THE CRITICAL NATURE OF ANAEROBIC PHASE IN COCOA MICRO-FERMENTATION METHODS TO REPRODUCE OPTIMUM COCOA FLAVOUR POTENTIAL SIMILAR TO LARGE SCALE FERMENTATION

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ABSTRACT

Successful cocoa fermentation is defined by development of the optimum flavour potentials of cocoa. Cocoa flavour precursors are developed during fermentation. The initial anaerobic phase of the microfermentation process is demonstrated to be essential for proper development of cocoa flavour attributes.

Key words: *flavour characteristic, fermentation, anaerobic phase*

INTRODUCTION (FERMENTATION PROCESSES)

In a large scale cocoa fermentation process, a sequence of enzymatic and microbial activities and biochemical reactions are involved to produce desirable qualities of cocoa flavour. During fermentation, successive growths of microorganisms are observed beginning with fungal species, followed by yeast and bacteria species (Acetobacter, Lactobacilli and Bacillus species). Cocoa fermentation is a crucial biochemical process required in development of flavour precursors. Fermentation is regarded as being essential for initiating the reactions that lead to the formation of substances that confer characteristic of chocolate flavour (Lehrian and Patterson, 1983).

The flavour precursors (amino acids, peptides and reducing sugar) developed during fermentation undergoes Mallaird or non-enzymatic browning reaction during roasting to produce typical cocoa flavour compounds. There are 462 compounds found from several sources of cocoa and there is no single compound that produces an aroma that can be described as "cocoa" or "chocolate". It is more a balance of pyrazines, aldehydes, alcohols, volatile acids and esters, which make up the overall flavour (Macdonlad *et al.*, 1994).

The initial anaerobic phase in cocoa fermentation is crucial for flavour development. During the initial anaerobic phase in the first 24 hours of fermentation, pulp sugars are converted by yeast to ethanol, which is later converted into acetic acid by bacteria species. The conversion of ethanol to acetic acid is an exothermic reaction which increases the temperature of the fermenting mass. A combination of acetic acid levels and heat generated from the exothermic reactions during fermentation kill the cocoa beans causing a disruption of cell membranes; thus releasing enzymes and substrates which react to produce flavour precursors.

Similar processing conditions to large scale fermentation have to be reproduced when fermenting small quantities of beans (microfermentation) to produce desirable optimum flavour attributes. A reliable laboratory microfermentation method is required in breeding programs to address the critical problem of the small number of pods available from each genotype at any one time. Hence, the wet beans cannot be fermented as per normal commercial practice. A successful microfermentation method would allow rapid and reliable assessment of flavour potentials of genotypes. The method has to be less cumbersome, time and labour consuming and more reliable to reproduce basic physical and organic chemical attributes and microbiology similar to that of large scale fermentation.

Various microfermentation methods for processing small quantities of beans have been developed (Clapperton *et al.*, 1991; Quesnel and Lopez 1975; Challot, 1977; Perkins, 1982; Bridgland, 1984; Jacquet *el al.*, 1981). Most of these procedures produce beans of abnormal qualities, while other methods developed are cumbersome, time and labour consuming and unreliable.

From a detailed literature review (Hollywood, 1994); it was revealed that the anaerobic phase of microfermentation was ignored by most researchers when developing microfermentation methods to ferment small quantities of beans,

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though it is a critical factor that contributes towards production of optimum cocoa flavours similar to beans processed by large scale fermentation. Consequently, the microfermentation methods developed produce beans of abnormal qualities.

The work reported here was intended to validate the importance of anaerobic phase in large scale cocoa fermentation that needs to be reproduced in microfermentation methods to produce desirable cocoa flavour attributes similar to beans processed by large scale fermentation. This hypothesis was tested by microfermenting cocoa with or without an anaerobic phase in the first 24 hours of a microfermentation process described. The cocoa flavour profiles of the two treatments (with or without inclusion of anaerobic phase) were compared statistically to verify whether the anaerobic phase of fermentation is necessary in microfermentation process to develop desirable flavour attributes.

OBJECTIVE

The objective was to compare the flavour profiles of cocoa processed by a microfermentation method with or without anaerobic phase.

MATERIALS AND METHODS

Experimental Design

The cocoa genotypes assessed for flavour characteristics were collected from a cocoa breeding trial conducted at the Papua New Guinea Cocoa and Coconut Research Institute (PNGCCRI).

Sample collection & preparation

Treatment 1

Microfermenting cocoa under an anaerobic phase using an anaerobic jar inserted with a campy pak and microaerophilic gas generator.

A kilogram of wet beans was collected from Trinitario clones (K20) from a cocoa breeding trial. The beans were inoculated with 10 g pulp from day 1 commercial fermented beans and placed inside an anaerobic jar inserted with campy pak and microaerophilic gas generator to create an anaerobic phase in the first 24 hours of fermentation, mimicking a condition similar to a large fermentation. The beans were removed from the anaerobic jar after 24 hours and placed in a buchner funnel mounted on an erlenmeyer flask, which allowed draining of sweating and placed inside a thermostatically controlled incubator set at the following temperature range from day 0 (D0) to 6:(D6) D0-D1, 30 ^{0}C; D1-D2, 35 ^{0}C; D2-D3, 40 ^{0}C; D3-D4, 46 ^{0}C; D4-D5, 47^{0}C; D5-D6, 47^{0}C. The bean sweating collected inside the anaerobic jar and in the erlenmeyer flask were discarded. The moisture levels in the beans were maintained by sealing the buchner funnel with plastic wrappings. Aeration in the buchner funnel was controlled by stirring the beans daily. For both treatments, after six days of fermentation, the beans were solar dried (Hollywood *et al.*, 1996) for three days and drying was completed by drying the beans in cabinet dryer at 60 °C. The dried beans were cooled under room temperature before bagged and stored under dry condition for sensory assessment.

Treatment 2

Microfermenting cocoa without an anaerobic phase

For treatment 2, same procedure as for treatment 1 was applied except exclusion of the anaerobic phase of fermentation in the first 24 hours of microfermentation. Instead of placing the beans inside a campy pak with microaerophilic gas generator, the wet beans were placed straight into a buchner funnel mounted on an erlenmeyer flask and fermented after inoculation with 10 g pulp from day 1 commercial fermented beans. After fermentation and drying, the dried beans were bagged for sensory assessment.

Sample preparation

Approximately 2 kg of dried beans were collected from three replicates of each treatment. The sampled beans were dried inside an oven (Cotherm, New Zealand) at 115 °C for 15 min to standardize the moisture content of the beans to less than 7 %, cooled under room temperature before being emptied into a mixing container and thoroughly mixed. Any foreign materials and debris were removed. The samples were processed and packed following a procedure described by Sukha, 2001. The dried cocoa samples were sent to Queensland Department of Primary Industry (QDPI) and their basic flavour attributes were rated.

Flavour assessment procedure

Cocoa samples were assessed using standard rating test (AS2542.2.3-1988). Data were collected using a fully integrated software system Compusense five version 2.2 (Compusense Inc, Canada) and statistically analysed using appropriate techniques. The samples were rated for the basic flavour attributes of chocolate, acidity, bitterness, astringency and fruitiness.

Statistical Analysis

The data generated were analysed as a factorial using analysis of variance with tasters as a blocking factor and the two treatments assessed three times.

RESULTS & DISCUSSION

Table 1: Factorial analysis of variance, comparing cocoa flavours produced by microfermentation methods with or without an anaerobic phase.

Source	*Significant (p)*
Taster (Chocolate)	0.0050*
Taster (Acid)	0.0050*
Taster (Astringency)	0.0428 – NS
Taster (Bitterness)	0.0000*
Taster (Fruitiness)	0.0000*
Taster others (chemical, phenolic, overripe fruit)	0.1635 – NS

* Significant difference at 5 % level
NS No significant difference at 1 % level

Taster refers to a taste panel differentiating levels of flavour intensity between samples generated by two treatments.

Table 2: LSD pairwise comparisons of cocoa flavour produced by microfermentation methods with or without anaerobic phase.

Microfermentation method	Chocolate mean*
1	42.3
2	32.1
	Acid mean*
1	47.1
2	22.8
	Astringency mean NS
1	13.6
2	11.4
	Bitterness mean*
1	19.2
2	9.6
	Fruitiness mean*
1	54.2
2	27.1
	Others (chemical, phenolic, overripe fruit) mean NS
1	10.9
2	3.1

* Significant difference at 5 % level
NS No significant difference at 1 % level.

1. denotes microfermentation with anaerobic phase (treatment 1)
2. denotes microfermentation without anaerobic phase (treatment 2)

The analysis of variance and LSD pairwise comparisons of cocoa flavour intensities (Tables 1-2) indicate that the six variables analyzed showed significant (5 %) differences in their levels of flavours between treatment one and two except for astringency which was not significant. Chocolate and other flavours were significant at the 5 % level. Intensities of all flavours were higher for treatment one which is from beans processed by the micro-fermentation method; that include an anaerobic phase using anaerobic jar inserted with a campy pak and microaerophilic gas generator. Other flavours observed were described as overripe fruit, phenolic or chemical. The results indicate the need for an anaerobic phase in any micro-fermentation method as part of the initial fermentation process.

The initial anaerobic phase of the micro-fermentation process is crucial to reproduce the basic physical and organic chemistry attributes and microbiology of large scale fermentation. Exclusion of an anaerobic phase in cocoa fermentation would result in processing cocoa with undesirable flavours or the flavour potential of cocoa may not be fully developed. Successful microfermentation methods would allow rapid and reliable assessment of the flavour potential of genotypes to cater for small numbers of pods available from each genotype at any one time which may not be possible to ferment using normal commercial practice. The successful microfermentation method would support progressive breeding programs to select planting material with superior quality attributes for the industry.

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