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by Anak Agung Nanak Antarini, Ni Putu Agustini Gusti Putu Sudita Puryana,ni Komang Wiardani

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Identification of Microbes, Chemical, and Organoleptic Characteristics towards *Teh Wong* during Fermentation

Anak Agung Nanak Antarini[1], Ni Putu Agustini[1], I Gusti Putu Sudita Puryana[1], Ni Komang Wiardani[1], Anwar Mallongi[2]

[1]*Lecturer in Department of Nutrition, Health Polytechnic, Ministry of Health Denpasar Bali,*
[2]*Department of environmental health, faculty of Public Health, Hasanuddin University*

ABSTRACT

Teh wong is fermented of sweetened tea water using a starter of *tuak wong*. If *tuak* was left for six months will form a clot that can be used as a starter in making *teh wong*. The present study was intended to determine the characteristics of microbes, chemicals, and organoleptic towards *teh wong*. Microbial characteristics were determined by TPC and Total BAL, with gram staining and catalase test. For chemical characteristics was at knowing pH, total acid, alcohol, and sugar content. Organoleptic qualities of *teh wong* with hedonic test and quality in taste and its flavor. This research was conducted with five treatment that was *teh wong* was saved for 0 days, 3 days, 6 days, 9 days, and 12 days. The study result was indicated that there was a significant difference in total bacteria and LAB. The study result was obtained on pH, acid total, alcohol, and sugar content showed most significantly different ($p<0,01$), and the organoleptic test was significantly different ($p<0,05$) and was favored by the panelists on the 9 days retention of *teh wong* with slightly sweet sour taste.

Keywords: *teh wong, fermentation duration, microbiology, chemistry, organoleptic.*

INTRODUCTION

Fermentation is defined as a gradual change by enzymes from some bacteria, yeasts, and fungi in the growth medium. The chemical changes fermentation included *e.g.,* milk acidification, starch decomposition, and sugar to alcohols and carbon dioxide, as well as the oxidation of organic nitrogen compounds[1]

Lactic Acid Bacteria (LAB) is a group of gram-positive bacteria, no spore, round or stem and can convert carbohydrates into lactic acid[2]. LAB has an essential role, almost all food, and beverage fermentation processes.

Balinese traditional drink is hereditarily made and consumed by the Balinese people *e.g., tuak, arak, brem*, and *teh wong*. *The wong* is one of Balinese traditional drink commonly consumed by the people in Guwang, Sukawati Gianyar. *Teh wong* (fungi tea) is made from sugar water (sweetened tea water) fermented by a starter from *tuak* fungi. Fungi *tuak* is living in water containing sugar to maintain its life and is usually kept in sweet tea water. This fermented water is consumed by using sweet tea water as the basic ingredient of fermentation. Then, the resulting water is called *teh wong* (fungi tea). The fermentation will convert sugar into alcohol. Thus, the bacteria will turn alcohol into acid. Then, the sweet tea water turns into a slightly sweet acid. *Teh wong* is usually consumed by the people in Guwang Sukawati as a drink.

MATERIALS AND METHOD

This research is an experimental research with an experimental design using *Randomized Block Design (RBD)*, five treatments and four replications, then organoleptic test. Acidity level (pH) with pH meter[3]. Total sugar with refractometer; alcohol level with alcoholmeter[4]; total acid content with titration; for LAB confirmation *i.e.,* isolates, catalase, and gram staining are performed. Microbe total calculates with Total Plate Count (TPC), Processing and analysis of the data with *Analisis Of Varians (Anova).* If it is obtained different results will be followed by the *Least Square Difference (LSD)* test.

This research consists of five treatments, they are P1: fermentation period of *teh wong* for zero days,

P2: fermentation period of *teh wong* for three days, P3: fermentation period of *teh wong* for six days, P4: fermentation period of *teh wong* for nine days, P5: fermentation period of *teh wong* for 12 days.

Teh wong beverage procedure is: Three grams tea brewed in hot water about 1000 ml for three minutes then added 20% b/v stirred evenly until the sugar melted entirely; After the cold tea water with 30°C temperature (room temperature) then enter *nata wong* (fungi) as a starter; Then it is fermented according to treatment for 0 days, 3 days, 6 days, 9 days, 12 days; Then *teh wong* is conducted a microbial identification, chemical, and organoleptic test.

The organoleptic test is conducted by a panelist rather trained 30 people from the society in Guwang Sukawati who are familiar and used to consume *teh wong* with five hedonic scales.

RESULTS

Total microbial is expressed in *Total Plate Count (TPC)* on *teh wong* products. In the study, it is shown that total microbial in *teh wong* with fermentation period for zero to 12 days was 1.53 x 10^4 to 1.95 x 103 cfu/ml.

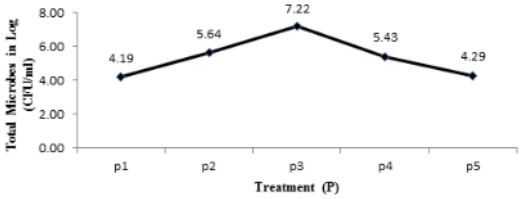

Figure 1.Total microbial of *teh wong* based on fermentation period

Figure 1. shows that the total bacteria increased from zero days (1.53 x 10^4 cfu/ml) to the fermentation period of *teh wong* in the 6th day is (1.64 x 10^7 cfu/ml). Then, a slight decrease at the end of the observation is fermentation for 9 days (2.66 x 10^5 cfu/ml) and 12 day (1.99 x 10^4 cfu/ml).

Total LAB on *teh wong* product showed that total LAB fermentation period for zero to 12 days is 5.92 x 10^3 to 2.86 x 10^6 cfu/ml.

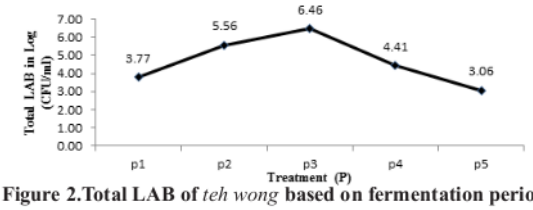

Figure 2.Total LAB of *teh wong* based on fermentation period

Figure 2. shows that the total bacteria increased from zero days (5.92 x 10^3 cfu/ml) to the fermentation period at the 6th day is (2.86 x 10^6 cfu/ml) then a slight decrease at the end of observation that is fermentation period at the 12th day (1.15 x 10^3 cfu/ml).

Acidity level (pH) towards *teh wong* based on fermentation period decreasing from pH 3.62 to pH 2.14 after 12 days fermentation.

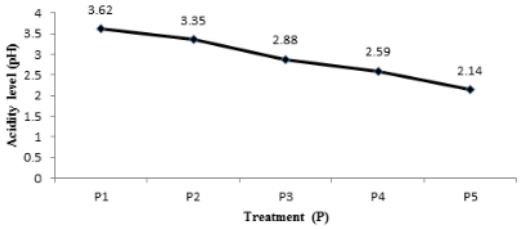

Figure 3. pH teh wong based on fermentation period

Total acid level for *teh wong* based on fermentation period increased from 0.18% to 0.50% after 12 days fermentation.

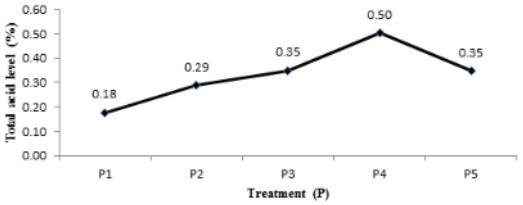

Figure 4. Total acid level of *teh wong*

Alcohol level towards *teh wong* based on fermentation period increase from 0% to 0.17 % after 9 days fermentation then decrease alcohol level to 0.13% after 12 days fermentation.

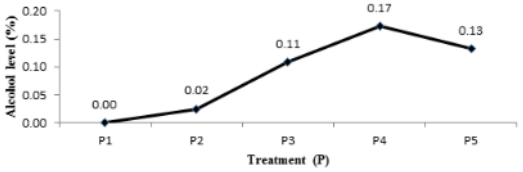

Figure 5. Alcohol level towards teh wong

Total sugar towards *teh wong* based on fermentation period decreased from 19.1%% to 1.35% after 12 days fermentation.

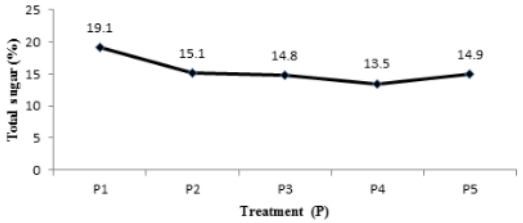

Figure 6.Total sugar towards *teh wong*

The organoleptic test for *teh wong* included flavor, flavor, and color as well as overall acceptance with a hedonic test with five hedonic scales and for hedonic quality on taste quality and flavor quality with three hedonic quality scale. Based on the analysis result regarding on organoleptic test variety for flavor, taste and color and acceptance in whole at *teh wong* that there is significan (P <0,01).

The research results of organoleptic test for *teh wong* with fermentation period 9 days (P4) is most favored by panelists, especially the overall acceptance of flavor and flavor, as well as supported by chemical analysis pH 2.59, total acid 0,50%, 0.17% and sugar level of 13.5% supported by total Lab 2.57 x 10^4 cfu/ml. For more details, *teh wong o*rganoleptic test with 5 fermentation treatments can be seen in Figure 7.

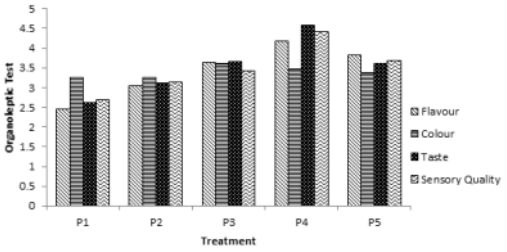

Figure 7. Organoleptic test of *teh wong*

DISCUSSION

Based on the research that is conducted, it is known that the long treatment of fermentation influences total microbial *i.e.,* TPC on zero-day (1.53 x 10^3 CFU/ml) and keeps increasing on the fermentation period to 6 (P3) is 1.64 x 10^7 CFU/ml) for fermentation time to 6 days (2.66 x 10^5 cfu/ml) and 12 days (P5). There is a slight decrease. On zero day fermentation, microbes still experience the phase of adaptation than on the fermentation period to 6 days is increasing due to this phase of microbial splitting rapidly influenced by the medium of growing places unlike nutrient content, pH, and environmental conditions as well as during the fermentation process and still occur has reached a static growth phase followed by a slight decrease in fermentation duration to 9 days and 12 days with phase to the end due to reduced substrate concentration, high population density, and toxic metabolism products *e.g.,* lactic acid, acetic acid, and propionate acids[5,6]. This is due to the influence of product viability, thus, due to the total bacteria amount to decrease on the fermentation period for 9 days.

LAB result is obtained that there is an increase until the fermentation period for 6 days (2.86 x 10^{6c} cfu/ml), then decreased on 9 days (2.57 x 10^4 cfu/ml), this is due to the high production of lactic acid and the decrease of pH environment to 2.59 during fermentation. The lactic acid level is an important factor in LAB viability due to the high production of lactic acid inhibits bacterial growth , stated that *the wong* fermentation for 7 days with a sugar concentration is 15% b/v with a pH value of 2.96, total acid 0.14% and total LAB 5.4 x 104 colony/ml. The main factors have decreased the viability of Lactobacillus strains is decreased pH of the media and accumulation of organic acids as a fermentation result[7-9].

The research result with Cat Gram identification is to produce positive Cat Gram in the form of basil, and this bacterium has negative catalase properties. However, there is some positive catalase. The negative LAB catalase test is taken from a clean white bulbous colon rather prominently at 10^{-1}, 10^{-2}, 10^{-3} dilution. In a negative catalase test on MRSA media with no air bubbles. If it is suspected to be H_2O_2 suspected lactic acid bacteria, this was supported by[10] opinion that stated a lactic acid bacteria is a negative bacterium producing catalase enzymes due to lactic acid bacteria is an anaerobic facultative bacterium that produces peroxidase enzymes that will break down H_2O_2 into organic compounds and H_2O, and not to produce air bubbles.

Acidity level (pH) is one of the intrinsic factors of food that can affect microbial growth. Based on research conducted, it is known that pH in each treatment more day is decreased. The lowest pH of *teh wong* is found in fermentation for 12 days and the highest in fermentation for 0 days (3.62). Microbes in one medium initially undergo a phase of adaptation with the substrate and environmental conditions. The change in pH is strongly influenced by microbial activity during the fermentation

process. The lowest pH on fermentation for 12 days and the highest on fermentation for 0 days this is due to the total microbial increased. Therefore, the substrate decomposition of the microbial sugar increased, especially into alcohol and CO_2, and metabolized further into organic acids, especially lactic acid. The total acid value is inversely proportional to pH value in the fermentation, the higher total acid value is the lower fermentation pH as well as vice versa. In the present study that the fermentation period of *teh wong* has an effect on total acid. Due to in the fermentation process will produce organic acids that cause the taste to be acidic. The fermentation period has an effect on the increase of the total acid level of *teh wong*. Due to at the time of fermentation will be produced organic acids [11].The formation of organic acids, unlike lactic acid in fermentation products based on the glucose breakdown into lactic acid. Lactic acid produced by LAB will be excreted out of cells and will accumulate in the fermentation fluid, thereby, increasing the total acid level[12,13].

This is due to the activity of yeast in *teh wong* maximum P4 at optimum condition pH 3. In addition, the higher alcohol levels of beverages are also due to the starter used has the highest production capacity of alcohol at a certain optimum sugar concentration[14].

The addition of sugar level used in making *teh wong* concentration is 20% b/v with fermentation period has a significantly different effect on sugar level in *teh wong* drinking. The longer total sugar fermentation of *teh wong* has decreased. This is due to the microbes in the fermentation process will utilize sucrose through enzymatic and convert it to ethanol and acid[15].

A Taste regarding a range of dislikes to likes and *teh wong* who get the highest value is with 9 days fermentation for along as it is in accordance with the fermentation conducted generally in the society. During the fermentation period will change the nutrients components in *teh wong.* Therefore, the taste effect of *teh wong* flavors of sour *teh wong* is preferred and the sour taste is due to the bacteria that convert alcohol to acid. Lactic acid bacteria produce a number of lactic acids as the end result of carbohydrate metabolism, thereby, decreasing pH value of the growth sphere and causing a sour taste[5].

The acidic flavor is due to the fermentation of sugar into alcohol, which then the bacteria will turn alcohol into acid. The flavor of *teh wong* on zero-day fermentation is not acidic flavor. This is due to zero-day has not happened changes of nutrient components in tea solution that have not degraded by a microbe, therefore, the flavor is not acid (normal flavor of tea). Whereas the sixth fermentation has started with a rather acidic scent, this is due to the fermentation process resulted in the formation of alcohol and decreased pH and the other formation metabolites that will directly act as a precursor flavor in *teh wong*.

Teh wong color shows a brownish yellow. This is due to the tea used as a fermentation medium. The color of the brownish tea is due to the drying process in the manufacture of black tea leaves due to the enzyme phenolase. A tea color that plays a role in the formation of color and flavor typical of black tea is the change of caffeine compound into *the aflavin* and *thearubigin*[16]

Thus, the most preferred *teh wong* with for 9 days fermentation with an organoleptic test on flavor and flavor and supported by appropriate microbial and chemical characteristics and in accordance with other studies. In *teh wong* research for 9 days fermentation treatment, total microbial is 2,66 x 10^5 cfu/ml and total LAB is 2,57 x 10^4 cfu/ml with chemical analysis that is pH 2,59, total acid 0,50%. This is supported by the study[17,18] stated that *teh wong* fermented for 8 days with total microbial is 4.50 x 10^4 cfu/ml and total LAB 9.90 x 10^5 cfu/ml, for chemical characteristics pH 2.57 and total acid 0.13%.

CONCLUSIONS

Based on the results of data analysis and discussion, it can be concluded the research results as follows: 1) Microbiological characteristics of total microbes towards *teh wong* based on increasing TPC of zero fermentation day is 1.53 x 10^3 cfu/ml to fermentation for 6 days is 1.64 x 10^7 cfu/ml. Then, there is a slight decrease in P4 and P5 is 2.66 x 10^5 cfu/ml and 1.95 x10^4 cfu/ml. For LAB identification with positive Gram painting with negative catalase test. 2) Chemical characteristic is included pH, total acid, and alcohol level and in *teh wong* based on fermentation period is an increase from zero-day fermentation (P1) to 9 days (P4). Then, the decrease is its fermentation for 12 days (P5). For pH decrease until 12 days fermentation. 3) Organoleptic characteristics of *teh wong* based on different fermentation period on

organoleptic test on flavor, flavor, color, and Overall Acceptance (OA) is significantly different while for the quality of flavor and taste of *teh wong* is very real. 4) The most *teh wong* preferred characteristic is 9 days fermentation.

Conflict of Interest: All authors declare that there is no any conflict of interest within this research and publication including the final agreement.

Etichal Clearence: Ethical Clearance obtained from the university committee and respondent assignment.

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