Cocoa Fermentation in Ghana and Malaysia

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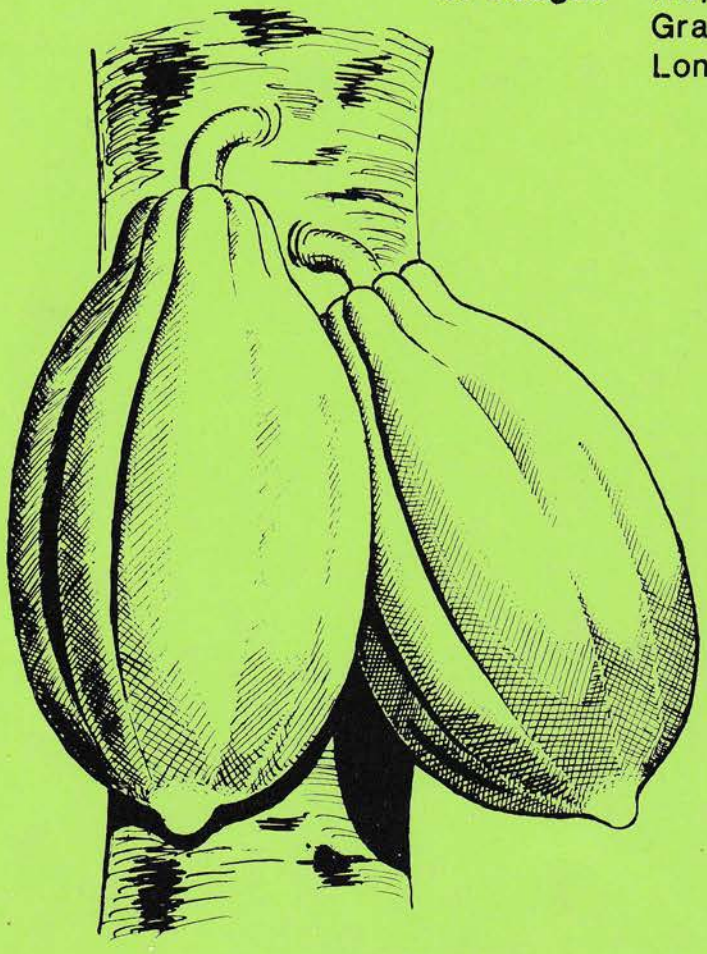

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INTRODUCTION

As a result of a preliminary survey of the methods of cocoa fermentation in Ghana and Malaysia certain recommendations were made (The Cocoa Bean Acidity Problem in Malaysia, Carr and Dougan, 1977) as follows:

1. The investigation of field factors such as delay between pod harvest and splitting and between splitting and fermentation on the course of the fermentation.

2. Investigations into fermentation with particular reference to: (a) dissimilation of individual sugars; (b) ethanol production, the occurrence of acetic acid and non-volatile acids throughout the fermentation; (c) Rohan's nitrogen and sugar index; (d) changes in pH and temperature.

3. A study of artificial drying.

4. A study of the microbiology.

5. Collaboration with SABAH and other organisations.

During the course of the work to be described in this report 2(a)(b) and 4 were studied in some depth; 1 and 3 were examined in less detail and 5 was not achieved.

The period of work in each country lasted approximately 5 weeks. In Ghana 2 heaps and a box fermentation were studied. It was hoped that a heap might be studied in Malaysia to give a direct comparison between the methods used in each country; however, the number of variations of the Malaysia box fermentation required to be studied in the available time made this impossible.

The prime objectives of these studies were: to study cocoa fermentation in Ghana and in Malaysia and to determine the chemical and microbiological differences between the two countries; to explain in the light of discoveries the cause of excess acidity in Malaysian cocoa and to suggest remedies.

During the course of this work a large volume of information has been accumulated so that it is proposed to publish a document in two parts. The first of these will describe methods briefly and give an overall picture of the situation in Ghana and Malaysia, whereas the second part will describe in much more detail the methods used and data obtained.

GHANA

Some five weeks were spent at the Cocoa Research Institute of Ghana. During this period two heaps and a box fermentation of Upper Amazon hybrid cultivars were studied.

The heaps were constructed in traditional fashion with radial slats at the bottom to aid drainage. Plantain leaves covered the heap over the base and sides. Dial thermometers were inserted in the top, middle and bottom. The dimensions of the heaps and box and details of the quantities of cocoa fermented are recorded on the information sheets preceding the description of each fermentation.

The dimensions of the boxes used in Africa were considerably smaller than those used in Malaysia. The beans were also turned less frequently than in the standard Malaysian procedure. Due to difficulties with the operation of the UNICAM SP600 spectrophotometer, the lack of skilled assistance, power cuts, shortage of distilled water due to a drought, it was not possible to analyse each sample chemically as it arrived at the laboratory. To save the situation any attempts to analyse samples from the first heap, except for that of the last day, were abandoned. Samples from the box fermentation and the second heap were immersed in 0.2% benzoic acid solution and air freighted to the UK for analysis at the Tropical Products Institute. Most of the apparatus for microbiological analysis was imported into Ghana and the only difficulty experienced was in finding a microscope in which the high power objective had not been obscured by mould growth. Because of this the examination of stained films of the fresh isolates was severely limited.

Samples were taken daily from the top, middle and bottom of the heaps or box using sterile disposable gloves. Samples weighed between 400 and 500 gms and each was divided respectively into beans for microbiological and chemical analyses. Temperatures were recorded 3 times daily and these are given in Figs 1-9. Sampling continued through the drying period, beans from Heap 1 and the Box being sun dried whilst those from Heap 2 were dried artificially.

Microbiological analyses

Twenty grams of beans were placed in sterile bags containing 100 ml of sterile water and pulverised in a stomacher. This crushed material was serially diluted, usually up to 10^6, and various dilutions were spread onto

apple juice yeast extract plates (AJYE composition described in Part 2). This medium was chosen since it has a low pH of 4.8 and is known to support the growth of the kind of organisms expected in the acid environment of cocoa beans. Plates were incubated both aerobically and anaerobically. Most incubations were done at room temperature which varied over a 24 h period from 25-30°C. Later samples were also incubated at 45°C to see whether there were organisms that could grow more readily at the elevated temperatures of the first fermentations (approximately 50°C) than at the chosen room temperature incubation.

When colonies appeared they were counted and these are recorded in Tables 1, 3 and 7. In addition the different types of organism present at each stage were assessed and each kind scored out of 10. This gave an idea of the relative proportion of organisms present and these figures are recorded in Tables 2, 4 and 8.

Selected colonies were described and the organisms were transferred to small bottles of liquid AJYE and there allowed to grow. When grown, the liquid culture was stabbed into AJYE agar in small screw-capped bottles which were incubated. When visible growth occurred in the stab, the bottles were stored in a refrigerator until transported to this country. On arrival the surviving micro-organisms were put through a series of tests aimed at identifying them and also elucidating some of their biochemical activities. Most of these very detailed results will be recorded in Part 2.

Chemical analyses

Fifty beans were taken from each sample and peeled; the pulp and testa and cotyledons were analysed separately. The pH and moisture content of both pulp and testa and cotyledons were determined in Ghana. The pulp and testa and cotyledons from 20 beans were homogenised separately in 200 ml of 0.2% benzoic acid solution. The samples were centrifuged and stored in a refrigerator. After the end of the second heap fermentation, the sample solutions and 1 Kg of dried product from each fermentation was air freighted to the UK. At TPI the samples were analysed for total sugars, acetic acid, lactic acid and ethanol by procedures which have already been made available to the Cocoa Quality Sub-Committee. In the light of the experience gained in Ghana and Malaysia the analytical methods have been improved. Part 2 will include the modified procedures.

Heap 1 - General information

Period of the experiment: 31.10.77 - 6.11.77

Original weight of wet beans: 555 Kg

Mixture of Upper Amazon hybrids harvested on 26/10 - some replaced with freshly harvested pods on 31/10 to replace rotten pods

Size: Circumference 540 cm; diameter 172 cm; height 60 cm

Turned: After sampling on 2.11.77 at 11.30 am and
after sampling on 4.11.77 at 11.30 am

Sampling: Top at apex; middle 45 cm; bottom taken from just below the surface

Final weight of beans: 528 Kg

Drying: Sun - Trinidad drier - rained 11 am 11.11.77

All numerical and graphical information referring to this section is in Tables 1 and 2 and Figs 1, 2 and 3

TABLE 1

Ghana - Heap 1

Organisms per gram of beans x 10^6

	0 h		4 h		24 h		48 h		72 h		96 h		120 h		144 h	
	Ae	An	Ae	An	Ae	An	Ae	An	Ae	An	Ae	An	Ae	An	Ae	An
Top	25	16	64	95	38	43	18	>	15	27	>	267	27	14	101	163
Middle			44	25	46	65	92	27	6	13	4	17	7	9	19	149
Bottom			80	158	130	145	86	91	>	>	>	>	>	>	293	135

> = > 1 x 10^7

TABLE 2

The frequency of micro-organisms in Heap 1 expressed as a percentage

		0 h		4 h		24 h		48 h		72 h		96 h		120 h		144 h	
		Ae	An	Ae	An	Ae	An	Ae	An	Ae	An	Ae	An	Ae	An	Ae	An
TOP	Yeasts	50	50	50	46	-	-	-	-	1	-	-	-	-	-	-	-
	Other yeasts	-	-	-	5	9	9	1	-	1	-	1	-	-	1	-	-
	Acetics	-	-	50	32	73	73	87	100	94	-	83	83	77	66	59	53
	Lactics	50	50	-	17	18	18	12	-	4	-	16	17	15	27	-	10
	Bacillus	-	-	-	-	-	-	-	-	-	-	-	-	8	6	41	37
	Punctiforms	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MIDDLE	Yeasts	50	50	31	10	-	-	5	6	1	1	1	1	-	-	-	-
	Other yeasts	-	-	3	20	5	8	3	3	-	-	1	1	1	1	-	-
	Acetics	-	-	63	70	10	15	16	34	14	33	47	40	71	71	71	62
	Lactics	50	50	3	-	38	77	22	57	25	46	9	20	21	21	7	19
	Bacillus	-	-	-	-	-	-	-	-	-	-	-	6	7	7	22	19
	Punctiforms	-	-	-	-	47	-	54	-	60	20	42	32	-	-	-	-
BOTTOM	Yeasts	50	50	46	53	-	-	-	1	1	-	1	-	-	-	-	-
	Other yeasts	-	-	-	-	6	6	1	2	2	1	-	-	1	3	-	-
	Acetics	-	-	46	37	31	31	33	9	75	90	76	83	50	61	30	30
	Lactics	50	50	8	10	63	63	66	88	22	9	23	17	27	30	30	30
	Bacillus	-	-	-	-	-	-	-	-	-	-	-	-	3	6	8	8
	Punctiforms	-	-	-	-	-	-	-	-	-	-	-	-	19	-	32	32

Ae = Aerobic incubation
An = Anaerobic incubation

Fig.1 Ghana - Heap 1 : Top

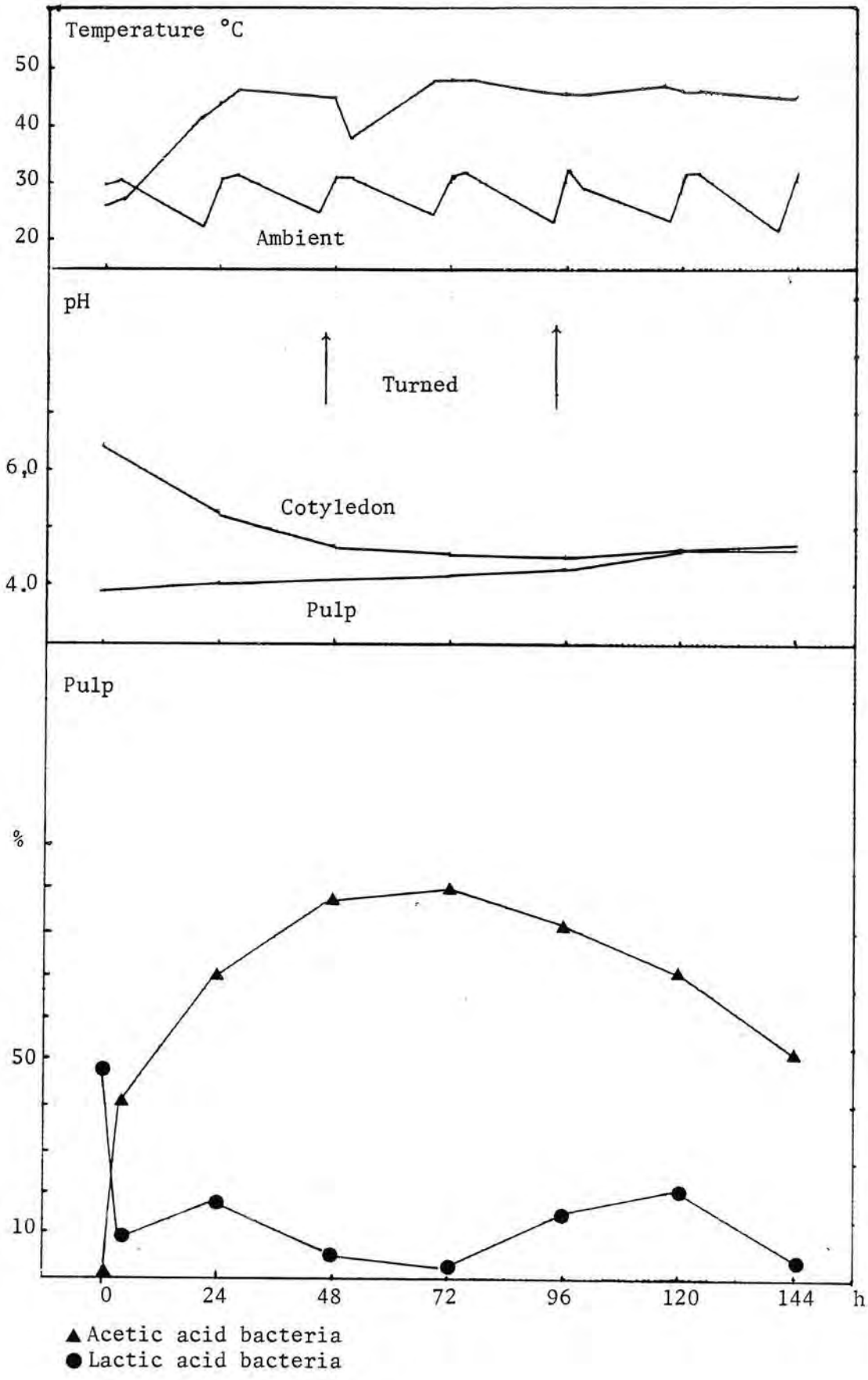


Fig.2 Ghana - Heap 1 : Middle

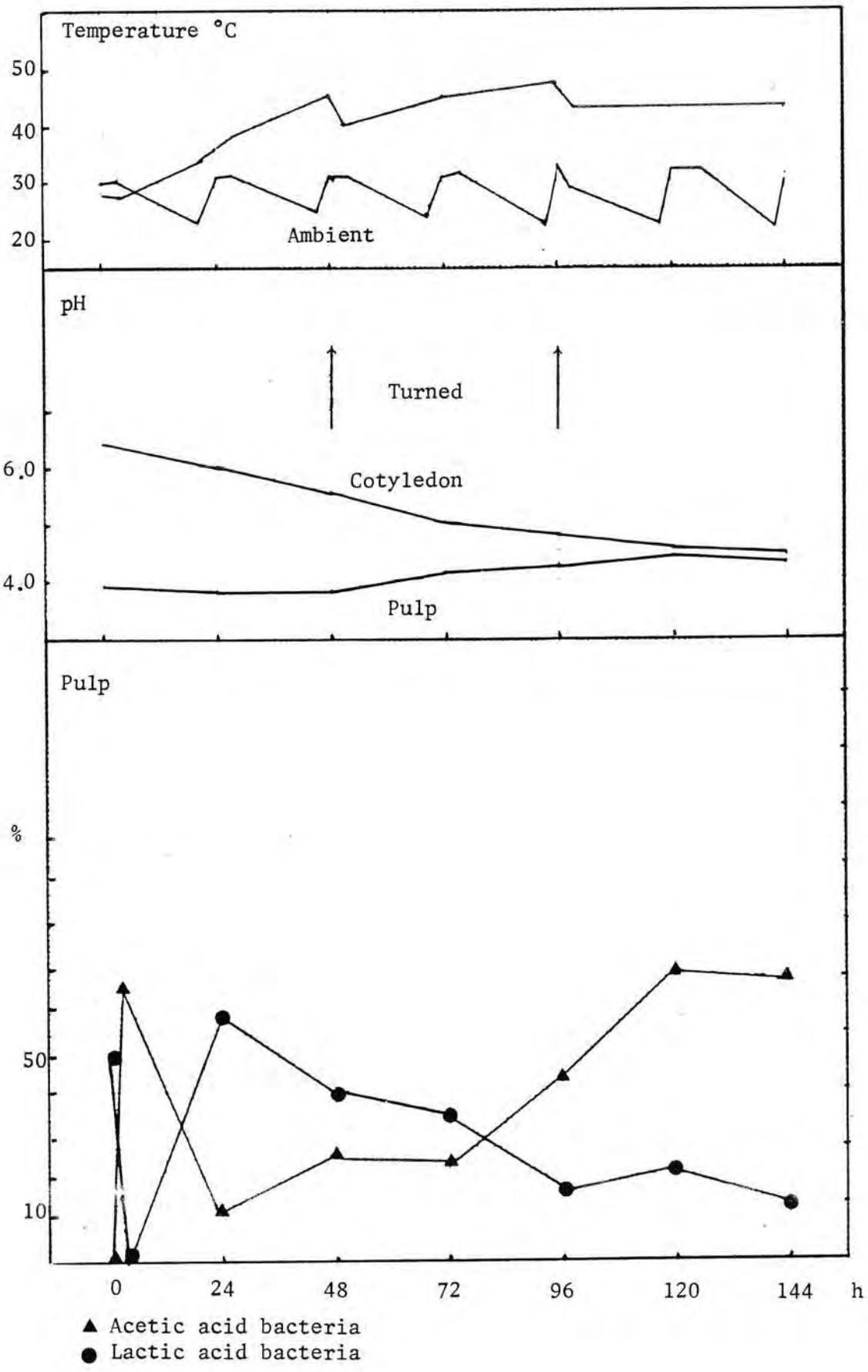
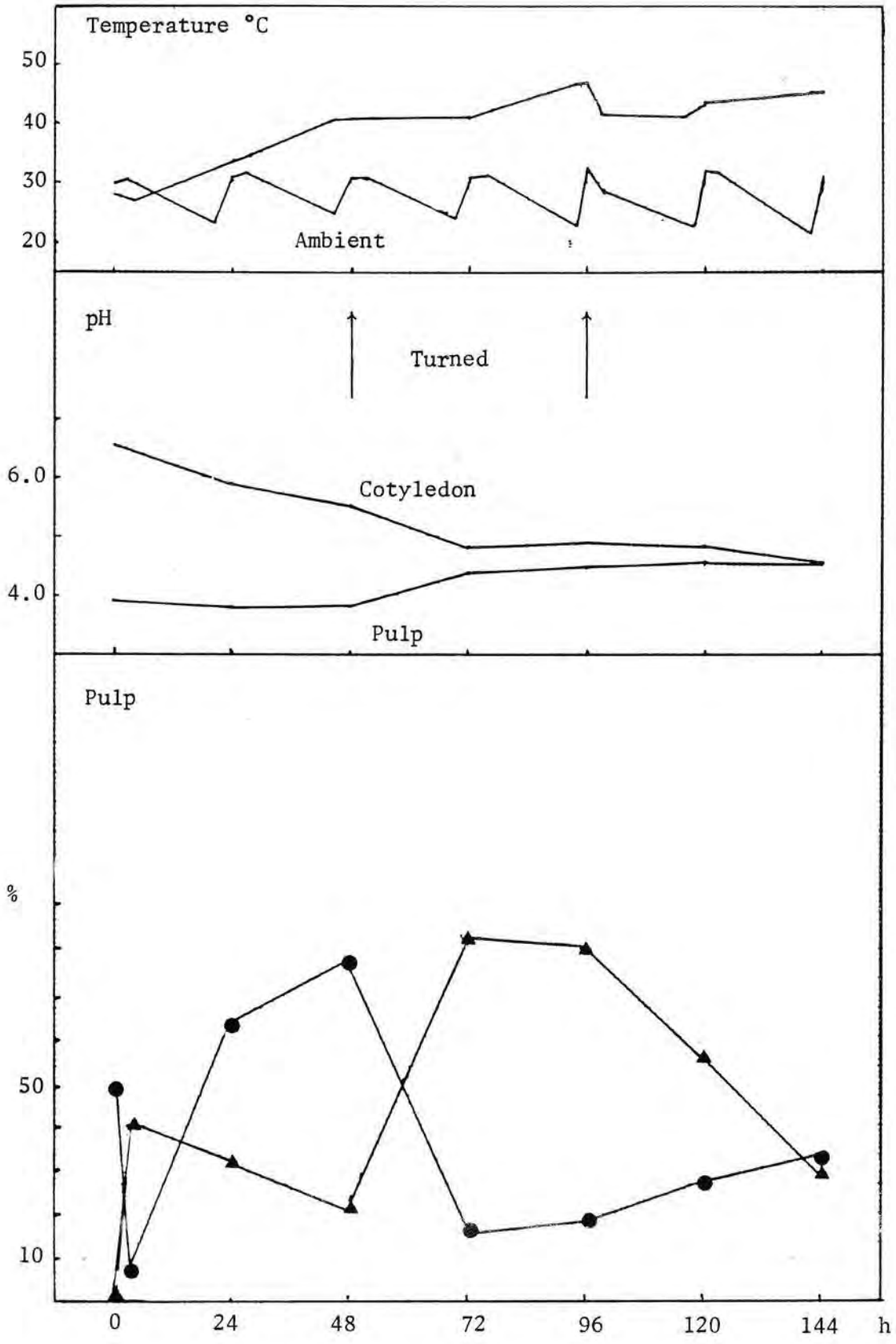


Fig.3 Ghana - Heap 1 : Bottom



Heap 1 - General comments

There are no chemical results for this experiment available, apart from that of the last day and the samples drawn during sun drying. The microbiological results are shown in Table 1, which lists the actual numbers per gram of organisms counted.

Top sample

The most predominant organisms in the early stages of fermentation are the fermenting yeasts accompanied by lactic and acetic bacteria. The fermenting yeasts do not, however, remain very long and by 24 hours after pod breaking they have virtually disappeared and are only detectable sporadically in other samples. The reasons for the rapid decline of the fermenting yeasts are 3-fold. Firstly, by 24 hours a substantial part of the sugars have disappeared, and secondly, they have been replaced by ethyl alcohol. The latter is toxic to yeasts and would tend to suppress them. However, the third reason for their decline is the conversion of ethanol to acetic acid by the acetic acid bacteria. It is this acid that kills fermenting yeasts. The acetic and lactic acid bacteria survive throughout the fermentation but it is the former that are present in larger numbers. Towards the end of the fermentation species of the genus *Bacillus* occur and these were associated with a somewhat unpleasant ammoniacal smell. Other yeasts not affected by acetic acid are to be found often but in small numbers and are not thought to contribute significantly to the chemical changes that take place.

Middle sample

The fermenting yeasts are more persistent in this position and can be detected as late as 96 hours after pod breaking. The acetics are the predominant organisms throughout the fermentation in spite of the more anaerobic conditions. The lactic acid bacteria persisted throughout the fermentation but are usually present in smaller numbers than the acetic acid bacteria. Again the *Bacillus* species are to be found within the final stages of fermentation. A new type of organism was detected in considerable numbers. They were tiny pinpoint colonies with an irregular outline. When isolated in pure culture they failed to survive and so it was not possible to investigate these organisms. However, since they occur so frequently and in such large numbers they have been named irregular punctiforms.

Bottom sample

The pattern of micro-organisms is much the same as within the other two positions, the early disappearance of the fermenting yeasts, the predominance of the lactic and acetic acid bacteria and the late appearance of *Bacillus* spp. along with the irregular punctiforms. It is interesting to note that at all levels the acetic acid bacteria do not appear until 4 hours after pod breaking and it is probably a reflection on the availability of ethanol, the substrate on which the acetic acid bacteria seem to flourish. It may seem surprising to find acetic acid bacteria growing in relatively anaerobic conditions. Although they are classified as aerobic, many strains can function under what are fairly severe anaerobic conditions.

Heap 2 - General information

Period of the experiment: 10.11.77 - 16.11.77

Original weight of wet beans: 547 Kg

Mixture of Upper Amazon hybrids left 3 days before pod splitting - typical treatment

Size: Circumference 554 cm; diameter 176 cm; height 50 cm

Turned: After sampling on 12.11.77 at 11.00 and
after sampling on 14.11.77 at 11.00 am

Sampling: Top at apex; middle 45 cm; bottom 45 cm

Final weight of beans: 528 Kg

Drying: 18.11.77 - artificial in hot air dryer

All numerical and graphical information referring to this section is in Tables 3, 4, 5 and 6 and Figs 4, 5 and 6

TABLE 3

Ghana - Heap 2

Organisms per gram of beans x 10^6

	0 h			4 h			24 h			48 h			72 h			96 h			120 h			144 h		
	Ae	An	45	Ae	An	45	Ae	An	45	Ae	An	45	Ae	An	45	Ae	An	45	Ae	An	45	Ae	An	45
Top	15	4	0	70	37	0	2	12	12	0	1	45	2	6	0	15	4	2	13	14	5	125	94	220
Middle	44	30	0	56	16	0	142	251	>	108	13	80	1	1	1	3	2	1	0	4	11	19	25	3
Bottom	24	3	1	17	10	0	186	-	2	4	6	3	7	17	19	29	6	25	27	17	13	172	>	44

> = > 1 x 10^7

\- = no result

TABLE 4

The frequency of micro-organisms in Heap 2 expressed as a percentage

		0 h			4 h			24 h			48 h			72 h			96 h			120 h			144 h		
		Ae	An	45	Ae	An	Ae	An	45	Ae	An	45	Ae	An	45	Ae	An	45	Ae	An	45	Ae	An	45
TOP	Yeasts	37	52	-	50	62	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Other yeasts	1	1	17	1	1	-	-	-	1	-	1	-	-	-	1	-	-	1	1	-	-	1	-
	Acetics	52	31	-	30	25	29	17	16	94	91	1	100	90	77	83	53	56	37	29	50	17	44	24
	Lactics	10	16	-	19	12	-	82	8	-	9	8	-	10	-	8	5	-	21	12	25	56	55	40
	Bacillus	-	-	83	-	-	-	-	-	5	-	8	-	-	23	8	42	6	41	58	25	27	-	4
	Punctiforms	-	-	-	-	-	71	-	76	-	-	82	-	-	-	-	-	38	-	-	-	-	-	32
MIDDLE	Yeasts	48	29	-	30	45	15	7	-	3	9	1	-	-	-	-	1	-	-	-	-	-	-	-
	Other yeasts	5	1	-	1	1	1	4	1	3	4	1	1	-	-	-	-	-	-	-	-	-	-	-
	Acetics	38	59	-	37	32	10	15	15	6	1	7	99	71	71	91	76	56	5	15	-	19	59	35
	Lactics	9	11	-	32	22	25	73	8	29	86	21	-	29	7	-	15	17	-	8	-	19	12	44
	Bacillus	-	-	-	-	-	-	1	-	-	-	-	-	-	7	9	7	6	95	77	100	62	29	21
	Punctiforms	-	-	-	-	-	49	-	76	59	-	70	-	-	15	-	1	21	-	-	-	-	-	-
BOTTOM	Yeasts	57	52	-	32	41	3	18	-	1	8	-	5	6	-	5	5	-	-	-	-	-	-	-
	Other yeasts	5	1	53	1	1	1	1	4	4	8	1	1	1	1	-	1	-	3	-	-	-	-	-
	Acetics	24	31	-	40	29	27	23	4	9	4	-	52	58	32	48	43	42	67	39	53	67	50	35
	Lactics	14	16	-	27	29	69	58	8	86	80	16	42	35	1	5	17	-	3	6	-	13	35	50
	Bacillus	-	-	47	-	-	-	-	-	-	-	1	-	-	3	-	-	5	27	55	47	20	15	15
	Punctiforms	-	-	-	-	-	-	-	84	-	-	82	-	-	63	42	34	53	-	-	-	-	-	-

Ae = Aerobic incubation
An = Anaerobic incubation
45 = Aerobic incubation at 45°C

TABLE 5

Analyses of bean pulp and testa from Heap 2

Sampling			pH	Sugars %	Ethanol %	Acetic acid %	Lactic acid %
10/11	0 h	T	4.05	10.20	0	0	0.02
		M	4.05	9.83	0	0	0.02
		B	4.05	11.54	0	0	0.02
	4 h	T	3.95	9.37	0.10	0.42	0.01
		M	4.00	8.02	0.15	0.32	0.02
		B	4.15	8.64	0.09	0.30	0.04
11/11	24 h	T	3.90	3.71	0.10	1.86	0.13
		M	4.05	1.88	0.39	0.49	0.41
		B	4.15	6.87	0.54	0.66	0.92
12/11	48 h	T	4.05	2.10	0.28	2.39	0.12
		M	3.95	1.00	1.05	1.06	0.72
		B	3.85	4.34	0.47	0.58	0.77
13/11	72 h	T	4.30	1.72	0.05	2.07	0.16
		M	4.30	1.31	0.13	1.47	0.34
		B	4.10	1.24	0.10	1.48	0.51
14/11	96 h	T	4.35	1.86	0.04	1.75	0.21
		M	4.40	2.20	0.13	1.62	0.45
		B	4.20	1.49	0.23	1.70	0.88
15/11	120 h	T	4.50	1.48	0.05	1.70	0.33
		M	4.35	1.78	0	2.20	0.21
		B	4.50	1.78	0	2.20	0.29
16/11	144 h	T	4.50	1.75	0	1.99	0.25
		M	4.50	1.78	0	1.86	0.29
		B	4.55	1.37	0	1.83	0.31

TABLE 6

Analyses of cotyledons from Heap 2

Sampling			pH	Sugars %	Ethanol %	Acetic acid %	Lactic acid %
10/11	0 h	T	6.45	1.76	0	0	0.005
		M	6.50	1.99	0	0	0.005
		B	6.65	1.63	0	0	0.005
	4 h	T	6.45	1.54	0.15	0.19	0.005
		M	6.45	1.47	0.13	0.13	0.005
		B	6.55	1.44	0.20	0.13	0.005
11/11	24 h	T	5.05	1.36	0.09	0.80	0.02
		M	6.25	1.62	0.82	0.13	0.01
		B	6.45	1.95	0.23	0	0.01
12/11	48 h	T	4.65	1.44	0.03	1.18	0.02
		M	5.60	1.57	0.96	0.40	0.04
		B	5.85	1.58	0.47	0.20	0.05
13/11	72 h	T	4.45	1.24	0.10	1.27	0.09
		M	4.60	1.31	0.25	1.23	0.09
		B	4.95	1.30	0.40	0.81	0.12
14/11	96 h	T	4.60	1.12	0.16	1.39	0.09
		M	4.65	1.20	0.28	1.26	0.22
		B	4.65	1.17	0.25	1.18	0.16
15/11	120 h	T	4.60	0.73	0.09	0.99	0.12
		M	4.45	1.02	0	1.36	0.12
		B	4.55	1.02	0	1.40	0.12
16/11	144 h	T	4.55	0.98	0.04	1.38	0.10
		M	4.45	0.85	0.14	1.21	0.15
		B	4.45	0.90	0	1.06	0.17

Fig.4 Ghana - Heap 2 : Top

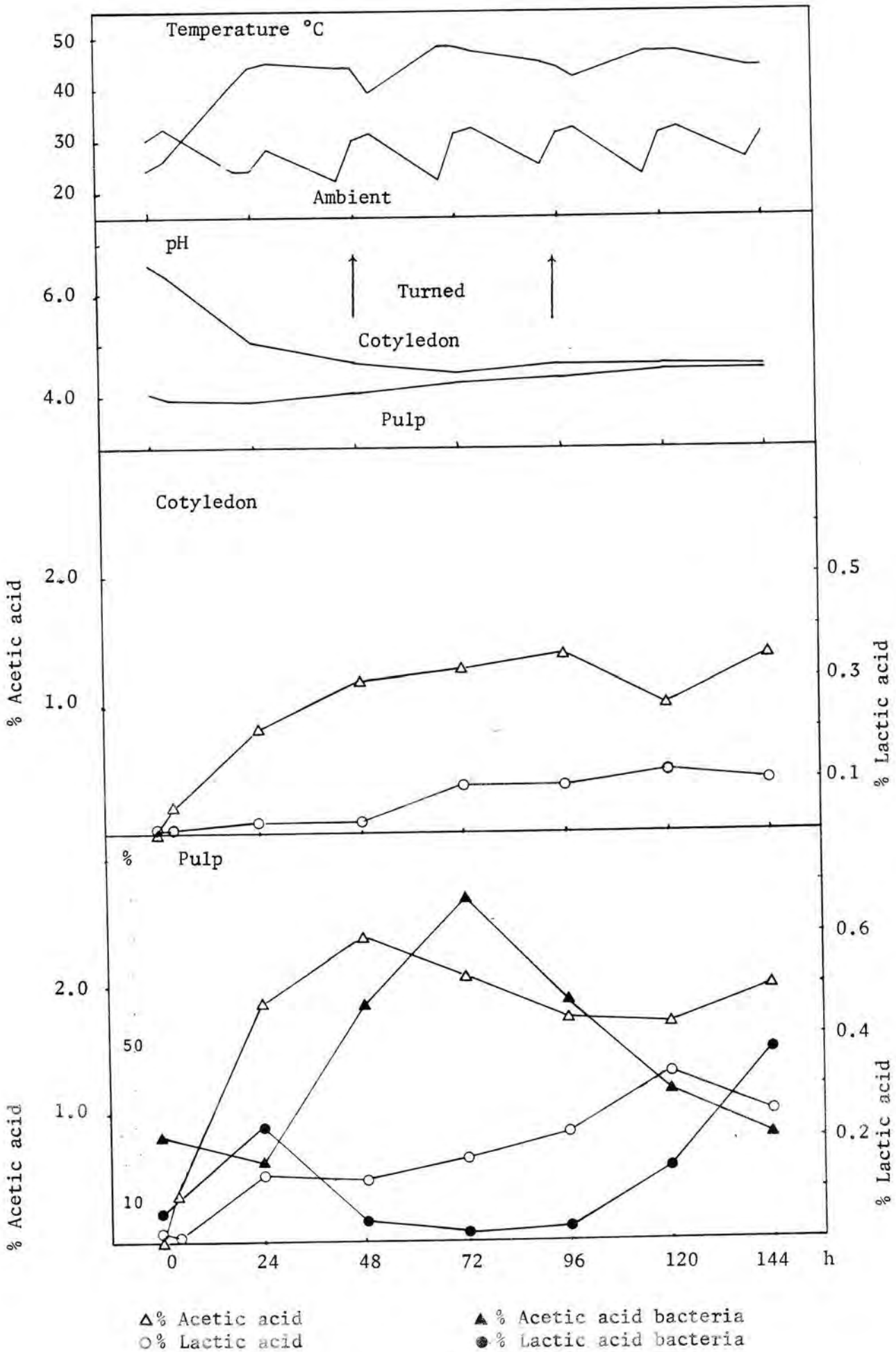


Fig.5 Ghana - Heap 2 : Middle

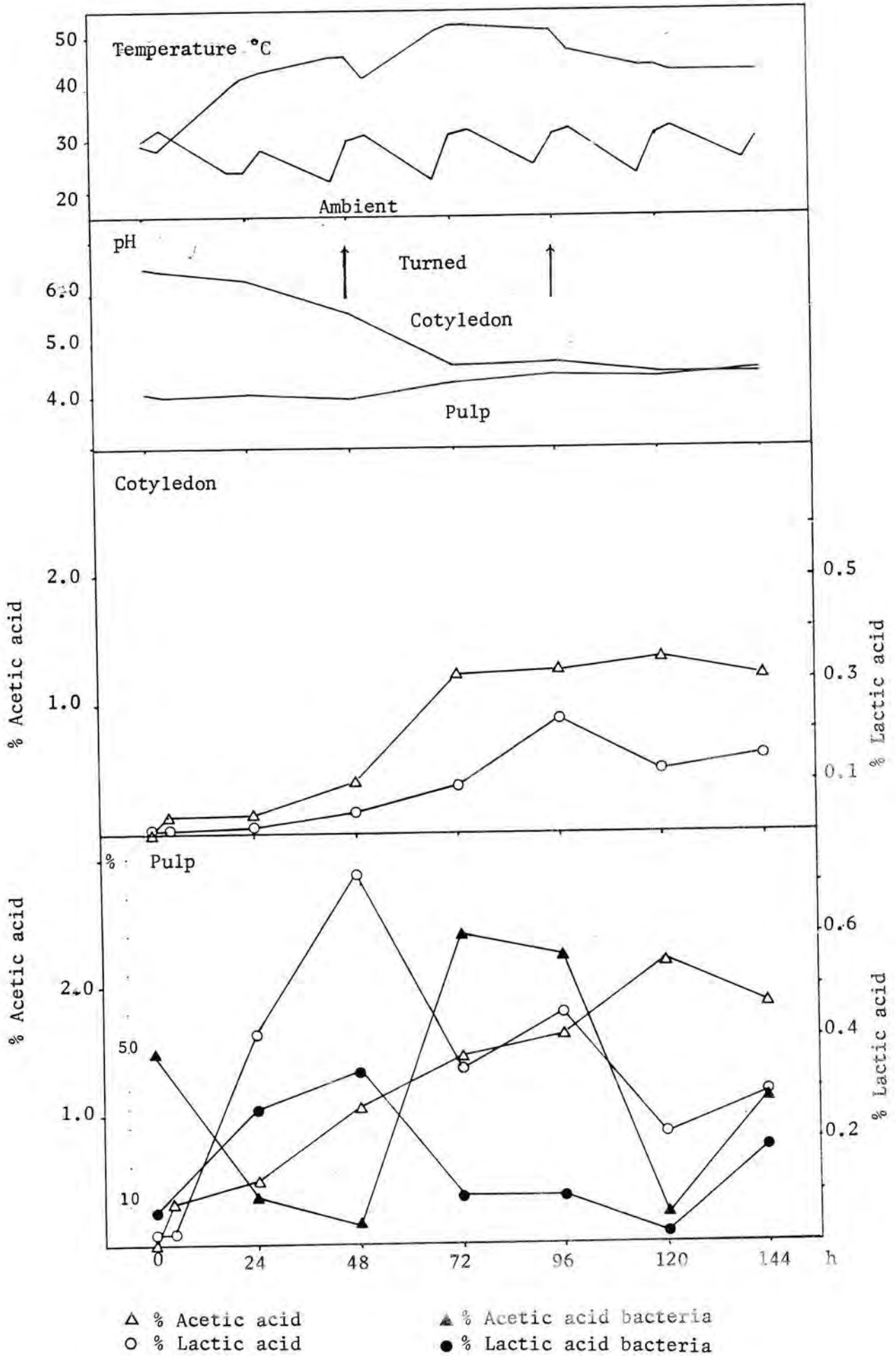
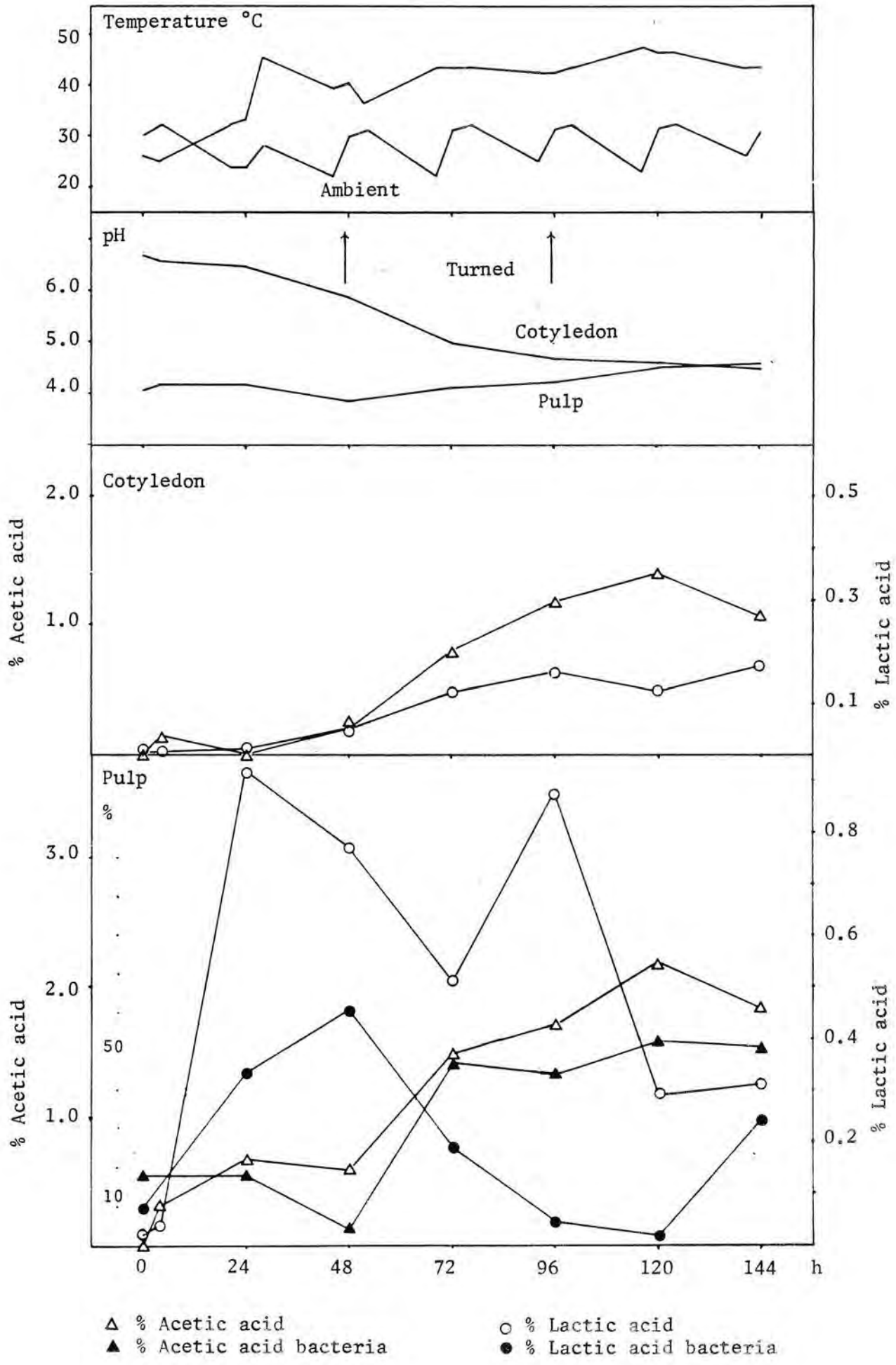


Fig.6 Ghana - Heap 2 : Bottom



Heap 2 - General comments

Top sample

During the first 48 hours the sugars rapidly diminish. Most of this takes place within the first 24 hours when the fermenting yeasts are most active (Table 4). Peak ethanol production occurred at 48 hours. At no time did the alcohol ever reach the theoretical level that can be calculated from the amount of sugar fermented and it can only be surmised that it is constantly being removed from the beans by the action of the acetic acid bacteria that occur as a large proportion of the microflora throughout the fermentation (Table 4).

The presence of these bacteria was reflected by an early rise in the pulp acetic acid, thereafter followed by a slight drop. The cotyledon acetic acid level increased rapidly up to 48 hours and thereafter showed little change. Lactic acid is produced in the pulp from 4 hours and gradually increases throughout the remainder of the fermentation. The lactic acid bacteria were present as a smaller proportion of the microflora (Table 4).

Middle sample

The fermenting yeasts persist longer as a higher proportion of the total population than in the top sample. As usual the amount of sugar drops rapidly to leave a small residue which remains at about the same level throughout the remainder of the fermentation. Much larger quantities of ethanol are found in the pulp and cotyledons. Peak pulp ethanol production occurs at 48 hours. Lactic and acetic acid bacteria alternate throughout the fermentation as to which forms the largest proportion of the population. Acetic acid production in the pulp and diffusion into the cotyledons occurs at a much slower rate than that found in the top sample and both tend to increase throughout the fermentation. Pulp lactic acid rises rapidly, peaking at 48 hours and then dropping. Lactic acid in the cotyledons reaches its maximum concentration at 96 hours when the pulp lactic acid is falling. Pulp lactic acid concentration is affected by the turning schedule. At its peak the heap is turned and there is a drop in lactic acid production. This recovers and starts moving upwards until the next turn which causes another drop. Finally there is a recovery and it starts moving upwards until the end of the fermentation. *Bacillus* spp. are detected at an earlier

stage in the middle site and the top site and towards the end of fermentation form a major part of the microflora. Irregular punctiforms occur sporadically in the middle part of the fermentation and only on plates incubated at 45°C do they form a majority of the microflora. This growth at 45°C probably indicates that irregular punctiforms are thermophiles and this would appear to be confirmed by the increase of their colony size at the elevated temperature.

Bottom sample

The disappearance of sugar is considerably slower in this site than the others and this is reflected by the persistence of fermenting yeasts until 96 hours.

Ethanol production in the pulp is spread out to 96 hours. As usual the acetic acid bacteria are persistent throughout the bean fermentation. The acetic acid levels in the pulp and cotyledon increase slowly initially and after 48 hours behave in a manner similar to that of the middle sample. Pulp production of lactic acid increases rapidly from 4 hours to levels much higher than found in the top or middle sites. Two peaks of pulp lactic acid production occur at 24 and 96 hours. Although these large quantities of lactic acid are present in the pulp they are not reflected in the quantities found in the cotyledon. The production of lactic acid in high concentrations probably reflects the more anaerobic condition at the bottom of the heap and this would seem to be confirmed by the larger proportion of lactic acid bacteria occurring in the middle of the fermentation. *Bacillus* spp. were found mainly towards the end of the fermentation and it is noticeable that when present as a large part of the microflora the irregular punctiforms do not grow. This last named organism only occurs in the majority when incubated at 45°C, again confirming its thermophilic nature.

Incubation of plates at 45°C

This was done because it was thought that an elevated temperature approaching that of the heaps was necessary to distinguish thermophylic organisms. It is interesting to note that of all the organisms listed the fermenting yeasts were the only ones never to grow at 45°C.

Summary

The principal differences between the top, middle and bottom sites are as follows:

1. Pulp sugar disappearance in the bottom site is very much slower than in either the top or the middle sites.

2. Ethanol production in the pulp in the middle sample was higher than that of either the top or the bottom samples.

3. Acetic acid production in the pulp and diffusion into the cotyledon of the top sample occurred at a much more rapid rate than that found in either the middle or bottom sample.

4. Pulp lactic acid production in the bottom sample is at all times much greater than that found in either of the top or middle sites.

The slower utilisation of the sugars in the middle and bottom sites is due to the existence of less aerobic conditions. In the top site the yeasts reproduce faster, produce ethanol faster and they then die. In the other two sites the fermentation is more protracted and this accounts for the fermenting yeasts living for longer periods.

The rapid production of ethanol coupled with the more aerobic conditions at the top of the heap enables an early and rapid rise in acetic acid to occur. In the middle and bottom sites where conditions are less aerobic the production of acetic acid is initially slower but rises steadily throughout the fermentation.

The larger amount of lactic acid in the pulp of the bottom sample depends on the fact that conditions in this site tend to be less aerobic and thus encourage the growth of lactic acid bacteria and this can be seen by comparing the number of lactic acid bacteria in top and bottom sites in Table 4.

It is not known what chemical changes are brought about by the irregular punctiforms but their presence should not be discounted, since they do occur in such large numbers. Similarly, with *Bacillus* spp. their exact role is not known. It is, however, known from experiments to be reported in Part 2 that they can break down a range of organic acids.

Box - General information

Period of the experiment: 1.11.77 - 7.11.77

Original weight of wet beans: 562 Kg

Mixture of Upper Amazon hybrids. Top covered with plantain leaves

Size: Height 88 cm; length 120 cm; width 105 cm

Bean depth: 45 cm

Turned: Into Box 2 after the 11.30 am sample on 3.11.77 and to Box 3 after 11.30 am sample on 5.11.77

Sampling: Top-surface sample; middle - 20 cm below the top; bottom - slide up trap door and take sample from the surface

Final weight of beans: 520 Kg

Drying: 7.11.77 - 18.11.77 - Trinidad sun drier raked 6 times a day

All numerical and graphical information referring to this section is in Tables 7, 8, 9 and 10 and Figs 7, 8 and 9.

TABLE 7

Ghana - Box

Organisms per gram of beans x 10^6

	0 h		4 h		24 h		48 h		72 h		96 h		120 h		144 h	
	Ae	An	Ae	An	Ae	An	Ae	An	Ae	An	Ae	An	Ae	An	Ae	An
Top	60	22	91	82	133	102	4	2	2	2	94	20	79	132	>	>
Middle	86	68	39	60	84	77	325	39	1	100	2	4	16	7	1	29
Bottom	47	23	75	42	>	28	246	245	>	>	>	>	304	215	>	>

> = > 1 x 10^7

TABLE 8

The frequency of micro-organisms in the Ghana Box expressed as a percentage

		0 h		4 h		24 h		48 h		72 h		96 h		120 h		144 h	
		Ae	An	Ae	An	Ae	An	Ae	An	Ae	An	Ae	An	Ae	An	Ae	An
TOP	Yeasts	27	27	30	30	1	1	1	1	-	-	-	-	-	-	-	-
	Other yeasts	7	7	5	5	1	1	1	-	-	-	-	-	-	-	1	1
	Acetics	53	53	43	43	48	37	66	71	99	99	99	94	63	56	47	53
	Lactics	13	13	22	22	25	61	17	28	-	-	-	1	-	39	19	36
	Bacillus	-	-	-	-	-	-	-	-	1	1	1	5	37	5	33	10
	Punctiforms	-	-	-	-	25	-	15	-	-	-	-	-	-	-	-	-
MIDDLE	Yeasts	25	25	37	43	6	7	1	1	-	-	-	-	-	-	-	-
	Other yeasts	19	19	-	-	1	-	1	1	-	-	-	-	-	-	-	-
	Acetics	50	50	42	42	39	35	24	42	99	99	71	91	50	23	67	23
	Lactics	6	6	21	15	54	58	34	56	-	-	21	-	-	-	7	-
	Bacillus	-	-	-	-	-	-	-	-	1	1	8	9	50	77	26	77
	Punctiforms	-	-	-	-	-	-	40	-	-	-	-	-	-	-	-	-
BOTTOM	Yeasts	27	27	71	56	1	1	1	-	-	-	1	-	-	-	-	-
	Other yeasts	13	13	1	7	1	1	1	1	1	-	1	-	-	-	-	-
	Acetics	53	53	7	17	50	32	45	59	90	90	70	65	98	77	71	77
	Lactics	7	7	21	20	48	66	33	35	9	10	21	32	1	15	7	8
	Bacillus	-	-	-	-	-	-	-	-	-	-	7	3	1	8	22	15
	Punctiforms	-	-	-	-	-	-	20	5	-	-	-	-	-	-	-	-

TABLE 9

Analyses of bean pulp and testa from the Box

Sampling			pH	Sugars %	Ethanol %	Acetic acid %	Lactic acid %
1/11	0 h	T	3.90	9.99	0	0.27	0.02
		M	3.85	7.96	0	0.27	0.02
		B	4.00	8.67	0	0.27	0.03
2/11	24 h	T	3.70	1.28	0.20	1.89	0.19
		M	3.92	5.17	1.55	0.58	0.45
		B	3.85	1.49	2.37	1.06	0.41
3/11	48 h	T	4.00	1.31	0.72	0.66	0.23
		M	3.85	2.17	0.79	0.83	0.36
		B	3.85	0.72	0.28	2.39	0.20
4/11	72 h	T	4.00	1.39	0.15	2.12	0.23
		M	4.15	2.18	0.16	1.38	0.36
		B	4.10	0.77	0	0.50	0.12
5/11	96 h	T	4.15	0.89	0.08	0.49	0.07
		M	4.25	1.30	0.17	1.19	0.58
		B	4.35	0,60	0	0.27	0.04
6/11	120 h	T	4.30	0.27	0	0.85	0.35
		M	4.35	1.95	0.04	1.99	0.42
		B	4.40	1.65	0	0.93	0.20
7/11	144 h	T	4.40	0.40	0	0.89	0.33
		M	4.50	1.43	0	1.72	0.49
		B	4.50	0.86	0	0.48	0.17

TABLE 10

Analyses of cotyledons from the Box

Sampling			pH	Sugars %	Ethanol %	Acetic acid %	Lactic acid %
1/11	0 h	T	6.25	2.83	0	0	0.005
		M	6.35	2.67	0	0	0.005
		B	6.40	2.76	0	0	0.005
2/11	24 h	T	5.45	1.02	0.25	1.78	0.01
		M	6.10	1.14	0.39	0.13	0.01
		B	5.90	1.29	0.77	0.23	0.01
3/11	48 h	T	4.60	1.44	0.23	1.46	0.02
		M	5.75	0.96	0.81	0.42	0.03
		B	5.85	1.08	0.23	0.20	0.05
4/11	72 h	T	4.55	0.91	0.20	1.46	0.12
		M	4.70	0.84	0.21	1.27	0.13
		B	4.85	0.77	0.01	0.82	0.05
5/11	96 h	T	4.35	0.64	0.09	1.46	0.05
		M	4.75	0.82	0.45	0.80	0.07
		B	4.80	0.73	0	0.46	0.14
6/11	120 h	T	4.60	0.60	0	0.42	0.14
		M	4.55	0.60	0.04	1.21	0.17
		B	4.75	0.65	0	1.10	0.18
7/11	144 h	T	4.60	0.55	0	0.49	0.16
		M	4.40	0.61	0.03	1.15	0.19
		B	4.75	0.58	0	0.33	0.15

Fig.7 Ghana Box : Top

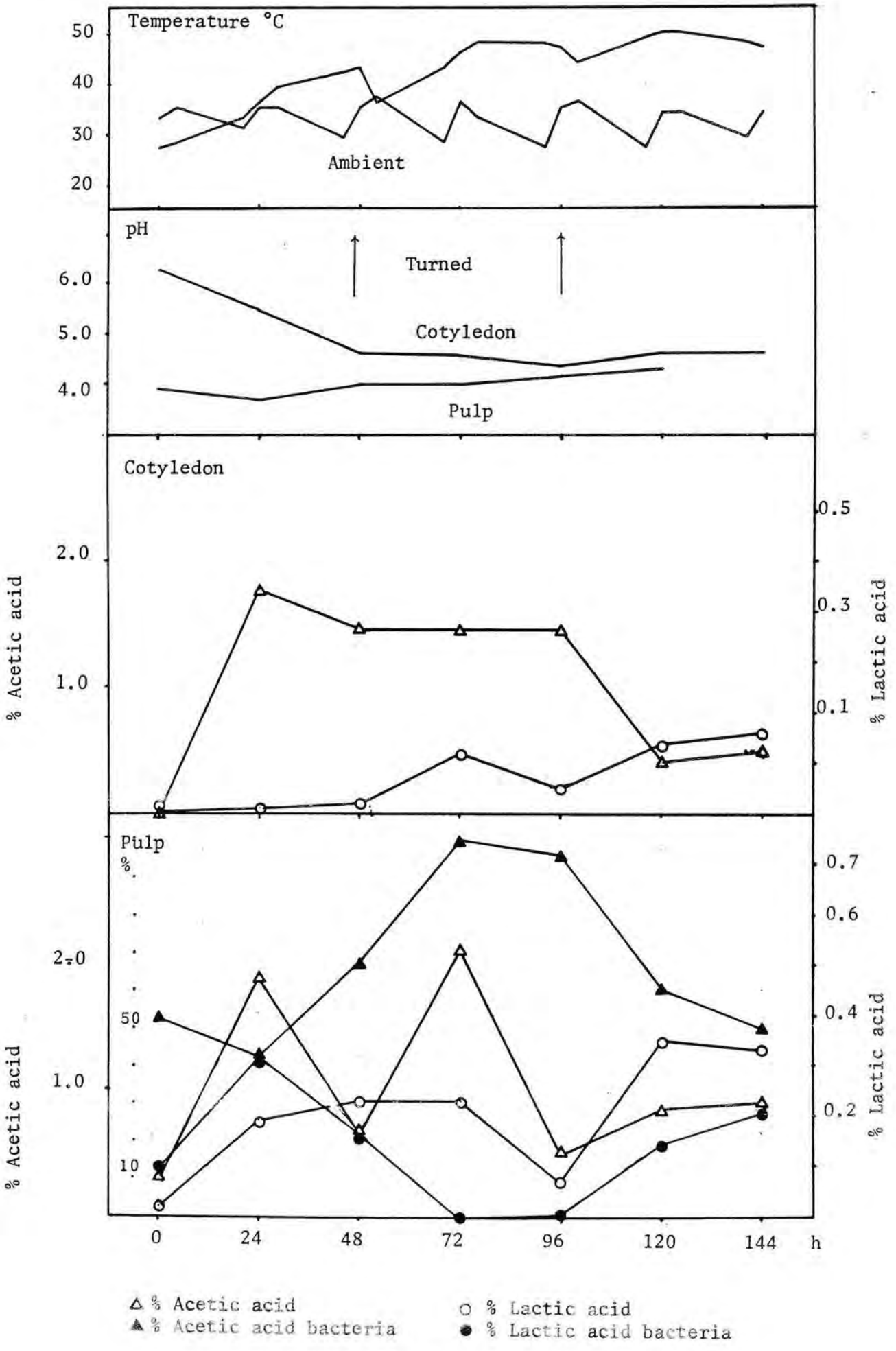


Fig.8 Ghana - Box : Middle

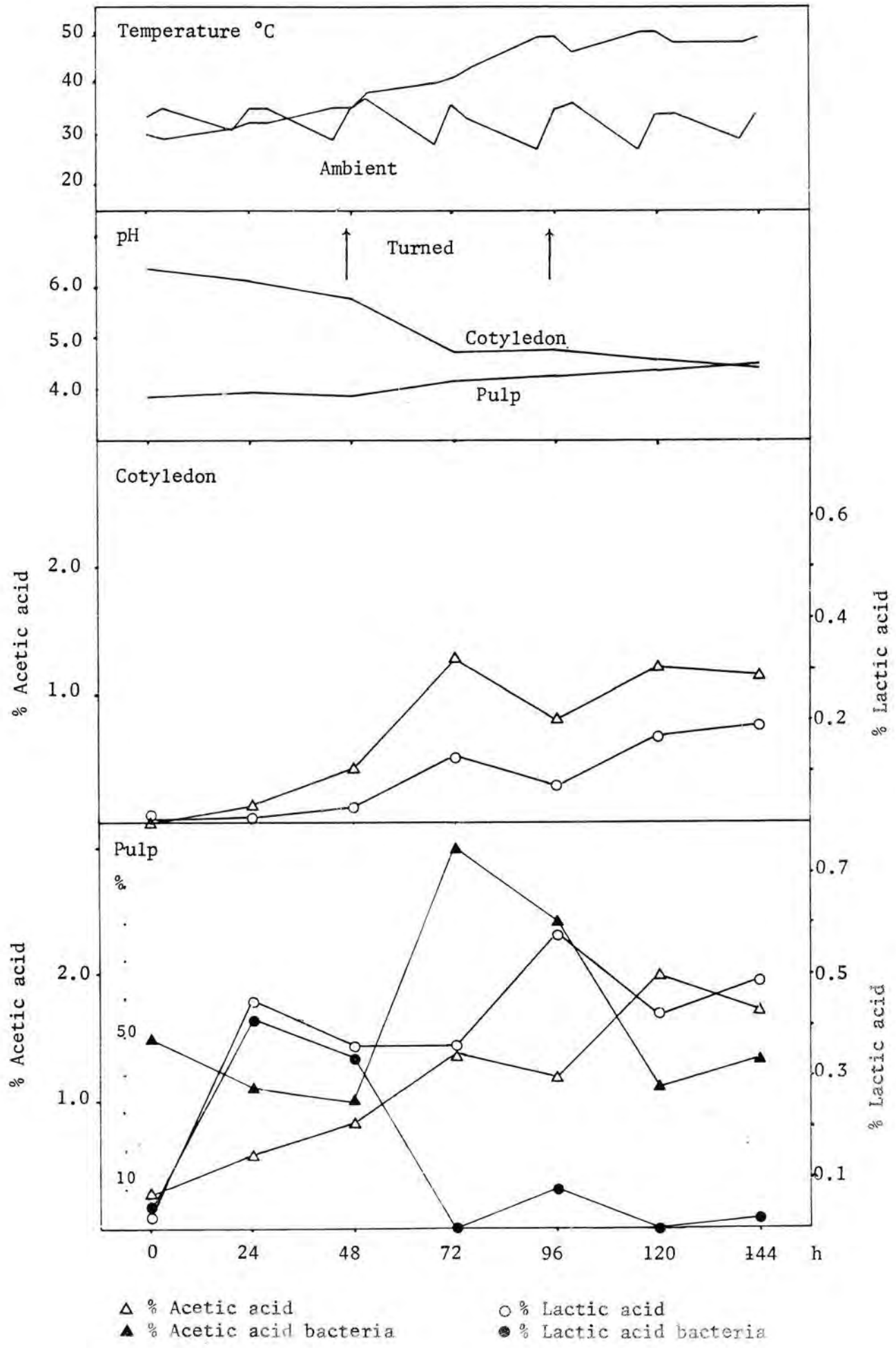
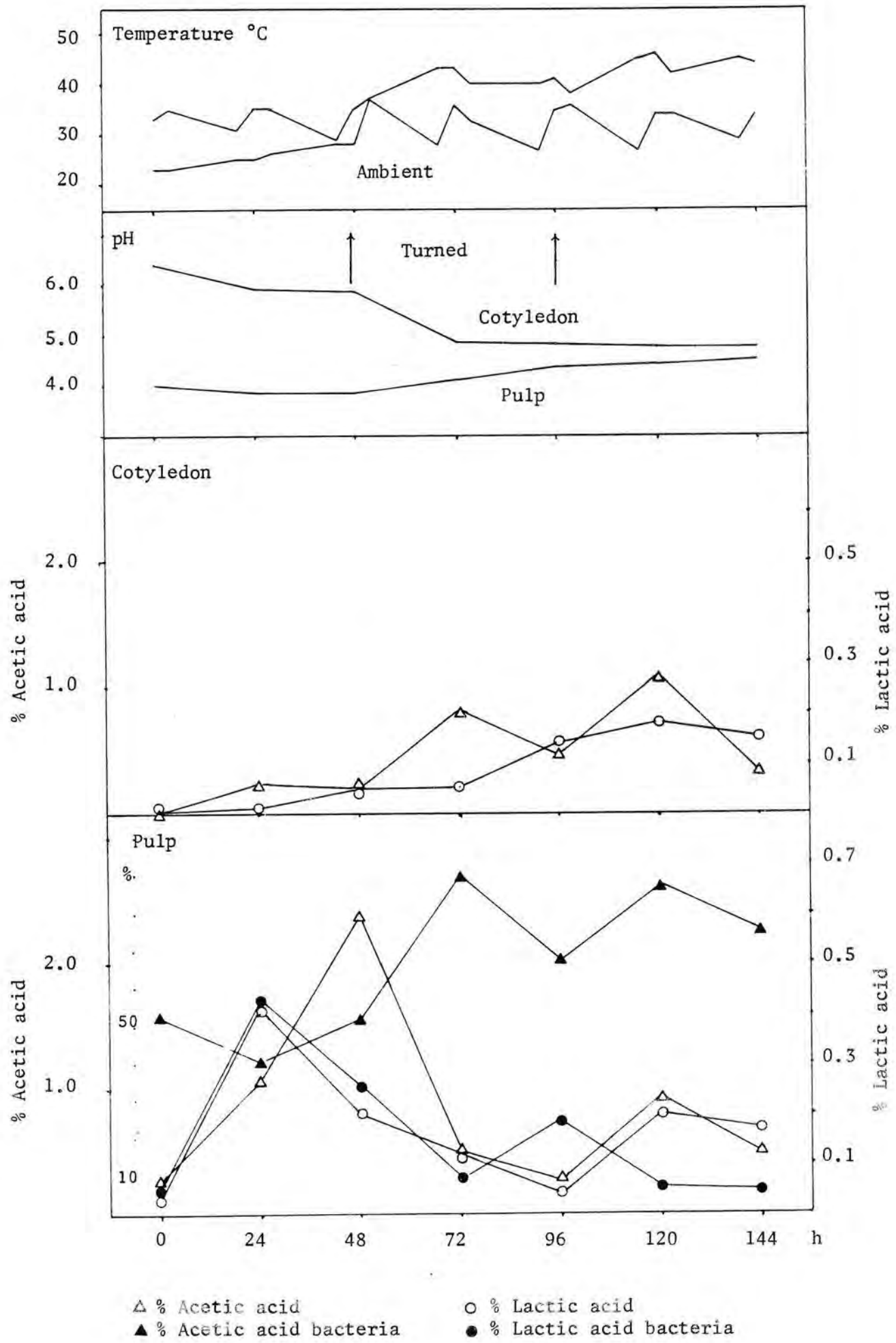


Fig.9 Ghana - Box : Bottom



Box fermentation

Top sample

The pulp sugar content drops rapidly in the first 24 hours and then the rate of disappearance slows. This is reflected, to some extent, by the low proportion of the microflora formed by the fermenting yeasts. Maximum pulp ethanol concentration occurs at 48 hours. Acetic acid production in the pulp and diffusion into the cotyledon begins immediately at the commencement of fermentation. Pulp acetic acid reaches a peak at 24 hours, declines at 48 hours, reaches a maximum at 72 hours and thereafter declines throughout the fermentation. The acetic acid bacteria appear at a high level throughout the fermentation. Cotyledon acetic acid reaches a maximum at 24 hours, stabilises until 96 hours and declines thereafter. There is no obvious relationship between the concentrations of acetic acid in the pulp and cotyledon. The lactic acid bacteria never form a very large proportion of the microbial population and this is reflected in the slow rise and fall of pulp lactic acid up to 96 hours only showing a resergence between 96 and 120 hours. Cotyledon lactic acid tends to increase throughout the fermentation from 24 hours.

Middle sample

The sugar in this site drops very gradually indeed whereas the alcohol shows a higher peak than most previous ones. Maximum production of pulp ethanol occurs at 24 hours. There is a slow build up of acetic acid in the pulp and cotyledon at the beginning of the fermentation. The concentrations tend to increase throughout the remainder of the fermentation. Acetic acid bacteria are present in fairly high numbers throughout the fermentation. The behaviour of pulp lactic acid is similar to that observed in Heap 2, showing 2 peaks in the early stages (24/48 hours) and 96 hours. This is reflected by the fairly high numbers of lactic acid bacteria between 4 and 48 hours. Diffusion of lactic acid into the cotyledons begins at 24 hours and the increase in concentration continues throughout the fermentation. *Bacillus* spp. are present in high numbers towards the end of the fermentation and it is once again noticeable that no irregular punctiforms grew at that time. This is similar to the situation at the top. Irregular punctiforms were only detected once at 48 hours. There were, however, no plates

incubated at 45°C and it is thought that a truer reflection of this organism's numbers would have been obtained had 45°C plates been used.

Bottom sample

Pulp sugars fall gradually to a low level and then increase at 120 hours. Production of ethanol in the pulp begins immediately, reaching a maximum at 24 hours. There is more ethanol in the bottom sample than either the top or middle samples. The fermenting yeasts do not survive in any significant quantity beyond 24 hours. The acetic acid bacteria as usual form the majority of the micro-flora with the lactic acid bacteria equally persistent forming a smaller proportion. Acetic acid production in the pulp reaches a maximum at 48 hours and then declines thereafter. Pulp lactic acid levels reach a maximum at 24 hours then decline to 96 hours and increase throughout the rest of the fermentation. Cotyledon lactic acid tends to increase throughout the fermentation from 24 hours. *Bacillus* spp. as usual occurred at the end of the fermentation and irregular punctiforms were detected only at 48 hours.

Summary and comparison with Heap 2

The Ghana Box middle sample is very similar to Heap 2 middle sample. The principal differences are the maximum ethanol production in the pulp middle sample is at 24 hours and at 48 hours in the Heap 2 middle sample. Pulp lactic acid increases at a greater rate in the Heap 2 middle sample than the Box middle sample and decreases to lower levels at the end of the fermentation. Heap 2 sample and the Box top sample profiles were again very similar. There is a greater variation in the pulp acetic content in the Box sample than in the Heap sample. The final acetic acid value in the cotyledon of the Box top sample was half that of the top of Heap 2. Diffusion of lactic acid into the cotyledon of the top Box sample starts at 48 hours.

The profiles of the bottom Box samples are very different from the bottom Heap 2 sample. There is a much greater reduction in pulp sugars in the first 24 hours in the Box bottom sample than in the Heap bottom sample. There is a much greater ethanol content in pulp and cotyledon of the Box bottom sample than the Heap 2 bottom sample. The rapid increase in pulp lactic acid found in the bottom of Heap 2 in the initial

stages of fermentation does not occur in the Box bottom sample. The behaviour of the cotyledon with respect to lactic acid is, however, very similar, both increasing from 24 hours. There is a much greater increase in pulp acetic acid at the onset of fermentation in the bottom Box samples. The cotyledon acetic acid values are generally lower in the Box bottom samples than in the Heap samples.

These differences can be explained as follows. The Boxes in Africa were bored with a considerable number of holes which must have led to the bottom layer being more aerobic than is usual in a Box. The early rise of acetic acid in the bottom is typical of the pattern found in the top of Heap 2 and the Box. In addition there is a very marked difference in viable counts between top and bottom. The latter contains considerably more organisms throughout fermentation.

MALAYSIA

The work in Malaysia lasted about 5 weeks and was carried out in 3 places. All samples were collected from the fermentary of the Flemington Estate. Chemical analyses were carried out in the well equipped laboratory of UNITATA. This meant that with the assistance of the skilled laboratory staff and use of the available equipment it was possible to complete the chemical work in Malaysia.

The microbiological work was done some 10 miles from UNITATA by courtesy of MARDI at their agronomical research station at Hilir Perak. The accommodation consisted of a mobile caravan laboratory which was air conditioned and suitable for one worker. In addition the microbiologists were allocated another room in the main building which was not air conditioned but had a large suspended fan. When this fan was operating it caused so much air turbulence that no work with open petri dishes was possible and in consequence much of the work done in this room had to be carried out at ambient temperature. About half way through the work, staff of the research station became involved in an intensive exercise of chopping up and weighing large quantities of freshly harvested coconuts. From this time our plates became progressively contaminated with an orange-coloured mould, probably of the genus *Neurospora*. Towards the end of our stay the counting and isolation of cocoa micro-organisms became virtually impossible due to their overgrowth with this and other moulds.

Two young female microbiologists were seconded to us from MARDI. During their stay they were shown the techniques we used and participated in the various phases of the work. It was never clear, however, whether they had been sent to render assistance or to learn the techniques.

The methods of sampling and subsequent handling of the beans for microbiological observations were virtually the same as those employed in Ghana. The methods of sampling the beans for chemical analysis was the same as that employed in Ghana. The chemical analyses were the same as those previously described but carried out on site.

Trial 1 - General information

Harvesting: 50,000 pods were harvested on Saturday, 18th February on all three divisions and delivered to the factory. The pods were heaped both inside and outside the factory.

Splitting: Pods were split from 7 am to 12 midday on Monday, 20th February. Beans were initially kept in polythene bags and transferred to perforated collection boxes at midday.

Fermentation boxes: Two days in boxes 472 cm x 168 cm x 61 cm and the balance in boxes 259 cm x 168 cm x 99 cm.

Loading to fermentation box: Loading of 4071 Kg wet beans into the fermentation box was completed at 2.30 pm on Monday, 20th February.

Turning: Beans were turned on the mornings of 21st, 22nd, 23rd, 24th, 25th and 27th at approximately 9.00 am.

Pre-drying: Beans were loaded into the pre-drier (1097 cm x 366 cm x 12.7 cm) on 27th at 9.30 am. The drier was started at 11.00 am running at 79°C and dried for 4 hours.

Resting: Beans were left in the pre-drier until 3 pm on 28th February.

Final drying: Beans dried from 3 pm until 8 pm on 28th February and 7 am to 10 am on 1st March (8 hours), i.e. till sufficiently dry ± 6% moisture.

All numerical and graphical information referring to this section is in Tables 11, 12, 13 and 14 and Figs 10, 11 and 12

TABLE 11

Malaysia - Trial 1

Organisms per gram of beans x 10^6

	0 h		4 h		24 h		48 h		72 h		96 h			120 h		144 h		168 h		
	Ae	An	Ae	An	Ae	An	Ae	An	Ae	An	Ae	An	45	Ae	An	Ae	An	Ae	An	45
Top	15	6	15	12	218	>	125	16	>	22	128	5	30	41	65	21	-	50	50	6
Middle			20	11	37	>	>	304	119	11	5	5	1	16	18	44	13	-	-	-
Bottom			16	5	>	>	85	100	2	13	49	2	61	-	-	-	-	-	-	3

> = > 1 x 10^7

\- = no result

TABLE 12

The frequency of micro-organisms in Trial 1 expressed as a percentage

		* 0 h		4 h		24 h		48 h		72 h		96 h			120 h		144 h		168 h		
		Ae	An	Ae	An	Ae	An	Ae	An	Ae	An	Ae	An	45	Ae	An	Ae	An	Ae	An	45
TOP	Yeasts	85	70	50	15	40	20	30	-	29	5	15	-	-	-	1	-	-	-	-	-
	Other yeasts	-	5	10	15	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-
	Acetics	15	25	40	30	10	10	30	36	59	36	70	50	18	90	62	100	-	95	90	49
	Lactics	-	-	-	5	-	70	40	64	12	55	15	50	9	5	-	-	-	5	10	20
	Bacillus	-	-	-	-	-	-	-	-	-	-	-	-	9	-	-	-	-	-	-	1
	Punctiforms	-	-	-	35	50	-	-	-	-	4	-	-	64	5	36	-	-	-	-	30
MIDDLE	Yeasts			47	55	20	20	20	20	12	5	-	-	-	-	-	-	-	-	-	-
	Other yeasts			6	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Acetics			47	15	20	10	20	-	77	70	90	40	80	90	50	99	100	-	-	-
	Lactics			-	25	60	70	20	80	11	25	5	-	10	5	-	-	-	-	-	-
	Bacillus			-	-	-	-	-	-	-	-	-	-	-	5	-	1	-	-	-	-
	Punctiforms			-	-	-	-	40	-	-	-	5	60	10	-	50	-	-	-	-	-
BOTTOM	Yeasts			45	70	40	5	20	10	15	10	-	-	-	-	-	-	-	-	-	-
	Other yeasts			5	-	-	5	-	-	-	-	-	-	-	-	1	-	-	-	-	-
	Acetics			18	10	15	10	-	-	-	-	80	80	9	26	79	100	-	-	-	-
	Lactics			32	20	45	80	30	90	15	90	10	10	9	4	-	-	-	-	-	-
	Bacillus			-	-	-	-	-	-	-	-	-	-	2	4	-	-	-	-	-	-
	Punctiforms			-	-	-	-	50	-	70	-	10	10	80	66	20	-	-	-	-	100

* Time from pod breaking

TABLE 13

Analyses of bean pulp and testa from Trial 1

Sampling			Pulp/testa per bean (g)	pH	Sugars %	Ethanol %	Acetic acid %	Lactic acid %
20/2	Fresh pulp 0 h		n.d-	3.80	15.72	Nil	Nil	0.01
After pod breaking	2 h		n.d	3.84	12.24	0.06	Nil	0.02
	4 h	T		3.98	10.10	0.13	0.34	0.03
		M	n.d	3.89	10.45	0.21	0.19	0.15
		B	n.d	3.90	8.79	0.18	1.93	0.04
21/2	24 h	T	n.d	3.92	2.30	1.06	1.57	0.43
		M	n.d	3.43	4.58	0.67	1.91	0.45
		B	n.d	3.94	4.59	0.74	1.85	0.48
22/2	48 h	T	0.68	3.92	1.75	Nil	3.60	0.10
		M	0.66	3.85	0.92	0.38	1.11	0.16
		B	0.67	3.84	0.41	1.41	1.29	0.73
23/2	72 h	T	0.61	4.50	0.96	Nil	1.59	0.22
		M	0.60	4.20	0.64	0.48	1.50	0.42
		B	0.67	4.14	0.58	0.91	1.09	0.87
24/2	96 h	T	0.58	4.58	1.12	Nil	1.90	0.29
		M	0.69	4.25	0.91	0.27	1.81	0.44
		B	0.63	4.30	0.58	0.45	1.81	0.50
25/2	120 h	T	0.59	4.45	0.70	Nil	1.89	0.69
		M	0.66	4.35	0.52	0.26	1.96	0.72
		B	0.64	4.30	0.43	0.47	1.58	0.66
26/2	144 h	T	0.59	4.60	1.92	Nil	1.46	0.48
		M	0.66	4.30	0.54	0.08	2.03	0.77
		B	0.63	4.25	0.43	0.25	1.55	0.71
27/2	168 h	T	0.57	4.65	0.72	Nil	1.11	0.46
		M	0.66	4.30	0.45	0.29	3.72	0.86
		B	0.64	4.30	0.38	0.36	1.46	0.65

TABLE 14

Analyses of cotyledons from Trial 1

Sampling			Average bean weight (g)	pH	Sugars %	Ethanol %	Acetic acid %	Lactic acid %
20/2	Fresh pulp 0 h		n.d	6.30	2.47	Nil	Nil	0.01
After pod breaking	2 h		n.d	6.44	2.22	Nil	Nil	0.01
	4 h	T	n.d	6.42	2.31	Nil	0.40	0.01
		M	n.d	6.49	2.42	0.19	0.14	0.01
		B	n.d	6.46	2.25	0.01	0.13	0.01
21/2	24 h	T	n.d	6.25	2.20	0.34	0.25	0.01
		M	n.d	6.20	2.32	0.44	0.18	0.01
		B	n.d	6.33	2.09	0.33	0.13	0.01
22/2	48 h	T	1.40	4.74	1.92	0.12	0.82	0.03
		M	1.33	5.00	1.72	0.30	0.33	0.12
		B	1.36	5.32	2.07	0.49	0.38	0.11
23/2	72 h	T	1.44	4.65	1.27	0.12	1.95	0.13
		M	1.41	4.68	1.31	0.76	0.95	0.27
		B	1.49	4.75	1.46	0.79	0.78	0.11
24/2	96 h	T	1.46	4.65	1.22	0.03	1.07	0.13
		M	1.47	4.49	1.09	0.19	2.38	0.18
		B	1.41	4.43	1.25	0.54	0.90	0.18
25/2	120 h	T	1.37	4.48	1.24	0.08	1.05	0.27
		M	1.46	4.40	0.96	0.29	1.07	0.28
		B	1.50	4.48	1.06	0.64	1.01	0.35
26/2	144 h	T	1.43	4.40	1.22	0.02	1.09	0.20
		M	1.46	4.30	1.00	0.14	1.04	0.17
		B	1.34	4.30	1.03	0.30	0.98	0.20
27/2	168 h	T	1.35	4.50	0.80	Nil	0.84	0.32
		M	1.38	4.32	0.58	0.29	2.00	0.23
		B	1.35	4.35	0.94	0.30	2.18	0.22

Fig.10 Malaysia - Trial 1 : Top

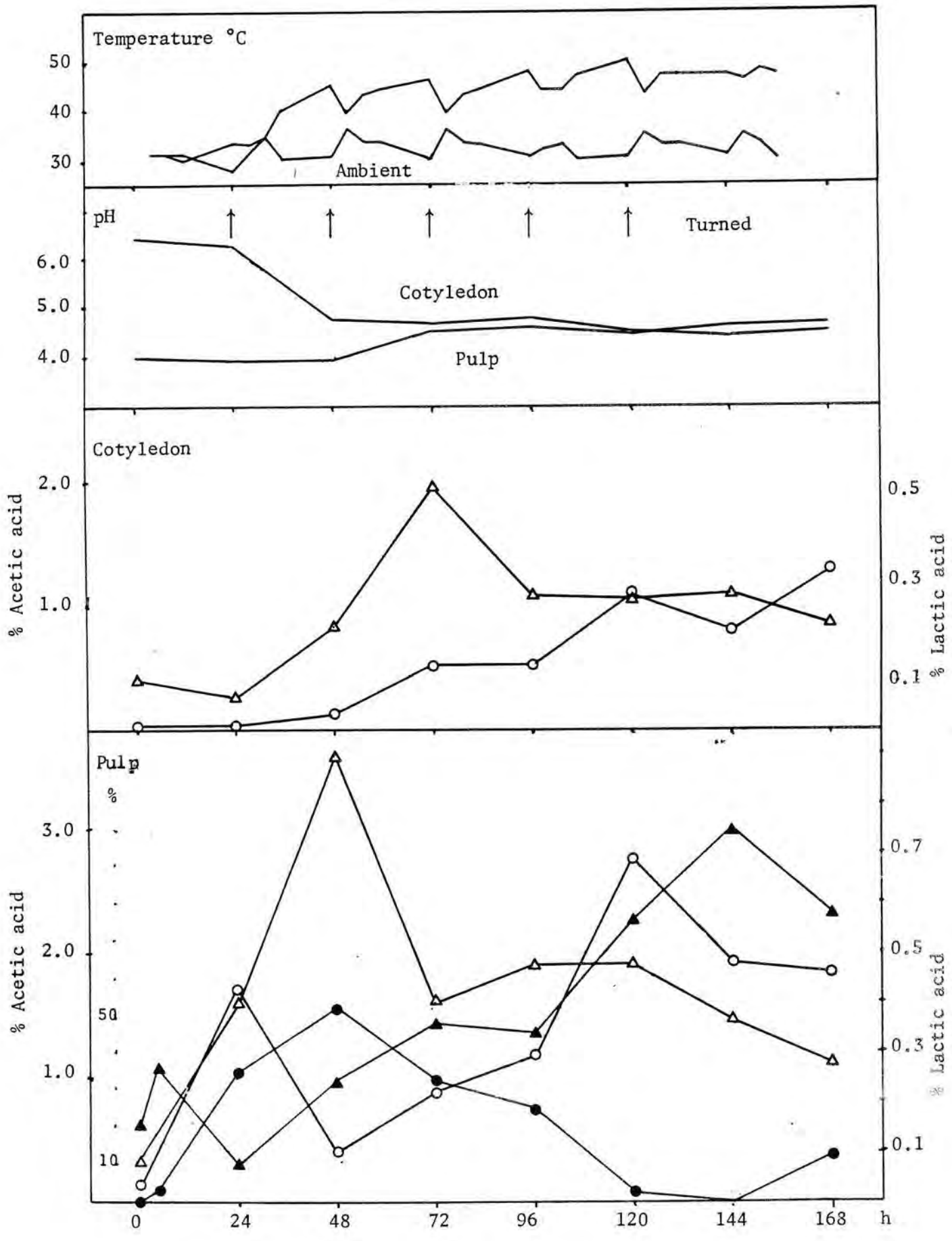


Fig.11 Malaysia - Trial 1 : Middle

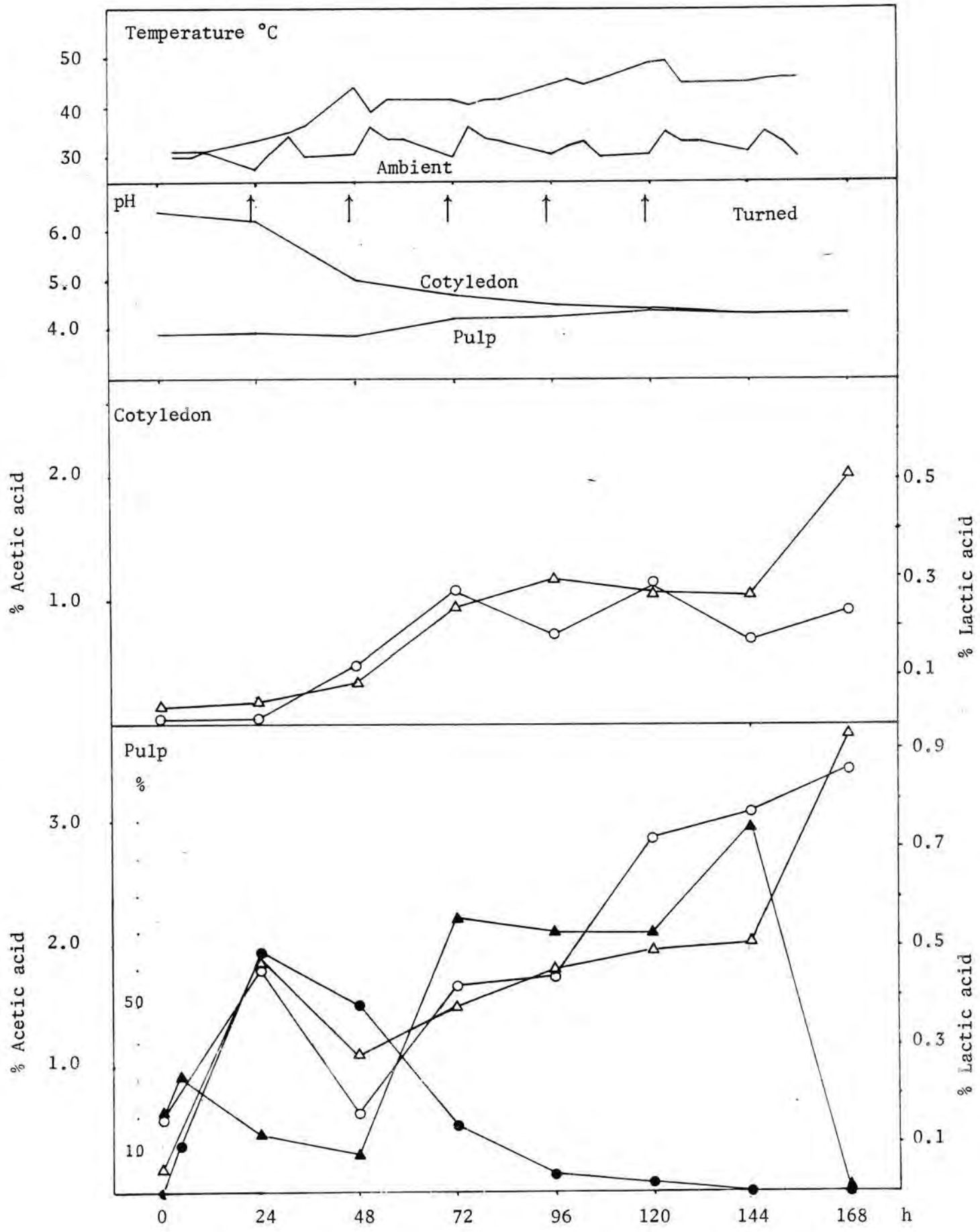
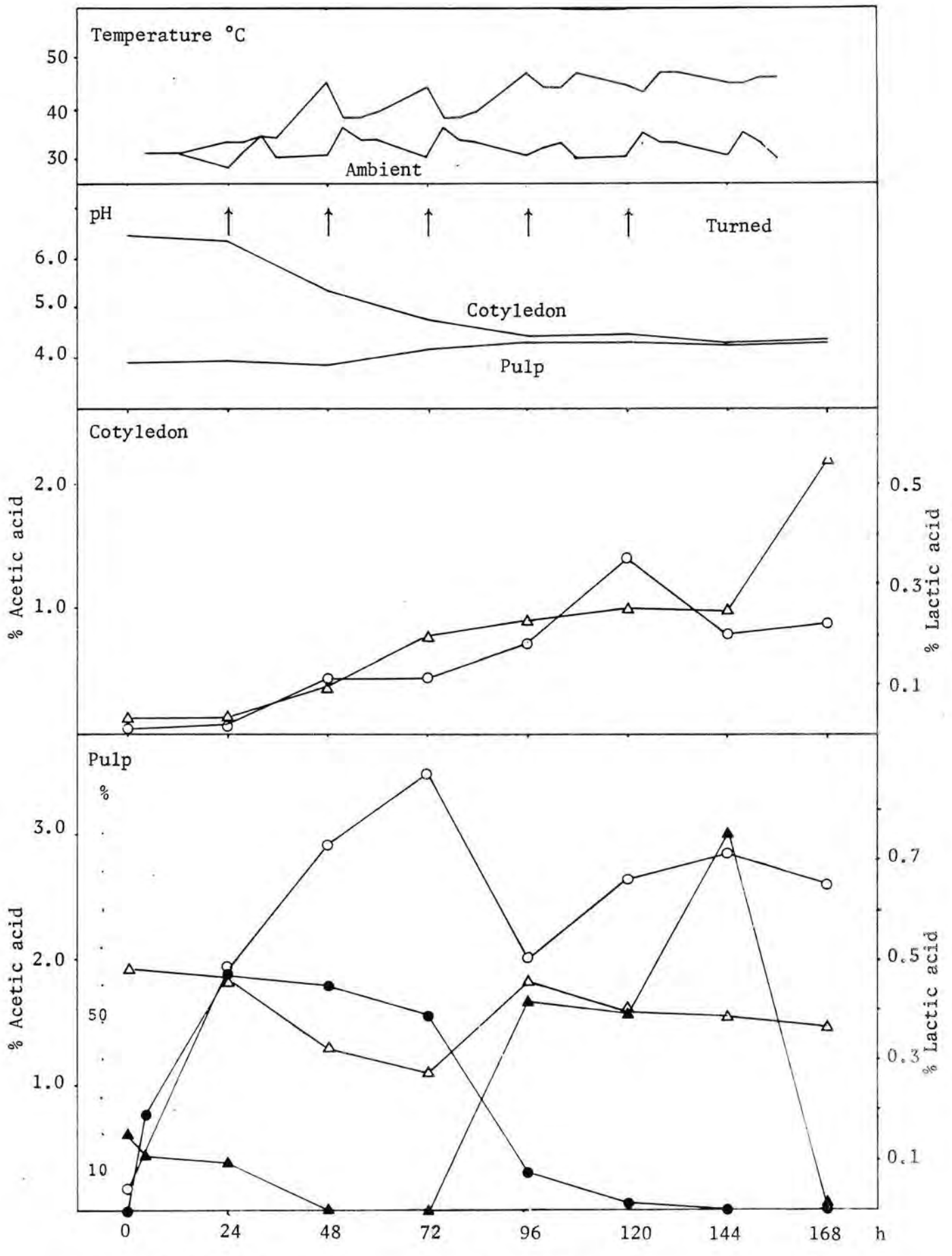


Fig.12 Malaysia - Trial 1 : Bottom



Trial 1 - Normal

Top sample

In the first 24 hours pulp sugars are rapidly reduced corresponding to the presence of high numbers of fermenting yeasts which eventually diminish as the temperature increases and pulp sugars are reduced to a fairly consistent low level from 72 hours onwards. Pulp ethanol reaches a maximum at 24 hours and declines thereafter and cannot be detected after 48 hours. Cotyledon ethanol levels decline gradually after 24 hours but can still be detected at the end of the fermentation. Yeasts persist much longer than hitherto noted and significant numbers can be detected at 96 hours.

Acetic acid in the pulp is produced from the beginning of fermentation and increases up to 48 hours, thereafter it declines gradually throughout the remainder of the fermentation. Cotyledon acetic acid levels increase after 24 hours reaching a maximum at 72 hours and then decline slowly. Acetic acid bacteria are to be found throughout the fermentation and the maximum levels from 72 hours onwards correspond with the decline in the amount of acetic acid. This decline in acetic acid concentration corresponds to the acetic acid bacteria metabolising this acid. Maximum activity of these organisms is indicated by a maximum temperature at 120 hours.

After the initial increase in lactic acid production in the pulp up to 24 hours there is an apparent decline to 48 hours. From 48 hours to 120 hours there is a gradual increase and then a decline to the end of the fermentation. The first increase is marked by an increase in the number of lactic acid bacteria as is the second rise which takes off steeply at 96 hours. The decrease in pulp lactic acid is marked by the detection of small numbers or no lactic acid bacteria at all. After 24 hours the cotyledon lactic acid levels near that of the pulp with equal distribution of lactic acid between pulp and cotyledon. *Bacillus* spp. were only detected very occasionally and irregular punctiforms occurred sporadically but were always found to be on plates incubated at 45°C.

Middle sample

The usual rapid decline in sugars occurs within the first 48 hours and during this period the ethanol shows a distinct increase. This

corresponds to the presence of fermenting yeasts that declined gradually and disappeared after 96 hours. In contrast to the top sample ethanol persists in the pulp until the end of fermentation. This is probably due to the more anaerobic conditions at this site.

Pulp acetic acid increases from the onset of fermentation and reaches a peak at 24 hours; after a slight drop to 48 hours it increases throughout the remainder of the fermentation.

After 24 hours cotyledon acetic acid increases throughout the fermentation. The increase and decline in the pulp acetic acid corresponds to similar increases and reduction in the number of acetic acid bacteria present. Pulp lactic acid levels increase rapidly at the beginning of fermentation reaching a maximum at 72 hours and gradually reducing thereafter. Lactic acid concentration in the cotyledon increases from 24 hours at a rate independent of the high concentrations found in the pulp. After 120 hours there is a slight reduction in cotyledon lactic acid levels. Lactic acid bacteria show a similar pattern to that of acetic acid bacteria. There is not, however, the marked correspondence of numbers of lactic acid bacteria to the increase in pulp lactic acid. *Bacillus* spp. and irregular punctiforms appear with about the same frequency as found in the top sample.

Bottom sample

Pulp sugars disappear in a manner similar to that observed in the middle sample. Ethanol production in the pulp starts immediately, reaching a maximum at 48 hours and declining thereafter. Fermenting yeasts are found in fairly high numbers initially but gradually decline and have disappeared by 96 hours. Ethanol persists in the pulp and cotyledons throughout the fermentation.

Pulp acetic acid is initially at a high level, probably due to sweatings derived from upper layers. This high level of pulp acetic acid is maintained for 24 hours followed by a reduction to 48 hours then an increase to 96 hours and thereafter with little change. The initial numbers of acetic acid bacteria are not high. None could be detected at either 48 or 72 hours and this corresponds with the initial decline in pulp acetic acid. The numbers of these organisms increased between 72 and 96 hours and were not detected at 168 hours. The initial high level of pulp acetic acid is not reflected in the

initial cotyledon level which does not increase until after 24 hours.

Pulp lactic acid increases rapidly from the onset of fermentation until 72 hours gradually reducing thereafter. The lactic acid bacteria are present in fairly high numbers initially and then show a decline from 72 hours, finally disappearing at 144 hours. Cotyledon lactic acid increases from 24 hours onwards at a rate independent of the high concentrations found in the pulp. After 120 hours there is a slight reduction in cotyledon lactic acid level.

Summary

1. Pulp sugar reduction in the top sample is much faster than either the middle or bottom samples which are similar.

2. Peak ethanol production in the top sample occurs at 24 hours; after 48 hours no ethanol could be detected in the pulp. Maximum ethanol production in the pulp of the bottom site occurs at 48 hours. There is no clearly defined maximum in the middle sample. Ethanol persists in both pulp and cotyledon throughout the fermentation in the middle and bottom samples.

3. Pulp acetic acid in the top site is distinguished by an early increase to a maximum level at 48 hours and a tendency to decrease thereafter, whilst acetic levels in the middle site tend to increase throughout the fermentation. There is little change in pulp acetic acid levels in the bottom site throughout the fermentation.

4. Pulp lactic acid in the top and middle samples behaves in a similar manner excepting that in the middle sample pulp lactic acid continues to increase throughout the fermentation, whilst there is a tendency to decline after 120 hours in the top sample. There is very much greater production of pulp lactic acid in the initial stages of fermentation in the bottom site. The build up of lactic acid in the cotyledon in the top, middle and bottom sites is very similar, starting from the onset of fermentation; this is surprising in view of the large differences in pulp lactic acid levels between the top, middle and bottom sites.

This fermentation shows the usual pattern seen in the African fermentation Heap 2, namely, that there is an early rapid rise in acetic acid which eventually falls. The fall is probably due to the fact that the *Acetobacter* spp. are able to metabolise acetic acid,

particularly in the absence of ethanol. The middle and bottom sites tend to show the pattern of acetic acid production more closely associated with a lower oxygen tension, namely, steady rise throughout the fermentation. This is more clearly evident in the middle of the Box.

The high pulp lactic acid levels found in the bottom site is most likely due to the more anaerobic conditions which would encourage the micro-aerophilic lactic acid bacteria and tend to discourage aerobic organisms. It is less easy to find an explanation for the early peak in pulp lactic acid levels observed in the top and middle of the Box. It is possibly due to a response by these organisms to a substrate which at the time is full of nutrients and also a response to the slight rise in temperature that occurs then.

Trial 2 - General information

Harvesting: Approximately 3319 Kg wet beans were delivered to the factory from 2.30 pm on 22nd February, having been harvested and split on that day.

Pressing: Beans in steel boxes 110 cm x 91.5 cm x 76 cm were subjected to pressure with the use of lorry jacks until volume was reduced by ± 22%. Steel bars were inserted in the boxes to maintain the pressure overnight.

Fermentation: Beans were fermented in the usual manner, turned daily on 23rd, 24th, 25th, 27th and 28th February, 1st and 2nd March (fermentation was extended by 1 day to a full 8-day ferment).

Drying: Dried in the pre-drier on Friday, 3rd March, rested for 24 hours after pre-drying and dried to 6% moisture on Saturday, 4th March.

All numerical and graphical information referring to this section is in Tables 15, 16, 17, 18 and 19 and Figs 13, 14 and 15.

TABLE 15

Malaysia - Trial 2

Organisms per gram of beans x 10^6

	0 h		24 h			48 h			72 h			96 h			120 h			144 h			168 h			192 h			216 h		
	Ae	An	Ae	An	45	Ae	An	45	Ae	An	45	Ae	An	45	Ae	An	45	Ae	An	45	Ae	An	45	Ae	An	45	Ae	An	45
Top	0	0	2	122	50	1	0	0	13	1	0	4	5	0	1	0	3	15	-	18	99	0	4	1	1	15	25	1	13
Middle			>	388	>	0	6	0	0	9	0	-	0	2	-	-	0	1	-	0	83	50	111	155	1	0	(mixed sample)		
Bottom			>	190	3	22	81	71	>	13	0	-	-	0	3	-	0	0	0	>	350	5	>	1	1	5			

> = > 10^7

- = no result

TABLE 16

The frequency of micro-organisms in Trial 2 expressed as a percentage

		0 h		24 h			48 h			72 h			96 h			120 h			144 h			168 h			192 h			216 h		
		Ae	An	Ae	An	45	Ae	An	45	Ae	An	45	Ae	An	45	Ae	An	45	Ae	An	45	Ae	An	45	Ae	An	45	Ae	An	45
TOP	Yeasts	30	20	5	15	10	10	11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Other yeasts	40	50	5	5	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Acetics	10	10	45	20	-	45	58	50	99	-	-	60	-	-	90	100	-	50	-	-	100	-	20	49	-	20	80	60	-
	Lactics	20	20	35	60	5	45	31	-	1	-	-	-	70	10	-	-	-	20	40	40	-	10	20	-	30	10	19	10	19
	Bacillus	-	-	-	-	-	-	-	50	-	-	-	-	-	-	-	-	-	30	-	-	-	-	10	1	-	20	1	-	1
	Punctiforms	-	-	10	-	80	-	-	-	-	-	-	40	30	90	10	-	100	-	60	60	-	90	50	50	70	50	-	30	80
MIDDLE	Yeasts			21	15	-	10	30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
	Other yeasts			5	5	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
	Acetics			-	-	5	45	-	-	100	90	-	60	60	-	-	-	-	5	-	5	30	40	-	40	-	-			
	Lactics			21	80	5	45	70	50	-	10	-	-	-	-	-	-	-	5	-	5	5	20	60	-	90	-			
	Bacillus			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	90	-	90	5	-	10	20	-	30			
	Punctiforms			53	-	30	-	-	50	-	-	100	40	40	100	-	-	-	-	-	-	60	40	30	40	10	70			
BOTTOM	Yeasts			10	15	-	10	20	-	70	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
	Other yeasts			10	5	5	2	5	-	30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
	Acetics			-	-	5	39	-	-	-	95	-	60	-	40	100	100	40	40	10	-	70	50	30	40	-	20			
	Lactics			80	80	14	49	70	30	-	5	-	-	-	-	-	-	-	20	80	5	10	20	60	-	30	5			
	Bacillus			-	-	-	-	-	-	-	-	-	-	-	-	-	-	10	10	-	25	5	-	10	60	-	15			
	Punctiforms			-	-	76	-	5	70	-	-	100	40	-	60	-	-	50	30	10	70	15	30	-	-	70	50			

TABLE 17

Analyses of bean pulp and testa from Trial 2

Sampling			Pulp/testa per bean (g)	pH	Sugars %	Ethanol %	Acetic acid %	Lactic acid %
22/2	Prior to pressing		1.15	3.82	12.99	0.01	0.32	0.03
23/2	24 h	T	0.83	3.98	9.25	0.39	0.45	0.63
		M	0.72	3.95	8.25	0.31	0.73	0.65
		B	0.65	3.98	8.30	0.42	1.07	0.65
24/2	48 h	T	0.54	4.05	0.86	0.46	1.36	0.18
		M	0.62	4.08	1.42	0.70	0.43	0.30
		B	0.59	4.10	0.76	0.67	0.95	0.17
25/2	72 h	T	0.55	4.29	1.75	0.16	1.16	0.28
		M	0.59	4.42	1.74	0.29	1.38	0.39
		B	0.56	4.44	1.65	0.53	1.45	0.53
26/2	96 h	T	0.54	4.30	1.27	0.01	2.01	0.27
		M	0.48	4.30	1.50	0.03	1.35	0.29
		B	0.49	4.40	1.58	0.05	1.68	0.23
27/2	120 h	T	0.51	4.43	1.39	0.10	1.04	0.22
		M	0.53	4.45	1.65	0.03	1.81	0.29
		B	0.47	4.52	1.06	Nil	1.48	0.40
28/2	144 h	T	0.46	4.55	0.96	Nil	1.77	0.50
		M	0.47	4.46	1.52	Nil	2.28	0.51
		B	0.38	4.92	1.18	Nil	1.69	0.49
1/3	168 h	T	0.44	4.75	0.45	Nil	1.67	0.60
		M	0.44	4.56	0.75	Nil	2.26	0.55
		B	0.40	5.20	0.81	Nil	1.77	0.51
2/3	192 h	T	0.43	4.70	0.60	Nil	2.08	0.81
		M	0.43	4.80	0.69	Nil	2.48	0.69
		B	0.43	4.68	0.65	Nil	2.81	0.69
3/3	216 h	T	0.33	4.98	0.73	Nil	1.26	0.77
		M	0.32	4.92	0.50	Nil	1.42	0.72
		B	0.40	4.70	0.72	Nil	2.53	0.90

TABLE 18

Analyses of cotyledons from Trial 2

Sampling			Average bean weight (g)	pH	Sugars %	Ethanol %	Acetic acid %	Lactic acid %
22/2	Prior to pressing		1.36	6.44	2.07	0.02	Nil	0.01
23/2	24 h	T	1.44	6.38	2.29	0.21	0.29	0.03
		M	1.37	6.35	2.15	0.20	0.21	0.03
		B	1.35	6.25	1.94	0.25	0.37	0.03
24/2	48 h	T	1.34	5.02	2.33	0.56	1.06	0.10
		M	1.33	5.05	1.97	0.63	0.74	0.19
		B	1.50	5.15	1.66	0.79	0.64	0.12
25/2	72 h	T	1.38	4.55	1.50	0.36	1.72	0.15
		M	1.56	4.65	1.63	0.41	0.49	0.15
		B	1.50	4.82	1.74	0.46	1.20	0.19
26/2	96 h	T	1.54	4.50	1.14	0.12	0.63	0.12
		M	1.36	4.40	1.13	0.14	1.27	0.14
		B	1.38	4.45	1.20	0.10	1.37	0.21
27/2	120 h	T	1.26	4.48	1.03	0.12	1.38	0.10
		M	1.46	4.50	1.08	0.06	1.42	0.15
		B	1.28	4.59	1.20	Nil	1.21	0.20
28/2	144 h	T	1.38	4.68	1.32	Nil	1.22	0.17
		M	1.33	4.60	1.36	Nil	1.56	0.20
		B	1.29	4.72	1.24	Nil	0.93	0.23
1/3	168 h	T	1.51	4.82	1.15	Nil	1.03	0.17
		M	1.33	4.60	1.10	Nil	1.76	0.26
		B	1.56	4.82	1.25	Nil	1.28	0.24
2/3	192 h	T	1.39	4.70	1.24	Nil	0.74	0.33
		M	1.44	4.78	1.09	Nil	1.25	0.28
		B	1.44	4.65	0.98	Nil	0.67	0.30
3/3	216 h	T	1.45	4.85	0.99	Nil	0.58	0.31
		M	1.32	4.75	0.99	Nil	0.71	0.36
		B	1.49	4.62	0.97	Nil	1.31	0.39

Fig.13 Malaysia - Trial 2 : Top

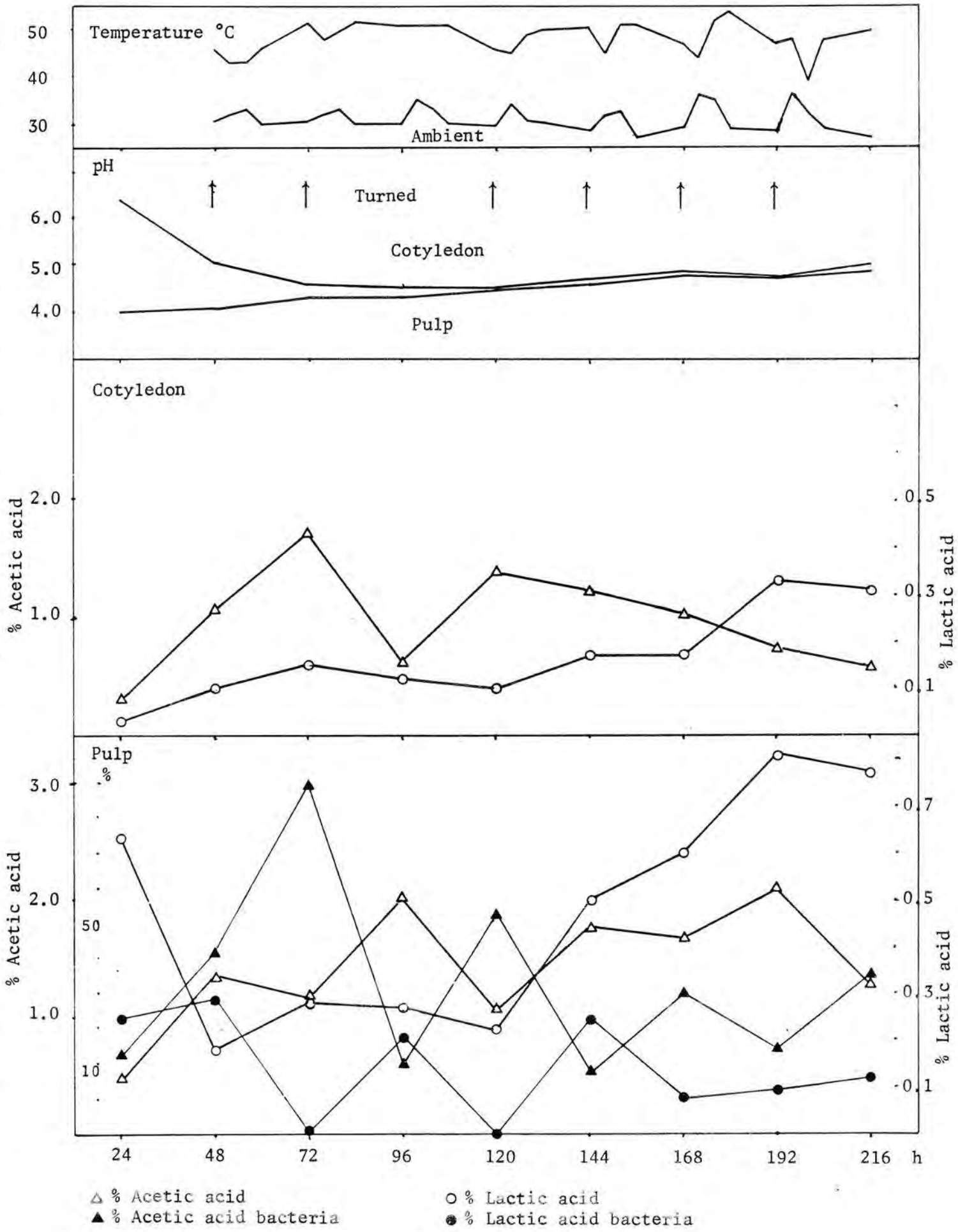
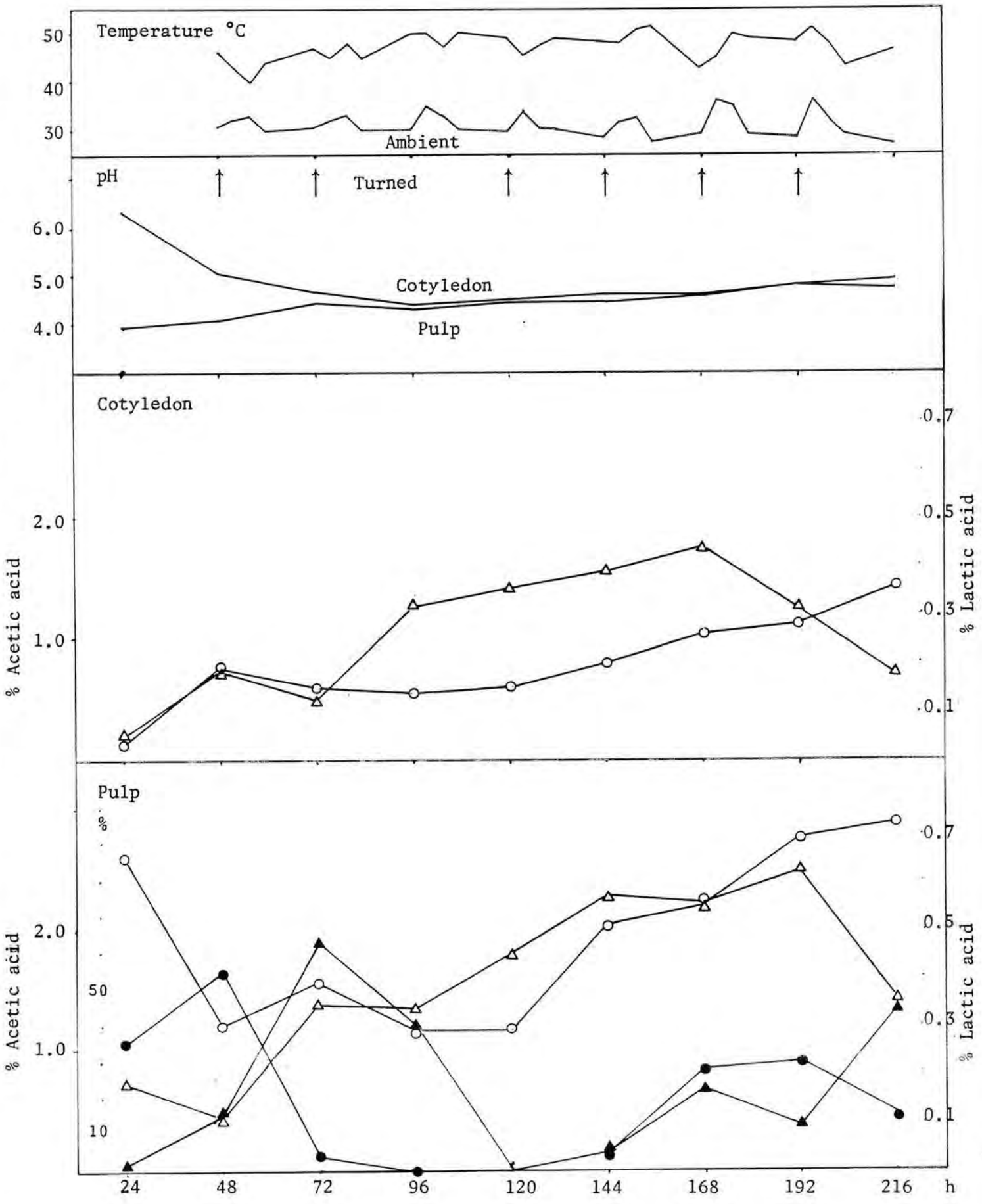
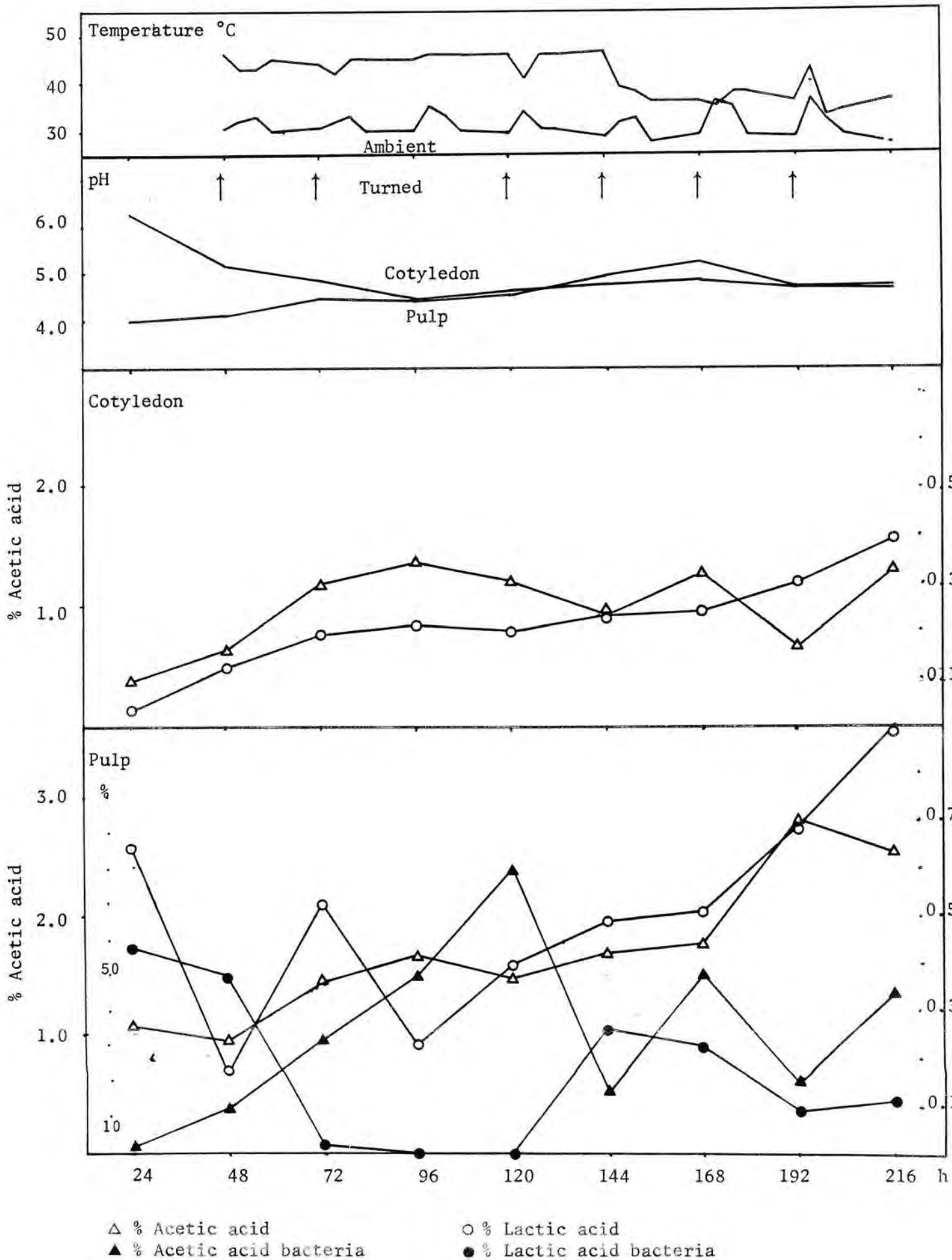


Fig.14 Malaysia - Trial 2 : Middle



△% Acetic acid ○% Lactic acid
▲% Acetic acid bacteria ●% Lactic acid bacteria

Fig.15 Malaysia - Trial 2 : Bottom



Trial 2 - Pressing

Top sample

There is rapid disappearance of sugars within 48 hours and a corresponding rise in alcohol. Ethanol levels in pulp and cotyledons reach a maximum at 48 hours, declining thereafter. After 120 hours ethanol could not be detected in either the pulp or cotyledon. Yeasts are only present in moderate numbers and have disappeared by 72 hours.

The profile of acetic acid production in the pulp is very variable showing a general tendency to increase throughout the fermentation until 192 hours. Cotyledon acetic acid increases from the beginning of fermentation and shows a steady and consistent decline after 96 hours. Acetic acid bacteria occur throughout the fermentation and are usually present in moderate to large numbers.

Pulp lactic acid which is initially at a high level falls during the first 24 hours of fermentation, stabilises and increases after 120 hours at a rapid rate for the remainder of the fermentation. The lactic acid profile of the cotyledon mirrors the behaviour of that of the pulp. The occurence of lactic acid bacteria, although not present in very large numbers, corresponds to the chemical observations. Virtually none were found at 72 hours and they were completely absent at 120 hours. *Bacillus* spp. occur in large numbers and earlier than in Trial 1 and many were detected on the 45°C plates. Irregular punctiforms were detected at all times except at 48, 72 hours, and were in particularly large numbers on the 45°C plates.

Middle sample

There is the usual rapid disappearance of pulp sugars during the first 24 hours with concomitant appearance of alcohol. The ethanol levels in the pulp and cotyledons reach a maximum at 48 hours, declining rapidly thereafter. After 120 hours ethanol could not be detected in the pulp or cotyledon. Yeasts were present in moderate numbers and survived only 48 hours. Pulp acetic acid increased from the onset of fermentation, only on the last stage there is a tendency for a reduction in levels. Build up of acetic acid in the cotyledons is very similar to that found in the pulp except that there is a tendency for a reduction in cotyledon levels

from 168 hours. Acetic acid bacteria occur at very high levels particularly at 72 hours. At 120 hours no acetic acid bacteria were detected and lesser numbers occurred for the remainder of the fermentation.

Pulp lactic acid levels again are at a high level at the onset of fermentation and the pattern of lactic acid production in both pulp and cotyledon are identical to that found in the top sample. Lactic acid bacteria were found to be present in moderate to large numbers except at 96 and 120 hours. *Bacillus* spp. were present in fairly large numbers especially towards the end of the fermentation and some grew well at 45°C. Irregular punctiforms were found at all stages excepting 120 and 144 hours. Particularly good growth of these organisms occurred on the 45°C plates.

Bottom sample

Rapid disappearance of pulp sugars and parallel production of ethanol again occurred. Maximum production of ethanol occurred at 48 hours and it could not be detected after 120 hours. Fermenting yeasts are at a low level except at 72 hours, after which they could not be detected.

The profile of acetic acid build up in the pulp is very similar to that found in the middle sample, although generally the actual level of acetic acid is lower in the bottom sample than in the middle sample. After an initial increase in cotyledon acetic acid level up to 96 hours there seems to be little change thereafter. Acetic acid bacteria were present throughout the fermentation, sometimes as a very high proportion of the total microflora.

Lactic acid levels and profiles are very similar to that found in the middle sample. Lactic acid bacteria also showed a similar profile to the middle site. These organisms were present in moderate to high levels except at 96 and 120 hours. *Bacillus* spp. present towards the end of the fermentation and some are to be found on the 45°C plates. Irregular punctiforms are found at all times mainly on the 45°C plates.

Summary

1. The main effect of pressing is to render the fermentation much more homogeneous as compared to Trial 1.

2. In Trial 2 maximum ethanol levels in the pulp and cotyledon all occur the same time, viz. 48 hours and is not detectable after 120 hours. This behaviour could be contrasted with Trial 1 where generally speaking ethanol could be detected in both pulp and cotyledons throughout the fermentation. Less ethanol is produced in Trial 2 than in Trial 1.

3. The acetic acid profile of pulp and cotyledon in Trial 2 is completely different from those found in Trial 1. Acetic acid levels in the pulp of Trial 2 samples is generally higher than that found in Trial 1 samples. It is noted that only in the top of Trial 1 samples is there a tendency for acetic acid reduction in the cotyledon during the later stages of fermentation, whereas this effect is apparent in the top of Trial 2 and the middle of Trial 2.

4. Cotyledon lactic acid levels in the top, middle and bottom samples of Trial 2 are all higher than comparable samples found in Trial 1. It should be noted, however, that Trial 2 was fermented for 48 hours longer than Trial 1. If one compares lactic acid cotyledon levels on the last day of Trial 1 with the appropriate day in Trial 2, cotyledon lactic acid levels are comparable in the top and middle samples; cotyledon lactic acid levels in the bottom of Trial 2 are higher than those found in Trial 1. This demonstrates that prolonging the fermentation increases the lactic acid content of the cotyledons.

This technique does not appear to have altered the microflora markedly. The yeasts are perhaps in slightly smaller numbers but remain for about the same time as usual. The lactic and acetic acid bacteria are present in their usual proportions. *Bacillus* spp. occur in greater numbers towards the end of the fermentation and the irregular punctiforms are to be found fairly consistently mostly on the 45°C plates. The highest viable counts were obtained in the bottom and this is the same as several of the other fermentations. Bean pressing was introduced as a procedure for reducing substrate and thereby limiting the total amount of acetic acid produced. A comparison of the top, middle and bottom pulp sugar levels and moisture contents of Trial 1 and the comparable samples for Trial 2 after pressing, show that little reduction in initial substrate levels has occurred (Table 19). Bean pressing ubdoubtedly has a significant effect on the final acetic acid content of the cotyledon and this can be rationalised by considering that compression of the bean causes the fermentation to be more anaerobic at least in the initial stages. This will reduce the amount of ethanol produced and delay the onset of acetification.

TABLE 19

A comparison of Trials 1 and 2

	T		M		B	
	Moisture %	Sugars %	Moisture %	Sugars %	Moisture %	Sugars %
Trial 1	80.5	10.1	80.5	10.45	79.9	8.79
Trial 2	78.4	9.25	77.1	8.25	77.0	8.30

Trial 3 - General information

Harvesting: Approximately 3820 Kg of wet beans were delivered to the factory by 2 pm on Monday, 27th February, having been harvested and split on that day and kept in well drained boxes.

Air-blasting: Six boxes of beans were sent by lorry to Kuala Bernam Estate on 28th February, leaving the estate at 9.10 am and arriving at Kuala Bernam at approximately 9.45 am. These beans were loaded into three bin-driers, 457 cm diameter and ± 819 cm deep. They were subjected to air-blasting for 3 hours, removed from the circular driers and returned to Flemington.

Fermentation: Beans were lifted into the fermentary on Tuesday and turned on Thursday, Saturday and Monday. They were loaded into the drier on Tuesday.

Drying: Beans were part dried on Tuesday, 7th March, with final drying to ± 6% moisture on 8th March.

All numerical and graphical information referring to this section is in Tables 20, 21, 22 and 23 and Figs 16, 17 and 18.

TABLE 20

Malaysia - Trial 3

Organisms per gram of beans x 10^6

	0 h			4 h			24 h			48 h			72 h			96 h			120 h			168 h			192 h		
	Ae	An	45	Ae	An	45	Ae	An	45	Ae	An	45	Ae	An	45	Ae	An	45	Ae	An	45	Ae	An	45	Ae	An	45
	Split pods			Lorry																							
Top	0	0	0	4	500	500	2	1	50	18	0	1	-	0	0	2	-	8	1	0	0	22	3	1	5	500	25
Middle							17	14	0	12	5	500	-	7	0	5	1	0	-	0	0	0	0	0	0	0	0
Bottom							5	41	0	9	7	500	35	190	500	5	5	0	6	1	0	8	5	0	50	33	0

- = no result

TABLE 21

The frequency of micro-organisms in Trial 3 expressed as a percentage

		Split pods		Lorry 19 h			26 h			48 h			72 h			96 h			120 h			168 h			192 h		
		Ae	An	Ae	An	45	Ae	An	45	Ae	An	45	Ae	An	45	Ae	An	45	Ae	An	45	Ae	An	45	Ae	An	45
TOP	Yeasts	30	20	30	20	-	50	50	15	20	30	-	10	30	-	-	-	-	-	-	-	-	-	-	-	-	-
	Other yeasts	-	-	10	-	10	10	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Acetics	-	-	30	-	-	20	10	-	80	-	-	90	-	30	60	-	-	100	20	-	99	99	98	100	80	80
	Lactics	60	70	30	70	30	20	30	15	-	-	80	-	70	10	10	100	10	-	-	-	-	-	-	-	20	15
	Bacillus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	1	-	-	-
	Punctiforms	10	10	-	10	60	-	-	70	-	70	20	-	-	60	30	-	90	-	80	100	-	-	1	1	-	5
MIDDLE	Yeasts						50	60	-	30	20	-	60	10	-	-	-	-	-	-	-	-	-	-	-	-	-
	Other yeasts						10	-	40	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Acetics						10	-	-	-	-	5	30	-	-	99	-	-	-	-	-	99	98	98	99	98	-
	Lactics						30	40	60	70	80	21	10	90	10	1	100	-	-	100	-	-	-	-	-	-	-
	Bacillus						-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	1	1	1	1	2	10
	Punctiforms						-	-	-	-	-	73	-	-	90	-	-	100	-	-	100	-	1	1	-	-	90
BOTTOM	Yeasts						40	70	-	30	50	-	15	10	5	-	-	-	-	-	-	-	-	-	-	-	-
	Other yeasts						-	-	90	-	-	-	-	-	5	-	-	-	-	-	-	-	-	-	-	-	-
	Acetics						20	-	-	-	-	-	5	-	-	95	80	20	60	-	-	90	60	60	99	95	35
	Lactics						40	30	10	70	50	20	80	90	20	5	20	-	-	20	-	-	10	10	-	5	-
	Bacillus						-	-	-	-	-	21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5
	Punctiforms						-	-	-	-	-	59	-	-	70	-	-	80	40	80	100	10	30	30	1	-	60

TABLE 22

Analyses of bean pulp and testa from Trial 3

Sampling			Pulp/testa per bean (g)	pH	Sugars %	Ethanol %	Acetic acid %	Lactic acid %
27/2	Fresh pod 0 h		1.11	4.00	13.51	Nil	0.18	0.02
28/2	19 h after pod breaking Lorry		0.96	4.00	11.31	0.10	1.15	0.36
28/2	26 h	T	0.91	3.92	9.86	0.24	0.41	0.43
		M	0.89	3.98	10.21	0.17	0.26	0.48
		B	0.75	3.98	0.46	0.13	0.53	0.39
1/3	48 h	T	0.66	3.80	1.11	0.15	1.69	0.27
		M	0.69	3.80	2.22	1.12	0.72	0.80
		B	0.70	3.78	4.87	0.88	0.68	0.68
2/3	72 h	T	0.63	4.20	2.28	0.09	2.71	0.21
		M	0.71	3.92	0.24	1.85	0.41	0.65
		B	0.70	3.70	1.08	1.35	1.30	0.93
3/3	96 h	T	0.59	4.20	1.19	0.12	1.62	0.42
		M	0.65	4.22	0.82	0.36	1.65	0.51
		B	0.57	4.28	0.97	0.52	2.10	0.49
4/3	120 h	T	0.56	4.20	1.32	0.08	3.07	0.24
		M	0.65	4.38	1.33	0.36	2.53	0.46
		B	0.55	4.30	1.06	0.06	3.98	0.38
5/3	144 h	T	0.62	4.20	1.11	0.10	3.00	0.39
		M	0.62	4.38	1.20	0.34	2.45	0.40
		B	0.58	4.35	1.04	0.06	3.00	0.45
6/3	168 h	T	0.64	4.35	1.20	0.09	3.30	0.29
		M	0.67	4.45	1.20	0.22	2.16	0.42
		B	0.62	4.42	1.08	0.09	2.63	0.48
7/3	192 h	T	0.59	4.52	0.76	Nil	1.91	0.74
		M	0.63	4.35	1.10	0.12	2.06	0.57
		B	0.61	4.55	1.11	Nil	2.15	0.48

TABLE 23

Analyses of cotyledons from Trial 3

Sampling			Average bean weight (g)	pH	Sugars %	Ethanol %	Acetic acid %	Lactic acid %
27/2	Fresh pod 0 h		1.32	6.32	2.80	Nil	Nil	0.01
28/2	19 h after pod breaking Lorry		1.26	6.25	2.38	0.07	Nil	0.004
28/2	26 h	T	1.35	6.30	2.19	0.10	0.17	0.01
		M	1.42	6.30	0.21	0.08	0.22	0.01
		B	1.26	6.38	0.21	0.08	0.16	0.01
1/3	48 h	T	1.32	4.98	2.01	0.29	0.66	0.04
		M	1.26	5.70	2.12	0.45	0.37	0.04
		B	1.21	6.20	1.86	0.48	0.16	0.04
2/3	72 h	T	1.41	4.40	1.73	0.19	1.09	0.08
		M	1.41	5.25	2.19	Nil	0.95	0.16
		B	1.25	5.50	2.02	0.56	0.75	0.13
3/3	96 h	T	1.35	4.45	1.43	0.13	2.10	0.20
		M	1.36	4.50	1.31	0.53	1.60	0.20
		B	1.29	4.65	1.33	0.53	0.85	0.17
4/3	120 h	T	1.26	4.40	1.50	0.21	2.64	0.14
		M	1.35	4.50	1.45	0.66	1.75	0.21
		B	1.23	4.50	1.16	0.11	2.04	0.17
5/3	144 h	T	1.28	4.30	1.47	0.17	2.39	0.20
		M	1.34	4.48	1.07	0.42	2.08	0.25
		B	1.19	4.50	1.23	0.11	1.57	0.23
6/3	168 h	T	1.35	4.42	1.06	0.15	1.99	0.20
		M	1.37	4.50	1.02	0.29	1.41	0.17
		B	1.34	4.45	1.09	0.08	1.72	0.26
7/3	192 h	T	1.36	4.45	0.94	0.02	1.09	0.25
		M	1.26	4.38	1.07	0.16	1.59	0.25
		B	1.36	4.50	0.88	Nil	1.37	0.22

Fig.16 Malaysia - Trial 3 : Top

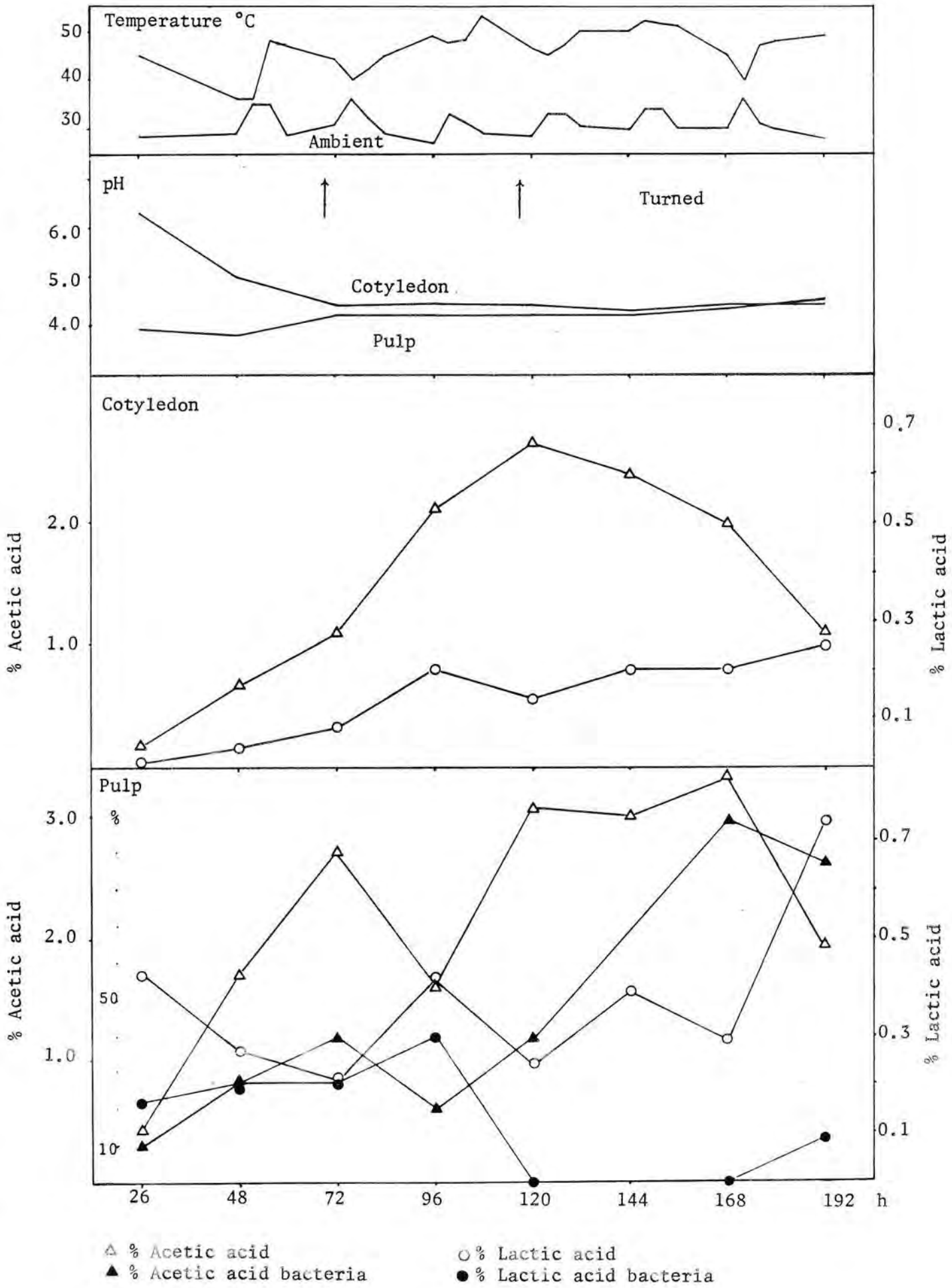


Fig.17 Malaysia - Trial 3 : Middle

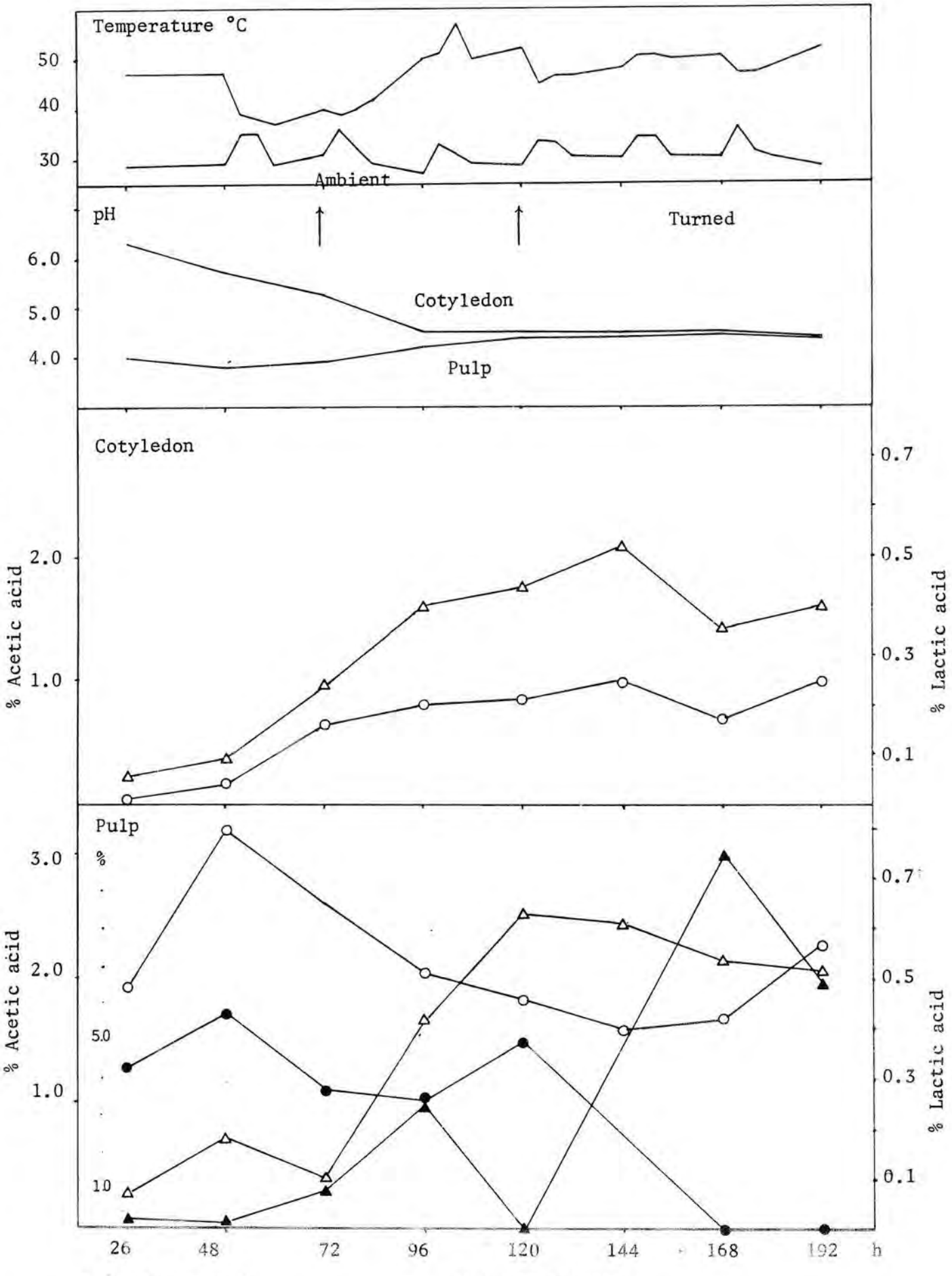
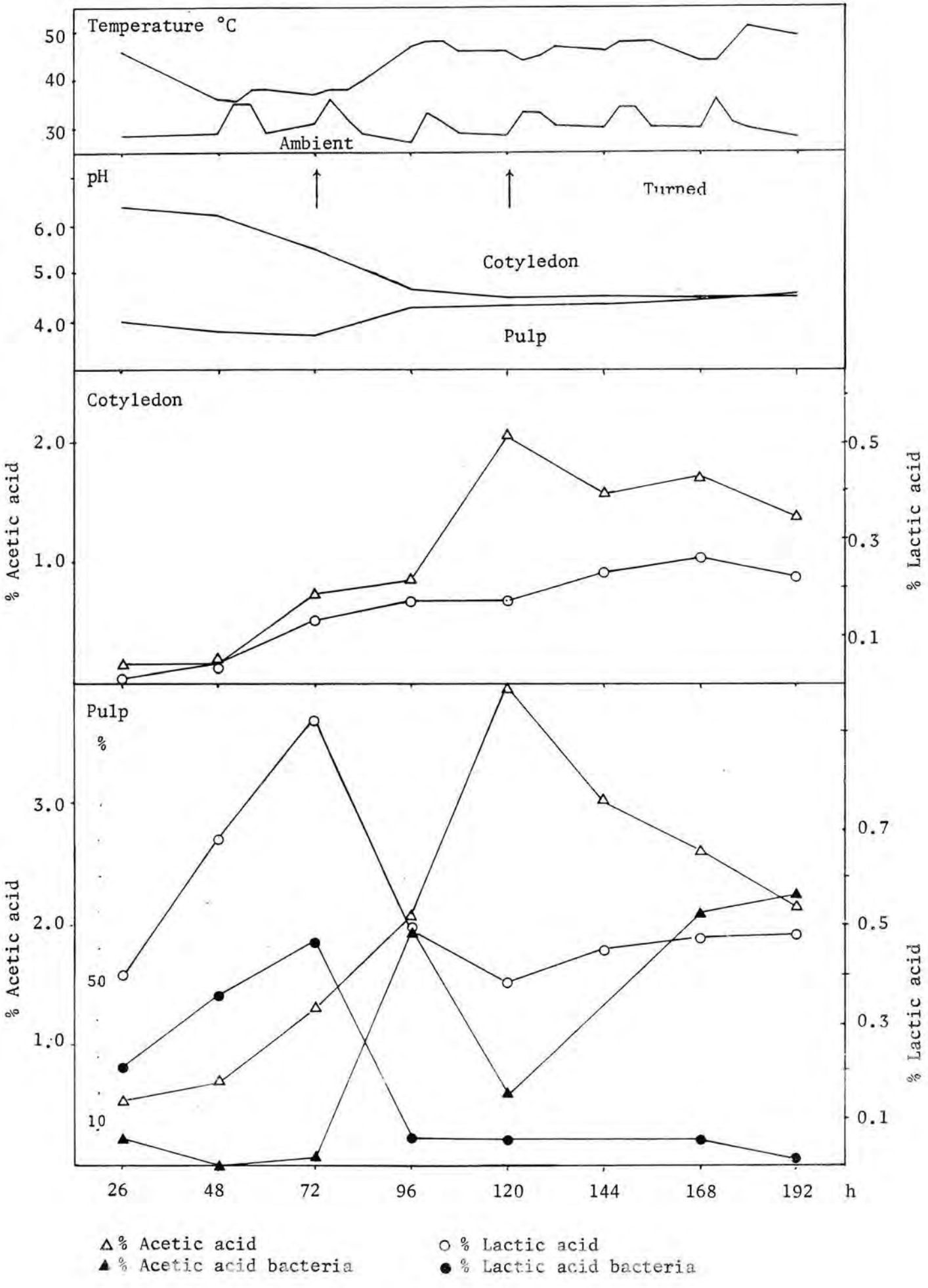


Fig.18 Malaysia - Trial 3 : Bottom



Trial 3 - Air blasting

Top sample

Rapid dissipation of pulp sugars occurs in a similar manner to that found in Trial 1. Pulp and cotyledon ethanol levels do not have a clearly defined maximum. Ethanol does, however, persist right through the fermentation, unlike the top sample in Trial 1. Fermenting yeasts persist for somewhat longer but at no time do they form a major part of the microflora. By 96 hours they have disappeared.

Pulp acetic acid increases rapidly from the onset of fermentation to 72 hours; thereafter there is a decline to 96 hours and a subsequent increase which is maintained up to 168 hours, followed by a decline up to the end of fermentation. Acetic acid bacteria gradually increase in number throughout the fermentation. Pulp lactic acid, which is at a high initial level, falls and eventually increases to a maximum at 192 hours. Lactic acid bacteria are present in fairly large numbers until 96 hours, disappearing between 120 and 168 hours. They reappear, however, at 168 hours in small numbers.

Bacillus spp. detected in very small numbers towards the end of fermentation. Punctiforms are present throughout and they are especially numerous on the 45°C plate.

Middle sample

There is a less rapid disappearance of pulp sugars than that found in the top sample. Pulp ethanol levels in the middle site of Trial 3 are much larger than those found in Trial 1; maximum ethanol production occurs at 72 hours. Yeasts are present in relatively high numbers in the initial stages of the fermentation and survive until 72 hours.

The behaviour of the pulp acetic acid in the middle site of Trial 3 is very similar to that found in Trial 1. However, there is more pulp acetic acid formed in the initial stages of Trial 1 than Trial 3, whilst there is more acetic acid formed in Trial 3 after 72 hours than that found in Trial 1. Acetic acid bacteria show a gradual build up towards the end of the fermentation, corresponding in the main to the increase of acetic acid. The pattern of acetic acid build up in the cotyledon samples of Trial 3 is very similar to that found in the middle site of Trial 1. Pulp lactic acid levels

are initially very high but after 24 hours show a steady decrease throughout the remainder of the fermentation. Cotyledon lactic acid levels mirror that of the pulp. The profile of the build up of lactic acid in the pulp in the middle site of Trial 3 is completely different from that found in the middle site of Trial 1. This does not, however, appear to affect the build up of lactic acid in the cotyledon of the middle site in Trial 3, the profile and levels of which are identical with that found in Trial 1. Lactic acid bacteria are present in high numbers up to 120 hours and thereafter are not detected. *Bacillus* spp. found only in very small numbers at the end of fermentation. Punctiforms are found at most times, especially on the 45°C plate.

Bottom sample

The disappearance of pulp sugars and the build up of ethanol are very similar to that found in the bottom site of Trial 1. Yeasts are present in diminishing numbers and are not detected after 96 hours. The pulp acetic acid profile and levels in Trial 3 are very different from those found in Trial 1. Much larger quantities of acetic acid are found in Trial 3. Cotyledon acetic acid levels and pattern are very similar to those found in Trial 1. Acetic acid bacteria are present in low numbers initially but increase to higher levels after the yeasts have disappeared. There they remain at a high level until the end of the fermentation.

The pulp lactic acid profiles of Trial 3 and Trial 1 bottom sites are similar to each other since they peak at 72 hours. Cotyledon lactic acid levels and profiles are very similar to Trial 1 and Trial 3 bottom sites. Lactic acid bacteria are present in moderate numbers initially but by 96 hours they begin to diminish. They did, however, remain at a low level until the end of fermentation. *Bacillus* spp. were found sporadically but only on the 45°C plates. Punctiforms are found consistently except for the first sample. These are found by incubation at 45°C.

Summary

1. Whilst there are major differences in the profile of the various substrates measured in Trials 1 and 3 the end result appears to be the same.

2. Only in the top sample of Trial 3 is there a clearly defined tendency for cotyledon acetic acid to decrease throughout the latter stages of fermentation; in this respect its behaviour is very similar to that of the Trial 1 top site.

There is much less pulp ethanol found in the top sample of Trial 3 as compared with Trial 1. There is more ethanol found in the pulp in the middle of Trial 3 than in the middle sample of Trial 1. Pulp ethanol level in the bottom samples of Trials 1 and 3 are comparable.

Trial 4 - General information

Harvesting: Beans were harvested, split and hoisted into the fermentary on Friday, 24th January.

Fermentation: Normal fermentation with daily turning (except Sunday) was carried out until Day 6, i.e. Thursday, 2nd March. On Day 6 ± 1134 Kg wet beans were transferred to a smaller fermentary. Beans were turned every 2½ hours during daylight on Days 6 and 7.

Drying: A large sample ± 16.6 Kg was dried in the rotary drier on Saturday, 4th, and Sunday, 5th March, to the usual ± 6% moisture.

All numerical and graphical information referring to this section is in Tables 24, 25, 26 and 27 and Fig. 19.

TABLE 24

Malaysia - Trial 4

Organisms per gram of beans x 10^6

	Day 6									Day 7								
	9.00			12.30			17.30			9.30			12.30			17.00		
	Ae	An	45	Ae	An	45	Ae	An	45	Ae	An	45	Ae	An	45	Ae	An	45
Mixed sample	38	6	8	120	-	2	63	1	5	5	5	63	1	5	500	-	-	5

- = no result

TABLE 25

The frequency of micro-organisms in Trial 4 expressed as a percentage

	Day 6									Day 7						
	9.00			12.30			17.00			9.30			12.00			17.00
	Ae	An	45	Ae	An	45	Ae	An	45	Ae	An	45	Ae	An	45	45
Powdery yeast	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
Acetics	70	-	15	60	-	-	99	-	-	25	-	-	60	-	-	-
Lactics	5	10	3	19	100	10	1	1	-	5	1	1	10	8	1	1
Bacillus	5	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-
Puncitforms	20	90	80	20	-	90	-	99	100	70	99	99	30	92	99	99

TABLE 26

Analyses of bean pulp and testa from Trial 4

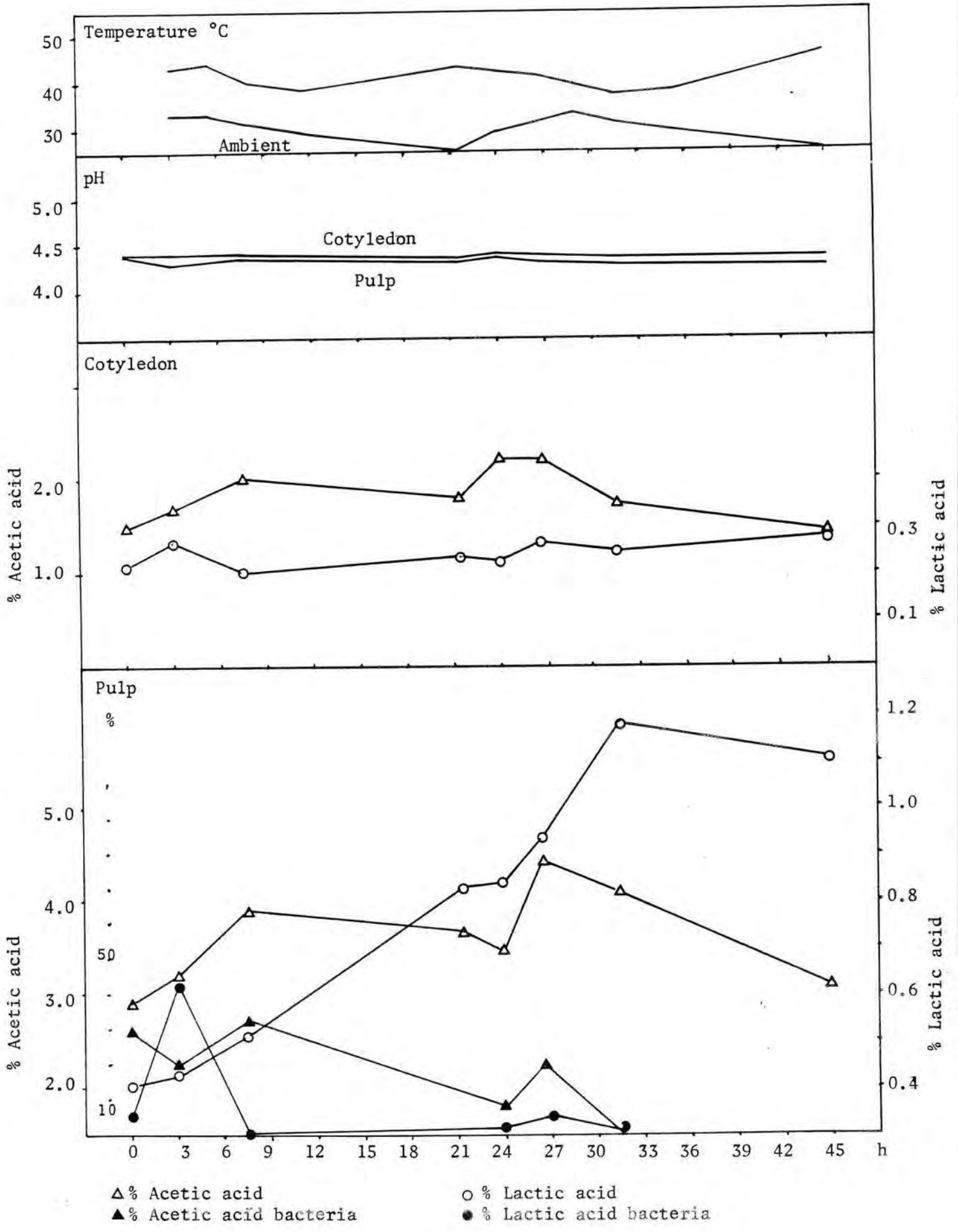
	Time	Pulp/testa per bean (g)	pH	Sugars %	Ethanol %	Acetic acid %	Lactic acid %
2/3 Day 6	9.00	0.68	4.38	1.07	0.05	2.90	0.40
	12.30	0.68	4.28	1.08	0.05	3.21	0.42
	17.30	0.67	4.35	1.08	Nil	3.89	0.51
3/3 Day 7	7.00	0.66	4.30	0.55	Nil	3.66	0.83
	9.30	0.62	4.35	0.66	Nil	3.45	0.84
	12.30	0.60	4.30	0.42	Nil	4.42	0.93
	17.00	0.62	4.28	0.40	Nil	4.10	1.18
4/3 Day 8	7.00	0.64	4.25	0.37	Nil	3.08	1.10

TABLE 27

Analyses of cotyledons from Trial 4

		Time	Average bean weight (g)	pH	Sugars %	Ethanol %	Acetic acid %	Lactic acid %
2/3	Day 6	9.00	1.57	4.40	0.97	0.20	1.48	0.21
		12.30	1.54	4.40	0.94	0.12	1.67	0.27
		17.30	1.43	4.40	1.10	0.11	2.01	0.20
3/3	Day 7	7.00	1.45	4.35	1.05	0.03	1.78	0.23
		9.30	1.49	4.40	0.93	Nil	2.20	0.22
		12.30	1.49	4.38	1.09	Nil	2.20	0.26
		17.00	1.50	4.35	1.07	Nil	1.73	0.24
4/3	Day 8	7.00	1.41	4.35	1.19	Nil	1.43	0.28

Fig.19 Malaysia - Trial 4



Trial 4 - Maturation

The principal effect of multiple turning is to promote the production of lactic acid in the pulp. It is interesting to note that the cotyledon lactic acid level is totally unaffected by the level in the pulp. The increase in the pulp lactic acid level coincides with a decrease in pulp sugar level from which it is undoubtedly derived. No pulp ethanol could be detected after the initial turning. There is a slight increase in the acetic acid levels of both pulp and cotyledon which is not maintained during the latter stages of turning; there is a tendency for the pulp and cotyledon acetic acid to fall. Turning has the effect of equally distributing acetic acid between pulp and cotyledon.

Since the lactic acid bacteria occur in small numbers it is difficult to explain the high levels of lactic acid observed in these samples, unless it is assumed that another major group of bacteria can produce this acid. Such a group is the irregular punctiforms which from their colony appearance and apparent thermophilic mode of growth, appear to resemble *Lactobacillus acidophilus*, as described in *Bergey's Manual of Determinative Bacteriology. 8th Edition* (1974). In this particular fermentation the increase of growth of the irregular punctiforms closely mirrors the considerable increase in lactic acid.

Drying in Ghana and Malaysia

Chemical changes

A study of pH, acetic and lactic acids during sun drying of Heap 1 and Box fermentation in Ghana revealed that:

1. No pH changes occurred in 264 hours of drying.
2. No loss of acetic and lactic acids occurred from the cotyledon.
3. Acetic acid only was lost from the shell.

A similar study of the artificial drying of cocoa from Trial 1 in Malaysia achieved the same findings.

Microbiological changes

In Ghana beans from Heap 1 and the Box were sun dried and in spite of exposure to ultraviolet rays micro-organisms survived. Organisms at 24 hours were respectively 99.8×10^6 on the aerobic plates and 169×10^6 on the anaerobic plates. These gradually diminished until at 240 hours the numbers were, aerobic 1.8×10^6 and anaerobic 5.5×10^6. A similar picture was observed in beans from the Box which were at 24 hours aerobic 4.25×10^6, anaerobic 17×10^6, whereas at 288 hours they were aerobic 0 and anaerobic 1.5×10^6. Heap 2 beans were dried by hot air so the organisms survived for only a short time. At the beginning of drying there was a whole range of micro-organisms including yeasts, acetic acid, lactic acid, bacteria and bacilli. Only the bacilli survived until the end, as might be expected, since they produce spores resistant to heat and desiccation. It was considered that these organisms were superficial and not altering the beans significantly and this is borne out by the lack of chemical changes during this period.

In Malaysia all beans were dried by hot air and in spite of the elevated temperatures, organisms survived until drying was complete. As in Africa the ultimate survivors were representatives of the genus *Bacillus*.

TABLE 28

Variation of cotyledon acetic and lactic acid on a dry weight basis during sun drying of Heap 1

	Drying time (h)											
	24	48	72	96	120	144	168	192	216	240	264	288
% Acetic acid	0.85	0.89	0.82	0.89	0.74	0.85	0.86	1.06	0.95	1.03	0.98	1.06
% Lactic acid	0.36	0.22	0.23	0.30	0.33	0.41	0.26	0.30	0.24	0.29	0.28	0.35

DISCUSSION

The comparative microbiology of Africa and Malaysia

Table 29 shows that the type of organisms isolated from fermenting cocoa beans in both countries was remarkably similar, there being one or two species different in each category. Whilst there was individual variation between one experiment and another in each of the countries the overall numbers of viable organisms between Africa and Malaysia were about the same.

One noticeable difference between the fermentations of Africa and Malaysia was the occurrence of *Bacillus* spp. These usually formed a larger proportion of the microflora in Africa and sometimes occurred earlier. This is well illustrated by Heap 2 in Africa (Table 4).

The fact that African fermentations were only turned twice made the microbiological pattern fairly predictable. In Malaysia the daily turning of the beans mixed the microflora more so that the plates often contained small numbers of micro-organisms that could not readily be placed within the major categories.

As mentioned earlier, the identity of the irregular punctiforms remains speculative. They occurred in the fermentations in both countries. They are probably thermophilic and when isolated on plates incubated at 45°C usually formed the major part of the microflora. Any future work should aim to isolate and identify these bacteria and to ascertain their biochemical role in cocoa fermentation.

One group of micro-organisms not considered in this survey were the moulds. They certainly do occur as they can be seen growing on the Heaps and in the corners of the Boxes. Any future microbiological work should also include a study of their role in cocoa fermentation.

The comparative chemistry of Africa and Malaysia

It must be stressed that since there was insufficient time to replicate samples in Ghana and Malaysia there is no statistical validity for assuming that any differences found are real. The variation in fermentation practices between the two countries makes it extremely difficult to ascribe any differences to a particular cause. The failure in Ghana to monitor the chemistry of Heap 1 was particularly unfortunate since a comparison of results from each stage of Heaps 1 and 2 would

TABLE 29

Organisms found in the two countries

AFRICA	MALAYSIA
Yeasts	
Hansenula	*Hansenula*
Kloeckera	*Kloeckera*
Torulopsis	*Torulopsis*
Saccharomyces	*Saccharomyces*
Candida	*Candida*
Pichia	*Hanseniospora*
Schizosaccharomyces	*Rhodotorula*
	Debaryomyces
Acetic acid bacteria	
Acetobacter rancens	*Acetobacter rancens*
A. xylinum	*A. xylinum*
Gluconobacter oxydans	*Gluconobacter oxydans*
A. ascendens	
Lactic acid bacteria	
Lactobacillus collinoides	*Lactobacillus collinoides*
L. plantarum	*L. plantarum*
L. fermentum	
L. mali	
***Bacillus* spp.**	
Bacillus cereus	*Bacillus cereus*
B. subtilis	*B. subtilis*
B. licheniformis	*B. licheniformis*
B. coagulans	

have provided valuable information on inter-fermentation differences. Differences between a Heap and Box fermentation in Ghana have already been noted. Since fermentation in Heaps in Ghana and Boxes in Malaysia are the standard practices, it is only proposed to compare Trial 1 with Heap 2.

Top samples from Heap 2 and Trial 1

Initial pulp sugar levels are comparable. Pulp sugar levels are more rapidly reduced in Trial 1 than in Heap 2. After 48 hours in both fermentations there is little change in pulp sugar but it is noted that the level of pulp sugars in Heap 2 tends to be higher than those found in Trial 1.

Peak ethanol production in Trial 1 occurs at 24 hours and in Heap 2 at 48 hours, larger quantities of ethanol being found in Trial 1 than in Heap 2.

The pulp lactic acid profile in both fermentations is somewhat different. The Heap lactic acid profile is smoother and less subject to the variations that occur in Heap 2. In both fermentations there is a rapid growth in the production of lactic acid in the first 24 hour period. In Trial 1 the pulp and lactic acid level decreases after 24 hours to 48 hours and thereafter increases to 120 hours, whereas in Heap 2 there is a continuous increase in pulp lactic acid to 120 hours. After 120 hours in both fermentations the pulp lactic acid level declines. There is a considerably larger quantity of lactic acid produced in the pulp of Trial 1 than in Heap 2.

Differences occur in the pattern of acetic acid production in the pulp in both fermentations. The fermentations are characterised by a rapid increase in pulp acetic acid levels from the onset of fermentation. After 48 hours in both fermentations there is a tendency for the reduction of acetic acid levels in the pulp. More pulp acetic acid is generated at every stage in Trial 1 as compared with Heap 2.

Cotyledon acetic acid levels in both fermentations tend to follow the behaviour of the pulp levels. More acetic acid is to be found in the Trial 1 samples than Heap 2 samples. After 72 hours in Trial 1 there is a general tendency for a reduction in the cotyledon acetic acid levels; in contrast in Heap 2 after 72 hours there is little change in cotyledon acetic acid levels.

Middle samples from Heap 2 and Trial 1

In the initial stages of the fermentation of Heap 2, pulp sugar reduction proceeds at a faster rate than that found in Trial 1; subsequent pulp sugar levels are generally higher in Heap 2 than in Trial 1.

Pulp and cotyledon ethanol levels are comparable in both fermentations. Production and dissimilation of ethanol is concentrated over a 72 hour period in Heap 2 and a 96 hour period in Trial 1.

Pulp acetic acid levels are consistently higher in Trial 1 than Heap 2. The initial rate of increase of acetic acid in the pulp of Trial 1 is higher, much higher than that found in Heap 2. The amount of pulp acetic acid in the pulp of the Trial 1 and Heap 2 samples at 120 hours is comparable; after this time in Trial 1 pulp acetic acid tends to increase whilst in Heap 2 there is a tendency to reduce.

In both fermentations the cotyledon acetic acid level tends to follow that of the pulp acetic acid. The profile of the cotyledon acetic acid in Trial 1 and Heap 2 are virtually identical.

Pulp lactic acid profile in both fermentations are completely different. Heap 2 is characterised by a rapid build up of lactic acid from the onset of fermentation to 48 hours with a reduction thereafter. In Trial 1 pulp lactic acid tends to increase throughout the fermentation.

Bottom samples from Heap 2 and Trial 1

Pulp sugar reduction in Trial 1 is a much more rapid process than that found in Heap 2. Again pulp sugar levels can be higher in the Heap 2 bottom sample than in the Trial 1 sample during the latter stages of fermentation.

Pulp ethanol levels are higher in Trial 1 than in Heap 2. Maximum ethanol production in the pulp in Trial 1 occurs at 48 hours. There is no clearly defined maximum in the Heap 2 fermentation sample.

Initial levels of acetic acid in Trial 1 are higher than those found in Heap 2, but subsequent profiles and levels are virtually identical.

The build up of acetic acid in the cotyledon of Trial 1 and Heap 2 is also virtually identical.

Both Trial 1 and Heap 2 are distinguished by very rapid increases in the pulp lactic acid levels during the initial stages of fermentation. After 96 hours in Heap 2 there is a rapid reduction of pulp lactic acid levels; in Trial 1 pulp lactic acid levels tend to stabilise at a higher level in the latter stages of fermentation.

In Heap 2 cotyledon lactic acid tends to increase after 24 hours in a similar manner to that found in Trial 1. If one discounts the high cotyledon lactic acid level at 120 hours in Trial 1 the correspondence in cotyledon lactic acid level between the two fermentations is remarkable. In both fermentations cotyledon lactic acid levels increase with time.

Sensory evaluation

Cured cocoa from each of the trials was submitted to Cadbury Ltd, UK, for quality evaluation and the results are given below:

Fermentation	Acidity score	Difference from control
Trial 1	2.2	-2.4
Trial 2	1.4	-2.0
Trial 3	1.8	-2.8
Trial 4	2.6	-3.0
Heap 1	0.7	-1.9
Heap 2	1.3	-1.9
Box	1.1	-1.6
Ghana control	0.3	-
BP10	0.4	-2.4

It can be seen that all fermentations produced cocoa more acid than the Ghana control, which was also the preferred sample in every instance. The cocoa samples from each trial were analysed at Tropical Products Institute for volatile and non-volatile acids, the results of which are given in Table 30. An analysis of BP10 and the results obtained at the Unitata laboratories in Malaysia have been included for comparison.

It is evident that reducing the acetic acid level of the cured bean improves the acidity score and that a level of 0.4% should be the final target. It is tempting to speculate from these results that lactic acid has little influence on acidity but the sensory results relate only to the small-scale chocolate preparation. In a full-scale production run,

TABLE 30

Acids of cured cocoa

Fermentation	pH	Acetic acid %	Lactic acid %	Citric acid %	Oxalic acid %	Succinic acid %	Malic acid %
Heap 1	4.65	0.82	0.20	0.39	0.23	0.034	0.021
Heap 2	4.60	1.15	0.18	0.47	0.24	0.03	0.026
Box	4.75	0.56	0.28	0.51	0.30	0.038	0.029
Trial 1	4.60	1.08	0.67	0.67	0.26	0.074	0.036
Unitata	-	2.77	0.52	-	-	-	-
Trial 2	4.85	0.69	0.58	0.83	0.43	0.03	0.048
Unitata	-	1.07	0.68	-	-	-	-
Trial 3	4.5	0.89	0.54	0.61	0.29	0.046	0.035
Unitata	-	1.40	0.60	-	-	-	-
Trial 4	4.36	2.18	0.71	0.54	0.32	0.044	0.034
Unitata	-	2.62	0.58	-	-	-	-
BP10	5.30	0.38	0.26	0.75	0.37	0.035	0.042
Ghana control	5.74	0.39	0.05	0.74	0.33	0.03	0.032
Torkington normal	-	0.89	0.35	-	-	-	-
Torkington BP trial	-	0.29	0.25	-	-	-	-

a conching process is included which will dispel free acetic acid leaving lactic acid and combined acetic acid residues, i.e. residual acidity.

It is hoped to carry out an experiment in the future to relate the sensory evaluation of small and full-scale chocolate preparations. Statistical analysis of a series of 22 cocoas of commerce are currently being carried out and indications are that both acetic and lactic acids contribute equally to acidity; pH is a function of acetic acid concentration, and agreement between pH and acidity is not a strong one. The only advice we can offer at this stage is that efforts should be made to reduce lactic acid levels to the values found in normal West African cocoa, that is, 0.1%.

Sampling of cured cocoa

The TPI analytical results for volatile and non-volatile acids were based on 100 g samples as received. There is a marked discrepancy with the analyses performed at Unitata. The data has been checked and no source of error detected. In view of the lack of homogeneity found throughout fermentations in Ghana and Malaysia it is vital that due regard is paid to obtaining a representative sample from the dryer by coning and quartering. Sampling in Malaysia by hand from a sack has proven to be inadequate. The analytical methods used are capable of a precision of better than ± 10% for acetic and lactic acids.

Fermentation of Amazon hybrid cultivars

Chemical and microbiological studies in Ghana and Malaysia and the sensory data of the final product can only lead to the proposition that the Amazon hybrid material is itself responsible for the acidity effect. While the African cocoas were less acidic than the Malaysian ones it must be recalled that the quantity of cocoa fermented in Ghana was only about one tenth of that fermented in Malaysia and there were other differences in practice which might have influenced the results.

It was unfortunate that whilst in Africa we were unable to compare the fermentation of Amazon and Amelonado cocoa. Dr Adomako of the Cocoa Research Institute of Ghana was requested to carry out comparative fermentations of Amazon and Amelonado Cocoa. Samples were taken from the middle sites of the fermentations and sent to Tropical Products Institute for analysis; the results are given in Table 31 and Figs 20 and 21.

TABLE 31

Results of Amelonado and Amazon fermentations - July 1978

			0 h	10 h	24 h	48 h	72 h	96 h	120 h	144 h
AMELONADO	Sugars %	A*	1.98	1.89	1.68	1.05	1.44	1.18	1.40	1.03
		B	6.25	9.43	3.33	1.45	0.53	2.91	1.59	0.70
	Lactic acid %	A	0.02	0.015	0.012	0.027	0.025	0.027	0.047	0.079
		B	0.006	0.023	0.038	0.035	0.150	0.047	0.237	0.309
	Acetic acid %	A	0.045	0.084	0.28	1.497	1.35	0.85	0.45	0.43
		B	0.114	0.810	1.35	0.23	1.99	0.98	0.63	0.80
	Ethanol %	A	0.05	0.06	0.36	0.79	0.28	0.015	0.017	0.013
		B	Trace	0.84	0.29	0.43	0.46	0.015	0.011	0.011
AMAZON	Sugar %	A	1.54	2.34	1.66	1.57	1.02	0.77	0.84	0.68
		B	10.65	15.22	5.38	1.00	0.54	1.75	1.28	0.88
	Lactic acid %	A	0.018	0.113	0.018	0.038	0.071	0.043	0.06	0.054
		B	0.019	0.116	0.22	0.118	0.165	0.188	0.10	0.20
	Acetic acid %	A	Trace	Trace	Trace	1.729	1.68	1.59	1.22	0.99
		B	0.194	0.37	0.51	2.66	1.39	2.33	1.68	1.49
	Ethanol %	A	0.005	0.085	0.462	0.68	0.18	0.25	0.026	0.015
		B	0.014	0.343	1.50	0.50	0.22	0.13	0.029	0.028
	Citric acid %	B	0.942	0.689	-	0.063	0.076	0.271	0.184	0.135
	Malic acid %	B	0.126	0.078	0.054	0.043	0.03	0.056	0.06	-

* A = cotyledon B = pulp

Fig.20 Ghana - Amelonado fermentation : July 1978

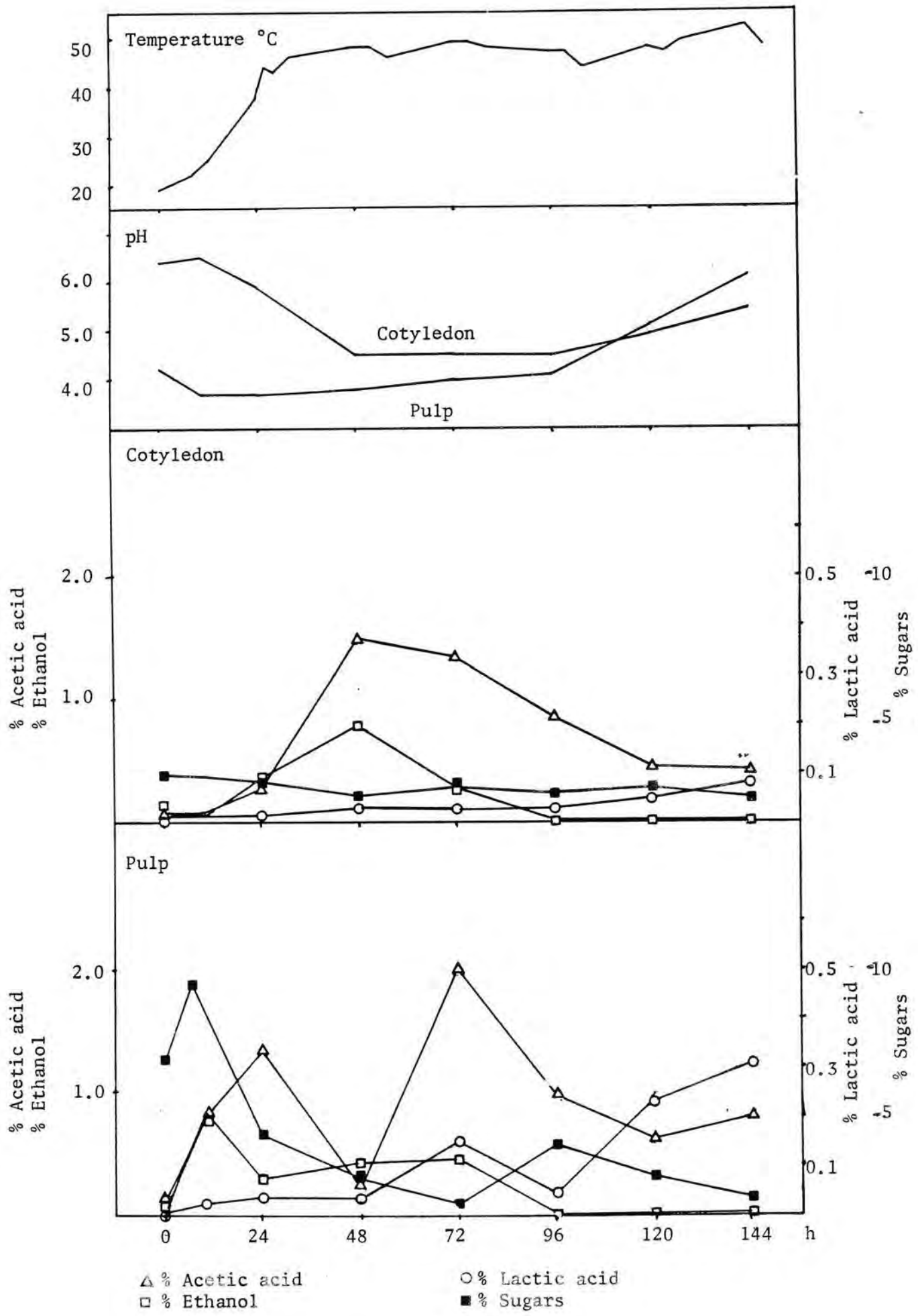
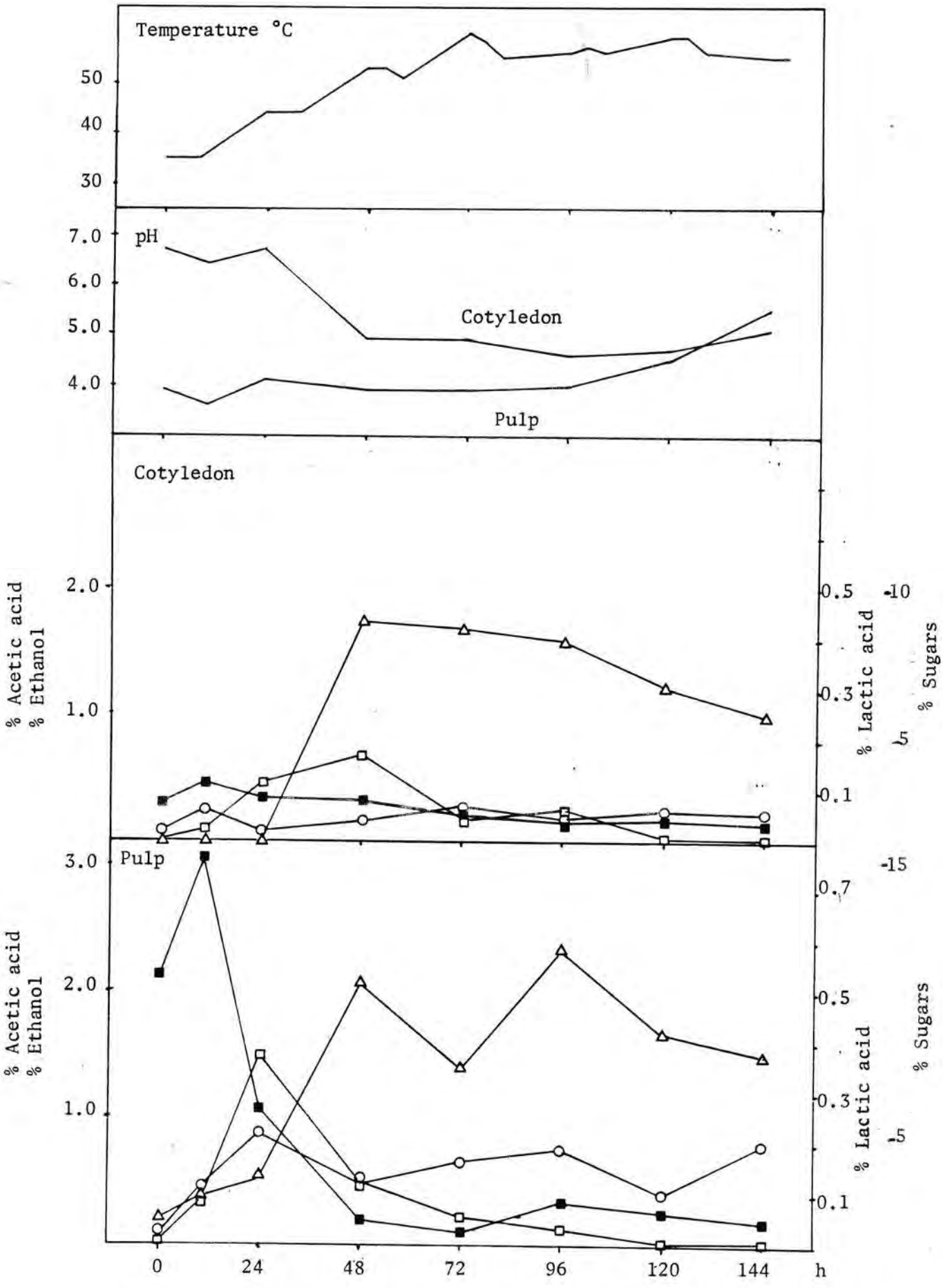


Fig. 21 - Ghana - Amazon fermentation : July 1978



It can be seen that an Amazon fermentation leads to a more acidic bean at the end of fermentation. It should also be noted that little lactic acid is generated in either fermentation. This experiment tends to confirm our Ghanaian and Malaysian conclusions that Amazon cultivars are liable to produce more acidic fermentations. In addition there appears to be a relationship between the quantity of lactic acid generated and the quantity of cocoa fermented, as may be seen below:

Fermentation	Quantity (Kg)	Cotyledon lactic acid
Trial 1	4,080	0.67
Heap 1	555	0.20
Heap 2	547	0.18
Box	562	0.28
Adomako Amazon	51	0.60
Adomako Amelonado	52	0.04

Cause of acidity

Accepting that Amazon cultivars produce acid cocoa and that microbiological differences between cultivars are minimal (yet to be proven), it is to the pulp that we must turn our attention for the probable cause. No comparative information on the composition of the pulp from different cultivars is available. The quantity of fresh pulp/testa per bean in grammes for both cultivars is given below:

Amazon Malaysia	Amazon Ghana	Amelonado Ghana
1.12	1.16	0.75

The greater quantity of pulp of the Amazon cultivar can have two effects:

1. The greater the quantity of pulp the more difficult will be the entry of air into the fermentation. The importance of aeration has been frequently stressed: 2. Larger quantities of pulp/bean means in principle more sugars, citric acid, other essential microbiological nutrients and water. Which effect is dominant can readily be identified by carrying out small-scale fermentation in a cool box (Quesnel, V.C. and Lopez, A. 1975. *Trop. Agric. (Trinidad)*, 52, No.4, 309) of under-ripe cocoa of low initial pulp sugar content and comparing acetic and lactic acid levels on the last day of fermentation with that of a control. We are assuming that the pulp/bean ratio will be the same in under-ripe cocoa and control.

The observed effect that the quantities of acetic acid and lactic acid produced in Ghana is a function of the quantity of cocoa fermented (i.e. a function of surface area/volume ratio) supports premise No.1.

The recent experiments carried out in Malaysia at Torkington Estate on a normal fermentation control and one subjected to pressing and air blasting indicates that perhaps pre-fermentation treatment can influence acetic and lactic acid. The normal fermentation produced beans superior to any of our Malaysian trials (Table 22), and very similar to the Ghana Box fermentation. These comparisons were based on final acetic and lactic acid levels. It is noted that Torkington beans have a lower pulp/bean ratio. Any differences between Torkington and Flemington cultivars and fermentation practice should be identified.

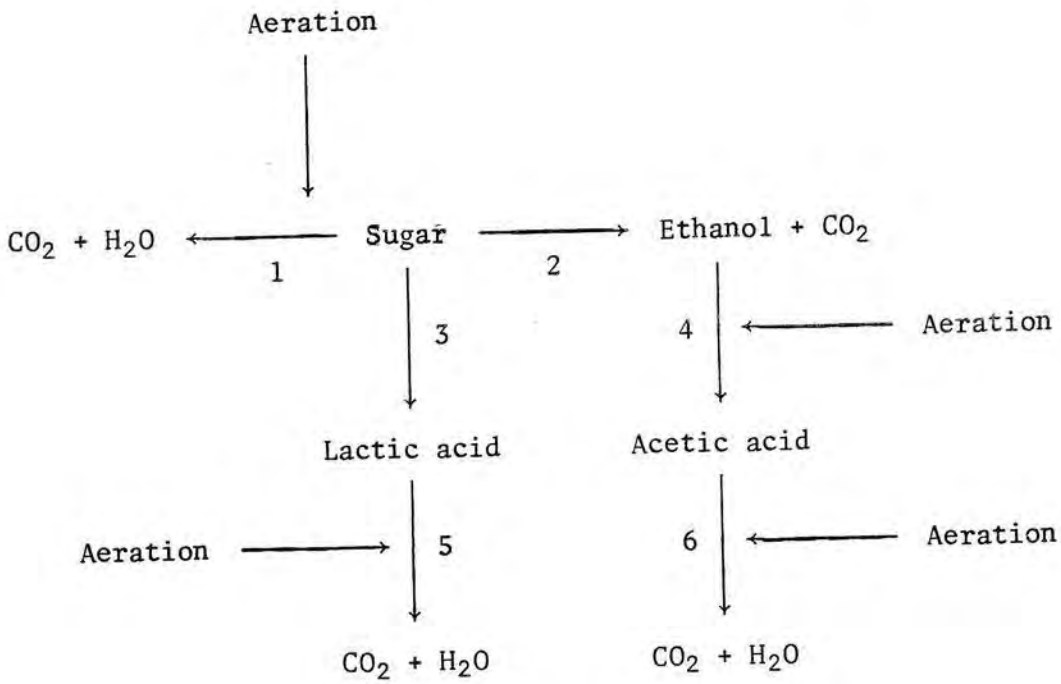
The BP technique as practised at Torkington Estate leads to a reduction in acetic and lactic acid levels. The reduction of acetic acid level is caused by two effects: 1. Pressing renders cocoa fermentation more homogeneous with maximum ethanol production occurring simultaneously throughout the Box, rendering any subsequent aeration more efficient (see Trial 1). 2. Air blasting entrains ethanol reducing the quantity available for acetic acid production. The decrease in lactic acid is by virtue of a shorter fermentation time since it has already been noted that cotyledon lactic acid levels increase with fermentation time.

It is interesting to speculate that since the Torkington normal practice produces a less acidic bean than Flemington Estate, air blasting may not be necessary.

Aeration

Fig.22 shows the importance of aeration on the metabolism of a single available substrate, mainly glucose. Intense aeration would cause the yeasts to proliferate and to respire the sugar to CO_2 and H_2O. In conditions of more limited air, pathway 2 will be followed and the substrate converted to ethanol and CO_2. Less aeration tends to encourage the lactic acid bacteria to grow and convert the substrate to lactic acid. Aeration subsequent to the formation of lactic acid may encourage the acetic acid bacteria to oxidize it to CO_2 and H_2O. Aeration subsequent to the formation of ethanol produces acetic acid in considerable quantities. Further aeration, probably after ethanol is exhausted, will induce the acetic acid bacteria to oxidize acetic acid to CO_2 and H_2O.

Fig.22 Effect of aeration on various phases of cocoa fermentation



Although yeast can operate anaerobically a certain amount of air is required for the cells to reproduce. Intense anaerobic conditions will severely limit or even stop yeast growth. This accounts for the depression of the quantity of ethanol produced in Trial 2.

Aeration, as seen in Fig.22, will have different effects according to when applied. If applied towards the end of fermentation when ethanol is depleted and lactic and acetic acids are the only remaining products from sugar, the tendency is for the acetic acid bacteria to convert them into CO_2 and H_2O, which will have the effect of reducing acidity. It is a noticeable feature of most top fermentations that the acetic acid shows a drop towards the end. This tendency is not so marked for lactic acid however.

RECOMMENDATIONS

Ghana

1. A study of a number of fermentations of Amelonado and Amazon cultivars to confirm the acid tendency of the latter. The studies should include measurement of the degree of aeration and be continued through the sun drying stage. 2. Irregular punctiforms should be isolated, identified, and biochemical characteristics studied.

Malaysia

Any future study of fermentation should include analysis of aeration conditions in the fermentation. A good introduction to gas analysis is contained in a textbook of Quantitative Inorganic Analysis by Arthur, I. 3rd Edition, Longmann's (1962). A range of portable oxygen analysers is commercially available.

1. The standard Flemington pressed fermentation should be aerated by:
(a) reducing bean depth and turning daily, or turning after 48 and 72 hours;
(b) multiple turning; (c) forced ventilation not air blasting.

These variations should take place 48 hours after bean pressing. Acetic and lactic acid should be monitored in these experiments at least twice - on the last day of fermentation and before the variation is introduced.

2. The bean pressing technique should be fully characterised with regard to applied pressure and pressure within the Box. Various pressing times should be investigated to ascertain their effect on acidity. If samples were drawn from top, middle and bottom sites for analysis, sampling integrity should be maintained during drying. Samples from 3 sites should be taken on the last day of fermentation, sun dried and the results compared with the bulked artificially dried cocoa.

3. Pathway No.1 (Fig.22) should be forced by making the fermentation as aerobic as possible at the beginning, thus having the effect of reducing the amount of ethanol produced and consequently reducing the amount of acetic acid. By diverting some of the sugar to CO_2 and H_2O the amount available for the production of lactic acid is simultaneously diminished. One way of achieving better aeration is to harvest the pods and then allow

them to remain for about 3 days. This has the effect of reducing the moisture content of the beans and thus rendering them less sticky and, therefore, improving their aeration characteristics. This can be accentuated by spreading the beans in thin layers for several hours. The amount of pulp around the beans and sugar content should be monitored throughout this period.

4. The Torkington trials should be repeated including a pressing only trial.

5. Small-scale trials on the effect of the addition of pectinolytic enzymes on pulp/bean ratio and pulp sugars should be evaluated.

6. Different yeasts should be evaluated for their ability to reduce pulp/bean ratios.

7. A complete analysis of Amazon and Amelonado pulp should be carried out.

8. Some work on improving the loss of acetic acid from the cotyledon during drying should be investigated.

9. Collaborative analysis between Unitata and Tropical Products Institute should be undertaken.

10. Study of the rate of diffusion of acids into the cotyledons.