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The effect of yeast concentration and fermentation time on the physical, chemical, and flavor characteristics of cocoa beans

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Abstract

Indonesia is the largest cocoa producer, yet most of the cocoa production at the farmer level remains unfermented due to its low capacity. Therefore, this study aimed to examine the effect of yeast concentration and fermentation time on cocoa beans' physical, chemical, and flavor characteristics. A total of 10 kg beans was fermented in individual styrofoam boxes, each with a centimeter hole on each side. It was carried out using a completely randomized design with a factorial pattern of two factors. The first factor comprised the yeast treatment, including 1% and 2% baker yeast (BY), 1% and 2% tapai (fermented cassava) yeast (TY), and a control group without yeast-the second factor involved four fermentation time intervals of 3, 4, 5, and 6 days. The parameters observed included temperature, pH of the pulp, fermentation index, slaty beans, pH of the beans, total acid and flavor. The results showed that fermentation with 1% and 2% TY significantly improved the quality of cocoa beans compared to the control and BY. The pH of beans fermented with 1% TY was slightly higher than 2% TY, while other parameters did not show significant differences between the two yeast concentrations. Furthermore, the fermentation time of cocoa beans had no significant effect on chemical quality, except for flavor, which improved on the 5th day of fermentation.

1. Introduction

Indonesia is the fifth-largest cocoa producer in the world, following Ivory Coast, Ghana, Ecuador, Cameroon, Nigeria and Brazil (Rahmadona *et al.,* 2023). The country has significant competitive advantages, including low costs, high production capacity, efficient infrastructure, an open trading system (business environment), and superior taste. There is evidence supporting the higher taste potential of Indonesian cocoa beans compared to the other countries in East Asia. Indonesian cocoa beans typically exhibit a higher average weight and butter content, approximately 8.0% and 9.0% more than those from Papua New Guinea. Additionally, they have an average of about 20.0% and 25.0% higher weight and butter content compared to Chinese dried beans (Gu *et al*., 2013). Despite experiencing remarkable growth in cocoa production over the past few decades, its sustainable competitiveness is threatened by inconsistent and low-quality production. Cocoa beans exported by Indonesia are considered of low quality due to the significant proportions of unfermented beans. These unfermented beans, which generally come from smallholder plantations, account for about 90% of cocoa exports (Manalu, 2018).

Fermented dry cocoa is the principal raw material for chocolate production. The process of cocoa bean fermentation plays a significant role in determining the taste, aroma, color, composition, and flavor of chocolate (De Vuyst and Weckx, 2016; Di Mattia *et al*., 2017). Indonesia has a wide area for cocoa plantations, but approximately 80% of these plantations are managed by smallholder farmers. Due to the limited production capacity per household, the yield of each farmer is often insufficient to meet the fermentation standard of 40 kg (Tarigan and Tajul, 2017). Consequently, the majority of farmers do not engage in bean fermentation. Other factors hindering the adoption of fermentation include the need for labor and time-consuming processes. For instance, cocoa of the Forastero variety usually takes five to seven days to undergo fermentation (Hatmi *et al*., 2015).

The optimum performance of the fermentation

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process is influenced by several factors, such as the minimum weight of the seeds, stirring (reversal), fermentation time, and the shape of the fermentation box (Sabahannur and Nirwana, 2017). The minimum gross weight of the seed is one of the requirements to obtain the ideal temperature for an effective fermentation process (Hidayat *et al.*, 2022). In the fermentation box, a biological and chemical process occurs, breaking down the beans and causing a temperature rise. The presence of heat and acetic acid inactivates the beans and some enzymes present in the seeds (proteases and polyphenol oxidases), leading to the separation of substrates (anthocyanins, flavanols, phenols, and protein reserves). Increasing the fermentation process by the inoculation of microorganisms such as *Saccharomyces cerevisiae* and certain bacterial cultures can improve the fermentation performance of cocoa beans (Meersman *et al*., 2016). The addition of yeasts and molasses increases the number of microbes and substrates that can be converted into ethanol (Khoshkho *et al*., 2022).

Several studies evaluated the effect of fermentation using small amounts of cocoa beans, including the addition of starters in the form of pure or mixed cultures and aeration with stirring (Jans *et al*., 2017; Kristanto *et al*., 2017; Aryani *et al*., 2018). Furthermore, various efforts have been made to optimize the small-scale fermentation process (<40 kg), including the addition of starters in the form of pure or mixed cultures, the combination of sucrose and Tapai yeast (TY), and the use of sucrose with baker yeast (BY) (Sabahannur *et al.*, 2018; Sabahannur, 2019). A previous study reported that fermenting 25 kg of seeds per box with one-time stirring led to temperatures reaching 44°C while fermentating 15 kg with three times stirring elevated the temperature to 45°C (Sabahannur and Nirwana, 2017). The addition of 1% yeast during cocoa fermentation may reduce the fermentation time to 48 hrs and produce a higher cocoa bean quality due to the rapid and complete degradation of the pulp sugar process. Various yeast species exhibiting relevant pectinolytic activity (Schwan and Wheals, 2004; Nielsen *et al*., 2005), such as *Kluyveromyces marxianus*, *S. cerevisiae* var. *chevalieri*, *Candida rugopelliculosa,* and *Kluyveromyces thermotolerans*, were identified from the natural fermentation of cocoa beans. However, the insufficient activity of wild pectinolytic strains in natural fermentation limited the amount of pulp melting. Inoculation of fermented cocoa beans under laboratory conditions with *S. cerevisiae* var. *chevalieri* and *Kluyveromyces fragilis* possessing pectinolytic activity was observed to increase pulp liquefaction and enhance the quality of the final product (Buamah *et al*., 1997).

Aprianto *et al*. (2017) found that increasing the fermentation process through the inoculation of *S. cerevisiae* and several bacterial cultures improved the fermentation performance of cocoa beans. However, the addition of a microbial inoculum, particularly pure culture, was considered impractical when applied to cocoa fermentation in the field, as it requires starter preparation, a sterile environment, and aseptic conditions (Purwanto *et al*., 2019). The addition of different types of yeast to accelerate the fermentation of cocoa beans affects the microbes involved in the process. These variations in microorganisms subsequently impact the attributes of the resulting fermented cocoa beans. One commonly used yeast for fermentation is TY, which is frequently applied in the fermentation process of traditional foods and drinks (Law *et al*., 2011).

TY contains a consortium of microorganisms, such as mold, yeast, and bacteria (Barus, 2013). The microorganisms include *Amylomyce rouxii*, *Mucor* sp. and *Rhizopus* sp., yeast S. *fibuligera*, *Saccharomycopsis malanga*, *Pichia burtonii*, *S. cerevisiae, Candida utilis*, *C. pelliculosa, C. guillermondii, C. magnoliae* and bacteria species such as *Pediococcus* sp. and *Bacillus* sp. (Yuwono, 2015). TY and BY also contains *Acetobacter aceti* and various microbes including *Endomycopsis* sp., *Hansenula* sp., *Amylomyces* sp., *Aspergillus* sp., *Fusarium* sp., *Mucor* sp., *Rhizopus* sp. and *S. cerevisiae* (Nuraida and Owen, 2014; Vellanki *et al*., 2018; Wahyuni *et al*., 2018; Nisa *et al*., 2020; Delva *et al*., 2022; Hidayati *et al*., 2022). Those may undergo selection, mutation, or hybridization to increase their ability to properly ferment sugar in the dough and grow rapidly (Pelczar and Chan, 1988). BY can be directly used as an inoculum in ethanol production, eliminating the need for special inoculum preparation (Salsabilah *et al*., 2013).

Although yeast diversity in the fermentation of cocoa beans has been reported in several studies, most of these studies were conducted on beans weighing more than 40 kg. There are limited studies investigating the effect of fermentation on the quality of cocoa beans (Pereira *et al*., 2012). Therefore, it is necessary to evaluate the use of TY and BY in the fermentation of cocoa beans as a source of microbes (starter) due to their ease of acquisition, storage, and application. This study aimed to examine the effect of yeasts (TY and BY) and fermentation time on cocoa beans' physical, chemical, and flavor characteristics.

2. Materials and methods

This study was conducted at the Laboratory of Postharvest Technology, Faculty of Agriculture, Universitas Muslim Indonesia, Makassar, South Sulawesi, Indonesia. A completely randomized design

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with a factorial pattern of two factors was used. The first factor consisted of yeast type and concentration levels, which included 1% and 2% TY, 1% and 2% BY, and a control group without yeast. The second factor included four fermentation time intervals of 3, 4, 5, and 6 days, with each treatment replicated two times. The yeast concentrations were administered based on a study by Putra and Wartini (2016) using 0.5-1.5% TY. This occurs because adding 0.5-1.5% TY provides better conditions for decomposing pulp sugar into organic acids. Research by Agung *et al*. (1998) indicated that adding TY to the fermentation of cocoa beans with a range of 1.0% can shorten the fermentation time to 4 days from 6 days in natural fermentation, resulting in dry cocoa beans of first quality (Satryo *et al*., 2015). Fermentation time duration of 3 (72 hrs) to 6 days (144 hrs) was done based on research by Sabahannur (2015), fermentation of cocoa beans in various types of cocoa clones takes 3-6 days.

2.1 Cocoa bean preparation

Cocoa beans of the local cultivar, the Masamba Cocoa Clone 02, were obtained from the North Luwu plantation, South Sulawesi, Indonesia, and used in this study. Cocoa beans were harvested from the trees using a machete, and the surrounding pulp was scooped out using sterile gloves and placed in plastic bags. The beans were stored in sacks in the laboratory before treatment.

2.2 Yeast preparation

The yeast used in this study was commonly used daily for fermentation. It was obtained from the market in solid form. Tape yeast was crushed into powder then weighed 10 g and mixed into 990 g of wet cocoa (1% concentration). The mixture was than stirred for 10 mins to ensure even distribution. The same method was used to prepare 2% TY (20 g and 980 g of wet cocoa beans). Bread yeast weighed 10 g and was directly mixed into 990 g of wet cocoa (1% concentration) beans because the form was already powder. The same method was done to prepare 2% BY (20 g in 980 g of wet cocoa beans).

2.3 Treatment procedures

The fermentation processes were conducted in the field laboratory, and the cocoa beans were placed in a 30 × 30 × 40 cm^3 fermentation box made of styrofoam, with a centimeter-sized holes on each side of the boxes. The fermentation box was covered with banana leaves and gunny sacks to retain heat. Subsequently, fermentation was conducted for 3, 4, 5, and 6 days. During the first 48 hours of fermentation, stirring was carried out. After fermentation, cocoa beans were dried until they reached 6–7% water content. Temperatures and pH were monitored every 24 hours during the fermentation process. The pH of dried cocoa beans was observed according to the Association of Official Analytical Chemists (AOAC) International, 2006.

2.4 Slaty beans evaluation

Slaty beans were evaluated with the cut test method by cutting into two to show the entire surface of the cotyledon. The color of both halves of the seeds was visually observed under sunlight to analyze the level of fermentation. The potential flavor of cocoa was evaluated based on fermentation quality using a fermentation index. The fermentation index was determined using the Misnawi method, which involved using a UV-vis Shimadzu UV-1601 spectrophotometer with a mixture of 50 mL methanol and HCl (97:3) (Misnawi *et al*., 2003).

Total acid was measured using titration, while bean taste was evaluated by two certified panelists at the Coffee and Cocoa Research Center (Puslitkoka) in Jember. Taste of cocoa beans was evaluated on the result of fermentation using tapai yeast because, of its fermentation index consistency and low slaty beans. One to ten characteristics of cocoa bean quality were assessed to express the taste of cocoa. These include cocoa (chocolate) aroma, acidity, bitterness, astringency, fresh fruit, brown fruit, floral, spicy, nutty, and roasted taste (Sari *et al.,* 2022). The panelists' assessments ranged from 0 to 10 (0: nil, 1-2: weak; 3-4: moderately weak; 5-6: moderately strong; 7-8: strong; 9-10: very strong).

2.5 Data analysis

Data on most variables (pH of the pulp, Fermentation index, Slaty beans, pH of bean, Acid level) were subjected to analysis of variance. Data of Fermentation temperature was analyzed descriptively because the result was compared with standard (44°C). All statistical tests performed using SPSS version 18. Differences between treatments were examined using a difference test, the test results were considered significant if $P<0.05$.

3. Results and discussion

The results showed a significant effect of yeast treatments on all parameters, while the effect of treatment time was only significant on slaty beans. The interaction of the two factors was significant for the fermentation index, slaty beans, and pH of beans (Table 1).

The anaerobic phase initiated the fermentation process, which occurred in the first 24 to 36 hrs after the fruit was opened. During this phase, seeds and pulp were fermented with several microorganisms. The microbial

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ecology of cocoa fermentation involves a variety of organisms, including yeast, bacteria (Lactic acid and Acetic acid bacteria, *Bacillus* spp.), and filamentous fungi.

The fermentation process may occur spontaneously, due to the presence of microorganisms naturally in cocoa beans and the environment. The microorganisms involved in the spontaneous fermentation of cocoa beans are yeast, acetic acid bacteria, and lactic acid bacteria (Jespersen *et al*., 2005; Guehi *et al.,* 2010; Pereira *et al.,* 2012). Yeast breaks down sucrose, glucose, and fructose into ethanol, and further assists in the degradation of pulp through the action of pectinolytic enzymes during fermentation (Jespersen *et al*., 2005; Ho *et al.,* 2014).

Table 1. Summary of analysis of variance results among chemical and physical variables.

Dependent variables	Yeast treatment (Y)	Treatment time (T)	Interaction (Y*T)
pH of the pulp	14.09^{**}	2.55^{ns}	1.55^{ns}
Fermentation index	72.52^{***}	0.18^{ns}	3.02^{*}
Slaty beans	34.84^{***}	6.01^{**}	3.90^{**}
pH of bean	15.53^{**}	0.74^{ns}	4.05^{**}
Acid level	12.38^{**}	2.49^{ns}	1.33^{ns}

*P≤0.05, **P≤0.01, *** P≤0.001, ns: not significant

3.1 Fermentation temperature

In the TY treatment, the temperature tended to increase, reaching a peak on days 3 (2% TY) and 6 (1% TY and control). In the treatment with BY, the temperature only slightly increased but remained below 33°C (Figure 1). The average treatment temperature of 1% TY and 2% TY resulted in a standard temperature of 44°C. This shows that the addition of TY is better than spontaneous fermentation and fermentation with BY. TY consists of various microorganisms that thrive at about 30°C and a pH of 4.8. Meanwhile, fermentation with BY resulted in a lower temperature. The fermentation process of *S. cerevisiae* is performed optimally for up to 24 hrs. Furthermore, the fermentation was replaced by other microorganisms. The suboptimal performance of BY was thought to be caused by the fermentation process being less synergistic with other microorganisms.

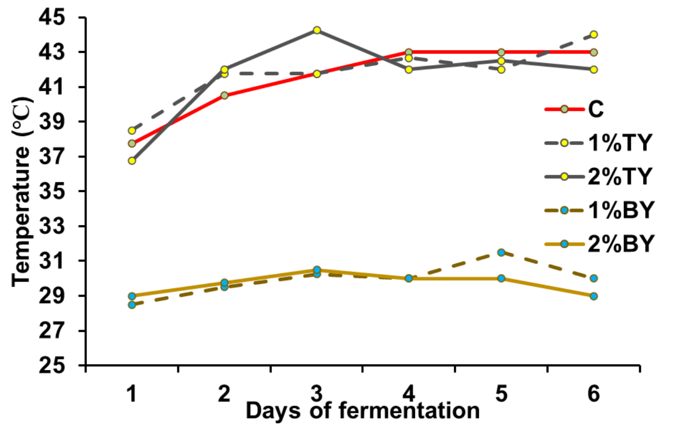

Figure 1. Temperature of bean fermentation among the treatments.

A successful fermentation is considered to be when the temperature reaches 44°C for at least 6 hrs (Abubakar *et al.,* 2022). The initial alcohol fermentation was an exothermic reaction, releasing 93.3 kJ of energy and causing an increase in temperature of up to 25°C to 45°C. By the second day, the pulp started to break down due to pectinolytic yeast. Within 4 days of fermentation, the temperature of cocoa beans reached 42°C to 52°C. The three main types of microbes that play a role in the fermentation process of cocoa beans are yeast, lactic acid bacteria, and citric acid. Yeast degrades sugar-rich pulp into ethanol and releases heat. Lactic acid bacteria convert sugar and citric acid which are free organic acids in fresh cocoa pulp, into lactic and acetic acid, while acetic acid bacteria oxidize ethanol to acetic acid, releasing greater heat (Schwan and Wheals, 2004). The release of energy through anaerobic respiration activity by yeast during the fermentation process caused temperature changes (Kristanto *et al*., 2017).

3.2 Pulp pH

In the 1% TY and 2% TY treatments, the pH of the pulp increased significantly compared to other treatments, approaching a value of 5 by the 6th day. In the 2% BY treatment, the pH increased, with the final value remaining below 4.4. However, the pH remained unchanged in the 1% BY treatment and control (Figure 2). The optimal pulp pH of 4.85 was achieved with the addition of 2% TY, while 1% TY produced cocoa beans with a very low pH of 3.87. A high pH indicates a lower total acid, and vice versa (Ariefta *et al*., 2016).

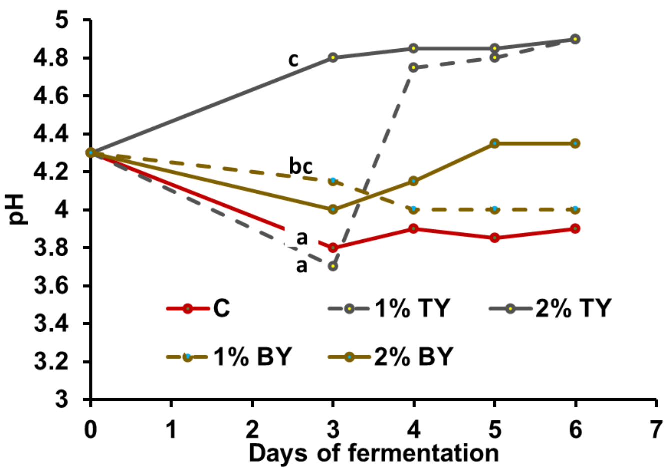

Figure 2. pH of the pulp during fermentation among the treatments. C: control, TY: tapai yeast, BY: baker yeast.

The higher the concentration of TY and the length of fermentation, the more carbohydrates are broken down into glucose, alcohol, lactic acid, and other compounds.

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Microbes transform the sugar compounds in the pulp into organic acids during fermentation. These acids diffuse into the seeds and induce enzymatic reactions to form compounds that contribute to taste, aroma, and color (Afoakwa, 2014).

3.3 Fermentation index

The fermentation index of cocoa beans in the 1% TY treatment and control tended to increase, while in other treatments it tended to decrease. The 1% TY, 2% TY, and 1% BY treatments were significantly different compared to controls. The addition of yeast significantly affected ($P<0.05$) the fermentation index of the beans, while the fermentation time and the interaction of both parameters had no significant effect ($P>0.05$). In addition, the fermentation index in the 1% BY treatment fell below all other treatments (Figure 3).

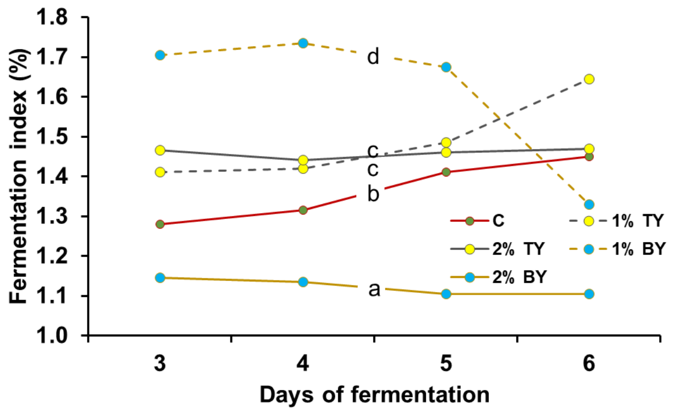

Figure 3. Value of fermentation index of cocoa beans among the treatments. Lines with different notations are statistically significantly different. C: control, TY: tapai yeast, BY: baker yeast.

The average fermentation index value of cocoa beans was above 1 (IF>1), with the highest value of 1.74 in the 1% BY treatment with a fermentation time of 4 days. In the 1% TY treatment during 6 days of fermentation, the fermentation index value was 1.65. In the control and TY treatment, the value increased with time. Meanwhile, the fermentation index in the treatment of BY tended to decrease on day 5 (Figure 3), which was consistent with previous studies. In the 1% BY treatment with sucrose, a higher fermentation index than that of 1% TY was observed (Sabahannur *et al*., 2018; Sabahannur, 2019). The addition of TY to fresh cocoa beans at the beginning of fermentation increased microbial activity during the fermentation process. This yeast culture starter significantly increased the process of removing the slimy pulp and produced more metabolic products, increasing the fermentation index (Cempaka *et al*., 2014). The addition of 1% yeast to fresh cocoa beans was sufficient to increase microbial activity during fermentation.

The fermentation index can indirectly be used as a measurement of the anthocyanin content in cocoa beans. Anthocyanin is one of the main components of polyphenols, which produce a red to purple color with a maximum absorbance of 500-50 nm in acidic conditions (Misnawi *et al*., 2003). Conventionally, fermented cocoa beans have a fermentation index ≥ 1 caused by the formation of the brown-yellow color of the oxidized polyphenols (Nazaruddin *et al*., 2006). The absorbance at 530 nm indicates the level of anthocyanin present (Jinap *et al*., 2002). Polyphenols oxidation can be detected at a wavelength of 460 nm (Romero-Cortes, 2012).

The fermentation index is a measure of the degree of chemical fermentation. It relies on the absorbance level of the fermented compounds and their formation, making it a more objective chemical measurement compared to the cut test. The potential for cocoa flavor can be determined from the quality of the fermentation through a color index called the fermentation index (Kongor *et al*., 2013).

3.4 Slaty beans

The sample showed that the beans were 1.29% slaty, 90.27% dark brown, 6.75% partially brown, and 1.69% defective. The percentage of slaty beans in 1% and 2% BY treatment tended to decrease from days 3 and 5. The average levels in 1% TY, 2% TY, and control treatments were not significantly different. The value in the 2% TY treatment was the same as the control treatment at 1% from day 3 to 6. Furthermore, the 1% TY treatment percentage was significantly lower than in other treatments (Figure 4).

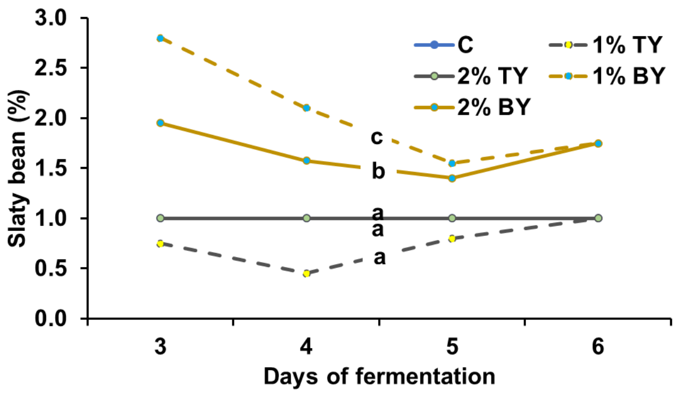

Figure 4. Slaty beans score among the treatments. Lines with different notations are statistically significantly different. C: control, TY: tapai yeast, BY: baker yeast.

The physical quality assessment of cocoa beans has been traditionally conducted through the cut test, which is a visual assessment of the development of fermentation by using an accurate representative sampling. The addition of 1% TY to the 3-day, 4-day, and 5-day fermentations resulted in low-slaty beans with values of 0.75%, 0.45%, and 0.8%, respectively. Slaty beans refer to unfermented cocoa beans with over half of

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their sliced surface exhibiting a greyish slate or blue-gray appearance and a dense cheese-like texture. According to the Indonesian standard, the permissible limit is 3% (quality I - B).

Yeast growth and activity are essential for the success of the cocoa bean fermentation process (Kristianto, 2017). This process generally occurs naturally due to microorganisms present in the fermentation atmosphere (Hii *et al.*, 2006). Fully fermented seeds are characterized by a dark brown color covering 80% of the outer skin and cotyledons and the presence of small pores within the seeds, while partial fermentation may result in different characteristics.

3.5 The pH of cocoa beans

The pH of cocoa beans tended to fluctuate for all treatments. The pH in 1% TY, 1% BY, and 2% BY treatments was significantly higher than in 2% TY and control treatments. The pH in the 2% BY, the 1% TY, and the 1% BY treatment peaked on the 3rd, 5th, and 6th day, respectively (Figure 5).

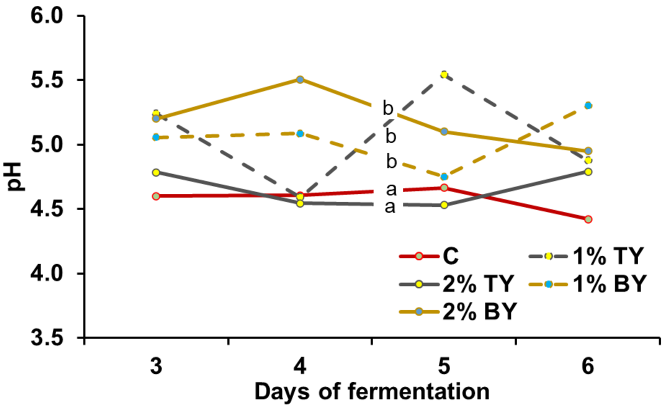

Figure 5. pH of dried cocoa beans among the treatments. Lines with different notations are statistically significantly different. C: control, TY: tapai yeast, BY: baker yeast.

pH of unstored beans fluctuates through the fermentation process. Initially, the pH decreased during 4 from 6.7 to 4.9 and then increased slightly again to 5.3 by the sixth day (Afoakwa *et al.,* 2011). The optimal pH for cocoa beans manufactured in Europe and America ranged from 5.1 to 5.8 (Sabahannur and Nirwana, 2017). A pH higher than 5.1 resulted in relatively better quality (Binh *et al*., 2012). Sabahannur *et al.* (2023) added that a pH of 5.2 was the most preferred. Acidity values expressed in pH units ranging from 5.20-5.50 or an acid titration value of 0.12-0.15 meq/g were considered optimal by chocolate manufacturers. Previous studies reported that the pH in the fermentation of cocoa beans ranged from 5.04 to 5.16 in 5 days (Guehi *et al*., 2010). The use of starter TY and BY at various concentrations showed similar pH patterns, particularly at 1% and 2% concentrations. According to Hii *et al.* (2009), low pH values were usually associated with high acidity levels of cocoa beans, as observed in spontaneous fermentation, where the pH value only reached 4.48 (Figure 5).

3.6 Total acid

Total acid also showed fluctuations across all treatments. The total acid in the 1% TY and 1% BY treatments were significantly lower than those in the 2% TY, 2% BY treatment, and controls (Figure 6).

During fermentation, a consortium of natural yeasts grows, including acetic and lactic acid bacteria responsible for producing alcohol and acids that diffuse into the seeds. This process leads to complex biochemical reactions (Menezes *et al.,* 2016), resulting in changes in the pH or acidity and the formation of precursors. The fermentation process usually reduces levels of organic acids (acetic, lactic, and citric acid) due to the unpleasant aroma of these compounds in cocoa beans (Hernani and Haliza, 2013). Adding a starter produces more microbes and ethanol, degrading into acetic acid. Consequently, the more acid in the dried cocoa beans, the lower the quality of cocoa beans (Apriyanto *et al*., 2016).

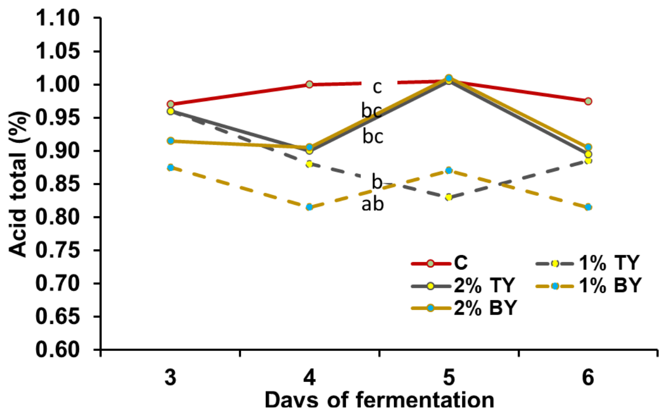

Figure 6. Total acid percentage produced by the treatments. Lines with different notations are statistically significantly different. C: control, TY: tapai yeast, BY: baker yeast.

Sionek *et al.* (2023) stated that acids were produced by microorganisms during fermentation, resulting in an increase in acidity and a subsequent decrease in pH. The organic acids produced during fermentation induce an enzymatic reaction in the seed cotyledons resulting in biochemical changes. Most of these are compounds that give cocoa beans a specific taste, taste, and color (Apriyanto *et al*., 2017).

3.7 Taste of cocoa beans

The results of taste evaluation showed a significant effect of yeast treatments on eight variables, while the effect of treatment time was significant for all variables except for spicy. The interaction of the two factors was significant for cocoa (chocolate), fresh fruit, floral, nutty, and roasted aromas (Table 2).

Table 2. Average temperature range of fermented cocoa beans treated with tapai and beaker yeast.

Dependent variables	Yeast treatment (Y)	Treatment time (T)	Interaction (Y*T)
aroma of cocoa (chocolate)	28.05 **	13.66 **	8.85 **
acidity	22.86 **	18.91 **	1.451 ns
bitterness	2.27 ns	8.21 **	0.84 ns
astringency	1.47 ns	6.26 **	1.32 ns
fresh fruit	43.35 **	13.65 **	13.62 **
brown fruit	11.39 **	4.61 *	2.62 ns
floral	4.40 *	3.47 *	12.97 **
spicy	7.97 **	1.61 ns	2.96 ns
nutty and roasted	4.22 *	22.89 **	20.94 **

*P≤0.05, **P≤0.01, *** P≤0.001, ns: not significant

The sensory test results showed that fermentation with the addition of 2% TY and spontaneous fermentation with a fermentation time of 5 days resulted in a strong cocoa/chocolate aroma (score 6-7), very weak acidity (score 2), weak taste of bitterness and weak astringent (score 3). There were additional aromas in the form of fresh fruit and brown, floral, very weak nutty (score 2) to weak spicy (score 3), and medium roasted (score 5) to strong caramel (score 6) (Figure 7). According to Kusumaningrum *et al*. (2014), South Sulawesi cocoa paste has the most similar aroma and taste profile to cocoa paste from Ghana. This was consistent with the results obtained from this study since cocoa beans used came from cocoa farms in South Sulawesi.

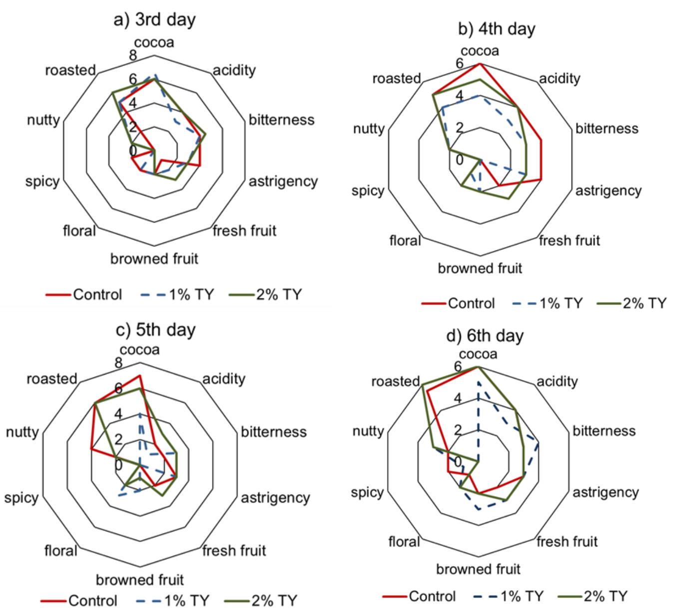

Figure 7. The score of chocolate flavor produced by treatment without yeast (C), 1% (1% TY) and 2% tapai yeasts (2% TY) from a) 3rd day, b) 4th day, c) 5th day, and d) 6th day of the fermentation process.

Previous studies indicated that Indonesian cocoa generally had a weak chocolate taste and a sour and fruity aroma. This taste is very different from Ivory Coast cocoa which has a good brown character, is not too bitter, has a weak sour aroma, but has a fruity and nutty aroma. Meanwhile, Ghanaian cocoa has a very strong chocolate aroma. In terms of melting point, Indonesian chocolate has a higher melting point than chocolate from other countries. It is also known to have a unique taste, which varies depending on the region. This is influenced by geographical diversity, such as the condition of nutrients in the soil and vegetation around cocoa. Therefore, premium quality Indonesian chocolate has a chocolate, bitter, sour, astringent, nutty, and caramel taste (Kusumaningrum *et al*., 2014).

The chelating and bitter taste of cocoa beans is attributed to the high levels of unoxidized polyphenols. This bitterness is influenced by the polyphenols and alkaloids content (Camu *et al*., 2008; Aikpokpodion and Dongo, 2010). The acidity of the beans is a crucial aspect of chocolate flavor due to its association with sour taste and the formation of flavor compounds, particularly during roasting (Cortes *et al*., 2012). Nutty flavor comes from the trimethylpyrazine compound, caramel aroma has a positive correlation with nutty, bitter and sour taste, while astringency has a positive correlation with chocolate aroma (Kusumaningrum *et al*., 2014). Among the taste attributes of chocolate, sourness contributes significantly to the taste of chocolate products. The presence of a slightly sour taste contributes to the balance of chocolate flavor, but the excessive sourness is considered a taste defect (Jinap *et al*., 1994).

One of the most valued characteristics of cocoa beans is their aroma and flavor, which contribute to the final taste of chocolate and other derivative products. Dried cocoa beans are tested for strong cocoa flavor or chocolate flavor, residual acidity, bitterness, astringency, and the presence of unpleasant flavors and positive additional flavors, such as fruity or floral (Quelal-Vásconez *et al*., 2019). A specific aroma of cocoa is the nutty and chocolate flavor that comes from trimethyl pyrazine. In contrast, the acid taste comes from 3-methyl -butanoic acid, caramel comes from 3-hydroxy-2 butanone, and earthy from the 2-ethyl-3.5-dimethylpirazin compound (Kusumaningrum, 2014). Another study stated that cocoa-vicilin-derived peptides were essential precursors of both cocoa and nutty-specific aroma components (Voigt *et al*., 2018).

A close relationship was found between pH and the formation of volatile compounds. Low pH (≤5) supported the formation of pyrazine, while a higher pH supported methyl pyrazine. Dried cocoa beans with 3 days of fermentation had a bitter taste (Ofosu-Ansah *et al*., 2013), and the presence of excessive acid usually

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correlated with the development of chocolate flavor. The entry of acetic acid into the seeds kills the seeds and activates enzymes to prevent chemical changes that improve the taste and aroma of cocoa. Seed acidity is a very important aspect of chocolate flavor because it is related to sour taste and determines the course of reaction of flavor compound formation, particularly during roasting (Romero-Cortes, 2012).

This study showed that the best taste was identified in cocoa beans resulting from a 5-day fermentation period, evident in the control and treatment with 2% TY. The astringency and bitter taste are attributed to polyphenolic lactic acid compounds generated during the fermentation process, which impart undesirable flavors to chocolate products. The compounds responsible for taste are polyphenols, theobromine, and organic acids. Meanwhile, the aroma component is formed from the hydrolysis of protein and sucrose in cocoa beans, producing smelly gasses such as ammonia. One of the factors influencing the level of bitterness is the presence of polyphenol compounds consisting of anthocyanin, leucoanthocyanins, catechins, complex polyphenols, and polyphenols oxidized to form quinones and diquinones. During the fermentation process, the enzyme activity hydrolyzes polyphenols, and exhibits significant polyphenol content (120-180 g/kg) (Bonvehí, 2005), contributing to the formation of a bitter taste in processed products (D'Souza *et al*., 2017). Fermentation for five days was shown to reduce the concentration of polyphenols by about 53.4% of the initial content.

4. Conclusion

In conclusion, fermentation of small-sized cocoa beans (10 kg/box) with 1% and 2% TY improved the physical, chemical and flavor qualities compared to spontaneous fermentation (control) and BY. The pH when fermented with 1% TY was slightly higher than that of 2% TY. Other parameters, including the pH of pulps, fermentation index, and total acid, were not significant between fermentation using 1% and 2% of TY. Additionally, fermentation time had no significant effect on the chemical quality of the cocoa beans. The fermentation time had a significant effect on flavor on the 5th day of fermentation.

Conflict of interest

The authors declare no conflict of interest.

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