DEVELOPING A NEW SMALL-SCALE LABORATORY MICROFERMENTATION METHOD THAT CAN SUCCESSFULLY FERMENT SMALL QUANTITIES OF COCOA FOR A RAPID AND RELIABLE GENOTYPE FLAVOUR PROFILING

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ABSTRACT

A method of fermenting small quantities of cocoa in a thermostatically controlled incubator was developed. The method is referred to as CCRI microfermentation method. This method reproduces the basic physical and organic chemical attributes and microbiology similar to a commercial fermentation. Statistical analysis of data indicated no significant difference between cocoa flavours attributes generated by the microfermention and commercial fermentation methods.

Key words: *microfermentation, genotype, cocoa flavour attributes*

INTRODUCTION

Early production of cocoa in Papua New Guinea (PNG) was from the low yielding, largely unselected Trinitario variety which gave an average yields of 0.7 t/ha and 0.3 t/ha for large and small-scale holders respectively. In the early 1980s, the National Department of Primary Industry (DPI) developed and distributed new series of high yielding hybrids from Trinitario x Amazonian crosses with potential yields of more than 2 t/ha. Since then, more extensive breeding programs have been carried out by Papua New Guinea Cocoa and Coconut Research Institute (PNGCCRI), developing hybrid planting materials with substantially improved yields.

However, the major concern of the breeding program has been that as the genetic make-up of hybrids is modified to improve yield, concomitants changes may occur that alters their flavour profiles which may no longer meet end-users requirements. Some sections of the industry were concern that the introduction of hybrids has resulted in reduced levels of cocoa/chocolate flavours in PNG cocoa. To address this problem, sensory assessments by chocolate manufacturers have been incorporated into most breeding programs. However, some doubts have been cast on the reliability of the sensory data obtained.

Yield is determined by changes in pod production and this can be assessed from unprocessed cocoa. However, to assess changes in flavour profile, the wet bean must be fermented and dried so that chocolate tasters can be processed and evaluated.

A limitation in most breeding trias is that only a small number of pods are available from each genotype at any one time, hence the wet beans cannot be fermented as per normal commercial practices. Various procedures for processing small quantities of beans have been developed and tested (Clapperton et al., 1991; Quesnel et al., 1975; Challot, 1977; Perkins, 1982; Bridgland, 1984; Jacquet, 1981). But, most of these procedures produce beans of abnormal qualities.

However, one procedure that has been developed and widely used to ferment small quantities of cocoa is by placing a muslin or nylon mesh bag containing the wet beans into the centre of commercial size ferment (large cocoa mass) for normal duration of fermentation. This method has received criticism because of the likely effects of the larger mass of commercial ferment on the small samples. The transfer of liquid through the sample bag allows the characteristic of the larger mass to overwhelm those of the small samples. Such method of flavour assessment would result in selecting genotypes with undesirable flavour characteristic because the samples could be overpowered by those of the large mass. Under this condition, the true characteristic of the genotypes may not be identified at the initial stage of selection until commercial production which could result in serious losses to the industry if the geno-

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types selected have undesirable characteristics. Incorrect procedures may also cause rejection of progenies that may be promising.

Clapperton *et al.*, 1991 described a method of fermenting small quantities of cocoa in a bag placed in heap fermentation. This method has been demonstrated to give good assessment of flavour characteristic of genotypes. It has also been demonstrated to reproduce the flavour characteristic of genotype similar to commercial size fermentation. Although, this methodology has been well assessed and represents a valid technique, it is still cumbersome and time and labour consuming. Therefore, there is a need to develop a microfermentation method that allows rapid and reliable assessment of flavour potential true to their sensory properties, less cumbersome, time, labour consuming and reliable.

This paper discusses development of a new microfermentation method that reproduces flavour profiles similar to normal commercial fermented beans.

OBJECTIVES:

Develop a microfermentation method that can reproduce basic physical and organic chemical attributes and microbiology similar to a commercial fermentation. The method should be relatively cheap, rapid and reliable with the potential of high output to support breeding programs.

MATERIALS & METHODS

From a detailed literature review, the following important factors were considered critical to the designing of reliable and effective microfermentation techniques.

Temperature regime

In a commercial fermentation, the temperature of bean is generally between 24-30 ^{0}C at the start of fermentation and rises to 50-51 ^{0}C by the third and fourth day, and generally remains at this temperature until the end of fermentation. Therefore a similar microfermentation temperature regime is required.

Size and type of inoculums

The microflora occurring in microfermentation should be similar in type and level to that occurring in a commercial fermentation.

Organic acid and ethanol production

Organic acids and ethanol production need to be similar to that occurring in a commercial fermentation. These factors affect pH, titrable acidity (TA) and death and subsequent release of flavour precursors and enzymes in the cotyledon of the bean.

Moisture levels of beans

Moisture level must be maintained to prevent small quantities of beans from excessive drying during fermentation.

Regulating degree of aeration

Aeration regime affects both microbial and chemical processes during fermentation therefore it has to be regulated.

Adequate drying

Drying method is important during the fermentation process because the rate of moisture loss and time of oxidative process during drying greatly affects cocoa chemistry and flavour.

Genotypes

The selected cocoa genotypes assessed for their flavour characteristics were collected from a cocoa breeding trial conducted at Papua New Guinea Cocoa & Coconut Research Institute.

Microfermentation method

Following the preliminary assessment (Hollywood, 1994) of using different inoculums, manipulating of temperature regime, regulation of oxygen concentration and drying regimes; the following microfermentation method was developed.

A kilogram of wet beans was collected from each of the 13 Trinitario/Amazonian hybrid materials (Table 1). The beans were inoculated with 10 g pulp from a day 1 commercial fermented beans and placed inside an anaerobic jar with campy pak and a microaerophilic gas generator creating an anaerobic phase in the first 24 hours of fermentation. The beans were removed from the anaerobic jar after 24 hours and placed in a Buchner funnel mounted on an erlenmeyer flask which allows draining of sweating. The beans were placed inside a thermostatically controlled incubator set at the following temperature range

Cocoa genotypes were selected based on other quality characteristic (such as high fat content, large bean size and reasonable bean shell content and agronomical factors (such as high yielding, vigour and expressing a certain degree of disease resistance etc.

from day 0 to 6: 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 beans sweating collected inside the anaerobic jar and in the Erlenmeyer flask were discarded. The moisture level in the bean was maintained by sealing the Buchner funnel with plastic wrappings. Aeration in the Buchner funnel is controlled by stirring the bean daily. The fermented beans were solar dried (Hollywood *et al*., 1996) for three days and drying was completed in a cabinet dryer at 60 °C . The dried beans were bagged and stored for sensory assessment.

Sample collection & preparation

The dried beans collected from each genotype were dried in 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. Approxi-

mately 2 kg of dried beans were sampled from each genotype collected over a crop season (12 months). The dried cocoa samples were sent to Nestle (UK) and their basic flavour attributes were rated.

Flavour profile assessment

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, fruitiness and floral fragrant. The flavour rating scale is from 1-10, with 1 being weak and 10 being strong. The average flavour attributes of the selected genotypes and commercial fermented export quality beans were compared.

RESULTS AND DISCUSSION

Table 1: Basic flavour attributes of selected genotypes

Genotype	Chocolate Flavour	Acidity	Bitterness	Astringency	Fruitiness	Floral/ Fragrant
17/31	4.7	5.4	4.1	3.9	2.9	0.6
36-3/1	5.1	4.7	3.4	3.3	3.8	0.3
23-6/1	4.1	5.7	3.6	3.4	2.8	0.5
37-13/1	4.2	5.2	3.6	3.3	3.9	0.8
16-3/2	4.6	5.8	3.9	3.1	4.1	0.2
34-13/2	5.3	5.3	3.7	3.2	3.5	1.5
16-2/3	4.9	6.1	3.7	3.8	3.8	0.2
63-7/3	4.0	5.8	3.5	3.6	2.7	0.2
33-8/3	5.1	6.0	3.2	3.3	4.1	0.3
38-10/3	5.2	5.3	3.6	3.1	3.7	0.5
17-7/4	4.7	5.3	3.2	3.5	3.9	0.5
17-14/4	4.7	6.3	3.3	3.3	3.6	0.5
34-14/4	4.8	5.9	3/6	3.5	4.0	0.4
***Control mean**	**4.9**	**5.0**	**3.4**	**3.5**	**3.5**	**0.3**
Significant (p)	*0.20*	*0.03*	*0.03*	*0.2*	*0.5*	*0.10*

*Control mean represents mean flavour sensory attributes of commercial fermented PNG export cocoa assessed over the years by Nestle, UK. All flavour results including the 13 genotypes were evaluated by Nestle, UK. All genotype samples showed no significant difference at 1 % level against the control sample.

The results (Table 1) indicate that there is no significant difference in flavour of samples generated by microfermentation method described as compared to conventional fermentation which is indicated by the *p* values ($p > 0.005$). Many of the other previously developed microfermentation methods produced undesirable results when compared to this method. The difference between this method and other previously developed methods is the exclusion of anaerobic phase in the initial phase of fermentation. The inclusion of anaerobic phase in the first 24 hours of fermentation has demonstrated to be essential for the development of proper cocoa flavour attributes.

The development of flavour begins from the start of the fermentation process. During the initial anaerobic phase, pulp sugars are converted to etha-

Conventional fermentation box (120 x 90 x 90 cm)2 recommended for cocoa fermentation by Papua New Cocoa Board

nol by yeast, 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. The initial temperature of bean is generally between 24-30 °C at the start of fermentation and increases to 50-51 °C by the third and fourth day, and generally remains at that temperature until the end of fermentation. A combination of acetic acid levels and heat generated during fermentation are responsible for killing the cocoa beans causing a disruption of cell membrane thus releasing enzymes and substrates which react to produce flavour precursors.

For this microfermentation method, an anaerobic condition is created by using an anaerobic jar inserted with a campy pak and microaerophilic gas generator. The use of inoculums (10 g pulp from a day 1 commercial fermented beans) supplied the initial microflora required to initiate the fermentation process. Also the temperature regime was regulated artificially using thermostatically controlled incubator from 30 to 37 °C to generate temperature regime similar to normal fermentation since small quantities of beans microfermented can not generate sufficient heat to kill the beans. The daily turning of the beans promotes aeration which is necessary for ethanol to be converted into acetic acid. The plastic wrapping of the Buchner funnel keeps the microfermented beans moist hence prevents excessive drying that would lead to beans producing undesirable flavours. The beans were dried initially on a solar dryer to promote slow drying which allows sufficient time for continuation of oxidative reactions after fermentation to promote flavour developments and also allow adequate time for evaporation of acids from the bean cotyledon thus reducing bean acidity.

The microfermented method developed reproduces basic physical and organic chemical attributes and microbiology of a commercial fermentation. The method is relatively cheap, with the potential of high output to support the rapid breeding programs. The method is used for rapid and reliable assessment of flavour potential of genotype assessment.

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