MICROFLORA ASSOCIATED IN THE PROCESSED COCOA BEANS

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ABSTRACT

The quantitative and qualitative enumeration of microflora associated with the processed cocoa beans were investigated. Among the fungal flora (moulds) the species of *Aspergillus* and *Mucor* were predominant. More attention was given on moulds, since it is considered to tell upon the quality of cocoa beans, as it affects the flavour of the manufactured cocoa products. Further the infection of moulds on the butter fat content of the beans were studied. This suggests that, the packing and moisture content of the beans were important in the storage of the cocoa beans.

INTRODUCTION

Cocoa is being grown as one of the important plantation crops in our country. It is mainly grown as a mixed crop in coconut and arecanut gardens. The increase in area under cocoa cultivation has increased the production of cocoa in our country. Since the demand for the internal consumption of cocoa products is less, the surplus produce has to be exported. Some countries have adopted the Iternational Cocoa Standard which comprise the "Model ordience and code of practice" (Wood, 1979). Cocoa of marketable quality is required to be free from smoky beans. Further it is required to be reasonably free from living insects, broken beans and pieces of shell. In addition, cocoa is graded on the basis of limits to its content of particular defects viz. mouldy beans, slaty beans, germinated beans and flat beans. Mould has been described as worst defect of cocoa beans, as it affects the flavour. It is possible to detect the mould off-flavour in the samples with as little as 4 percent mouldy beans. The other effects of moulds are (i) It increases the free fatty acid content of the cocoa butter (Kavanagh *et al*, 1970; Guenot *et al*, 1977) (ii) The growth of some moulds may produce mycotoxins (Feuell, 1966). Moulds can develop in the beans during the process of fermentation or drying or during storage. Several types of moulds

are reported in the processed cocoa beans by many workers (Broadbent, 1967; Broadbent and Oyeniran, 1967; and Oyeniran, 1972). Apart from mould, yeast, bacteria and actionomycetes were also reported in the beans (Lopez and Quesnel, 1973). In the present investigation, the authors report the presence of ectophytic and endophytic microorganisms associated with the processed cocoa beans.

Materials and Methods

Sample collection—The processed cocoa bean samples were collected from two places in Karnataka, namely (i) C.P.C.R.I. Vittal with improved method of processing. (ii) CAMPCO processing unit, Puttur. The two samples of processed beans collected from Kerala, represents Trichur Distirct, (C.P.C.R.I. Peechi), and Alleppey District. The samples were collected in Polythene bags and stored before use.

pH and moisture content of the beans

Twenty g. of cocoa beans were taken and ground to powder using mortar and pestle and 50 ml. of hot water was added to it. The pH was then determined.

Ten g. of cocoa bean samples crushed roughly with the help of mortar and pestle were dried in an oven regulated at 103 $\pm$ 2°C for 16 hrs and percentage moisture was calculated. (Anon, 1967).

Smoke contamination—100 g of whole cocoa bean was soaked in 33 ml of ethyl alcohol (96 percent) for 5 minutes with constant agitation. Then the ethanol was poured out, filtered and the resulting yellow coloured compound was assessed both visually and spectrophotometrically. (Alvim, 1975).

Ectophytic mycoflora

The fungi associated on the surface of processed cocoa beans were studied by blotter technique. For each sample 50 beans were placed on moist blotting paper kept in sterile petridishes. The plates were incubated at room temperature (32$\pm$2°C). The fungal growths on the bean surface were examined after 5 days.

The purified cultures were maintained on Potato Dextrose Agar (PDA) slants for identification. The percentage occurrence of mould was calculated.

Endophytic micoflora

The studies on the presence of endophytic fungi were carried out by removing the testa of the beans after surface sterilization in 0.1% $HgCl_2$ solution for 3 minutes and washed 4 times in sterile water. Then each bean was cut longitudinally by using sterile scalpel into 2 halves and plated on Potato Dextrose Agar (PDA). The petridishes were incubated at room temperature (32 $\pm$2°C). The observations on fungal growth were taken after 5 days. The purified cultures were maintained for identification.

Quantitative microflora

Beans surface washing method

Ten g. of cocoa beans were transferred to 250 ml. flask containing 100 ml. of sterile water and shaken for 10 minutes. The serial dilution plating method was followed for the enumeration of microflora. Nutrient Agar (NA) and NA + 15% been extract agar media were used for the bacterial flora estimation. The fungal propagules were estimated using Potato Dextrose Agar and PDA + 15% bean extract agar media. The plates were incubated under laboratory condition to count bacteria after 48 hrs. and fungi after 72 hrs. The frequently occurring bacteria and fungi were isolated for identification.

The surface washed beans were repeatedly passed through several changes of sterile water. Then the beans were placed on the petridish containing PDA medium to find out the mycelial form of fungi present on the surface of the beans.

Bean maceration method

Ten g. of cocoa beans were macerated and dilution plating method was followed to count the microorganisms inhabiting on the surface and inside the beans. The method followed was same as explained for the surface washing.

Qualitative microflora

The bacterial and fungal colonies were purified and transferred to slants for identification. The percentage occurrence of fungi were calculated. The fungal colonies were tentatively identified upto generic level. The bacterial colonies were subjected to morphological, physiological and biochemical tests to group them to genus level.

The effect of mould infection on the butter fat content of beans

Three species of *Aspergillus* and a species of *Mucor* were inoculated separately into the cocoa beans by pin prick method. The beans were placed in petridish with moist blotting paper for 5 days. 3 g of powdered beans were used for the estimation of butter fat using solvent petroleum ether (40–60°C) in a soxhlet extraction apparatus for 10 hrs. The solvent was evaporated in an oven at 105°C and dried to constant weight. The increase in weight of the flask was the butter fat content of the beans. The cocoa bean with out mould infection was used as check.

Results and Discussion

The data pertaining to the general analysis of the processed cocoa bean samples for pH, moisture content and smoke contamination are presented in Table 1. The pH of the cocoa bean samples varied between 4.9 and 5.7 and a pH increase was noted in the Vittal sample. The moisture content of the cocoa beans ranged from 5.4 percent to 6.8 percent. No smoke contamination was detected in the samples since the drying of beans was done in an electric oven/sun drying.

The moisture content of the beans and atmospheric humidity are important in the storage of the beans. The moisture content of the beans should be between 6 and 7 percent. When the moisture percent exceeded 8, the mould growth occurs on the beans (Wilbaux, 1965). The relative humidity during storage should not exceed 80 percent. Checking the humidity is essential not only at the port of embarkment but also when the beans are brought from the farmer or during storage.

Table 1. Description of four samples, determination of pH, moisture and Smoke contamination of Processed cocoa beans

Sl. No.	*Sample collected*	*Fermentation method*	*Drying method*	*pH*	*Moisture*	*Smoke contamination*	
						Visual	*Spectrophotometer*
1	CPCRI Vittal	Improved Box method with 2nd & 4th day turning "Maturation." in the 5th & 6th day of fermentation.	Electric Oven	5.2—5.7	5.8	+	0.05
2	CAMPCO Puttur	Box fermentation with 2nd & 4th day turning	Electric Oven	4.9—5.2	5.4	+	0.06
3	CPCRI Peechi	Heap method of fermentation, turning 2nd and 4th day	Electric Oven	4.9—5.1	6.0	+	0.09
4	Alleppey	Heap fermentation, turning 2nd and 4th day	Sun drying	5.0	6.8	+	0.07

The analysis of the beans for the ectophytic mycoflora. The maximum mould growth was observed on the beans collected from Alleppey district (100 percent) and the least being in the samples collected from Puttur (6.67 percent). The mould growth in Peechi samples was 83 percent and that of Vittal was 24.39 percent. The mould growth observed on the surface of the beans are 5 species of *Aspergillus,* 2 species of *Mucor,* one species of *Rhizopus* and a few non sporulating fungi. The frequency of mould occurrence in four samples were calculated (Table 2). The *Aspergillus* sp. was observed in more number, followed by *Mucor* sp.

The occurrence of endophytic mycoflora is presented in Table 3. The bean samples collected from Puttur did not show the presence of fungi inside the beans whereas the other three samples indicated the presence of mould in the beans. The mould growth was observed more in the sample collected from Alleppy (36 percent).

In the Vittal sample the internal fungal growth observed was up to the extent of 12 percent. The mould growth inside the beans are tentatively identified as two species of *Aspergillus* and a species of *Mucor.* The percentage occurrence of mould indicated the presence of *Aspergillus* sp in more numbers followed by *Mucor* sp. Similar observation was reported by the earlier workers (Broadbent, 1968 and Oyeniran, 1972). The maximum mouldy beans allowed as per the cut test followed in the international market, is 3 percent for Grade I and 4 percent for Grade II. Twenty eight mould species have been isolated from the commercial cocoa in Nigeria (Broadbent, 1968 and Oyeniran, 1972). The growth of mould in cocoa beans produced mycotoxin (Feuell, 1966).

The dilution plating method for the quantitative enumeration of fungal and bacterial flora are presented in Table 4. The use of cocoa bean extract in the medium has no influence on the counts of fungi and bacteria. The surface washing and bean maceration method has decreased the total bacterial counts considerably in all the samples. Among the four samples used, the cocoa beans collected from Puttur showed least count both for fungi and bacteria.

Table 2. The Ectophytic mycoflora in the Processed Cocoa beans

Mould (Percent)	*Vittal*	*Puttur*	*Peechi*	*Alleppey*
Mould	24.39	6.67	83.00	100.00
Aspergillus	10.50	4.50	52.70	80.00
Mucor	5.00	2.17	17.00	15.00
Rhizopus	2.00	—	—	—
Others				
(No sporulation)	6.84	—	13.30	5.00

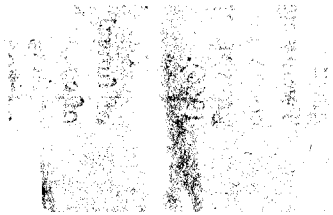


Table 3. The Endophytic mycoflora in the Processed cocoa beans

	Vittal	*Puttur*	*Peechi*	*Alleppey*
Mould Growth (Percent)	12.00	0.00	17.85	36.00
Aspergillus Sp. (Black)	4.00	0.00	3.57	12.00
Aspergillus Sp. (Brown)	4.00	0.00	7.14	18.00
Mucor Sp.	4.00	0.00	7.14	—
Others	—	—	—	—
Cut test	1.33	0.00	4.66	5.33

Table 4. The Quantitative Microflora in the Processed cocoa beans

Sl. No.	*Samples*	*Fungi* (10^3)		*Bacteria* (10^5)	
		Surface washing	*Maceration*	*Surface washing*	*Maceration*
1	Vittal	49.66	62.00	29.13	16.00
2	Puttur	33.33	15.33	22.33	12.60
3	Peechi	47.33	55.33	24.20	14.33
4	Alleppey	53.66	58.00	25.86	18.63

The qualitative fungal flora and their percentage occurrence indicated the presence of 5 types of *Aspergillus* and 2 types of *Mucor* which are predominantly found in the cocoa beans(Table 5). Few non-sporulating fungi were also observed on Potato dextrose agar plates. Three isolates of *Aspergillus* were tentatively identified as A. *niger, A. flavus* and *A. fumigatus.* The *Mucor* was identified as *Mucor pusillus.* The *Aspergillus* sp was frequently occurring in the processed cocoa beans followed by *Mucor* sp. The other species of fungi observed were *Fusarium, Penicillium* and *Rhizopus.* The yeast and actinomycetes colonies were seen on the plates. The bacteria present in the beans are the three species of *Bacillus* and 2 unidentified.

The effect of mould infection on the butter fat content of processed cocoa beans is shown in Table 6. The butter fat content in check without mould infection was 56 percent. The infection of *Aspergillus* increased the butter fat content of the beans, whereas the infection by *Mucor* sp decreased the butter fat content of the beans. The free fatty acid content by mouldy beans exceeded that of sound beans as reported by Kavanagh *et al* (1970).

Cocoa is generally harvested during wet periods when atmospheric humidity is continuously high. The moisture content of the beans must not exceed 7.5 percent for the purpose of trade outside the producing country (Wood, 1979). All cocoa beans are hygroscopic but some are more hygroscopic than others (Wood, 1965). The testa (shell) and cotyledons (nib) differ in hygrosopicity and consequently the moisture content. The damage to testa (shell) will favour the development of mould inside the cocoa beans. If the moisture content is below 7.5 percent and the testa of the beans is intact, the presence of mould on the surface of the beans will not be able to affect the quality.

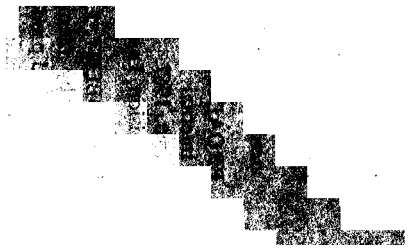
The *Mucor pusillus* growth was seen in three samples collected from Vittal, Peechi, and Alleppey. The mould growth is possible in the black pod (*Phytophthora palmivora*) infected cocoa pods, as the saprophytes develop after the invasion of the pathogen. The *Mucor* growth is more often observed in the black pod infected pods during the fermentation. This observation is in conformity with Rohan (1963). Beans from black pod disease (*Phytophthora*

Table 5. Qualitative fungal flora in the Processed cocoa beans

Sl. No.		*Vittal*		*Puttur*		*Peechi*		*Alleppey*	
		Surface washing	*Macera-tion*	*Surface washing*	*Macera-tion*	*Surface washing*	*Macera-tion*	*Surface washing*	*Macera-tion*
1.	Aspergillus (Brown)	19.3	18.0	11.66	5.00	13.33	15.3	14.66	18.3
2.	Aspergillus (Blue)	10.3	15.0	10.00	3.3	11.66	8.3	10.3	12.3
3.	Aspergillus (Black)	2.3	4.5	6.0	2.0	8.33	10.3	9.3	5.3
4.	Mucor	7.3	2.0	—	—	4.0	8.3	6.0	8.0
5.	Others (Un identified)	10.34	22.50	5.67	5.33	7.34	13.00	13.67	15.10

Table 6. The effect of mould infection in the butter content of the beans

Sl. No.	*Sample*	*Butter Fat content (Percent)*
1	Control	56.00
2	Aspergillus (Brown)	62.26
3	Aspergillus (Blue)	58.85
4	Aspergillus (Black)	62.26
5	Mucor Sp.	50.35



palmivora and charcoal pod rot (*Botryodiplodia theobromae*) affected cocoa pods will be black in colour even after fermentation.

The adoption of suitable fermentation and drying process can reduce the microbial load in the processed cocoa beans. The dried beans when rubbed with fingers should produce a *crakling* noice. The beans have to be stored in polythene bags or gunny bags lined with ploythene cover. Methyl bromide or phosphine fumigation against stored pests during storage is recommended for the control of insect infestation. The development of mould either during drying or in storage has to be prevented in the maintenance of quality of the cocoa beans.

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