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The Change of Characteristics and Antioxidant Activity of Cocoa Vinegar During Fermentation of Pulp Liquids by Adding *Tape* Yeast

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Abstract: The pulp liquids as a by-product of cocoa beans fermentation is potential to be used as a raw material for making cocoa vinegar, but unfortunately the content of acetic acid is relatively low and there have been no studies related to antioxidant activities. The objectives of the present study were to examine the effect of addition of tape yeast and fermentation time of pulp liquids to the changes of characteristics and antioxidant activities of cocoa vinegar and to define the best treatments of the addition of tape yeast and fermentation time for making cacao vinegar. The experiment in this study used factorial Group Randomized Design (GRD) with 2 factors. Factor I is the addition of tape yeast consisting of 5 levels, namely: without tape yeast (control), addition of 0.05; 0.10; 0.15 and 0.20% (w/v) and factor II is fermentation time which consists of 6 levels, namely: 5, 10, 15, 20, 25 and 30 days. The characteristics of cacao vinegar observed were as stated in SNI 01-437-1996, including: acetic acid, acidity (pH), total soluble solid (TSS), total sugar, and alcohol; and the antioxidant activity, namely: total phenolic and antioxidant capacity. The results showed that: (1) the changes in the characteristics of cocoa vinegar consisting of acetic acid content, acidity (pH), total soluble solids (TSS), total sugar content, and alcohol content; and the antioxidant activity based on the total phenolic content and antioxidant capacity occurred during fermentation with the addition of tape yeast, (2) the best treatment for making cocoa vinegar is the addition of 0.10% tape yeast and 25 days fermentation time, with its characteristics namely: 4.09±0.01% acetic acid content, acidity (pH) of 3.40±0.00, TSS of 5.05±0.07 (^{o}Brix), 0.49±0.02% total sugar content, and 0.00% alcohol content; and antioxidant activities, namely: total phenolic content of 78.94±6.54 (mg/100g GAE) and antioxidant capacity of 12.50±0.14 (mg/L GAEAC).

Keywords: Cocoa Vinegar, Pulp Liquids, *Tape* Yeast, Quality Characteristics, Antioxidant Activities

1. Introduction

Indonesia is the third largest cocoa producer in the world, after Ivory Coast and Ghana. Indonesia cocoa production reached 777,500 tons of dry beans in 2016 [1]. Cocoa processing is basically the conversion of cocoa pods to dry cocoa beans that meet the quality standard and produce the best flavor of cocoa. The most important step to produce the best quality cocoa is fermentation [2]. The process includes decomposition of pulp and facilitates biochemical reaction, which contribute to the formation of precursor of flavor and brown color [3]. Decomposed pulp would be easily separated from the seeds and forming pulp liquid dripping out from mass of seeds. Pulp liquid is a by-product of fermented cocoa beans.

During fermentation, cocoa beans produce 10-15% pulp liquids from their total weight [4-6]. Pulp liquids as a by-product contain acetic acid, lactic acid, alcohol and sugar. Organic acids are formed from the fermentation of sugars contained in the pulp of cocoa beans. Cocoa pulp is white slimy membrane that encloses the cocoa beans, there are about 25-30% of the weight of the seeds, which contain 82-

87% water, 10-15% sugar (60% sucrose and 39% is a mixture of glucose and fructose), 2-3% pentose, 1-3% citric acid, and 1-1.5% pectin, but it also contains protein, amino acids, vitamins (especially vitamin C) and minerals that can be rich media for microbial growth [2, 7, 8]. Economic potential of the pulp liquids is large enough but so far they have only been discarded around the place of processing which adversely pollute the surrounding environment. Cocoa pulp which can be used to make jam, jelly, and juice, can also be processed into fermented beverages. Pulp liquid could actually be used to make a fermented beverage, such as acetic acid [9], cocoa wine [10], and a new cocoa-based kefir drink [11]. Vinegar cocoa produced from liquid pulp also contains bioactive phenolic compounds [12], so it can give positive functional effects that are beneficial to health.

Vinegar in the market can be natural as well as synthetic vinegar. The natural vinegar is a better food additive than synthetic vinegar as it carries essential amino acids from its fruit source and is reported to act as medicine for many illnesses. The acetic acid in vinegar elicits beneficial effects by altering metabolic processes in the gastrointestinal tract and in the liver [13]. The natural vinegar is produced from coconut water, apple, bit, pineapple. Acetic acid or vinegar is mainly used as a flavor and aroma provider [14], for the preservation of fruits and vegetables as it inhibits the microbial growth and contributes to sensory properties to a number of foods such as sauces and mayonnaise, and is used as a food marinade ingredient (acidulan) [15]. Natural vinegar has many advantages compared to synthetic acetic acid because acetic acid synthetic compounds have no acetoin, diacetyl, ethanol, and several kinds of acetic ester [16]. Acetic acid fermentation results do not contain harmful substances (heavy metals), like one of the processes of making acetic acid in synthetic use of heavy metals as catalysts.

A study to optimize the formation of acetic acid by development of further fermentation methods of the pulp liquids substrate is required. This need demands industrial fermentation systems capable of producing a large amount of vinegar. Acetic acid can be made through two-phase fermentation (anaerobic and aerobic). Anaerobic fermentation produces alcohol by adding the inoculum of yeast *Saccharomyces cerevisiae* to substrate whereas aerobic fermentation to convert alcohol into acetic acid uses bacteria *Acetobacter aceti*. Previous studies have shown that cacao vinegar production can be carried out by further fermentation methods with the addition of inoculum *Saccharomyces cerevisiae* and *Acetobacter aceti* [17], but it is still not optimal and rather difficult to apply to smallholder farmers. This is related to the preparation of inoculums which require aseptic equipment and conditions. Therefore, it is necessary to study the process of making cacao vinegar which is more practical by using commercially available inoculums, such as *tape* (Indonesian fermented rice desert) yeast.

Tape yeast contains various kinds of microbes including *Candida* sp., *Endomycopsis* sp., *Hansenula* sp., *Amylomyce*s sp., *Aspergillus* sp., *Fusarium* sp., *Mucor* sp. and *Rhizopus* sp. [18], which plays an important role in the fermentation process. However, according to [19], tape yeast contains *Saccharomyces cerevisiae*, besides that in tape yeast there are microorganisms that on anaerobic conditions it will produce amylase and amyloglucosidase enzyme, both of which are responsible for breaking down carbohydrates into glucose and maltose. *Tape* yeast is a mixed population consisting of the genera *Aspergilius*, *Saccharomyces*, *Candida*, *Hansenulla*, and bacteria *Acetobacter*. The use of *tape* yeast has been tried before on the fermentation of cocoa beans with the result that the addition with a range of 1.0% can shorten the fermentation time, from 6 days to 4 days (Unpublished).

So far there have been several studies on the manufacture of cocoa vinegar from pulp liquids sources but they have not been optimal and there have been no studies related to their antioxidant activities. The study of adding *tape* yeast to further fermentation in cocoa vinegar production was based on the amount of pulp liquids produced during the fermentation of cocoa beans, which is about 10%. For this reason, this research was carried out with the addition of *tape* yeast by 10% from the treatment of cocoa beans fermentation. The objectives of the present study were to examine the effect of addition of *tape* yeast and fermentation time of pulp liquids to the changes of characteristics and antioxidant activities of cocoa vinegar and to define the best treatments of the addition of tape yeast and fermentation time for making cacao vinegar.

2. Materials and Methods

2.1. Materials and Equipment

The research material was a pulp liquid by-product of fermentation of cocoa beans for 1-2 days, which was carried out by farmers in Angkah Village, West Selemadeg District, Tabanan Regency, and Bali Province. The sample was kept in sterile container and directly brought to the laboratory. Another ingredient were *tape* yeast (brand of "NKL"), alcohol, standard of Gallic acid, methanol, Pholin Ciocalteu's, Na_2CO_3, DPPH solution, 1% pp. indicator, glucose standard, nelson reagent, HCl, and Arsenomolybdat reagent.

The equipment used included fermentation containers (gallons and plastic jars), magnetic stirrers (HP 220), pH meters (SCHOTT®), spectrophotometers (GENESYS 10S UV-VIS), hand refractometer (Portable Refractometer N-80), oven incubators (MEMMERT), filter paper, and aquarium aerators.

2.2. Experimental Design

The experiment in this study used factorial Group Randomized Design (GRD) with 2 factors. Factor I is the addition of *tape* yeast consisting of 5 levels, namely: without *tape* yeast (control), addition of 0.05; 0.10; 0.15 and 0.20% (w/v) and factor II is fermentation time which consists of 6 levels, namely: 5, 10, 15, 20, 25 and 30 days. Each treatment combination (30 combinations) was made in 2 groups so were obtained 60 units of the experiment.

2.3. Methods

2.3.1. Preparation of Pulp Liquids

The pulp liquids as a by-product of cocoa beans fermented for 1-2 days as much as 50 liters was prepared for each experimental group. Then pulp liquids were filtered through a filter cloth to separate the impurities.

2.3.2. Cocoa Vinegar Fermentation

The pulp liquid substrate was included in a 5 liter gallons fermentation container and added *tape* yeast according to the treatment levels, i.e. without *tape* yeast (control), addition 0.05; 0.10; 0.15 and 0.20 (% w/v). The *tape* yeast used was in 40 mesh powder form and was first dissolved in the pulp liquids substrate. The fermentation of cocoa vinegar was carried out through two stages, namely anaerobic and aerobic fermentation at room temperature. Anaerobic fermentation was carried out for 10 days. Furthermore, it was aerobically fermented for 30 days in a plastic jar container. Aerobic condition was made by flowing air through a plastic hose using aquarium aerators. The sampling of cocoa vinegar from anaerobic and aerobic fermentation was done for 5, 10, 15, 20, 25, and 30 days then they were analyzed.

2.4. Analysis of Characteristics and Antioxidant Activity of Cocoa Vinegar

Analysis of the characteristics of cocoa vinegar was based on the procedures of Indonesian National Standard [20], including: acetic acid, acidity (pH), total soluble solid (TSS), total sugar, and alcohol. In addition, an analysis of antioxidant activity was also carried out, namely: total phenolic by spectrophotometric methods [21] and antioxidant capacity with DPPH method [22].

2.5. Statistical Analysis

The data were evaluated using one-way analysis of variance (ANOVA) in SPSS 16.0 (SPSS Inc., Chicago, IL, USA). The results were presented as the mean and standard error (SE) of the mean. Differences between treatment means were determined by Duncan's multiple comparison tests (DMRT). Significance was assessed at $P \leq 0.05$.

3. Results and Discussion

3.1. The Characteristics of Cocoa Vinegar

3.1.1. Content of Acetic Acid

Acetic acid contents of cocoa vinegar were highly significantly affected ($P \leq 0.01$) by the treatment of adding yeast *tape*, fermentation time and interaction between treatments. The changes in the average value of acetic acid contents of cocoa vinegar during fermentation with the addition of *tape* yeast are presented in Table 1.

Table 1 shows that the higher percentage of addition of *tape* yeast and the longer the fermentation time causes the acetic acid content to increase, but the addition of *tap*e yeast is more than 0.10% and the fermentation time is more than 25 days the acetic acid content tends to decrease. The highest acetic acid content was obtained in the treatment of the addition of *tape* yeast 0.10% and fermentation time of 25 days, which was 4.09 ± 0.01%, higher than the acetic acid content of the sample of cocoa pulp liquids which was 1.16 ±0.06%.

This happened because the addition of *tape* yeast initially increased the amount of microbes that worked to transform the sugar into ethanol, then ethanol would be converted to acetic acid. However, the higher percentage of adding *tape* yeast tended to reduce the levels of acetic acid vinegar. Due to microbial competition, the same substrate conditions become less optimal for the process of reforming the substrate. The transformation process also requires time, there longer the fermentation time, the higher the acetic acid contents (until the 25th day). However, the acetic acid tended to decrease because it had been formed and further transformed, as explained by [23] that in further fermentation, acetic acid was transformed into H_2O and CO_2.

Table 1. *The average value of acetic acid contents (%) of cocoa vinegar during fermentation with the addition of tape yeast.*

Addition of *tape* yeast (%, w/v)	**Fermentation time (day)**					
	5	**10**	**15**	**20**	**25**	**30**
0.00	1.69±0.03^{o}	2.60±0.02^{kl}	3.43±0.14^{gh}	3.55±0.05^{ef}	3.60±0.02^{ef}	3.63±0.02^{ef}
0.05	1.72±0.05^{no}	2.65±.04^{kl}	3.38±0.02^{hi}	3.52±0.01^{efg}	3.85±0.01^{c}	3.71±0.11^{d}
0.10	1.90±0.07^{m}	2.97±0.02^{j}	3.72±0.00^{d}	3.88±0.06^{bc}	4.09±0.01^{a}	3.99±0.06^{ab}
0.15	1.85±0.08^{m}	2.67±0.07^{k}	3.37±0.03^{hi}	3.50±0.01^{efg}	3.93±0.05^{bc}	3.69±0.07^{de}
0.20	1.82±0.07^{mn}	2.55±0.12^{l}	3.28±0.01^{i}	3.52±0.01^{efg}	3.84±0.03^{c}	3.58±0.11^{ef}

Note: letters different in the superscript mean ± SE (2 replications) show a significant difference in 5% DMRT ($P \leq 0.05$)

Adding 0.10% *tape* yeast with 25 days fermentation time can produce cacao vinegar with 4.09±0.01% acetic acid content. These results are higher than the results of the survey by [24] in the process of making acetic acid traditionally in Indonesia and the Philippines, which is about 2%. These results also meet the standard acetic acid levels on SNI for fermented vinegar [20], which is a minimum of 4%.

3.1.2. Acidity (pH)

Acidity (pH) of cocoa vinegar were highly significantly affected ($P \leq 0.01$) by the treatment of adding yeast *tape*, fermentation time and interaction between treatments. The changes in the pH average value of cocoa vinegar during fermentation with the addition of *tape* yeast are presented in Table 2.

Table 2 shows that the higher the percentage of addition of *tape* yeast and the longer the fermentation time causes the pH of cocoa vinegar is lower, but the addition of *tape* yeast more than 0.10% pH tends to be higher. The lowest pH of cocoa vinegar was obtained in the treatment of adding 0.10% *tape* yeast and fermentation time of 25 days or in all treatments of the addition of tape yeast with a time of more than 25 days, which was 3.40±0.00 lower than the pH of the sample of cocoa pulp liquid which was 4.15±0.07. This condition occurs because of the presence of acetic acid in cacao vinegar which changes in line with changes in pH. The higher the concentration of yeast added, the more microbes, so that the total acid produced increases. The increase in total acid is due to the formation of organic acids as the end result of fermentation in the form of acetic acid and lactic acid. These acids will affect acidity (pH) after fermentation [25].

Table 2. *The pH average value of cocoa vinegar during fermentation with the addition of tape yeast.*

Addition of *tape* yeast (%, w/v)	**Fermentation time (day)**					
	5	**10**	**15**	**20**	**25**	**30**
0.00	3.70±0.00^{cd}	3.68±0.04^{cd}	3.65±0.07^{de}	3.60±0.00^{ef}	3.58±0.04^{ef}	3.48±0.04^{gh}
0.05	3.73±0.04^{c}	3.70±0.00^{cd}	3.70±0.00^{cd}	3.60±0.00^{ef}	3.50±0.00^{g}	3.40±0.00^{h}
0.10	3.75±0.07^{c}	3.60±0.00^{ef}	3.50±0.00^{g}	3.50±0.00^{g}	3.40±0.00^{h}	3.40±0.00^{h}
0.15	3.85±0.07^{b}	3.68±0.04^{cd}	3.60±0.00^{ef}	3.58±0.04^{ef}	3.50±0.00^{g}	3.40±0.00^{h}
0.20	3.95±0.07^{a}	3.75±0.07^{c}	3.60±0.00^{ef}	3.55±0.00^{fg}	3.50±0.00^{g}	3.40±0.00^{h}

Note: letters different in the superscript mean ± SE (2 replications) show a significant difference in 5% DMRT ($P \leq 0.05$)

However, the pH range of cocoa vinegar produced is not too large, which is between 3.95 - 3.40. This condition is possible because acetic acid is classified as a weak acid with pKa 4.76 so that it does not affect the change in pH value too much in cocoa vinegar produced.

3.1.3. Total Soluble Solid (TSS)

Total soluble solid (TSS) of cocoa vinegar were highly significantly affected ($P \leq 0.01$) by the treatment of adding yeast *tape* and fermentation time, but the interaction between treatments only had a significant effect ($P \leq 0.05$). The changes in the TSS average value of cocoa vinegar during fermentation with the addition of *tape* yeast are presented in Table 3.

Table 3 shows that the higher the percentage of addition of *tape* yeast causes TSS tends to be higher, but the longer the fermentation time causes TSS tends to be lower. The lowest TSS of cacao vinegar was obtained in the treatment without the addition of *tape* yeast and fermentation time of 30 days, which was 4.33±0.04 (^{o}Brix), lower than the TPT of sample of cacao pulp liquid which was 6.70±0.14 (^{o}Brix). Higher TSS in the higher percentage of addition of *tape* yeast due to additional of *tape* yeast dissolved in the cacao vinegar was produced. Furthermore, in acetic acid fermentation, TSS levels in cacao vinegar also decreased, presumably caused during the fermentation process, sugar which is the dominant soluble solid component in the medium, besides pigments, vitamins and minerals, is utilized by bacteria as a carbon source [23]. In addition, it was stated by [26], that there was a decrease in TSS during the fermentation process by bacteria as well as yeast. This is made clear that the decrease in the TSS occurred during storage because sugar was changed to alcohol, aldehyde, and amino acids. The remnants of these organic acids, sucrose, and lactose dissolved are counted as TSS.

Table 3. *The TSS average value (^{o}Brix) of cocoa vinegar during fermentation with the addition of tape yeast.*

Addition of *tape* yeast (%, w/v)	**Fermentation time (day)**					
	5	**10**	**15**	**20**	**25**	**30**
0.00	6.25±0.07^{cd}	5.63± 0.04^{gh}	5.13±0.04^{lmn}	4.68±0.04^{o}	4.43±0.04^{p}	4.33±0.04^{p}
0.05	6.40±0.00^{b}c	5.70±0.00^{fg}	5.30±0.00^{jkl}	5.00±0.00^{mn}	4.68±0.25^{o}	4.93±0.13^{n}
0.10	6.58±0.04^{b}	5.90±0.00^{e}	5.40±0.00^{ijk}	5.20±0.00^{klm}	5.05±0.07^{mn}	5.00±0.14^{n}
0.15	6.78±0.04^{a}	6.13±0.04^{d}	5.55±0.00^{gh}	5.38±0.04^{ijk}	5.10±0.00^{lmn}	5.20±0.14^{klm}
0.20	6.90±0.00^{a}	6.20±0.00^{cd}	5.60±0.00^{gh}	5.50±0.00^{ghij}	5.30±0.00^{jkl}	5.45±0.18^{hij}

Note: letters different in the superscript mean ± SE (2 replications) show a significant difference in 5% DMRT ($P \leq 0.05$)

3.1.4. Total Sugar Content

Total sugar of cocoa vinegar were highly significantly affected ($P \leq 0.01$) by the treatment of adding *tape* yeast, fermentation time and interaction between treatments. The changes in the average value of total sugar content of cocoa vinegar during fermentation with the addition of *tape* yeast are presented in Table 4.

Table 4. *The average value of total sugar content (%) of cocoa vinegar during fermentation with the addition of tape yeast.*

Addition of *tape* yeast (%, w/v)	**Fermentation time (day)**					
	5	**10**	**15**	**20**	**25**	**30**
0.00	1.25±0.07^{a}	1.09±0.10^{bc}	0.89±0.08^{efg}	0.74±0.08^{hi}	0.51±0.01^{j}	0.37±0.06^{kl}
0.05	1.23±0.04^{a}	1.05±0.10^{bcd}	0.87±0.08^{efg}	0.80±0.08^{ghi}	0.51±0.01^{j}	0.39±0.06^{jkl}
0.10	1.16±0.01a^{b}	1.01±0.10^{cde}	0.88±0.08^{efg}	0.78±006^{ghi}	0.49±0.02^{jk}	0.34±0.05^{l}
0.15	1.08±0.04^{bc}	0.97±0.08^{cde}	0.87±0.06^{efg}	0.76±0.08^{gh}i	0.43±0.02^{jkl}	0.36±0.06^{kl}
0.20	1.00±0.07^{cde}	0.94±0.08^{def}	0.85±0.08^{fgh}	0.71±0.08^{i}	0.42±0.02^{jkl}	0.38±0.05^{jkl}

Note: letters different in the superscript mean ± SE (2 replications) show a significant difference in 5% DMRT ($P \leq 0.05$)

Table 4 shows that the higher the percentage of addition of *tape* yeast and the longer the fermentation time causes the total sugar content tends to be lower. The lowest total level of cacao vinegar sugar was obtained in the addition of 0.10% *tape* yeast and 30 days fermentation time, which was 0.34±0.05%, lower than the total sugar content of the sample of cocoa pulp liquids which was 1.46±0.11%. This happens as a result of decomposition of sugar during fermentation by microbes contained in *tape* yeast as a carbon source. According to [23], in acetic acid fermentation, carbon sources (usually glucose) are oxidized to CO_2 and H_2O.

3.1.5. Alcohol Content

Alcohol content of cocoa vinegar were highly significantly affected (P≤0.01) by the treatment of adding *tape* yeast, fermentation time and interaction between treatments. The changes in the average value of alcohol content of cocoa vinegar during fermentation with the addition of *tape* yeast are presented in Table 5.

Table 5. *The average value of alcohol content (%) of cocoa vinegar during fermentation with the addition of tape yeast.*

Addition of *tape* yeast (%, w/v)	Fermentation time (day) 5	10	15	20	25	30
0.00	0.96±0.01^{f}	1.07±0.01^{e}	0.59±0.02^{i}	0.00±0.00^{l}	0.00±0.00^{l}	0.00±0.00^{l}
0.05	1.04±0.01^{e}	1.24±0.01^{c}	0.67±0.01^{g}	0.00±0.00^{l}	0.00±0.00^{l}	0.00±0.00^{l}
0.10	1.06±0.01^{e}	1.36±0.01^{a}	0.52±0.02^{k}	0.00±0.00^{l}	0.00±0.00^{l}	0.00±0.00^{l}
0.15	1.10±0.01^{d}	1.29±0.01^{b}	0.64±0.02^{h}	0.00±0.00^{l}	0.00±0.00^{l}	0.00±0.00^{l}
0.20	1.12±0.01^{d}	1.27±0.01^{b}	0.55±0.01^{j}	0.00±0.00^{l}	0.00±0.00^{l}	0.00±0.00^{l}

Note: letters different in the superscript mean ± SE (2 replications) show a significant difference in 5% DMRT (P ≤ 0.05)

Table 5 shows that the higher percentage of addition of *tape* yeast causes the alcohol content of cacao vinegar to increase in 5 and 10 days fermentation, but then decreases, even undetectable in observations of the 20th, 25th and 30th days. The highest alcohol content (10 days fermentation) resulting in the addition of 0.10% *tape* yeast treatment compared to the addition of other *tape* yeast treatments. The highest alcohol content of cocoa vinegar was obtained in the treatment of adding 0.10% *tape* yeast and 10 days fermentation time, which was 1.36±0.01%, higher than the alcohol content of the sample of cocoa pulp liquids which was 0.55 ± 0.10%. This happens because in anaerobic fermentation sugar is broken down by *Saccharomyces cerevisiae*, one of the microbes contained in yeast tape, become alcohol and CO_2. Addition of yeast will increase the amount of microbes that work to break down sugar into alcohol. Furthermore, CO_2 produced in the alcoholic fermentation process can inhibit the activity of *Saccharomyces cerevisiae* itself so that the alcohol content decreases. According to [27], the production of CO_2 during the fermentation process, the growth of *Saccharomyces cerevisiae* will stop even though it is still alive.

The decrease in alcohol content also takes place because the alcohol is oxidized by the bacterium *Acetobacter* sp., producing acetic acid and H_2O. Decreasing alcohol content after the 15th day of fermentation for all treatments of adding *tape* yeast can occur because the yeast undergoes a phase of growth retardation due to reduced essential nutrients for the growth of glucose-decomposing microorganisms. The fermentation process after 10 days produced acetic acid formed from alcohol. In addition, changes in a compound are influenced by time, so that the longer the fermentation time causes the alcohol content of cocoa vinegar is lower, even undetectable from the 20th day. During acetic acid fermentation, alcohol is transformed into acetic acid so that the initial alcohol content is reduced, as stated by [28], that alcohol is a medium of acetic acid bacteria to live and is converted to acetic acid.

3.2. The Antioxidant Activity of Cocoa Vinegar

3.2.1. Total Phenolic Content

Total phenolic content of cocoa vinegar were highly significantly affected (P≤0.01) by the treatment of adding *tape* yeast, fermentation time and interaction between treatments. The changes in the average value of total phenolic content of cocoa vinegar during fermentation with the addition of *tape* yeast are presented in Table 6.

Table 6. *The average value of total phenolic content (mg/100g GAE) of cocoa vinegar during fermentation with the addition of tape yeast.*

Addition of *tape* yeast (%, w/v)	Fermentation time (day) 5	10	15	20	25	30
0.00	37.76±0.95^{m}	68.05±0.13^{hi}	74.33±2.52^{fg}	80.48±1.14^{cde}	77.78±1.78^{ef}	70.77±0.03^{gh}
0.05	43.73±0.88^{l}	75.39±0.66^{efg}	80.71±1.58^{cde}	86.68±4.24^{b}	79.48±1.46^{cde}	64.47±1.10^{i}
0.10	44.60 ±0.60^{kl}	76.87±0.06^{ef}	84.83±1.53^{bc}	92.56±4.11^{a}	78.94±6.54^{def}	52.82±3.28^{j}
0.15	46.94 ±2.05^{kl}	77.73±0.68^{ef}	83.00±0.57^{bcd}	88.20±4.60^{ab}	77.93±6.51^{ef}	47.99±2.67^{jkl}
0.20	48.87±2.57^{jkl}	77.77±0.63^{ef}	83.84±2.14^{bcd}	87.30±4.29^{ab}	76.02±0.63^{ef}	49.08±0.13^{jk}

Note: letters different in the superscript mean ± SE (2 replications) show a significant difference in 5% DMRT (P ≤ 0.05)

Table 6 shows that the higher percentage of addition of *tape* yeast and the longer the fermentation time causes the phenolic total contents of cocoa vinegar to increase until the fermentation time is 20 days, but then decreases. In the 20th day fermentation, the phenolic total content tended to be highest in the addition of 0.10% *tape* yeast compared to the addition of other *tape* yeast treatments. The highest phenolic total content of cacao vinegar was obtained in the treatment of the addition of *tape* yeast 0.10% and fermentation time of 20 days, namely 92.56±4.11 (mg/100g GAE), higher than the total phenolic content of cocoa pulp liquids samples, namely 31.46±0.11 (mg/100g GAE). This happens because the addition of *tape* yeast will increase the amount of microbes for the formation of phenolic compounds until the 10th day of fermentation, then the 15th and 20th days show that the addition of 0.10% *tape* yeast provides more optimal conditions than the other addition of *tape* yeast treatments. The decrease in phenolic total content after 20th day fermentation was thought to be due to further decomposition of the formed phenolic compounds.

As it is known that the cocoa pulp contains 0.17% phenolic compounds which are soluble in water and as much as 0.15% alcohol soluble [7, 29], so that they will dissolve in the pulp liquid. Phenolic compounds are allelopathy chemicals which can inhibit plant seed germination [30, 31]. According to [32], phenol compounds have an effect on hydrolytic enzymes which play a role in breaking down the substrate into compounds that are ready to be metabolized.

3.2.2. Antioxidant Capacity

The antioxidant capacity of cocoa vinegar were highly significantly affected (P≤0.01) by the treatment of adding *tape* yeast, fermentation time and interaction between treatments. The changes in the average value of the antioxidant capacity of cocoa vinegar during fermentation with the addition of *tape* yeast are presented in Table 7.

Table 7 shows that the higher the percentage of addition of *tape* yeast causes the antioxidant capacity of cacao vinegar to increase until the fermentation time of 20 days and the antioxidant capacity tended to be highest in the addition of 0.10% *tape* yeast compared to the other addition of *tape* yeast treatments. During fermentation, the longer the fermentation time increases the antioxidant capacity until the 20th day, but then at the fermentation time of the 25th and 30th days there is a decrease. The highest antioxidant capacity of cacao vinegar was obtained in the treatment of adding 0.10% *tape* yeast and 20 days fermentation time, which was 13.94±0.43 (mg/L GAEAC), higher than the antioxidant capacity of cocoa pulp liquids samples, which was 3.71±0.24 (mg/L GAEAC). This is possible because the content of polyphenols total in cacao vinegar is produced. The higher of phenolic total contents cause the higher its antioxidant capacity. In line with the results of the research of [33], that the highest total phenolic content showed the strongest antioxidant capacity. This is also shown by [34] that polyphenol compounds are antioxidants that give hydrogen atoms derived from hydroxyl groups so that a stable compound is formed.

Table 7. *The average value of antioxidant capacity (mg/L GAEAC) of cocoa vinegar during fermentation with the addition of tape yeast.*

Addition of *tape* yeast (%, w/v)	**Fermentation time (day)** 5	10	15	20	25	30
0.00	4.32 ± 0.02^{l}	5.43 ± 0.02^{jk}	11.02 ± 1.10^{cd}	12.30 ± 0.02^{bc}	10.47 ± 0.45^{d}	4.88 ± 0.45^{kl}
0.05	4.63 ± 0.06^{l}	5.71 ± 0.14^{ijk}	12.62 ± 0.02^{bc}	13.21 ± 0.76^{ab}	11.66 ± 0.02^{c}	6.50 ± 0.01^{ghi}
0.10	5.71 ± 0.05^{ijk}	6.00 ± 0.07^{hij}	12.86 ± 0.05^{bc}	13.94 ± 0.43^{a}	12.50 ± 0.14^{bc}	7.34 ± 0.02^{efg}
0.15	6.33 ± 0.29^{hij}	7.02 ± 0.08^{fgh}	12.96 ± 0.02^{abc}	13.86 ± 0.15^{a}	13.28 ± 0.17^{a}	8.25 ± 0.02^{e}
0.20	7.53 ± 0.18^{efg}	7.97 ± 0.22^{ef}	13.07 ± 0.21^{ab}	13.89 ± 0.19^{a}	13.23 ± 0.15^{ab}	8.33 ± 0.04^{e}

Note: letters different in the superscript mean ± SE (2 replications) show a significant difference in 5% DMRT (P ≤ 0.05)

4. Conclusion

The changes in the characteristics of cocoa vinegar consisting of acetic acid content, acidity (pH), total soluble solids (TSS), total sugar content, and alcohol content; and the antioxidant activity which were based on the total phenolic content and antioxidant capacity occurred during fermentation with the addition of *tape* yeast. The best treatment for making cocoa vinegar is the addition of 0.10% *tape* yeast and 25 days fermentation time. The treatment produced cocoa vinegar with the following characteristics: 4.09±0.01% acetic acid content, acidity (pH) of 3.40±0.00, TSS of 5.05±0.07 (^{o}Brix), 0.49±0.02% total sugar content, and 0.00% alcohol content; and antioxidant activity, namely: total phenolic content of 78.94±6.54 (mg /100g GAE) and antioxidant capacity of 12.50±0.14 (mg/L GAEAC).

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