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Research article

Aroma precursor enhancing in dried cocoa beans fermentation using enzyme and heat addition

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Abstract: Changes in amino acids and reducing sugars in cocoa beans during fermentation were investigated using a 3 × 3 full complete factorial design using different enzyme additions (cellulase, papain and control–no enzyme) and water bath temperatures (40, 45 and 50 ℃) as variables over three days of fermentation. Aroma precursors (reducing sugars and free amino acids) developed inside the bean by enzymatic mechanisms during fermentation are converted into volatile compounds such as pyrazines and aldehydes during roasting. This study aimed to improve the fermentation process of dried beans by adding acetic acid, heat and enzymes, because there is insufficient pulp for the ideal spontaneous fermentation process. Samples were analyzed for fermentation index, cut bean, reducing sugar amino acid composition and volatile aroma composition profile using headspace solid phase microextraction (HS-SPME) and gas chromatography mass spectrometry (GC-MS). The results showed that the fermentation index was significantly affected by the addition of enzymes and water temperature. Although amino acids rose to 200%–300%, the composition contained several acidic amino acids because the pH utilized less than 4. Adding cellulase enzymes increases the amount of reducing sugars and amino acids but does not result in the formation of various amino acids.

Keywords: Amino acid; cut test; cellulase; cocoa fermentation; reducing sugar; volatile aroma

1. Introduction

The cocoa fermentation purpose is to initiate biochemical events in the beans that lead to the formation of aroma precursors, enhance flavor and color, reduce bitterness and improve the physical appearance of cocoa [1]. Cocoa bean fermentation works through two stages. The first involves pulp processes, whereas the second involves many hydrolytic reactions occurring inside the bean [2]. The first phase is carried out between 25 and 45 ℃, whereas the second phase is carried out at 42–52 ℃ [3]. Microorganisms such as yeast, lactic acid bacteria (LAB) and acetic acid bacteria (AAB) conduct the fermentation of cocoa pulp and generate acids and ethanol during the first stage of fermentation [4]. The degree and duration of exposure to acids in the cotyledons during fermentation have a major influence on bean quality. These acids damage the cotyledon bean and cause a chain of metabolic processes in the bean that produce aroma precursors [5] such as reducing sugars and free amino acids by enzymatic mechanisms during fermentation.

In Indonesian smallholder plantations, dried cocoa beans are frequently unfermented. Farmers are hesitant to ferment cocoa beans because the price of fermented cocoa beans, which require an additional 6 days, is the same as the price of non-fermented cocoa beans. Furthermore, the usual harvest is only 10–20 kg/ha, which is still insufficient for spontaneous fermentation. The government encouraged the industry to absorb non-fermented cocoa beans by banning imports and increasing the import cost of fermented cocoa beans by 5%. The unfermented and fermented cocoa beans were subsequently combined by a local cocoa processing company. However, limitations in the importation of fermented cocoa beans have resulted in a scarcity of raw materials and limited market development.

Another method of fermenting cocoa beans is to re-ferment dried beans [6]. It was suggested that re-fermentation, in which dried beans from farmers are rehydrated and fermented again, should occur at the sub-district level [7]. The lack of pulp in the dry cocoa beans used in the re-fermentation process prevents the development of acid and heat. Therefore, acetic acid must be added to the re-fermentation system. In the cocoa fermentation simulation process, the addition of acetic acid and alcohol as artificial pulp media resulted in a decrease in polyphenols and an increase in volatile compounds, such as phenylethyl alcohol, 2-phenylethyl acetate, acetoin and 3-methyl butyl acetate [8]. Maintaining heat in a jacketed system with a small capacity (10 kg) during the final days of fermentation (72–120 hours) can result in cocoa beans with a good aroma and quality comparable to spontaneous fermentation with a capacity of 300–500 kg at 6 days in terms of the fermentation index value [9].

Frequently, farmers mistakenly ferment cocoa beans by temporarily putting them in sacks for one day in the afternoon or when it rains, resulting in partially fermented cocoa beans. Partially fermented cocoa beans have lower enzyme effectiveness than unfermented dry cocoa beans [10]. If the re-fermentation process is performed on dry cocoa beans, the fermentation process is not optimal because of a decrease in the concentration of enzymes inside the cocoa beans. The addition of protease or cellulase enzymes to the fermentation system is designed to increase the efficiency of converting proteins into amino acids and sugars into reducing sugars when using partially fermented cocoa beans.

In this study, dry cocoa bean fermentation was performed by soaking the cocoa beans in water, adding acetic acid, protease or cellulase enzymes, and reheating them without immersion. Three replicates of a 3 × 3 factorial treatment with enzyme type and heating temperature were performed. Each stage of fermentation changes daily until it is completed after three days, after which the product was dried and roasted. The primary indicators are the fermentation index and contents of amino acids and volatile chemicals. The fermentation index is used in the analysis to track the changes in

anthocyanin levels that fall during fermentation. The 460 nm/530 nm ratio of the cocoa methanolic acid extract has been demonstrated to be a reliable indicator of the fermentation index, with a non-linear relationship to the sensory cut test [11]. This study aims to improve the fermentation process of dried cocoa beans by adding heat and enzymes.

2. Materials and methods

Scavina 6 (Forastero cocoa varieties) were supplied by the Indonesian Industrial and Beverage Crops Research Institute (IIBCRI). Dried cocoa beans were prepared by fermenting fresh cocoa beans for one day and then sun-drying for four days. The acidic liquid was made by combining the remaining liquid from harvesting nata de coco and residual water from soaking nata de coco, which was filtered and stored in a refrigerator. The utilized nata de coco liquid has a pH of 4, but it can be made more acidic by adding glacial acetic acid solution.

2.1. Fermentation procedure

Rehydrating dried cocoa beans involves soaking the beans in water for eight hours. After soaking, the cocoa beans were placed in a plastic container with an acidic liquid with a pH of 4, the container was closed, and they were aerated with air. The plastic container was then immersed in warm water at 45 ℃ for one day for incubation. Except for the control group, which used water, after one day, the fluid was removed and replaced with an enzyme solution for incubation one day later. A complete 3 × 3 factorial design using different enzyme additions and water bath temperatures is presented in Table 1. After enzyme incubation, the cocoa beans were heated to the treatment temperature for one day without water.

Table 1. Dry bean fermentation research design.

Enzyme treatment	Temperature		
	40 ℃	45 ℃	50 ℃
Cellulase (0.04 g/ml)	P1-40	P1-45	P1-50
Papain (0.04 g/ml)	P2-40	P2-45	P2-50
Control (Without Enzyme)	P3-40	P3-45	P3-50

After the fermentation process, the cut test and fermentation index were used to determine the quality of the fermentation results. Fermented beans were dried in an oven at 55 ℃. Each treatment was carried out in triplicate, and the fermentation process took three days to complete. The best treatment for the fermentation index was turned into a cocoa paste after roasting, which was then tested using GC-MS. Amino acid tests were also conducted.

2.2. Fermentation index

The fermentation index was calculated using the absorbance ratio at 460 nm/530 nm and the extraction of cocoa bean pigment with methanol-HCL [12]. A dark container was filled with 50 ml of 97:3 v/v methanol / hydrochloric acid solution, followed by cocoa powder (0.5 grams). Subsequently, the solution was cooled for 16–18 hours at 8 ± 2 ℃. This solution was filtered using a Whatman

No. 4 filter, yielding a clear extract that was quantified using a spectrophotometer.

2.3. Bean cut test

The bean cut test can also be used to determine the degree of cocoa bean fermentation. Thirty beans were cut down the middle lengthwise. Both sides could be slaty (not fermented), purple (not fermented enough), purple brownish (moderately fermented), and brown (well fermented). Each treatment was performed in triplicate.

2.4. Determination of amino acids

Amino acid composition analysis was performed using ultra-performance liquid chromatography (UPLC) to measure amino acid levels [13,14]. UPLC analysis began with the preparation of a calibration standard as an internal standard using the Waters Amino Acid Hydrolysate Standard. Waters Amino Acid Hydrolysate Standard is a mixture of protein hydrolysate standard, containing 2.5 mM of each of the following amino acids dissolved in 0.1 N HCl: L-alanine, L-arginine, L-aspartic acid, L-glutamic acid, L-glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tyrosine and L-valine.

Subsequently, sample preparation was performed by weighing 1 gram of sample into a 20 ml headspace vial followed by hydrolysis with 5–10 ml of 6 N HCl in an oven at 110 ℃ before being cooled to room temperature. The hydrolyzed sample was then transferred to a 50 ml volumetric flask. Double distilled water was added to the mark and homogenized. The solution was filtered using a 0.2 µm syringe filter, and the resulting filtrate was collected. Internal standard were added, and the samples were derivatized using the AccQ-Fluor reagent kit. The solution was then injected into the UPLC system. The amino acid content of the sample was obtained from interpretation using the ratio of the analyte area to the internal standard area. The formula used was as follows:

$$\text{Amino acid (mg/kg)} = \frac{\frac{L_1}{L_2} x \frac{C_{asam\ amino}}{1000000} x\ BM_{asam\ amino} x\ V_a\ x\ F_p}{W_{smpl}}. \quad (1)$$

L_1 = Area of an amino acid analyte,
L_2 = Area of standard analyte,
$C_{asam\ amino}$ = Concentration of standard amino acid solution (pmol/µL),
BM = Amino acid molecular weight,
V_a = Final sample volume (µL),
F_p = The dilution factor,
W_{smpl} = Sample weight (g).

2.5. Reducing sugar

Dinitrosalicylic acid (DNS) from the Miller method [15] was utilized for sugar reduction with modifications. A standard glucose stock solution was used to create a standard curve for decreasing sugar content. A spectrophotometer with a wavelength of 540 nm was used to measure the absorbance of the solution. The reducing sugar content was determined after mixing 3 mL of DNS reagent into a test tube containing 1 mL of supernatant (sample), followed by incubation at 100 ℃ for 15 minutes,

cooling and then measurement using a spectrophotometer with a wavelength of 540 nm. The reducing sugar content of the sample (mg/mL) was calculated using the mathematical equation of the conventional linear regression curve of the reducing agent.

2.6. Volatile aroma composition

Volatile aromas were analyzed in the Indonesian Center for Rice Research (ICRR) flavor laboratories in Sukamandi-Subang, West Java. Samples of 50 grams were roasted in a BRZ GT-2 Probat roaster at an initial temperature of 130 ℃. The airflow was adjusted to a half opening with a medium heat level and a 2 mm bar pressure in the device for the first 5 minutes. At 150 ℃, the roasting process was completed after 10 minutes in the medium roast, and then they were deshelled. Cooled roasted beans were ground to obtain the cocoa liquor.

The testing procedure for volatile aroma composition was carried out using solid phase micro-extraction (SPME) fibers composed of divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) and GCMS [9]. The linear retention index (LRI) was calculated for each peak using the standard n-alkane retention time data (C9-C33), with injection under identical conditions as the sample. A comparison was made between the LRI value calculation and identification of volatile components in the National Institute of Standards and Technology (NIST) 14 database. Principal component analysis (PCA) was performed using XLSTAT version 20 on the peak areas of the selected aroma components, using an average of three replicates. The data were mean-centered by subtracting the mean estimation and scaling of each variable to obtain the unit difference for each factor (automatic scale).

2.7. Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA), followed by the post-hoc Duncan multiple range test ($p < 0.05$) and Tukey's honestly significant difference (HSD) test ($p < 0.05$). Data are expressed as means ± standard deviation (mean ± SD).

3. Results and discussion

3.1. Fermentation index of fermented cacao made from dried beans

The fermentation index (FI) values were determined for the three heating temperatures (Figure 1), and the dried cocoa bean cut test results supported the findings. FI values greater than one indicate that the beans have been fully fermented, whereas lower values suggest that the beans have been less fermented [12,16].

Due to the anthocyanin concentration, unfermented beans have a high absorbance at 530 nm, resulting in a low FI of approximately 0.5 in raw cocoa. The rate of anthocyanin hydrolysis is different at high temperatures (45 ℃ and 50 ℃) and lower temperatures (40 ℃); therefore, the final value of the fermentation index is different.

This decrease in anthocyanin degradation-hydrolysis could be related to lower glycosidase activity in cocoa beans due to lower temperatures [17]. Temperature and the presence of water during incubation may have activated the residual glycosidase and polyphenol oxidase, causing the beans'

polyphenols to hydrolyze and oxidize, resulting in the reduction of purple beans and development of brown beans. In Figure 1, the vertical bars reflect the standard deviations of three separate determinations, followed by different superscripts where differences were statistically significant (Duncan, $p < 0.05$).

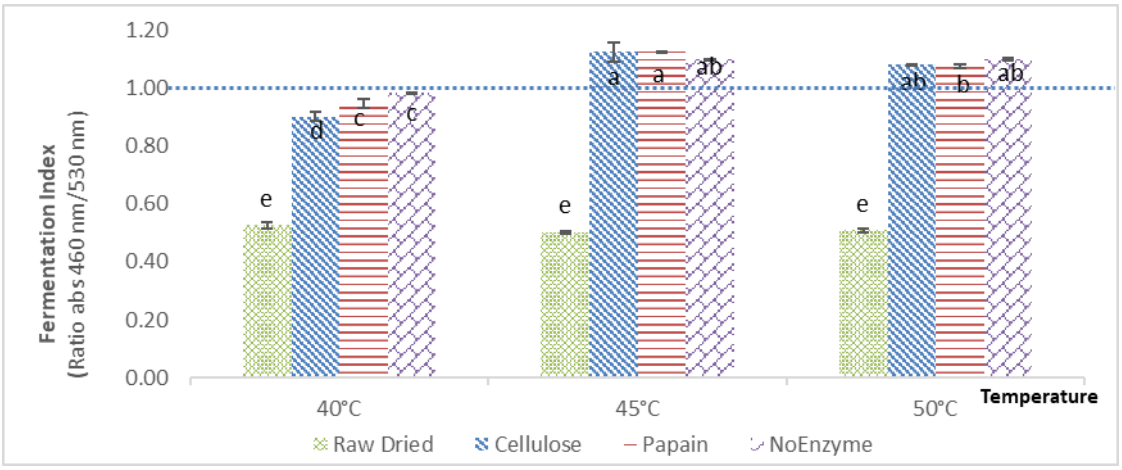

Figure 1. Fermentation index after three days of fermentation in triplicate.

Note: Different superscripts indicate significant differences (Duncan, $p < 0.05$).

Levene's test revealed that the variances of the groups were unequal ($F(11,24) = 8.138$, $p = 0.000$). Using a one-way factorial ANOVA, the influences of enzyme type and water temperature, as well as their interactions, on the dry cocoa fermentation index were investigated. The influence of enzyme type and water temperature was statistically significant ($p = 0.001$). The main impact of the enzyme type was significant at 0.992, suggesting that enzyme type could explain 99.2% of the variance in the fermentation index ($F(3,24) = 963.168$, $p = 0.0001$). Duncan's test showed that raw dry beans had a much lower fermentation index than the other enzyme treatments. The primary effect of water temperature was 0.866, accounting for 86.4% of the variation in the fermentation index ($F(2,24) = 76.084$, $p = 0.000$). A water temperature of 40 °C had a lower fermentation index than the others. We also computed a factorial ANOVA for each treatment, as shown in Figure 1 ($F(11,24) = 284.364$, $p = 0.000$). The reduction in the fermentation index at 50 °C compared to 45 °C in the enzyme treatment was attributed to temperature-enzyme interactions and color changes related to tannin accumulation. The more tannin molecules there are, the darker the cocoa color.

3.2. The bean cut test scores were used to classify cocoa beans based on their appearance.

The degree of fermentation in cocoa beans can be determined quickly using a bean cut test score. Based on these results, the samples were classified as slaty, violet or brown, and their transitional stages were based on their appearance. The results showed that slaty, dark purple, purple-brown and brown colors were significantly different ($p < 0.05$) between the raw dry beans and all treatments in the dry fermented bean cut test (Table 2). A more significant percentage of purple-brown seeds was incubated at 50 °C water temperature with cellulase enzymes than purple-brown seeds incubated at 45 °C. According to Takrama [18], purple-brown cocoa beans are not defective, and their appearance

is not a serious issue because they are close to brown, which may take a little longer in the fermentation process [19].

Table 2. Bean cut test of dry bean fermentation.

Code	Bean cut test (%)			
	Brown	Purple-brown	Deep purple	Slaty
Raw dried	5.6 ± 1.1^{c}	8.9 ± 2.2^{c}	63.3 ± 1.9^{a}	22.2 ± 4.0^{a}
P1-40	42.2 ± 2.2^{ab}	50.0 ± 3.3^{ab}	7.8 ± 1.1^{b}	0.0 ± 0.0^{c}
P2-40	46.7 ± 3.3^{ab}	47.8 ± 1.1^{ab}	5.6 ± 2.9^{bc}	0.0 ± 0.0^{c}
P3-40	48.9 ± 1.1^{ab}	45.6 ± 1.1^{ab}	0.0 ± 0.0^{c}	5.6 ± 1.1^{b}
P1-45	53.3 ± 3.3^{a}	42.2 ± 6.8^{b}	4.4 ± 4.4^{bc}	0.0 ± 0.0^{c}
P2-45	53.3 ± 6.7^{a}	41.1 ± 8.0^{b}	5.6 ± 2.2^{bc}	0.0 ± 0.0^{c}
P3-45	52.0 ± 1.1^{ab}	44.4 ± 1.1^{ab}	2.2 ± 1.1^{bc}	1.1 ± 1.1^{c}
P1-50	36.7 ± 8.4^{b}	62.2 ± 8.9^{a}	1.1 ± 1.1^{bc}	0.0 ± 0.0^{c}
P2-50	45.6 ± 9.5^{ab}	54.4 ± 9.5^{ab}	0.0 ± 0.0^{c}	0.0 ± 0.0^{c}
P3-50	57.8 ± 2.2^{ab}	38.9 ± 2.9^{b}	2.2 ± 2.2^{bc}	1.1 ± 1.1^{c}

Note: Columns with different superscripts have significant differences (Duncan's test, $p < 0.05$).

The results of the three-day cut test for the dry bean fermentation process were similar to those of the six-day spontaneous fermentation process. According to Afoakwa [20], pod storage (0 days) and a fermentation duration of 6 days resulted in bean cut test findings of purple-brown 32% and brown 58%. The cell walls of cocoa beans shrink and partially crack during the drying process, resulting in the release of anthocyanins and a color change to purple. Anthocyanins are also released when the cell walls of dried beans are enlarged and broken during soaking and acidification processes. Polyphenols and polymers oxidize proteins via hydrogen bonding to generate condensed tannin complexes, which are insoluble high-molecular-weight compounds that are brown pigments [21]. The polyphenol polymerization process considerably reduces its concentration, bitter taste, astringency of cocoa, and it promotes the development of chocolate color. Polyphenol polymerization changes the color of cocoa beans to brown rapidly during dried bean fermentation.

3.3. Determination of amino acids in fermented cocoa beans

Enzyme activity in cocoa beans breaks down proteins, resulting in free peptides and amino acids [22]. Peptides and amino acids react with reducing sugars during roasting to form the aromatic components of chocolate (Maillard reaction). Unfermented cocoa beans contain low levels of free amino acids [23]. During fermentation, the overall amino acid content increased by roughly 150%–200% [24,25], but the final concentration was highly dependent on the pH. The dried bean fermentation approach yielded more acidic amino acids (aspartic acid and glutamic acid), possibly because of the use of additional acid at pH 4, resulting in over-acidification, as indicated in Table 3. Low pH at the beginning of the fermentation process lowers the flavor precursors and results in over-acidification of the product [26]. Amino acid precursors in fermented cocoa beans (cellulase enzyme and heat treatment) increase the amount of amino acids by two to three times. These amino acid results were still more significant than that of Sabahannur [27], who used the same analytical technique with a 6-day spontaneous fermentation procedure.

Table 3. Amino acid profile of unfermented and enzyme-fermented dried bean fermentation at 45 °C.

Amino acid	Code	Raw Dried mg/g	P1-45 mg/g	P2-45 mg/g
Hydrophilic				
Serine	Ser	0.61 ± 0.03^{c}	1.46 ± 0.07^{a}	0.84 ± 0.04^{b}
Threonine	Thr	0.50 ± 0.02^{c}	1.20 ± 0.08^{a}	0.71 ± 0.04^{b}
Cysteine	Cys			
Asparagine	Asn			
Glutamine	Gln			
Tyrosine	Tyr	0.37 ± 0.06^{c}	0.94 ± 0.07^{a}	0.62 ± 0.05^{b}
Acidic				
Aspartic acid /aspartate	Asp	0.95 ± 0.05^{c}	2.14 ± 0.14^{a}	1.26 ± 0.08^{b}
Glutamic acid / glutamate	Glu	1.57 ± 0.11^{c}	3.76 ± 0.24^{a}	2.03 ± 0.08^{b}
Hydrophobic				
Glycine	Gly	0.55 ± 0.06^{c}	1.32 ± 0.10^{a}	0.77 ± 0.05^{b}
Alanine	Ala	0.46 ± 0.04^{c}	1.03 ± 0.06^{a}	0.60 ± 0.04^{b}
Valine	Val	0.60 ± 0.07^{c}	1.42 ± 0.06^{a}	0.80 ± 0.05^{b}
Leucine	Leu	0.71 ± 0.07^{c}	1.65 ± 0.09^{a}	0.95 ± 0.05^{b}
Isoleucine	Ile	0.41 ± 0.04^{c}	0.97 ± 0.06^{a}	0.55 ± 0.04^{b}
Proline	Pro	0.53 ± 0.04^{b}	1.26 ± 0.12^{a}	0.69 ± 0.05^{b}
Phenylalanine	Phe	0.79 ± 0.06^{c}	1.70 ± 0.11^{a}	1.16 ± 0.05^{b}
Methionine	Met			
Tryptophan	Trp			
Others				
Arginine	Arg	0.79 ± 0.09^{b}	1.93 ± 0.20^{a}	1.13 ± 0.12^{b}
Lysine	Lys	0.60 ± 0.06^{b}	2.23 ± 0.14^{a}	0.67 ± 0.05^{b}
Histidine	His	0.28 ± 0.06^{b}	0.64 ± 0.06^{a}	0.40 ± 0.03^{b}
Total		9.78 ± 0.81	23.67 ± 0.33	13.22 ± 0.13
The ratio of hydrophobic/acidic free amino acids (FAA)		1.61	1.58	1.68

Note: Rows with different superscripts have significant differences (Tukey's HSD, $p < 0.05$).

Table 3 shows two hydrophobic amino acids with high final concentrations following enzyme addition at 45 °C, leucine and phenylalanine. The most abundant hydrophobic amino acids were alanine, phenylalanine, leucine, valine, tyrosine and isoleucine, which are essential for the synthesis of typical cocoa aroma components after roasting in the presence of reducing sugars [28,29]. According to Rohsius [23], after fermentation, high-quality cocoa beans should have an amino acid content of 8–14 mg / g total dry matter, and the hydrophobic to acid ratio ranges between 2.0 and 2.3 when the total amount of free amino acids is low. The highest ratio of 4.6 to 4.8 was found in the samples with high free amino acids. Despite the significant amount of amino acids produced, the hydrophobic/acidic ratio in each dried bean fermentation treatment was still lower than the results of Rohsius [23]. A low hydrophobic/acidic ratio in dried beans indicates that the development of amino acids depends on the performance of the internal enzymes of the cocoa and the acid (pH) that activates them.

3.4. Determination of reducing sugar in fermented cocoa beans

The yield of reducing sugars produced by cellulase enzymes in dry bean fermentation was higher than in any other treatment (Table 4). Reducing sugars may contain more types of glucose than fructose because of the conversion mechanism of the cellulase enzyme. In spontaneous fermentation, the fructose/glucose ratio can be used to measure how well the fermentation process works [30]. Rottiers [30] also found that a fructose/glucose ratio of 2:1 in the Arriba/Trinitario variety provided a reasonably acceptable aroma.

Table 4. Reducing sugars (mg/g) in dry bean fermentation.

Enzyme treatment	Water temperature		
	40 °C	45 °C	50 °C
Unfermented (mg/g)	4.63 ± 0.02^{h}	4.58 ± 0.02^{h}	4.62 ± 0.02^{h}
Cellulase (mg/g)	6.68 ± 0.19^{e}	14.75 ± 0.08^{a}	10.47 ± 0.19^{c}
Papain (mg/g)	6.18 ± 0.06^{fg}	8.97 ± 0.18^{d}	10.91 ± 0.11^{b}
Control (mg/g)	5.93 ± 0.21^{g}	6.12 ± 0.02^{g}	6.52 ± 0.05^{ef}

Note: Different superscripts indicate significant differences (Duncan, $p < 0.05$).

3.5. Volatile aroma composition of fermented cacao made from dried beans

Volatile cocoa compounds are formed from aroma precursors released during fermentation and drying. The typical chocolate flavor is obtained during the roasting stage via Maillard reactions and Strecker degradation of aroma precursors and their intermediates [31]. Approximately 600 volatiles have been identified in the cocoa taste [32]. After medium roasting, the volatile chemicals in the cocoa mass were 217 compounds (unfermented), 235 compounds (cellulase) and 243 compounds (papain and control).

A two-part PCA model was developed based on the GC–MS peak area to illustrate the impact of the varied enzyme treatment in warm water at 45 ℃ on the volatile aroma profile. The score plot in Figure 2 indicates the separation of samples due to variations in unfermented and enzyme treatments along PC2, with an evident variation of 98.55%. Unfermented samples were separated by 14 volatile chemicals on the positive y-axis, whereas the negative axis separated the other roasted samples. Some of the volatile compounds on the positive y-axis are 1) alcohol: 2-pentanol, 2-furan methanol and phenyl-ethyl alcohol and 2) pyrazine: methyl pyrazine, 2-ethyl-6-methyl-pyrazine, 2,6-dimethyl-pyrazine, 2-ethyl-5-methyl-pyrazine, 2,5-dimethyl-pyrazine, trimethyl-pyrazine. The number of aldehydes was not reduced by medium roasting for a short time (10 minutes). Aldehydes are essential reactants in the synthesis of heterocyclic compounds, pyrazines and flavor components [32].

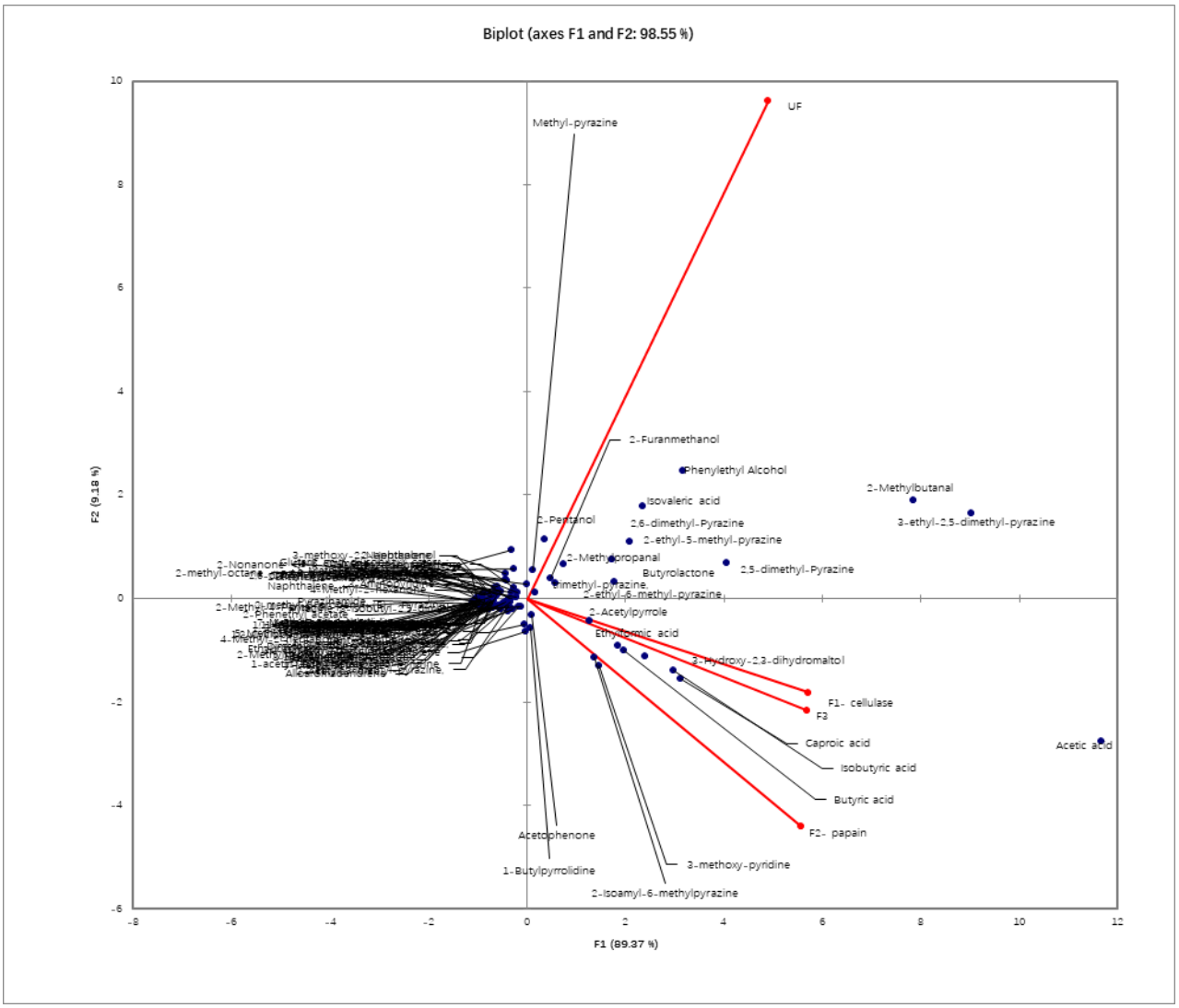

Figure 2. Separation of samples based on their volatile aroma profiles at 45 ºC.

There was a strong relationship between the volatile aroma composition of the medium roasting process (Figure 2) and the high acidic amino acids (Table 3). The addition of acetic acid at pH 4 during the processing stage turns the cocoa bean excessively acidic, resulting in the production of less hydrophobic amino acids. The carboxypeptidase enzyme produces optimal protein decomposition to hydrophobic amino acids at pH 5.2 [33], so that the pH conditions are not too acidic, which is required for its activation.

In Figure 2, the treatments with the cellulase enzyme (F1) and without the enzyme (F3) are in the same region. The use of cellulase enzymes did not result in the formation of new volatile compounds, even though they produced sufficient total amino acids and reduced sugars. Adding cellulase enzymes only helps optimize the function of internal enzymes by damaging the glucose network in the cell walls, so that acid penetration into the cells is more significant.

4. Conclusions

The fermentation index was significantly affected by the enzymes and temperature. Fermentation of amino acid precursors utilizing cellulase enzymes and a temperature of 45 ℃ increases the amount of amino acids up to 2–3 times the number of amino acids in unfermented cocoa beans. However, some amino acids remain acidic because the pH is too low during the process. The composition of volatile components, in addition to cellulase enzymes and the control, showed the same PCA pattern. The addition of cellulase enzymes does not form different amino acids and can increase the amount of aroma precursors (reducing sugars and amino acids).

Use of AI tools declaration

The authors declare they have not used Artificial Intelligence (AI) tools in the creation of this article.

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Conflict of interest

We declare no conflicts of interest.

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