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SHORT COMMUNICATION

# Microbiological and Biochemical changes during the Cacao Beans Fermentation.

Cacao bean pulp is an ideal medium for the proliferation of yeasts and certain groups of bacteria. Fermentation of cacao beans is an important step in the processing of cacao for the development of flavour and aroma precursors. During the course of fermentation, these microorganisms alter the fermenting medium. Although much work has been done on the fermentation and drying of cacao beans, the microorganisms associated with the fermentation have been examined only by a few workers (1-4). The physical and chemical composition of Indian cacao beans during fermentation has been studied by Malini *et al* (5). The present report deals with microbiological and biochemical changes that occur during the fermentation of cacao beans.

Ripe cacao pods were collected from the areca—cacao mixed cropping experimental plot of the Regional Station of Central Plantation Crops Research Institute, Vittal, India. The pods are broken open using a billet and the beans along with pulp were collected for fermentation, discarding the pod husk and placenta. Bean samples (200 g) were collected and after removing the adhering pulp were analysed for pH, total soluble solids (TSS) and titratable acidity (6). The TSS of the pulp extract was determined using Abbe refractometer. The titratable acidity was determined by titrating the aqueous extract (10g in 90 ml water) against 0.1N NaOH with phenolpthalein as indieator.

The Box (60cm × 60cm × 45cm size) method of fermentation, using 125 kg of wet beans in five lots was used. Five boxes were kept, raised 10cm from the ground level to allow the draining of sweat liquor and to provide aeration. The fermentation was carried out for six days. The temperature during the fermentation was recorded at a depth of 10cm using a thermometer daily. Since the beans were mixed on the 2nd and 4th day of fermentation, the temperature was recorded prior to the mixing.

Bean samples were collected from five boxes separately (200 g each) at 24 h intervals to monitor the pH of the pulp and for the enumeration of microflora. The pulp adhering to the beans was removed using a blade and to 10 g of pulp 90 ml boiling water was added. After cooling, the pH was recorded using a digital pH meter (6).

Total yeasts and bacteria were enumerated using the dilution and plate count method. Ten g beans along with the pulp was transferred to 250 ml flask containing 90 ml of sterile water and the flasks were shaken on a rotary shaker for 10 min. The supernatant was then diluted and plated. The media used for the enumeration of yeast and bacteria were yeast agar (7) and nutrient agar, respectively. Representative colonies of yeast and bacteria were purified and maintained for identification. The yeast isolates were identified using the proce-

dure of Barnett *et al.* (7) and the bacteria using Bergey's manual of determinative bacteriology (8). Total phenols (9), orthodehydroxy phenol (10) and anthocyanin pigment (11) content of the cotyledons were determined at 24 h intervals during the fermentation. Catechol was used as the standard for the determination of total phenols and orthodehydroxy phenol. For anthocyanin determination, 1 g sample was ground in 25 ml methanol containing 1% HCl (v/v), filtered and the OD at 530 nm of the extract was determined in a spectrophotometer.

Pulp analysis revealed that the pH and TSS prior to fermentation was 4.0 and 13.5, respectively. The titretable acidity was 5.4. The changes in temperature and pH of the pulp during the fermentation are given in Fig. 1. A sharp increase in temperature was seen on the 1st and 2nd day of fermentation. The maximum temperature of 48°C was recorded on the 5th day of fermentation. The pH of the pulp increased steadily during the fermentation and was 5.3 on the 6th day of fermentation.

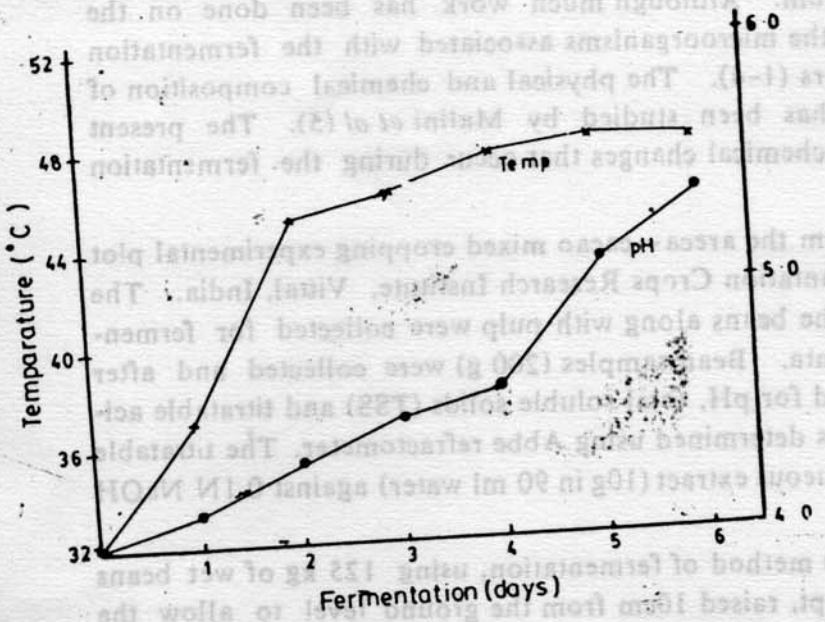


Fig. 1. Change in temperature and pulp pH during the fermentation of cacao beans.

The number of yeast and bacteria during the fermentation is given in Table 1. The yeasts dominated upto the 2nd day of fermentation and thereafter their number decreased. The bacterial number also showed a similar trend. Microscopic examination of pulp showed the presence of rod shaped bacteria in chains after 3 days of fermentation.

The yeast isolates belong to the genera of *Torulopsis* sp. *Saccharomyces* sp., *Rodotorula* sp. and *Schizosaccharomyces* sp. The bacteria were *Bacillus cereus*, *Bacillus subtilis*, *Bacillus licheniformis* and *Bacillus* sp. Forsyth and Rombouts (1) have reported the typical microbial sequence of yeasts, lactic acid bacteria and acetic acid bacteria during the fermentation in Trinidad

The total phenols and orthodehydroxy phenol in the cotyledons during the fermentation did not reveal any variation However. breakdown of anthocyanin pigment occurred during the fermentation (Table 1). Cacao beans are normally fermented without adding any external

inoculum. Generally, the yeast and certain groups of bacteria play an important role in this fermentation. Roelofsen (12) reported that many strains of yeasts, in addition to fermenting sugars, also had the ability to degrade the pulp. The breakdown of anthocyanin pigment during the fermentation is important for the development of chocolate aroma and flavour precursors and to know the extent of fermentation of beans (12).

Table 1. Microbial count and anthocyanin pigment during the fermentation of cacao beans.

| Days of fermentation | Yeast ( $\times 10^5$) | Bacteria | Anthocyanin content (OD 530 nm) |
|---|---|---|---|
| 0 | — | — | 1.45 |
| 1 | 29.2 | 22.3 | 1.55 |
| 2 | 32.0 | 30.6 | 1.50 |
| 3 | 13.2 | 11.6 | 1.05 |
| 4 | 12.4 | 18.5 | 0.75 |
| 5 | 15.8 | 10.6 | 0.60 |
| 6 | 10.6 | 1.3 | 0.55 |

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