# THE ROLE OF MICROBIAL AND ENDOGENOUS PECTOLYTIC ENZYMES IN COCOA FERMENTATION 1.2

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## ABSTRACT

The role of microbial and endogenous pectolytic enzymes in the degradation of cocoa pulp during fermentation was studied on the laboratory scale. Fermentation were carried out both under sterile and non-sterile (natural) conditions and by monitoring the rates and yields of sweatings over the first 12 hours.

Results indicated that both microbial and endogenous pectolytic enzymes were involved in the sweating process. Under conditions, sweating yields of the order of 130% over that under sterile conditions were observed.

Pectin esterase and polygalacturonase activities were detected in cocoa pulp under both conditions. Enzyme inhibition studies suggested that polygalacturonase played a more significant role in the fermentation process, than did pectin esterase. This was confirmed by observations of large increases in sweating yield and rate accompanied by drastic reduction in viscosity when a more pure commercial polygalacturonase was added to fermenting beans.

Key Words:

Microbial, cocoa sweatings, sterile, natural, pectolytic enzymes.

## INTRODUCTION

Although a substantial amount of work has been done on cocoa fermentation dating as far back as the 15th century, very little is known about the initial stages of the process, which includes the breakdown of the pulp material surrounding the bean resulting in the production of a pale yellowish liquid called sweatings. This initial process has been found to influence the quality of the fermented bean product and is thus considered to be essential for the proper curing of the beans [1].

Earlier studies have indicated the involvement of microbial enzymes in cocoa fermentation [2-5]. Secondly several micro-organisms have been found to be associated with fermenting cocoa beans [6-8] but the extent of their involvement in the breakdown of the pulp has not been established. This work aims at investigating the roles of microbial and non-microbial pectolytic enzymes in the fermentation of cocoa, in an attempt to elucidate the mechanism of the sweating process and how it can be manipulated to produce more sweating within a given time but with no adverse effect on the bean quality.

## EXPERIMENTAL

### Collection of sweatings

The method for the collection of sweatings was a modification of that described by Bediako [2]. For sweatings collection under sterile conditions, 6-10 pods were washed, weighed and sprayed with thymol. They were then immediately transferred to an inoculation chamber where they were finally sterilized by U.V. irradiation for one hour using sterilized gloves. The pods were cracked open and the beans removed with sterile spatula into the fermenting vessels and covered immediately with perforated aluminium foil.

Collection of sweatings under natural conditions was similarly done except that the entire process was carried out in the laboratory under non-sterile conditions.

### Determination of Pectin Esterase (P.E.) and Polygalacturonase (P.G.) activities in Cocoa Pulp.

Crude P.E. preparations were made by the method of Gamble [9] and beans were removed from fermenting vessels for analysis at 3 hour intervals.

Pectin esterase activity was determined by measuring the amount of methanol liberated [10]. Hobson's [11] procedure for the extraction and determination of P.G. activity was adopted in this work. Galacturonic acid monohydrate was used for the standard curve and enzyme activity was determined under both sterile and natural conditions.

### Enzyme Inhibition Studies

Pectin esterase activity was inhibited with 11 mg % of sodium lauryl sulphate detergent as described by McColloch [12].

Polygalacturonase inhibition was carried out using 5 ml of 0.5N NaOH [13] per 100 gm. of beans. Inhibition

studies were done both under natural and sterile conditions and the rate and yield of sweatings determined at 3 hour intervals.

### Effect of Commercial Polygalacturonase on Sweating yield and Relative Viscosity of Sweatings.

A commercial fungal P.G. preparation obtained from Sigma Company (USA) was employed in this experiment. Aliquots of the P.G. extract were made up to 40 ml. With double distilled sterile water to give the desired concentrations. These were added to beans fermenting under sterile conditions at the 6th hour. The amount of sweatings obtained were determined up to 12 hours after addition of enzyme, in another experiment using double distilled sterile water.

### Determination of Anthocyanin Content of Cocoa Beans

The method described by Kenton [14] was used in the estimation of the anthocyanin content of the fermented dried cocoa beans.

## RESULTS

The initial set of experiments were designed to investigate whether the sweating process is solely due to micro-organisms or whether endogenous enzymes play a role. Figures 1 and 2 show the results obtained when the sweating rate was determined over 144 hours under 3 different conditions. Sweating was found to occur even under sterile conditions, but the rate and yield were however lower than those obtained under natural conditions.

When beans fermenting under sterile conditions were transferred to natural conditions, there was a sudden burst of sweating equalling the rate under natural conditions by the 144th hour (Figure 1).

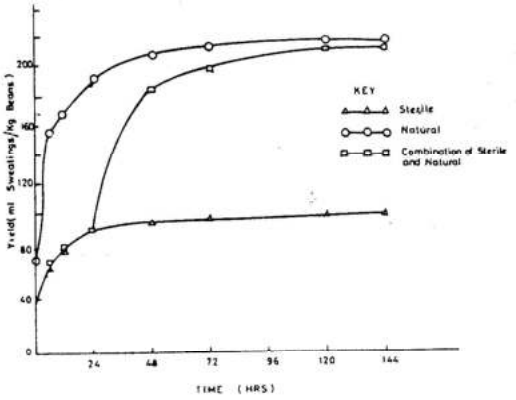



Fig. 1 Yield of Cocoa Sweatings under different fermentation conditions

The highest rate of sweating under both conditions occurred at 0 hour (30 minutes); 56ml/kg-hr and 134ml/kg-hr under sterile and natural conditions respectively (Figure 2). These rates decreased drastically reaching a mean of 2ml/kg-hr in both cases by the 6th hour.

The relative viscosities of sweatings collected under natural conditions were consistently lower than those under sterile conditions over the entire sampling period. (Figure 3).

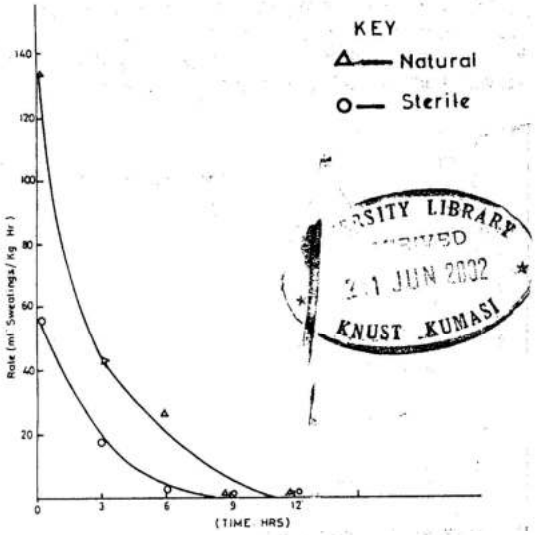





Fig. 2 Sweating rate of Cocoa under different fermentation conditions

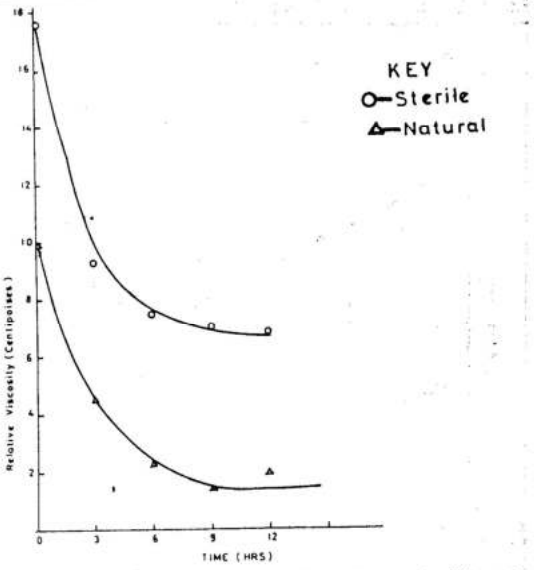
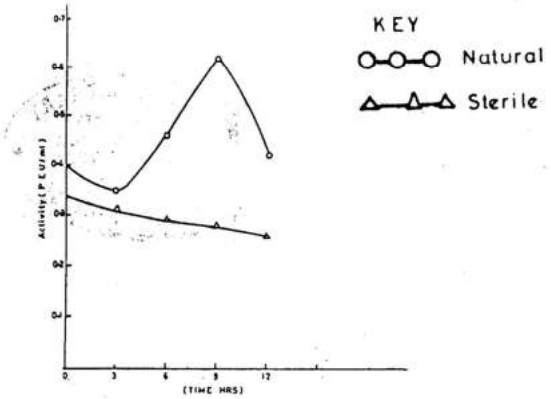



Fig. 3 Relative viscosity of Cocoa Sweating under different conditions of fermentation

## Pectin Esterase and Polygalacturonase Activities in Cocoa pulp.

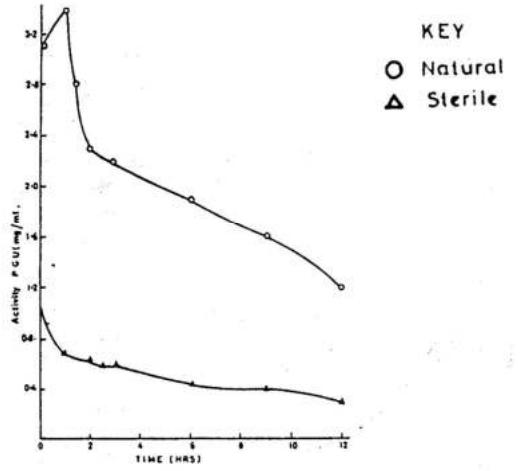
Figures 4 and 5 show the levels of activities of pectin esterase and polygalacturonase respectively, under natural conditions.

There was low but consistent degree of pectin esterase activity in cocoa pulp even under sterile conditions over the entire 12 hour sampling period (Figure 4). This activity declined from a mean of 0.344 P.E.U./ml at the 0 hour to 0.255 P.E.U./ml by the 12th hour under sterile conditions. The trend observed was however irregular under natural conditions, probably due to the activity of different micro-organisms in the pulp.




P.E.U. One P.E.U. represents the amount of pectin esterase that liberates one micro equivalent of methoxy groups in 1 min.

Fig. 4 Levels of P.E. activity in Cocoa Pulp




P.G.U. (mg ml) represents milligrams of reducing groups liberated from polygalacturonic acid per ml of enzyme extract

Fig. 5 Levels of Polygalacturonase activity in Cocoa Pulp under different fermentation conditions

Polygalacturonase activity was also clearly detected in cocoa pulp under sterile conditions declining from a mean of 0.920 P.G.U. (mg/ml) by the 12th hour (Figure 5). The corresponding figure under natural conditions being from 3.11 P.G.U. to 1.24 P.G.U. (mg/ml).

Inhibition of P.E. activity produced a significant reduction in sweating yield under natural conditions (Figure 6). Inhibition of P.G. by the application of NaOH, however abolished the sweating process within 40 minutes due to gel formation in the presence of NaOH (Figure 6).

The effect of added commercial fungal on cocoa pulp and sweatings is shown in Figure 7. Addition of the

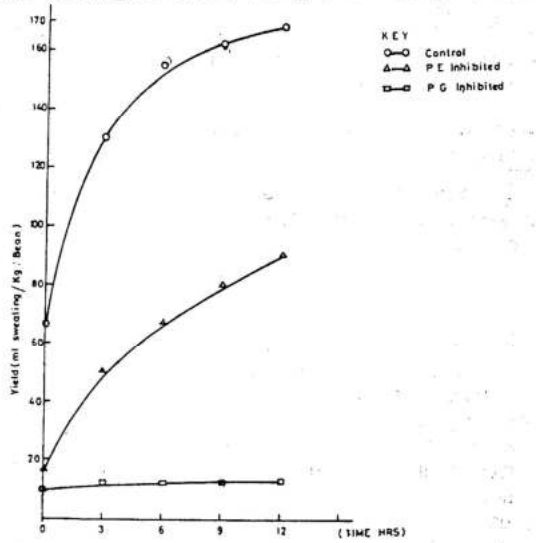
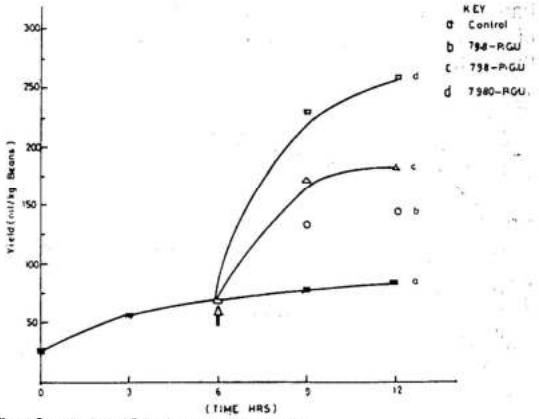



Fig. 6 Effect of P.E. and P.G. inhibition on yield of Sweatings under natural conditions




Different Concentrations of Polygalacturonase were employed per kg of Beans. One polygalacturonase unit (P.G.U.) is the amount of enzyme that liberates 1 Umol (0.194 mg) of reducing groups from polygalacturonic acid Arrow indicates point of enzyme application

Fig. 7 Effect of added Fungal Polygalacturonase on the yield of Cocoa Sweatings under sterile conditions

commercial P.G. at the 6th hour of fermentation resulted in a sudden burst of sweating yield. The effect was concentrations dependent and increases in yield of between 71% and 210% was observed for the lowest and highest concentrations respectively (Table 1). This was accompanied by large increase in sweating rate with a drastic reduction in the relative viscosity of the sweatings (Table 2).

**TABLE 1.**

**Effect of Polygalacturonase Concentration on sweating yield.**

| Enzyme concentration P.G.U. (mg/kg beans) | % Increase in sweating yield (0-12 hours) |
|---|---|
| 15.50 | 71.5 |
| 155.00 | 115.4 |
| 1550.00 | 210.7 |

**TABLE 2**

**Effect of Polygalacturonase on the Relative Viscosity of Sweatings Collected from 6th - 12th Hours of Fermentation Under Sterile Conditions.**

| Amount of Enzyme P.G.U. (mg/kg beans) | Relative Viscosity of Sweatings (Centipoises) ± S.D. | |
|---|---|---|
| | (6 - 9) Hour | (9 - 12) Hour |
| 0 | 7.29 ± 0.01 | 6.85 ± 0.01 |
| 15.50 | 1.50 ± 0.01 | 1.42 ± 0.1 |
| 155.00 | 1.45 ± 0.01 | 1.39 ± 0.0 |
| 1550.00 | 1.22 ± 0.01 | 0.08 ± 0.01 |

## DISCUSSION

The procedure used for sweating collection and fermentation were on laboratory scale, but they were designed to simulate the normal fermentation conditions as practised on cocoa farms as far as possible. Experiments were carried out under natural and sterile conditions.

The sterile conditions was used to determine the role of microorganisms in the fermentation process. This helped to elucidate the contribution of micro-organisms in sweat production and also to distinguish between the roles of microbial and non-microbial pectolytic enzymes in the fermentation process.

In general, the yield of sweatings under sterile conditions was consistently lower than that under natural conditions, over the entire sampling period (Figure 1). This implies that micro-organisms contribute more to sweat production than the endogenous enzymes. Furthermore, when beans fermenting under sterile environment were transferred to natural environment there was a sudden increase in the rate and yield of sweatings (Figure 1) confirming the significant role played by micro-organisms. Throughout the experiment, the yields and rates were found to be within the first hour of collection.

In a previous investigation [15] no P.G. activity was detected in cocoa pulp. P.G., however, is known to be so labile that when extracts of it are stored overnight, even in the cold, it loses activity [16]. In this work, the extraction period was limited to 3 hours at 22°C whereas the previous worker stored the extract overnight in the cold before enzyme activity determinations.

Over a six hour period, the inhibition of P.E. activity resulted in a significant reduction in sweating yield under natural conditions (Figure 6). This suggests a partial involvement of this enzyme in the sweating process. Furthermore, P.G. activity was lowered slightly when P.E. was inhibited (data not shown).

Although the inhibition studies on P.G. was inconclusive due to the formation of a gel in the presence of NaHO, the major role played by this enzyme can be inferred from the large increase in sweatings obtained when a commercial P.G. was added to cocoa bean (Figure 7).

In addition, the drastic reduction observed in the viscosity of sweatings (Table 2) when pure P.G. was added to fermenting cocoa beans confirms the role of this enzyme in the degradation of cocoa pulp into sweatings. A third piece of evidence came from histological studies of the enzyme's action on cocoa pulp. Microscopic observation of a piece of pulp tissue from fresh cocoa fermenting under sterile conditions revealed that addition of P.G. resulted in the disintergration of the pulp cells as a result of the breakdown of their pectin, (Figures 8 and 9), in the cell wall.

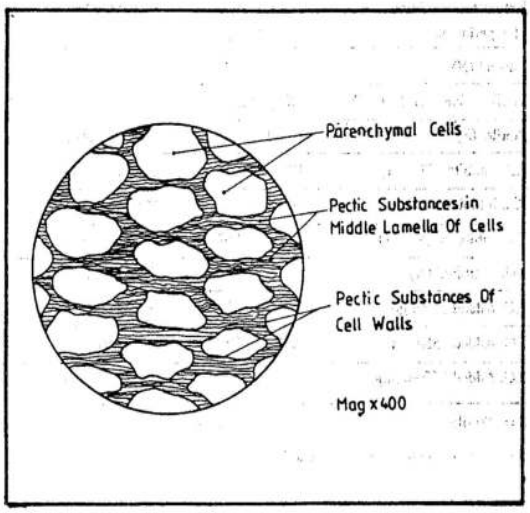



Fig. 8 Cocoa Pulp Tissue Parenchymal Cells

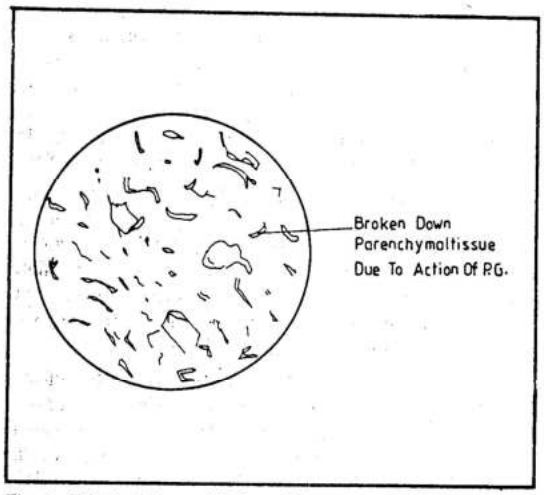



Fig. 9 Effect of Fungal PG on Parenchymaltissue of Pulp

Analysis of the fermented bean product indicates that despite the over 100% increase in yield of sweatings the beans were of acceptable quality but above 200% increase in yield the beans were not acceptable as judged by the anthocyanin content (Table 3). It can therefore be inferred that probably the presence of some amount of pulp on cocoa bean is essential for proper fermentation and therefore excessive sweating during fermentation should be avoided.

**TABLE 3**

**Fermented Bean Quality Under Various Experimental Conditions.**

| Nature and condition of experiment | Anthocyanin content (%) | Acceptability |
|---|---|---|
| Natural (N) | 3.70 ± 0.17 | Acceptable |
| Sterile + Natural (S + N) | 4.20 ± 0.33 | Acceptable |
| Sterile (S) | 43.00 ± 0.67 | Not acceptable |
| P.E. Inhibited (S + N) | 18.16 ± 0.33 | Just acceptable |
| P.E. Inhibited (N) | 10.05 ± 0.17 | Acceptable |
| P.G. Inhibited (S + N) | 47.05 ± 0.67 | Not acceptable |
| P.G. Inhibited (N) | 43.00 ± 0.50 | Not acceptable |
| P.G. Added 15.5mg/kg | 7.35 ± 0.00 | Acceptable |
| P.G. Added 155mg/kg | 6.40 ± 0.13 | Acceptable |
| P.G. Added 1550mg/kg | 18.12 ± 0.20 | Just acceptable |

S = Sterile N = Natural

S + N = Combination of both conditions

# CONCLUSION

This work has investigated the relative roles of microbial and endogenous pectolytic enzymes in cocoa fermentation.

Both types of enzymes were found to be present during cocoa fermentation. Pectin esterase and polygalacturonase activities were detected under both sterile and natural conditions. The microbial enzymes appear to contribute more to the sweating process than endogenous enzymes since much higher yields of sweat were obtained under natural conditions than under sterile conditions.

Of the two pectolytic enzymes in the pulp, polygalacturonase appears to contribute more to the sweating process than pectin esterase.

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