the foods contaminated by *Salmonella* do not usually show any modification in their sensory characteristics, ev though the pathogens within have reached very high levels, concretely harmful to human health. The occurrence of *E. coli* in the samples indicated recent fecal pollution of human origin [13].

3.3. pH
Data pH of juice pastes and condiment paste can be seen in Figures 4 and 5,

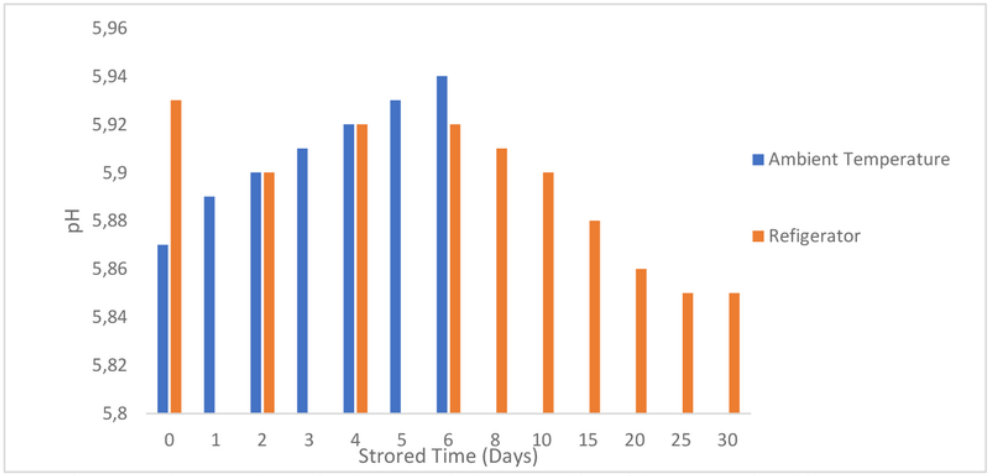

Figure 4. The pH values of Juice paste during stored in refrigerator and ambient temperatures

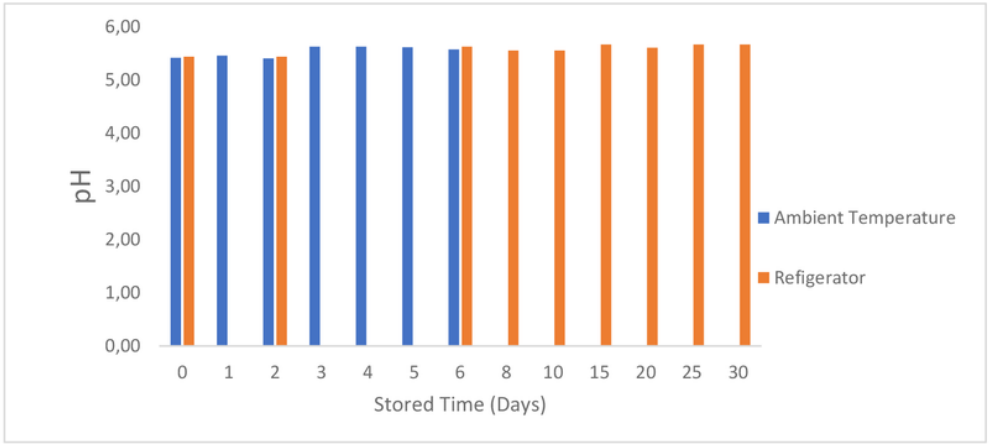

Figure 5. The pH values of Condiment paste during stored in refrigerator and ambient temperatures

Figure 4. showed that during stored in ambient temperature, pH of juice paste slightly increased, while those stored in refrigeration, the pH of paste slightly decrease. The pH juice paste stored at ambient temperature range between 5.90-5.93 and in refrigerator range between 5.85-5.97. Figure 5, showed that during stored at refrigerator and ambient temperatures, pH only increased slowly. The pH of condiment paste stored in ambient temperature range between 5.40–5.62 and in refrigerator range between 5.3-5.7. *Staphylococcus aureus* has minimum, optimum, and maximum pH for growth i.e. at 4.2, 17-7.5 and 9.3 respectively. *Vibrio parahaemolyticus* has optimum pH for growth ranged between 7.8-8.6, although it can grow in the pH range of 4.8-11.0 [14]. Vibrio parahaemolyticus and *E. coli* can grow in the pH 6.0-8.1 [15]. The decreased in pH value on the first few weeks of fermentation was also observed in the study of [16]. and [17]. That is mean, during stored in ambient temperature and in refrigerator, no fermentation process happened.

3.4. Water Content

Data of water content can be seen in Figures 6 and 7.

Figure 6. Water content of juice paste during stored in refrigerator and ambient temperatures

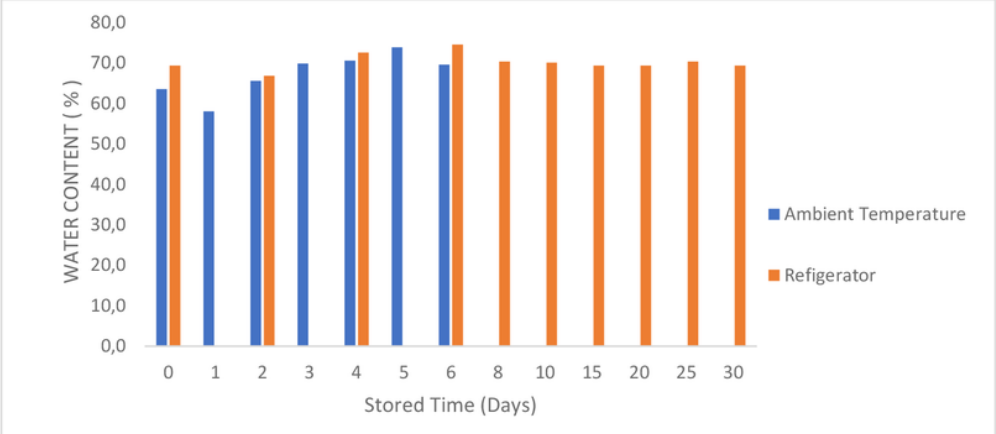

Figure 7. Water content of condiment paste during stored in refrigerator and ambient temperatures

From Figure 6 and 7, can be seen that water content of juice pastes during stored at ambient temperature ranged between 76%-78,8%, water content of juice pastes stored at refrigerator ranged between 59.3%-73.8 %. From TPC data, it can be seen that during stored in ambient temperature and in refrigerator, no spoilage happened yet, because even though the water content quite high but probably the water activity (Aw) of the pastes were low and prevented bacteria to be growth optimally.

4. Conclusion

The results shown that juice paste stored in refrigerator still good until 30 days (TPC $1.5x10^4$ CFU/g), and in ambient temperature still good until 6 days ($2.0x10^4$ CFU/g). Condiment paste stored in refrigerator still good until 30 days ($6.5x10^3$ CFU/g), and in ambient temperature still good until 6 days ($1.17x10^4$ CFU/g). There were no pathogenic bacteria found in samples, both stored in ambient temperature for 6 days and in refrigerator for 30 days. Good hygiene practice should be inforced in each step of producing semi preserved pastes natural condiment, in order to eliminate the post processing contamination.

5. Recommendation

For the safety reason, can be recommended that condiment paste can be stored in ambient temperature only for maximum 4 days and juice paste can be stored in ambient temperature only for maximum 5 days. During transportation from mini plant to condiment factory, the pastes should be put in cool box with ice.

Acknowledgement

This research was funded by The Indonesia Endowment Fund for Education (Lembaga Pengelola Dana Pendidikan/LPDP).

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