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THE EFFECTS OF SELECTED MICROBIAL INOCULUM ON MICROBIAL AND BIOCHEMICAL CHANGES DURING COCOA FERMENTATION

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Thesis Submitted in Fulfilment of the Requirements for the Degree of Master of Science in the Faculty of Food Science and Biotechnology, Universiti Pertanian Malaysia

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Dedicated to my beloved wife,

Daeng Elysa Putri Mambang

and my children

Healthy Aldriany Prasetyo
Yayuk Putri Rahayu
Mauli Aldriano Aquarino Prasetyo

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TABLE OF CONTENTS

Page

Page

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

Abstract of the thesis submitted to the Senate of Universiti Pertanian Malaysia as fulfilment of the requirement for the degree of Masters of Science.

THE EFFECTS OF SELECTED MICROBIAL INOCULUM ON MICROBIAL AND BIOCHEMICAL CHANGES DURING COCOA FERMENTATION

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AUGUST 1990

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Faculty : Food Science and Biotechnology.

Fermentation and drying play an important role in influencing the overall quality of fermented cocoa beans. A study was conducted to look into the effect of various microbial inocula (Saccharomyces cerevisiae, Hansenula anomala and Acetobacter aceti in different combination) on changes in mass temperature, pH, ethanol, ethyl acetate, lactic and acetic acid contents and the degree of fermentation index (FI) of cocoa beans fermented over a period of six days. The dried treated cocoa beans were also analysed for pH, titratable acidity, lactic and acetic acids, ethyl acetate content and fermentation index.

The temperature of bean mass inoculated with microbial inoculum was found to increase to a maximum ranging from 47 to 48°C. The pH of cocoa cotyledon was found to decrease from pH

6.8 to 5.0 - 5.3, while the fermentation index (F.I.) of fermented beans was found to increase from 0.56 to 1.44. Beans inoculated with yeasts (S. cerevisiae + H. anomala) were found to yield higher ethyl acetate (0.5639 g/100 g) and lower levels of acetic and lactic acids (0.0748 g/100 g and 0.0443 g/100 g, respectively) as compared to the controlled sample at the end of 6 days. The controlled bean sample was found to have 0.2436 g/100 g ethyl acetate and 0.1669 g/100 g, 0.2565 g/100 g of acetic and lactic acid content, respectively after a 6 day fermentation period.

Cocoa beans inoculated with yeasts (S. cerevisiae + H. anomala) were found to have fully brown internal colour with a F.I value of 1.44 as compared to the controlled bean (with no inoculum) having dark brown colour with a F.I value of 1.26. There was no significant difference in pH, titratable acidity, lactic and acetic acids, ethyl acetate content and F.I. values between the freshly fermented and dried fermented cocoa beans.

Abstrak tesis yang dikemukakan kepada Senat Universiti Pertanian Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains.

KESAN-KESAN INOKULUM MIKROORGANISMA YANG DIPILIH KE ATAS PERUBAHAN MIKROORGANISMA DAN BIOKIMIA SEWAKTU PENAPAIAN BIJI KOKO

By

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OGOS 1990

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Fermentasi dan pengeringan memainkan peranan penting dalam mempengaruhi mutu keseluruhan biji koko yang telah difermentasi. Satu kajian telah dijalankan untuk melihat kesan kegunaan inokulum mikroorganisma (Saccharomyces cerevisiae, Hansenula anomala dan Acetobacter aceti dengan kombinasi berbeza) ke atas perubahan suhu, pH, kandungan etanol, etil asetat, asid asetik dan laktik dan indeks fermentasi biji koko semasa difermentasi selama enam hari. Biji koko yang disuntik dengan mikroorganisma dikeringkan dan dikaji perubahan pH, jumlah asid asetik dan laktik, etil asetat dan indeks fermentasi.

Biji koko yang disuntik dengan inokulum mikroorganisma telah didapati mencapai suhu maksimum antara 47°C dan 48°C, dan

pH kotiledon didapati menurun dari pH 6.8 ke 5.0 - 5.1 dan indeks fermentasi (F.I.) didapati naik dari F.I 0.56 ke 1.44 di akhir fermentasi. Biji koko yang disuntik dengan yis (S. cerevisiae + H. anomala) didapati mempunyai kandungan etil asetat yang tinggi (0.5639 g/100 g) dan kandungan asid asetik dan laktik yang rendah (0.0748 g/100 g dan 0.1443 g/100 g, masing-masing) berbanding dengan sampel kawalan selepas menjalani enam hari proses fermentasi. Sampel kawalan mempunyai 0.2436 g/100 g etil asetat dan 0.1660 g/100 g, 0.2565 g/100 g asid asetik dan laktik masing-masing, selepas enam hari proses fermentasi.

Biji koko yang disuntik dengan yis (S. cerevisiae + H. anomala) berwarna coklat dengan indeks fermentasi 1.44 berbanding dengan biji kawalan yang berwarna coklat gelap dengan indeks fermentasi 1.26. Didapati tidak ada perbezaan bererti pada pH, asid tertitrat, kandungan asid asetik dan laktik, etil asetat dan indeks fermentasi di antara biji koko segar yang telah difermentasi dan biji koko yang telah dikeringkan selepas difermentasi.

CHAPTER 1

INTRODUCTION

Cocoa fermentation naturally involves the growth of mixed population of microorganism of the pulp. The chemical and physical nature of the pulp makes it an ideal medium for the development of yeasts, bacteria and fungi (Rohan, 1963). The presence of sugar and high acidity (pH 4) provides excellent condition for the development of microorganisms. The process of cocoa bean fermentation is a prerequisite to the formation of chocolate flavour and aroma (Allison and Rohan, 1958). During this curing process, the involvement of microbial activities on the cocoa bean mucilage or pulp contributes to the formation of alcohol, esters, lactic acid and acetic acid. These components together with the heat generated may eventually lead to the death of the cocoa bean (Forsyth and Quesnel, 1963; Quesnel, 1965; Maravalhas, 1972). The diffusion of pulp fermentation products (acetic acid and ethanol) into the bean causes it to swell and simultaneously triggers biochemical reactions in the bean, resulting in the formation of flavour precursors which eventually produce chocolate flavour upon roasting. Absorption of acid by the beans during fermentation provides an ideal condition for the development of flavour precursors such as reducing sugars, amino acids, and flavonoids which are produced

as a result of enzymatic reaction occuring during the fermentation process. The reaction of most enzymes such as protease, glucosidase, and polyphenol oxidase found in the bean are responsible for the development of flavour precursors.

The flavour and acids of fermented cocoa beans play a major role in influencing the overall quality of the beans. Cocoa produced by some countries, like Malaysia and Brazil, are described by the world market as being characteristically acidic. Malaysian cocoa beans have an average pH value of 4.89 as compared to 5.26 with that of Ghanian beans (Selamat, 1987). According to Lopez (1983), free fatty acids especially acetic acid is responsible for the acidity of cocoa beans. The high residual acidity in Malaysian cocoa beans has been associated with the primary processing practices and planting materials which are different from those of West Africa. Most of the larger estates in Malaysia ferment their beans in deep boxes and use artificial dryers for drying. Cocoa pods are often harvested earlier which to the West African standard are underripe. All these factors countribute to excessive acid formation during fermentation. Another possible factor that may be associated with the bean acidity is the degree of fermentation. Selamat (1987) reported that highly acidic cocoa beans have a high fermentation index. This indicated that the beans which undergo fermentation for a long period (6 to 7 days in Malaysia) could accumulate more acid in the beans.

The importance of cocoa seed acidification in cocoa flavour precursor development and subsequently in chocolate flavour potentials has been discussed in details elsewhere (Biehl and Adamako, 1983; Brunner et al., 1985). The technology to improve the flavour of Malaysian cocoa based on slow seed acidification has been recently developed by Said et al., (1988).

A lot of efforts has been made to mitigate the acidity of cocoa beans. Countries such as Malaysia and Brazil, which are known for supplying high acid beans, modify the traditional methods of fermentation to control excessive acidity in beans. The modified methods are based on two principles: 1) reducing the amount of pulp by pressing, draining, or by using microbial inoculum and enzymes to liquify the pulp; and 2) increasing the aeration during the fermentation process by using the shallow box fermentation, as well as by using perforated boxes, air blasting and turning the bean mass frequently. Some of the modification methods have been successful; however, some have yielded inconsistent results and were impractical and thus abandoned.

The purpose of this study is to find the effects of using various microbial inocula on microbial and biochemical changes during the fermentation of cocoa beans. The objectives of the experiment include:

(1) To study the effect of various microbial inocula on changes of microbial population, biochemical changes and fermentation index of cocoa beans fermented for a period of six days.

(2) The evaluation of acidic characteristics of dry beans.

(3) The evaluation of fermentation index and ethyl acetate concentration of dried fermented cocoa nibs.

CHAPTER 2

LITERATURE REVIEW

Fermentation of cocoa beans plays an important role in determining the overall quality of fermented beans needed in processing of various chocolate products. During fermentation various biochemical and microbiological changes take place in the bean.

The Importance of the Fermentation Process of Cocoa Beans

The origin of the fermentation process is lost in antiquity, but it is generally assumed that fresh cocoas are primarily heaped to affect removal of the pulp and facilitate drying. It is fortuitous, therefore, that this pulp removal should be accompanied by enzymic reactions inside the cotyledon which gives rise to the production of chocolate flavour precursors.

The `sweating' process is now a common practice and essential to the production of chocolate flavour and is in fact the first and most critical stage in chocolate flavour development. The chemical processes involved are not entirely fermenting reactions. The changes which occur in the combined