
FERMENTATION OF TAPAI AND ALCOHOL CONTENT RELEASED FROM TAPAI.

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Abstract

Background: Tapai is a well-known dessert with slight alcoholic flavour and a sweet sour aroma. Normally, glutinous rice and cassava roots are always used to make Tapa. Both of them are almost similar in taste. However, Tapai needs to be consumed immediately because it is a perishable product. So we need to preserve Tapai by using either natural or synthetic preservatives. In this study, we will review turmeric essential oil as natural preservative to preserve Tapai. If we let it to be fermented for too long, its alcohol content might increase and it may turns the Tapai becomes too alcoholic. So, this review study is conducted to study how to detect alcohol released from Tapai.

Methods: Different research studies have been done on detecting alcohol released from Tapai by using different kind of methods such as specific-gravity method, refractive index and GC-MS methods. But this review article is focusing more on alcohol biosensor. Other than that, we will observe the changes in biochemical properties of Tapai such as pH and total lactic acid.

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1. Introduction

Tapai is one of the famous traditional snacks in Asian countries. Tapai has different names in certain countries. In Malaysia and Brunei, we call it as Tapai. In Indonesia, they call it as Tape. This was reported by (Mughtaridi, Musfiroh, Hambali, & Indrayati, 2019) Since Tapai is a product that easily spolt, so it needs to be eaten between three to four days after it achieves optimum fermentation level (Law, Abu Bakar, Mat Hashim, & Abdul Hamid, 2011). Previous study reported that Kuning Cassava is the best option for choosing cassava due to its sweetness and low alcohol content. Meanwhile, the best size for preserving Tapai as table food is 2x2x2 cm in the cubic form (Candra, Suprpto, & Ardian, 2014). The objectives for this research study are to study the effect of natural and synthetic preservative on Tapai and to detect alcohol released from Tapai by using

different kind of methods from different research studies. To enhance good fermentation of Tapai, a starter culture is needed to trigger large numbers of at least one microorganism cells to accelerate and steer the fermentation process (Brandi & Biotechnology, 2008). Ragi is a common starter culture for fermentation of Tapai and is used in many countries under different names. Moulds in the inoculums *ragi* break down the carbohydrates compounds in rice or cassava, supported by yeast's role to convert simple sugar alcohol. Some acids will be produced through this process the raw material will be softens along the fermentation process. The reaction of the acids and alcohols produces a good aroma of the fermented food. Since Tapai is perishable product, we need to ensure Tapai can last longer than its normal shelf life, we will preserve Tapai by using turmeric essential oil as our natural preservative (Manzan, Toniolo,

Bredow, & Povh, 2003).

Tapai was sweet when ripe although no sugar added before fermenting it. This is due conversion of enzyme to glucose into ethanol in an anaerobic fermentation. Alcohol content increased as the level of glucose is higher. This is one of the factors that causing good aroma of alcoholics on Tapai. There have been different methods of primitive alcohol detection, of which the alcoholmeter-based densitometry is generally better known. Many types of effective instrument-based methods such as HPLC, GC, FTIR, and IR have been developed over the years (Thungon, Kakoti, Ngashangva, & Goswami, 2017). Other than that, study from (Kuswandi, 2014) was carried on ethanol determination based on enzyme biosensor, using alcohol dehydrogenase (ADH) and alcohol oxidase (AOx). Oxidation of aliphatic short-chain alcohols is catalysed by AOx, just like methanol, while ADH has high ethanol activity and low precision for aromatic and aliphatic alcohol (Thungon et al., 2017). Besides alcohol content, we will discuss of other biochemical changes of Tapai that can be observed such as pH and lactic acid. Study from (Rajkovic, Novakovic, & Petrovic, 2007) shown the titratable acidity in white wine was determined by titrating the wine with bromthymol blue as indicator, titration with phenolphthalein as an indicator, and potentiometric titration. Other than that, study from (Yusmarini et al., 2019) observed the parameters of probiotic Tapai such as pH value, total lactic acid, and also alcohol level.

2. Turmeric Essential Oil as natural preservative to preserve Tapai.

Turmeric is a rhizomatus herbaceous perennial plant, known as *Curcuma longa* from *Zingiberaceae* family contributes wide application in industries especially in food, pharmaceutical and textile industries (Hewlings & Kalman, 2017). Turmeric is safe in quantities that are usually used in food, and it is declared as generally recognized as safe (GRAS) to use as food additive by FDA (Santos-Sánchez, Salas-Coronado, Valadez-Blanco, Hernández-Carlos, & Guadarrama-Mendoza, 2017). Other than that, turmeric also can be used in medicinal purpose because it can reduce the cholesterol level, anti-inflammatory and anti-arthritis activities. Curcuminoids act as bacteriostatics to against *Staphylococcus* and *ar-Tumerone*, component that found in turmeric essential oil has the function to act as an anti-venom agent. It can be said that the

quality of any product that is derived from turmeric is based on the presence of curcuminoids and its essential oil (Manzan et al., 2003).

2.1. Mechanism of Action of Turmeric Essential Oil to Tapai.

i) Antioxidant

One of the pigments that found in turmeric is curcumin. 2-5% of turmeric is estimated consists of curcumin. Curcumin has antioxidant properties (Hewlings & Kalman, 2017) which it can helps to prevent oxidation of other compounds in Tapai, and neutralize free radicals (Yashin, Yashin, Xia, & Nemzer, 2017). Study from (Tanvir et al., 2017) shown that flavonoid content in aqueous turmeric extract between 0.29% and 0.67% but in ethanolic extracts was higher which it is (between 4.28% and 9.66%). Flavonoids in turmeric have the ability to scavenge free radicals (Yashin et al., 2017), to delay the substrates oxidation that caused by prooxidants such as ROS and NRS (Tanvir et al., 2017). When added to Tapai, antioxidants will regulate the production of rancidity, inhibit the formation of toxic oxidation products, preserve nutritional quality, and extend the Tapais shelf-life.

ii) Antimicrobial

(Gul & Bakht, 2015) stated that preserving food by using natural antibacterial compound like extracts of spices and herbs is getting more interest among researches. It is found that turmeric extract can be successfully applied by combining with other preservatives technique for meals that are ready to eat, same goes to Tapai. It has been said that turmeric extract can against *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus* very well by the help of the presence of curcuminoid, a phenolic compound (Gul & Bakht, 2015).

2.2. Extraction of Essential Oil

Generally, essential oil can be extracted by different kinds of method such as hydrodistillation (Ali Talati, 2017), steam distillation method (Ali Talati, 2017), Soxhlet extraction (Mottahedin, Haghghi Asl, &

Khajenoori, 2017), subcritical water extraction (Mottahedin et al., 2017) and solvent extraction (Aziz et al., 2018). This article will review the conventional extraction methods of essential oil.

2.2.1 Hydrodistillation.

The aromatic plant is packed to extract essential oil by hydrodistillation process and sufficient water is added and later on will be boiled or we can inject live steam into the plant charge (Ali Talati, 2017). The essential oil in the plant is released from the oil gland because of the effect of hot water and steam. The vapour mixture of oil and water will be condensed by cooling indirectly with water. Distillate discharges from the condenser and enters the separator, while oil will automatically be extracted from distillate water. This extraction technique is considered as an unique method to extract plant materials like wood or flower and is typically used for extractions involving natural hydrophobic plant material with a high boiling point (Aziz et al., 2018).

2.2.2. Steam Distillation

Steam distillation method is the widest technique applied in extraction of plant essential oil. The amount of essential oils being extracted by this technique is 93% and the remaining 7% can be further extracted by different methods (Aziz et al., 2018). The plant material is heated using steam which is supplied from steam generator. The efficiency of plants' structures break down is determined by sufficient amount of heat which then it will release the aromatic component or essential oil.

2.2.3. Soxhlet Extraction

It is a method of extraction in which the Soxhlet extractor is used. A Soxhlet extractor considered as part of laboratory equipment which Franz von Soxhlet invented in 1879. It was initially for lipid removal from solid substance. However, a Soxhlet extractor is not only used for lipid extraction. Usually, a Soxhlet extraction is needed only where the desired compound has minimal solvent solubility, and the solvent's impurity is insoluble. If the desired compound has a significant solubility in a solvent then a simple

insoluble substances can be distinguished from compounds by using simple filtration (Papias, n.d.).

2.2.4 Subcritical Water Extraction

SWE is a modern and effective approach at temperatures ranged of 100 to 374°C and pressure is high enough to sustain the state of the liquid. The definition of subcritical stage of liquid is the time when liquid reaches pressure higher than the critical pressure, P_c and lower than the critical temperature, T_c or *vice-versa* (Aziz et al., 2018). As temperature increases, the permittivity declines drastically and consistently, the diffusion rate increases but the viscosity and surface tension decreases. Consequently, more polar target materials with high water solubility under ambient conditions are efficiently extracted at lower temperatures, while mild polar and non-polar targets need a less polar medium caused by elevated temperature (Haghighi & Khajenoori, 2013).

2.2.5. Solvent Extraction

Generally, the plant samples are blended with solvents to be extracted by mildly heating the mixture, then the solvents will be filtrated and evaporated. The filtrate contains a resin (resinoid), or the mixture of wax, fragrance, and essential oil. Alcohol is combined with the filtrate mixture so that we can dissolve the essential oil into it and it will undergo distillation process at low temperature. During the distillation process, the alcohol absorbs the pleasant aroma from the essential oil and is evaporated while the aromatic absolute oil remains in the pot residue. Compared to other methods, this method is more difficult for essential oils extraction, and as a result, time-consuming and more expensive (Aziz et al., 2018).

3. Analytical Method to Detect Alcohol From Tapai.

Tapai has been chosen as one of popular desserts in Malaysia. The consumption of Tapai, however, had brought up the issue of Halal for Muslim people, as its alcohol level is approximately equivalent to beer which is 5%. This reported by Abdul Hamid et al. (2009).

Other study stated showed that we can identify the compounds that contain alcohol by coupling headspace to gas chromatography flame ionization detector (HSGC-FID). The Tapai was let to undergo fermentation process for three days at temperature of 300°C using inoculum that can be acquired from Malaysian Agricultural Research and Development Institute (MARDI). After three days, the measured alcohols were short chain and long chain alcohol. Ethanol was a short chain alcohol, and 1-propanol, 2-methyl-1-propanol and 3-methylbutanol were the long chain alcohols.

Chromatography and its combination with numerous spectrometric techniques were used to detect alcohol accurately and sensitively. But, these conventional techniques are complex costly, prolonged and difficult to use in on-site computation. More alcohol sensors have been invented through independent and interdisciplinary approaches to physical, chemical and biological concepts.

3.1. Conventional instruments based analysis of alcohol

There have been different methods of primitive alcohol detection, among which the densitometry based on alcoholmeter is kindly better known. Many types of effective instrument-based methods such as HPLC, GC, FTIR, and IR have been developed over the years (Thungon et al., 2017). Gass chromatography was driven to measure highly sensitive alcoholic samples by combining them with capillary-column detectors and highly accurate detectors such as hypothermic negative surface ionization, sensor arrays and flame ionization detectors (Thungon et al., 2017),(Ridder, Hendee, & Brown, 2005) .

3.2. Biosensor to detect alcohol

The limitations of modern approaches and the poor precision of chemical and physical methods became a factor to the study of alcohol detection biosensors. Enzymes are well-recognized as an element of bio recognition because of their high selectivity and behaviour under moderate experimental conditions, extensive available sources and the variety of molecular techniques. A biosensor is an analytical that consists of two essential components, and when it has

an intimate contact with a transducer, it will convert a biological signal to electrical signal. Study from (Kuswandi, 2014). was carried on ethanol determination based on enzyme biosensor, using alcohol dehydrogenase and alcohol oxidase. It can be concluded that ethanol can be determined efficiently determined by using enzyme alcohol biosensor such as ADH, and AOx.

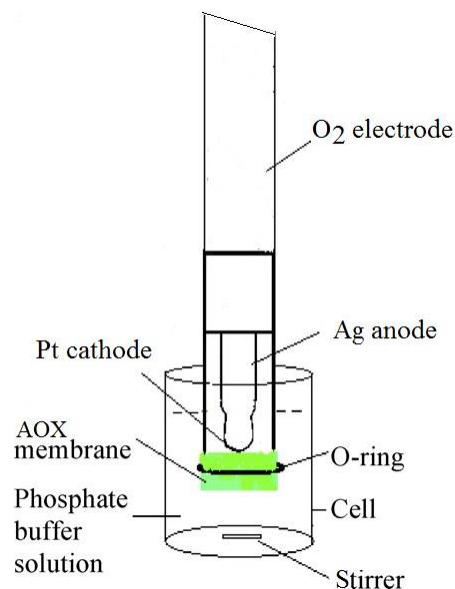


Figure 1: The schematic diagram of proposed biosensor for alcohol detection.

3.2.1 Enzyme based alcohol biosensor.

There are two most widely used enzymes for the production of alcohol biosensors which are alcohol dehydrogenase (ADH) and alcohol oxidase (AOx) (Thungon et al., 2017). The oxidation of aliphatic short-chain alcohol is catalysed by AOx, just like methanol, while ADH has high ethanol activity and low for both aromatic and aliphatic alcohol selectivity. Also mentioned was about the pyrroloquinolinequinone alcohol-dependent dehydrogenase (PQQ-ADH) for detecting alcohol. Nevertheless, the unavailability of the raw enzyme and its poor stability disable its uses (Thungon et al., 2017) .

3.2.2 ADH based alcohol biosensor

Yeast ADH is one the most enzymes that have been mostly used in alcohol biosensors' development. When reacted with ethanol, it shows that the activity of yeast ADH is the most active. As the molecules' size of alcohol increases, its activity

decreases. The catalyzed ADH reaction is used mainly through electrochemical or optical methods to detect alcohol (Thungon et al., 2017). Since electrochemical transducer-based alcohol biosensor has the characteristic of its high sensitivity, simple adaptability for in-situ analysis and low reagent requirements, it gained lot of recognition from worldwide researchers. Polymer conductive polyaniline (PANI) gives a great benefit for enzyme immobilisation due to its bio-compatibility, strength and manageable electrochemical properties. The optical biosensors based on ADH were mostly investigated using NADH's fluorescence signal (at ~ 459 nm).

3.2.3 AOX based alcohol biosensor

Alcohol oxidase (AOx) is an octameric protein composed of a tightly bond Flavin adenine dinucleotide coenzyme in each subunits. The irreversible catalytic conversion of carbonyl products from alcohol and the non-requirement of additional co-factors to the reaction medium make AOX an attractive bio-recognition feature for the application of alcohol biosensing. The first AOX based amperometric alcohol biosensor was invented by crosslinking AOX on the surface of a Clark-type O₂ electrode. Although the biosensors based on O₂ are free from electrochemical interference and have low sensitivity, duplicability and high detectable concentration (Thungon et al., 2017).

4 Biochemical Changes of Tapai.

Besides detecting alcohol content released from Tapai, we also observe some biochemical changes of Tapai. Study from (Yusmarini et al., 2019) set parameters of probiotic Tapai that must be observed which are pH values, total lactic acid, alcohol level, total LAB, and total yeast. But we will only review parameters of pH values, total lactic acid and glucose concentration.

4.1. pH value

In process of making Tapai, pH value is one of the main parameters that need to be considered.

(ARYANTA, 2000) stated that the pH of *tape ketela* changed from 5.65 to 5.15 during first 4 days of fermentation while study (Yusmarini et al., 2019) showed that pH ranged from 3.3 to 3.85 for sticky rice tapai and 4.0 to 4.6 for cassava Tapai. Basically the pH of Tapai will decline day by day if we let to the room temperature. This is because low pH level (higher number of acids) affected by the increase in yeast consent used. Yeast will break down sugar becomes alcohol to acid by increment of acid-forming bacteria (Rahman, 2018). Study from (Yusmarini et al., 2019) stated that pH value of Tapai was measured by using pH meter. We also can use pH paper to know value of pH. But, to get more accurate reading, it is recommended to use pH probe and meter. This is because the reading of pH probe and meter will give you significant pH value. pH paper only help you to determine the alkalinity or the acidity of your solution. We need to calibrate the pH probe or meter before using it by testing the meter in a solution with a known pH value. As an example, we can use pure or distilled water by knowing the pH value is 7. Adjust the meter if required. Rinse the probe and meter with clean water and dry with a clean tissue before running the test. This is because we want to avoid any error of the pH reading on the pH meter and probe.

4.2. Total Lactic Acid

Rajkovic et al.,(2007) conducted his study in determining total lactic acid in white wine by using *titratable acidity* method, where it is determined by wine titration by a strong base (after CO₂ removal) till the end point of titration. Titration acidity (acidity) contains acids that can be titrated by the strong base solution and these are their free carboxylic functions (-COOH). Other than that, Tapai's lactic can also be determined by titrating the filtrates, mix well 10 g sample in 90 ml distilled water with standardized 0.1 N sodium hydroxide to end point using phenolphthalein as indicator (Chiang, Chye, & Ismail, 2006). Lactic acid bacteria (LAB) is crucial in food industries because the characteristic of LAB can act as natural preservatives as well as flavour enhancers. During fermentation process, sugar will be converted to alcohol by the help of yeast, while lactic acid

bacteria predominantly ferment sugar into organic acid, especially lactic acid (Yusmarini et al., 2019). It can be said that total lactic acid was inversely proportional to pH value, which means the higher the total lactic acid, the lower the pH value.

4.3. Glucose Concentration

Initially fermentation process begins with degradation of starch components into dextrin and sugars which are slowly will be turned into alcohol and acids causing a sweet taste in the tapai product that will also give the sweetness of Tapai fluid (Rahman, 2018). Production of amylase enzyme causing the degradation of sugar compounds into alcohol. (Palaniveloo & Vairappan, 2013) stated in their study about rice wine, the glucose level of wine from glutinous rice varied between $300.27 \pm 0.28 \sim 440.14 \pm 29.97$ mg/ml, double the concentration of glucose in typical rice wine. Rahman (2018) 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 hydrolysis process of starch by amylase enzyme produced by amylolytic microbes, resulting in producing sugar. However, the sugar content will decrease until fermentation process reaches 72 hours and will decrease drastically during fermentation of 120 hours (Rahman, 2018). Vonach et al (1998) said that glucose concentration in Tapai fluid solution can be analysed by combining of high- performance liquid chromatography (HPLC) with Fourier – transform infrared spectroscopy (FTIR). For the sample preparation, it needs to be diluted in distilled water and more processes are needed to carry out.

5. Conclusion

It can be concluded that fermentation of Tapai is will affect the biochemical properties of Tapai itself. Basically, the pH of Tapai will decrease along the fermentation as the yeast takes place in converting sugar becomes alcohol to acid by increment of acid-forming bacteria. It can be said that total lactic acid was reciprocal to pH value, which means the higher the total lactic acid, the lower the pH value. However, the sugar content will decrease until fermentation process reaches 72 hours and will decrease drastically during as it achieve fermentation period of 120 hours.

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