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Effect of Bromelin Enzyme in Pineapple (Ananas comosus L.) on Caffeine Content and Flavour of Arabica Coffee

Ach. Ariful Maulidana[a], Ayu Puspita Arum[a], Susan Barbara Patricia Sembiring[a], dan Dyah Ayu Savitri[a]

[a] Agricultural Science Study Programme, Faculty of Agriculture, University of Jember, Indonesia

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Corresponding Author:
name author
[a] Agricultural Science Study Programme, Faculty of Agriculture, University of Jember, Indonesia
*email: ayu.puspita@unej.ac.id

A B S T R A C T

Coffee is one of the main plantation commodities in Indonesia. One of the efforts to produce quality coffee is during the processing of the coffee. Quality coffee is coffee that has a bitter and sour flavour that is not excessive and has a low caffeine content. This is because high caffeine content is not good if consumed in excess. To reduce the caffeine content in coffee, you can use natural ingredients in the form of pineapple which contains the enzyme bromelain which is mixed during the fermentation process. The purpose of this research was to determine the effect of bromelain enzyme concentration and fermentation time on caffeine content and flavour in Arabica coffee. This study used a randomised complete factorial design. The first factor is the concentration of pineapple pulp, namely 0%, 40%, and 80%. The second factor was the length of fermentation time, namely 24 hours, 36 hours and 48 hours. Data were analysed using ANOVA analysis of variance if significantly different, then further tested by Duncan with a 5% error rate. The results showed that the interaction between the concentration of pineapple pulp and the length of fermentation had a very significant effect on the observation variable of caffeine content with the best results E2P1. The effect of pineapple pulp concentration had a significant effect on the pH parameter and a very significant effect on the caffeine content parameter. The effect of fermentation duration had a very significant effect on the observation variable of caffeine content.

INTRODUCTION

Coffee is one of the plantation commodities that has an important role in economic activities in Indonesia. Indonesia is the fourth largest coffee producer in the world after Brazil, Vietnam, and Colombia. Total production in Indonesia is quite large. In 2020 it reached 757.4 thousand tonnes and increased in 2021 with coffee production reaching 780 thousand tonnes per year, an increase of 3.12% (BPS RI, 2022). The characteristics of coffee grown in Indonesia

have a distinctive aroma and flavour. There are 3 types of coffee grown, namely robusta, arabica and liberica coffee. Arabica coffee is the most popular type of coffee among coffee lovers. This is because arabica coffee has a slightly sour taste and fragrant aroma. The caffeine content contained in arabica coffee is smaller than the caffeine content of raobusta coffee, the caffeine content contained in arabica coffee is 1.77% (Aryadi et al., 2020). Caffeine is a type of compound found in various types of coffee. Caffeine is an alkaloid psychostimulant purine type compound in the form of a white powder or shiny needle shape, lumpy, odourless, bitter taste, and has a melting point at 235°-237°. Caffeine is poorly soluble in water, ethanol and ether. However, in chlorophyll and dilute acids it will dissolve more easily. Caffeine compounds have a dependence effect if consumed in excess. In addition, excessive caffeine consumption can cause abnormal heartbeat, headaches, reduced memory, and cause stomach and digestive disorders. However, in addition to providing negative effects, caffeine also provides a positive impact if consumed in moderation. A good dose of caffeine for the human body is ≤ 400 mg, with this dose the positive effects of consuming caffeine can increase passion, excitement, peace and pleasure. According to SNI 01-7152-2006 the maximum recommended limit of caffeine consumption is 150 mg/day or 50 mg/serving. In addition to caffeine compounds coffee also contains few nutrients and more contains thousands of natural chemicals such as lipids, carbohydrates, nitrogen compounds, minerals, alkaloids, vitamins, and phenolic compounds. The compounds found in coffee can be healthy and some others are potentially harmful to health. Caffeine is one of the alkaloid compounds that is potentially harmful to health (Riyanti et al., 2020).

The high caffeine content is certainly not good for health, therefore there is a need for innovation in reducing caffeine levels by decaffeinating. Decaffeination is a process of reducing caffeine levels or even eliminating caffeine levels found in Arabica coffee. Decaffeination can be done with the help of enzymes mixed during the fermentation process. The enzymes used are proteolytic enzymes that can break down the compounds found in arabica coffee. The proteolytic enzyme used is the bromelain enzyme found in pineapple using various concentrations, namely 0%, 40%, and 80% with a fermentation time of 24 hours, 36 hours and 48 hours. This can be seen in research (Wicaksono & Kurniawati, 2023) which conducted a fermentation process with a smaller concentration of pineapple with the use of a faster fermentation time, and found that the use of the highest concentration of pineapple produced the lowest caffeine content. The concentration of pineapple used was 80% with a fermentation time of 36 hours which resulted in the lowest caffeine content.

Based on the above considerations, a study will be conducted on coffee fermentation using pineapple pulp with concentrations of 0%, 40%, and 80% with 200mL water mixed. The weight of coffee used is 250g. The fermentation time used is 24 hours, 36 hours, and 48 hours. Drying was done by drying in the sun. With the difference in treatment, it is expected that the results obtained can reduce caffeine levels lower. The purpose of the study was to determine whether pineapple affects the chemical properties of arabica coffee. To determine whether the length of fermentation time affects the chemical properties of Arabica coffee.

METHODS

Location and Time

This research was conducted at the Chemistry and Food Laboratory at the Faculty of Agricultural Technology, University of Jember. This research was conducted in November 2023 - February 2024.

Materials and tools

This study uses green bean arabica coffee material derived from coffee farmers Argopuro mountain slopes, pineapple fruit with harvest age 6-8 months, water, Aquades, MgO, Aquades, H2SO4, and KOH. The tools used in this research are scales, stopwatch, NORKIT-250 roasting machine, oven, elenmeyer, cup, furnace, pH meter, mobile phone, captis bowl, stationery, basin, sieve, grate, measuring cup, stirring spoon, and filter paper.

Method of Collecting Data

This study used a factorial Completely Randomised Design (CRD) consisting of 2 factors with 3 replications. The first factor was pineapple pulp concentration (0%, 40%, and 80%) and fermentation duration (24 hours, 36 hours, and 48 hours). The first factor is pineapple concentration which consists of 3 levels E0: 0%, E1: 40%, and E2: 80%. The second factor is the duration of fermentation which consists of 3 levels: P1 : 24 hours, P2: 36 hours, and P3: 48 hours.

Data Analysis

The data obtained was analysed using analysis of variance with a confidence level of 95% and if there were significant differences between treatments, further tests were carried out using Duncan's multiple range test with an error level of 5%. The research method includes several stages, including the preparation of materials to be used, namely green bean arabica, pineapple pulp, and water which will later be fermented. The fermentation process was carried out for 24 hours, 36 hours, and 48 hours by adding pineapple concentrations of 0%, 40%, and 80%. Grinding coffee beans using a grinder to obtain uniform ground arabica coffee and then analysing caffeine content, moisture content, pH, and organoletpik test.

Analysis of caffeine content determines the maximum wavelength of the standard caffeine solution by making a solution with a concentration of 4 ppm and measuring its absorbance in the wavelength range of 250-300 nm. Then, make a calibration curve of caffeine standard solution with concentrations of 2, 2, 4, 6, 8, 10 mg/L. After that, the absorbance was measured at the maximum wavelength of the caffeine standard solution. One gram of caffeine sample was extracted first as much as one gram which was then dissolved in 150 mL of hot distilled water. Then, it was filtered and the filtrate was taken which was then put into a separating funnel and added with 1.5 g of CaCO3. After that, it was extracted 4 times by adding chlorophom with the size of 25 mL each. The extract was then evaporated using a rotary evaporator until the chlorophyllome evaporated and the remaining caffeine extract was diluted using distilled water 10 times. Then the solution was measured for absorbance using a UV-Vis

Spectorphotometer at the maximum wavelength. Precision and accuracy were made by making artificial samples with a level of 10 ppm by replicating 9 times. Then the solution was measured for absorbance using UV-Vis Spectrophotometry at the maximum wavelength (Fajriana & Fajriati, 2018).

Analysis of the moisture content of arabica coffee samples as much as two grams put into an aluminium cup that has been dried using an oven at 105 ° C for 2 hours and the cup has known weight. After that, the sample that has dried to a constant weight in a desiccator and then weighed. The difference in weight before and after drying can be calculated by the following formula:

$$\text{Moisture Content (\% b/b)} = \frac{(\text{m1}-m2)}{(m1-m0)} X100 \text{ (SNI, 2008).}$$

Measuring pH using a pH meter the first thing to do is rinse the electrode with mineral-free water. Next dry with a fine tissue and then dip the electrode into the sample to be tested until the pH meter shows the colour. After that match the colour to the pH meter to show the pH level tested. Record the results and scale or numbers on the display of the pH meter then record the temperature results at the time of pH measurement, rinse the electrode again with mineral water after measurement (Wicaksono & Kurniawati, 2023).

The organoleptic test is carried out with the type of test carried out in this pre-organoleptic test is to use the method of the panelists' level of preference for the taste, aftertaste, and aroma produced by each treatment. The senses that play an important role in organoleptic tests are the senses of sight, smell, taste and touch. Panelists are indispensable in carrying out organoleptic assessment. The organoleptic test was conducted by 30 non-standardised panelists to determine the feasibility level of colour, taste and aroma of the treated samples. The organoleptic test uses the hedonic testing method (liking) which includes: taste, aroma, and aftertaste, with a liking level of 5 (very like), 4 (like), 3 (somewhat like), 2 (dislike), and 1 (very dislike) (Fitriyah et al., 2021).

RESULTS AND DISCUSSIONS

Caffeine Content

The results of the analysis of variance in table 4.1 show that the interaction between pineapple concentration and fermentation duration showed significantly different results on the parameters of moisture content and pH. On the parameter of caffeine content showed significantly different results. The results of the average test of the effect of the interaction of pineapple pulp concentration and fermentation duration on the parameter of caffeine content using the Duncan test at the 5% error level are presented in table 1.

Table 1. Results of Duncan's distance test 5% of the interaction effect of pineapple pulp concentration and fermentation duration on the parameter of caffeine content (%)

Pineapple pulp extract concentration (%)	Fermentation time (hour)		
	P1 (24 hour)	P2 (36 hour)	P3 (48 hour)
E0 (0%)	0.98 (a)	0.96 (b)	0.97 (b)
	A	C	B
E1 (40%)	0.97 (b)	0.98 (a)	0.98 (a)
	B	A	A
E2 (80%)	0.96 (c)	0.98 (a)	0.96 (c)
	B	A	B

Description:

-Numbers followed by the same lowercase letter (vertical) show no significant difference in the simple effect of pineapple pulp concentration at the same fermentation duration level.

-Numbers followed by the same capital letters (horizontal) show that the simple effect of fermentation time at the same concentration level of pineapple pulp extract is not significantly different.

It can be seen in Table 1 that Arabica ground coffee that has the lowest caffeine content is found in the interaction of pineapple pulp concentration (80%) and fermentation time (24 hours) (E2P1) which has an average value of caffeine content of 0.96%. The highest caffeine content was found in the treatment of 0% pineapple concentration with 24 hours fermentation time (E0P1). From the data obtained, the value of caffeine content fluctuated. Data from the results showed that there was a decrease in caffeine levels in arabica coffee powder where the caffeine content in arabica coffee powder without concentration had a caffeine content of 1.2% and after fermentation using pineapple decreased to 0.96%. Based on these data, the caffeine content meets the SNI-01-3542-2004 standard with a maximum powder caffeine content of 2%.

One of the chemical compounds found in coffee is caffeine. Caffeine has a negative effect on health if consumed in excess. It is better to consume coffee with lower caffeine levels, therefore the need for a decaffeination process using enzyme assistance during the fermentation process. The use of enzymes used bromelain enzyme which is still included in proteolytic enzymes that are able to hydrolyse the content of compounds contained in coffee. The use of bromelain enzyme found in pineapple can certainly reduce caffeine levels in coffee (Oktadina et al., 2013). According to (Wicaksono & Kurniawati, 2023) the more pineapple concentration and the longer the fermentation time, the lower the caffeine content in Arabica coffee. This is because caffeine in coffee beans is found in the cell membrane, which according to (Rosalinda et al., 2021) the cell membrane in coffee beans contains 40% fat, 52% protein, and 8% carbohydrates. The largest component found in the cell membrane of coffee beans is protein, so the process of reducing caffeine levels requires breaking down the components contained in

the cell membrane. Protein itself can be hydrolysed by an enzyme that can break down proteins called proteolytic enzymes. The bromelain enzyme found in pineapple is included in proteolytic enzymes that can hydrolyse proteins contained in cell membranes, so that by mixing pineapple pulp extract during the fermentation process can reduce caffeine levels in coffee beans.

In addition, the decrease in caffeine content was also caused by the increasing microbial activity during the fermentation process. During the fermentation process, microbes can produce the enzyme a-amylase which will utilise the pineapple pulp substrate which is broken down into simple sugars in its metabolism. The results of microbial metabolism help break down the caffeine contained in coffee beans by oxidising the caffeine component, the main mechanism by which microbes can break down caffeine into simpler compounds so that there can be a decrease in coffee caffeine levels (Pratiwi et al., 2023).

Moisture Content

The results of the analysis of variance of the effect of pineapple pulp concentration (E) showed the results were not significantly different on the parameters of water content. The average value of water content showed a difference in the average value between the treatments of pineapple pulp extract Eo (No pineapple concentration), E1 (40%), and E2 (80%). The average results of the effect of factor (E) in Figure 1.

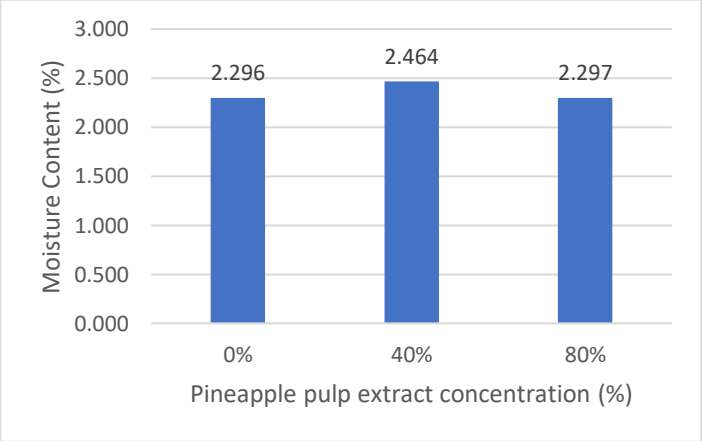

Figure 1. Effect of pineapple pulp concentration on the observation variable of moisture content (%)

Figure 1 shows that the E0 (control) treatment provides a decrease in coffee moisture content of up to 2.296%, E1 (40%) treatment provides a decrease in coffee moisture content of up to 2.464%, and E2 (80%) treatment provides a decrease in coffee moisture content of up to 2.297%. It can be seen from the results of the average value that the E0 (control) treatment provides the highest decrease in water content compared to the E1 (40%) and E2 (80%) treatments. giving a high concentration of pineapple pulp extract, a protein breakdown process will occur so that the pores in the coffee beans become open and water molecules are used to enter them (Rosalinda et al., 2021). In addition, there are also other factors that cause high water

content in coffee with the addition of pineapple extract, namely the content of the ingredients used during the fermentation process, namely the water content contained in pineapple pulp. Almost the entire content contained in pineapple fruit is 83.5% water from 100 grams, causing an increase in the water content of coffee during the fermentation process (Aditya et al., 2021).

The results of variance analysis (F-count) in table 4.1 that the effect of factor (P) showed different results were not significantly different on the parameters of water content. The average value of water content shows a difference in the average value between the treatment of fermentation duration P1 (24 hours), P2 (36 hours), and P3 (48 hours). The average results of the influence of factor (P) can be seen in Figure 2.

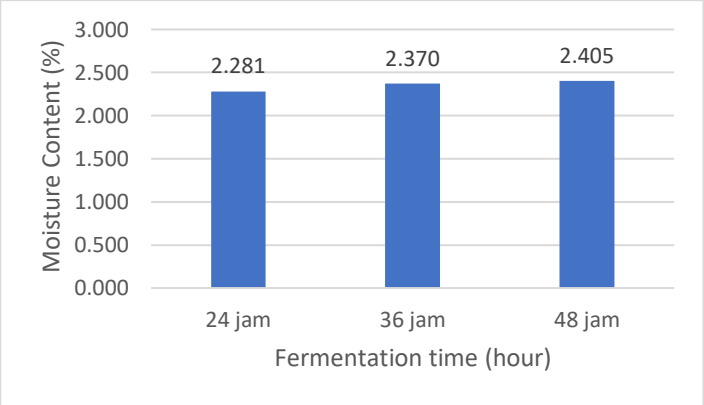

Figure 2. Effect of fermentation duration on the observation variable of water content.

Figure 2 shows that the fermentation time treatment P1 (24 hours) provides a decrease in coffee moisture content of up to 2.28%, P2 treatment (36 hours) provides a decrease in coffee moisture content of up to 2.37%, and P3 treatment (48 hours) provides a decrease in coffee moisture content of up to 2.40%. It can be seen from the results of the average value that the P1 (24 hours) treatment provides the highest decrease in water content compared to the P2 (36 hours) treatment and the P3 (48 hours) treatment. The highest water content is found in the 48-hour fermentation length treatment (P3), this is because when fermenting the time used is very long so that the water content in the pineapple pulp is absorbed so much by the beans that it causes the water content and pH in coffee to be very high. According to (Budiman et al., 2021) during the fermentation process, the water content in the enzyme will dissolve in the coffee beans so that the longer the fermentation time used will affect the water content of the coffee.

pH

The results of the analysis of variance of the factor (E), namely the concentration of pineapple pulp extract, showed significantly different results on the pH parameter, so it is necessary to conduct a further Duncan test using the 5% level to determine the effect of the factor (E) concentration of pineapple pulp extract on the pH parameter. The results of the 5% Duncan's further test can be seen in Figure 3.

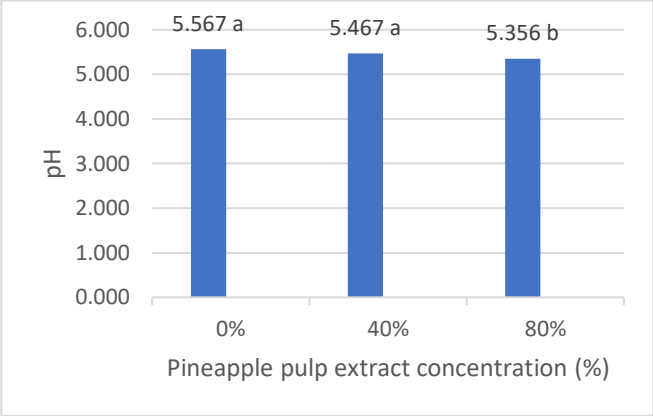

Figure 3. Effect of factor (E) pineapple pulp concentration on pH of ground arabica coffee

Figure 3 above shows that the effect of pineapple pulp extract concentration factor (E) on treatment E2 (80%) shows a significant difference by producing a pH size of 5.356, while in other treatments, namely E0 (control), and E1 (40%) pineapple pulp extract concentration factor (E) shows different results not significantly. So it can be seen that the use of a high concentration of pineapple, the pH of the coffee beans decreases. pH decreased compared to coffee fermented without using pineapple pulp extract so that the more pineapple concentration used, the acidity of the coffee will increase and the pH of the coffee decreases. This is in accordance with the statement (Noviar et al., 2016) that the decrease in pH in coffee occurs because during the fermentation process organic acids such as lactic acid are formed which affect the acidity of coffee, the more organic acids formed, the acidity of coffee will increase and the pH value will decrease. The decrease in pH occurs because the accumulation of organic acids has increased due to the breakdown of amino acids caused by the metabolism of local bacteria contained in coffee beans (Pratiwi et al., 2023).

Organoleptic Test

Observations in the organoleptic test were carried out using several panellists who were asked to score the level of liking on the form provided, where when observing the organoleptic test there were three parameters that had to be assessed by panellists in each treatment, namely aroma, flavour, and aftertaste. The following is a table of results from organoleptic observations using 30 panellists, namely:

Table 2. Organoleptic test observation results conducted by 30 panellists

Organoleptic Test					Description
Treatment	aroma	flavour	After taste	Score	
E0P1	3,63	4,57	4,13	4,11	Neutral
E0P2	4,17	4,27	4,00	4,14	Neutral
E0P3	4,43	4,33	3,60	4,12	Neutral
E1P1	4,27	4,07	4,07	4,13	Neutral
E1P2	5,03	5,17	5,07	5,09	like
E1P3	3,67	4,37	4.03	4,02	Neutral
E2P1	4,93	4,87	5,20	5	like
E2P2	4,20	3,97	4,07	4,08	Neutral
E2P3	4,03	4,10	4,03	4,06	Neutral

Description: 1 (Strongly dislike), 2 (dislike), 3 (Somewhat like), 4 (neutral), 5 (like), 6 (strongly like)

The table above shows the results of the organoleptic of all treatments carried out by 30 panelists. There are differences in scores ranging from aroma, flavor, and after taste, where from these three parameters, there are score results from each parameter averaged to produce a final score that determines the category of this organoleptic test. Based on the table above, there are 2 treatments that produce a taste with the category like, namely the treatment using 40% pineapple pulp extract with a fermentation time of 36 hours which has a score of 5.03 aroma score, 5.17; flavor and 5.07 aftertaste. The second treatment that produced a like category was the treatment using 80% pineapple pulp extract with a fermentation time of 24 hours, which had a score of 4.93 aroma score, 4.87 flavors, and 5.20 aftertaste. As for the other treatments, the score is below 5 which is included in the neutral category. So the treatment of 40% pineapple pulp extract with a fermentation time of 36 hours and 80% pineapple pulp extract treatment with a fermentation time of 24 hours are treatments that are preferred by panelists compared to other treatments. Based on the results of the study, the average value of the 1-6 scale hedonic test conducted by 30 non-standardized panelists by conducting aroma, flavour, and aftertaste tests as follows:

Aroma

Aroma is the smell of coffee that comes out after the coffee has been brewed for 4 minutes (Saleh et al., 2020). Aroma is one of the most important attributes in assessing the quality of brewed coffee. The fermentation process makes the aroma of coffee stronger and more distinctive. this is due to the high acidity level and pyrolysis where complex compounds contained in coffee beans break down into simple products when heated so that there are volatile compounds that cause a stronger coffee aroma. The treatment most favoured by panellists was the E1P2 treatment (40% pineapple pulp concentration and 36 hours fermentation time) with an average organoleptic test value of 5.03. While the other best response was in the treatment of 80% pineapple pulp concentration (E2) and 24 hours fermentation time (P1) with a total value of 4.93. The aroma produced there is a distinctive aroma of pineapple pulp which in the treatment is produced by volatiles, namely ethyl and daisies (Wibowo et al., 2014).

Flavour

Flavour is the taste that comes out after the coffee is cleaned, the measure of flavour is not how delicious the coffee is when tried but how rich the taste is in the coffee (Saleh et al., 2020). Based on the average taste test results obtained in table 4.3, it shows that the panelists gave the highest score to the 40% pineapple concentration treatment with a fermentation time of 36 hours, which was 5.03, which in this treatment had a slightly sour and bitter taste. The appearance of this flavour is not only caused by the fermentation process, but also caused by the roasting process of coffee. The result of the roasting process causes the degradation of several compounds such as carbohydrates, alkaloids, chlorogenic acid, molatyl compounds, caffeine and trigonelline. These degraded compounds cause the bitter taste found in coffee (Purnamayanti et al., 2017). In terms of the overall liking parameter of the sample, panellists preferred sample E1P2 by getting the highest average value compared to other samples. Where panelists prefer black coffee with low acidity.

Aftertaste

Aftertaste is the taste of coffee when tasted left at the base of the tongue or not (Saleh et al., 2020). Based on the taste test that has been carried out with several panelist treatments, the highest score obtained is in the 40% pineapple concentration treatment with a fermentation time of 36 hours (E1P2), which is 5.09 (table 2). The flavour impression left was bitter and sour. Acidity and bitterness are formed from non-volatile components contained in coffee beans (Asiah et al., 2017). The content of non-volatile compounds contained in coffee beans is caffeine, chlorogenic acid, and nutritional compounds consisting of carbohydrates, proteins, fats and minerals. Chlorogenic acid is one of the non-volatile compounds that is decomposed gradually along with the formation of volatile aroma and polymer compounds. The addition of enzymes during fermentation of coffee beans causes a decrease in caffeine levels, where caffeine itself contributes to the bitter taste of ground coffee. This is due to the hydrolysis of protein into free amino acids (Budiman et al., 2021).

CONCLUSIONS

The interaction of 80% pineapple pulp concentration with 24-hour fermentation time (E2P1) showed the best results in producing the lowest caffeine levels and the most preferred flavor by panelists. Giving pineapple pulp concentration in the E2 treatment (80%) gave the best results on caffeine levels and pH. The length of fermentation time in the P3 treatment (48 hours) gave the lowest decrease in caffeine levels, so it showed the best results. In the organoleptic test using 30 panelists, the best results were obtained in the E1P2 treatment with a score of 5.09 with a description of liking. Based on the results obtained from this study, it can be concluded that the best recommendation from all treatments is found in the E2P1 treatment with a combination of 80% pineapple concentration treatment with 24 hours of fermentation time.

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