World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 5.088

THE PRESENCE OF NATURAL MICROFLORA IN TAPAI FLUID OF CASSAVA (*MANIHOT UTILISSIMA* POHL.) FROM TRADITIONAL MARKET IN PADANG

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Article Received on 29/05/2018 **Article Revised on 19/06/2018** **Article Accepted on 10/07/2018**

ABSTRACT

The research related to the presence of natural fermentative microflora in tapai fluid. Tapai fluid is one of by-product that produced during tapai fermentation process. Based on previous research, in tapai have been found yeast, bacteria, mould, and other natural fermentative microflora. It can be assumed if tapai fluid still occur natural fermentative microflora. Survey method was used and the data was alnalyzed descriptively. The aim of the research was to determine the presence of natural fermentative microflora in tapai fluid of cassava (*Manihot utilissima* Pohl.) and to know the characteristic of Tapai fluid based on alcohol level, residual sugar content, pH, and temperature. The results showed tapai fluid contain colony of natural fermentative microflora (yeast and bacteria), that was isolated by *GPA* and *YEA* medium and obtained alcohol level (2.5-11%), a residual sugar content (15.3-22.0% Brix), pH (3.43-3.87), with temperature (15.3-22.0%).

KEYWORDS: Alcohol Fermentation, Natural Microflora, Tapai Fluid, Yeast.

INTODUCTION

Tapai fluid produced during the fermentation tapai of cassava by using yeast. The amylolytic microbes contained in the tapai will convert the carbohydrates into a simple glucose bond which will be decomposites into alcohol by yeast (Rikana and Adam, 2010). According to Dwijeseputro in Tarigan (1988) in yeasts of tapai there are found various species of genus *Aspergillus*, *Saccharomyces*, *Candida*, *Hansenulla*, and *Acetobater*. *Aspergillus* produced a glucoamylase enzyme that will simplify starch into glucose units, while *Saccharomyces*, *Hansenulla*, and *Candida*, are decompose sugars into alcohol and any other organic substances. The formation of tapai fluid occurs during the process of starch hydrolysis to produce simple glucose and will release some water as a result of metabolic process. Increased of water content in tapai is also influenced by *Acetobacter acetii* which plays a role in converting alcohol into acetic acid and water (Saono, 1981 *cit* Finallika and Widjanarko, 2015). Related research about tapai fluid has been done before by Finallika *et al*. (2015) it was found that the water content produced during the fermentation process of cassava tapai was 14.49%. Meanwwhile, fermentation of black tapai ketan produces higher water content because of the starch content in black rice is higher than cassava.

Indonesian people in general consume water fluid as one of the healthy drinks that are safe for consumption. It is known that in the water fluid found various kinds of good bacteria, that why tapai fluid can be classified as a source of probiotics for the human body The content of lactic acid bacteria in water fluid as much as ± 1 million per mililiter or gram. This fermented product was trusted to provide healthful effects for the body, especially for the digestive system, because it can increase the amount of good bacteria in the body (Asnawi, Sumarlan, and Hermanto, 2013). In addition, the fermentation process occurs in the fomation of water fluid also be a factor of improving the nutriotional value of water fluid. Wasito (2005) states that fermentation not only to preserve food but also can increase the anti-nutritional substances or toxins contained in a foodstuff.

Alcohol fermentation in tapai fluid was occurs spontaneously by the indigenous microflora that present in tapai fluid. In general, yeast is a main producer of alcohol. Yeast is widely used as commercial for alcohol fermentation more that bacteria, this is due to the yeast can ferment alcohol in larger quantities. During the fermentation process, yeast fermented glucoses form into alcohol in anaerobic condition. Khamir has enzymes known as zymase which play role in fermentation of glucose compounds into alcohols and carbon dioxide (Wanto dan Arif, 2004).

The information about the presence of natural microflora in tapai fluid of cassava is still limited there for the further research is needed. This study aims to determine the presence of natural microflora in tapai fluid of cassava (*Manihot utilissima* Pohl.) and to know the characteristic of tapai fluid based on alcohol level, residual sugar content, pH, and temperature.

MATERIALS AND METHODS

This research was conducted by using survey method which results presented descriptively. The stages of this research are the isolation of yeast in the *Yeast Extract Agar* medium and isolation of microflora used *Glucose Peptone Agar* medium. Parameters observed included microfolora total and yeast total. Biochemical analysis of residual sugar content, pH value, temperature, and alcohol level.

Tapai fluid samples are taken in fresh condition, which is each sample are from several traditional market in Padang, that sample code are Lubuk Buaya Market (LBM), Raya Padang Market (RPM), and Bandar Buat Market (BBM) where samples are collected from two different producers each market.

Table 1: Sampling Location of Water Fluid of Cassava at Traditional Market in Padang.

No.	Sample Code	Location
1	LBM	Lubuk Buaya Market St. Adi Negoro, Koto Tangah, Lubuk Buaya, Padang
2	RPM	Raya Padang Market St. Ps. Raya, Kp. Jao, Padang Baru, Padang
3	BBM	Bandar Buat Market St. Raya Indarung, Bandar Buat, Lubuk Kilangan, Padang

The isolation media consists of *GPA*, *YEA*, and *PDA* medium. The *Glucose Peptone Agar* medium *(GPA)* was used for counting the natural microflora total (bacteria and yeasts) in a tapai fluid sample, it composed of glucose, peptone, and agar. Yeast Extract Agar medium (*YEA*) used to see the presence of yeasts in a tapai fluid sample, which composed yeast extract, peptone, and agar. *Potato Dextrose Agar* medium *(PDA)* was used for purification and maintenance of yeast, composed of potato extract, dextrose, and agar with pH 5.6.

RESULTS AND DISCUSSION

The results of observation that has been done to the existence of microflora in tapai fluid of cassava sample, showed that in six samples of tapai fluid there are yeast colony and bacteria. The existence of microflora in tapai fluid can be seen on *Glucose Peptone Agar* medium. Meanwhile, the existence of yeasts in tapai fluid can be seen on *Yeast Extract Agar* medium, the existence of yeast was supported by the occurrence of spontaneously fermentation process in tapai fluid.

The existence of microflora in tapai fluid can be seen in Figure 1. from the picture can be seen the colony of yeast and bacteria. Result of isolation of six samples of tapai fluid not found the existence of mold because in tapai fluid has been dominated by yeast and bacteria thus suppress the growth of mold in tapai fluid. In addition, the spontaneous fermentation process produces alcohol, the alcohol level that contained in tapai fluid inhibits mold growth itself. This is supported by Waluyo (2007) the mold growth will run slowly compared to the growth of bacteria and yeasts. If conditions allow for all organisms to grow, in that competition the mold growth will be suppressed by the growth of yeasts and bacteria.

Presence and Proportional Natural Microflora in Tapai Fluid

Tabel 2: Average of Yeast and Microflora Total in Tapai Fluid Samples from Several Traditional Market in Padang.

No.	Sample Code	Parameters		Percentage of Yeast (%)
		Microflora Total (x 10^7 cfu/ml)	Yeast Total (x 10^7 cfu/ml)	
1	LBM_1	16.2	3.2	19.8
2	LBM_2	19.3	3.4	17.6
3	RPM_1	15.7	1.7	10.8
4	RPM_2	12.5	1.6	12.8
5	BBM_1	12.0	3.7	30.8
6	BBM_2	8.6	2.5	29.1

Information: LBM_1 (Lubuk Buaya Market Sample 1), LBM_2 (Lubuk Buaya Market Sample 2), RPM_1 (Raya Padang Market Sample 1), RPM_2 (Raya Padang Market Sample 2), BBM_1 (Bandar Buat Market Sample 1), BBM_2 (Bandar Buat Market Sample 2).

In Table 2 it appears the microflora total (yeasts and bacteria) and yeasts total in each sample were different The differences of presence of microbial that found in each sample caused by the metabolism of organic materials, nutrition, and the variant of environment of microflora growth. This is supported by the opinion of Syamsu (2005) which states that the growth of microbes is influenced by various environmental factors, such as temperature, pH, water activity, the presence of oxygen, and the availability of food. Meanwhile, the proportion of yeast that grow can be seen from the percentage comparison of the growing microflora with yeast growing on *Yeast Extract Agar* medium *(YEA).*

The percentage of yeasts grown in *YEA* medium sample BBM_1 was higher when it compared with other proportional samples that 30.8%. This is due to the yeast grows in the medium optimum in degradation the sugar. In line with Fardiaz (1989) in case fermentative yeasts can degrade 70% of the glucose present in the substrate to alcohol and generate CO_2. Meanwhile, the remaining 30% will be converted into a saving product as reserves to be reused through fermentation if glucose in the medium is depleted.

The proportional magnitude of tapai fluid yeast in fermenting glucose and producing alcohol causing death of non-alcohol resistant microbes It was supported by statement of Waluyo (2007) alcohol that was produced by yeasts in fermentation may be inhibit the growth of the microbes. When alcohol and acids produced by fermentative microbes are high enough, it will push down proteolytic and lipolytic microbes.

Yeast can grow in medium containing carbohydrates that can be fermented as a provider of energy and carbon sources. Carbohydrates as carbon sources can be monosaccharides such as glucose and fructose, in addition to monosaccharides, disaccharides (such as sucrose and maltose) can also be fermented. Substrate containing glucose, fructose, and sucrose rapidly will be used by yeast in the early stages of fermentation. Sucrose is hydrolyzed by an invertase enzyme located outside the cell membrane and bounded by a cell wall. While the existing glucose and fructose will enter the cell (Umbreit, 1959).

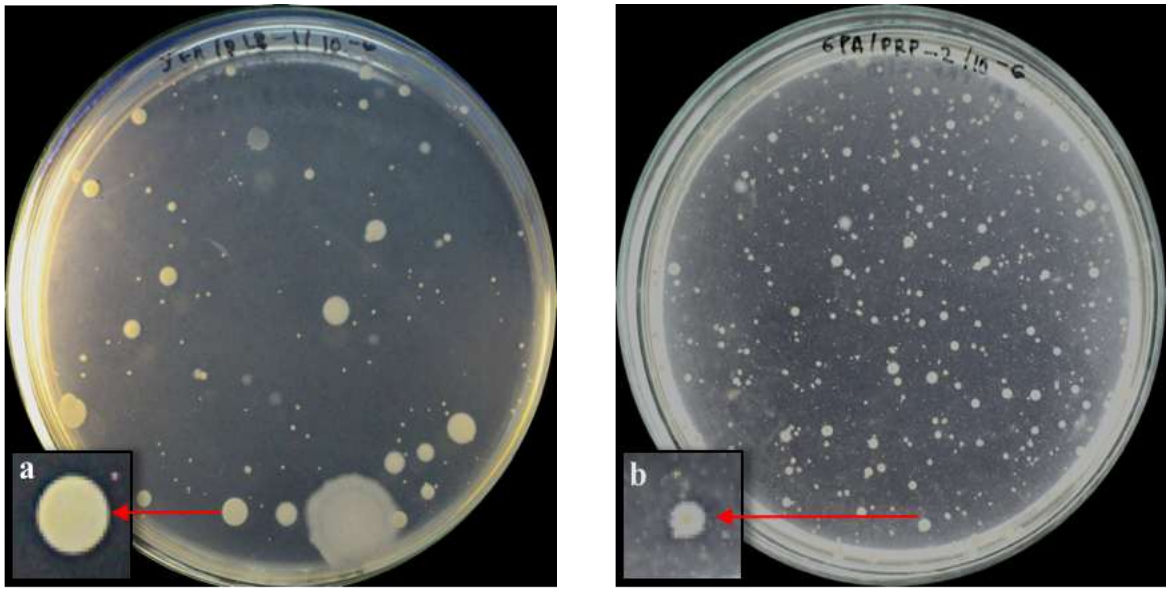

Figure 1: Natural Microflora of Tapai Fluid in *Yeast Extract Agar* medium (left) and *Glucose Peptone Agar* medium (right); a. yeast, b. bacteria.

Sugar content, pH value, alcohol level, and temperature of Tapai Fluid samples Biochemical analysis of tapai fluid sample was observation on several measurement parameters such as measurement of sugar content, pH value, measurement of alcohol level, and samples temperature. Measurement of various parameters was used to know the initial condition of tapai fluid sample before the natural fermentation process. Various parameters can affect the fermentation process that occurs in the tapai fluid, because the physical and chemical properties can affect the growth of microorganisms that exist in tapai fluid samples. In line with the opinion of Berg and Tymozkco (2002) environmental factors may affect the existence and growth of microbes, the factors such as oxygen, moisture, temperature, and Ph.

Table 3: Sugar Content, pH, Temperature, and Alcohol Level some Tapai Fluid from Seeveral Traditional Market in Padang.

No.	Sample Code	Parameters			
		Sugar Content (% Brix)	pH	Temperature (^{0}C)	Alcohol Level (%w/v)
1	LBM_1	17.4	3,80	27	2,5
2	LBM_2	17.5	3,69	28	3
3	RPM_1	22	3,57	28	6
4	RPM_2	20,5	3,43	28	3,5
5	BBM_1	15,3	3,50	29	4,5
6	BBM_2	17,5	3,87	30	11

Information: LBM_1 (Lubuk Buaya Market Sample 1), LBM_2 (Lubuk Buaya Market Sample 2), RPM_1 (Raya Padang Market Sample 1), RPM_2 (Raya Padang Market Sample 2), BBM_1 (Bandar Buat Market Sample 1), BBM_2 (Bandar Buat Market Sample 2).

In Table 3 showed that difference of sugar content, pH, temperature and alcohol level of each tapai fluid sample. This difference occurs because of differences in the composition of organic materials composing tapai fluid such as carbohydrates, residula sugars, cyanide content and water content. In addition, the amount of yeast in tapai fluid also gives effect to the composition of tapai fluid, because yeast cells also have a chemical composition. This is consistent with the opinion of Suriawiria (1990) the chemical composition present in the yeast cell compound almost 7.5-8.5% nitrogen, 2-6% fat, 6-12% nucleic acid, and 5-9, 5% ash. The difference of producer of cassava tapai is also suspected to affect tapai water, because basically will differentiate raw material, yeast type, and fermentation process. The initial process of fermentation begins with the degradation of starch components into dextrin and sugars which are subsequently overhauled into alcohols or acids, causing a sweet taste in the tapai product that will also give the sweetness of the tapai fluid later Followed by a splash into alcohol, acetic acid, which produces carbon dioxide and water. Degradation of sugar compounds into alcohol occurs due to the amylase enzyme produced by yeasts. In line with De Mot (1990) said that yeasts one of them *Saccharomyces cerevisiae* is an organism that has enough potential as an amylase producer, in addition to bacteria and molds. According to Rose and Harrison (1993) amilolytic yeasts play an important role in starchy products. Van derr Maarel *et al.* (2002) further argues that the role of amylolytic yeasts is due to the activity of the amylase enzyme, especially the isoamylase enzymes which can hydrolyze α-1.6 bonds in amylopectin. In addition, (McCann and Barnett (1986); Ardhana and Fleet (1989); De Mot (1990) added another role of yeast is in producing ethanol from starch in rice fermentation for the production of low-rate beverages and foods as well as tapai.

The results of the measurement of sugar content in tapai fluid samples showed that the water content of tapai fluid ranged from 15-22% Brix. Sugar reduction in tapai fluid increases along with the fermentation process. This is due to the process of hydrolysis of starch by the amylase enzyme produced by amylolitic microbes, resulting in the production of sugar (Saono, Winamo and Karjadi, 1982). The results obtained in line with research conducted by Finallika *et al.* (2015) sugar reduction of tapai fluid of cassava with fermentation process for 2 days is 26.53%. Also supported by Putriyanti (1990) said that incisive increase of sugar content in 48 hours fermentation process, the sugar content will decrease until fermentation process for 72 hours, and will decrease drastically during fermentation 120 hours.

The alcohol content of cassava tapai fluid ranges from 5-22%. The highest alcohol content was found in PBB_2 sample, as much as 22%. High levels of alcohol of tapai fluid are in line with the high proportional yeasts as well. The amount of yeasts in the tapai fluid in line with the length of time fermentation the longer the fermentation process the amount of yeasts will be more increase and ethanol production will become higher. This process will stop if the increase in ethanol levels can not be tolerated again by the yeast cells. The high content of ethanol will inhibit the growth of yeasts, so the alcohol-tolerant microbes can grow (Hambali, 2008). Widyaningrum (2009) states that the high levels of alcohol produced during the fermentation process are closely related to the number of yeasts present, the growth of yeasts is related to the presence of amylase enzymes and maltases that convert starch into glucose. Glucose will then convert yeast into alcohol and CO_2.

The enviroment temperature will affect the temperature during the fermentation process and the growth of yeast cells as a fermenting microbe. This is in line with the opinion of Adrados *et al*., (2005); Palmqvist and Hahn-Hagerdal (2000) the process of fermentation and degradation of simple sugars into inhibitor compounds is influenced by temperature, time, and acid concentration. After the sample temperature measurement, the temperature ranges from 27-30^{0}C. The temperature is very possible for the growth of the yeast cells in the tapai water sample. This is in accordance with Fardiaz (1992) assertion that in general the yeast grows in the same temperature range with the optimum temperature of mold growth ie 25-30^{0}C and the maximum yeast can grow to a temperature ranging from 35-47^{0}C.

The measurement of the pH value of the tapai fluid sample showed that the pH of the tapai water ranged from 3.4-3.8. Based on research that has been done by Finallika *et al*. (2015) obtained total acid in cassava tapai water by 0,65%. Further Santosa *et al.* said that in his study more acid levels (lower pH) are in line with the increase in yeast consent used. This is because the ability of yeast in breaking down sugar becomes alcohol are bigger and faster so, the reaction of breaking alcohol to acid by acid-forming bacteria will increase.

The effect of pH value on yeast cell growth can be seen from the results of the study, where the RPM sample having a lower pH has lower proportional yeast as compared with other tapai fluid samples. This is presumably because the RPM sample has been dominated by the growth of lactic acid bacteria, thus suppressing the growth of the yeast. During the fermentation process the breakdown of starch to the simpler sugars, at the same time the process of reshuffling of glucose into alcohol by yeast, then the alcohol formed will be converted into organic acids by bacteria to produce a slightly acidic taste (Widjajaseputra, 1998). Further Suwaryono, Oyon, and Ismaeni (1987); Chiang (2006) argues that the alcohol produced from yeast fermentation in the presence of O_2 will be further fermented by *Acetobacter acetii* become acetic acid. In addition there are several species of fermentative bacteria in tapai fluid are *Lactobacillus brevis, L. plantarum,* dan *Pediococcus* sp. which can break down the sugar into lactic acid.

Differences in the characteristics of tapai fluid of cassava are not only influenced by sugar content, pH, alcohol level, and sample temperature, but also influenced by yeast, substrate, process, and length of fermentation process. This is in accordance with Astawan and Mita (1991); Sahratullah, Jekti, and Zulkifli (2017), that the type of yeast used in the process of tapai production can affect the condition of the tapai fluid produced during the fermentation process, because the difference in raw materials in yeast production can affect the quality of yeast and the diversity of microorganisms present in the material raw. In addition, susbtrat used can also affect the fermentation process, it is also closely related to the process undertaken during substrate processing from the preparation of the substrate to the tapai fermentation process itself. The length of fermentation can also affect the tapai fermentation process, which can affect the concentration of tapai fluid content produced during the process.

CONCLUSION

Based on the research that has been done, it can be concluded as follows

1. The highest of proportional presence of natural microflora tapai fluid on sample LBM_2 ($19.3x10^7$ cfu/ml), while the highest of natural microflora total were found in sample BBM_1 ($3.7x10^7$ cfu/ml). The highest percentage of tapai fluid in sample BBM_1 (30, 8%).
2. In the observation of biochemical analysis of tapai fluid samples obtained the highest alcohol level in sample BBM_2 (11%), the highest residual sugar content in sample RPM_1 (22, 0% Brix), the highest pH value in sample BBM_2 (3, 87), and the samples temperature ranged was between 29-30^{0}C.

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