



UNIVERSITI PUTRA MALAYSIA

**STORAGE OF COCOA (THEOBROMA CACAO L.)
SEEDS AND CHANGES ASSOCIATED WITH THEIR
DETERIORATION**

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STORAGE OF COCOA (*THEOBROMA CACAO L.*)
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DETERIORATION

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TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS	ii
LIST OF TABLES	vi
LIST OF FIGURES	viii
LIST OF PLATES	x
LIST OF APPENDICES	xiii
LIST OF ABBREVIATIONS	xv
ABSTRACT	xvi
1. INTRODUCTION	1
2. REVIEW OF LITERATURE	4
2.1 STORAGE OF COCOA SEEDS	4
2.2. CHANGES ASSOCIATED WITH SEED DETERIORATION	12
3. MATERIALS AND METHODS	35
3.1 SEED MATERIALS AND SAMPLING	36
3.2. EXPERIMENTAL PROCEDURES	36
3.2.1. Seed treatment	36
3.2.2. Packaging and storage	37
3.2.3. Regulation of seed moisture content	38
3.2.4. Preparation of axes for analysis	39
3.2.5. Preparation of sections for observation under the light microscope ...	39
3.2.6. Preparation of sections for examination under the transmission electron microscope	41
3.3. MEASUREMENTS AND OBSERVATIONS	43
3.3.1. Moisture content	43



3.3.2.	Germination	44
3.3.3.	Seedling height and dry weight	46
3.3.4.	Conductivity of seed leacheate	47
3.3.5.	Incidence of storage fungi	47
3.3.6.	Uptake of oxygen during respiration	47
3.3.7.	Incorporation of ¹⁴ C-leucine into protein	48
3.4.	EXPERIMENTS	49
3.4.1.	Selected factors affecting cocoa seed storage	49
3.4.2.	Changes associated with cocoa seed deterioration	58
3.5.	STATISTICAL ANALYSIS	60
4.	RESULTS	61
4.1.	SELECTED FACTORS AFFECTING COCOA SEED STORAGE	61
	<u>Experiment 1a.</u> Effect of drying method on seed viability and storability	61
	<u>Experiment 1b.</u> Effect of high and low seed moisture and storage temperature on the storability of cocoa seeds	66
	<u>Experiment 1c.</u> Effect of high seed moisture and moderate storage temperature on the storability of cocoa seeds	69
	<u>Experiment 1d.</u> Effect of imbibed storage of cocoa seeds in combination with germination inhibitors	73
	<u>Experiment 1e.</u> Effect of biocides on the storability of cocoa seeds	77



<u>Experiment 1f.</u> Effect of benlate-thiram applied as a dust and a soak on the storability of cocoa seeds.	81
<u>Experiment 1g.</u> Effect of periodic retreatment with benlate-thiram on the storability of cocoa seeds	91
4.2. CHANGES ASSOCIATED WITH COCOA SEED DETERIORATION .	96
<u>Experiment 2a.</u> Physiological, biochemical and structural changes associated with dehydration damage	96
<u>Experiment 2b.</u> Physiological, biochemical and structural changes associated with chill injury	114
5. DISCUSSION	129
5.1. SEED DRYING AND VIABILITY	129
5.2. SEED MOISTURE AND STORABILITY	131
5.3. TEMPERATURE AND STORABILITY	134
5.4. STORAGE FUNGI AND STORABILITY	137
5.5. DETERIORATION DUE TO DEHYDRATION INJURY	143
5.6. DETERIORATION DUE TO CHILL INJURY	147
5.7. DEATH OF COCOA SEEDS AND PROSPECTS FOR STORAGE ...	154
6. SUMMARY AND CONCLUSIONS	163
7. BIBLIOGRAPHY	169
8. APPENDICES	185



LIST OF TABLES

		Page
Table I	Effect of drying methods on the rate of moisture reduction and germination of cocoa seeds	63
Table II	Percentage germination of cocoa seeds exposed to different drying methods and subsequently stored for various periods in the air-conditioned room	63
Table III	Percentage germination of cocoa seeds stored for different periods at 32% and 27% moisture and 30°C, 22°C and 15°C temperature	67
Table IV	Percentage germination of cocoa seeds stored for different periods at 35%, 33.5% and 32% moisture and 22°C, 17°C, 15°C and 13°C temperature	71
Table V	Percentage germination of cocoa seeds treated with different biocides and stored for various periods	78
Table VI	Percentage moisture of cocoa seeds treated with different biocides and stored for various periods	80
Table VII	Percentage germination of cocoa seeds dusted or soaked with benlate-thiram and stored for different periods	82
Table VIII	Percentage germination of cocoa seeds initially dusted or soaked in benlate-thiram and subsequently with or without retreatment at six weekly intervals	92



Table IX	Moisture content of cocoa seeds initially dusted or soaked in benlate- thiram and subsequently with or without retreatment at six weekly intervals	92
Table X	Effect of dehydration on the physiological indices of cocoa seeds	97
Table XI	Effect of dehydration on the biochemical indices of cocoa seeds	97
Table XII	Effect of chilling on the physiological indices of cocoa seeds	116
Table XIII	Effect of chilling on the biochemical indices of cocoa seeds	116



LIST OF FIGURES

		Page
Figure 1.	Effect of drying methods on the moisture of cocoa seeds	62
Figure 2.	Effect of drying methods on the germination of cocoa seeds	62
Figure 3.	Moisture content of cocoa seeds exposed to different drying methods and subsequently stored for various periods in the air-conditioned room.	65
Figure 4.	Effect of seed moisture reduction on the germination of cocoa seeds	65
Figure 5.	Percentage germination of cocoa seeds stored for various periods at 30 ^o C, 22 ^o C and 15 ^o C	68
Figure 6.	Moisture variation of cocoa seeds with initial moisture content of 32% and 27% after different storage periods	68
Figure 7.	Percentage germination of cocoa seeds stored for various periods at 22 ^o C, 17 ^o C, 15 ^o C and 13 ^o C.	72
Figure 8.	Moisture variation of cocoa seeds with initial moisture content of 32%, 33.5% and 35% after different storage periods....	72
Figure 9.	Percentage radicle emergence of cocoa seeds stored unimbibed or imbibed in various concentrations of abscisic acid, sodium chloride and polyethylene glycol 1500	74



Figure 10.	Percentage germination of cocoa seeds after different periods of storage as unimbibed or imbibed seeds in 0.5 M sodium chloride and 35% polyethylene glycol 1500	76
Figure 11.	Moisture variation of cocoa seeds soaked or dusted with benlate-thiram and stored for different periods	82
Figure 12.	Percentage of untreated, benlate-thiram dusted and benlate-thiram soaked seeds infected with various fungi after different periods of storage	89
Figure 13.	Percentage of untreated, benlate-thiram treated and benlate-thiram retreated seeds infected with various storage fungi after different periods of storage.	94
Figure 14.	Effect of hours of dehydration on the time course of respiration of cocoa seed axes	99



LIST OF PLATES

		Page
Plate 1.	Categories of seeds and seedlings identified in the germination test	45
Plate 2.	Infection of untreated, benlate-thiram dusted and benlate-thiram soaked cocoa seeds of storage fungi after four weeks storage	84
Plate 3.	<i>Penicillium citrinum</i> isolated from stored cocoa seeds	84
Plate 4.	<i>Penicillium cyclopium</i> isolated from stored cocoa seeds	85
Plate 5.	<i>Aspergillus niger</i> isolated from stored cocoa seeds	85
Plate 6.	<i>Aspergillus flavus</i> isolated from stored cocoa seeds	86
Plate 7.	Pycnidiospores of <i>Botryodiplodia theobromae</i> isolated from stored cocoa seeds	86
Plate 8.	Clamydospores of <i>Botryodiplodia theobromae</i> isolated from stored cocoa seeds	87
Plate 9.	<i>Rhizopus arrhizus</i> isolated from stored cocoa seeds	87
Plate 10.	<i>Paecilomyces variotii</i> isolated from stored cocoa seeds	88
Plate 11.	Changes in cocoa seed structures after different hours of dehydration in the air-conditioned room	102



Plate 12.	Structure of the embryonic axis of cocoa seeds	102
Plate 13.	Cellular structure of epicotyl and radicle from embryonic axis of fresh cocoa seeds	104
Plate 14.	Cellular structure of epicotyl and radicle seeds subjected to 16 hours of dehydration in the air-conditioned room ...	105
Plate 15.	Cellular structure of epicotyl and radicle of cocoa seeds subjected to 40 hours of dehydration in the air- conditioned room	106
Plate 16.	Cellular structure of epicotyl and radicle of cocoa seeds subjected to 64 hours of dehydration in the air- conditioned room	107
Plate 17.	Ultrastructure of fresh radicle cells	109
Plate 18.	Ultrastructure of radicle cells dehydrated for 16 hours in the air- conditioned room	110
Plate 19.	Ultrastructure of radicle cells dehydrated for 40 hours in the air- conditioned room.	111
Plate 20.	Ultrastructure of radicle cells dehydrated for 64 hours in the air- conditioned room	112
Plate 21.	Changes in cocoa seed structures after different periods of chill treatment at 10°C	119



Plate 22.	Cellular structure of epicotyl and radicle of cocoa seeds subjected to 10 minutes of chill treatment at 10 ^o C	120
Plate 23.	Cellular structure of epicotyl and radicle of cocoa seeds subjected to 40 minures of chill treatment at 10 ^o C	121
Plate 24.	Cellular structure of epicotyl and radicle of cocoa seeds subjected to 160 minutes of chill treatment at 10 ^o C	122
Plate 25.	Ultrastructure of radicle cells chilled for 10 minutes at 10 ^o C	124
Plate 26.	Ultrastructure of radicle cells chilled for 40 minutes at 10 ^o C	125
Plate 27.	Ultrastructure of radicle cells chilled for 160 minutes at 10 ^o C	126



LIST OF APPENDICES

	Page
Appendix 1. Preparation of chemicals	185
Appendix 2. Mean square values of initial moisture, initial germination, final germination and duration of drying of cocoa seeds as influenced by drying methods - Experiment 1a	190
Appendix 3. Mean square values of germination and moisture of cocoa seeds as influenced by storage after different methods of drying - Experiment 1a	190
Appendix 4. Mean square values of germination and moisture of cocoa seeds as influenced by storage at 32% and 27% seed moisture and 30°C, 22°C and 15°C storage temperature - Experiment 1b	191
Appendix 5. Mean square values of germination and moisture of cocoa seeds as influenced by storage at 35%, 33.5% and 32% seed moisture and 22°C, 17°C, 15°C and 13°C, storage temperature - Experiment 1c	192
Appendix 6. Mean square values of cocoa seed germination as influenced by imbibed storage in various germination inhibitors - Experiment 1d	193
Appendix 7. Mean square values of germination and moisture of cocoa seeds as influenced by storage after different biocide treatments - Experiment 1e	193



Appendix 8.	Mean square values of germination and moisture of cocoa seeds as influenced by storage after different applications of benlate-thiram - Experiment 1f	194
Appendix 9.	Mean square values of germination and moisture of cocoa seeds as influenced by storage with different methods of benlate-thiram retreatment - Experiment 1g	194
Appendix 10.	Mean square values for physiological indices of cocoa seeds subjected to various duration of dehydration treatment - Experiment 2a	195
Appendix 11.	Mean square values for biochemical indices of cocoa seeds subjected to various duration of dehydration treatment - Experiment 2a	195
Appendix 12.	Mean square values for time course of respiration of cocoa seeds subjected to various duration of dehydration treatment - Experiment 2a	196
Appendix 13.	Mean square values for physiological indices of cocoa seeds subjected to various duration of chill treatment - Experiment 2b	196
Appendix 14.	Mean square values for biochemical indices of cocoa seeds subjected to various duration of chill treatment - Experiment 2b	197



LIST OF ABBREVIATIONS

The following abbreviations were used in the text :

ABA	=	Abscisic acid
PEG	=	Polyethylene glycol
LSD	=	Least significant difference
DMRT	=	Duncan's multiple range test
RH	=	Relative humidity
ER	=	endoplasmic reticulum
ETOH	=	ethyl alcohol
TBA	=	tertiary butyl alcohol
w/w	=	weight by weight
v/v	=	volume by volume
wb	=	fresh weight basis
db	=	dry weight basis
h	=	hour
min	=	minute
°C	=	degree centigrade
cm	=	centimetre
mm	=	millimeter
u	=	micron, micrometre
kg	=	kilogram
g	=	gram
mg	=	milligram
ug	=	microgram
l	=	litre
ml	=	millilitre
ul	=	microlitre
M	=	molar
mM	=	millimolar
uCi	=	microcurie



ABSTRACT

An abstract of the thesis presented to the Senate of Universiti Pertanian Malaysia in partial fulfilment of the requirements for the Degree of Doctor of Philosophy.

STORAGE OF COCOA (*THEOBROMA CACAO L.*)

SEEDS AND CHANGES ASSOCIATED WITH THEIR DETERIORATION

by

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Cocoa (*Theobroma cacao L.*) seeds are recalcitrant and rapidly lose their viability during preparation and within two to three weeks of storage. To improve their storability, the responses of a cultivar of cocoa seeds (NA 33) to different drying methods and storage factors including seed moisture, storage temperature and fungi were investigated. Physiological, biochemical and structural changes associated with seed death caused by dehydration in the air-conditioned room (22°C, 55% RH) and chilling at 10°C were also monitored.



Freshly harvested seeds were best dried in the air-conditioned room. The critical seed moisture content was 26% to 27%, but for storage a moisture content of 33.5% to 35% was optimal. Temperatures below 15°C were lethal and storage in the air-conditioned room at 22°C is recommended. Seed dusting with 0.2% w/w of an equal benlate-thiram mixture was essential since untreated seeds were rapidly killed by storage fungi such as *Penicillium* spp., *Aspergillus* spp. and *Botryodiplodia theobromae*. Treated seeds maintained germination for at least six weeks and retreatment with fresh fungicides either as a dust or a soak did not prevent rapid seed death after this period. For optimal storage, cocoa seeds at 33.5% to 35% moisture and dusted with 0.2% w/w of an equal benlate-thiram mixture should be stored in thin perforated polythene bags in loosely closed plastic boxes in the air-conditioned room. Germination of at least 80% could be maintained for two to three months.

Changes associated with dehydration damage and chill injury of cocoa seeds were different. Although germination and seedling growth were rapidly decreased in both cases, axial respiration and protein synthesis were unaffected by the chill treatment, but were reduced significantly in axes moderately damaged by dehydration. Loss of membrane integrity as evidenced by increased leachate conductivity also occurred in seeds moderately damaged by dehydration, but was not detected in chilled seeds until they were totally killed. Progressive damages to cell organelles including cell membranes, mitochondria,



ribosomes and nuclei were observed with increasing dehydration damage. Conversely, organelles were essentially unchanged in chilled seeds except for severe derangement of the plasmalemma and tonoplast. The data suggest that death caused by dehydration is progressive and involves damage to many biological processes including respiration, protein synthesis and function of cell organelles; culminating finally in total cell collapse. Death caused by low temperature is more abrupt and may be triggered by only a few vital processes resulting mainly in severe degeneration of cell membranes and their related functions. Respiration, protein synthesis and other cell organelles were essentially unaffected.



1. INTRODUCTION

Cocoa, *Theobroma cacao*, is one of several species belonging to the family *Sterculiaceae*. It originates from the tropical rain forest of Central America where its seeds were used for concocting a drink popular with the Mayas and Aztecs. Today the seed is used for manufacturing a wide range of beverages and confectioneries. This was supported by a world production of 1.67 million tonnes of cocoa beans in 1981 (FAO, 1982).

The main producers of cocoa are localised in the tropical Americas and Africas. These include Brazil, Ghana, Nigeria and the Ivory Coast. There is less emphasis on the crop in tropical Asia, probably because of the pre-eminence of other plantation crops such as rubber (*Hevea brasiliensis*) and oil palm (*Elaeis guineensis*). In Malaysia, small areas of the crop were grown, but owing to disease, poor management and lower profitability, it did not expand as rapidly as rubber and oil palm. However, in the early 1970's crop diversification was emphasised and this together with higher price for cocoa resulted in an expansion of the crop. This is evident from the increased area from which the crop is harvested, from 9,000 hectares in 1970 to 72,000 hectares in 1981 (FAO, 1982).

With the expansion of the crop, the demand for planting materials has also increased. In Malaysia, the crop is largely propagated by seed although vegetative propagation is possible. The demand for planting materials exists throughout the year, but



seed production is bimodal and generally peaks during the months of February to March and October to November. Such seasonal supply in the face of continual demand emphasises the need for a suitable method of seed conservation during the peak seasons.

However, cocoa seeds are recalcitrant in that they do not withstand dehydration and low temperature (Swarbrick 1965, Barton, 1965). They deteriorate rapidly when exposed to humid tropical conditions. The maximum period of storage reported is only approximately three to four weeks. Even under such short storage period, a high percentage of the seeds was already pregerminated (Evans, 1950; Swarbrick, 1965). If handled like orthodox seeds, they lose their viability even faster in a matter of two to three days. Because of this, much planting materials have been lost as a result of poor handling and storage before the seeds were planted.

The recalcitrant nature of the seed has also made it difficult to conserve the genetic resources of cocoa. As very short term storage is only currently available, the main method for conserving cocoa germplasm is by the planting out method. With the current rate of depletion of our world natural reserves, the loss of germplasm materials of cocoa and other recalcitrant seeds is very real.

The need to improve the storability of cocoa seeds has prompted the first objective of this study, which is to investigate the effects of various seed and environmental factors on the storability of cocoa. It is hoped that with a clearer



understanding of the behaviour of cocoa seeds under various conditions, an improved method of storage may be devised to prolong their viability.

The second objective of the study is a follow up of the first, and involves investigations into the physiological, biochemical and structural changes associated with the deterioration of cocoa seeds. It is aimed at elucidating the changes involved as seed viability decreases during storage. It is hoped that these studies will provide a better understanding of seed deterioration and point out venues for improving further the storability of cocoa seeds.



2. REVIEW OF LITERATURE

2.1. STORAGE OF COCOA SEEDS

Based on the behaviour of seeds during storage, Roberts (1973 a) categorised them as orthodox or recalcitrant. Orthodox seeds are those which can be dried to a relatively low moisture of 4% (Harrington, 1970) without loss of viability. On the other hand, recalcitrant seeds are unable to withstand excessive dehydration and rapidly lose their viability at relatively high moisture levels of 12% - 31% (Roberts, 1973 a). Many recalcitrant seeds also do not tolerate low temperature and are often injured at temperatures of less than 10°C - 15°C. Because of their susceptibility to drying and low temperature, cocoa seeds are also considered to be recalcitrant.

Maintenance of the viability of recalcitrant seeds is more difficult since they have no inherent quiescent stage and are not storable at low moisture and temperature (Tang and Tamari, 1973; Chacko and Singh, 1971; Chin 1975; Teng 1977 b; Ang, 1976; and Chin *et al*, 1983). A review of the literature on the storage of cocoa seeds illustrates clearly the problems involved in storing these seeds, especially when they are unable to withstand cold conditions. Such studies began in the 1930's, but initial works were concentrated mainly on pod storage. Subsequent studies shifted the emphasis to the storage of the extracted seeds.