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Physico-chemical and Microbial changes in Cocoa Beans during Different Methods of Fermentation

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Abstract: Fermentation is a crucial step in the post-harvest processing of cocoabeans. The present investigation was to analyze the effect of four types of fermentation method such as tray, box, heap and basket method and it was compared with three different environmental conditions (ambient condition, poly house and artificial fermentation chamber). The final recommendation based on physico-chemical and microbial analysis. The fermentation of cocoa beans of all methods in the artificial fermentation chamber was the best among the other conditions for the production of good quality cocoa. Similarly, the box method was found the best among the other methods for the production of good quality cocoa.

Keywords: Cocoa beans, Fermentation, Basket method, Box method

Cocoa (*Theobroma cacao L.*) is an important plantation crop in the world, belongs to Malvaceae family. It is a tropical crop, grows within 15-20^0 latitude from equator and native to the amazon region of South America. The cocoa production procedure encompasses harvesting of cocoa pods, stripping the beans from the pods and thereby fermentation emerges out to be the first step in cocoa processing. The quality of value added product from cocoa depends on cocoa fermentation. In traditional method the beans are tumbled in heaps, boxes, baskets and trays covered with plantain leaves and left to ferment for 5-7 days (Fowler, 1999).The fermentation process accomplishes several other desirable changes such as prevention of germination, release of enzymes in the beans, reduction of astringency, richness of colour, altered texture of the seed coat and most important factor is the growth of microorganisms (Schwan and Wheals, 2004). During fermentation, due to exothermic oxidation reaction, the temperature of fermenting bean mass increases to 45–50°C.This is considered inevitable for the successful fermentation and the development of chocolate flavor. During fermentation the microbial succession of a wide range of yeasts, lactic-acid, and acetic-acid bacteria and microbial products, such as ethanol, lactic acid and acetic acid kill the beans embryo and cause production of flavor precursors. Increase in acetic acid leads to a rise in bacilli and filamentous fungi that can cause off-flavors. Very limited studies on the effect of different methods and conditions of fermentation were reported. In the present paper results were reported on fermentation of cocoa beans was conducted by four methods under three different environmental conditions namely ambient, polyhouse and fermentation chamber. The effects of treatments on dependent variables like temperature, pH, moisture content and different microorganisms such as bacteria and yeast during their fermentation was optimized.

MATERIAL AND METHODS

Raw materials: The freshly harvested cocoa pods procured from farmer's field at Karuvarakundu, Malappuram district. The maturecocoa pods were broken by developed cocoa pod breaker and their beans were separated from placenta by developed beans extractor. Fermentation was carried out in 12 batches by four methods using bamboo baskets(22cm × 12cm×27.5cm),wooden box(21.5 cm × 20m × 14 cm), heaps on concrete ground (55cm×170cm) and plastic (High-density polyethylene) trays (27.5 cm × 22.5 cm ×6.5 cm) with sound beans.

Experimental set up: The wooden box, tray and bamboo baskets were lined with one or two layers of plantain leaves to prevent any heat and moisture loss during fermentation. The bottom of the wooden box, tray and bamboo baskets facilites in draining acidic liquid resulted from liquefaction of mucilaginous pulp during fermentation. In order to study the effect of temperature on fermentation, studies were conducted in fermented chamber, poly house and ambient condition. A low tunnel type polyhouse of length 4m, breadth 1.5m and height 1.25m was set up. It was completely covered using 200 micron polythene sheet (HDPE) A fermentation chamber is rectangular chamber made of wood with dimension 1.90 m × 0.85 m × 0.85 m. It consists of a heating unit and drying chamber. Four 100 W electric bulbs placed diagonally 150mm from the corner of the rectangular

chamber act as heating source. The temperature inside the chamber was regulated by using a thermistor (Laser 56ON-22)

The experimental treatments were as follows;

T_1 = Tray method (ambient condition) T_2 = Box method (ambient condition)

T_3= Heap method (ambient condition) T_4 = Basket method (ambient condition)

T_5 = Tray method (polyhouse) T_6 = Box method (polyhouse)

T_7 = Heap method (polyhouse)T_8 = Basket method (poly house)

T_9 = Tray method (fermentation chamber) T_{10} = Box method (fermentation chamber)

T_{11} = Heap method (fermentation chamber) T12 = Basket method (fermentation chamber)

Physico-chemical analysis: The physico-chemical analysisof the samples were carried out Daily at 6 hours interval.

pH of pulp and bean : The pH of fermenting mass was drawn from three different positions such as top, center and bottom. pH of cotyledons (10 g) or pulp and test a (5g) were measured (Hii et al 2009) using a digital pH meter (ELICO-612 model).

Temperature profile of the fermenting mass: The ambient temperature and the temperature of the fermented cocoa mass was monitored by inserting a mercury glass thermometer (0-60°C range) at three different positions (Sunilkumar 2005).

Moisture content of fermenting beans: Moisture content of samples were determined by the method of Lagunes-Galvez et al 2007.

Microbial enumeration: For the enumeration of bacteria and yeast in fermented cocoa mass was determined by method of Ardhana and Fleet (2003). The incubation period for bacteria 24 – 48 h and 3 -5 days for yeast and fungi respectively.

RESULTS AND DISCUSSION

Temperature: The temperature of fermenting mass increased under ambient (35.5°C to 40.54°C), polyhouse (38.1°C to 42.01°C) and artificial fermentation chamber (40.5°C to 43.07°C)up to fourth day of fermentation thereafter slightly decreased.The present observations corroborate Ardhana and Fleet (2003). Among the four methods higher temperature of fermenting mass was observed in box method (44°C) at fermentation chamber due to exothermic reaction, produced by microbial activity will lead to an increase in temperature.

Ambient condition: In all the four methods (heap, tray, box and basket) temperature increased due to the heat that is formed of several reactions, namely sugar ethanol + CO_2+ 18 calorie, ethanol acetate acid + H_2O + 235 calorie and, acetate acid H_2O+ CO_2 + 419 calorie. The temperature rise in the fermentation process takes place periodically. In the first day, the temperature varied between 30and 36°C. At that time there is a process of fermentation by yeasts pulp, converted into ethanol and organic acids. The second day, the temperature ranged between 36-39°C. Ethanol and dead yeast converted into acid by bacteria. This process continues until the third day thereafter temperature reaches maximum of 39°-41°C during fourth day (high temperature observed in

Table 1. Effect of ambient conditions on temperature profile of basket, box, and heap and tray fermentation

Fermentation conditions	Treatments	Fermentation days					Mean
		1st	2nd	3rd	4th	5th	
Open condition	T1	35.50	37.87	38.90	39.94	39.14	38.27
	T2	36.54	38.70	39.10	40.54	39.90	38.96
	T3	35.52	36.97	38.53	39.54	38.95	37.90
	T4	35.50	36.9	38.80	39.98	39.50	38.14
Polyhouse	T5	38.50	39.07	40.24	41.85	41.15	40.16
	T6	39.42	39.85	40.75	42.01	41.78	40.10
	T7	38.10	39.04	40.35	41.74	41.25	40.76
	T8	38.53	39.00	40.53	41.52	41.00	40.12
Fermented chamber	T9	40.54	41.53	42.23	42.93	42.75	42.00
	T10	41.03	42.16	42.33	43.07	42.95	42.31
	T11	40.70	42.14	42.20	42.82	42.71	42.11
	T12	40.80	41.75	42.06	42.80	42.85	42.05
	Mean	38.39	39.58	40.50	41.56	41.16	40.24

box method). On the third day and fourth day the success factor of the fermentation process was air circulation well, the cocoa beans were converted intolactic acid [anaerobic] or to acetate acid [aerobic].Thereafter temperature decreases at the end of fermentation below 40°C. Cocoa beans will die (not germinate) when the temperature over 40°C and will decompose when the temperature over exceeds (Hatmi et al 2015).

Polyhouse: Fermentation of cocoa beans in the polyhouse by four methods (heap, tray, box and basket) produce temperature an average 36°C.In the second and third day temperature reached to39 to 41°C maximum during fourth days (41-42°C) .The proper air circulation and high humidity in polyhouse causes increases in temperature during fermentation thereafter decreases in fifth day below 40°C. This resulted in a fermentation of microbiological and enzymatic imperfect and the quality of cocoa beans will be lower. Well fermentation can only take place at temperatures of more than 40°C and less than 42°C. The air circulation takes place very quickly due to holes so the aerobic process (acetic acid) and anaerobic (lactic acid) inhibited.

Fermentation chamber: In the process inhibited chamber, box shows a slight rise of temperature differences in the upper, middle and bottom. The top and middle of the box generate an average rise of temperature and was quite stable. In the fermentation chamber the average temperature was more than 40°C, except at the bottom (39.17°C and 38.17°C) in all methods such as heap, tray, box and basket. The bottom of the box has the lowest temperature due to melting pulp, due to this maximum temperature observed in fermentation chamber.

pH: Cocoa fermentation is a complex biochemical reaction due to aerobic or anaerobic hydrolytic process (Table2).

Mucilage pulp (pH): The pH of mucilage pulp increased with increase in fermentation time. The average pH of the mucilage pulp that undergone fermentation ranged from3.88 to 4.50. The maximum pH of mucilage pulp of 4.99 was for T_{10} t (box method) in artificial fermentation chamber may be due to artificial fermentation chamber condition can enhance the optimal activity of the endogenous enzymes and may increase fermentation temperature and pH of beans (Hansen et al 1998). The pH at the end of fermentation ranged between 4.31 (T_1) and 4.39 (T_2), in different methods. The maximum pH was in poly house and artificial fermentation chamber (Fig. 2a, 2b and 2c). The higher pH was for box method (T_2, T6 and T_{10}) in three environmental conditions and due to frequent mixing and turning of coco beans increase the pH. The lower pH was observed for heap treatments (T_3, T_7 and T_{11}) in all environmental conditions (ambient, polyhouse and artificial chamber) due to absence of turning the cocoa pulp. The initial low pH of cocoa mucilage pulp was due to the presence of citric acid that favored the yeast to grow. However, as fermentation proceed, the dominant yeasts with good pectinolytic activity degraded the mucilage pulp and removed the citric acid to allow subsequent bacteria to grow resulted in higher pH (Ganeswari et al 2015). An increase in pH towards the end of fermentation was caused by citric acid conversion. During fermentation, the sugar present in cocoa mucilage pulp gets converted to ethyl alcohol, lactic acid and acetic acid. A portion of acid is drained out from fermenting mass as sweating leads to increase in pH (Sunil Kumar 2005, Sarkar et al 2014).

Coco bean (pH): The pH of cocoa beans decreased by

Table 2. Effect of ambient conditions on pulp pH of basket, box, heap and tray fermentation

Fermentation conditions	Treatments	Fermentation days					Mean
		1st	2nd	3rd	4th	5th	
Open condition	T1	3.86	3.99	4.11	4.20	4.31	4.09
	T2	3.95	4.07	4.16	4.25	4.39	4.16
	T3	3.85	3.96	4.12	4.23	4.32	4.10
	T4	3.96	4.05	4.14	4.21	4.37	4.15
Polyhouse	T5	3.86	4.15	4.19	4.21	4.37	4.16
	T6	3.92	4.21	4.22	4.32	4.42	4.22
	T7	3.91	4.11	4.23	4.29	4.33	4.17
	T8	3.79	3.96	4.11	4.19	4.29	4.07
Fermented chamber	T9	3.85	4.12	4.33	4.66	4.96	4.38
	T10	3.91	4.30	4.51	4.78	4.99	4.50
	T11	3.82	4.11	4.27	4.57	4.82	4.32
	T12	3.90	4.25	4.37	4.71	4.90	4.43
	Mean	3.88	4.11	4.23	4.39	4.50	4.23

increasing the days of fermentation whereas reverse trend was observed for mucilage pulp (Table 3). The minimum pH (4.09) of beans was for T_6 in poly house followed by T_6, T_7 and T_8 (basket method) in poly house at the end of fermentation. The minimum pH at ambient condition and artificial fermentation chamber was between4.21 (T_2) and 4.49 (T_{12}) respectively. Rohsius et al (2006) observed avoidance of mixing and absence or inadequate aeration of beans in fermentation favored the development of anaerobic conditions, thus the onset of bacterial activities producing organic acids in the beans, which reduced the pH to the lower point of 4.5. The sugars present in cocoa mucilage pulp get converted to ethanol, lactic acid and acetic acid. A portion of acid intruded into the beans which reduced the pH of beans (Sunil Kumar2005).Higher pH of coco beans was observed for try methods ($T_{1, T5}$ and T_9) in three environmental condition and may be try boxes provided an adequate oxygenation due to turning practice increases the pH.

Moisture content: The fermentation time had a significant effect on moisture content. High moisture content causes a short shelf life and is vulnerable to pests and diseases (Table 4). The moisture content decreased by increasing the fermentation days (one to five days) in all treatments (ambient condition, polyhouse and artificial fermentation chamber).

Ambient condition: In first day of fermentation T_1 and T_4 has

Table 3. Effect of ambient conditions on beans pH of basket, box, heap and tray fermentation

Fermentation conditions	Treatments	Fermentation days					Mean
		1st	2nd	3rd	4th	5th	
Open	T1	5.30	5.18	4.75	4.42	4.33	4.80
	T2	5.15	5.05	4.58	4.32	4.21	4.66
	T3	5.20	5.11	4.60	4.49	4.38	4.76
	T4	5.26	5.16	4.62	4.45	4.36	4.77
Polyhouse	T5	5.33	5.18	4.72	4.51	4.37	4.82
	T6	5.21	5.09	4.50	4.27	4.09	4.63
	T7	5.23	5.14	4.63	4.39	4.19	4.72
	T8	5.30	5.19	4.54	4.39	4.22	4.73
Fermented chamber	T9	5.60	5.43	4.85	4.61	4.45	4.99
	T10	5.41	5.21	4.71	4.58	4.32	4.85
	T11	5.55	5.31	4.89	4.68	4.43	4.97
	T12	5.58	5.25	4.82	4.6	4.49	4.95
	Mean	5.34	5.19	4.68	4.48	4.32	4.80

Table 4. Effect of ambient conditions on moisture content of beans on basket, box, heap and tray fermentation

Fermentation conditions	Treatments	Fermentation days					Mean
		1st	2nd	3rd	4th	5th	
Open	T1	59.95	57.53	55.23	52.98	51.27	55.39
	T2	57.00	56.40	54.00	52.82	51.18	54.28
	T3	58.20	57.92	55.75	53.09	51.95	55.38
	T4	59.75	57.54	56.90	53.19	52.05	55.89
Polyhouse	T5	57.50	56.23	55.13	53.19	51.85	54.78
	T6	57.22	56.12	54.23	52.98	51.03	54.32
	T7	57.40	56.80	54.31	53.62	52.94	55.01
	T8	58.85	56.92	55.03	54.98	52.94	55.74
Fermented chamber	T9	57.50	55.45	54.12	52.09	50.94	53.12
	T10	56.27	55.24	52.89	51.05	50.17	54.02
	T11	56.86	55.31	54.65	52.13	50.64	53.92
	T12	57.27	56.50	54.87	52.15	50.25	54.21
	Mean	57.81	56.50	54.76	52.86	51.43	54.67

high moisture content around 60%, whereas T_2 and T_3 had lower moisture. In second day fermentation T_3(heap method) showed high moisture thanT_1, T_2 and T_4 around 58% due to weather condition and open atmosphere (Galvez et al 2007). During third and fourth day T_3 and T_4 has high moisture content of 53 to 57% than other treatments due to uneven drying of fermentation mass. Finally, fifth day all the samples moisture was lowered down to around 50% moisture. This may be due to the high temperature causes uneven drying and partial removal of moisture

Polyhouse: In polyhouse chamber maximum fermentation moisture was 58% (T_8) during first day of fermentation. Second day of fermentation all the sample moisture slightly reduced among all the samples T_6 and was below 55% moisture due to control condition of drying in box method. Thereafter third and fourth day of fermentation moisture reaches up to 50% and lowest moisture has been observed for T_6 treatment. During final day of fermentation the moisture has reduced after completing the fermentation. The moisture reduced, more in polyhouse compared to ambient condition due to whether condition and controlled removal of moisture .

Fermentation chamber: Among the three methods, lowest moisture observed in artificially fermentation chamber around 57%. First day of fermentation the moisture content of all the samples T_9, T_{10}, T_{11} and T_{12} was similar around 56 to 58%. The after moisture content ofT_9, T_{10}, T_{11}sampleswere reduced whereas in T_{12}there was not much variation due to basket aeration in the chamber. During fourth and fifth days of fermentation all the treatment (T_9, T_{10}, T_{11} and T_{12}) moisture was reduced to 50%.

Microbial Population

The microbial population (yeast and bacteria)was increased significantly during the first 3 days of fermentation thereafter decreased at the end of fermentation may be due to proper aeration in first three day favors the growth of microorganism (Table 5).

Ambient condition: The maximum population of yeast was observed on third day of fermentation. The yeast count for T_1, T_2, T_3 and T_4 under ambient condition at the third day of fermentation was3.31×10^5, 5.3×10^5, 3.3×10^5 and 4.3×10^5cfu/ml, respectively. Similarly, maximum bacteria (lactic acid bacteria and acetic acid bacteria)population estimated under ambient condition at the third day of fermentation was 47.34×10^5, 27.65×10^5, 23.34×10^5 and 28.36×10^5cfu/ml, respectively, This may be due to the excellent nutrients contained in the cocoa beans as well as the biochemical changes inside in the beans also play significant role in the above scenario. Ardhana and Fleet (2003) observed a yeast count of 10^4–10^5cfu/g in cocoa beans is able to initiate cocoa bean fermentation. Yeasts, lactic acid bacteria and acetic acid bacteria are the main micro floras involve in spontaneous cocoa bean fermentation as each of them are responsible to the synthesis and production of related metabolites such as ethanol, lactate, acetate, heat and also volatile precursors (Lopez and Dimick 1995). The initial phases of the fermentation growth of yeasts are favored due to the high sugar content, low pH and limited oxygen availability in the mucilage pulp (Thompson et al 2001). Cempaka et al (2014) observed that the optimal temperature and pH for yeast growth was 30ºC and 4.5,

Table 5. Population of yeast and bacteria of different treatments

Fermentation conditions	Treatments	Fermentation days					
		Yeast (10^5cfu ml^{-1})			Bacteria (10^5cfu ml^{-1})		
		1st	3rd	5th	1st	3rd	5th
Ambient condition	T_1	1.21	3.33	2.10	6.66	47.34	33.24
	T_2	2.60	5.33	4.20	7.33	27.65	20.33
	T_3	1.40	3.30	2.60	5.66	23.34	13.60
	T_4	2.50	4.63	3.30	5.30	28.36	18.66
Polyhouse	T_5	7.30	19.33	14.66	4.36	13.67	9.40
	T_6	4.34	25.60	12.30	4.66	15.32	8.30
	T_7	4.15	14.36	9.29	5.50	11.02	9.60
	T_8	5.30	21.61	11.35	6.30	18.60	15.24
Fermentation	T_9	6.34	20.3	15.3	2.13	5.34	3.60
	T_{10}	9.07	27.30	19.61	9.33	15.33	10.6
	T_{11}	6.70	25.32	23.60	3.40	9.60	4.33
	T_{12}	8.25	26.33	21.30	4.60	12.60	6.60
	Mean	4.93	16.39	11.63	5.43	19.01	12.77

respectively. The yeast fermentation produces various organic acids, such as acetate and citrate. It normally produces ethanol as metabolic product. The increase in ethanol concentration may also have ceased the yeast growth after 30 hours of fermentation

Polyhouse: The maximum yeast count was observed from tray (T_5) followed by box (T_6), heap (T_7) and basket (T_8) method under poly house at the third day of fermentation. The bacteria population also showed the same trend.

Fermentation chamber: The maximum population of yeast and bacteria (lactic acid bacteria and acetic acid bacteria) was observed from T_9 (tray) followed by T_{10} (box), T_{11} (heap) and T_{12} (basket method) under artificial fermentation chamber at the third day of fermentation. The increase of the microbial load during fermentation is due to the proliferation of dominating micro floras especially filamentous fungi, yeasts or those lactic and acetic acid producing bacteria. Moreover, the excellent nutrients contained in the cocoa beans as well as the biochemical changes inside in the beans also play significant role in the increase of microbial population (Camu et al 2007). The slight decrease of microbial load during end of the fermentation might attribute by the depleting of essential nutrients as most of the sugars originated from the mucilage pulp were converted into alcohol and other metabolites.

CONCLUSION

The mean temperature of fermenting mass increased whereas pH and moisture content decreased from the initial to the fifth day of fermentation. The yeast population is more in artificial fermentation chamber as compared to other two conditions (poly house and ambient condition).The fermentation of cocoa beans kept under artificial fermentation chamber was the best among the other conditions for the production of good quality cocoa and also, the box method was the best among the other fermentation methods.

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