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*Research article*

# Effects of the maturity level and pod conditioning period of cocoa pods on the changes of physicochemical properties of the beans of Sulawesi 2 (S2) cocoa clone

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**Abstract:** Cocoa quality largely depends on postharvest processing, including fruit maturity levels and practices, such as ripening (pod conditioning), fermentation, drying, and roasting. This study aimed to identify the effects of the maturity level and pod conditioning period of cocoa pods on the changes in the physicochemical properties of the beans of Sulawesi 2 (S2) cocoa clone from Pinrang Regency, South Sulawesi, Indonesia. Two treatment variables were applied and assessed: fruit maturity levels (treatment A) and duration of pod conditioning (treatment B). Results indicated that pulp weight ranged from 29.69–47.96 g/100 g fresh beans, pulp moisture was 74.43%–83.29%, total sugar content was 4.28%–11.91%, sucrose content was 0.33%–0.88%, glucose/fructose was 3.84%–11.09%, and cocoa pulp pH was 3.27–3.79. The bean moisture was 37.13%–58.67%, bean weight was 54.19–72.4 g/100 g fresh beans, and polyphenol content ranged from 4.76–13.05 mg/g in gallic acid equivalents. Statistical analyses indicated that fruit maturity level did not have a significant effect on the weight, moisture, total sugar content, glucose/fructose content or pH of the pulp or the moisture content and weight of the bean. However, fruit maturity level significantly affected the polyphenol and pulp sucrose contents of the bean. Furthermore, pod conditioning had significant effects on the weight, total sugar content, sucrose content, and glucose/fructose content of the pulp as well as the weight and polyphenol contents of the beans. The effects of pod conditioning on the moisture and pH of the pulp and the bean moisture content were insignificant. Pod conditioning for 6

days with an A2 maturity level not only reduced the acidity of the cocoa beans, but it also increased the polyphenol content and the weight of the beans. Increased bean weight will increase the yield of cocoa beans. Therefore, pod conditioning for 6 days with an A2 maturity level can be applied to the cocoa processing industry, especially before carrying out the cocoa bean fermentation.

**Keywords:** cocoa; pulp; beans; pod conditioning; pod maturity

## 1. Introduction

Cocoa beans are the raw material for the chocolate manufacturing industry. Cocoa beans are processed into cocoa butter, powder, cake, and liquor. High-quality cocoa beans produce excellent chocolate products and their derivatives. Cocoa bean quality largely depends on the postharvest process, which includes fruit maturity at the time of harvest, and postharvest practices, such as ripening (pod conditioning), fermentation, drying, and roasting [1]. The maturity of cocoa fruit affects the sugar content and microorganisms. A fully mature fruit has a high content of sugar, which functions as a substrate for microbial activities during pod conditioning and fermentation. In addition, the ripeness of the fruit affects the dry bean yield, bean appearance and dry bean quality. Fruit storage is a pulp conditioning method applied to freshly harvested cocoa pods. Farmers store cocoa pods for a few days before splitting these pods to obtain a taste, improve the uniformity of fruit ripening, reduce the amount of pulp covering wet cocoa beans, and promote the removal of cocoa beans from cocoa pods [2].

The fermentation method of cocoa beans determines the quality of the resulting product, especially the flavor. Research on postharvest fruit storage revealed an enhancement in chocolate flavor and a reduction in acidity, bitterness, and astringency [3]. Variations in fermentation conditions, such as pod conditioning before fermentation, affect pH, temperature, enzyme activity, and flavor development. Fermentation is fast when the cocoa beans are obtained from a ripened fruit [4]. Cocoa beans that have not undergone pod conditioning have fruity, floral and spicy aromas, and those with prolonged pod conditioning contain cocoa and nut aromas [5]. Different types of cocoa can produce different flavor components [6]

The physical and chemical changes that occur in cocoa beans due to pod conditioning before fermentation are not yet fully understood. Hence, studying the changes in pulp and cocoa beans during pod conditioning is necessary. Although the potential of pod conditioning before pod opening and fermentation to enhance the quality of cocoa beans has been explored [7], assessments of the combined influence of pod conditioning and fruit maturity levels on pulp and bean properties are lacking. Changes in the pulp and beans during pod conditioning can affect fermentation conditions and the quality of the produced cocoa beans. One of the successes of a fresh cocoa bean fermentation process is determined by the maturity level of the fruit [8]. Therefore, the current work aimed to analyze the changes in pulp and cocoa beans during pod conditioning at three different levels of pod maturity.

## 2. Material and methods

### *2.1. Materials*

The cocoa pod sample used in this study was the S2 clone of Sulawesi cocoa (var. *Trinitario*). Sulawesi cocoa clone S2 is local Sulawesi cocoa (*Trinitario* variety) resulting from crosses of *Criollo* and *Forastero* varieties. The samples were obtained from the Bukit Tinggi Cocoa Farmers Group, Tapporang Village, Batullappa District, Pinrang Regency, South Sulawesi Province, Indonesia. The fruits were harvested at a local cacao farm by cutting the fruit stem from the branch. Cocoa pods were harvested at three levels of maturity: level A1 at the early-maturity stage (reddish-purple fruit color with slight color change in fruit grooves), level A2 at the medium-maturity stage (red to orange color), and level A3 at the fully-mature stage (yellow to slightly orange color). The appearance of fruits at the three levels of maturity is shown in Figure 1. The experiment was conducted using a completely randomized design with three replications. The treatments used in this study were pod maturity level (A1, A2 and A3 level) and ripening (pod conditioning) period (0, 1, 2, 3, 4, 5 and 6 days corresponding to B0, B1, B2, B3, B4, B5 and B6, respectively). Pod conditioning was performed under shade to avoid rain and direct sunlight with a surrounding temperature of about 26–31 ℃. The measured chemical and physical parameters were moisture content, weight, total sugar, sucrose, glucose/fructose and pH of the pulp or the moisture, polyphenol content and weight of the cocoa beans per 100 g of fresh cocoa beans (covered with pulp). Chemical analyses were performed at the Department of Chemical Engineering, State Polytechnic Ujung Pandang, South Sulawesi, Indonesia.

### *2.2. Measurement of pH*

Pulp pH was measured using the method outlined by the Association of Official Analytical Chemists [9]. In brief, 10 g of pulp sample was placed in a beaker, followed by the addition of 90 mL of hot distilled water with a temperature of 70–80 ℃. The mixture was then stirred using a magnetic stirrer at a low speed until a homogeneous suspension was formed, which was then filtered. The filtrate was allowed to cool to the ambient temperature of 27 ± 2 ℃, and its pH was measured using a pH meter (Metrohm AG, Swiss).

### *2.3. Pulp and bean weight*

Pulp was removed from 100 g of cocoa beans by scraping using a sharp cutter. The pulp and beans were weighed using an analytical balance (accuracy ± 0.001 g).

### *2.4. Moisture content using gravimetric method*

Moisture content was measured using the gravimetric method [10]. Cocoa bean samples were first cut into small pieces. Approximately 2 g of the sample was then dried in an oven at 105 ℃ until a constant weight was achieved. Water content was calculated as the weight loss of the sample ($M_w$) during drying by using Equation (1).

$$\text{Mwb} = \frac{\text{Mw}}{\text{Ms}} \times 100\ \% \quad (1)$$

Description:
$M_{wb}$ = moisture content on wet basis, %
$M_w$ = weight of water in the cocoa bean samples, g
$M_s$ = total the cocoa bean samples weight, g

### *2.5. Determination of total phenolic content*

Total polyphenol content was determined using the Folin–Ciocalteu method described by Christova-Bagdassrian et al. [11] with slight modifications. In brief, 1 g of ground cocoa beans was freed of fat by extraction with 25 mL of hexane at room temperature for 24 h. The extract was then centrifuged at 2500 rpm for 10 min. This procedure was repeated twice. The phenolic content of the fat-free sample was then extracted twice using 25 mL of a 70% methanol solution by sonicating for 25 min. After each sonication event, the mixture was centrifuged at 3500 rpm for 15 min and the liquid phase was separated. After all obtained liquid phases were combined, 0.25 mL of the mixture was transferred to a 10 mL volumetric flask. The extract was mixed with 0.5 mL of Foli–Ciocalteau (0.2 N) solution and 2 mL of 20% $Na_2CO_3$. Distilled water was added to the mixture to obtain a final volume of 10 mL; it was then homogenized by allowing it to stand for 30 min. Sample absorption was measured by using the UV-visible spectrophotometer, Orion AquaMate 8000 (Orion, Nagano Japan) at wavelengths of 720–740 nm. For the determination of the total polyphenol content, a standard curve for gallic acid solution (2–20 mg/L) was first prepared using the same instrument. The results of the absorption readings of each sample were plotted against the standard curve, and the concentrations of polyphenols in the sample were expressed as gallic acid equivalents.

### *2.6. Reducing sugar test procedure with Luff–Schoorl method*

Reducing sugar was measured using the Luff–Schoorl method [12]. In brief, 10 g of sample was weighed and placed in a 250 mL volumetric flask and mixed with 50 mL of distilled water. A Pb–acetate solution was added dropwise until the reagent stopped causing further precipitation. $Na_2CO_3$ was added to remove excess Pb and obtain a clear Pb-free filtrate. Distilled water was added to the filtrate to obtain a final volume of 250 mL, and the mixture was shaken and filtered.

In brief, 100 mL of Pb-free filtrate was pipetted into a 250 mL volumetric flask and diluted by adding distilled water up to the mark. About 25 mL of the diluted Pb-free filtrate was pipetted into a 250 mL Erlenmeyer flask and mixed with 25 mL of Luff–Schoorl solution. In addition, a blank consisting of 25 mL of Luff–Schoorl solution with 25 mL of distilled water was prepared. After a few grains of boiling stone were added, the Erlenmeyer flask was connected to the back cooler and brought to a boil for 10 min. After cooling, 15 mL of 20% KI and 25 mL of 26.5% $H_2SO_4$ were added. The liberated iodine was titrated against 0.1 N $Na_2S_2O_3$ solution with 2–3 mL of the starch indicator. The endpoint of the titration was reached when the color of the solution changed from blue to white. Reducing sugar content before inversion (glucose/fructose) was calculated based on the *z* value, where *z* = glucose blank titration volume (*Vb*)−sample titration volume (*Vs*). For example, *z* = 8.9 mL, where 8 mL of $Na_2S_2O_3$ was equivalent to 19.8 mg of glucose as indicated by the Luff–Schoorl table [13], 0.9 mL was the excess titration volume, and Δ sugar of 2.6 mg was determined

using the Luff–Schoorl table. The reducing sugar content before inversion was calculated using Equation (2).

$$\%\ \text{Rs before inversion} = \frac{(\text{fp} \times \text{Ybi})}{\text{mg sample}} \times\ 100\% \quad (2)$$

where fp is the dilution factor, Rs is the reducing sugar content, and Ybi is the glucose/fructose (mg) presented in the Luff–Schoorl table + (excess titration volume × Δ sugar).

### *2.7. Determinations of sucrose content*

Sucrose content was determined using the Luff–Schoorl method described by Pradnyana et al. [14]. Diluted Pb-free filtrate solution (50 mL) was pipetted into a 100-mL Erlenmeyer flask and mixed with 25 mL of distilled water and 10 mL of 30% HCl (specific gravity 1.15). The solution was heated over a water bath at 67–70 ℃ for 10 min. After cooling to 20 ℃, the solution was neutralized with 45% NaOH, placed into a 100-mL volumetric flask, and squeezed with distilled water to the delimiting mark of the flask.

A total of 25 mL of this solution was pipetted into a 250 mL Erlenmeyer and mixed with 25 mL of Luff–Schoorl solution. In addition, a blank was also prepared, which consisted of 25 mL of Luff–Schoorl solution with 25 mL of distilled water. After adding a few boiling stones, the Erlenmeyer flask was connected to the back cooler and brought to a boil. The boiling process was maintained for 10 min. After cooling, 15 mL of 20% KI and 25 mL of 26.5% $H_2SO_4$ were added to the solution. The iodine thus liberated was titrated with 0.1 N $Na_2S_2SO_3$ solution and 2–3 mL of the starch indicator. Starch was added at the end of the titration to clearly see the color change, which was stopped upon the change of color from blue to white. Reducing sugar content after inversion (total sugar) was calculated using Equation (3).

$$\%\ \text{Rs after inversion (total sugar)} = \frac{(\text{fp x Yai})}{\text{mg sample}} \times\ 100\% \quad (3)$$

where *fp* is the dilution factor, *Rs* is the reducing sugar content, and *Yai* is the amount of fructose (mg) found from the Luff–Schoorl table + (excess titration volume × Δ sugar). Sucrose content was calculated using Equation (4).

$$\%\ \text{Sucrose} = (\text{Sugar after inversion} -\ \text{sugar before inversion}) \times\ 0.95 \quad (4)$$

### *2.8. Statistical analysis*

Data were statistically analyzed by two-way analysis of variance (ANOVA) using SPSS 22.0 software (SPSS, Inc., Chicago, IL, USA). Tukey test was performed for results with a significant or very significant effect. ANOVA was carried out to determine the effects of fruit maturity level and pod conditioning duration, as well as the interaction of these two factors on the moisture, weight, total sugar content, sucrose content, glucose/fructose content and pH of the pulp and the moisture, weight and polyphenol content of the bean.


**Figure 1.** Color appearance of S2 cacao clones at different stages of maturity, i.e., early-maturity stage (A1), medium-maturity stage (A2), and full maturity stage (A3) (Photograph courtesy of N. Laylah, 2021).

## 3. Results and discussion

### *3.1. Pulp weight*

Pulp weight is related to the thickness of the pulp that covers the cocoa bean. The thicker the pulp layer, the greater the weight of the pulp. In this work, the weight of bean pulp decreased with the increase in the duration of pod conditioning, except for the early-maturity stage beans, A1 (Figure 2). The weight of the bean pulp increased with the fruit maturity level (Figure 2). The decrease in pulp weight at A2 and A3 maturity levels during pod conditioning was due to pulp degradation caused by the breakdown of pectic polysaccharides in the pulp, which corroborated the report of Meersman et al. [15]. At the early-maturity stage (A1), there was a slight increase in pulp weight because at maturity level A1 it was still in the growth stage of the cells that form the pulp and include the formation of pectin polysaccharides in the pulp (Figure 2).

ANOVA results are presented in Table 1 pulp weight was significantly affected by pod conditioning ($p$ = 0.002) but not by fruit maturity ($p$ = 0.219). The effect of pod conditioning duration on pulp weight was highly pronounced at A2 and A3 levels of maturity where pod conditioning for 4 or more days led to significantly lower pulp weight than the original value. The main components of cocoa pulp are water and sugar. Afoakwa [16] reported that cocoa pulp contains about 82%–87% water and 10%–15% sugars. Nunes et al. [17] indicated that the water and sugar contents of cocoa pulp are about 86% and 18%, respectively. The respiration process that still occurs after harvest (during pod conditioning) used sugars as a substrate in the metabolic reaction, resulting in the degradation of sugars in the pulp, such as the breakdown of sugar into simple compounds, forming $CO_2$, $H_2O$, and energy [18]. Similarly, Hinneh et al. [5] reported a decrease in pulp volume with the increase in pod conditioning duration. Figure 2 shows that pulp degradation was highly

pronounced at the A3 pod maturity level as shown by the significant decrease in pulp weight from day 1 to day 5 of pod conditioning. These results agreed with Biehl et al. [19], who reported that the pulp volume of Malaysian cocoa pods varies from 2.68 mL/seed for unripe cocoa to 1.38 mL/seed and 1.12 mL/seed for ripe and overripe cocoa pods, respectively. Although the measurement in the current work was based on pulp weight, the obtained results can be directly compared with those reported by Hinneh et al. [20] and Biehl et al. [19] because weight and volume are positively correlated. Hence, a decrease in pulp weight corresponds to a decrease in pulp volume.

**Table 1.** *p*-values for the effects of experimental variables (ANOVA recapitulation).

| No | Parameter | Maturity Level (A) | Pod Conditioning (B) | Interaction (A*B) |
|---|---|---|---|---|
| 1 | Pulp weight | 0.219 | 0.002 | 0.617 |
| 2 | Pulp moisture content | 0.464 | 0.903 | 0.342 |
| 3 | Pulp total sugar | 0.680 | 0.004 | 0.002 |
| 4 | Pulp sucrose | 0.042 | 0.000 | 0.549 |
| 5 | Pulp glucose/fructose | 0.722 | 0.011 | 0.002 |
| 6 | Pulp pH | 0.737 | 0.073 | 0.273 |
| 7 | Bean moisture content | 0.076 | 0.834 | 0.589 |
| 8 | Bean weight | 0.194 | 0.003 | 0.689 |
| 9 | Bean polyphenol content | 0.000 | 0.000 | 0.000 |

The interaction fruit maturity level (A factor) and pod conditioning period (B factor) did not significantly affect the weight of the pulp ($p$ = 0.617). The pulp weight, particularly before pod conditioning (pod conditioning 0 days), ranged from 41.03–47.96 g/100 g of wet seeds. These values were lower than the weights recorded by Biehl et al. [19], who measured pulp weight before pod conditioning to be 72 and 54 g for ripe and overripe Malaysian cocoa pods, respectively. In the current work, only pod conditioning duration affected the pulp weight.

The Tukey test in Table 2. shows that the samples under pod conditioning day 5 (B5) significantly differed from those under pod conditioning day 0 (B0), day 1 (B1) and day 2 (B2). Meanwhile, the samples under pod conditioning day 5 (B5) were not significantly different from those under pod conditioning day 3, day 4, and day 6. This phenomenon was due to pulp degradation caused by the breakdown of pectic polysaccharides in the pulp during pod conditioning [15]. The lowest pulp weight was found on day 5 (B5) at 32.55 g/100 g of wet seeds. On the 6th day of pod conditioning, there was an increase because the pulp from maturity level A1 was not easily decomposed and even still experienced cell growth that included forming pectin polysaccharides in the pulp (Figure 2).

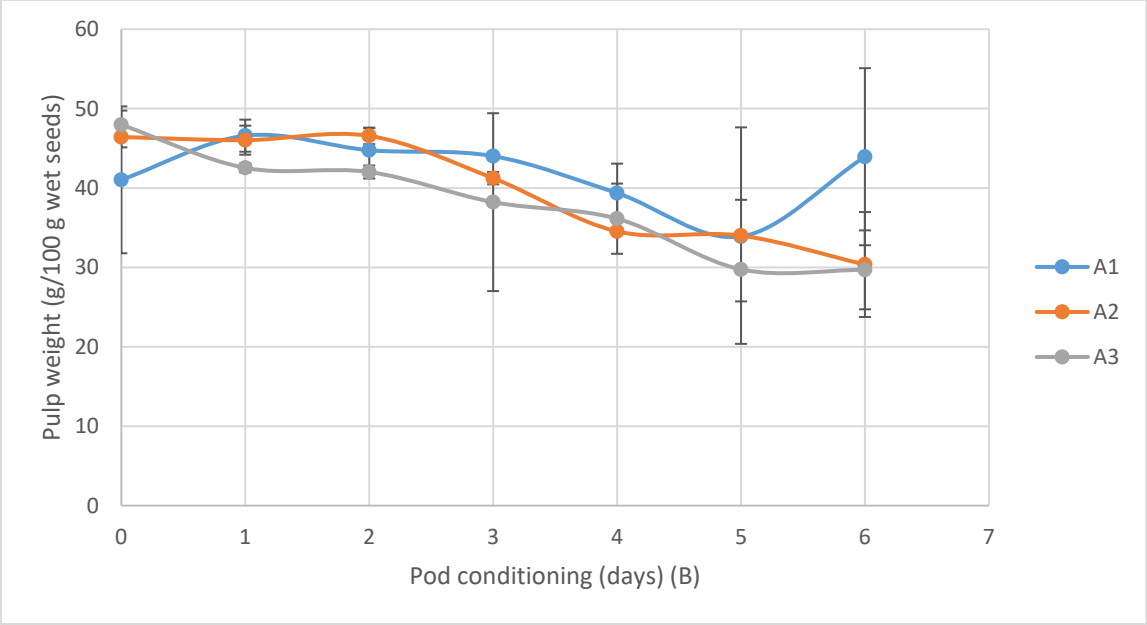


**Figure 2.** Weight of cocoa pulp.

**Table 2.** Tukey test results on the effect of pod conditioning duration on pulp weight.

| Pod conditioning (days) | Pulp weight average (g/100 g of wet seeds) |
|---|---|
| B5 | 32.55 b |
| B6 | 34.67 ab |
| B4 | 36.67 ab |
| B3 | 41.16 ab |
| B2 | 44.45 a |
| B1 | 45.05 a |
| B0 | 45.14 a |

Note: values written in different letters indicate the potential for significantly different results at the 5% level.

### *3.2. Pulp moisture content*

At the A3 pod maturity level, the moisture content of the pulp decreased from 80.87% on day 0 to 74.43% on day 6 of pod conditioning. At the A2 pod maturity level, the moisture content of the pulp also decreased from 81.35% on day 0 to 79.60% on day 6 of pod conditioning. Meanwhile, at treatment level A1, the moisture content of the pulp increased from 78.33% on day 0 to 83.12% on day 6 of pod conditioning. These findings illustrated that cocoa pods at maturity level A1 increased their pulp moisture content during the 6-days of pod conditioning, and the cocoa pods with maturity levels A2 and A3 decreased their pulp moisture content during pod conditioning. These results were in line with previous observations on pulp weight, where pulps with maturity levels A2 and A3 showed a decrease in weight from day 0 to 6 of pod conditioning due to a decrease in water content. Biehl et al. [19] similarly reported that the water content in ripe pods' pulp is not significantly changed in ripe or overripe storage pods, and that the pulp water and dry weight per seed are

significantly lower in seeds from overripe pods. The moisture content of the pulp during pod conditioning is shown in Figure 3.

As indicated by the *p*-value from the ANOVA results summarized in Table 1, the moisture content of the pulp was not significantly affected by pod maturity levels ($p > 0.05$ and $p = 0.464$) and pod conditioning ($p = 0.903$ and $p > 0.05$). The interaction between fruit maturity level and pod conditioning also did not significantly affect the moisture content of the pulp ($p = 0.342$ and $p > 0.05$).

Cocoa fruit is a non-climacteric fruit, that is, it does not show any sudden change in respiration patterns similar to a climacteric fruit [21]. The pattern of sudden changes in respiration in climacteric fruit increases the water content in the fruit and causes the fruit texture to soften. This phenomenon does not occur in cocoa pods, so no significant change in the moisture content of the pulp was observed between the three different maturity levels different pod conditioning durations or as a result of the interaction of fruit maturity level and pod conditioning.

The water content of the pulp ranged from 74.425% (A3B6) (sample with A3 maturity level on day 6 of pod conditioning) to 83.285% (A1B1) (sample with A1 maturity level on day 1 of pod conditioning), which was lower than that reported by Amoa-Awua et al. [[22] and Afoakwa et al. [23] who observed pulp moisture in the range of 82–87%. Pettipher [24] also reported that the water content in West African (Ivorian and Nigerian) and Malaysian cocoa pulps is 83% and 86%, respectively. Pulp is a crucial component in cocoa fermentation; the water in the pulp is essential for the growth of fermenting microorganisms and aids in the dissolution of sugars, making them readily available as substrates for fermentation.

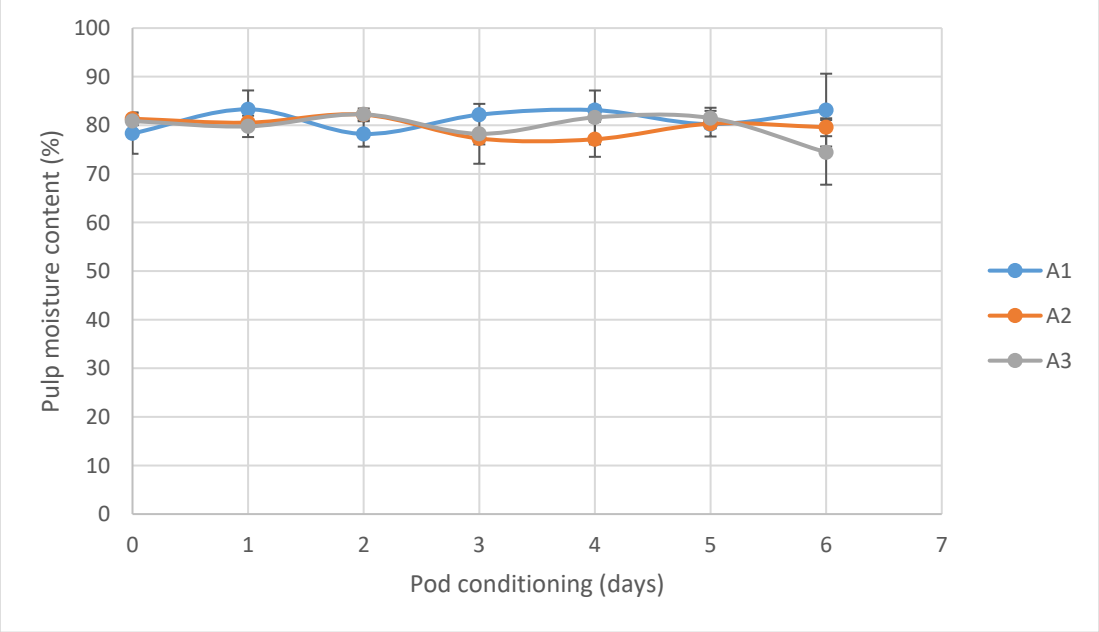


**Figure 3.** Moisture content of cocoa pulp.

### *3.3. Pulp total sugar*

In general, the total pulp sugar decreased during pod conditioning. Fruits with maturity level A1 had the lowest total sugar (4.28%) after pod conditioning on day 6. These results were in agreement

with Biehl et al. [19], who reported a smaller final amount of pulp sugar and higher percentage loss during postharvest pod conditioning compared with those with a higher degree of ripening. The total sugar produced in the cocoa beans in the current study was lower than that reported by Afoakwa in fresh pulp, i.e., 10%–15% [16]. The trend of change in total sugar in the pulp during pod conditioning is shown in Figure 4.

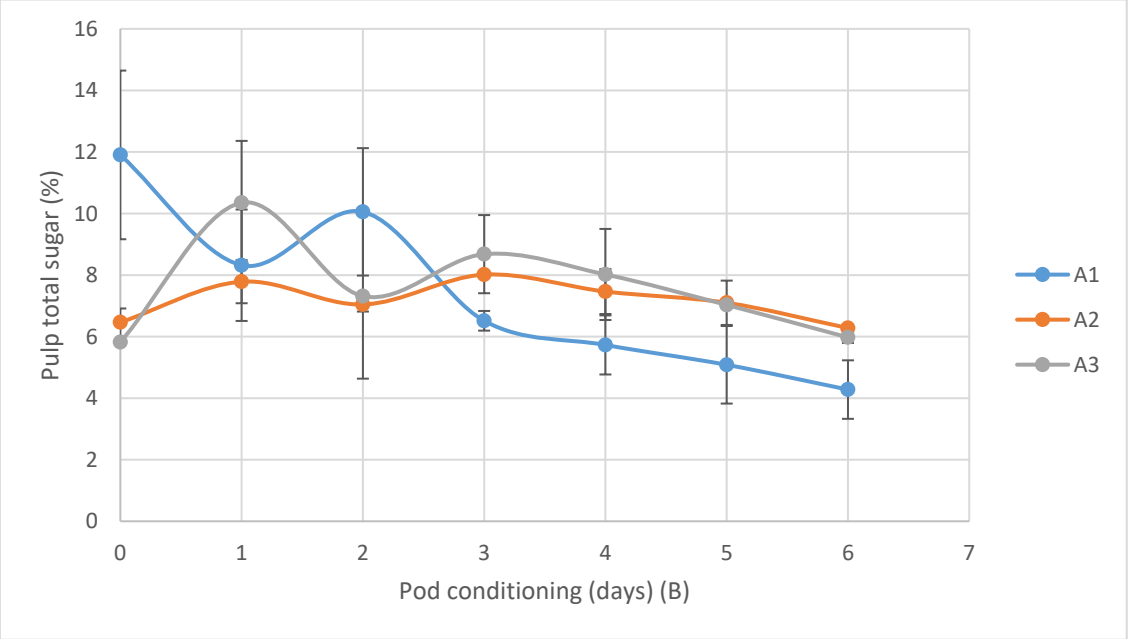


**Figure 4.** Total sugar content in cocoa pulp.

Figure 4 presents a decrease in the total sugar content of pulp during pod conditioning, which can be attributed to respiration in the cocoa pods and the breaking down of sugar into simple compounds. Recapitulation of the *p*-values from ANOVA (Table 1) shows that the total sugar of the pulp was significantly influenced by pod conditioning ($p < 0.05$ and $p = 0.004$) but not by maturity level ($p > 0.05$ and $p = 0.680$). According to Tukey's analysis, B1 (pod conditioning day 1) had the highest total sugar and B6 (pod conditioning day 6) had the lowest total sugar. The longer the pod conditioning duration, the lower the total sugar of the pulp.

**Table 3.** Tukey test results on effect of pod conditioning on the total sugar content of pulp.

| Pod conditioning period (days) | Pulp total sugar average (%) |
|---|---|
| B6 | 5.51 b |
| B5 | 6.40 ab |
| B4 | 7.07 ab |
| B3 | 7.73 ab |
| B0 | 8.06 a |
| B2 | 8.13 a |
| B1 | 8.81 a |

Note: values written in different letters indicate the potential for significantly different results at the 5% level.

Table 3 shows the effect of pod conditioning on the average total sugar pulp of fresh cocoa beans. The average total sugar content of the pulp following 6 days of pod conditioning was not significantly different from that following 5, 4 and 3 days of pod conditioning. However, the average total sugar content of the pulp subjected to 6 days of pod conditioning was significantly different from that resulting from the 2-day (B2), 1-day (B1) and 0-day (B0) pod conditioning. This phenomenon can be attributed to an increase in the temperature of the environment during pod conditioning. The ambient temperature when pod conditioning was performed on the 6th day from 12.00 to 2.00 PM was at an average of 31 ℃. Meanwhile, the temperature on the previous days of day 0 to day 5 from 12.00 to 2.00 PM was at an average of 28 ℃. At high temperatures, amylase is active in breaking down carbohydrates into glucose, fructose and sucrose. At the same time, sucrose breaks down into glucose and fructose. The formed glucose is then oxidized to produce $CO_2$, $H_2O$ and energy. Here, the lowest total sugar content of 5.51% was found on the 6th day of pod conditioning. The longer the pod conditioning duration, the lower the pulp total sugar. The interaction between fruit maturity level (A factor) and pod conditioning (B factor) significantly influenced the total sugar of the pulp ($p = 0.002$ and $p < 0.05$).

**Table 4.** Tukey test results on the interaction between the fruit maturity level of cocoa pods and the pod conditioning on the total sugar of pulp.

| Fruit Maturity Level | Pod conditioning period (days)(B) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 day(B0) | 1 day (B1) | 2 days (B2) | 3 days (B3) | 4 days (B4) | 5 days (B5) | 6 days (B6) |
| A1 | 11.905 a | 8.320 abcd | 10.055 abc | 6.515 bcd | 5.730 bcd | 5.085 cd | 4.280 d |
| A2 | 6.465 bcd | 7.785 abcd | 7.045 abcd | 8.015 abcd | 7.465 abcd | 7.100 abcd | 6.280 bcd |
| A3 | 5.820 bcd | 10.35 ab | 7.315 abcd | 8.680 abcd | 8.020 abcd | 7.030 abcd | 5.975 bcd |

Note: values written in different letters indicate the potential for significantly different results at the 5% level.

Table 4 describes the effect of the interaction between fruit maturity level and pod conditioning on the total sugar of pulp. The effect of the interaction in A1B0 treatment significantly differed from that in A1B3, A1B4, A1B5 and A1B6. Similarly, the effect of the interaction in A1B0 treatment significantly differed from that in A2B0, A3B0, A2B6 and A3B6. The samples in A1B0 treatment had the highest total sugar and had not been ripened (pod conditioning). Pod conditioning reduced the total sugar content and glucose in the pulp. Glucose produced from the decomposition of sucrose was oxidized to $CO_2$, $H_2O$ and energy. Low total sugar was produced on the 6th day of pod conditioning, which is beneficial because it will produce low acetic acid during the fermentation of cocoa beans. Low acetic acid will produce less acidic cocoa beans.

The sugars in cocoa pulp are mainly glucose and fructose with a small amount of sucrose. During fermentation, these sugars are metabolized by microorganisms, such as yeast and acetic acid bacteria. The sugars in the pulp are metabolized by yeast into alcohol, which is then converted by acetic acid bacteria to acetic acid. Alcohol and acetic acid cause the beans to die, releasing and diffusing polyphenols from their cells throughout the seed tissues. Upon contact with air,

polyphenols turn into brown quinones under the action of polyphenol oxidase. Afoakwa [16] indicated that chemical processes occur during fermentation, such as the formation of flavor precursors, color development and a significant reduction in astringent and bitter tastes. In addition, pulp digestion during fermentation liquefies the pulp, releasing it from the beans. Overall, such processes induce changes in appearance (color and cleanliness) and increase the drying rate of cocoa beans.

### *3.4. Sucrose content in the pulp*

Sucrose is the common sugar that we know and use every day. Apart from traditional sources, i.e., sugar cane and beets, sucrose is found in other plants, such as pineapples, carrots, and cocoa pulp. Sucrose undergoes hydrolysis to produce glucose and fructose [25].

Sucrose was present in the tested cocoa pulp in small amounts ranging from 0.33% to 0.88%. This content was lower than that observed by Pettipher [24], who reported that Ivorian, Nigerian and Malaysian fresh cocoa pulps have 4.35%, 1.92% and 1.35% sucrose, respectively. According to Table 2, pod maturity level significantly affected the sucrose content of cocoa pulp ($p < 0.05$ and $p = 0.042$). Cocoa fruit with an A1 maturity level had the highest sucrose content at the beginning (0.8%) and end (0.416%) of pod conditioning. The smallest amount of sucrose was found in fruits with maturity level A3 (0.33%) at the end of pod conditioning. Therefore, the riper the fruit, the lower its sucrose amount. The decrease in sucrose content caused by the degradation of complex compounds occurs; for example, sugars are broken down into simple compounds. Here sucrose was likely degraded into glucose and fructose, which are simple sugars (monosaccharides).

Table 1 shows that pod conditioning significantly affected the sucrose content in the pulp ($p < 0.05$ and p = 0.000). The longer the pod conditioning, the lower the sucrose content of the pulp. The decrease in sucrose content was due to its degradation into glucose and fructose during pod conditioning.

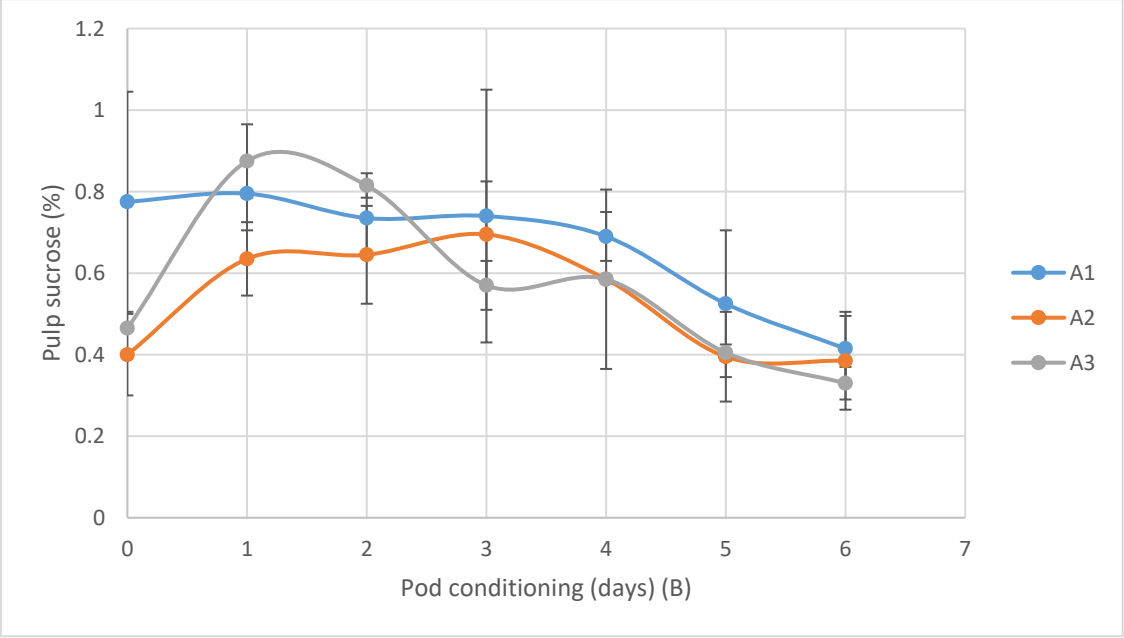


**Figure 5.** Sucrose content in cocoa pulp.

### *3.5. Pulp glucose/fructose content*

Pulp glucose/fructose content was not affected by fruit maturity level ($p$ = 0.722, where $p$ > 0.05). From day 3 to 6 of pod conditioning, the highest glucose levels were observed in fruits with A3 maturity level, followed by fruits with A2 and A1 maturity levels. Pulp glucose/fructose content was affected by pod conditioning ($p$ < 0.05 and $p$ = 0.011). The longer the pod conditioning time, the lower the glucose level. At the completion of pod conditioning, fruits with the maturity level A2 had the highest glucose levels, followed by fruits with A3 and A1 maturity levels (Figure 6). The longer the pod conditioning time, the lower the glucose content. This observation was attributed to respiration in cocoa pods during pod conditioning when the glucose in the fruit reacted with oxygen to produce $CO_2$ and $H_2O$.

The fluctuation in glucose/fructose content shown in Figure 6 was similar to the results of Hinneh et al. [20], who reported that the concentration of glucose increased marginally from day 0 of pod conditioning (0.264%) to day 3 of pod conditioning (0.30%) but slightly decreased at day 7 of storage (0.211%).

The glucose/fructose content in cocoa pulp varied between 3.84% and 11.09%. These sugar concentrations were higher than those reported by Pettipher [24], who reported 3.00%, 5.06%, and 4.90% in Ivorian, Nigerian and Malaysian fresh cocoa pulps, respectively.

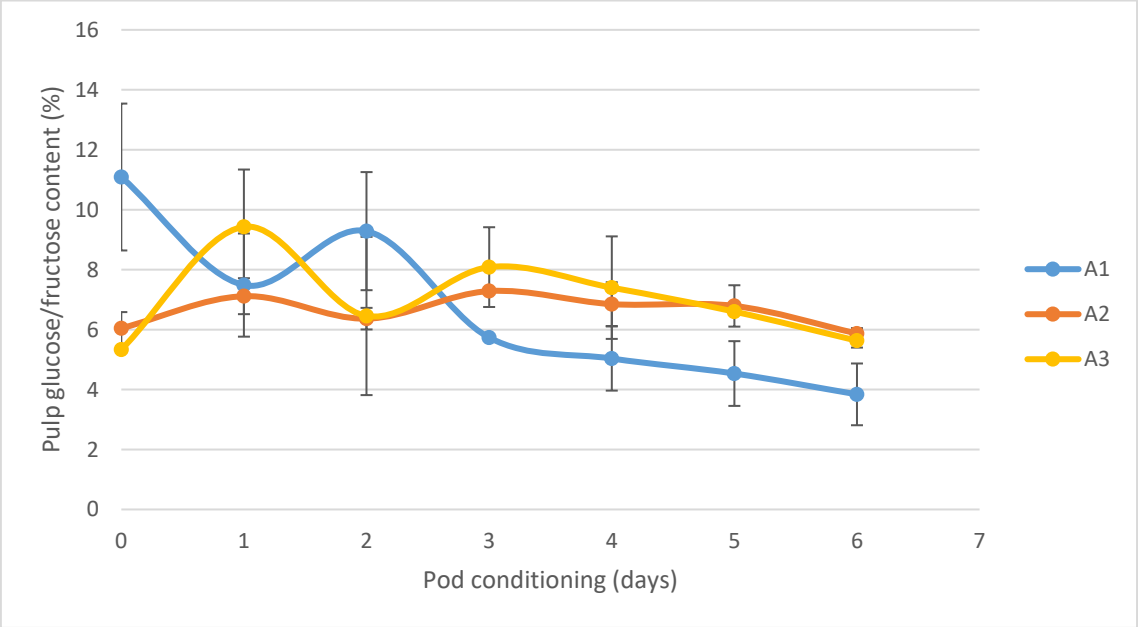


**Figure 6.** Glucose/fructose content in cocoa pulp.

Fruits that have a thick skin layer have a low respiration rate, and in young tissue metabolic processes will be more active than in older tissue [26]. Cocoa pods with maturity level A1 are considered young fruit compared to pods with maturity levels A2 and A3. Because A1 tissue is younger, its metabolic processes are more active than A2 and A3. Because fruit with maturity level A1 has a more active metabolic process, this fruit consumes more glucose/fructose than fruit A2 and A3 during pod conditioning.

### *3.6. pH of cocoa pulp*

The pH of the cocoa pulp ranged from 3.272 (A1B1) (cocoa beans with A1 maturity level at 1 day of pod conditioning) to 3.792 (A3B5) (cocoa beans with A3 maturity level on day 5 of pod conditioning). These values were lower than those reported by Afoakwa [16], who observed the pH of fresh pulp to be between 3.94 and 4.12, and those of Pettipher [24], who reported the pH values of Ivorian, Nigerian and Malaysian fresh cocoa pulp at 3.3, 3.6 and 3.9, respectively.

Table 1 shows that the pH of the cocoa pulp was not significantly affected by fruit maturity level ($p$ = 0.737, where $p$ > 0.05 means that A1, A2, and A3 maturity levels were not different) or pod conditioning period ($p$ = 0.073, where $p$ > 0.05 means that Bo, B1, B2, B3, B4, B5 and B6 pod conditioning periods were not different). The interaction between fruit maturity level and pod conditioning period did not significantly affect the pH of cocoa pulp ($p$ = 0.273, where $p$ > 0.05 means no difference in the pH value according to the combination of maturity level and pod conditioning).

Cocoa fruit is a non-climacteric fruit, as it does not experience a spike in respiration rate during pod conditioning; so, the water produced during respiration does not affect the pH of the pulp. Therefore, the pH values of the pulp from the three levels of maturity were not significantly different additionally the duration of pod conditioning did not affect the pH of the pulp. The change in the pH of cocoa pulp during pod conditioning is shown in Figure 7.

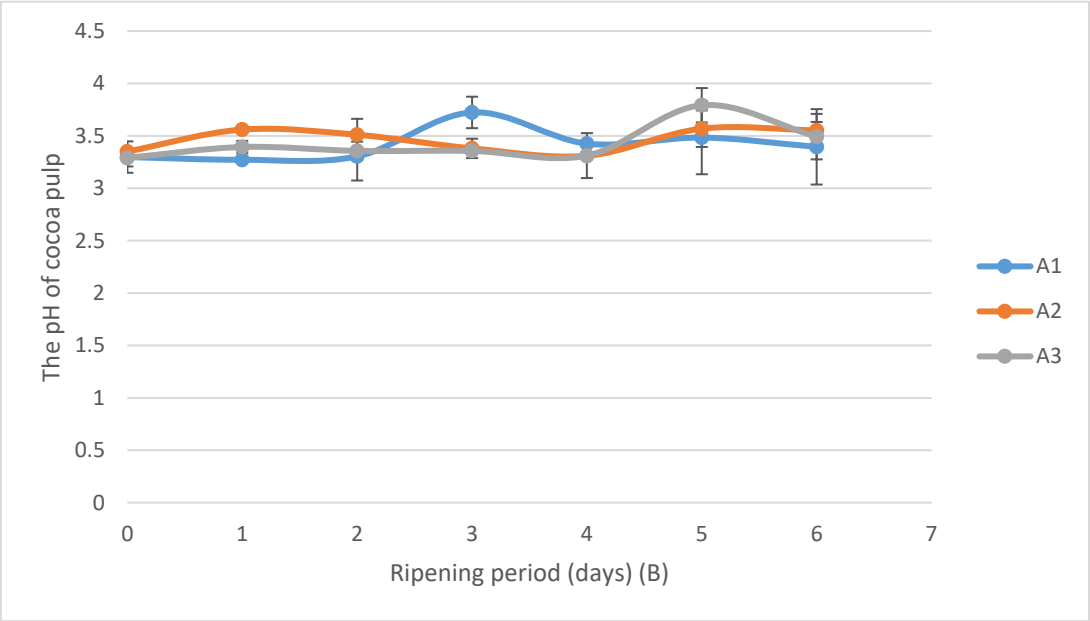


**Figure 7.** pH of cocoa pulp.

### *3.7. Cocoa bean moisture content*

Cocoa beans essentially consist of a shell (testa), which comprises 10–14% of the bean's dry weight. The major portion is the kernel or cotyledon (nib) (86–90%), which confers a characteristic flavor and imparts the aromas of chocolate [16]. One-third of the cotyledons are composed of water, and another one-third comprises fat (cocoa butter). The remainder consists of phenolic compounds, starch, sugar, theobromine, nonvolatile acids and many other components in small quantities [16].

Table 1 indicates that the moisture content of cocoa beans was not influenced by fruit maturity level ($p$ = 0.076 indicates that A1, A2 and A3 maturity levels were not different) or pod conditioning ($p$ = 0.834 means that Bo, B1, B2, B3, B4, B5 and B6 pod conditioning periods were not different). The interaction between fruit maturity level (A factor) and pod conditioning (B factor) did not significantly affect the moisture content of cocoa beans ($p$ = 0.589 means no difference in the moisture content value of cocoa beans according to the combination of maturity level and pod conditioning).

Cocoa fruit is a nonclimacteric fruit, that is, it does not show any sudden change in respiration patterns similar to a climacteric fruit [20]. These sudden changes in respiration in climacteric fruit increase the water content in the fruit and soften its texture. This phenomenon does not occur in cocoa pods, so no significant change in the moisture content of the cocoa beans was observed between the three different maturity levels, different pod conditioning duration or different levels of interaction of maturity level and pod conditioning.

The moisture content of fresh cocoa beans during pod conditioning ranged from 37.125% (A2B1) to 58.665% (A1B6). The moisture content of fresh cocoa beans was higher than the value reported by Afoakwa [16], i.e., 32%–39% in fresh cocoa beans without fermentation. The change in cocoa bean moisture content during pod conditioning is shown in Figure 8.

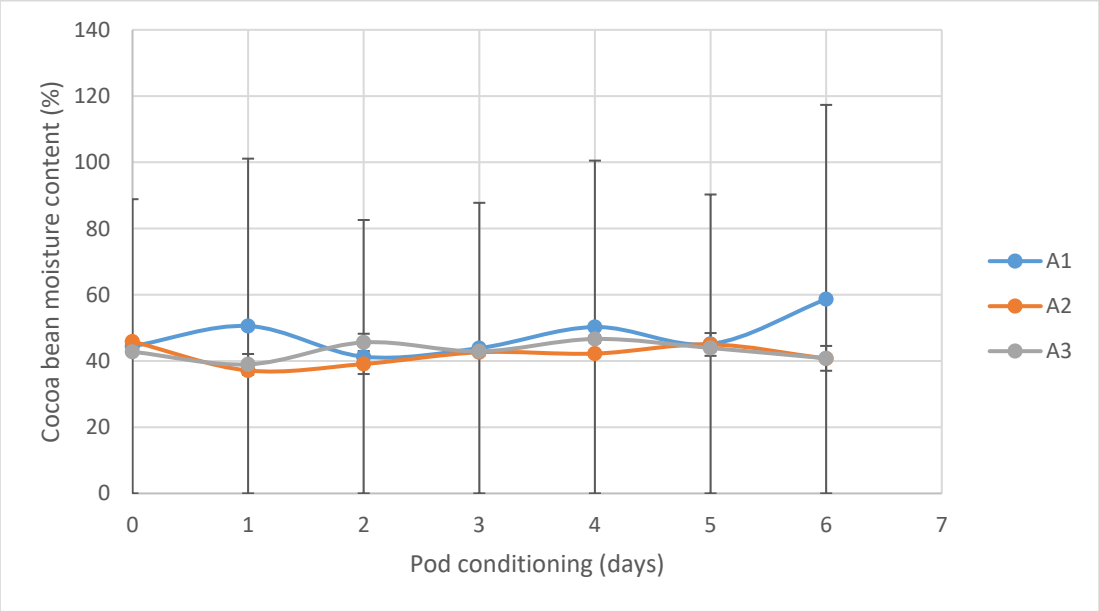


**Figure 8.** Moisture content in fresh cocoa beans.

### *3.8. Cocoa bean weight*

The weight of cocoa beans increased from day 0 to 6 of the pod conditioning ($p$ = 0.003 and $p$ < 0.05) as shown in Table 1, indicating that the pod conditioning period significantly affected the weight of cocoa beans. The results of Tukey's analysis in Table 5 reveal significant difference in the weight of cocoa beans between pod conditioning on day 1 (B1) and day 5 (B5)/day 6 (B6), between pod conditioning on day 0 (B0) and day 5/6, and between pod conditioning day 2 (B2) and day 5 (B5). In general, the longer the pod conditioning time, the higher the weight of the cocoa beans. The reason is that the cocoa bean shell is similar to a semipermeable membrane. Water from the pulp can

enter the cocoa bean, slightly increasing the weight of the cocoa bean during pod conditioning. As a result of imbibition or water absorption, the cocoa bean skin becomes soft and cracked. The formation of new cells in the embryo is succeeded by the differentiation of cells to form the radicle, which is the ovule of the root, to the plumule, which is the ovule of the stem and leaf. These two halves increases in size so that the seed eventually germinates [27].

A decrease in cocoa pulp weight was accompanied by an increase in bean weight during pod conditioning. Biehl et al. [19] reported an increase of 8.9% in fresh seed weight during pod conditioning. The recapitulated ANOVA results presented in Table 1 show that fruit maturity level did not significantly affect the weight of cocoa beans ($p > 0.05$ and $p = 0.194$).

**Table 5.** Tukey test results on the effect of pod conditioning on bean weight.

| Ripening period (days) | Bean weight average (g/ 100 g of wet seeds) |
|---|---|
| B1 | 56.27 c |
| B0 | 56.30 c |
| B2 | 57.64 bc |
| B3 | 60.34 abc |
| B4 | 64.50 abc |
| B6 | 66.98 ab |
| B5 | 68.82 a |

Note: values written in different letters indicate the potential for significantly different results at the 5% level.

The weight of cocoa beans ranged from 54.19 g (A3B0) (cocoa beans with A3 maturity level on day 0 of pod conditioning) to 72.4 g (A3B6) (cocoa beans with A3 maturity level after 6 days of pod conditioning) per 100 g of fresh cocoa beans. The increasing weight of cocoa beans on the 6th day of pod conditioning was likely to increase the yield of dry cocoa beans. However, on the 5th and 6th days of pod conditioning, one seed was germinated each day. These seeds were obtained from two split cocoa pods. Before fermentation, these sprouted beans should have been separated and not mixed with the cocoa beans being fermented.

One of the purposes of fermentation in cocoa processing is to kill the beans so that the polyphenols in the cell can come out and diffuse into cotyledon. Upon contact with air, polyphenols convert polyphenols into brown quinones with the help of polyphenol oxidation enzymes [21]. If the germinating cocoa beans are not separated during fermentation, then they will continue to grow and will not experience seed death. Therefore, polyphenols cannot get out of the cells and do not diffuse throughout the cotyledon tissue; thus, they do not come into contact with air, and polyphenols cannot be converted by polyphenol oxidation enzymes into brown quinones. Seeds that have been germinated cannot be fermented, resulting in purple slaty seeds.

Pod conditioning for 6 days can be applied because the longer the pod conditioning time, the higher the seed weight except for the A1 maturity level. A1 is considered a young fruit, so fruit development is not yet perfect at harvest; also, seed weight gain stops after the 5th day of pod conditioning and even decreases on the 6th day. It is suspected that cocoa beans from level A1 will not be able to germinate even if pod conditioning is continued, so they cannot become good cocoa seeds for planting.

Of all of the seeds that opened, only one seed germinated out of the six cocoa pods opened on the 5th and 6th days. The change in the weight of cocoa beans during pod conditioning is shown in Figure 9.

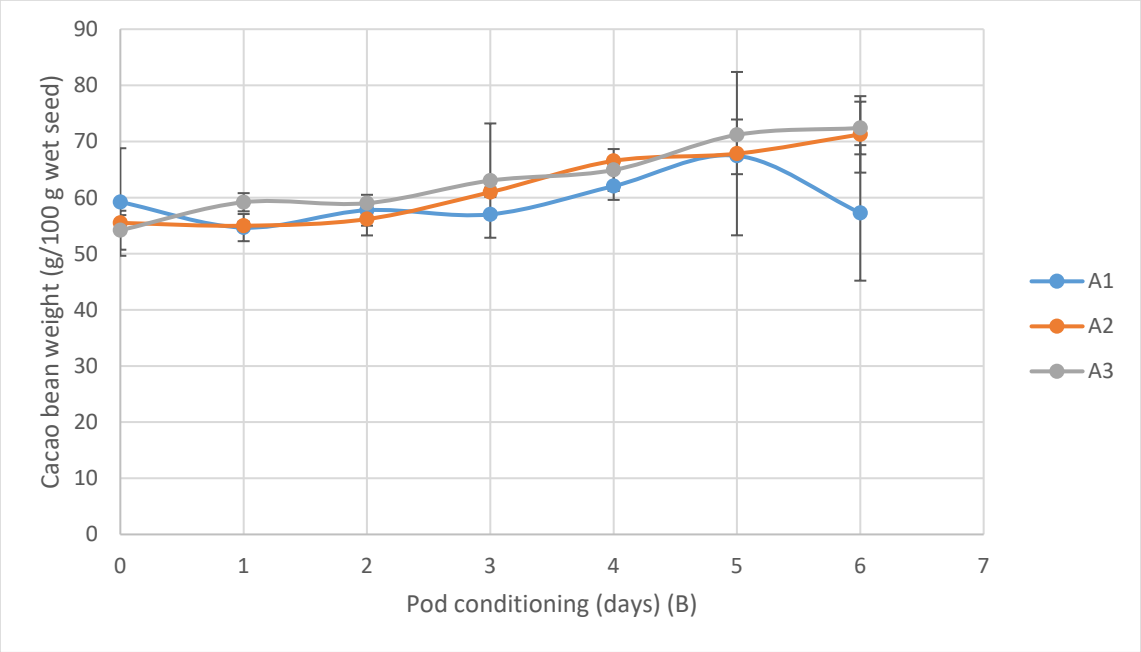


**Figure 9.** Cocoa bean weight.

### *3.9. Polyphenol content of cocoa beans*

Fresh cocoa beans contain polyphenols at approximately 12%–18% of their dry weight. These compounds are largely responsible for cocoa's distinctive flavor and color. Roughly 35% of the total polyphenols in unfermented *Forastero* cocoa beans are flavonoids, particularly (-)-epicatechin. The content of (-)-epicatechin in fresh cocoa beans varies from 34.65 mg/g to 43.27 mg/g of fat-free cocoa beans depending on the cocoa variety and region of origin [28].

The polyphenol content in fresh cocoa beans increased with the pod conditioning period. On the basis of the recapitulated *p*-values from the ANOVA results presented in Table 1, the polyphenol content is significantly affected by the pod conditioning period ($p < 0.05$ and $p = 0.000$), fruit maturity level ($p < 0.05$ and $p = 0.000$) and the interaction between fruit maturity level and pod conditioning period ($p < 0.05$ and $p = 0.000$). Before pod conditioning (day 0 of pod conditioning), fruits with the A3 maturity level had the highest polyphenol content, followed by fruits with the A2 and A1 maturity levels. On days 3 and 6 of pod conditioning, fruits with the A2 maturity level showed the highest polyphenol content, followed by those with A3 and A1 maturity levels.

**Table 6.** Tukey test results on the effect of the interaction between the fruit maturity level of cocoa pods and the pod conditioning period on the polyphenol content of fresh cocoa beans.

| Fruit maturity level | Pod conditioning period (days) (B) | | |
|---|---|---|---|
| | B0 (0 day) | B3 (3 days) | B6 (6 days) |
| A1 | 4.755 e | 7.735 d | 9.665 bc |
| A2 | 4.90 e | 10.70 b | 13.05 a |
| A3 | 5.29 e | 8.835 cd | 10.50 b |

Note: values written in different letters indicate the potential for significantly different results at the 5% level.

The results of the Tukey test are shown in Table 6. The effects of interactions in A1B0, A2B0, A3B0, A2B3, A1B6 and A3B6 did not show any difference. Meanwhile, the effects of interactions in A1B0, A1B3, A1B6, A2B0, A2B3 and A2B6 showed a significant difference. The interaction in A2B6 treatment was the best treatment because it had the highest polyphenol content.

This finding can be explained as follows. The weight of fresh cocoa beans increased with the pod conditioning period. The weight gain of cocoa beans was due to the growth of cells in preparation for seed germination [27]. The growth of cells was accompanied by an increase in the content of the polyphenols in the cells. Therefore, the longer the pod conditioning time, the higher the polyphenol content.

The polyphenol content in the cocoa clone S2 from Pinrang district, South Sulawesi, Indonesia, ranged from 4.755 to 13.05 mg/g, which was deficient relative to the 140 mg/g value reported by Bonvehi and Coll [29] in fresh beans. The changes observed in the polyphenol content of fresh cocoa beans during pod conditioning are shown in Figure 10. Overall, our observations suggested that the polyphenol content can be enhanced significantly by pod conditioning or storing cocoa pods for 6 days.

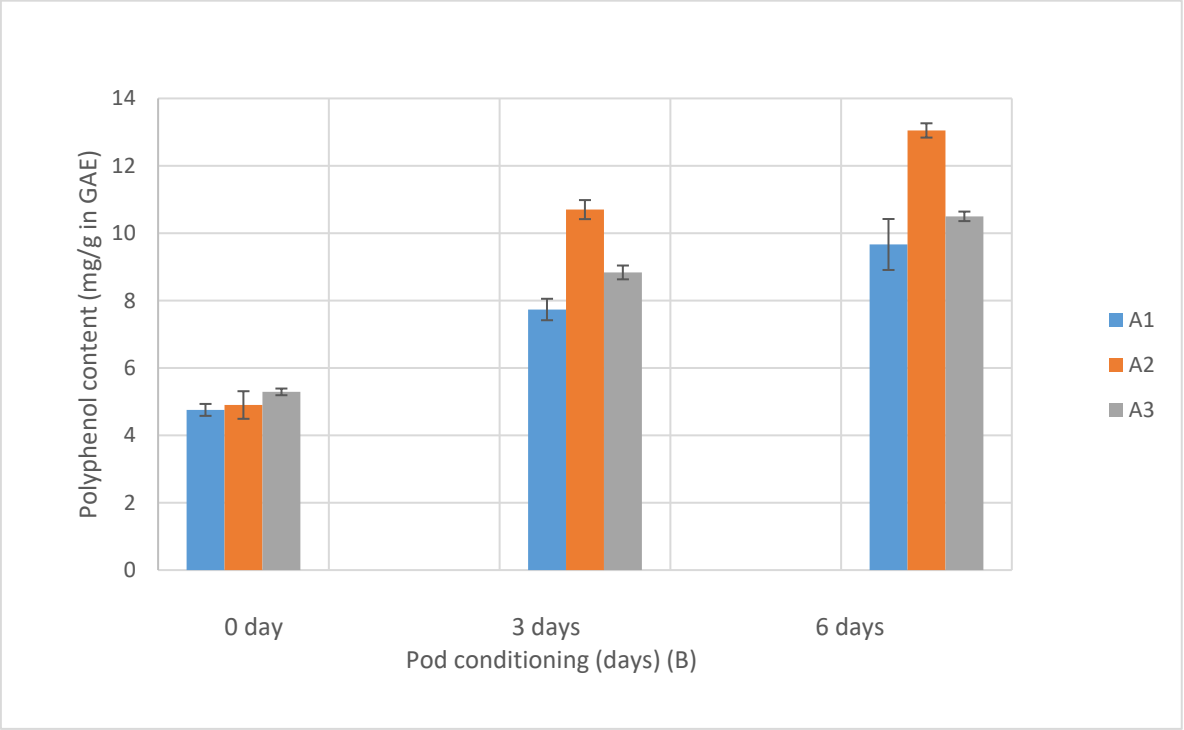


**Figure 10.** Polyphenol content of fresh cocoa beans.

## 4. Conclusions

In terms of pod conditioning time, the lowest pulp weight (32.55 g/100 g wet seeds) was obtained on the 6th day of pod conditioning. The highest pulp weight was obtained from the fruit without pod conditioning at 45.14 g/100 g wet beans. The moisture content of the pulp is not affected by fruit maturity level, pod conditioning or the interaction between pod conditioning and maturity level.

Judging from the duration of pod conditioning, the highest total pulp sugar (8.818%) was obtained after pod conditioning for 1 day, and then it decreased until the 6th day of pod conditioning (5.512%). The fruit maturity level did not affect the total cocoa pulp sugar. Judging from the duration of pod conditioning, the highest pulp sucrose (0.768%) was obtained on the 1st day of pod conditioning and then decreased until the 6th pod conditioning day (0.377%). Judging from the maturity level of the fruit, the highest pulp sucrose (0.667%) was obtained at the early maturity level (A1), and then it decreased to (0.534%) at the medium maturity level (A2) and (0.578%) at full maturity (A3).

Based on the duration of pod conditioning, the highest pulp glucose/fructose (8.1%) was obtained at one day of pod conditioning and then decreased until the 6th day of pod conditioning (5.113%). In terms of fruit maturity level, the highest glucose/fructose pulp (6.990%) was obtained at full maturity, while the lowest glucose/fructose pulp was obtained at medium maturity (6.617%). The pH of cocoa pulp is not affected by the degree of ripeness of the fruit, the duration of pod conditioning or the interaction between the degree of maturity and the duration of pod conditioning. The moisture content of cocoa beans is not affected by fruit maturity level, pod conditioning or the interaction between maturity level and pod conditioning.

Judging from the duration of pod conditioning, the highest weight of cocoa beans (72.4 g/100 g wet beans) was obtained on the 6th day of pod conditioning. The lowest weight of cocoa beans (54.19 g/100 g wet beans) was obtained for the fruits without pod conditioning. The fruit maturity level and interaction between the two treatments did not affect the weight of cocoa beans.

Based on the fruit maturity level, the highest polyphenol content was 9.55 mg/g in GAE obtained at the medium maturity level (A2), and it decreased by 14.13% at full maturity (8.2 mg/g in GAE). Judging from the duration of pod conditioning, polyphenols in fruit without pod conditioning increased by 83.67% when pod conditioning for 3 days; it then increased by 124.69% when pod conditioning for 6 days.

The lowest pulp glucose/fructose (6.617%) and the lowest pulp sucrose contents (0.534%) were both found at the medium maturity level (A2); therefore, this maturity level has a lower level of acidity than the others. Pod conditioning for 6 days with maturity level A2 reduces acidity and increases the polyphenol content and weight of cocoa beans. The increase in bean weight also increases the yield of cocoa beans. Therefore, 6 days of pod conditioning with the medium maturity level (A2) can be applied to the cocoa processing industry, especially before the cocoa beans are fermented.

## Use of AI tools declaration

We did not use artificial intelligence tools in the creation of this article.

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## Conflict of interests

The authors declare no conflict of interest.

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