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FULL-LENGTH RESEARCH ARTICLE

# Effect of Fermentation Methods and Turning Interval on the Quality of Cocoa Beans (*Theobroma cacao*)

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**Abstract** Cocoa (*Theobroma cacao* L.) is the main ingredient in the manufacture of chocolates and confectionery undergoes different unit operations, viz. fermentation, drying and roasting for a quality product. Fermentation followed by drying is an important farm level processing of cocoa. Fermentation studies were conducted by holding the cocoa beans (mixed F1 progeny varieties) in the bamboo basket, heaping on the floor and wooden box, and allowed to ferment for 6 days. During fermentation, the fermenting mass was manually turned at 12, 24 and 48h interval with a control. During the fermentation, pH of the pulp and beans, quantity of sweat collected temperature of the fermenting mass, moisture content of the mass and microbial population were determined following the standard procedures. After fermentation, the beans were dried by sun-drying, and the physical and biochemical qualities of dried cocoa beans, viz. bean count, number of beans per 100 g, bean texture, cut test, pH, titratable acidity and free fatty acid were determined. The method of fermentation and tuning intervals were non-significant on pH of pulp, moisture content, sweat produced, microbial population and free fatty acid. The method of fermentation and tuning intervals were significant on temperature profile, bean count, cut test and pH of bean. The microbial population varied with fermentation duration in all fermentation methods. In heap method with 48-h turning interval, pH, temperature and hardness (texture) were found higher than other fermentation methods. The per cent brown beans, considered as healthy beans, were at par with the turning interval and significant with the method of fermentation and were higher under the heap method at 12 and 24 h of turning intervals. Turning at 12h interval in heap method resulted in less acidic nature (titratable acidity) and desirable quality attributes, which is considered as optimum.

**Keywords** pH · Brown beans · Cut test · Bean texture · Bean count · Titratable acidity

## Introduction

Cocoa (*Theobroma cacao* L.) is an important ingredient in the manufacture of chocolates and confectionery. India is a leading grower and consumer of cocoa with area under cocoa increasing steadily from 94,000 ha. in 2018–2019 to 1,06,000 ha in 2021–2022 with production from 24,000 MT to 28,000 MT [2]. Cocoa being a perennial crop it can withstand different seasonal variations with good health and yield potential. It is cultivated at altitudes up to 1200 m above mean sea level (MSL) with an annual rainfall of 1000 mm to 2000 mm and a relative humidity of 80% with maximum 35 °C and minimum temperature of 15 °C. Cocoa is also grown as intercrop in coconut and arecanut gardens. Other applications of cocoa are found in beverages, cosmetics, pharmaceuticals and toiletry products [15]. Commercial cocoa is obtained from the beans originated as seeds from the ripe pods of the plant.

Farm level processing of cocoa includes breaking the pods, removal of beans, fermentation and drying [11]. The

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raw, unfermented cocoa bean is extremely bitter and astringent, completely lacking in chocolate flavour. After fermentation, it loses its bitterness and astringency, whereas on roasting, it develops the typical chocolate aroma and flavour. The fermentation stage plays a significant role in the development of microbial succession and various enzymatic, chemical and physicochemical reactions, among other processes, that contribute to the flavour and aroma profile of cocoa beans. Fermentation produces acids and alcohols that will also penetrate the cocoa bean and start the chemical reactions that will form the precursors of chocolate flavour [15]. Although fermentation is a natural process, it requires microbial activity to modify the components of the beans and reduce their bitterness and astringency.

The most common methods traditionally followed for the fermentation of cocoa beans are: heap [24, 35], box [6, 11, 12. 15, 38, 45], basket [8, 11, 12] and platform [8]. However, in most countries fermented cocoa is produced by using the box fermentation method and the batch quantity of cocoa beans processed varies between 5 and 2000 kg [12], which depends on the production capacity of the local cocoa farm. Also farmers use different methods such as plastic sacks, bamboo baskets, or wooden boxes lined and covered with banana leaves [12, 36]. By these methods, it takes 4–7 days to complete the fermentation [11]. Also, it is believed that the heap size, pod age, fermentation time, number and timing of turns during fermentation and drying method and time influence the quality of the fermented cocoa beans [15].

The fermentation time was possibly decreased by adding inoculums / starters [13, 32, 37]. Lactic acid bacteria [13], consortium of *Saccharomyces cerevisiae, Lactobacillus plantarum* and *Acetobacter aceti* [13, 39], *Saccharomyces cerevisiae* and *Lactobacillus plantarum* [12] and *Saccharomyces cerevisiae* and *Pichia kudriavzevii* [37] were used as starter cultures, which reduced the fermentation time to 48, 24 and 24 h, respectively. The starters lead to changes throughout fermentation, but provided fermented cocoa with similar pH, titratable acidity, reducing sugars and phenolic compounds. However, greater control and understanding of cocoa bean fermentation by employing an artificial fermentation system with controlled incubations is essential [30]. Also, the results of fermentation need to align with existing scientific knowledge, emphasizing the need to tailor fermentation practices to the specific cocoa type and intended processing [23].

The first phase of fermentation is dominated by anaerobic yeasts for 24–36 h with low oxygen and low pH (< 4), the second phase by lactic acid bacteria is for 48–96 h and in the third phase with acetic acid bacteria, the temperature increases up to 50 °C and above [8]. Mixing or turning the cocoa beans at various times during fermentation is essential to increase the aeration and achieve uniformity in the rate of fermentation through the mass, thus avoiding clumping of the beans which discourages fungal growth on the surface and/or corners of the fermentation box.

Though many researchers in various countries have evaluated the quality of the bean as influenced by the fermentation methods, it has been reported that the process is region specific. Also, the effect of turning the mass during fermentation and its frequency on the final quality of the beans has not been reported by the earlier researchers. Hence, in this study it has been aimed to carry out the performance of three methods of fermentation, viz., box, basket and heap, and the effect of the turning interval on the physical and chemical quality attributes of raw cocoa.

## Materials and Methods

### Raw Materials and Preparation for Fermentation

The freshly harvested mature cocoa pods of mixed F1 progeny varieties were obtained during November–December 2019, from a local farmer in Coimbatore, India (*latitude* of 11°1′0.64″ N; *longitude* of 76°57′21″ E and altitude of 411 m above MSL). Pods having crack or skin injuries and infestation were rejected by physical observation. Harvested pods were collected in gunny bags and transported to the laboratory. In the laboratory, pods were stored open at ambient condition (30 °C ± 1 °C and 65% to 70% relative humidity) and used in the experiments within 3 days. The well matured and ripe pods (bright yellow in colour) were broke opened manually using a piece of wood (mallet). The beans were removed carefully by hand from placenta to exclude any germinated, black or diseased beans or pieces of shell or placenta fragments. The beans are then mixed thoroughly and used in the fermentation studies.

### Fermentation of Cocoa Beans

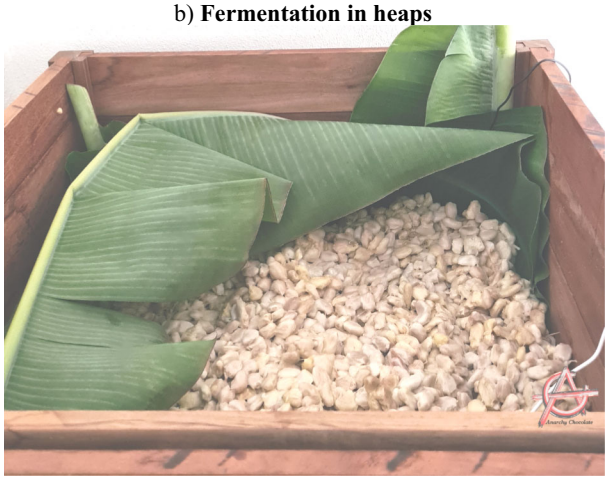
At the laboratory level, cocoa beans were fermented by the following methods. In each of these cases, about 5 kg of beans was taken for fermentation studies.

#### *Fermentation in Bamboo Baskets*

Cocoa beans were taken in bamboo baskets (Fig. 1a) of 450 mm diameter and 300 mm height, commonly used at farm, to its full level and placed under the shade [10].

a) **Fermentation in bamboo baskets**

b) **Fermentation in heaps**



c) **Fermentation in wooden box**

**Fig. 1** Cocoa fermentation by different methods

*Fermentation in Heaps*

Cocoa beans were heaped to 450 mm diameter on plantain (banana) leaves of length 600 to 900 mm and width 300 to 450 mm, placed on a level/mild slope floor under the shade (Fig. 1b). The height of the heap was in the range of 200 to 250 mm [15, 24, 35].

*Fermentation in Wooden Box*

Wooden boxes of 300 mm × 200 mm × 200 mm size were used to hold the cocoa beans for fermentation. Holes are provided at the bottom and sides of the box to facilitate easy draining of acidic liquid (sweat) from the fermenting mass [6, 11, 12, 15, 38, 45].

*Turning of Fermenting Mass*

To achieve uniform and faster fermentation, the fermenting mass is turned periodically. Thereby the heat generated in the mass will be distributed uniformly. The fermenting mass in all the methods of fermentations are tuned manually at 12-, 24- and 48-h interval with a control (without turning).

## Quality Assessment of Fermentation

*Sampling for Analysis*

From the fermenting mass, provisions were made to collect the draining acidic liquid (called sweat) resulted from liquefaction of mucilaginous pulp during fermentation. From the fermenting masses, randomly bean samples from different zones (top, centre and bottom), 25 g samples for microbiological analyses and 80 g samples for biochemical analyses were taken at 12-h interval for 144 h (6 days) and analysed.

*pH of Pulp and Bean*

Following the procedures of AOAC (970.21, 2016), pH of pulp, samples of cocoa beans drawn from three different positions (top, centre and bottom) of the fermenting mass and the dried cocoa beans were measured [3]. Cotyledons (10 g) or pulp and testa (5 g) were weighed into a 100-ml blender jar followed by the addition of 90 ml of boiling distilled water. After blending for 2 min, the resultant homogenate was filtered through a Whatman No. 4 filter paper and 50 ml were collected [3]. After cooling to 20–25 °C, pH was measured using a digital pH meter (ELICO-612 model).

*Temperature Profile of the Fermenting Mass*

The ambient temperature and the temperature of the fermenting cocoa mass were measured by inserting a long-stemmed mercury-filled glass thermometer (0–60 °C range with 0.5 °C least count) into the fermenting mass at three different positions (top, centre and bottom) separately. From each location three observations were taken and the average was reported.

*Moisture Content of Fermenting Beans*

Moisture content of the fermenting cocoa beans was determined as per ISO 2451 [29] by drawing whole cocoa beans at different stages of fermentation and locations. Triplicated samples were placed in a ventilated hot air oven at 103 ± 2 °C for 16 ± 1 h and the moisture content was determined and the mean was reported.

*Sweat*

The sweat from the fermenting mass of the cocoa beans was collected and measured (in ml.) at 24 and 36 h of fermentation from each method [42].

*Microbial Analysis*

Samples (25 g) of beans were aseptically scooped from three different zones (top, centre and bottom) of the fermenting mass at intervals of 12 h and were mixed with 25 ml of 0.1% peptone water and vigorously shaken for 3 min. to give a uniform homogenate. Samples (1 ml) of the homogenate were serially diluted in 9 ml of 0.1% peptone water from which aliquots (1 ml) were spread inoculated in duplicate over the surface of plates of agar media for the isolation and enumeration of specific organisms. Inoculated agar plates were incubated at room temperature for 1 to 4 days after which colonies were counted [5, 9, 22]. The morphological properties of the different colony types were recorded and counts made for each type. The colonies were observed after 48 h as slimy, dull white or white growth on the agar medium surface. All chemicals used were purchased from HiMedia, Mumbai. The results were expressed as:

Colony-forming unit per gram of sample = Number of colonies × Dilution factor

*Sun-drying of Fermented Beans*

The cocoa bean mass fermented by different methods and turning interval were dried under sun-drying (28 to 35 °C; 60 to 65% R.H.) method by spreading on a cement floor as a thin layer and turned at every 3-h interval to ensure faster and uniform drying. Cocoa beans were dried to about 8% (d.b.) as noted that the beans produced a rattling sound during raking and the shell became brittle. The sun-dried cocoa beans were further analysed for physical and biochemical qualities. The basic samples for physical and chemical analysis were obtained from 100 g of manually shelled beans, frozen in liquid nitrogen and ground in a mill to obtain a particle size of less than 0.5 mm.

**Physical and Biochemical Qualities of Cocoa Beans**

*Bean Count, Number OF Beans/100 g*

Three hundred grams of beans were weighed and the impurities, debris and broken beans removed. The waste weight was replaced (w/w) with whole beans taken at random from the rest of the sample, and then, the number of beans per 100 g was determined as described by [7].

*Bean Texture*

Texture of the bean was tested using a texture analyser (Model:TA.XT TEE32, Stable Microsystems, UK) by using a HDP/BSK blade set with knife at test speed of 0.5 mm/s with penetration distance of 2 mm. Trigger force was set to auto (5 g) with data acquisition rate of 400 Hz. The probe penetrated the flatter side of the beans at stable position. The hardness and fracturability were measured from the force deformation curve (force versus time). The maximum force exerted by the beans indicates the hardness and the linear distance along the curve is the fracturability. This measurement was taken with different triplicated samples.

*Cut Test*

Cut test is the first quality control of cacao beans and is done to assess the sanitary and fermentation quality of all cocoa samples as previously described [27, 35, 45]. One hundred numbers of dried cocoa beans were cut lengthwise through the middle using a penknife. Both halves of each bean were examined in full daylight according to the cross-sectional colour of the beans. Observations were made for mould infestation, insect damage, flat and germination as well as colour of the beans (slate, fully purple and fully brown). The cut test score is calculated using the following equation [35].

Cut test score = (10 × % brown) + (5 × % purple/brown) + (0 × % purple and slaty).

*Titratable Acidity*

The cocoa was assayed for titratable acidity (lactic acid and acetic acid) following the method of colorimetric acidity titration [16]. Equal parts of deionized distilled water were added to the solid samples and macerated in a blender at 100 rpm for 2 min before centrifuging for 5 min at 2500 rpm at room temperature. With 10 ml of this supernatant solution using phenolphthalein indicator, titration was done using 0.1N NaOH to get a faint but definite pink colour as end point. The titratable acidity is calculated using the equation:

$$\text{Titratable Acidity } (\%) = \frac{V \times N \times \text{meq.}wt \times 100}{1000 \times V_s}$$

where $V$ is volume (ml) of NaOH solution used for titration, $N$ is normality of NaOH solution, *meq.wt* is milliequivalent weights of acids (lactic acid = 90; acetic acid = 60), $V_s$ is sample volume = 10 ml.

The analysis was performed in triplicate to find the mean titratable acidity of each sample.

*Fermentation Index (FI)*

Ground cocoa nibs (0.5 g) were added to 50 ml of a mixture of methanol and HCl at a volume ratio of 97:3 and homogenized [18, 24]. The mixture was left in the refrigerator (at 8 °C temperature) for 16 to 19 h and vacuum-filtered using Whatman No. 1 filter paper. The filtrate was made up to volume in a 50-ml volumetric flask. The ratio of the absorbance at 460 nm and 530 nm was determined using a UV–visible spectrophotometer (Model: Genova, Jenway, Bibby Scientific Limited, Staffordshire, UK). Three replicate readings were obtained for each sample.

*Fat Content*

The fat content was determined as per AOAC Official method 963.15 [4]. To 5 g of ground deshelled nibs (particle size less than 0.5 mm), 45 ml of boiled distilled water and 55 ml of 8 M hydrochloric acid and silicon chips were added as anti-bumping agent and a homogenous mix was made in a beaker. The beaker covered with watch glass was slowly boiled for 15 min., and the digest is filtered through fluted filter paper. The wet filter paper with residue is transferred to an extraction thimble and dried at 100 °C for 6–8 h in an oven.

After drying, the thimble is placed in a Soxhlet apparatus. The digested residue is refluxed with petroleum ether for 4 h and the heat is adjusted in such a way that the extractor siphons more than 30 times per hour or 5 to 6 drops per second. After 4 h, the flask is removed and the solvent is evaporated in a steam bath and the fat collected is weighed.

The fat content is estimated using the following formula

$$\text{Fat, } \% = \frac{g, \text{Fat} \times 100}{g\,, \text{Test sample}}$$

*Free Fatty Acid Analysis (FFA)*

Free fatty acid content was analysed following the method [31] recommended by federation of cocoa commerce (FCC). Fat was extracted by taking 180 ml of hexane in a well-dried round-bottomed flask and 10 g of ground test sample in a thimble, by allowing for extraction for two hours in a Soxhlet apparatus. The solvent was concentrated into fat by evaporating the hexane using the rotary evaporator. The fat content was dried in the oven for 2 h and cooled. The weighed fat extract was dissolved in 50 ml ethanol/diethyl ether solution, 1/1 [*v*/*v*]. The mixture was titrated against 0.1 M sodium hydroxide using phenolphthalein indicator to the end point, and the FFA content is calculated as follows.

$$\text{FFA, } \% = \frac{282 \times V \times C}{10 \times M}$$

$$\text{M} = (\text{M}_2 - \text{M}_1)$$

$$C = \frac{W_p}{(M_p - V_p)}$$

where 282 is molecular mass of oleic acid, $V$ is volume (ml) of standardized sodium hydroxide used for titration, $C$ is concentration (mol/l) of the standardized sodium hydroxide used for titration, $Mp$ is molecular weight of hydrogen phthalate, $Vp$ is volume of sodium hydroxide solution, ml, $Wp$ is weight of sodium hydroxide phthalate, *g,* $M$ is mass of extracted fat, *g,* $M_1$ is mass of conical flask and pumice stones before extraction, *g,* $M_2$ is mass of conical flask after extraction, *g*.

*Statistical Analysis*

All the experiments / analysis were performed with three replications. The results obtained were analysed for one-way ANOVA and Duncan's multiple range test using SPSS statistical software (Version 16, SPSS, USA) at 95% confidence level.

## Results and Discussion

### pH of Pulp and Beans

The pH of the pulp recorded daily during fermentation in response to different fermentation methods, turning interval and fermentation duration is given in Table 1. The methods and turning interval had no significant difference on the pH of pulp. However, the fermentation duration had significant effect on pH of pulp. The pH of pulp increased from the first day to sixth day of fermentation as also indicated by darkening of colour of beans. The mean pH of pulp increased from the initial value of 3.57–4.20 on sixth day of fermentation.

The pH of beans (Table 1) decreased during the fermentation process. The mean pH, 5.38, recorded on first day of fermentation was reduced to 4.40 on sixth day of fermentation. Method of fermentation and fermentation duration had significant effect on the pH of beans, but the

**Table 1** pH of pulp and cocoa beans fermented by basket, heap and box at various turning intervals with fermentation duration

| Methods | Turning interval, h | pH of the pulp | | | | | | | | pH of the beans | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Fermentation duration, h | | | | | | | | Fermentation duration, h | | | | | | | |
| | | 0 | 24 | 48 | 72 | 96 | 120 | 144 | Mean | 0 | 24 | 48 | 72 | 96 | 120 | 144 | Mean |
| Basket method | 0 | 3.58 | 3.64 | 3.89 | 3.91 | 4.04 | 4.12 | 4.24 | 3.92 | 5.38 | 5.24 | 5.08 | 4.63 | 4.37 | 4.26 | 4.21 | 4.74 |
| | 12 | 3.60 | 3.67 | 3.72 | 3.83 | 3.98 | 4.06 | 4.18 | 3.86 | 5.38 | 5.21 | 5.17 | 4.82 | 4.74 | 4.58 | 4.53 | 4.92 |
| | 24 | 3.57 | 3.61 | 3.84 | 3.91 | 4.07 | 4.15 | 4.27 | 3.92 | 5.37 | 5.23 | 5.14 | 4.92 | 4.84 | 4.78 | 4.73 | 5.00 |
| | 48 | 3.59 | 3.65 | 3.76 | 3.81 | 3.91 | 3.99 | 4.11 | 3.83 | 5.36 | 5.24 | 5.12 | 4.78 | 4.66 | 4.62 | 4.57 | 4.91 |
| Heap method | 0 | 3.57 | 3.65 | 3.78 | 3.86 | 3.99 | 4.07 | 4.19 | 3.87 | 5.36 | 5.25 | 5.16 | 4.32 | 4.23 | 4.17 | 4.12 | 4.66 |
| | 12 | 3.58 | 3.62 | 3.85 | 3.93 | 4.02 | 4.10 | 4.22 | 3.90 | 5.39 | 5.23 | 5.13 | 4.97 | 4.75 | 4.68 | 4.63 | 4.97 |
| | 24 | 3.55 | 3.64 | 3.79 | 3.91 | 4.15 | 4.23 | 4.35 | 3.95 | 5.34 | 5.27 | 5.19 | 5.07 | 4.98 | 4.96 | 4.91 | 5.10 |
| | 48 | 3.57 | 3.66 | 3.81 | 3.95 | 4.18 | 4.26 | 4.38 | 3.97 | 5.39 | 5.21 | 5.17 | 5.04 | 4.99 | 4.99 | 4.94 | 5.10 |
| Box method | 0 | 3.56 | 3.64 | 3.73 | 3.80 | 3.97 | 4.05 | 4.17 | 3.85 | 5.38 | 5.26 | 5.16 | 4.47 | 4.35 | 4.33 | 4.28 | 4.75 |
| | 12 | 3.58 | 3.63 | 3.74 | 3.81 | 3.91 | 3.99 | 4.11 | 3.82 | 5.37 | 5.24 | 5.14 | 4.32 | 4.18 | 4.12 | 4.07 | 4.63 |
| | 24 | 3.57 | 3.65 | 3.71 | 3.78 | 3.85 | 3.93 | 4.05 | 3.79 | 5.39 | 5.23 | 5.05 | 4.17 | 4.02 | 3.93 | 3.88 | 4.52 |
| | 48 | 3.56 | 3.64 | 3.70 | 3.77 | 3.87 | 3.95 | 4.07 | 3.79 | 5.40 | 5.23 | 5.03 | 4.22 | 4.07 | 3.97 | 3.92 | 4.55 |
| | Mean | 3.57 | 3.64 | 3.78 | 3.86 | 3.99 | 4.08 | 4.20 | 3.87 | 5.38 | 5.24 | 5.13 | 4.64 | 4.52 | 4.45 | 4.40 | 4.82 |
| ANOVA | | M | T | F | MT | TF | MF | MTF | | M | T | F | MT | TF | MF | MTF | |
| | Prob | 0.06 | 0.97 | 0.00 | 0.82 | 1.00 | 1.00 | 1.00 | | 0.00 | 0.08 | 0.00 | 0.00 | 0.99 | 0.01 | 1.00 | |
| | Sig | NS | NS | ** | NS | NS | NS | NS | | ** | NS | ** | ** | NS | ** | NS | |
| | SEd | 0.05 | 0.05 | 0.07 | 0.09 | 0.14 | 0.12 | 0.24 | | 0.06 | 0.07 | 0.09 | 0.12 | 0.18 | 0.15 | 0.31 | |
| | CD | 0.09 | 0.11 | 0.14 | 0.18 | 0.28 | 0.24 | 0.48 | | 0.11 | 0.13 | 0.18 | 0.23 | 0.35 | 0.30 | 0.61 | |

*M* Fermentation method, *T* Turning interval (h), *F* Fermentation duration (h)

turning interval had no effect. Also there is no interaction among the treatments. Decrease in pH of cocoa beans was also reported in fermentation by box method in the range of 6.05–4.65) [6, 11] and in heap method from 6.5 to 4.9 [24]. So far no interaction between the type of cocoa clone and fermentation duration on pH has been reported [6].

The pulp contains sugar (fructose, glucose and sucrose) and during the fermentation, the sugars get converted to ethanol, lactic acid and acetic acid. A portion of this sugar drains out from the fermenting mass as sweating. This increases the pH of the pulp. A portion of the acids intrude into the beans and this reduces the pH of the pulp [42].

The decrease in pH of fermented cocoa was because of the decrease in citric acid concentration in the pulp [5] and migration of ethanol, lactic acid, acetic acid and other many organic acids produced by microbial activities, into cocoa beans [43]. After citric acid consumption and despite the production of acetic acid, the pH value of the mass of cocoa could increase in the range of 5 to 6 [40, 41]. On completion of fermentation usually the pH of pulp as well as beans reaches the almost same level. The pH of pulp in heap fermentation with 48-h turning interval increased from 3.57 to 4.38, which can be considered as an indication of proper fermentation.

## Temperature Profile of Fermenting Mass

The mean temperature of fermenting mass under basket, heap and box method of fermentation with 0-, 12-, 24- and 48-h turning interval during six days of fermentation is given in Table 2. As seen from the table, there is significant difference in temperature as influenced by the method of fermentation, turning interval and fermentation duration. With all the methods of fermentation, the temperature of the fermenting mass increased up to 2 to 4 days and started decreasing. Frequent turning in the early stages of fermentation has an advantage in causing more rapid temperature rise due to increased aeration. The increased temperature favours the activities of the acetic acid bacteria. Therefore, the difference in temperature rise among the treatments may be due to the variation in the degree and time of aeration of the cocoa mass which stimulates the exothermic reactions of the aerobic microflora. The temperature of the fermenting mass from the range of 29.5–32.5 °C increases to 36–41 °C during peak fermentation period and drops to 32.1–40.1 °C. The highest temperature is reached around 72–120 h of fermentation duration in all the methods of fermentation and turning intervals. Heap method of fermentation reached the highest temperature in the range of 38.5–41.5 in 48 h of

**Table 2** Temperature of fermenting mass of cocoa beans by different methods of fermentation and turning interval with fermentation duration

| Methods | Turning interval, h | Temperature, °C | | | | | | | Mean |
|---|---|---|---|---|---|---|---|---|---|
| | | Fermentation duration, h | | | | | | | |
| | | 0 | 24 | 48 | 72 | 96 | 120 | 144 | |
| Basket method | 0 | 31.0 | 34.5 | 35.5 | 36.5 | 38.5 | 37.0 | 36.1 | 35.6 |
| | 12 | 30.5 | 33.5 | 35.5 | 38.0 | 39.5 | 41.0 | 40.1 | 36.9 |
| | 24 | 31.0 | 33.0 | 35.0 | 35.5 | 38.0 | 38.5 | 37.6 | 35.5 |
| | 48 | 29.5 | 32.5 | 34.5 | 35.0 | 38.0 | 37.5 | 36.6 | 34.8 |
| Heap method | 0 | 31.0 | 36.5 | 38.5 | 37.0 | 34.0 | 33.0 | 32.1 | 34.6 |
| | 12 | 31.0 | 39.5 | 41.5 | 40.0 | 35.0 | 34.5 | 33.6 | 36.4 |
| | 24 | 30.5 | 38.0 | 40.0 | 39.5 | 34.0 | 34.0 | 33.1 | 35.6 |
| | 48 | 31.0 | 38.5 | 40.5 | 38.5 | 35.5 | 34.5 | 32.6 | 35.9 |
| Box method | 0 | 31.0 | 34.0 | 36.0 | 35.0 | 36.0 | 34.0 | 33.1 | 34.2 |
| | 12 | 31.5 | 33.5 | 36.5 | 37.5 | 37.0 | 35.5 | 34.6 | 35.2 |
| | 24 | 32.5 | 34.5 | 35.5 | 36.0 | 36.0 | 35.0 | 34.0 | 34.8 |
| | 48 | 30.0 | 33.5 | 35.5 | 35.0 | 36.5 | 35.0 | 34.1 | 34.2 |
| | Mean | 30.9 | 35.1 | 37.0 | 37.0 | 36.5 | 35.8 | 34.8 | 35.3 |
| ANOVA | | M | T | F | MT | TF | MF | MTF | |
| | Prob. | 0.02 | 0.03 | 0.00 | 0.70 | 1.00 | 0.00 | 1.00 | |
| | Sig. | * | * | * | NS | NS | * | NS | |
| | SEd | 0.42 | 0.49 | 0.65 | 0.85 | 1.29 | 1.12 | 2.24 | |
| | CD | 0.83 | 0.96 | 1.27 | 1.67 | 2.55 | 2.21 | 4.41 | |

*Significant at 5% level, *M* Fermentation method, *T* Turning interval (h), *F* Fermentation duration (h)

fermentation duration. This indicates that fermentation is faster in heap method.

The rise in temperature of the fermenting mass could be taken as an indication of adequate favourable biochemical reaction during fermentation and the lack of temperature development as a symptom of inadequate fermentation [1]. The earlier research findings reported the higher temperatures in the African countries in the range of 30–46.6 °C in box method [11] and 27–46 °C in heap method [24]. Fermentation in small holdings using plastic sack reported the increase in temperature up to 40 °C [28]. The raise in temperature only up to 41 °C in the present study may be due to the region specific, where moderate ambient temperature and relative humidity prevailed.

## Moisture Content of Fermenting Beans

Moisture content of the beans fermented by different methods and turning interval is given in Table 3. It is observed that the method of fermentation and turning interval had no significant difference on the moisture content of the samples and the fermentation duration had significant effect. From the initial moisture content of 53.7 to 58.4% (w.b), the beans reached the moisture content of 49.2 to 53.8% (w.b). on complete fermentation and dried to safe moisture content (below 8%).

Also there is no interaction among the method of fermentation, turning interval and fermentation time on the moisture content of beans during fermentation. It was reported by the earlier researchers that the moisture content of beans during fermentation reduced from an initial moisture content of 43.4% (w.b) to the final moisture content of 40.9% (w.b) [17].

## Quantity of Sweat Produced During Fermentation

Cocoa sweat, the pale yellowish liquid that drains off during fermentation, is the breakdown product of mucilage surrounding the fresh cocoa beans and constitutes about 10% of the weight of cocoa pod. Draining of the sweat during the fermentation is an indication of the progress of fermentation process. The sweat is identified to be a suitable raw material for production of wines, alcohol, marmalade, jam, syrup, etc. [14]. Its rapid collection in large quantity is the first step for its utilization on a commercial scale. However, its large-scale utilization needs exploration.

The quantity of sweat obtained from the fermenting mass under various treatments at 24 and 36 h of

**Table 3** Moisture content of fermenting cocoa beans with fermentation duration at different methods of fermentation and turning interval

| Methods | Turning interval, h | Moisture content, % (w.b.) | | | | | | | Mean |
|---|---|---|---|---|---|---|---|---|---|
| | | Fermentation duration, h | | | | | | | |
| | | 0 | 24 | 48 | 72 | 96 | 120 | 144 | |
| Basket method | 0 | 57.40 | 55.30 | 54.20 | 53.40 | 52.80 | 52.40 | 52.20 | 54.0 |
| | 12 | 53.70 | 52.90 | 52.30 | 51.60 | 51.50 | 51.30 | 49.20 | 51.8 |
| | 24 | 56.30 | 54.80 | 53.10 | 52.70 | 52.00 | 51.80 | 51.60 | 53.2 |
| | 48 | 55.20 | 54.10 | 53.40 | 52.30 | 52.00 | 51.80 | 51.60 | 52.9 |
| Heap method | 0 | 57.60 | 56.20 | 54.90 | 53.70 | 53.80 | 53.60 | 53.40 | 54.7 |
| | 12 | 55.70 | 54.10 | 53.70 | 52.20 | 51.50 | 51.30 | 51.10 | 52.8 |
| | 24 | 55.60 | 54.40 | 53.40 | 52.70 | 52.00 | 51.80 | 51.60 | 53.1 |
| | 48 | 56.30 | 55.20 | 54.30 | 53.20 | 52.50 | 52.30 | 52.10 | 53.7 |
| Box method | 0 | 58.40 | 55.70 | 54.60 | 54.20 | 54.20 | 54.00 | 53.80 | 55.0 |
| | 12 | 57.10 | 56.30 | 54.80 | 53.60 | 52.70 | 52.50 | 52.30 | 54.2 |
| | 24 | 56.60 | 56.40 | 54.50 | 52.30 | 52.00 | 51.80 | 51.60 | 53.6 |
| | 48 | 57.70 | 56.50 | 54.70 | 53.50 | 52.80 | 52.60 | 52.40 | 54.3 |
| | Mean | 56.5 | 55.2 | 54.0 | 52.9 | 52.5 | 52.3 | 51.9 | 53.6 |
| ANOVA | | M | T | F | MT | TF | MF | MTF | |
| | Prob. | 0.12 | 0.14 | 0.00 | 0.96 | 1.00 | 1.00 | 1.00 | |
| | Sig. | NS | NS | * | NS | NS | NS | NS | |
| | SEd | 0.63 | 0.73 | 0.97 | 1.27 | 1.94 | 1.68 | 3.35 | |
| | CD | 1.25 | 1.44 | 1.91 | 2.50 | 3.82 | 3.31 | 6.62 | |

*Significant at 5% level, *M* Fermentation method, *T* Turning interval (h), *F* Fermentation duration (h)

fermentations is given in Table 4. Irrespective of the methods, 51 to 95 ml/5 kg and 13 to 24 ml/5 kg, sweat were collected during 24 and 36 h of fermentation, respectively. From the table, it is seen that both the method and turning interval had significant effect on the sweating yield. In all the cases, the major portion of the sweat is collected during the first 24 h of fermentation. The total quantity of sweat produced for 5 kg of wet beans was high in heap fermentation at all the turning intervals.

Irrespective of the method of fermentation, the samples that are not turned produced less quantity of sweating and the samples turned at 12-h interval produced the highest quantity than other treatments. This may be due to the action of yeast, which dominates the fermentation process during first 38 h [42].

## Changes in Microbial Population

The population dynamics of yeasts, lactic acid bacteria (LAB) and acetic acid bacteria (AAB) were slightly influenced by turning but not by the method of fermentation. A model of the relationship between the fermentation duration and population is given in Fig. 1 for the heap method of fermentation. In general, simultaneous growth of yeasts, LAB and AAB took place. High yeast counts (7.35 $\log_{10}$ CFU/ml of pulp and beans) and high AAB counts (7.71 $\log_{10}$ CFU/ml) were observed [9] in the heap fermentation, indicating the use of well-ripened pods (Fig. 2).

The size of the yeast population increased during the first 12 to 24 h and grew to maximum populations of 7.15 to 7.30 $\log_{10}$ CFU/ml. Upon prolonged fermentation, the yeast population declined. In the case of turned heaps, the yeast population declined rapidly after turning, resulting in no yeast retrieval after 96, 120 and 144 h of fermentation in basket, heap and box method, respectively.

LAB grew during fermentation to maximum population of 8.15 to 8.30 $\log_{10}$ CFU/ml after 48 h. Afterwards, there was a slight decrease of the LAB population and stabilizing upon prolonged fermentation (5.05 to 5.46 $\log_{10}$ CFU/ml). It was remarkable that high counts of AAB were found after 72 h of fermentations (7.06 to 7.21 $\log_{10}$ CFU/ml). However, only in the fermentations with turning did a considerable increase of AAB due to better aeration and a remarkable stabilization upon prolonged fermentation take place. Every time the heap was turned, an increase in the AAB counts was noticed. In the turned treatments, maximum population densities were reached after 30 to 72 h of fermentation. It was also suggested that 72 h of fermentation of cocoa resulted in good quality beans [12, 33].

**Table 4** Amount of sweat collected from the fermenting cocoa beans with fermentation duration and method of fermentation

| Methods | Turning interval, h | Amount of sweating collected, ml/5 kg | | Total, ml |
|---|---|---|---|---|
| | | Fermentation duration, h | | |
| | | 24 | 36 | |
| Basket method | 0 | 63.0 | 19.0 | 82.0 |
| | 12 | 85.0 | 16.0 | 101.0 |
| | 24 | 70.0 | 19.0 | 89.0 |
| | 48 | 70.0 | 18.0 | 88.0 |
| Heap method | 0 | 72.0 | 21.0 | 93.0 |
| | 12 | 95.0 | 26.0 | 121.0 |
| | 24 | 84.0 | 28.0 | 112.0 |
| | 48 | 86.0 | 24.0 | 110.0 |
| Box method | 0 | 51.0 | 13.0 | 64.0 |
| | 12 | 67.0 | 17.0 | 84.0 |
| | 24 | 58.0 | 14.0 | 72.0 |
| | 48 | 59.0 | 15.0 | 74.0 |
| ANOVA | | M | T | MT |
| | Prob | 0.00 | 0.01 | 0.83 |
| | Sig | ** | ** | NS |
| | SEd | 3.21 | 3.71 | 6.42 |
| | CD | 6.63 | 7.65 | 13.26 |

*Significant at 5% level, *M* Fermentation method, *T* Turning interval (h), *F* Fermentation duration, (h)

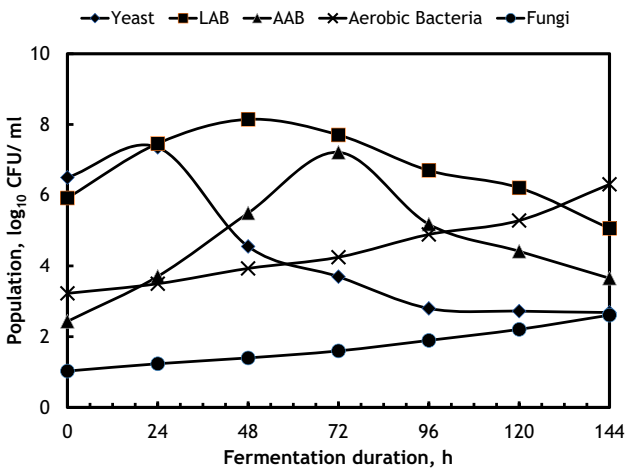



**Fig. 2** Effect of turning interval on microbial population in cocoa fermentation by heap method

The aerobic spore forming bacteria ranged from 3.21 $\log_{10}$ CFU/ml during the start of fermentation and reached a maximum population of 6.73 $\log_{10}$ CFU/ ml at the end of 144-h fermentation. The population of filamentous fungi was the least at the beginning of the fermentation (1.01 $\log_{10}$ CFU/ml) increased to a maximum count of 2.67 $\log_{10}$ CFU/ml at the end of 144 h fermentation. The similar trend was observed in basket and box methods of fermentation.

## Physical and Biochemical Qualities of Sun-Dried Cocoa Beans

The fermented cocoa beans spread in a single layer on cement floor, under sun-drying, took 43 h (6 days) to reduce from initial moisture content of 123.4% (d.b.) to a final moisture content of 8% (d.b.). The physical and biochemical qualities of dried cocoa beans are presented and discussed.

### *Bean Count*

Irrespective of the treatment, the bean count varied from 83 to 101 for 100 g of beans (Table 6) which is an acceptable limit for grade II as established by the Cocoa and Coffee Industry Board. Grade II cocoa bean has a bean count of 85/100 g with less than 4% commercial defects (defective beans) or a bean count of 100/100 g with less than 1% commercial defects [42]. The heap fermented samples recorded the lowest bean count of 83/100 g, whereas basket fermented sample recorded the highest number of beans per 100 g of sample (101) and the turning interval was significant. In the heap method, the turning interval of 12, 24 and 48 h was at par and in the box method the turning interval did not show any effect. Bean

**Table 5** Effect of fermentation method and turning interval on physical quality characteristics of dried cocoa beans

| Characteristics | Method of fermentation | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Basket method | | | | Heap method | | | | Box method | | | |
| | Turning interval, h | | | | Turning interval, h | | | | Turning interval, h | | | |
| | 0 | 12 | 24 | 48 | 0 | 12 | 24 | 48 | 0 | 12 | 24 | 48 |
| Bean count (No./100 g) | 97 ± 2.04b | 101 ± 8.52c | 89 ± 2.97a | 94 ± 6.32b | 85 ± 4.36a | 91 ± 9.87b | 90 ± 6.24b | 89 ± 7.08b | 83 ± 4.18a | 85 ± 4.94a | 84 ± 5.02a | 87 ± 9.44a |
| Bean texture (Hardness, N) | 74.5 ± 2.09a | 98.4 ± 8.30c | 82.1 ± 2.74b | 77.4 ± 5.20a | 82.5 ± 9.86b | 103.9 ± 11.27c | 78.6 ± 9.95a | 83.9 ± 6.68b | 80.0 ± 4.03b | 78.0 ± 4.53a | 77.1 ± 4.60a | 81.9 ± 8.88b |
| Fracturability, mm | 2.0 ± 0.02a | 2.5 ± 0.21c | 1.7 ± 0.06a | 1.9 ± 0.13a | 2.2 ± 0.26b | 2.9 ± 0.31d | 2.5 ± 0.32c | 2.3 ± 0.18b | 2.5 ± 0.13c | 2.3 ± 0.13b | 2.3 ± 0.14b | 2.4 ± 0.26c |
| Brown beans (%) | 78 ± 3.51a | 85 ± 3.06a | 84 ± 2.81a | 82 ± 3.67a | 82 ± 4.00a | 89 ± 4.36a | 88 ± 2.52a | 83 ± 3.03a | 79 ± 3.98a | 84 ± 4.88a | 83 ± 4.96a | 80 ± 2.00a |
| Purple beans (%) | 14 ± 0.13c | 9 ± 0.76a | 10 ± 0.33b | 13 ± 0.87c | 13 ± 1.55c | 7 ± 0.76a | 9 ± 1.14a | 12 ± 0.95b | 15 ± 0.75c | 10 ± 0.58b | 13 ± 0.78c | 14 ± 1.52c |
| Slaty beans (%) | 5 ± 0.05c | 4 ± 0.34b | 4 ± 0.13b | 3 ± 0.20a | 3 ± 0.36c | 2 ± 0.22a | 2 ± 0.25a | 3 ± 0.24c | 3 ± 0.15c | 4 ± 0.23c | 2 ± 0.12a | 4 ± 0.43c |
| Defective beans (%) | 3 ± 0.03b | 2 ± 0.17a | 2 ± 0.07a | 2 ± 0.13a | 2 ± 0.24a | 2 ± 0.22a | 1 ± 0.13a | 2 ± 0.16a | 3 ± 0.15b | 2 ± 0.12a | 2 ± 0.12a | 2 ± 0.22a |
| Cut Test Score | 853 ± 35a | 892 ± 34b | 890 ± 30b | 883 ± 41b | 885 ± 48b | 925 ± 47c | 928 ± 31c | 890 ± 35b | 865 ± 44a | 890 ± 52b | 895 ± 53b | 870 ± 28a |

Mean values presented within the row with the same following letter are not significantly different ($p > 0.05$)

count of 90 to 93/100 g was reported [24] for the cultivars of Belgium.

*Bean Texture*

Hardness is a kernel characteristic that influences both milling and processing. Heap fermented samples are significantly different from basket and box fermented samples and recorded high hardness value of 103.7 N and basket fermented samples recorded lowest hardness value of 74.5 N (Table 5). Higher hardness value was noted in the heap method at 12-h turning interval.

Fracturability is also a textural character of cocoa beans ranging 1.7–2.5 mm. A mixed response of significance among the treatments was noted (Table 5). Turning interval of 24 and 48 h is at par with control in basket method. In heap method, turning interval of 48 h is at par with control and significant with 12- and 24-h interval. Similar trend of at par was noted with control and 48 h, and 12 and 24 h of turning interval in box method. The earlier studies on fermentation have not reported much on bean texture. However, higher hardness was reported as attributed by the higher temperature developed during the heap fermentation compared to other methods, resulting the shell crinkly and stuck tightly to the nib [25].

*Cut Test*

Both the methods of fermentation and turning interval affect the quality of dried cocoa beans. Results of physical quality characteristics of the cocoa beans are presented in Table 5. From the table, it is observed that the per cent brown beans, considered as healthy beans, are at par with the turning interval and significant with the method of fermentation. The per cent content of purple beans, slaty beans and defective beans, which are considered as inferior, are less under the heap method at 12 and 24 h of turning intervals. All the beans showed no sign of insect damages and mould infestation irrespective of the turning interval and the methods of fermentation. It was reported that cocoa beans treated in heaps showed higher percentage of brown beans with 62% than both cocoa beans fermented in wooden and plastic boxes, which presented no pronounced difference in percentage of brown beans below 60% [20]. According to the official standard, a batch of cocoa beans with more than 60% fully brown colour beans is considered as good quality product.

Cut test score assessed based on per cent of brown, purple and slaty bean with respect to method and turning interval [26] ranged 853–928. Without turning in box and basket methods and turning at 48-h interval in box method are at par. At 12 and 48 h of turning interval, the values at heap method are at par yielding the highest (925 and 928).

**Table 6** Effect of fermentation method and turning interval on biochemical quality characteristics of dried cocoa beans

| Chemical quality characteristics | Method of fermentation | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Basket method | | | | Heap method | | | | Box method | | | |
| | Turning interval, h | | | | Turning interval, h | | | | Turning interval, h | | | |
| | 0 | 12 | 24 | 48 | 0 | 12 | 24 | 48 | 0 | 12 | 24 | 48 |
| pH | 4.17 ± 0.04d | 4.58 ± 0.39c | 4.52 ± 0.15c | 4.73 ± 0.32b | 4.88 ± 0.58b | 5.06 ± 0.55b | 5.04 ± 0.64b | 5.25 ± 0.42a | 4.15 ± 0.21d | 4.32 ± 0.25c | 4.36 ± 0.26c | 4.39 ± 0.48c |
| Titratable acidity (meq of NaOH per 10 g of sample) | 3.24 ± 0.03d | 2.16 ± 0.18b | 2.13 ± 0.07b | 2.36 ± 0.16c | 2.27 ± 0.27c | 1.98 ± 0.21a | 2.08 ± 0.26b | 1.76 ± 0.14a | 3.22 ± 0.16d | 2.93 ± 0.17c | 2.84 ± 0.17c | 2.87 ± 0.31c |
| Free fatty acids content ( % oleic acid equivalent) | 0.88 ± 0.01a | 0.91 ± 0.08a | 0.86 ± 0.03a | 0.82 ± 0.06a | 0.80 ± 0.10a | 0.76 ± 0.08a | 0.74 ± 0.09a | 0.71 ± 0.06a | 0.86 ± 0.04a | 0.84 ± 0.05a | 0.79 ± 0.05a | 0.81 ± 0.09a |

Mean values presented within the row with the same following letter are not significantly different ($p > 0.05$)

All the other treatment combinations were at par. Under the heap fermentation method, the reported cut test score of 933–950 [24] is in range with the present findings.

### *pH*

pH**,** an important biochemical quality attributed by fermentation, assessed for the dried beans is shown in Table 6. The pH of beans fermented during 5 days without turning in box and in basket methods was found more acidic with a value of 4.15 and 4.17, respectively, than beans fermented in heap, which recorded a value of 4.88 showing significant difference among the treatments. When two turnings per day were done, beans resulted from box became extremely acidic with pH 4.39 than beans fermented in basket and heap which became less acidic with pH 4.73 and 5.25, respectively. Among the methods, the pH of the beans with 48-h turning interval in basket method and 0, 12 and 24 h in heap method was at par. Also, the pH of the beans with 12- and 24-h turning interval in basket method and 12, 24 and 48 h in box method was at par. Similar results were also reported in box method [6, 11, 25] and heap method [24, 35]. Thus, the heap fermentation method produced less acidic beans with a high pH value. The cocoa beans fermented in plastic box were reported as more acidic than those obtained from wooden box and heaps on banana leaves, which led to the less acidic product [19, 20].

### *Titratable Acidity*

Titratable acidity (Table 6), as a better indicator of acidity than pH, showed a trend of decreasing value with an increase in pH [34] in all fermentation methods and in all turning interval. Titratable acidity ranged from 1.76 to 2.87 meq of NaOH per 10 g for fermentation in heap and box method with two turnings. Fermentation in box and basket without turning beans recorded high values of 3.22 and 3.24 meq of NaOH per 10 g, respectively. These results are comparable with the results reported in the earlier studies [19, 21]. Turning intervals of 12 and 24 h were at par in both basket and box methods. Titratable acidity in the range of 0.565–3.07 meq of NaoH per 10 g was found in box method for the Brazilian varieties [11].

### *Free Fatty Acid*

Free fatty acid content is not significantly different with the fermentation method and turning interval (Table 6). The values ranged from 0.71 to 0.91% oleic acid equivalent among the methods of fermentation and turning interval. For reasons of quality, the directive 73/241/EEC (European

Economic Communities Act, 1973) limits the maximum FFA content to 1.75% oleic acid equivalent in cocoa butter [31].

## Conclusions

The method of fermentation, fermentation duration and tuning intervals during fermentation of cocoa beans are the important parameters to achieve desirable qualities of the cocoa beans. The study suggests that fermentation by heap method for 5 to 6 days with turning the mass at 12-h interval resulted in desirable quality parameters of pH, temperature and hardness (texture). The higher quality attributes, viz. bean count (91/100 g), pH (5.06), brown beans (89%), cut test score (925), lower acidic nature (titratable acidity of 1.98 meq of NaOH per 10 g of sample) were obtained under the heap method of fermentation with 12 h of turning intervals. Scope for further studies on drying the fermented beans by different methods at farm level to enhance the quality attributes, and utilization of cocoa sweat for edible, medicinal and aromatic purposes are foreseen.

**Author Contributions** Dr. R. Arulmari did planning and design of experiments, sourcing raw materials, execution of experiments, data collection, data analysis and drafting the manuscript. Dr. R.Visvanathan done supervision, planning experiments, guiding the scholar, data interpretation, manuscript correction and editing.

**Declarations**

**Conflict of interest** Authors declare that they have no conflict of interest.

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