AN EVALUATION OF ANTIOXIDANT AND HYDRATION TREATMENTS FOR THE IMPROVEMENT OF THE STORABILITY OF SOYBEAN *[Glycine max (L.) Merr.]* SEEDS.

A thesis presented in partial fulfilment of
the requirement for the Degree of
Doctor of Philosophy
in Seed Technology
at Massey University
Palmerston North
New Zealand

NIT SAKUNNARAK

1992

Dedication

To my father, who inspired those to work hard.

AN EVALUATION OF ANTIOXIDANT AND HYDRATION TREATMENTS FOR THE IMPROVEMENT OF THE STORABILITY OF SOYBEAN *[Glycine max (L.) Merr.]* SEEDS.

Author: Nit Sakunnarak, Ph.D. Thesis, 1992.
Supervisors: Dr. Peter Coolbear, Dr. David W. Fountain

ABSTRACT

Antioxidant and hydration treatments were evaluated for their potential to improve the storability of soybean *cvs*. Amsoy and Davis under different ageing conditions (accelerated ageing at 40°C, 36-100% RH; controlled deterioration at 40°C, 20% seed moisture; or slow ageing at 35°C, 9 or 12% seed moisture).

Despite previous reports in the literature, no protective effects of treatment were found using 1% α-tocopherol, 0.1% butylated hyroxytoluene (BHT) in acetone solution for 16 h, β-mercaptoethanol 0.52-52 *ppm* in aqueous solution or 0.1-1% iodine in calcium carbonate. High concentrations of BHT (2.2%) or iodine (1%) caused toxic effects to germination performance (as determined by normal germination, viability, fresh and dry weights of normal seedling axes). Acetone, used as a carrier for antioxidant treatments, was toxic to soybean seeds. Initial seed moisture contents, levels of mechanical damage, treatment duration and seed lot variation were key factors affecting susceptibility to acetone toxicity. Tetrazolium staining showed that acetone did not cause damage to a specific tissue but rather increased the area of dead tissue which had been mechanically damaged.

Hydration-dehydration pre-storage treatments of soybean also showed damaging effects. Soaking treatments caused injury to low vigour lots immediately and reduced ageing resistance in high vigour material. Moisture equilibration (ME) for 24-48 h had no effects on seed performance, but an extended moisture equilibration period up to 72 h increased rates of germination loss during subsequent ageing. However, post-storage hydration treatments showed some capacity to repair damaged seeds.

Increased conductivity of seed leachate was always significantly correlated with loss of germination performance, suggesting that membrane damage was related to seed deterioration. Changes in lipid and membranes were assessed in seeds aged at 40°C, ~100%

RH or 35°C, 9% constant seed moisture. No changes in total lipid content due to ageing or treatments suggested that changes in storage lipid were not related to germination performance. Significant losses of phospholipid (PL) from cotyledons occurred 4 days after accelerated ageing. These losses were correlated with loss of seed performance, but no changes in PL contents from axes were detected throughout the ageing period. By itself, this result suggests that cotyledon damage may be an important contributing factor to seed deterioration, but the transmission electron microscopy (TEM) study indicated that damage occurring in axes due to accelerated ageing was more severe than in cotyledons.

Acetone or water soaking pretreatments increased rates of loss of germination performance which were associated with PL losses or ultrastructural abnormalities in both axes and cotyledons. In addition, ME treatment (72 h) applied to slowly aged seeds accelerated PL loss in axes but, this was unrelated to seed performance. These data therefore indicate that PL losses *per se* are not a fundamental cause of seed deterioration and may not be the first event in membrane damage. Also germinating seeds seem to be able to repair some damage of this type. No changes in proportions of polyunsaturated fatty acids were observed in either total lipid from seed tissues or microsome fractions, indicating that lipid peroxidation was not involved in seed deterioration due to ageing or treatments. This, of course, explains the ineffectiveness of the antioxidant treatments in this study and suggests that hydrolytic damage may be involved in PL loss and seed deterioration.

Possible reasons for the differences between these results and the small amount of other work published on seed treatments in soybean are discussed, but it was clear that chemical or hydration treatments of soybean are unlikely to be a commercially viable option for maintenance of soybean quality where poor storage conditions are inevitable. Nevertheless, this study indicates some interesting areas for future research into the critical events involved in soybean deterioration.

ACKNOWLEDGEMENTS

I gratefully acknowledge my chief supervisor, Dr *Peter Coolbear*, for his excellent supervision, understanding, warm encouragement throughout my study, especially for his enthusiasm to help, constructive criticism, endless patience in discussion and correction of my English. Without him, this work would not have been possible. I owe a great debt to him.

I also wish to express my sincere thanks to Dr *David W Fountain*, my co-supervisor, for his friendship, encouragement, valuable suggestions, guidance concerning lipid and membrane study and reading my manuscripts.

My gratitude and appreciation are also extended to:

Mr *Doug Hopcroft* of EM laboratory DSIR, for his excellent work in praparing samples for EM study and Mr *Raymond Bennette* for help. Mr *Craig Mcgill* and Mr *Richard G Burr* for their technical assistance, friendship and encouragement.

Prof *Murray J Hill*, director of the Seed Technology Centre, and Dr *John G Hampton* for their readiness to help, cheerful support, suggestions in conducting experiments, and other present and past staff of Seed Technology Centre and Botany and Zoology Department for their help in many ways, especially Mrs *Dulcie Humphrey* for her friendly encouragement and moral support particularly during my stress period.

Mrs *K Hamilton* for typing tables and references, Mr *G Donaldson* for the illustrations, and *Mr T Watts* and staff of central photographic unit for photographic work.

Mrs *Daphne Meech*, *Michael* and *Nittayaporn Hare*, Dr *Clive Cornford*, Dr *Wanchai and Siree*, *Phuengporn*, *Pontip* and *Apichai* for their help, friendship and constant moral support.

Finally, *the New Zealand Government* for financial assistance, and *the Thai Government* for granting study leave.

TABLE OF CONTENTS

CHAPTER 3
MATERIALS AND METHODS

CHAPTER 4
ANTIOXIDANT AND SOLVENT TREATMENTS

CHAPTER 7

CHANGES IN THE ULTRASTRUCTURE OF CELLS DURING ACCELERATED AGEING AND EFFECTS OF PRIOR ACETONE DAMAGE OR SOAKING INJURY

CHAPTER 8

GENERAL DISCUSSION AND SCOPE OF FURTHER WORK

LIST OF FIGURES

LIST OF PLATES

LIST OF TABLES

Page

LIST OF APPENDICES

ABBREVIATIONS

AA	= Accelerated ageing
AOSA	= Association of Official Seed Analysts
BHA	= Butylated hydroxyanisole
BHT	= Butylated hydroxytoluene
CD	= Controlled deterioration
d	= day
ER	= Endoplasmic reticulum
GL	= Glycolipid
GLC	= Gas liquid chromatography
h	= hour
ISTA	= International Seed Testing Association
kD	= kilo-Dalton
ME	= Moisture equilibration
μS	= micro-Siemens
mth	= month
NL	= Neutral lipid
PA	= Phosphatidic acid
PC	= Phosphatidyl choline
PE	= Phosphatidyl ethanolamine
PEG	= Polyethylene glycol
PG	= Phosphatidyl glycerol
PL	= Phospholipid
PUFA	= Polyunsaturated fatty acid
RCR	= Respiratory control ratio
RH	= Relative humidity
RQ	= Respiratory quotient
SA	= Slow ageing
SMC	= Seed moisture content
TEM	= Transmission Electron Microscopy
TL	= Total lipid
wk	= week
WSB	= Water saturated butanol