BIOCHEMICAL CHANGES IN COCOA PULP AND SWEATINGS DURING FERMENTATION OF COCOA BEANS

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ABSTRACT

Biochemical changes in the cocoa pulp and sweatings during the traditional heap and sweat-box methods of fermentation of cocoa beans were studied. Carbohydrate analysis showed the presence of fructose (5.06%), glucose (3.58%) and sucrose (6.17%) as the only free or fermentable sugars present in cocoa sweatings. Changes in levels of the free sugars in the sweatings were followed with fermentation time over 48 hours. Sucrose was found to invert rapidly to glucose and fructose which were converted to alcohol and then acetic acid by yeast and acetic acid bacteria. Alcohol production started after 12 hours of fermentation and reached its peak at 30 hours and then declined after 30 hours. The pH of the cocoa pulp gradually increased while that of the cotyledons decreased simultaneously but both reached an approximate value at the end of fermentation by the traditional heap and sweat-box methods. A high buffering power of the sweatings was observed.

Keywords: Cocoa Sweatings and pulp, fermentable sugars, alcohol, acetic acid, pH, fermentation.

INTRODUCTION

The so-called 'fermentation' of cocoa beans is possibly the most important stage in the treatment of the raw material in order to produce a stable product for the manufacture of chocolate and other cocoa products. Various physical and chemical changes go on in and around the cocoa beans during fermentation and much work has been done on the changes that take place especially within the cotyledons of the beans by Chatt (1) and others; these investigations have been reviewed in detail by Minifie (2) and Dimick and Hoskin (3). However much work needs to be done on the mucilaginous pulp around the beans and the sweatings (the liquid that drains off from fresh cocoa beans as a result of the breakdown of the pulp). The pulp together with the microbial activity in the fermenting mass must provide a medium at the correct temperature and pH for the enzymes to liquify the pulp and to create the reactions within the cotyledon so vital to the production of chocolate flavour. Howat et al. (4) argued that the exothermic fermentation of the sugars in the sweatings alone is not sufficient to account for the temperatures observed during normal fermentation and suggested that microbial activity in the fermenting mass supplies heat to the mass.

In Ghana and most other cocoa producing countries the cocoa sweatings have been regarded as a useless by-product of cocoa fermentation. Several workers (5,2,6) have investigated the chemical constituents of the cocoa sweatings and have produced on the laboratory scale alcoholic beverages and other products from the sweatings. Opeke and Jacob (7) and Agyeman et al. (8) have investigated the large-scale collection of cocoa sweatings and the processing of cocoa sweatings.

MATERIALS AND METHODS

Fresh cocoa pods of Amazonia, Hybrid and Amelonado varieties were harvested from the plantation of Kwadaso Agriculture Station, Kumasi, Ghana and used within one day of harvesting. Only fresh and healthy beans from greenish-yellow pods were used for the collection of the sweatings.

The traditional heap and sweat-box methods of fermentation were done at Kwadaso Agriculture Station while the thermostatically controlled oven fermentation and biochemical determinations were done at the Biochemistry Department of the University of Science and Technology, Kumasi.

Heap Fermentation

A basket-full of fresh Forastero cocoa pods containing about 10.0-12.0 kg of fresh cocoa beans plus pulp was laid on a foundation of plantain leaves and care-

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fully covered with the same material to exclude rain. Mixing of the beans was done on the 2nd and 4th days of fermentation.

Sweat Box Fermentation

Twelve basket-full each containing about 10.0-12.0kg of fresh Forastero cocoa beans plus pulp were put in a 3 tier sweat-box of dimensions 80 x 70 x 75 cm. The beans were covered with plantain leaves and wooden planks. The beans were mixed by transferring them from the top box of the 3 tier sweat-box system to the 2nd lower box after 2 days and then the 3rd lowest box after 4 days. Fermentation of the beans by both the heap and sweat-box methods took 6 days.

Oven Fermentation

A small sweat-box of dimensions 25 x 20 x 13 cm was filled with 2.2 kg of fresh cocoa beans plus pulp which had previously been exposed to the atmosphere for two hours with intermittent mixing as to be uniformly contaminated with natural microbial flora. The beans were covered with plantain leaves and the box placed in a termostatically controlled oven. Mixing of the beans was done on the 2nd and 4th days of fermentation and two 100 ml beakers containing saturated lime solution were placed in the oven to absorb any CO_2 that might be produced in the course of the fermentation. Excess CO_2 in the atmosphere surrounding the fermenting mass of beans has an effect of producing cocoa with undesirable flavour (9). Meticulous care was taken in the control of the temperature never to allow the temperature of the fermenting mass of beans to exceed 50°C the temperature above which the normal fermentation process is affected (10). The oven temperature was set at 35°C for the first day and after mixing of the beans on the second day of fermentation the oven temperature was set at 45°C and this temperature was maintained for the remaining fermentation period. The beans were fermented for 5 days.

Cocoa sweatings were collected from the heap fermentation method and the sweat-box fermentation method as described by Agyeman et al. (8) Cocoa sweatings were also collected by a modification of a laboratory apparatus designed by Maclean and Wickens (11). The fermentable sugars in the sweatings were identified by paper chromatographic analysis on Whatman No.1 papers using solvent systems of Patridge and Westall (12) and quantitatively determined in the samples by a combination of chemical and enzymatic methods employing the techniques of Johnson et al (13).

Alcohol in the 0-48 hour samples of the sweatings was determined as ethanol by volume from specific gravity (14). An indirect method of determination of volatile acidity (as acetic acid) described by Joslyn (15) was used.

The pH values of the sweatings, pulp and cotyledons of the beans were measured by a pH meter. A method recommended by the Office International du Cacao et du Chocolat (16) was used in preparing the solutions for measuring the pH values of the pulp and cotyledons of the beans.

RESULTS AND DISCUSSION

Paper chromatographic analysis identified glucose, fructose and sucrose as the only free sugars in cocoa sweatings from Amelonado, Amazonia and Hybrid fermenting masses of cocoa beans. Chatt (1) also reported finding glucose, fructose and sucrose as the only free sugars in cocoa pulp from which the cocoa sweatings are formed. The concentrations of the free fermentable sugars in the sweatings that drained off from fermenting masses of Amelonado, Amazonia and Hybrid Cocoa beans are tabulated in Table 1. The initial total fermentable sugars obtained for Amelonado, 14.32%, Amazonia, 15.37% and Hybrid, 14.82% compare favourable with the pulp sugars reported by Minifie (2) as 14.07%. As shown in Figure 1 there was a rapid fall of sucrose between 0-18 hours and this was parallel by an equally sharp rise of levels of glucose and fructose. This trend suggests an inversion of sucrose to glucose and fructose most likely by enzymatic hydrolysis through probably the enzyme invertase or by acid hydrolysis or by both processes. This negative correlation between the sucrose on one hand and the glucose and fructose on the other was offset between 18 hours to 36 hours of the fermentation time during which time there was a rapid drop in concentrations of glucose and fructose. This decrease of sugar levels with fermentation time could be attributed to their conversion to alcohol by microorganisms.

Table II shows the results obtained for changes in alcohol, fermentable sugars and acetic acid concentrations with fermentation time in the sweatings from the fermenting mass of Hybrid cocoa beans. Alcohol production was observed in the sweatings after 12 hours reaching its peak at 30 hours. After 30 hours a decrease in alcohol content was observed until 48 hours when the alcohol content decreased to 2.35%. The decrease in alcohol content was accompanied simultaneously by a gradual rise of volatile acidity (mainly acetic acid) from 0.07% at zero hours to 2.70% at 48 hours. Furthermore a rapid decrease of total fermentable sugars was observed after 18 hours (Figure 2). Roelofsen (17) studying the fermentation of cocoa beans and pulp earlier reported a rapid upsurge of yeasts between 12-30 hours of fermentation of cocoa. The sugars therefore appear to be converted into alcohol by yeasts reaching the peak at 30 hours. The decrease in alcohol content after 30 hours was due to the conversion of alcohol to acetic acid by acetic acid bacteria the presence of which has been reported by Roelofsen (17) in the fermenting mass of cocoa beans.

Changes occurred in the pH of the pulp and the cotyledons of the cocoa beans during fermentation (Table III and Figures 3 and 4). Generally there was an increase in the pulp pH except in the oven fermentation with a concomitant decrease in the pH of the cotyledons. In both the heap and sweat-box methods of fermentation, the pulp and cotyledon pH changed more rapidly in the surface layer but reached approximately the same value of about pH 5 at the end of the fermentation. Rohan (9) reported that the pH values for cocoa pulp and cotyledons usually come between 4.5-5.0 at the end of fermentation. In the oven method of fermentation, the pH of the pulp and the cotyledons never reached an approximate value after 5 days of fermentation even though like the heap and the sweat-box methods there was a decrease in cotyledon pH (Figure 4) and a gradual increase in the pulp pH after a decrease in pH after the first day (Figure 4). Roelofsen (18) reported a small increase of pulp pH during fermentation of cocoa beans and attributed this increase to the dissimulation of citric acid content by yeasts and lactic acid bacteria and its replacement by less dissociated lactic acid and acetic acid. The decrease in cotyledon pH is not so easily accounted for but it may be partly explained on the basis of diffusion of acetic acid from the pulp and sweatings across the testa and partly on the basis of production of organic acids through hydrolytic breakdown of cocoa polyphenols during fermentation.

The small changes in pH values of the sweatings (3.62 to 3.72) with fermentation time even though there was an increase in fermentation products such as acetic acid indicate a strong buffering capacity exhibited by the sweatings. The organic acids in the presence of minerals as reported by Chatt (1) may serve as an efficient buffering system thus reducing any drastic changes in pH towards acid condition.

The chemical and physical changes that take place in the course of cocoa fermentation and sweating play important roles in the proper curing of the beans to give the beans the required chocolate flavour. The maintenance of optimum conditions such as temperature and pH are therefore of vital importance in the fermentation and sweating of the beans. There is a possibility of the passage of the products of pulp fermentation through the testa to the cotyledon and this could influence chocolate flavour. The rise in temperature and changes in pH help to kill the bean and much of the development of chocolate flavour may depend on this process. The exothermic fermentation of the sugars in the sweatings to produce alcohol and acetic acid account for much of the increase in temperature observed in normal cocoa fermentation.

The results give a picture of the high potentiality of cocoa sweatings to serve as a good natural medium for the production of alcohol and vinegar (acetic acid) by pure cultures of microorganisms. The sweatings contain suitable amounts of fermentable sugars and good natural conditions for the growth of microorganisms used in alcohol and vinegar production. Agyeman et al. (8) observed that over 70% of the total sweatings could be collected during the first 6 hours of fermentation time using the sweat-box method of fermentation. During the first 6 hours of fermentation time only a small amount of the fermentable sugars are converted to alcohol and acetic acid.

REFERENCES

1. **Chatt, E.M.** Cocoa: Cultivation, Processing, Analysis. Interescience Publishers Inc., New York, pp. 91-100, (1953)

2. **Minifie, B.W.,** Chocolate, Cocoa and Confectionery: Science and Technology. J. & A. Churchill, London, pp. 9-15, (1970).

3. **Dimick, P.S. and Hoskin, J.M.,** Chemico-Physical Aspects of Chocolate Processing - A Review. Can. Inst. Food Sci. Technol. J., 14 269-282, (1981).

4. **Howat, G.R., Powell, B.D. and Wood, G.A.R.,** Experiments in Cocoa Fermentation in West Africa, J. Sci. Fd. Agric., 8 65-72 - (1957)

5. **Forsyth, W.G.C.,** A Method of Studying the Chemistry of Cocoa Fermentation. Nature (London), 164, 25, (1949).

6. **Bediako, M.K.B.,** Utilization of Some Waste Materials from Cocoa - Biochemical Studies on Cocoa Sweatings and Production of Dextran. MSc Thesis, University of Science and Technology, Kumasi, pp. 117-133, (1973).

7. **Opeke, L.K. and Jacob, V.J.,** Cocoa Production in Nigeria and Suggestion to Improve the Quality of Cured Cocoa. Proc. 3rd Int. Cocoa Res. Conf. Tafo, Ghana, 23-29 Nov. 1969, p.634, (1969).

8. **Agyeman, K.O-G., Bediako, M.K.B. and Oldham J.H.**, Pilot Plant Studies on Collection of Cocoa Sweatings. Ghana Jnl. Agric. Sci., 9 65-69 (1976)

9. **Rohan, T.A.** Processing of Raw Cocoa for the Market. Food and Agriculture Organization of the United Nations, Rome, 74-101, (1963).

10. **Wood, G.A.R.**, Cocoa Longmans, London, pp. 233, 260, (1975)

11. **Maclean, J.A.R. and Wickens, R.**, Small Scale Fermentation of Cocoa. Cocoa Conference, London, p. 116, (1951).

12. **Patridge, S.M. and Westall, R.G.**, Filter-Paper Partition Chromatography of Sugars. Biochem. J., 42 238, (1948).

13. **Official Methods of Analysis of the Association of Official Analytical Chemists**, 11th ed., p. 18, (1970).

14. **Johnson, G., Lambert, C., Johnson, D.K. and Suderwith, S.G.**, Colorimetric Determination of Glucose, Fluctose and Sucrose in Plant Materials Using a Combination of Enzymatic and Chemical Methods. J. Agric, Fd. Chem., 12 216-219, (1964)

15. **Joslyn, M.A.**, Methods in Food Analysis. Academic Press, London, pp. 420-421, (1970).

16. **Official Report, Congress International des Fabricants, de Chocolat et de Cocoa, lausanne (1950) Office International du Cocoa et du Chocolat**, Brussel, (1960).

17. **Roelofsen, P.A.**, Advances in Food Research. 8 Academic Press, London, pp. 226-290, (1958).

TABLE 1: SUGAR COMPOSITION OF COCOA SWEATINGS FROM DIFFERENT VARIETIES OF COCOA

FERMENTATION TIME (HOURS)	% FREE SUGARS IN COCOA SWEATINGS											
	GLUCOSE			FRUCTOSE			SUCROSE			TOTAL FERMENTABLE SUGAR		
	HYB	AML	AMZ	HYB	AML	AMZ	HYB	AML	AME	HYB	AML	AMZ
0	4.21	2.71	3.82	4.31	5.41	5.55	6.30	6.20	6.00	14.82	13.32	[illegible]
6	5.63	5.32	4.85	5.22	5.51	5.43	4.00	3.30	4.20	14.85	14.13	[illegible]
12	6.84	6.65	5.92	5.41	5.91	5.62	3.80	2.90	4.10	15.05	14.46	15.64
18	7.00	6.44	6.87	7.76	7.32	8.50	1.30	1.01	1.32	16.06	14.77	16.70
24	5.80	6.00	5.54	6.43	6.11	6.01	0.90	0.48	0.65	12.13	12.59	12.20
30	3.20	3.39	2.45	3.00	3.49	2.32	1.00	0.31	0.33	7.20	7.19	5.10
36	0.71	0.85	1.71	1.03	1.06	0.93	0.37	0.26	0.30	2.11	2.17	1.94
42	0.55	0.25	0.69	0.79	0.61	0.65	0.28	0.14	0.21	1.62	1.00	1.55
48	0.39	0.10	0.41	0.35	0.42	0.33	0.11	0.07	0.10	0.85	0.59	0.84
54	0.23	0.10	0.10	0.11	0.20	0.11	0.04	0.01	0.02	0.38	0.31	0.23

* HYB = HYBRID COCOA; AML - AMELONADO; AMZ = AMAZONIA
The free sugars have been combined to give total fermentable sugars.
Each value above is an average of at least 12 determinations.

TABLE 11: VARIATION OF TOTAL FERMENTABLE SUGARS, ALCOHOL AND ACETIC ACID WITH FERMENTATION TIME

FERMENTATION TIME (HOURS)	TOTAL FERMENTABLE SUGARS(%)	ALCOHOL CONTENT (%)	VOLATILE ACIDS (ACETIC)(%)
0	14.82	0.00	0.07
6	14.85	0.00	0.10
12	15.05	0.33	0.20
18	16.06	1.20	0.22
24	12.13	3.81	0.57
30	7.20	4.01	0.78
36	2.11	3.95	1.50
42	1.62	2.70	2.60
48	0.85	2.35	2.70

Each value above is an average of at least 12 determinations.

TABLE III: CHANGES IN PULP AND COTYLEDON pH OF FORASTERO COCOA DURING SWEAT-BOX, HEAP AND OVEN FERMENTATIONS

FERMENTATION TIME (HOURS)	HEAP		SWEAT-BOX				OVEN	
	pH OF COTY-LEDONS	pH OF PULP	pH OF COTY-LEDONS		pH OF PULP		pH OF COTY-LEDONS	pH OF PULP
			SURFACE	CENTRE	SURFACE	CENTRE		
0	7.00	4.15	7.00	7.00	4.15	4.15	6.95	4.15
24	6.40	4.00	6.10	6.80	3.80	4.10	6.10	3.55
48	5.60	4.55	5.60	6.65	4.25	4.40	5.70	3.68
72	5.50	4.80	5.30	5.80	4.50	4.65	5.10	3.75
96	5.25	5.20	5.20	5.35	4.85	4.90	5.00	3.90
120	5.20	5.15	5.00	5.30	5.20	5.30	5.00	3.90
144	5.20	5.10	4.85	5.20	5.30	5.30	-	-

Each value above is an average of 3 determinations.

TABLE IV: CHANGES IN pH OF SWEATINGS DURING SWEAT-BOX FERMENTATION

FERMENTATION TIME (HOUR)	pH OF SWEATINGS
0	3.60
6	3.52
12	3.56
18	3.51
24	3.53
30	3.65
36	3.72

Each value above is an average of 3 determinations.

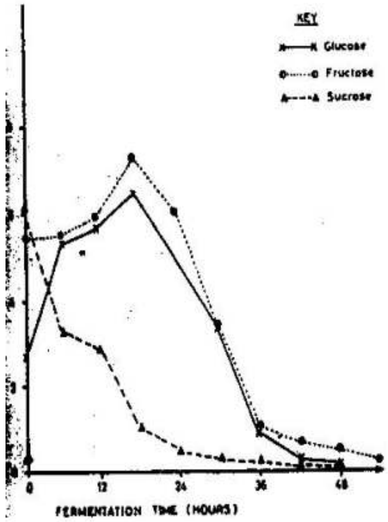

FIG. 1: VARIATION IN FREE SUGAR CONTENT OF COCOA SWEATINGS DURING FERMENTATION OF COCOA BEANS - AMELONADO

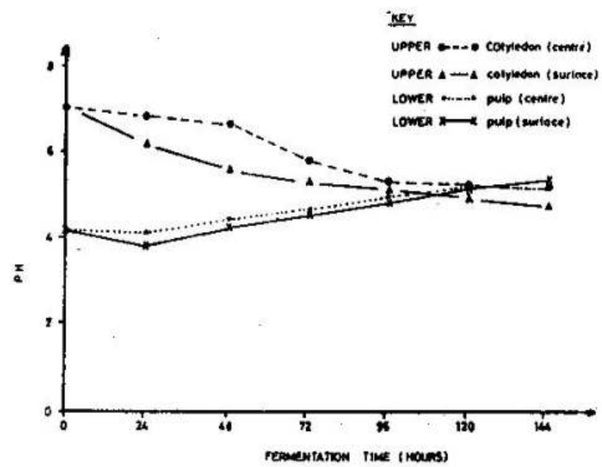

FIG. 3: CHANGES IN PULP pH AND COTYLEDON pH OF COCOA DURING SWEAT-BOX FERMENTATION

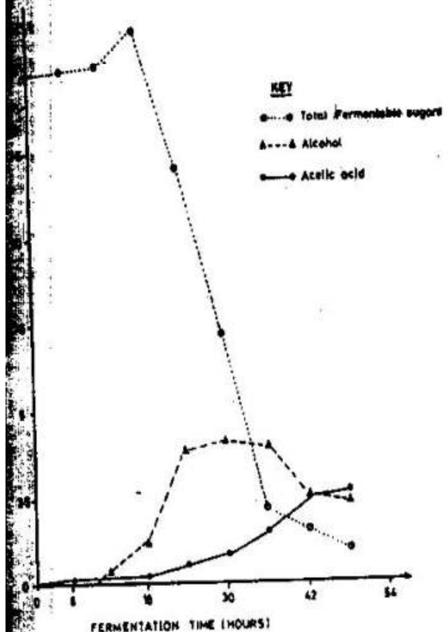

FIG. 2: VARIATION OF FERMENTABLE SUGARS, ALCOHOL AND ACETIC ACID WITH FERMENTATION TIME

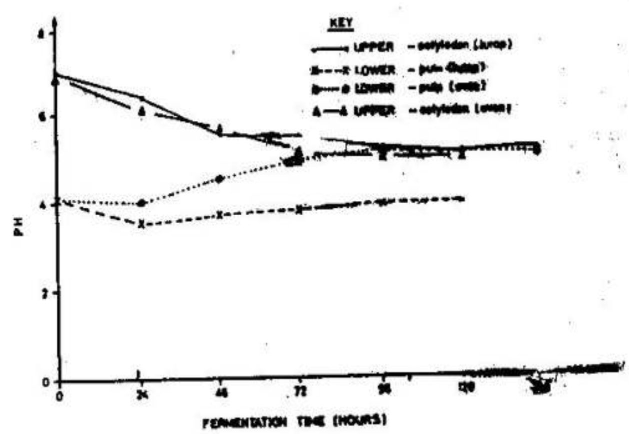

FIG. 4: CHANGES IN PULP pH AND COTYLEDON pH OF COCOA DURING HEAP OVER FERMENTATIONS