Changes of Antioxidants and Fatty Acid Composition in Stabilized Rice Bran During Storage.

Taisun Shin
Louisiana State University and Agricultural & Mechanical College

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CHANGES OF ANTIOXIDANTS AND FATTY ACID COMPOSITION IN STABILIZED RICE BRAN DURING STORAGE

A Dissertation

Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Doctor of Philosophy

in

The Department of Food Science

by

Taisun Shin
B.S., University of King Sejong, 1987
August 1995
ACKNOWLEDGEMENTS

The author wishes to express his most sincere and deepest appreciation to Dr. J. Samuel Godber, the author's major professor, for his care, inspiration, encouragement, patient guidance, and support which made this dissertation possible.

He wishes to thank Dr. Auttis M. Mullins, former head, Department of Food Science, and Dr. Douglas L. Park, head, Department of Food Science for facilitating the use of departmental facilities necessary to conduct this research.

Appreciation is extended to Dr. John A. Hebert, Professor, Department of Poultry Science, Dr. John H. Wells, Associate Professor, Department of Biological & Agricultural Engineering, Dr. Joseph A. Liuzzo, Professor, Department of Food Science, and Dr. Lalit R. Verma, head, Department of Biological & Agricultural Engineering for serving as members of the advisory and examining committee. Special thanks are given to Daniel E. Martin for preparation of extrusion rice bran samples. Appreciation is extended to Dr. Keunsoo Lee for his constant encouragement and friendship.

Finally, the author expresses his deepest indebtedness to his mother Mrs. Keumsub Choi and his father, Mr. Kabsuk Shin, for their nurturing of the investigator during the first 28 years of his life and for his father's prayers from another "world" that he would be successful during this study.
He wishes to acknowledge spiritual encouragement of his entire family which made this study in the United States possible.

The dissertation is dedicated to his wife, Whasoo Shin, who is his constant encouragement and inspiration, and to his children, Insik Shin and Hyensik Shin who unknowingly sacrificed the time with their father that this investigation occupied.

Thank You, Lord.
# TABLE OF CONTENTS

ACKNOWLEDGEMENTS ................................................................. ii

LIST OF TABLES ........................................................................ viii

LIST OF FIGURES ........................................................................ xi

LIST OF ABBREVIATIONS ............................................................ xiv

ABSTRACT ...................................................................................... xix

CHAPTER I
INTRODUCTION ............................................................................. 1

CHAPTER II
REVIEW OF LITERATURE ........................................................... 5
  A. Rice Bran and Rice Bran Oil ..................................................... 5
  1. Composition of Rice Bran ..................................................... 5
  2. Composition of Rice Bran Oil ................................................ 6
  3. Health Benefits of Rice Bran Oil ........................................... 7
  B. Methods of Stabilization of Rice Bran .................................... 11
  1. Refrigeration ........................................................................ 12
  2. Chemical Methods ................................................................ 12
  3. Irradiation ............................................................................. 12
  4. Heat Stabilization ............................................................... 13
  C. Antioxidants in Rice Bran ....................................................... 15
  1. Vitamin E Vitamers ............................................................. 15
  2. \( \gamma \)-Oryzanol ..................................................................... 18
  D. Factors that Influence Vitamin E and Fatty Acid Content from Various Sources ........................................... 21
  1. Drying ................................................................................ 21
  2. Acid Treatment .................................................................... 22
  3. Milling ................................................................................ 23
  4. Heating ............................................................................... 23
  5. Freezing ............................................................................. 24
  6. Storage ............................................................................... 25
  7. Irradiation ........................................................................... 26
  E. Analytical Method for Vitamin E Vitamers ......................... 27
  1. Thin-Layer and Paper Chromatography (TLC and PC) ........... 27
  2. Gas-Liquid Chromatography (GLC) ..................................... 28
  3. High-Performance Liquid Chromatography (HPLC) .......... 29
  F. Mathematical Modeling ......................................................... 31
  1. Reaction Order ................................................................. 31
  2. Temperature Dependence of Reaction Rates ....................... 33
CHAPTER III
MATERIAL AND METHOD

A. Experiment 1: Isolation of Four Tocopherols and Four Tocotrienols
   1. Analytical Approach
   2. Chemical and Material
   3. Extraction of Crude Oil
   4. Apparatus

B. Experiment 2: Development of a Mobile Phase System
   1. Analytical Approach
   2. Materials
   3. Preparation of Crude Rice Bran Oil
   4. High-Performance Liquid Chromatography

C. Experiment 3: Effect of Extrusion Cooking on Vitamin E and Oryzanol and Oryzanol in Rice Bran
   1. Analytical Approach
   2. Materials
   3. Sample Description
   4. Vitamin E Vitamers and Oryzanol Quantification
   5. Statistical Analyses

D. Experiment 4: Quality of Rice Bran Oil Extracted from Extruded Rice Bran
   1. Analytical Approach
   2. Material
   3. Lipid Analysis
   4. Statistical Analyses

E. Experiment 5: Effect of Microwave Heating on Vitamin E, Oryzanol, and Fatty Acids in Rice Bran
   1. Analytical Approach
   2. Microwave Heating
   3. Material, Analytical Methods, and Statistical Analyses

F. Experiment 6: Effect of Gamma-Irradiation on Vitamin E, Oryzanol, and Fatty Acids in Rice Bran
   1. Analytical Approach
   2. Gamma-Irradiation
   3. Material, Analytical Methods and Statistical Analyses
CHAPTER IV
RESULTS AND DISCUSSIONS ........................................... 53

A. EXPERIMENT 1: Isolation of Four Tocopherols and Four
Tocotrienols ................................................................. 53
1. Sample Preparation .................................................. 53
2. Isolation of Tocopherols and Tocotrienols ............... 55
3. Fraction Purification ............................................... 58
4. Purity Checks and Concentration Determination ...... 59
5. Gas Chromatography/Mass Spectrometry ................. 61
6. Analytical HPLC Analysis of Isolated and Purified
Vitamers ................................................................... 65

B. EXPERIMENT 2: Development of a Mobile Phase System 68

C. EXPERIMENT 3: Effect of Extrusion Cooking on Vitamin
E and Oryzanol in Rice Bran ........................................ 76
1. Separation of Oryzanol from Crude Rice Bran Oil .... 76
2. Recovery of Vitamin E Vitamers and Oryzanol ....... 78
3. Effect of Holding Time and Extrusion Temperature .. 80
4. Changes of Vitamers and Oryzanol in Raw Bran
During Storage ......................................................... 86
5. Changes of Vitamers and Oryzanol in Extruded Rice
Bran during Storage ................................................... 90
6. Kinetics of Vitamin E and Oryzanol Degradation
During Post Extrusion Processing .............................. 99

D. EXPERIMENT 4: Quality of Rice Bran Oil Extracted from
Extruded Rice Bran ...................................................... 109
1. Composition of Crude Rice Bran Oil ......................... 109
2. Free Fatty Acid in Extruded Rice Bran ..................... 113
3. Phospholipid in Rice Bran during Storage ............... 121
4. Neutral Lipid in Rice Bran during Storage ............... 127
5. Color Change in Extruded Rice Bran during Storage . 130

E. EXPERIMENT 5: Effect of Microwave Heating on Vitamin
E, Oryzanol, and Fatty Acids in Rice Bran .................... 132
1. Composition of Crude Rice Bran Oil ......................... 132
2. Free Fatty Acids in Rice Bran ................................... 132
3. Vitamin E Vitamers and Oryzanol in Microwave
Stabilized Rice Bran .................................................. 141
4. Phospholipid in Rice Bran ......................................... 149

F. EXPERIMENT 6: Effect of Gamma-Irradiation on Vitamin
E, Oryzanol, and Fatty Acids in Rice Bran .................... 152
1. Free Fatty Acids in Irradiated Rice Bran ................. 152
2. Tocopherols and Tocotrienols in Rice Bran ............. 160
3. Phospholipid in Rice Bran ......................................... 167
CHAPTER V
SUMMARY AND CONCLUSIONS ................................. 174

LITERATURE CITED ....................................................... 179

APPENDICES
Appendix 1. Structures of tocopherols and tocotrienols ........ 204

Appendix 2. Total vitamin E vitamers and oryzanol in rice bran extruded at 110°C with holding time 0, 3, and 6 min during storage. ................................. 205

Appendix 3. Total vitamin E vitamers and oryzanol in rice bran extruded at 120°C with holding time 0, 3, and 6 min during storage. ................................. 206

Appendix 4. Total vitamin E vitamers and oryzanol in rice bran extruded at 130°C with holding time 0, 3, and 6 min during storage. ................................. 207

Appendix 5. Total vitamin E vitamers and oryzanol in rice bran extruded at 140°C with holding time 0, 3, and 6 min during storage. ................................. 208

Appendix 6. Gas chromatogram of fatty acid in rice bran oil. Chromatographic conditions are given in the text. ........ 209

Appendix 7. Tocopherols and tocotrienols in rice bran treated with microwave for 4 min during 52 weeks storage. .......... 210

Appendix 8. Tocopherols and tocotrienols in rice bran treated with microwave for 8 min during 52 weeks storage. .......... 211

Appendix 9. Tocopherols and tocotrienols in rice bran treated with microwave for 12 min during 52 weeks storage. .......... 212

Appendix 10. Tocopherols and tocotrienols in rice bran irradiated at 5 kGy during 52 weeks storage. .................. 213

Appendix 11. Tocopherols and tocotrienols in rice bran irradiated at 10 kGy during 52 weeks storage. .................. 214

Appendix 12. Tocopherols and tocotrienols in rice bran irradiated at 15 kGy during 52 weeks storage. .................. 215

VITA ........................................................................... 216
## LIST OF TABLES

Table IV-1. Mass balance (g) of sample preparation ........................................ 54

Table IV-2. Mobile phase gradient program for mixture of soybean oil and wheat germ oil ................................................................. 54

Table IV-3. Mobile phase gradient program for latex lipid and wheat bran oil ....................................................................................... 57

Table IV-4. Analytical data for isolated and purified tocopherols and tocotrienols fractions from 6 g of a mixture of soy-bean oil and wheat germ oil and 8 g of a mixture of wheat bran oil and latex lipid ........................................................................... 63

Table IV-5. UV absorption maxima and molar absorbances of tocopherols and tocotrienols in ethanol solution taken from the literature ........................................................................................................... 63

Table IV-6. Molecular weights and major mass to charge ratios (m/z) of vitamin E vitamers in mass spectrum ........................................ 64

Table IV-7. Contents (mg/g) of tocopherols and tocotrienols in a mixture of soybean and wheat germ oil and a mixture of wheat bran oil and latex lipid ........................................................................... 67

Table IV-8. HPLC retention times (mean ± standard deviation) for vitamin E vitamers with various eluants on a Sulpelcosil LC-Si(25 cm x 4.6, 5μ) column ......................................................................................... 70

Table IV-9. Effect of post extrusion holding time on vitamin E vitamers and oryzanol ................................................................. 81

Table IV-10. The percentage of retention of vitamin E vitamers and oryzanol during extrusion stabilization of rice bran ......................... 82

Table IV-11. Effect of extrusion temperature on total vitamin E vitamers and oryzanol ................................................................. 85

Table IV-12. First order reaction rate constants (k) for retention of vitamin E and oryzanol during post extrusion holding time .......... 101

Table IV-13. Calculated E<sub>s</sub> and Q<sub>10</sub> values for vitamin E isomers and oryzanol ................................................................. 103
Table IV-14 Major lipid classes of crude bran oil extracted with a mixture of chloroform and methanol, and their fatty acid composition ........................................... 110

Table IV-15 Major lipid classes of crude bran oil extracted with petroleum ether and their fatty acid composition ......................................................... 112

Table IV-16. Effect of post extrusion holding time on free fatty acid during storage ........................................................................................................... 114

Table IV-17. Free fatty acid composition of raw bran and stabilized rice bran during storage .......................................................................................... 119

Table IV-18. Composition of neutral lipid in raw bran and stabilized rice bran during storage .......................................................................................... 120

Table IV-19. Composition of phospholipid in raw bran and stabilized rice bran during storage .......................................................................................... 128

Table IV-20. Neutral lipid contents in raw and stabilized rice bran during storage ........................................................................................................... 129

Table IV-21. Major lipid classes of crude bran oil extracted with a mixture of chloroform and methanol, and their fatty acid composition ........................................... 133

Table IV-22. Moisture and free fatty acid, and temperature of rice bran as affected by microwave treatment ................................................................. 135

Table IV-23 Free fatty acid composition of raw and microwave treated rice bran during 52 weeks storage ........................................................................... 140

Table IV-24 Tocopherol, tocotrienol, and oryzanol content of raw rice bran and rice bran after microwave treatment ................................................................. 142

Table IV-25 Composition of phospholipid in raw bran and rice bran heated by microwave during 52 weeks storage ........................................................................... 151

Table IV-26. Changes of free fatty acids and phospholipids in rice bran immediately following gamma irradiation ................................................................. 153

Table IV-27. Free fatty acid composition of raw and irradiated rice bran during 52 weeks storage ................................................................................. 159
Table IV-28. Tocopherol, tocotrienol, and oryzanol of raw and irradiated rice bran ....................................................... 162

Table IV-27. Retention of vitamin E vitamers in irradiated rice bran during 52 weeks storage ........................................ 165

Table IV-30. Composition of phospholipid in raw and irradiated rice bran during 52 weeks storage .......................... 168

Table IV-31. Composition of neutral lipid from raw and irradiated rice bran during 52 weeks storage .................... 172
LIST OF FIGURES

Fig. IV-1. Chromatogram of a mixture of a soybean oil and wheat germ oil; solid peaks represent fraction cuts. Chromatographic conditions are described in text and in table IV-2. .......................... 56

Fig. IV-2. Chromatogram of a mixture of a wheat bran oil and latex lipid; solid peaks represent fraction cuts. Chromatographic conditions are described in text and in table IV-3. .......................... 56

Fig. IV-3. Chromatograms at wavelengths 295 nm, 245 nm and the ratio of the two for (A) δ-tocopherol and (B) δ-tocotrienol. .... 60

Fig. IV-4. UV-visible spectra of isolated and purified tocopherols and tocotrienols. ................................................................. 62

Fig. IV-5. Gas chromatograms of isolated and purified α-tocopherol and α-tocotrienol. ................................................................. 66

Fig. IV-6. High-performance liquid chromatography chromatogram (HPLC) of standards of vitamin E vitamers. Chromatographic conditions: Supelcosil LC-Si, 250 x 4.6 mm 5μm column (Supelco, Bellefonte, PA); mobile phase, eluent 1; flow rate, 1.6 ml/min. ........................................................................................................... 71

Fig. IV-7. Changes of retention time of vitamin E for 72 samples. Twenty-four samples were analyzed each day for three consecutive days without reactivation of column. HPLC conditions as in Fig. IV-6. except for mobile phase, eluant 2. ................ 72

Fig. IV-8. Changes of retention time of vitamin E for two hundred consecutive sample analyses. Two hundred samples were analyzed continuously for about 63 hr without reactivation. HPLC conditions as in Fig. IV-6. except for mobile phase, eluent 3. ........................................................................................................... 73

Fig. IV-9. Chromatograms of rice bran samples. Chromatogram A is first injection and chromatogram B two hundredth injection. Chromatographic conditions as in Fig. IV-8. T, tocopherol; T3, tocotrienol. ........................................................................................................... 75

Fig. IV-10. UV-visible spectra of isolated and purified oryzanol. ...... 77
Fig. IV-11. Chromatogram of isolated and purified oryzanol
Chromatographic condition are described in text. .................. 77

Fig. IV-12. Chromatograms of rice bran samples. Chromatogram A is
method I and chromatogram B, method II. Chromatographic
conditions are described in text. ............................................... 79

Fig. IV-13. Tocopherols and tocotrienols in raw rice bran during
storage. ..................................................................................... 87

Fig. IV-14. Tocopherols and tocotrienols in rice bran extruded at
110°C with holding time 0 min during storage. ......................... 91

Fig. IV-15. Tocopherols and tocotrienols in rice bran extruded at
140°C with holding time 6 min during storage. ......................... 93

Fig. IV-16. Total vitamin E vitamers in rice bran extruded at 110,
120, 130, and 140°C with holding time 0 min during storage. .... 94

Fig. IV-17. Total vitamin E vitamers in rice bran extruded at 110,
120, 130, and 140°C with holding time 3 min during storage. .... 95

Fig. IV-18. Total vitamin E vitamers in rice bran extruded at 110,
120, 130, and 140°C with holding time 6 min during storage. .... 96

Fig. IV-19. Arrhenius plots for degradation of α-, β-, and γ-
tocopherol and α-tocotrienol in rice bran on post extrusion
processing (-- 95% confidence interval). .................................. 105

Fig. IV-20. Arrhenius plots for degradation of δ-tocopherol, γ- and δ-
tocotrienol, and oryzanol in rice bran on post extrusion
processing (--95% confidence interval). ................................. 106

Fig. IV-21. Arrhenius plots for degradation of total tocopherol and
total tocotrienol in rice bran on post extrusion processing (--
95% confidence interval). ....................................................... 107

Fig. IV-22. Total free fatty acid in raw rice bran and stabilized rice
bran stored for one year. ............................................................ 116

Fig. IV-23. Phospholipid in raw rice bran and stabilized rice bran
stored for one year. ................................................................. 122
Fig. IV-24. Regression of total vitamin E vitamer content on phospholipid content. The correlation is significant (p < 0.01); r=Correlation coefficient. ......................................................... 124

Fig. IV-25. Regression of total FFA content on phospholipid content. The correlation is significant (p < 0.01); r=Correlation coefficient. .......................................................... 125

Fig. IV-26. Regression of total vitamin E vitamer content on total FFA content. The correlation is significant (p < 0.01); r=Correlation coefficient. ......................................................... 126

Fig. IV-27. Hunter color values of stabilized rice bran stored at ambient temperature for one year. ................................................................. 131

Fig. IV-28 Total free fatty acid in raw rice bran and microwave treated rice bran during 52 weeks storage. ........................................................ 136

Fig. IV-29. Tocopherols and tocotrienols in raw rice bran during 52 weeks storage. ................................................................. 143

Fig. IV-30. Tocopherols, tocotrienols, and oryzanol in raw rice bran and microwave treated rice bran during 52 weeks storage. ... 146

Fig. IV-31. Phospholipid in raw rice bran and microwave treated rice bran during 52 weeks storage. ........................................................ 148

Fig. IV-32. Total free fatty acid in rice bran oil extracted from raw and irradiated rice bran during 52 weeks storage. .................. 156

Fig. IV-33. Total E vitamers and oryzanol in raw and irradiated rice bran during 52 weeks storage. ............................... 166

Fig. IV-34. Changes of phospholipid in rice bran oil extracted from raw and irradiated rice bran during 52 weeks storage. ........... 171
LIST OF ABBREVIATIONS

a.m.u  atomic mass unit
BHA  butylated hydroxy anisole
BHT  butylated hydroxy toluene
bp  boiling point
ch.  chapter
cis-  stereochemical isomer opposite of trans-
°C  degree celsius
d-  dextro (rotatory)
dl-  racemic mixture; optically inactive
DMP  2,2-dimethoxypropane
$E_{1cm}^{1%}$ the absorbance of a solution containing 1g/100mL contained in a cell having an absorption path of one centimeter.
ed.  edition
Ed(s)  editor(s)
et al.  (et alii) and others
etc.  et cetera
eV  electron volt
FAME  fatty acid methyl ester
FFA  free fatty acid
FIG.  figure(s)
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Meaning</th>
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<tbody>
<tr>
<td>g</td>
<td>gram</td>
</tr>
<tr>
<td>g/l</td>
<td>gram per liter</td>
</tr>
<tr>
<td>GC</td>
<td>gas chromatograph or gas chromatography</td>
</tr>
<tr>
<td>GC/MS</td>
<td>combined GC and mass spectrometer</td>
</tr>
<tr>
<td>GL</td>
<td>glycolipid</td>
</tr>
<tr>
<td>GLC</td>
<td>gas-liquid chromatography</td>
</tr>
<tr>
<td>HDL</td>
<td>high density lipoprotein</td>
</tr>
<tr>
<td>HPLC</td>
<td>high performance liquid chromatography</td>
</tr>
<tr>
<td>hr</td>
<td>hour</td>
</tr>
<tr>
<td>ID</td>
<td>inner diameter</td>
</tr>
<tr>
<td>IU</td>
<td>international units (of vitamins)</td>
</tr>
<tr>
<td>kg</td>
<td>kilograms(s)</td>
</tr>
<tr>
<td>KGy</td>
<td>kilogray (1 gray = 100 rad)</td>
</tr>
<tr>
<td>l-</td>
<td>levo (rotatory)</td>
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<tr>
<td>L-</td>
<td>levo (in configuration sense only)</td>
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<tr>
<td>L</td>
<td>liter</td>
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<tr>
<td>LDL-C</td>
<td>low density lipoprotein cholesterol</td>
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<td>m</td>
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<td>max</td>
<td>maximum, maxima</td>
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<td>MHz</td>
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T3 tocotrienols
TBA thiobarbituric acid
THF tetrahydrofuran
TL total lipid
TLC thin-layer chromatography
trans- stereochanical isomer opposite of cis-
UV ultraviolet
UV-vis ultraviolet-visible
vit. E vitamin E
VLDL very low density lipoprotein
vs versus
v/v volume-to-volume ratio of a solution
w/w weight-to-weight ratio
14:0 myristic acid (Tetradecanoic)
16:0 palmitic acid (Hexadecanoic)
18:0 stearic acid (Octadecanoic)
18:1 oleic acid (cis-9-Octadecenoic)
18:2 linoleic acid (cis,cis-9,12-Octadecadienoic)
18:3 linolenic acid (all cis-9,12,15-Octadecatrienoic)
20:0 arachidic acid (Eicosanoic)
\( \lambda_{\text{max}} \) UV absorption maxima
\( \mu \) micro

xvii
<table>
<thead>
<tr>
<th>Symbol</th>
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<tbody>
<tr>
<td>μg</td>
<td>microgram</td>
</tr>
<tr>
<td>μl</td>
<td>microliter</td>
</tr>
<tr>
<td>=</td>
<td>equals</td>
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<td>&gt;</td>
<td>greater than</td>
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<td>≥</td>
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ABSTRACT

The effects of extrusion temperature and post extrusion holding time, microwave heating, and gamma irradiation on antioxidants and lipid composition in rice bran were studied. Post extrusion holding time had no apparent effect on rice bran stability. Increased holding times reduced (p < 0.05) total vitamin E. Oryzanol concentration was lower (p < 0.05) only with 6 min holding time. Inactivation of lipolytic enzymes was obtained by extrusion temperatures of 120, 130, and 140°C. Increased extrusion temperatures reduced the retention of vitamin E and oryzanol during storage. An extrusion temperature of 110°C provided the highest retention of vitamin E and oryzanol during storage although FFA level was higher (p < 0.05) than that of the other extrusion temperatures from 105 to 375 days.

There were no significant (p > 0.05) decreases in individual vitamin E vitamers until 4 min microwave heating. After 8 min heating, individual E vitamers decreased significantly (p < 0.05) with exposure time. There was no significant increase (p > 0.05) in FFA level by 24 weeks of storage with microwave heating for 12 min. Microwave heating for 4, 8, and 12 min did not provided adequate inactivation of lipolytic enzymes. Total vitamin E loss was between 7 and 37% during microwave heating and a further 75-80% reduction occurred during storage.
FFA level in rice bran irradiated at 5 kGy was not significantly different from raw rice bran. Increases in irradiation dose of 10 and 15 kGy resulted in an increase in FFA levels. Gamma irradiation in rice bran did not inactivate lipolytic enzymes in the range used in this study. Gamma irradiation did have deleterious effect on lipid stability in rice bran during irradiation and storage. The decomposition of individual E vitamers increased with increased irradiation level. Decomposition of oryzanol increased ($p < 0.05$) with an increase from 5 to 10 kGy. The loss of total E vitamers and oryzanol occurred in two stages: 50-82% and 12-33% immediately following irradiation, and a further 10-35 and 39-42% during storage.
CHAPTER I
INTRODUCTION

Rice production in Louisiana in 1992 was about 1.44 million ton and the by-product from rice processing, rice bran was one hundred fifteen ton. Louisiana is the third leading state in production of rice, accounting for 14% of U.S production in 1992. National production of by-products from rice processing currently amounts to about 1.5 billion tons of rice hulls and 0.6 billion tons of rice bran (U.S. Department of Agriculture, 1990).

Rice bran has traditionally been used for livestock feed rather than for human use because the removal of bran from the grain mixes enzymes such as lipase, lipoxygenase, and peroxidase with oil in the bran that hydrolyses rapidly and can produce a high level of free fatty acids within hours (Linfield et al., 1985; Khor et al., 1986; Kim et al., 1987; Pillaiyar, 1995). These fatty acids produce acid bite and a soapy taste when they exceed about 15% in the oil (Young et al., 1991).

Rice bran oil has been reported to be one of the most effective vegetable oils for lowering serum cholesterol (Suzuki and Ochima, 1970; Sharma et al., 1987; Raghuram et al., 1989; Kahlon et al., 1989). In an animal study carried out by U.S Department of Agriculture scientists, it was found that regular full-fat stabilized or parboiled bran lowered serum cholesterol in hamsters with initial high cholesterol. However, the defatted
rice bran did not significantly lower serum cholesterol. In human studies (Gerhardt and Gallo, 1989; Hegsted et al., 1990) involving a combination of males and females with moderate to high cholesterol levels, it was also demonstrated that stabilized rice bran was equivalent to oat bran in reducing cholesterol.

Rice bran contains 10-23% oil. Three major fatty acid, palmitic, oleic, and linoleic, make up about 90% of the total fatty acids. Tocopherols and tocotrienols represent of 0.11% of crude rice bran oil (Sayre and Saunders, 1988). Tocopherols are effective antioxidants even at low levels and may be pro-oxidants at high levels. Another important class of antioxidant compounds in rice bran is ferulic acid esters, commonly called oryzanol. The unsaponifiable fraction of rice bran oil has 20% oryzanol. The excellent resistance of rice bran oil to oxidation is thought to be due not only to the tocopherol content but to esters of ferulic acid (Saunders, 1985). Tocotrienols are present in similar quantities and, in the case of barley oil, tocotrienols inhibit cholesterol synthesis in the liver. Barley oil tocotrienols have been shown to be a serum cholesterol lowering agents when included in the diet of chicks, pigs, and humans (Qureshi et al., 1986). In addition, tocotrienols have been shown to lower plasma glucose in normal and diabetic mice. Oryzanol is used in Japan for treatment of nervous system disorders and gastrointestinal problems, as a health food additive and as an ingredient in veterinary medicines (Young et al., 1991).
In addition to being a source of high quality vegetable oil, rice bran is an excellent source of many valuable derivatives. The distillates, soaps, and waxes produced in the oil-refining processes are sources for many potentially valuable and useful products used in pharmaceutical, cosmetic, and other applications. Among these are fatty acids, oryzanol, tocopherols, tocotrienols, gums and waxes, and inositol. Additionally, the defatted bran is a source of such compounds as dibasic calcium phosphate, phytic acid, calcium-magnesium phytate, inositol, vitamin B, and protein concentrate.

Utilization of rice bran for human consumption was not feasible until a practical method of stabilization was developed. Technology has been developed to stabilize rice bran by heating with an extrusion cooker, oven, or microwave, freezing, and treating with chemical, thereby preventing enzymatic hydrolysis of the oil in the bran (Young et al., 1991). The most common method of stabilization of rice bran is with the extrusion cooker. This process can stabilize the rice bran for several months (Haumann, 1989).

Stabilized rice bran can be used as a source of edible oil and the defatted rice bran can be used as an animal feed or human food. After it is stabilized, rice bran is usually stored for up to a several months until rice bran oil is extracted or marketed as a full-fat product. Even if rice bran is stabilized properly, compounds susceptible to oxidation lipids and antioxidant compounds would be subject to losses during storage. Most studies (Enochian et al., 1981; Rhee et al., 1984; Sayre, 1985; Saunders et al.,
evaluating changes of compounds in stabilized rice bran during short-term storage (within three months) have focused on free fatty acids, fatty acids and lipase. There is an urgent need to develop an appropriate stabilization and storage technology to maximize retention of the valuable components, in addition to lowering FFA level, during stabilization and post-stabilization storage. Otherwise, the many valuable components and efforts made in developing a successful rice bran stabilization technology will be wasted. No information is available on changes of vitamin E vitamers, oryzanols, and lipid composition in stabilized rice bran during long-term storage.

The specific objectives of the research were:

1. To develop analytical methods for vitamin E vitamers and oryzanols.
2. To determine stability of vitamin E vitamers and oryzanols to heat and irradiation.
3. To determine retention of vitamin E vitamers and oryzanols during storage.
4. To determine changes of composition of fatty acid in rice bran during storage.
5. To determine the relationship between fatty acids and antioxidants in rice bran during storage.
A. Rice Bran and Rice Bran Oil

1. Composition of Rice Bran

Rice bran protein (9-16%) is of relatively high nutritional value. Digestibility of protein in rice bran is reported to be 73%, whereas in the extracted concentrates, protein digestibility was greater than 90% (Saunders, 1990). The major carbohydrates are cellulose, hemicelluloses, and starch, 5-15%. Sugar in rice bran ranges from 3 to 8% (Saunders, 1985). The dietary fiber content of rice bran varies with the degree of milling and the amount of starch present in the bran. The ranges for soluble and total dietary fiber are 1.8-2.6% and 20-25%, respectively (Saunders, 1990). Rice bran typically contains 10-23% oil. The range in mineral and vitamin contents reflect rice variety, degree of milling, and growing environment. Sodium is relatively low, whereas potassium is high (Saunders, 1985; Sayre and Saunders, 1985). Vitamin A and C contents are very low, whereas B-complex vitamins are high (Sayre and Saunders, 1985). The occurrence of some antinutritional compounds has been reported. Among these are trypsin inhibitor, pepsin inhibitor, phytates, and an antithiamine factor (Saunders, 1985; Hargrove, 1990). Activities of these compounds are relatively low and can be inactivated by heat treatment.
2. Composition of Rice Bran Oil

The main component of crude rice bran oil, the triglycerides, make up approximately 80% of the oil. Palmitic, oleic, and linoleic acids comprise 90% of fatty acids in rice bran oil. Partial esters present as mono- and diglycerides and the free fatty acid are a reflection of the hydrolysis that has occurred (Saunders, 1990; Sayre and Saunders, 1985; Hargrove, 1990).

The minor constituents of an oil consist of phospholipids, glycolipids, and unsaponifiable matter. The phospholipids present are phosphatidylcholine, phosphatidylethanolamine, and phosphatidyl-inositol (Hargrove, 1990). The glycolipids are mainly galactose and glucose derivatives. At 4 - 5.2% of the crude oil, the unsaponifiable material in rice bran oil is unusually high compared to other vegetable oils. The unsaponifiable content of many common oilseed oils is normally only 0.4-0.6% (Sayre and Saunders, 1985; Gutifinger ant Letan, 1974). The broad class of unsaponifiable matter of rice bran oil consists of approximately 42% sterols, 24% higher alcohols, 20% ferulic acid esters, 19% hydrocarbons, and 2% unknown (Hargrove, 1990). The unsaponifilables are generally represented by the sterol fraction of the oils. These include free sterols, sterol ester, sterylglycosides, and acylsteryl glycosides. 8-Sitosterol is the most abundant sterol present (50% of total sterol) (Luh, 1991). Oryzanols, present at 0.96-2.9% of bran oil (Okada and Yamaguchi, 1983), are ferulic acid esters. Wax concentration in the crude oil is dependent on the extraction
method and origin of the bran. Wax may comprise about 3-9% of rice bran oil. Generally, the higher the extraction temperature, the greater the quantity of wax removed from the bran when hexane is used as the extraction solvent (Sayre and Saunders, 1985). Tocopherols are present in relatively high quantities at 0.07-0.12% (about 5% of the unsaponifiable fraction). \( \alpha \)-Tocopherol, \( \alpha \)-tocotrienol, \( \gamma \)-tocopherol, and \( \gamma \)-tocotrienol constitute the major fractions present.

3. Health Benefits of Rice Bran Oil

**Fatty Acid**: Numerous studies have demonstrated that diets high in saturated fatty acids raise total serum cholesterol and, in particular, low density lipoprotein cholesterol (LDL-C) levels (Schaefer et al., 1981). Diets high in unsaturated fatty acid (McDonald et al., 1989; Hegsted et al., 1965; Mattson and Grundy, 1985; Sirtori et al., 1986) lower LDL-C when replacing saturated fat. The mechanism(s) involved in the hypocholesterolemic action of the unsaturated fatty acids are not well understood, although studies (Nicolosi et al., 1989) would suggest that unsaturated fatty acids prevent the down regulation of the LDL receptor normally observed during intakes of saturated fat and cholesterol.

**Unsaponifiable Components**: The unsaponifiable materials in rice bran oil are largely sterols and cyclic triterpene alcohols of similar structures. Plant sterols have long been known to diminish absorption of cholesterol due to competition with cholesterol for incorporation into
micelles for transport across the intestinal cell wall, and most vegetable sterols are poorly absorbed (Sayre and Saunders, 1985; Grundy, 1989; Grundy and Mok, 1977; Lees et al., 1977; Heinemann et al., 1986). Three grams of β-sitosterol per day will lower cholesterol absorption by 50%, but higher levels do not increased the effect (Best et al., 1954). The beneficial effects of other unsaponifiables have also been demonstrated. For example, recent studies by Qureshi et al. (1991) in humans and various animal models (Qureshi et al., 1987; Qureshi et al., 1984) suggest that tocotrienol and an unsaponifiable component of palm oil, inhibits cholesterol synthesis and lowers serum cholesterol. Tocotrienols have also been shown to influence certain parameters of hemostasis (Abeywardena et al., 1991) and alter the occurrence of chemically induced tumors in rodent models (Tan and Chu, 1991; Could et al., 1991).

Another group of unsaponifiables, γ-oryzanol have also been demonstrated to have certain beneficial effects. When the unsaponifiable fraction of soy sterol was further purified and fractionated, both cyloartenol and 24-methylene cyloartenol, which are main constituents of the triterpene alcohols in soybean oil unsaponifiable matter, reduced plasma cholesterol and enhanced cholesterol excretion when fed to rats (Kiribuchi et al., 1983). Similar lipid-lowering oryzanols have inhibitory effects on platelet aggregation (Seetharamaiah et al., 1990) as has also been reported by Yoshino et al. (1989). When oryzanol was fed to human subjects at the rate
of 300 mg/day for three months, significant lowering of total serum cholesterol was found to be due mainly to reduction in the LDL fraction and there was a trend for HDL to increase. The conclusion was that the reduction in cholesterol concentration was due both to inhibition of cholesterol absorption from the intestine and to reduction of cholesterol biosynthesis in the liver (Yoshino et al., 1989). Also, oryzanol has been reported to have various hormone-like effects on the autonomic nervous system (Okada and Yamaguchi, 1983; Luh, 1991) with promotion of human and animal growth and facilitation of blood circulation. Oryzanol has been shown to maintain estrous cycle in rats, and to increase capillary development in skin (Sayre and Saunders, 1985; Saunders, 1990).

**Rice Bran Oil:** Sharma and Rukmini (1986) showed that rice bran oil fed to rats at a 10% level significantly lowered total cholesterol (-37%), very low density lipoprotein (VLDL+LDL-C) (-52%) and raised high density lipoprotein cholesterol (HDL-C) (+82%) compared to groundnut oil. These alterations in lipoprotein cholesterol were associated with a 136% increase in neutral steroids and a 47% increase in total bile acids in rats fed rice bran oil compared to groundnut oil. In a similar study of the hypocholesterolemic activity of rats fed rice bran oil compared to groundnut oil (Seetharamaiah and Chandrasekhara, 1989), decreases in serum total cholesterol (-38%) and VLDL + LDL-C (-39%), and a 27% increase in HDL-C were reported. Serum cholesterol levels were further reduced by 20% upon addition of
oryzanol to the diet. These changes in lipoprotein cholesterol levels were associated with decreases in liver cholesterol content (30%) in rats fed rice bran oil compared to groundnut oil. Raghuram et al. (1989) fed 35-40g of either rice bran oil or some combination of coconut oil, palm oil, or groundnut oil to human subjects and reported 25-30% reductions in total serum cholesterol levels in the rice bran oil group. Although interesting, these results need to be qualified because of the highly saturated fatty acid nature of coconut oil and the sparse information on peanut oil. However, a preliminary report in humans that compared rice bran oil to other vegetable oils as part of a blend of oils that are more normally consumed, confirmed the cholesterol-lowering properties of rice bran oil (Lichtenstein et al., 1991). They also suggested that factors other than fatty acids in rice oil were responsible for its cholesterol-lowering effects. A preliminary report (Nicolosi et al., 1991) demonstrated that rice oil fed to hamsters not only showed cholesterol-lowering properties but, in addition, had strong antioxidant characteristics and a striking reduction in aortic fatty streaks, the initiating event of atherosclerosis.

The explanation for the hypocholesterolemic action of rice bran oil is unknown, but many of its unsaponifiable components have cholesterol-lowering activity due to various mechanisms (Hargrove, 1990). For example, ferulate esters of plant sterols and triterpene alcohols such as oryzanol can inhibit dietary cholesterol absorption and enhance fecal sterol
and bile acid secretion. The high content of tocotrienols in rice bran oil suggests that cholesterol synthesis may be inhibited in individuals fed rice bran oil. Either one of these mechanistic responses can result in the deregulation of the LDL receptor, thereby increasing hepatic uptake of circulating LDL (Hargrove, 1990).

B. Methods of Stabilization of Rice Bran

The utilization of rice bran as a food is limited to some extent by its fiber content, but the major obstacle is its instability. Enzymes, both natural to the bran and of microbial origin, are the major cause of bran deterioration. Lipases are responsible for these deteriorative changes by promoting hydrolysis of the bran oil into glycerol and free fatty acids (FFA) (Houston, 1972; Young et al., 1991; Pillaiyar, 1995). Hydrolysis and oxidative rancidity are associated with this deterioration. The consumer experiences to bitterness and a soapy taste (Barber and de Barber, 1980). Within the intact rice kernel, lipases are localized in the testa-cross layer region, while the oil is localized in the aleurone and germ (Shastry and Raghavendar Rao, 1971). Once these regions are disrupted during the milling process, enzymatic hydrolysis is possible and proceeds rapidly. Microorganisms present on the surface of the kernel would also then have access to the bran oil (Duckworth and Dent, 1946).
1. Refrigeration

Cold storage of rice bran has been found effective in retarding the rise of free fatty acids (Kumar David et al., 1965). Keeping bran in a freezer for up to 60 days resulted in an increase of 1.5 per cent of FFA (Pillaiyar, 1995).

2. Chemical Methods

Use of chemicals for inactivating lipase was suggested by Loeb et al. (1949). Lipase activity was completely lost upon heating at 60°C with 5% alcohol for 20 min. At 20-30% alcohol, 100% enzyme inactivation was achieved in 2 min at 60°C (Duckworth and Dent, 1946). Lipase is sensitive to powerful oxidizing agents like iodine, H₂O₂, n-bromosuccinate o-nitrophenylsulfenyl chloride, etc. (Shastry and Raghavendar Rao, 1976). Heavy metals (Cu, Zn), CN and F also inactivate the lipase (Pillaiyar, 1995). Recently, a method for inactivating lipase with concentrated HCl has been successfully developed (Prabhakar and Venkatesh, 1986). The acid stabilization method utilizes the reduction or inhibition of activity of the lipase enzyme when the pH of the bran is reduced from 6.5 to 4.0.

3. Irradiation

The rate of FFA development in the stored bran separated from the rice kernel was much slower in the samples irradiated with gas plasma than in the control (Roseman et al., 1963); but the composition of oil extracted from the irradiated rice bran was found to be altered.
4. Heat Stabilization

Heat stabilization of rice bran has been widely used and found suitable (Young et al., 1991). Advantages of heat stabilization are that it can simultaneously kill bacteria, molds and insect eggs, that cause further spoilage (Pillaiyar, 1995). Heat stabilization has been carried out using an extruder, expeller, oven, microwave energy or steam. Three general types of heat stabilization procedures have been reported: retained-moisture heating, which requires that the bran be heated under pressure to prevent moisture loss until completion of the heating phase; added-moisture heating, in which the moisture content of the bran is increased during heating, followed by drying; and dry heating at atmospheric pressure.

Dry heating is attractive because of its simplicity of operation and availability of rice hulls as a fuel source, but reports on effectiveness of stabilization have been conflicting (Loeb et al., 1949). Equipment that retains the ambient moisture in the bran under pressure throughout heating and then utilizes the added heat to drive off some of the moisture as pressure is released appears to be effective and efficient (Sayre et al., 1982).

Added-moisture heating methods apply steam to increase the moisture content and heat the bran. In cases where steam is not available, water presumably could be added and allowed to equilibrate before application of heat. Studies have shown that the rate of enzyme inactivation increases as moisture content of the bran increases (Barber and de Barber, 1980). The
shorter, milder heat treatment does less nutritional damage to the bran proteins and vitamins than does dry heating, which may produce high and nonuniform temperatures. The most severe restriction on the use of added-moisture heat stabilization is the requirement for a steam boiler, and the cost of installing a boiler, associated plumbing, and satisfactory water supply. Another disadvantage is that the bran must be dried after heating (Sayre et al., 1982).

In retained-moisture heating methods, the ambient bran moisture is sufficient to aid in heat transfer enzyme denaturation, and sterilization. When the pressure is released after heating, part of the superheated moisture evaporates rapidly, and little or no subsequent drying is necessary. Two general retained-moisture heating methods have been described; extrusion cookers, and the sealed rotating drum. The advantages of both the extrusion cooker and the rotating drum systems are that they do not require an external steam supply, are relatively compact, and are simple to install and operate (Sayre et al., 1982). This infers that such stabilization systems can be added to existing rice mills with only minor modification and little or no additional cost beyond that for the stabilization equipment. A disadvantage is that power and heat is usually derived from utility electric service (Barber and de Barber, 1980).

Extrusion cooking has been used to stabilize rice bran and has been successful in rural environments in processing grain and legume seed.
Simplicity of installation and operation and no requirement for steam generation or subsequent drying of the bran are advantages of the process. Previous studies have indicated that the method may be economically viable in some countries under present conditions.

C. Antioxidants in Rice Bran

1. Vitamin E Vitamers

**Chemistry:** The name "tocopherol" comes from the Greek, meaning "to bear offspring". Tocopherols are methyl-substituted hydroxy chromans with an isoprenoid side chain (Lang et al., 1992). They are composed of two homologous series: the tocopherols, with a saturated side chain, and the tocotrienols, with a side chain unsaturated between carbons 3' and 4', 7' and 8', and 11' and 12'. The tocopherols and tocotrienols are designated alpha(α)-, beta(β)-, gamma(γ)-, and delta(δ)-, and differ according to the number and position of the methyl groups in the chromanol nucleus; the β- and γ-forms are positional isomers. Due to the presence of chiral centers at position 2 of the chroman ring and the 4' and 8' carbons of the terpenoid side chain, tocopherols can exist in eight diastereomeric forms; moreover, tocotrienol double bonds can form cis and trans isomers. To date, only all-trans-tocotrienols have been found in nature. α-Tocopherol occurs naturally only as the 2R, 4'R, and 8'R isomer (RRR-tocopherol, d-α-tocopherol), whereas pharmaceutical tocopherol supplements often contain synthetic α-tocopherol racemates. Commercial vitamin supplements often contain vitamin
E esters such as tocopheryl acetate or tocopheryl succinate (Lang et al., 1991). Tocopherols and tocotrienols in the pure form are pale yellow viscous oils. They are readily soluble in alcohol and other organic solvents, and can be found in many vegetable oils. Vitamin E acetates are less readily soluble in ethanol than free vitamin E (Nelis et al., 1985).

**Biological Activity:** Biological activities or biopotencies of vitamin E active compounds as determined by the classical rat fetal resorption test vary from less than 0.1 to 1.7 IU. The International Unit of activity (IU) was defined as the activity of 1 mg 2RS-α-tocopheryl acetate (d,l-α-tocopheryl acetate) (Nelis et al., 1985). Bioassays are notoriously difficult to standardize, and this is reflected in conflicting opinions as to the true bioactivity of the individual tocopherol and tocotrienol homologs and their stereomer (Parrish, 1980). There is agreement, however, that RRR-α-tocopherol (d-α-tocopherol) is the most potent compound. Esters of RRR-α-tocopherol have similar potencies; a value of 1.66 IU was suggested for 1 mg of RRR-α-tocopheryl acetate (Lang et al, 1992). The β and γ homologs are only half as active, and δ-tocopherol even less (Lang et al., 1992).

**Stability:** Vitamin E in the unesterified form is slowly oxidized by atmospheric oxygen to form mainly biologically inactive quinones, such as α-tocopherylquinone. Small amounts of various dimers and triradiiuses are also formed. Oxidation of vitamin E is accelerated by exposure to light, heat, alkaline pH conditions, and the presence of certain trace minerals, such as
iron (Fe$^{3+}$) and copper (Cu$^{2+}$) (Nelis et al., 1985; Lang et al., 1992). Ascorbic acid, if present, completely prevents the catalytic effect of ferric and cupric ions by maintaining the metals in their lower oxidation state (Cort et al., 1978).

Vitamin E vitamers are stable to heat and to alkali in the absence of oxygen and are unaffected by acids, up to 100°C. The vitamers are stable to visible light, but are destroyed by UV light, especially in the presence of oxygen (Association of Vitamin Chemists, Inc., 1966; Scott, 1978).

Natural Occurrence: The richest dietary sources of vitamin E are the cereal seed oils (Yuki and Ishikawa, 1976; Analytical Methods Committee, 1959; Syvaoja et al., 1986; Guzamn and Murphy, 1986, Hassapidou and Manoukas, 1993; Piironen et al., 1986a), and the margarines, salad dressings and other products made from them. The distribution of tocopherols and tocotrienols in different plant oils varies greatly. Vegetable materials and oils, nuts and seeds in particular, are rich sources of vitamin E. Tocopherols are widely distributed in animals, cereals, and fruits, but tocotrienols are mostly absent in nuts, fruits, feedstuffs, and vegetables (Folstar, et al. 1977; Parrish, 1980; Syvaoja et al., 1986; Barnes and Taylor, 1980; Wang et al., 1993; Piironen et al., 1986). Variable quantities of tocotrienols are found in carrots, sweetcorn, and cereal bran and germ oils (Govind Rao and Perkins, 1972; Syvaoja et al., 1986; Piironen et al., 1985; Piironen et al.,
1986). Rice bran oil and palm oil contain over 70% tocotrienol (Wilkinson, 1987; Cottrell, 1991).

Since animals and humans are unable to synthesize this vitamin, they have to rely on an external supply through their diet. Consequently, products of animal origin are relatively poor in this vitamin (Ball, 1995). d-α-Tocopherol is the predominant form in plasma and tissues. Until now, no detectable amounts of δ-tocopherol or tocotrienols have been found in humans or animals under physiological conditions (Lang et al., 1992). More stable dl-tocopheryl acetate and succinate esters have been synthesized and are commonly used to fortify foods and feeds. The recommended daily intake of vitamin E can be estimated at 5-15 mg (expressed as dl-α-tocopheryl acetate) (Ball, 1995).

2. γ-Oryzanol

Chemical Structure and Characteristics: In 1954, Kaneko and Tsuchiya separated a new compound (melting point(mp), 137.5-138°C) from rice bran oil and rice germ oil, which showed three absorption maxima at 230, 290, and 315 μm in heptane (Tanaka et al., 1971). Since it was isolated from rice bran oil (Oryza sativa L.) and contained a hydroxyl group, it was conveniently named oryzanol (Graf, 1992). Oryzanol was considered to be a pure compound at first. However, subsequent studies revealed that oryzanol is not a single compound but instead comprises a variety of ferulic acid esters called α-,β- and γ-oryzanol. Thus, oryzanol is mixtures of esters
of ferulic acid with sterols and triterpene alcohols, predominantly \( \beta \)-sitosterol, campesterol, cycloartenol, and 24-methylene-cycloartanol (Sayre and Saunders, 1985). Of these, \( \gamma \)-oryzanol has been the best characterized. The triterpene alcohol components of a typical \( \gamma \)-oryzanol consist primarily of cycloartenol and 24-methylene cycloartanol. The \( \gamma \)-oryzanol especially has beneficial health effects. The composition of ferulate in oryzanols extracted from crude rice bran oil by solvent fractional crystallization is 42.6% 24-methyl-cycloartanol, 35.2% cycloatenol, 14% campesterol, 8.1% \( \beta \)-sitosteryl, and trace amount of stigmasterol (Kim and Kim, 1991).

The physical properties of oryzanols such as melting point, color, and absorption maxima varies slightly from different source of rice bran. Tomataro et al. (1957) extracted oryzanols with mp 137.5-138.5°C, \( (E_{1cm}^{1%}=358.9) \), and mp 143-144°C, \( (E_{1cm}^{1%}=361.0) \) from different rice bran oils. Even though oryzanols are fat-soluble, they are minimally soluble in non-polar solvent such as petroleum ether, hexane, and isoctane which normally solubilize oil compounds. Oryzanols are extracted with less non-polar solvents because oryzanols are coextracted with oil components during extraction. Oryzanols are dissolved well in more polar solvents such as acetone, acetic acid, diethyl ether, any alcohol and alkaline solution. The oryzanols are classified as unsaponifiable even though they possess ester bonds because they are minimally hydrolyzed at normal saponification condition. During saponification or alkaline refining, the oryzanols remain
in aqueous solution and in an intermediate layer (Tomataro et al., 1957). Normal refining procedures remove about 80% of oryzanols from crude oil.

Some authors reported that antioxidant activity of oryzanols was similar to that of vitamin E (Sayre and Saunders, 1985; Luh, 1991). Others have reported that oryzanols are relatively weaker antioxidants than vitamin E (Ramarathnam et al., 1989; Ramarathnam et al., 1986). At 0.01% \( \alpha \)-tocopherol and oryzanols solution, \( \alpha \)-tocopherol had higher antioxidant activity than oryzanol (Okada and Yamaguch, 1983). With increasing concentration of \( \alpha \)-tocopherol up to 0.5%, the antioxidant activity did not increase much, but with a similar increase in oryzanols the activity increased proportionally. A mixture of \( \alpha \)-tocopherol and oryzanols did not show synergic effects. Oryzanols were found to be highly stable to heating compared to \( \alpha \)-tocopherol, butylated hydroxy anisole (BHA), and butylated hydroxy toluene (BHT) (Okada and Yamaguch, 1983).

\( \gamma \)-Oryzanol markedly inhibits oxidation of rice bran oil, malondialdehyde generation during iron-mediated microsomal lipid peroxidation, and formation of dienes during peroxidation of linoleic acid by UV irradiation. Due to its excellent emulsifying properties and high UV absorption, \( \gamma \)-oryzanol also is the active ingredient in various cosmetic preparations like skin creams, suntan lotions, and cosmetic soaps (Graf, 1992).
**Natural Occurrence:** Oryzanol comprises 1.5-2.9% of rice bran oil and rice germ oil. Corn oil contains about 0.5% oryzanol. But oryzanols are absent in soybean oil, cotton seed oil, rape seed oil, and other vegetable oils. Oryzanol contents in rice bran is dependent on variety, growing environment, and storage. Rice brans with high acid values contain higher amounts of oryzanol than those of low acid value (Okada and Yamaguchi, 1983).

**Preparation of Oryzanol:** There are many known methods for separation of oryzanols. These methods utilize pH adjustment, adsorbents (active alumina, active carbon, active earth, silica gel), ion exchange resin, distillation, solvent fractional crystallization, and acetylation of oryzanol. The methods using pH adjustment and adsorbents are generally applied to separation of oryzanols (Okada and Yamaguchi, 1983).

**D. Factors that Influence Vitamin E and Fatty Acid Content from Various Sources**

1. **Drying**

   Natural drying by exposure to the sun reduced the level of tocopherols in clover and corn leaves to zero in 4 to 5 days (Ames, 1972). When the drying was undertaken in the shade, the level fell more slowly; after fifty days in store, the level was 52 to 60% of the level in the fresh material. Thafvelin and Oksanen (1966) observed the effects of drying on the tocopherol, linolenic acid and peroxide values in timothy grass, red clover and tufted hair grass dried in the sun, or by artificial means. With hay dried on hay poles, the tocopherol loss was about the same (about 48%) as when
dried in cocks; when dried in swaths, the loss was about 60%. During forage cutting and drying, changes occur in fatty acid composition (Thafvelin and Oksanen, 1966; Vander Veen and Olcott, 1967). Losses of \(\alpha\)-tocopherol during commercial-scale dehydration in alfalfa production ranged from 5 to 33%, and larger losses (54 to 73%) occurred during storage. Adams et al. (1975) recorded vitamin E deficits in 16 samples of light-weight corn amounting to 21% below values for normal sound corn. Artificial drying of corn, when well controlled, can be effectively carried out without the destruction of vitamin E or unsaturated fatty acids.

2. Acid Treatment

Another approach to the preservation of high moisture grain is the addition of organic acids as an antifungal agent. When Madsen et al. (1973) added 1% propionic acid to moist barley grain and stored the grain for 6 months, the propionic acid-treated barley contained 0.3 mg of tocopherols as compared to 3.3 mg/100 g for untreated and dried barley. Komoda and Harada (1969) found that the addition of water and propionic acid to raw soybeans resulted in extensive oxidation of the tocopherols within 7 days. A rapid rise in the peroxide value of high-moisture grain as compared to that in dry grain may account for the destructive action of \(\alpha\)-tocopherol in most grains.
3. Milling

The degree of milling was recognized as an important contributor to vitamin E composition. Bauernfeind (1977) reported wheat flours of 100, 50, and 30% extraction (whole wheat to refined white flour) to contain progressively less tocopherol. Milling rye and triticale (Bauernfeind, 1977) also resulted in lower tocopherol containing flour fractions. Wet milling of corn was investigated by Howland et al. (1973). Commercial milling appears to have little effect on the content of \(\alpha\)- and \(\gamma\)-tocopherols in corn germ oil. The tocopherol composition of corn germ oil from hand-dissected germ was about the same as that of the germ recovered from a wet milling plant. According to Grams et al. (1971) the recoveries of \(\alpha\)- and total tocopherols were 68 and 73%, respectively, after dry milling, and 18 and 27%, respectively, after wet milling.

4. Heating

Various forms of heating maybe applied to many types of foods during processing or during preparation of food for table use. Chow and Draper (1974) investigated the stability of natural tocopherols and tocotrienols in corn and soybean oil heated at 70°C and aerated at 100 mL/min. In corn oil, \(\alpha\)-tocopherol and \(\alpha\)-tocotrienol were destroyed faster than \(\gamma\)-tocopherol and \(\gamma\)-tocotrienol. At an equivalent concentration of 0.020% in stripped corn oil, the order of antioxidant activity of the natural tocopherols was \(\gamma > \delta > \beta > \alpha\). Tocopherol oxidation and peroxide formation occurred more rapidly in
corn than in soybean oil. LeCoq (1944) showed that one-third of tocopherol was lost when wheat-germs were heated on a water-bath; but direct heating to 120 - 150°C caused a loss of 89% in 45 minutes. Feldheim and Thomas (1957) cooked wheat at 90°C for 795 minutes and found a loss of 19% of the vitamin E present. Ramanujan and Anantakrishnan (1958) heated groundnut oil to 175°C for a half and an hour and found a loss of 32%; but after adding 0.02% of propyl gallate or butylhydroxyanisole this loss was reduced to 11%. According to Morand and Silvestre (1960) grape-seed oil lost 20% of its total tocopherols after being heated for 130 - 200°C for half an hour. Parkhurst et al (1968) had reported the order of stability as γ > δ > α in a lard system at a temperature of 97°C.

The exact order of antioxidant activity is influenced by concentration (Lea and Ward, 1959; Moree and Bickford, 1952). Chow and Darper (1974) commented that the marked differences in the stabilities of raw and refined oils have been attributed to variations in the amount of antioxidants present and in fatty acid composition, but other organic and inorganic constituents are probably involved.

5. Freezing

Bunnell et al. (1968) were the first investigators to note that low storage temperatures do not prevent the oxidation of the tocopherols so that substantial losses can occur with time. There is a great loss of tocopherol.
in potato chips stored at room temperature and in french fried potatoes and potato chips stored frozen.

6. Storage

Storage of oils results in considerable destruction of tocopherol, depending on the oil, time, temperature, and concentration of antioxidants (Bauernfeind, 1977; Nazir and Magar, 1961; McLaughlin, 1979). The tocopherol content in castor oil was reduced 90% during storage at 10°C for 1 year, while that of coconut oil was only reduced 25% over a similar storage period. Safflower oil lost 45% of its tocopherol content after storage at 10°C for 6 months, 55% of the tocopherol after storage at room temperature for 3 months, and 70% of the tocopherol after storage at 37°C for 3 months. Tocopherols are apparently nature's choice of an antioxidant (Hove and Harris, 1951) as is indicated by a high correlation between the total tocopherol level and the combined levels of linoleic and linolenic acids in a number of oil-bearing plants. Kohler et al. (1955) demonstrated that the loss of tocopherol during storage depends on the temperature; rye-grass meal storage for six weeks at 3°C showed a loss of only 8% but the loss rose to 49% under storage at 60°C. Livingston et al. (1968) reported that the loss of tocopherol depends on the moisture content of dehydrated meals; the loss in lucerne meal rose from 54 to 73% when the moisture in meal rose from 2.5 to 12%. In freeze-dried meals, the vitamin E loss was only between 5% and 28%. The vitamin E level in wheat meal was reduced by 90% after 63
days in storage (Mühlefluh, 1963); and the level in wheat germ was reduced by 24-30% after 80 days in storage (Rothe et al., 1958). The level in wheat flour fell 58-65% after storage for 80 days. Hence, minimal mulling will result in higher vitamin E levels in animal feeds. Nordfeldt et al. (1962) found that wheat-germ held at 4°C and at 20°C lost only some 5 or 10% of its vitamin E after 6 months in storage. Tosic and Moore (1945) found a vitamin E loss in wheat germ oil of 83% (0.98 mg/100 g/month) after five months in storage. Nazir and Magar (1961) found that the level of vitamin E in groundnut oil (raw or refined) fell between 62% and 88% after four months in storage when the temperature rose from 10°C to 43°C.

7. Irradiation

The effects of irradiation on the vitamin E level in foods are complex because multiple reactions due to irradiation and processing occur simultaneously. Irradiating α-tocopherol dissolved in tributyrin in the presence of air to 10, 50, or 100 kGy produced a 51%, 78%, or 95% loss of tocopherol, respectively, (Diehl, 1971). The vitamin E content of wheat was decreased by irradiation at ambient temperatures in the presence of air (Vakil et al., 1973). Thayer et al. (1991) reported that the loss of tocopherol in oatmeal steadily increased as the irradiation temperature was increased from at -180°C 7% to at 50°C 46%. Irradiating food in the presence of air or oxygen usually results in greater destruction of α-tocopherol. The peroxides and hydroperoxides that are formed during irradiation of foods can destroy
α-tocopherol (Thayer et al., 1991). Kraybill (1960) reported that there was no loss of α-tocopherol in beef irradiated under an atmosphere of nitrogen; under an oxygen atmosphere, 60% and 80% losses of tocopherol were found when the beef was irradiated to 30 or 300 kGy. Oats that were packaged, irradiated to 1 kGy, and stored for 8 months under nitrogen lost only 5% of their tocopherol content compared with a 56% loss in oats irradiated and stored in air (Diehl, 1971). Vacuum packaging was as effective as nitrogen in protecting tocopherol for the first 3 months of storage but was less effective after 3 months. Packaging under carbon dioxide rather than air provided no protection to α-tocopherol. Kung et al. (1953) reported tocopherol destruction to the extent of 29 to 61% in fluid milk irradiated with gamma rays at 1 to 4 KGY. The tocopherol content of Manitoba wheat, English wheat (Tipples, 1965), and German oats (Bauernfeind, 1977) decreased with increasing radiation. Ramarathnam et al. (1989) found that the level of α-tocopherol in rice seeds decreased 72 to 78% after γ irradiation.

E. Analytical Method for Vitamin E Vitamers

1. Thin-Layer and Paper Chromatography (TLC and PC)

Before the advent of gas-liquid chromatography, TLC and PC were the best general methods to separate tocopherols from one another. TLC was a significant advancement from earlier colorimetric vitamin E assays, such as the Emmerie-Engel method (Bro-Rasmussen and Hjarde, 1957; Quackenbush
et al., 1963; Parker and McFarlane, 1940; Eggitt and Norris, 1956), which were nondiscriminating for tocopherols and were subject to interferences from other reducing substances, including antioxidants present for the protection of tocopherols (Parrish, 1980). Today, TLC has value as a straightforward and inexpensive qualitative or semiquantitative assay method. For quantitative analyses, high-performance liquid chromatography (HPLC) or gas chromatography (GC) has surpassed TLC, as quantitative TLC assays lack precision, are labor intensive, and are difficult to automate (Nelis et al., 1985; Lang et al., 1992). TLC systems are capable of separating tocopherol and tocotrienol homologs. One-dimensional systems are often satisfactory (Rao and Rao, 1965); additional resolution can be achieved by developing in a second dimension (Whittle and Pennock, 1967). Quantitation can be done in situ by densitometry or by the more traditional approach of eluting the spots from the plate and performing colorimetric assays (Yuki and Ishikawa, 1976; Herting and Drury, 1969; Cohen and Michel, 1978; Ames and Tinkler, 1962; Booth, 1961). TLC is also popular as a sample cleanup for other quantitative procedures, such as GC (Meijboom and Jongenotter, 1979; Erickson et al., 1973; Lovelady, 1973; Strohecker and Henning, 1966).

2. Gas-Liquid Chromatography (GLC)

Gas-liquid chromatography as a method to separate and determine tocopherols originated in the early 1960s and was developed rapidly over the next 10 years. The transition from packed columns to capillary columns has
brought more resolving power to separate isomeric tocopherols and
tocotrienols (Slover et al., 1983), improved separation from interfering
compounds, and lower detection limits. Capillary columns have evolved from
coated columns to chemically bonded phases, which are more durable, and
give more reproducible chromatography than coated capillary columns or
packed columns. Tocopherols can be assayed by GC with or without prior
derivatization. Tocopherols and their derivatives are conveniently monitored
by flame ionization detectors (Govind Rao and Perkins, 1972; Diplock;
1985). Mass spectrometric detection (GC-MS) provides additional
identification of GC peaks through characteristic mass spectra and superior
selectivity in the selected ion monitoring (SIM) mode (Govind Rao and
Perkins, 1972; Der Greef et al., 1986; Snyder et al., 1993; Rogers et al.,
1993).

Although GC can separate all tocopherols and tocotrienols, it's
application is limited to a simple sample matrix and requires saponification
and derivatization of sample, and over 40 minutes analysis in time.

3. High-Performance Liquid Chromatography (HPLC)

High-performance liquid chromatography (HPLC), also called high-
pressure, high-precision, high-speed, etc. liquid chromatography, has
developed as a procedure to determine vitamin E in the early 1970s. HPLC
is carried out on normal phase columns of silica gel with a single
hydrocarbon solvent or gradient mixtures of an alkane (hexane, heptane,
isoctane) with more polar solvents (ethanol, isopropanol, butanol, tetrahydrofuran, methyl, t-butyl isopropyl) as a mobile phase. Reversed-phase columns made up of hydrocarbon chains bonded to silica can also be used employing a mixture of acetonitrile, methanol, isopropanol or water as mobile phase (Parrish, 1980).

Reversed phase offers some practical advantages, e.g., column stability, reproducibility of retention times, and fast equilibration over normal phase, and, hence, is preferable for routine analysis of biological samples (Nelis et al., 1991). In addition, it is ideally suited to separate homologs, since retention on this type of support is essentially governed by the number of carbon atoms in a molecule. But Polar bonded phases have found only limited application for vitamin E liquid chromatography because they are generally inferior to normal phase with regard to the resolution of positional isomers (β- and γ-isomers) (Nelis et al., 1985; Ball, 1995).

Unlike reversed-phase, normal phase chromatography on a polar stationary column allows fast and easy differentiation of positional isomers. This is in agreement with the well-known stereochemical selectivity of silica acid for isomers. In 1974, the first separation of all eight natural tocopherols and tocotrienols was accomplished on a pellicular Corasil II Column (particle size, 37-50 μm) with diisopropyl ether-hexane (5:95, v/v) (Cavins and Inglett, 1974). Substitution of the pellicular by a microparticulate column (particle size, 3-5 μm) resulted in a dramatic improvement (Thompson and
Hatina, 1979; Tan and Brzusiewicz, 1989; Rammell and Hoogenboom, 1985; Cort et al., 1983). The same separation was now completed in 15 min as opposed to 112 min on the pellicular column.

F. Mathematical Modeling

Thermal processes are important methods for extending the storage life of foods and have been used extensively in combination with other preservation techniques. The basic function of a thermal process wherein the temperature of the product is elevated above ambient temperature is to eliminate or reduce microorganisms and/or enzymes that would, upon storage, result in deterioration of the food or would endanger the health of the consumer. The term "thermal process" can be applied to any process in which heat energy is transferred to or from the product. If one is to predict the extent of deterioration of important nutritive factors during processing, a knowledge of the reaction rate as a function of temperature and moisture content is needed (Labuza, 1972).

1. Reaction Order

Reaction order is the sum of the exponents of the reactant concentration terms in the rate equation.

Zero-Order Reactions:A characteristic of a zero order reaction is a linear relationship between the concentration of the reactant or product and the time of the reaction.
\[
\frac{dA}{dt} = k
\]
or
\[
A = A_o + kt
\]

Where \(A_o\) = Concentration of A at time = 0
\(A\) = Concentration of nutrient or chemical at time = \(t\)
\(k\) = rate constant with units of \((\text{time})^{-1}\)
\(t\) = Reaction time

First-Order Reactions: \(A_o\) is the concentration of A at time = 0. A first-order reaction is characterized by a logarithmic change in the concentration of a reactant with time. Most of the reactions involved in the processing of foods are first-order reactions; thiamin degradation in meats (Holdsworth, 1985); carotene degradation in liver (Wilkinson et al., 1981); ascorbic acid degradation in peas (Rao, 1981); ascorbic acid in wheat flour and infant cereals (Vojnovich and Pfeifer, 1970); nonenzymatic browning in milk (Burton, 1954); chlorophyll degradation in peas (Holdsworth, 1985); sensory quality loss in various foods (Lund, 1977); polyphenol oxidase in mushrooms (McCord and Kilara, 1983).

\[- \frac{dA}{dt} = kA\]
or
\[
\ln \left( \frac{A}{A_o} \right) = kA
\]
2. Temperature Dependence of Reaction Rates

The Arrhenius Equation: The activated complex theory for chemical reaction rates is the basis for the Arrhenius equation, which relates reaction rate constants to the absolute temperature. The Arrhenius equation is:

\[ k = k_o \exp \left( - \frac{E_a}{RT} \right) \]

where:
- \( k \) = rate constant in the nutrient or chemical destruction reaction
- \( k_o \) = constant, pre-exponential or frequency factor, independent of temperature
- \( E_a \) = activation energy
- \( R \) = ideal gas constant, 1.986 cal/mole °K,
- \( T \) = absolute temperature

Taking the natural log of both sides,

\[ \ln k = \ln k_o - \frac{E_a}{R} \left( \frac{1}{T} \right) \]

yields a linear equation of the form \( Y = A + BX \). The activation energy can therefore be determined by the slope of the plot of \( \ln(k) \) vs. \( 1/T \). The negative sign is placed on the exponent of the Arrhenius equation so that a positive activation energy will indicate an increasing reaction rate constant with increasing temperature (Toledo, 1991). The higher the activation energy the greater the effect an increase in temperature has on the reaction (Labuza, 1972). Typical values of activation energy for different food constituents have been reported (Labuza, 1972; Lund, 1972). Degradation of ascorbic acid during heat processing had an \( E_a \) of 10 to 30 Kcal/mole, lipid oxidation
an $E_a$ of 15 to 25 Kcal/mole, nonenzymatic browning an $E_a$ of 25 to 50 Kcal/mole, Enzyme destruction an $E_a$ of 12 to 100 Kcal/mole, and hydrolysis an $E_a$ of 15 Kcal/mole. However, Arrhenius relationships have limited applicability because the range of validity and the influence of other factors on activation energy must be considered (Tannenbaum, 1975).

The $Q_{10}$ Value: The $Q_{10}$ value of a reaction is often used for reporting the temperature dependence of biological reactions. It is defined as the multiplicable change in reaction rate with a 10°C change in temperature. If a reaction rate doubles with a 10°C change in temperature, $Q_{10} = 2$. A rule of thumb in food storage is that a 10°C reduction in storage temperature will increase the shelf life by a factor of 2. The relationship between the $Q_{10}$ value and the activation energy is derived as follows:

$$\ln Q_{10} = 2.3 \log \frac{k(T+10)}{k_T} = \frac{E_a 10}{R \left[\frac{T}{T+10}\right]}$$

or

$$\log Q_{10} = \frac{2.189 E_a}{[T][T+10]}$$

where $k_T$ = rate constant at absolute temperature, $T$
$k_{(T+10)}$ = rate constant at absolute temperature($T$) + 10
$E_a$ = activation energy
$R$ = ideal gas constant, 1.986 cal/mole °K,

The $Q_{10}$ value is temperature dependent and should not be used over a very wide range of temperature.
CHAPTER III
MATERIAL AND METHOD

A. Experiment 1: Isolation of Four Tocopherols and Four Tocotrienols

1. Analytical Approach

Pure standard grade tocopherols (α, γ, δ) are available commercially, but tocotrienol standards are not. Recently released standards for γ- and δ-tocopherol (ICN Biochemicals, Division, CA, U.S.A) are expensive and/or of lower purity. Research on rice bran composition and oxidative stability required analysis of endogenous tocopherols and tocotrienols. The purpose of the present study was to develop a procedure for isolation of highly purified tocopherols and tocotrienols as analytical standards from natural sources using semi-preparative liquid chromatography.

2. Chemical and Material

All solvents were HPLC grade from Mallinckrodt (Paris, KT, U.S.A). L-ascorbic acid was from Sigma (St. Louis, MO, U.S.A.). Rubber latex was obtained from Malaysia, and soybean oil, wheat bran, and wheat germ were purchased from a local grocery.

3. Extraction of Crude Oil

Rubber latex was used as a source for α-, γ-, and δ-tocotrienols, soybean oil for α-, γ-, and δ-tocopherol, wheat bran for β-tocotrienol, and wheat germ for β-tocopherol. To facilitate isolation, a mixture of soybean oil
and wheat germ oil was used for tocopherols, as was a mixture of rubber latex lipid and wheat bran oil for tocotrienols.

For extraction of crude wheat germ and bran oil, 20 g of wheat germ or 40 g of wheat bran was placed in a 500 mL Erlenmeyer flask with 200 mL ethanol and 5 g ascorbic acid. The mouth of the flask was covered with a beaker and placed in a 60°C water bath for 10 min. Then 1.2 mL of 80% KOH was quickly added and mixed by vortexing. The sample was saponified for 10 min at 80°C. During saponification, the sample was agitated using a wrist type shaker. After saponification, the flask was placed in an ice bath, and 30 mL water and 50 mL hexane were added. The mixture was vortexed, transferred to centrifuge bottles, and centrifuged at 120 x g for 1 min. The upper layer was transferred to a 500 mL separatory funnel. Extraction of the sample with 50 mL hexane was repeated twice. The pooled hexane layer was washed three times with 30 mL water to remove residual KOH, filtered through Na₂SO₄, and then evaporated to dryness on a rotary evaporator. The crude oil sample was diluted with 10 mL methanol and the mixture was allowed to stand overnight at -20°C. The mixture was centrifuged, 12,000 x g, at -20°C for 30 min and the supernatant filtered through a 0.45 μm filter. The filtrate was diluted with 20 mL water and extracted with 20 mL hexane twice. Solvent was evaporated to dryness under a stream of nitrogen and sample diluted with a known amount of hexane and placed under nitrogen at -20°C. For extraction of latex oil, the Whittle method (Whittle et al.,
1966) was employed with modification. Samples of latex (50 mL) were added to 500 mL chloroform with stirring and then homogenized in a Tissumizer (Tekmar Company, Cincinnati, OH, U.S.A.) for 5 min. To this mixture 250 mL methanol was added and stirred until the rubber coagulated. The coagulated rubber was filtered, then dried on a rotary evaporator under reduced pressure.

Saponification and crystallization (Whittle et al., 1966) of latex oil (4 g) and commercial soybean oil (4 g) were as described previously.

4. Apparatus

Semi-Preparative HPLC: The semi-preparative HPLC system that was used consisted of Waters (Milford, MA, U.S.A.) M-45 and 510 pumps, a Waters 680 automated gradient controller, a Waters 470 scanning fluorescence detector with 18 nm spectral bandwidth for excitation and emission, a Hewlett Packard (San Fernando, CA, U.S.A.) UV-VIS diode-array detector (series 1050), and a Waters 715 Ultra WISP injector equipped with a 2000μl loop and 200μl syringe. Chromatograms were recorded and peaks determined using a Baseline 810 Chromatography workstation (Waters, Milford, MA, U.S.A.). Concentrated extracts were injected into a 25 cm x 10 mm diameter column of 10 μm Alltech Econosil Silica (Deerfield, IL, U.S.A.). The column was used with 5 cm X 4.6 mm ID guard column packed with 40 μm Supelco pellicular silica (Bellefonte, PA, U.S.A.). The mobile phase consisted of a gradient of 0-15% tetrahydrofuran in hexane at a flow
rate of 8 - 9 mL/min, and the eluate was monitored from the fluorescence detector at 290 nm excitation and 330 nm emission. Eluates considered as α-, β-, γ-, and δ-tocopherols and tocotrienols in each experiment were collected into 250 mL amber bottles with teflon cap using a Gilson Model 202 Fraction Collectors (Beltline-Middleton, WI, U.S.A). Collection bottles were placed in a 50°C water bath and solvent evaporated in the dark using ultra high purity nitrogen. The pooled specimens of α-, β-, γ-, and δ-tocopherols and tocotrienols were rechromatographed until the specimens were chromatographically and spectrophotometrically pure. The pooled specimens were also concentrated. To check purity of each isomer, the absorption ratio of 295 nm to 245 nm wavelength with 4 nm bandwidth and 390 nm reference wavelength were compared using a diode array detector. The concentrations of fractionated vitamin E vitamers were determined using a Gilford UV-VIS Spectrophotometer (Oberlin, OH, U.S.A).

For semi-preparative HPLC, solvent A was 40% THF in hexane (V/V), and solvent B was 100% hexane. The Solvents were filtered through Millipore 0.45 μm membranes prior to use. Tetrahydrofuran was distilled (Riddick and Bunger, 1973) to remove peroxides that might form during storage prior to mobile phase incorporation.

Analytical HPLC: The analytical HPLC system was similar to the semi-preparative system with modifications as follow. Samples (0.5-5μl) were injected using a 200 μl loop and 25 μl syringes into a 25 cm length x
4.6 mm diameter column of 5 μm Sulpecosil™ LC-Si (Supelco, Bellefonte, PA, U.S.A.). The column was preceded by a 5 cm X 4.6 mm ID guard column packed with 40 μm pellicular silica. The mobile phase consisted of 2.4% ethyl acetate in isoctane at a flow rate of 2.0 mL/min, and the eluate was monitored from the fluorescence detector. Isolated and purified tocopherols and tocotrienols were used as standards.

Gas Chromatography/Mass Spectrometry (GC/MS): Purities of isomers were determined using a J&W Scientific (Folsom, CA, U.S.A.) 95% dimethyl-(5%)-diphenylpolysiloxane capillary column (0.25-μm stationary phase thickness, 30 m x 0.25 mm I.D.) on a Hewlett-Packard (San Fernando, CA, U.S.A.) 5890 gas chromatograph equipped with a split/splitless capillary inlet system and a flame ionization detector. Column oven temperature was programmed to increase from an initial temperature of 40°C to a final temperature of 280°C. Oven temperature was maintained at the initial temperature for 3 min after injection and then increased at 20°C/min to a temperature of 280°C for 40 min. Other operation parameters were as follows: injector temperature, 300°C; detector temperature, 350°C; helium carrier gas flow, 30 cm/second; split ratio, 1/50. A Maxima 820 Chromatography workstation was used to determine peak areas (Waters, Milford, MA, U.S.A.).

Mass spectra of tocopherols and tocotrienols were obtained by a Hewlett-Packard 5890 series II gas chromatograph/5971A mass spectrometer
with splitless (holding time, 0.75 min) injection. GC column and temperature program was as described previously. Additional conditions were as follows: ionization voltage, 70 eV; electron multiplier voltage, 1800; scan range, 40-450 a.m.u.; interface temperature, 280°C; injector temperature, 250°C.

B. Experiment 2: Development of a Mobile Phase System

1. Analytical Approach

Normal-phase high performance liquid chromatography (HPLC) allows fast and easy separation of positional isomers that reversed-phase HPLC does not. However, the reversed-phase approach is preferred over normal-phase in more than 70 percent of recently published procedures (Lang et al., 1992). The reason is that reversed phase chromatography offers certain practical advantages, e.g., column stability, reproducibility of retention times, and quicker equilibration as discussed in a previous chapter. On the other hand, reversed-phase systems, using water and methanol in mobile phase, have the disadvantages of pronounced deterioration of the peak shape of lipophilic compounds and may cause partial precipitation of lipophilic compounds (Nelis et al., 1985). Normal-phase columns would provide superior separation of vitamin E vitamers if the problems of column deactivation by water or polar compounds could be overcome. All organic solvents contain an inherent amount of water in the ppm range that is sufficient to deactivate normal-phase columns during long term usage. The purpose of this
experiment was to develop a mobile phase system using 2,2-dimethoxypropane (DMP) that would improve column stability and reproducibility on normal-phase columns for analysis of multiple rice bran samples.

2. Materials

Isooctane, ethyl acetate, and glacial acetic acid were purchased from Mallinckrodt (Paris, KY). DMP (98%) was obtained from Aldrich Chemical Company (Milwaukee, Wis). L-ascorbic acid was purchased from Sigma (St. Louis, MO). Tocopherols and tocotrienols were prepared by semi-preparative HPLC from natural sources, as described in a previous study.

3. Preparation of Crude Rice Bran Oil

Five hundred mg sample was placed in a 15 mL test tube with 5 mL ethanol and 0.1 g ascorbic acid. The test tube was placed in an 80°C water bath for 10 min, following which 0.15 mL of 80% KOH was added. The sample was saponified for 10 min at 80°C. After saponification, the flask was placed in an ice bath and 5 mL water and 5 mL hexane were added. The mixture was transferred to centrifuge bottles and centrifuged at 120 x g for 1 min. The upper layer was transferred to a 125 mL separatory funnel. Extraction of the sample with 5 mL hexane was repeated twice. The pooled hexane layer was washed three times with 5 mL water, filtered through Na₂SO₄ and then evaporated under a stream of nitrogen. The crude oil sample was diluted with 1 mL isooctane.
4. High-Performance Liquid Chromatography.

The HPLC system consisted of Waters (Milford, MA) M-45 pump, 715 Ultra WISP injector, 470 scanning fluorescence detector with excitation at 290 and emission at 330, A Sulpecosil™ (Supelco, Bellefonte, PA) LC-Si, 5 μm, column, 15 cm x 4.6 mm i.d. was used. Eluant 1 was isooctane:ethyl acetate (97.5: 2.5), eluant 2 was isooctane:ethyl acetate:acetic acid (97.3 :1.8:0.9), and eluant 3 was isooctane:ethyl acetate:acetic acid:DMP (98.15:0.9: 0.85:0.1) with a flow rate of 1.6 mL/min. To reactivate the column, it was flushed with methylene chloride, then isopropanol, and methanol, following which the solvent sequence was reversed. Alternatively, isooctane:ethyl acetate:acetic acid:DMP (87:10:1:2) was used to reactivate the column, instead of the previous method, with a flow rate of 2.5 mL/min for 20 min.

C. Experiment 3: Effect of Extrusion Cooking on Vitamin E and Oryzanols in Rice Bran

1. Analytical Approach

The purpose of this experiment was to determine the stability of vitamin E vitamers and oryzanols at 110, 120, 130, and 140°C extrusion temperature and to examine the effect of holding time (0, 3, and 6 min) on stability of vitamin E vitamers and oryzanols. Also, retention of vitamin E vitamers and oryzanols was determined during one year storage.
2. Materials

Isooctane, ethyl acetate, and glacial acetic acid were purchased from Mallinckrodt (Paris, KY). 2,2-Dimethoxypropane (DMP), 98%, was obtained from Aldrich Chemical Company (Milwaukee, Wis). L-ascorbic acid was purchased from Sigma (St. Louis, MO). Tocopherols and tocotrienols were prepared by semi-preparative HPLC from natural sources, as previously described. Oryzanol standards were isolated from crude rice bran oil using solvent fractional crystallization (Kim and Kim, 1991).

3. Sample Description

Raw Rice Bran: Three-hundred grams of raw rice bran from each treatment combination was packed in whirl plastic bag (Koch supplies inc., Kansas City, Mo) and stored at ambient temperature (23 - 24°C). Zero day samples were immediately vacuum packed and placed in an ultra-low freezer at -80°C until analyzed. The rest of the bran was randomly sampled at 7 days, 165 days, 210 days, and 375 days, vacuum packed and stored in an ultra low freezer until analyzed.

Extruded Rice Bran: The extrusion and sample randomization procedure used was as described by Martin et al. (1991). A Food-Ex model 1002L (Food Ex. Corp., Extrusion Eldorado hills, CA) fitted with a thermocouple to monitor bran exit temperature was used in this study. At the beginning of extrusion, the extruder was brought to a temperature of 110°C and allowed 15 min warm up period. At the exit port of the extruder, an
insulated auger was used for maintenance of temperature during holding. For zero post extrusion holding time, 300 g of rice bran were placed into whirl plastic bags without going through the auger, and at this point sampling and replication were randomized. When indicated in the randomization scheme, the holding auger speed was adjusted for a retention time of three and six minutes. After 110°C extrusion was completed, the temperature was raised to 120, 130, and 140°C, and at each temperature a procedure similar to that used at 110°C was followed for holding and sampling.

4. Vitamin E Vitamers and Oryzanol Quantification

Sample Preparation for HPLC: 1) The first analytical method was based on the method reported by Shin and Godber (1993). Five hundred mg rice bran was placed in a 15-mL test tube with 5 mL ethanol and 0.1 g ascorbic acid. The test tube was placed in an 80°C water bath for 10 min, after which 0.15 mL of 80% KOH was added. The sample was saponified for 15 min at 80°C. After saponification, the flask was placed in an ice bath, and 3 mL water and 5 mL hexane were added. The mixture was transferred to centrifuge bottles and centrifuged at 120 x g for 1 min. The upper layer was transferred to a 125 mL separatory funnel. Extraction of the sample with 5 mL hexane was repeated twice. The pooled hexane layer was washed three times with 5 mL water, filtered through Na₂SO₄ and then evaporated under a stream of nitrogen. The crude oil sample was diluted with 1 mL isoctane.
ii) A second analytical method which differed from the first in that 4 mL of water was added and 6 mL diethyl ether instead of 3 mL water and 5 mL hexane was also used to determine vitamin E loss.

Recovery Determination: Recovery studies were performed by adding tocopherols and tocotrienols into the 0.5 g rice bran sample. Amounts added were 65, 7, 42, and 5 μg for α-, β-, γ-, and δ-tocopherol and 50, 150, and 20 μg for tocotrienols, and 3600 μg for oryzanol. These amounts were similar to the contents of tocopherols, tocotrienols, and oryzanol in 0.5 g fresh rice bran. To determine retention of tocopherols, tocotrienols and oryzanol in extracted rice bran, the aqueous layer was discarded after three extractions with hexane, then 5 mL ethanol and 0.15 g ascorbic acid were added. The test tube was placed in an 80°C water bath for 3 hr, after which 0.15 mL of 80% KOH was added. The sample was saponified for 15 min at 80°C. Further extraction conditions were as described previously in sample preparation.

High-Performance Liquid Chromatography: The HPLC system consisted of Waters (Milford, MA) M-45 pump, 715 Ultra WISP injector, 470 scanning fluorescence detector with excitation at 290 nm and emission at 330 nm, Sulpecosil™ (Supelco, Bellefonte, PA) LC-Si, 5 μm, column, 15 cm x 4.6 mm i.d. was used. The mobile phase was isooctane:ethyl acetate:acetic acid:DMP (98.15 :0.9: 0.85:0.1) with a flow rate of 1.6 mL/min.
5. Statistical Analyses

A statistical analysis of the results was performed using the SAS General Linear Model for analyses of variance and covariance analysis. Differences were considered significant when means of compared sets differed at the p < 0.05 level of significance.

D. Experiment 4: Quality of Rice Bran Oil Extracted from Extruded Rice Bran

1. Analytical Approach

The aim of this study was to determine the effect of extrusion temperature and post extrusion holding time on free fatty acid composition, neutral lipid and phospholipid in rice bran oil during one year storage.

2. Material

Methanol, chloroform, hexane, petroleum ether, and acetone were purchased from Mallinckrodt (Paris, KY). Sodium chloride (NaCl) and potassium bicarbonate (KHCO₃) were obtained from EM Science (Gibbstown, New Jersey). Sodium sulphate and heptadecanoic acid methyl ester, 90% were purchased from Sigma (St. Louis, MO). The methyl ester mixture (Mixture Kit No. 2) and methanolic HCl reagent kits were purchased from Alltech (Deerfield, IL).

3. Lipid Analysis

Extraction: Rice bran (15 g) was extracted overnight at 3°C with a 40 mL mixture of chloroform and methanol (2:1, v/v) twice, and the extract was filtered and evaporated under vacuum on a rotary evaporator at 40°C to
obtain the crude oil. Also, crude rice bran oil was extracted by Soxhlet extractor with petroleum ether to compare the fatty acid composition in cruded oil with the above method.

Degumming and Dewaxing: The crude oil (2 g) was heated to 60°C and 1% water was added while stirring the oil gently. The hydrated gums were allowed to settle overnight after which oil was centrifuged at 100 x g. The supernatant oil was decanted. The degummed oil was chilled at 3°C for 48 hr and then was centrifuged at 3°C at 100g. The supernatant oil was decanted. This degummed and dewaxed oil was used for neutral lipid and free fatty acid fractionation.

Fractionation

1) Column Chromatography (Chan and Taniguchi, 1985)

The crude lipid extracts were chromatographed into neutral lipid (NL), glycolipid (GL), and phospholipid (PL) fractions by a silica gel column (25 mm i.d. x 30 cm). A loading ratio of 2 g lipid extract/100 g silica gel was used. Before packing, the silica gel was prepared by first washing with methanol, then with H₂O to remove fines. The silica gel was then activated overnight in an oven at 120°C, mixed with the first elution solvent (CHCl₃) and packed in the column as a gel. The NL was eluted with 12 mL CHCl₃/g silica gel, the GL with 48 mL acetone/g silica gel, and the PL with 12 mL CH₃OH/g silica gel at flow rates of 1, 0.5, and 1 mL/min, respectively.
These solvents were removed in a rotary evaporator under reduced pressure at 40°C.

ii) Liquid-Liquid Fractionation (Ramarathnam et al., 1989; Lee and Mattick, 1960)

Phospholipid Fraction: The acetone-soluble fraction was made up mainly of neutral lipid (NL), glycolipid (GL) and free fatty acid (FFA), and unsaponifiable matter. The acetone-insoluble matter fraction comprised the phospholipids (PL). The crude oil (1.0 g) was treated with HPLC grade acetone (20 mL x 2) to separate the acetone-soluble fraction from the acetone-insoluble material. The mixture stood at 0°C for 30 min and was filtered. The insoluble PL was dissolved in a 20 mL mixture of chloroform and methanol (2:1, v/v) and was filtered, and the filtrate was evaporated to dryness in a stream of nitrogen at 40°C. The residue of PL was transferred to sample bottles, dried and weighed.

Free Fatty Acid and Neutral Lipid: The FFA was separated as sodium salts using sodium bicarbonate from degummed and dewaxed oil and then the sodium salts are acidified with H₂SO₄ to obtain the free fatty acid form.

The soluble mixture of FFA and NL was evaporated to dryness, transferred to a separatory funnel and treated with a 30 mL mixture of diethyl ether and hexane (1:1, v/v) and 0.5% Na₂CO₃ (5 mL). The contents were shaken vigorously for 30 seconds and allowed to stand for 20 min. The
bottom aqueous layer was drawn into another separatory funnel. The upper portion, diethyl ether layer was treated with 0.5% Na$_2$CO$_3$ (5 mL). The contents were shaken vigorously for 30 seconds and allowed to stand for 20 min. The bottom aqueous layer was pooled into a separatory funnel. The aqueous layer was acidified with five mL of 10% H$_2$SO$_4$ and was extracted with 10 mL diethyl ether three times. The diethyl ether extracts were pooled, washed with distilled water until free from acid and dried to dryness. The FFA samples were transferred into sample bottles, dried and weighed. The diethyl ether layer left after separation of the FFA was washed with 20 mL distilled water twice, dried over anhydrous Na$_2$SO$_4$, filtered, and evaporated in a stream of nitrogen at 40°C.

Preparation of Fatty Acid Methyl Ester (FAME): The rice bran oil (up to 50 mg) was dissolved in hexane (1 mL) in a 15 mL test tube and 5% methanolic hydrogen chloride (2 mL) and 1 mL (1μg/μL) heptadecanoic acid methyl ester was added as internal standard. The mixture was refluxed for 8 hr at 80°C, then water (5 mL) containing 5% sodium chloride was added and the required esters were extracted with 5 mL hexane twice using Pasteur pipettes to separate the layers. The hexane layer was washed with 10 mL 5% potassium bicarbonate twice and dried over anhydrous sodium sulphate. The solution was filtered and the solvent removed in a stream of nitrogen at 40°C water bath (Christie, 1982).
Analysis of Methyl Ester: Analysis of the FAME, in duplicate, was carried out on a Hewlett-Packard (San Fernando, CA, U.S.A.) 5890 gas chromatograph equipped with a split/splitless capillary inlet system and a flame ionization detector with Supelco SP-2380 (Supelco, Bellefonte, PA) capillary column (0.20-μm stationary phase thickness, 30 m x 0.25 mm I.D.). Other operation parameters were as follows: injector temperature, 250°C; detector temperature, 250°C; helium carrier gas flow, 20 cm/second; split ratio, 1/100. A Maxima 820 Chromatography workstation was used to determine peak areas (Waters, Milford, MA, U.S.A.).

4. Statistical Analyses

Statistical analyses were the same as experiment 3.

E. Experiment 5: Effect of Microwave Heating on Vitamin E, Oryzanol, and Fatty Acids in Rice Bran

1. Analytical Approach

The objective of this experiment was to determine the effect of microwave heating on stability of vitamin E isomers and oryzanols in rice bran and to determine the effect of microwave energy on quality of rice bran oil. Also, retention of vitamin E vitamers and oryzanols during storage was measured.

2. Microwave Heating

Freshly milled raw rice bran was transported from the Riviana Mill in Abbeville, LA. in 200 kg containers on dry ice. Fifty gram batches of samples with 10.5 % moisture content were heated in the microwave oven for
0, 2, 4, 6, 8, and 12 min. The microwave oven, rated at 450 watts (2450 MHz), was operated at full power. Oven temperatures were monitored with thermocouples during heating and sample temperature was measured immediately after each treatment. To obtain uniform heating, the samples were placed in a cylindrical shell container of 0.8 cm thickness and 20 cm inner diameter and placed on a turntable. Samples were stored at ambient temperature (22 - 26 °C) for 0, 1, 3, 7, 24, and 52 weeks. At the end of the storage periods, samples were placed in an ultra-low freezer (-85°C) until analyzed.

3. Material, Analytical Methods, and Statistical Analyses

Material and analytical methods for E vitamers and oryzanols were the same as experiment 3 and those for lipid analysis were the same as experiment 4. Also, the statistical analyses were the same as experiment 3.

F. Experiment 6: Effect of Gamma-Irradiation on Vitamin E, Oryzanol, and Fatty Acids in Rice Bran

1. Analytical Approach

The objective of this study was to examine the effect of gamma-irradiation on stability of vitamin E isomers and oryzanols in rice bran and to determine the effect of gamma-irradiation on fatty acid composition of rice bran oil. Also, retention of vitamin E vitamers and oryzanols during storage was measured.
2. Gamma-Irradiation

Freshly milled raw rice bran was transported from the Riviana Mill in Abbeville, LA. in 200 kg containers on dry ice. The samples were exposed to irradiation from a cobalt-60 source. The radiation dose was computed by taking into account the strength of the source and the time of exposure. One hundred gram samples of 10.5% moisture were irradiated at doses of 0, 5, 10, and 15 kGy. All irradiation treatments were carried out at 20°C and the dose rate was 0.98 kGy/hr. Samples were taken immediately for determination of baseline levels of free fatty acids and oxidative degradation products. Samples were stored at ambient temperature (22 - 26 °C) for 0, 1, 3, 7, 24, and 52 weeks. At the end of the storage periods, samples were placed in an ultra-low freezer (-85°C) until analyzed.

3. Material, Analytical Methods and Statistical Analyses

Material and analytical methods for E vitamers and oryzanols were the same as experiment and those for lipid analysis were the same as experiment 4. Also, the statistical analyses were the same as experiment 3.
A. EXPERIMENT 1: Isolation of Four Tocopherols and Four Tocotrienols

1. Sample Preparation

Saponification of oils concentrated vitamin E vitamers and removed interfering glycerides and other hydrolyzable materials. At the same time, saponification liberated tocopherols and tocotrienols from esters that may have been present. Chow et al. (1969) reported that rubber latex lipid contains about 68% esterified tocotrienol. The unsaponifiable matter contains higher aliphatic alcohols (waxes), sterols, pigments, and hydrocarbons. In wheat germ and soybean oil, the major components of the unsaponifiable matter are sterols (Tamura and Matsumoto, 1973). Some sterols may be removed by precipitation at low temperature and filtration (Thompson and Hatina, 1979; Chow et al., 1969; Tamura and Matsumoto, 1973; Pennock et al., 1962). During sample preparation it is important to reduce mass of sample as much as possible to increase sample loading, reduce analysis time, and improve column stability. Table IV-1 shows changes in mass balance of sample during separation steps. With saponification and crystallization, over 95% of sample weight could be reduced from commercial soybean oil and extracted oils.
Table IV-1. Mass balance (g) of sample preparation

<table>
<thead>
<tr>
<th>Sample</th>
<th>Soybean oil</th>
<th>Wheat germ</th>
<th>Wheat bran</th>
<th>Latex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample before extraction</td>
<td>2</td>
<td>20</td>
<td>40</td>
<td>20</td>
</tr>
<tr>
<td>Sample after extraction</td>
<td>-</td>
<td>0.0471*</td>
<td>0.0395*</td>
<td>0.2194b</td>
</tr>
<tr>
<td>Sample after saponification</td>
<td>0.0165</td>
<td>-</td>
<td>-</td>
<td>0.07635</td>
</tr>
<tr>
<td>Sample after crystallizationc</td>
<td>0.00907</td>
<td>0.0164</td>
<td>0.0146</td>
<td>0.05008</td>
</tr>
</tbody>
</table>

* Extracted with saponification.

b Extracted without saponification.

c Crystallized at -20°C for 12 h and centrifuged at -20°C.

Table IV-2. Mobile phase gradient program for mixture of soybean oil and wheat germ oil

<table>
<thead>
<tr>
<th>Time(min)</th>
<th>Flow(mL/min)</th>
<th>Solvent A(%)</th>
<th>Solvent B(%)</th>
<th>Curvec</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>8</td>
<td>30</td>
<td>70</td>
<td>*</td>
</tr>
<tr>
<td>0.5</td>
<td>8</td>
<td>6</td>
<td>94</td>
<td>5</td>
</tr>
<tr>
<td>2.0</td>
<td>8</td>
<td>10</td>
<td>90</td>
<td>5</td>
</tr>
<tr>
<td>9</td>
<td>8</td>
<td>10</td>
<td>90</td>
<td>6</td>
</tr>
<tr>
<td>13.7</td>
<td>8</td>
<td>35</td>
<td>65</td>
<td>6</td>
</tr>
<tr>
<td>14.5</td>
<td>8</td>
<td>35</td>
<td>65</td>
<td>6</td>
</tr>
<tr>
<td>20</td>
<td>8</td>
<td>30</td>
<td>70</td>
<td>6</td>
</tr>
</tbody>
</table>

a Hexane:THF = 60:40.
b Hexane(100%).
c Pre-programmed gradient curve in Waters 680 Automated Gradient Controller.
2. Isolation of Tocopherols and Tocotrienols

Fig. IV-1 shows a chromatogram of vitamin E vitamers from soybean and wheat germ oil. Before sample injection, the column was flushed with a gradient system (Table IV-2) for 25 min. The mixture had a concentration of 479 mg/mL in 2.5% THF in hexane, and 60 µL (about 28.8 mg) was injected. Injection of over 35 mg of the mixture decreased vitamers' resolution. A flow rate between 8 and 9.99 mL/min, which was the maximum flow rate of our controller, had no influence on resolution. Peaks of α-, β-, γ-, and δ-tocopherol in Fig IV-1 represent amounts of 0.68, 0.29, 2.02, and 0.84 mg per injection, respectively. α-Tocotrienol eluted between α-tocopherol and β-tocopherol, and β-tocotrienol eluted between γ-tocopherol and δ-tocopherol. After 17 min, more polar compounds such as sterols eluted. Tocotrienols and most late eluates came from wheat germ oil. An injection of soybean oil alone produced level base line after δ-tocopherol eluted. Sterols could not be removed completely with precipitation at low temperature. To remove sterols completely, crystallization and digitonin precipitation were used (Whittle et al., 1966).

The column required cleaning between injections to obtain constant retention time and pure fractions. Hexane with 14% THF (Table IV-2, from 13.7 to 14.5 min) was used to remove late eluting compounds and stabilize retention time. A higher concentration of THF could be applied to accelerate elution of late compounds, but analysis time would be similar because the
Fig. IV-1. Chromatogram of a mixture of a soybean oil and wheat germ oil; solid peaks represent fraction cuts. Chromatographic conditions are described in text and in table IV-2.

Fig. IV-2. Chromatogram of a mixture of a wheat bran oil and latex lipid; solid peaks represent fraction cuts. Chromatographic conditions are described in text and in table IV-3.
Table IV-3. Mobile phase gradient program for latex lipid and wheat bran oil

<table>
<thead>
<tr>
<th>Time(min)</th>
<th>Flow(mL/min)</th>
<th>Solvent A(%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Solvent B(%)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Curve&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>8</td>
<td>30</td>
<td>70</td>
<td>*</td>
</tr>
<tr>
<td>0.7</td>
<td>8</td>
<td>8</td>
<td>92</td>
<td>6</td>
</tr>
<tr>
<td>1.1</td>
<td>8</td>
<td>5</td>
<td>95</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>8</td>
<td>5</td>
<td>95</td>
<td>6</td>
</tr>
<tr>
<td>13</td>
<td>8</td>
<td>7</td>
<td>93</td>
<td>5</td>
</tr>
<tr>
<td>14.5</td>
<td>8</td>
<td>8</td>
<td>92</td>
<td>4</td>
</tr>
<tr>
<td>16.5</td>
<td>8</td>
<td>10</td>
<td>90</td>
<td>9</td>
</tr>
<tr>
<td>20</td>
<td>8</td>
<td>15</td>
<td>85</td>
<td>8</td>
</tr>
<tr>
<td>22</td>
<td>9</td>
<td>35</td>
<td>65</td>
<td>10</td>
</tr>
<tr>
<td>25</td>
<td>9</td>
<td>35</td>
<td>65</td>
<td>6</td>
</tr>
<tr>
<td>27</td>
<td>8</td>
<td>30</td>
<td>70</td>
<td>8</td>
</tr>
</tbody>
</table>

<sup>a</sup> Hexane:THF = 60:40.
<sup>b</sup> Hexane(100%).
<sup>c</sup> Pre-programmed gradient curve in Waters 680 Automated Gradient Controller.
column would require reequilibration to the 12% THF mobile phase. Solvent A was prepared with 40% THF in hexane rather than pure 100% THF to improve control of the gradient system.

Fig.IV-2 shows the chromatogram of a mixture of a latex oil and wheat germ oil. The mixture of latex oil and wheat germ oil with concentration of 485 mg/mL in hexane containing 2.5% THF was prepared, and 180 µL (about 87.3 mg) was injected. Injection of over 95 mg decreased resolution of tocopherols and tocotrienols. The eluate between α-tocotrienol and β-tocotrienol was β-tocopherol. The peak between γ-tocotrienol and δ-tocotrienol was presumably δ-tocopherol. But δ-tocopherol was not detected in wheat bran oil and latex lipid using an analytical column. Peaks of α-, β-, γ-, and δ-tocotrienol in Fig.IV-2 represent amounts of 0.78, 0.55, 3.37, and 0.77 mg per injection, respectively. The sample matrix of wheat germ oil and wheat bran oil was complex, so isolation of β-tocopherol or β-tocotrienol from wheat germ oil took over 20 min.

3. Fraction Purification

Purities of first fraction were over 88% and those of second fraction over 97% by analytical HPLC. Isocratic mobile phase systems were used during purification steps. The concentrations of THF in hexane as a modifier solvent ranged from 7% for α-tocopherol to 18% for δ-tocotrienol. To reduce the purification time in the last steps, the amounts of THF in hexane were increased.
4. Purity Checks and Concentration Determination

To characterize fraction purity to a greater extent, the ratio of UV absorptions (295/245 nm) was determined using a UV diode-array detector (Fig.IV-3). Saito and Yamauchi (1990) used the ratio of 230 to 295 nm to check purities of α- and β-tocopherol fraction. Absorption at 230 nm represents tocopherols, fatty acids, and their ester, and the absorption at 295 represents only tocopherols. Presumably, our samples were free from fatty acids and triglycerides, so we used 245 nm to check for sterols in isomer fractions. Eluates from the fluorescence detector were passed through the UV detector with HPLC conditions similar to the purification procedure, except injection quantity was reduced to 5-20 μL. As shown in Fig.IV-3a, the δ-tocopherol peak produced a constant signal ratio (295 nm /245 nm) throughout the peak’s elution. However, the δ-tocotrienol peak evidenced a change in the signal ratio (295 nm / 245 nm). Impurities in the δ-tocotrienol fraction eluted at a similar time, so purification with the column could not be obtained. The impurities were not identified.

Fractionated δ-tocotrienol were scanned at UV-vis wavelengths from 200 to 350. The chromatogram had high absorption values in the wavelength range around 260 nm (minimum wavelengths of δ-tocotrienol). The fraction of δ-tocotrienol after three HPLC passes still contained impurities, which were then chromatographed two additional times using thin-layer chromatography with silica gel G (250 μ) and 20 % di-isopropyl ether in
Fig. IV-3. Chromatograms at wavelengths 295 nm, 245 nm and the ratio of the two for (A) δ-tocopherol and (B) δ-tocotrienol.
petroleum ether (Whittle et al., 1966). After TLC fractionation the purity was satisfactory as was shown by Table IV-4.

Each fractionated solution in the last purification steps was evaporated to dryness, weighed, and diluted with a known amount of hexane. A known amount of each tocopherol and tocotrienol solution was evaporated to dryness and diluted with ethanol to determine the concentration of vitamin E vitamers in hexane solution by published molar absorbance values \( (E_{1%}^{\text{abs}}) \) in Table IV-5. However, absorption maxima and molar absorbance values found in the literature differ. Absorption maxima that matched published \( E_{1%}^{\text{abs}} \) were chosen in accordance with UV-vis spectrum maxima of purified vitamers in Fig.IV-4. For \( \beta \)-tocotrienol, two \( E_{1%}^{\text{abs}} \) values at 294 nm have been published, so the average value of 86.4 was used for calculation of \( \beta \)-tocotrienol concentration. The concentrations of purified vitamin E vitamers as determined were used as standards for HPLC assays.

5. Gas Chromatography/Mass Spectrometry

Table IV-6 shows molecular weights and major peaks \( (m/z) \) of purified vitamin E vitamers in mass spectra. The major mass fragmentations of \( \beta \)- and \( \gamma \)-tocopherol and \( \beta \)- and \( \gamma \)-tocotrienol were the same, since these are positional vitamers having the same molecular weights. Also, they had a
Fig. IV-4. UV-visible spectra of isolated and purified tocopherols and tocotrienols.
Table IV-4. Analytical data for isolated and purified tocopherols and tocotrienols fractions from 6 g of a mixture of soy-bean oil and wheat germ oil and 8 g of a mixture of wheat bran oil and latex lipid

<table>
<thead>
<tr>
<th>Sample</th>
<th>α-T</th>
<th>β-T</th>
<th>γ-T</th>
<th>δ-T</th>
<th>α-T3</th>
<th>β-T3</th>
<th>γ-T3</th>
<th>δ-T3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purity(%)</td>
<td>99</td>
<td>99</td>
<td>99</td>
<td>99</td>
<td>99</td>
<td>99</td>
<td>99</td>
<td>99</td>
</tr>
<tr>
<td>Recovery(%)</td>
<td>79</td>
<td>75</td>
<td>83</td>
<td>78</td>
<td>76</td>
<td>72</td>
<td>81</td>
<td>54</td>
</tr>
<tr>
<td>Yield (mg)</td>
<td>112.2</td>
<td>45.22</td>
<td>350.4</td>
<td>136.5</td>
<td>54.16</td>
<td>33.96</td>
<td>250.5</td>
<td>38.2</td>
</tr>
</tbody>
</table>

* T = tocopherol; T3 = tocotrienol.
* By gas chromatography.

Table IV-5. UV absorption maxima and molar absorbances of tocopherols and tocotrienols in ethanol solution taken from the literature

<table>
<thead>
<tr>
<th>Compound</th>
<th>λ_max(nm)*</th>
<th>Molar absorbance*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tocopherol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-</td>
<td>292</td>
<td>292.1, 292.4, 292.5</td>
</tr>
<tr>
<td>β-</td>
<td>296</td>
<td>296.1</td>
</tr>
<tr>
<td>γ-</td>
<td>298</td>
<td>298.1</td>
</tr>
<tr>
<td>δ-</td>
<td>298</td>
<td>298.1</td>
</tr>
<tr>
<td>Tocotrienol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-</td>
<td>292</td>
<td>292.5, 292.5, 292.5</td>
</tr>
<tr>
<td>β-</td>
<td>294</td>
<td>294.1, 294.5, 294.5</td>
</tr>
<tr>
<td>γ-</td>
<td>296</td>
<td>296.1, 296.5</td>
</tr>
<tr>
<td>δ-</td>
<td>297</td>
<td>297.1, 297.5</td>
</tr>
</tbody>
</table>

* Isolated and purified vitamers in Fig.IV-4.
* Boldface value used to determine the concentration of vitamers.
Table IV-6. Molecular weights and major mass to charge ratios (m/z) of vitamin E vitamers in mass spectrum

<table>
<thead>
<tr>
<th>Compound</th>
<th>M.W</th>
<th>m/z</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tocopherol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-</td>
<td>430</td>
<td>43</td>
</tr>
<tr>
<td>β-</td>
<td>416</td>
<td>43</td>
</tr>
<tr>
<td>γ-</td>
<td>416</td>
<td>43</td>
</tr>
<tr>
<td>δ-</td>
<td>402</td>
<td>43</td>
</tr>
<tr>
<td>Tocotrienol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-</td>
<td>424</td>
<td>41</td>
</tr>
<tr>
<td>β-</td>
<td>410</td>
<td>41</td>
</tr>
<tr>
<td>γ-</td>
<td>410</td>
<td>41</td>
</tr>
<tr>
<td>δ-</td>
<td>396</td>
<td>41</td>
</tr>
</tbody>
</table>
similar retention time on the GC. However, using HPLC, differences in absorption maximum and retention time were noted (Table IV-5). The peak of m/z 205, 191, or 177 (M.W's - 255 or 219) indicates the loss of a side chain (C₁₆H₃₃ for tocopherols or C₁₆H₂₇ for tocotrienol), and peak of m/z 165, 151, or 137 (205, 191, or 177 - 40) originated from the cleavage of the side chain accomplished by the breakdown of chroman structure with hydrogen rearrangement and loss of a methyl acetylene CH₃-C≡CH fragment (Govind Rao and Perkins, 1972).

Fig.IV-5 shows chromatograms of purified α-tocopherol and α-tocotrienol. As can be seen, the chromatograms have a single peak and clear base line. Other chromatograms for β- + γ- and δ-tocopherols and tocotrienols had similar characteristics. Purities of vitamers were over 99% based on the peak area percentage.

6. Analytical HPLC Analysis of Isolated and Purified Vitamers

Baseline resolution was obtained for all vitamers in the mixtures of soybean oil and wheat germ oil, and wheat germ oil and latex. Table IV-7 shows the concentrations using analytical HPLC. Concentrations of tocopherols and tocotrienols were calculated from peak areas and corresponding standard curves ranging from 0.1 to 1.5 µg/mL. Table IV-4 shows recoveries, purities, and yield of isolated and purified vitamers.
Fig. IV-5. Gas chromatograms of isolated and purified α-tocopherol and α-tocotrienol.
<table>
<thead>
<tr>
<th>Sample</th>
<th>α-T</th>
<th>β-T</th>
<th>γ-T</th>
<th>δ-T</th>
<th>α-T3</th>
<th>β-T3</th>
<th>γ-T3</th>
<th>δ-T3</th>
</tr>
</thead>
<tbody>
<tr>
<td>mixture of Soybean oil and wheat germ oil*</td>
<td>23.66</td>
<td>10.05</td>
<td>70.29</td>
<td>29.171</td>
<td>0.114</td>
<td>0.491</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>mixture of Wheat bran oil and latex lipidb</td>
<td>4.164</td>
<td>0.612</td>
<td>-</td>
<td>-</td>
<td>8.914</td>
<td>5.901</td>
<td>38.66</td>
<td>8.846</td>
</tr>
</tbody>
</table>

* Soybean oil: wheat germ oil = 1:1 (g/g) in 2.5% THF in hexane.

b Wheat bran oil: latex lipid = 1:2 (g/g) in 2.5% THF in hexane.
Purities of all vitamers were over 99% by GC and HPLC. Chromatograms of analytical HPLC were clearer than those of GC due to the high selectivity of fluorescence detection for vitamin E vitamers. γ-Tocopherol and tocotrienol had higher recovery than other vitamers because concentrations of these vitamers in source oils were higher than those of other vitamers. Recovery of δ-tocotrienol was lower than the other vitamers because it required additional TLC procedures to attain purification.

Four vitamers of tocopherol and tocotrienol were isolated from a mixture of natural sources by semi-preparative HPLC for use in analytical HPLC. Recovery was higher than conventional TLC. The semi-preparative HPLC column is useful to obtain small amounts of pure tocopherols and tocotrienols as an alternative to conventional methods that tend to be tedious and time consuming, such as distillation, extraction, crystallization, and thin-layer chromatography. Isolation also was more efficient because all tocopherols and tocotrienols can be obtained from a mixture of several natural sources simultaneously and each fraction collected and rechromatographed to obtain pure standards.

B. EXPERIMENT 2: Development of a Mobile Phase System

The effect of modifiers on the retention times for seven vitamers of vitamin E is shown in Table IV-8. Eluent 1 was used as a control mobile phase to compare eluent 2 and 3, which were developed in our study to
improve column stability. The maximum number of injections with eluant 1 was 24, after which poor separation of early eluating compounds occurred. Seventy-two injections were possible with eluant 2 before resolution was reduced. Resolution remained acceptable after two hundred injections were made with eluant 3.

Ethyl acetate at a concentration of 2.5% (eluant 1) produced good separation of eight vitamers with a maximum retention time of 23 min (Fig.IV - 6). Decreasing retention times for each compound with increasing injection numbers caused a large standard deviation. The retention time of δ-tocotrienol in 1st injection was 23.09; after 24 samples were injected, the retention time decreased to 19.85. Decreasing retention times with eluant 1 are typical of normal-phase columns.

Comparatively shorter retention times were achieved with modification of eluant 1 with acetic acid. Addition of acetic acid especially reduced retention times of late eluting compounds. Also, acetic acid increased column stability relative to eluant 1, as indicated by smaller standard deviations with injection of 24 samples. Acetic acid presumably competes with water and polar material for binding to hydroxyl groups in the column. However, with higher number of injections, retention time gradually decreased, due to column deactivation (Figure 2). Adding 0.1% DMP (eluant 3) produced retention times that were similar to eluant 2. As shown in Figure 3, eluant 3 produced relatively constant retention times for each vitamer
Table IV-8. HPLC retention times (mean ± standard deviation) for vitamin E vitamers with various eluants on a Sulpelcosil LC-Si(25 cm x 4.6, 5μ) column*

<table>
<thead>
<tr>
<th>Compound</th>
<th>Eluant 1c</th>
<th>Eluant 2c</th>
<th>Eluant 3c</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 injections</td>
<td>24 injections</td>
<td>72 injections</td>
</tr>
<tr>
<td>α-T</td>
<td>5.39 ± 0.38</td>
<td>5.07 ± 0.07</td>
<td>4.67 ± 0.36</td>
</tr>
<tr>
<td>α-T3</td>
<td>6.36 ± 0.48</td>
<td>5.85 ± 0.08</td>
<td>5.38 ± 0.43</td>
</tr>
<tr>
<td>β-T</td>
<td>7.72 ± 0.59</td>
<td>7.40 ± 0.09</td>
<td>6.78 ± 0.54</td>
</tr>
<tr>
<td>γ-T</td>
<td>9.10 ± 0.64</td>
<td>8.04 ± 0.10</td>
<td>7.37 ± 0.59</td>
</tr>
<tr>
<td>β-T3</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>γ-T3</td>
<td>14.56 ± 0.71</td>
<td>9.61 ± 0.10</td>
<td>8.87 ± 0.66</td>
</tr>
<tr>
<td>δ-T</td>
<td>18.07 ± 0.79</td>
<td>11.35 ± 0.11</td>
<td>10.55 ± 0.67</td>
</tr>
<tr>
<td>δ-T3</td>
<td>22.26 ± 0.89</td>
<td>13.61 ± 0.12</td>
<td>12.45 ± 0.83</td>
</tr>
</tbody>
</table>

* From Supelco (Bellefonte, PA), with 25cm x 5 μm column.

b T=tocopherol; T3=tocotrienol.

c Eluant 1, isooctane:ethyl acetate (97.5:2.5); Eluant 2, isooctane:ethyl acetate:acetic acid (97.3:1.8:0.9); Eluant 3, isooctane:ethyl acetate:acetic acid:DMP (98.15:0.9:0.85:0.1).
Fig. IV-6. High-performance liquid chromatography chromatogram (HPLC) of standards of vitamin E vitamers. Chromatographic conditions: Supelcosil LC-Si, 250 x 4.6 mm 5 μm column (Supelco, Bellefonte, PA); mobile phase, eluent 1; flow rate 1.6 ml/min.

Response

- α-Tocopherol
- α-Tocotrienol
- β-Tocopherol
- γ-Tocopherol
- β-Tocotrienol
- γ-Tocotrienol
- δ-Tocopherol
- δ-Tocotrienol

Minutes
Fig. IV-7. Changes of retention time of vitamin E for 72 samples. Twenty-four samples were analyzed each day for three consecutive days without reactivation of column. HPLC conditions as in Fig. IV-6, except for mobile phase, eluant 2.
Fig. IV-8. Changes of retention time of vitamin E for two hundred consecutive sample analyses. Two hundred samples were analyzed continuously for about 63 hr without reactivation. HPLC conditions as in Fig. IV-6. except for mobile phase, eluent 3.
during 200 injections. Figure 4 depicts the chromatograms of the first (A) and two hundredth (B) injection for eluant 3. Little difference exists between these chromatograms. Changes of retention times with eluant 3 were most likely due to conditions and sample matrix, i.e., retention times of each vitamer extracted at the same time were similar. However, it was important to completely remove water with $\text{Na}_2\text{SO}_4$ from extracts because samples containing higher water would elute earlier due to an effect of methanol and acetone produced from water through the reaction with DMP.

In our laboratory, vitamin E vitamers in over 600 rice bran samples had been analyzed with eluant 2 prior to development of eluant 3. After every 48 injections, the column was reactivated with methylene chloride, isopropanol, and methanol. The conventional procedure took three hours. After eluant 3 was developed, column reactivation was obtained within 20 min. The need for long and tedious reactivation of columns was avoided by employing this mixture.

Use of a small amount of acetic acid and 2,2-dimethoxypropane in the mobile phase improved column stability and reproducibility of retention times in analyses of vitamin E vitamers on normal-phase columns. A similar modification may also benefit other analyses of lipophilic compounds in normal-phase columns, such as vitamin A.
Fig. IV-9. Chromatograms of rice bran samples. Chromatogram A is first injection and chromatogram B two hundredth injection. Chromatographic conditions as in Fig. IV-8. T, tocopherol; T3, tocotrienol.
C. EXPERIMENT 3: Effect of Extrusion Cooking on Vitamin E and Oryzanol in Rice Bran

1. Separation of Oryzanol from Crude Rice Bran Oil

Fig. IV-10 shows the UV spectra of isolated and purified oryzanol. The maximum wavelengths, 230, 290, and 315 nm, match published values (Okada and Yamaguchi, 1983; Ishitani, 1980). The oryzanol recovery from crude rice bran oil was 13% and the purity of oryzanol was 99.1% at 315 nm ($E_{1%}^{1%} = 358.9$) in n-haptene. The oryzanol obtained from the laboratory of Kim and Kim (1991) in Korea did not exhibit a maximum wavelength under similar conditions. During fractional crystallization at 0°C, over 60% of oryzanol was lost. According to Kim and Kim (1991), steryl ferulates such as stigmasteryl ferulate, campesterol ferulate, and $\beta$-sitosteryl ferulate were lost to a greater extent than cycloartenyl ferulates during the fractional crystallization purification step. Steryl ferulates are relatively less polar than cycloartenyl ferulates. The cycloartenyl ferulates precipitated selectively during fractional crystallization. They reported the composition of purified oryzanol was 7.12, 4.26, 26.12, and 62.50% for campesterol, stigmasteryl, cycloartenyl, and 24-methyl-cycloartanyl ferulate, respectively. The first peak in oryzanol group in Fig. IV-11 was suspected to be steryl ferulates and second peak, cycloartenyl ferulates in light of polarity and compositional aspects of oryzanol.
Fig. IV-10. UV-visible spectra of isolated and purified oryzanol.

Fig. IV-11. Chromatogram of isolated and purified oryzanol. Chromatographic conditions are described in text.
2. Recovery of Vitamin E Vitamers and Oryzanol

Recovery with hexane extraction was 100, 98, 99, and 99% for α-, β-, γ-, and δ-tocopherol (T), 95, 96, 98, and 99% for α-, γ-, and δ-tocotrienol (T3), respectively, and 14% for oryzanol (Fig. IV-12). Recovery with diethyl ether was 95, 96, 93, and 94% for α-, β-, γ-, and δ-tocopherol, 92, 93, and 93% for α-, γ-, and δ-tocotrienol, respectively, and 95% for oryzanol. The extracts with hexane had higher recoveries for tocopherols and tocotrienols than the those with diethyl ether. The extracts with diethyl ether had a higher recovery for oryzanol. The oryzanols are minimally soluble in less non-polar solvents such as hexane and petroleum ether, but are soluble in more polar solvents such as ethanol, acetone, and acetic acid (Tomataro, 1957). As shown in Fig.IV-12B, the leading peak and baseline are higher in sample extracted with diethyl ether than those with hexane. Diethyl ether extracted a greater amount of compounds such as free fatty acid and oryzanol from the aqueous layer. Some compounds extracted with diethyl ether resulted frequently in column degradation. The first method of extraction was mainly used for sample preparation and the second method was used to determine recovery of oryzanol. After vitamin E vitamers and oryzanol were extracted from rice bran, the residual amount in bran was 3.2, 2.8, 1.4, and 1.6% for α-, β-, γ-, and δ-tocopherol, 3.1, 1.8, and 1.7% for α-, γ-, and δ-tocotrienol, and 2.5% for oryzanol, respectively.
Fig. IV-12. Chromatograms of rice bran samples. Chromatogram A is method I and chromatogram B, method II. Chromatographic conditions are described in text.
3. Effect of Holding Time and Extrusion Temperature

Table IV-9 shows the effect of post extrusion holding time at all extrusion temperatures on vitamin E vitamers and oryzanol content in extruded rice bran. Increased holding times reduced (p < 0.05) total vitamin E vitamers in rice bran. Oryzanol concentration was lower (p ≤ 0.05) only after 6 min holding time. Vitamin E vitamers are stable to higher temperature in the absence of oxygen. However, oxidation of E vitamers is accelerated by heat at normal conditions (Ball, 1995; Lang et al., 1992).

Table IV-10 shows the percentage of vitamin E vitamers retention during extrusion stabilization. γ-Tocotrienol was the most sensitive to heat. Rice bran extruded at 140°C with 6 min holding time lost 24.7% of its γ-tocotrienol. The order of stability of vitamin E vitamers in extruded rice bran for all extrusion temperature was γ-T3 > α-T > α-T3 > γ-T > δ-T3 > β-T > δ-T. At an equivalent concentration of 0.02% in stripped corn oil, the order of losses of the tocopherols was γ > δ > β > α (Bauernfeind, 1977). Also, Parkhurst et al. (1968) had reported an order of γ > δ > α in a lard system at a temperature of 97°C. Lehmann and Solver (1976) studied relative stabilities of tocopherols and tocotrienols in methyl myristate and methyl linoleate under photolytic and autooxidative conditions. Under photolytic conditions, stabilities in increasing order in methyl myristate were γ-T3 < α-T3 < δ-T < α-T < γ-T < δ-T and in methyl linoleate were α-T
Table IV-9. Effect of post extrusion holding time on vitamin E vitamers and oryzanol

<table>
<thead>
<tr>
<th>Holding time (minute)</th>
<th>Mean value$^b$</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>vitamin E vitamers</td>
<td>Oryzanol</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>300.31$^a$</td>
<td>3101.89$^a$</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>290.80$^b$</td>
<td>3070.13$^a$</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>280.62$^c$</td>
<td>3019.51$^b$</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ mg/kg rice bran.

$^b$ Means (n = 6) with the same letter are not significantly (p < 0.05) different.
Table IV-10. The percentage of retention of vitamin E vitamers and oryzanol during extrusion stabilization of rice bran\textsuperscript{a, b, c}

<table>
<thead>
<tr>
<th>Temperature holding time</th>
<th>α-T</th>
<th>α-T3</th>
<th>β-T</th>
<th>γ-T</th>
<th>γ-T3</th>
<th>δ-T</th>
<th>δ-T3</th>
<th>Ory</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>91.3</td>
<td>92.1</td>
<td>95.2</td>
<td>92.9</td>
<td>90.9</td>
<td>95.8</td>
<td>94.1</td>
<td>95.9</td>
</tr>
<tr>
<td>110</td>
<td>89.1</td>
<td>90.4</td>
<td>93.4</td>
<td>91.3</td>
<td>88.7</td>
<td>94.5</td>
<td>91.5</td>
<td>95.6</td>
</tr>
<tr>
<td>120</td>
<td>86.1</td>
<td>87.7</td>
<td>90.6</td>
<td>88.1</td>
<td>85.3</td>
<td>93.0</td>
<td>89.7</td>
<td>94.4</td>
</tr>
<tr>
<td>130</td>
<td>84.0</td>
<td>86.2</td>
<td>88.6</td>
<td>86.2</td>
<td>83.3</td>
<td>90.9</td>
<td>86.7</td>
<td>92.6</td>
</tr>
<tr>
<td>140</td>
<td>82.5</td>
<td>85.1</td>
<td>87.9</td>
<td>85.6</td>
<td>82.9</td>
<td>91.2</td>
<td>86.2</td>
<td>93.0</td>
</tr>
<tr>
<td>150</td>
<td>80.2</td>
<td>82.5</td>
<td>84.7</td>
<td>82.8</td>
<td>79.9</td>
<td>89.0</td>
<td>84.5</td>
<td>91.4</td>
</tr>
<tr>
<td>160</td>
<td>78.0</td>
<td>81.7</td>
<td>83.6</td>
<td>82.4</td>
<td>80.3</td>
<td>88.8</td>
<td>84.1</td>
<td>90.6</td>
</tr>
</tbody>
</table>

\textsuperscript{a} % of raw rice bran.
\textsuperscript{b} Based on mean of six analyses.
\textsuperscript{c} T, tocopherol; T3, tocotrienol.
< $\alpha$-T3 \leq \gamma$-T3 \leq \beta$-T \leq \gamma$-T \leq \delta$-T. Under autooxidative conditions, stabilities in increasing order in methyl myristate were $\alpha$-T = $\alpha$-T3 < $\beta$-T3 < $\gamma$-T3 < $\delta$-T < $\delta$-T = $\beta$-T and in methyl linoleate were $\alpha$-T < $\alpha$-T3 < $\gamma$-T3 < $\beta$-T < $\gamma$-T < $\delta$-T. They found tocopherols were much more stable during autoxidation in methyl myristate than in methyl linoleate. The exact order of antioxidant activity or stability of vitamin E vitamers in foods might be influenced by their concentration rather than the absolute antioxidant activity or stability in pure solution. Oryzanol is relatively stable to heat (Okada and Yamaguchi, 1983). Oryzanol molar concentration in rice bran was about five times higher than total E vitamers. However, only 10.8% was lost at 140°C extrusion temperature with 6 min holding time (Table IV-10). Kim and Kim (1991) reported heat treated rice bran oil had a different composition of oryzanol from control rice bran oil. Cycloartenyl ferulates were relatively less stable to heat than steryl ferulates. During extrusion stabilization, the decomposition rate of cycloartenyl ferulates were possibly higher than the other ferulates.

The decomposition rates of vitamin E isomers increased with increasing holding time except at 130°C extrusion temperature. As holding time increased from 0 min to 3 min, the increase in average decomposition was 1.9, 2.3, and 2.9%, at 110, 120, and 140°C respectively. As holding time increased from 3 to 6 min, decomposition increased by 2.5, 2.8, and
3.7%. At 130°C extrusion temperature, increasing holding time from 0 to 3 min caused a 2.9% reduction in vitamin E concentration and a 2.5% reduction when increasing holding time from 3 to 6 min. Miyagawa et al. (1991) reported that the decomposition rate of tocopherols decreased with increasing heating time in tempura-frying oil. After frying oil was heated for 28 min, 10% α-tocopherol was lost. When the frying oils were heated for 9 hr, 17% α-tocopherol was lost. The decomposition rates of vitamin E vitamers differ with the amount of antioxidants, heating time, heating method, and food composition. Yoshda et al. (1991) found that vegetable oils heated with microwave had different decomposition rates of tocopherols depending on vitamin E vitamer contents and fatty acid composition in vegetables. Du Plessis and Van Niekerk (1981) showed that the order of the decomposition rates of α- and γ-tocopherol changed with heating time (α < γ with 26 hr heating and γ < α with 103 hr heating in peanut oil and γ < α with 26 hr and 103 hr heating in cottonseed oil). In rice bran, the decomposition rate of vitamin E vitamers with increasing holding time was not significantly increased except at 140°C.

Table IV-11 shows the effect of extrusion temperatures on total vitamin E vitamers and oryzanol. Vitamin E vitamers in rice bran stabilized at higher extrusion temperature were significantly lower. Oryzanol content
<table>
<thead>
<tr>
<th>Extrusion temperature (°C)</th>
<th>Mean value</th>
<th>Oryzanol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vitamin E vitamer</td>
<td></td>
</tr>
<tr>
<td>110</td>
<td>304.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3132.19&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>120</td>
<td>297.96&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3089.58&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>130</td>
<td>286.21&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3052.52&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>140</td>
<td>273.91&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2981.09&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> mg/kg rice bran.

<sup>b</sup> Means (n = 6) with the same letter are not significantly (p < 0.05) different.
in stabilized rice bran was not significantly different between 110 and 120°C extrusion temperature and between 120 and 130°C extrusion temperature.

Håkansson et al. (1987) showed that white flour lost 70% and 83% of total vitamin E vitamers with mild (148°C) and severe (197°C) extrusion processing, respectively. Håkansson and Jägerstad (1990) demonstrated that 92% and 96% of total E vitamers in whole meal and white flour, respectively, were lost when drum dried at 165°C. Non-enzymic lipid oxidation was likely to occur since the concentrations of pro-oxidative substances such as copper and iron, originating either from the processing water or from the processing equipment, increase markedly during the drum-drying and extrusion processing (Håkansson and Jägerstad, 1990; Schweizer et al., 1986; Håkansson et al., 1987). Moisture contents and particle size might affect the losses of E vitamers during heat processing (Galliarc, 1986). However, extrusion cooking caused extensive destruction of vitamin E compared with conventional cooking (Noble et al., 1971; Miyagawa et al., 1991; Bauernfeind, 1977).

4. Changes of Vitamers and Oryzanol in Raw Bran During Storage

Few studies have been done to determine retention of vitamin E during storage. Most of the studies determined only α-tocopherol content in feeds and vegetable oils within several months (Frazer and Lines, 1967; Chow et al., 1969; Chu and Lin, 1993). Fig IV-13 shows the retention of vitamin E
Fig. IV-13. Tocopherols and tocotrienols in raw rice bran during storage.
vitamers and oryzanol in raw rice bran during one year storage. The initial contents of vitamin E vitamers in raw bran were 68.2, 7.7, 42.0, and 3.8 mg/kg rice bran for α-, β-, γ-, and δ-tocopherol, and 50.7, 145.3, and 21.9 mg/kg rice bran for α-, γ-, and δ-tocotrienol. Raw rice bran lost 44% of its total vitamin E vitamers after 35 days. The order of losses of vitamin E vitamers and oryzanol in raw rice bran after 35 days was α-T (57.7%) ≥ α-T3 (56.8%) > γ-T3 (39.6%) > γ-T (34.9%) ≥ δ-T3 (34.0%) ≥ β-T (33.4%) ≥ δ-T (32.9%) > oryzanol (16.4%). This established that α-tocopherol and α-tocotrienol were less stable than the other E vitamers during storage. Wennermark and Jägerstad (1992) showed α-tocopherol and α-tocotrienol had lower retention than β-tocotrienol in wheat fraction, during one year storage. Upon extrusion stabilization, γ-Tocotrienol was the least stable vitamer to heat. However, α-tocopherol and α-tocotrienol had less oxidative stability than γ-tocotrienol during storage. The order changed slightly during one year storage. Beyond 35 days storage, the decomposition of α-tocopherol and α-tocotrienol slowed during the remainder of storage. On the other hand, γ-tocotrienol and oryzanol exhibited steady decomposition rate during storage (Fig. IV-13). After one year storage, 73% of total vitamin E vitamers was lost. The order of losses of vitamin E vitamers and oryzanol in raw rice bran for one year storage was γ-T3 (77.9%) > α-T (74.7%) ≥ α-T3 (72.4%) > δ-T3 (69.0%) > oryzanol (62.7%) > γ-T
(60.1%) ≥ β-T (58.6%) ≥ δ-T (57.0%). These losses were higher than other cereal grains and brans during storage. This high destruction of vitamin E vitamer may be related to the amount of free fatty acid which was hydolyzed by lipolytic enzymes in rice bran. Lipase in rice bran produce over 50% free fatty acid after 35 days storage. Lipoxygenase in bran catalyze peroxidation of free polyunsaturated fatty acids that originate from the lipase-catalyzed hydrolysis of wheat bran lipids (Galliard, 1986a). Vitamin E vitamers are lost if peroxidizing lipids are present (Håkansson and Jägerstad, 1990). Thus, E vitamers are oxidized by the co-oxidation reaction of lipoxygenase (Galliard, 1986b; Nicolas and Drapron, 1983). Also, E vitamers are oxidized directly (Slover and Lehmann, 1972). It is known that lipid oxidation is dependent on moisture content, temperature, humidity, and microbial growth during storage (Loeb et al., 1949; Loeb and Mayne, 1952; Galliard, 1986a). High moisture content and long storage of soybeans resulted in lower tocopherol content in the crude oils, with moisture content being more important than storage time in the reduction of tocopherol content of oils (Chu and Lin, 1993). Soy bean oil from stored beans with 15±1% moisture content led to a more significant decrease in the tocopherol content than did oil from stored beans with low (12%) or high (18%) moisture contents.

It appears that α-tocopherol and α-tocotrienol in raw rice bran played a protective role againsts lipid oxidation primarily during the initial storage
stage, while \(\gamma\)-tocotrienol and oryzanol losses occurred throughout storage. Wennermark and Jägerstad (1992) reported that wheat bran stored at 20°C for 12 months contained 72% of its total vitamin E vitamers. However, wheat flour stored in an open tin lost 64% of its total vitamin E vitamers during 190 day storage. Rice bran stored for 12 months lost 73% of its total E vitamers and 62% oryzanol. Raw rice bran is highly oxidative during storage even though it contains a large concentration of additional antioxidant.

5. Changes of Vitamers and Oryzanol in Extruded Rice Bran during Storage

The conditions of temperature, pressure and shear employed during extrusion can have an effect on lipid stability, and can induce lipid oxidation (Rao and Artz, 1989). Smith (1976) had suggested that lipid stability may increase as a result of extrusion due to lipid binding by starch. Daniels et al. (1970) suggested that bound lipids were protected against peroxidation, since lipid peroxides were formed predominantly from free lipids. Thus, changes relative to lipids, as a result of extrusion, were expected to affect E vitamer composition in rice bran during storage.

Fig.IV-14 shows the degradation of individual vitamin E vitamers in rice bran extruded at 110°C with 0 min holding time (110°C-0 min). Total E vitamers concentration was reduced by 21 and 46% after 7 day and 105 day storage, respectively. Degradation rates among individual E vitamers during
Fig. IV-14. Tocopherols and tocotrienols in rice bran extruded at 110°C with holding time 0 min during storage.
105 day storage were not significantly different. Thereafter, all E vitamers except γ-tocotrienol and δ-tocotrienol had a similar degree of decomposition. γ-Tocotrienol and δ-tocotrienol had a relatively constant degree of decomposition from 7 day to 210 day storage. The ratio of tocopherol to tocotrienol in rice bran was dependent on the degree of decomposition of γ-tocotrienol during storage. Oryzanol had a constant level of decomposition throughout storage. Losses of vitamers after 105 day storage were marginal. Similar degradation patterns were found for 110°C-3 min and 110°C-6 min treatments. At the end of storage, E vitamer retention between 110°C-0 min and 110°C-3 min were not significantly different even though total E vitamers and oryzanol were significantly different initially.

Fig.IV-15 shows degradation of individual E vitamers in rice bran extruded at 140°C with 6 min holding time (140°C-6 min). The decomposition rate of individual E vitamers was relatively low compared with 110°C-0 min during 7 day storage. However, all individual E vitamers and oryzanol had relatively degree of constant decomposition from 7 day to 210 day storage. Thereafter, the decomposition rate of all E vitamers was reduced. Similar observations were made for 120, 130, and 140°C extrusion temperature with all holding times (Fig.IV-16 - Fig.IV-18). Setlhako-Tlhomelang (1993) reported TBA values for the bran samples obtained in this
Fig. IV-15. Tocophers and tocotrienols in rice bran extruded at 140°C with holding time 6 min during storage.
Fig. IV-16. Total vitamin E vitamers in rice bran extruded at 110, 120, 130, and 140°C with holding time 0 min during storage.
Fig. IV-17. Total vitamin E vitamers in rice bran extruded at 110, 120, 130, and 140°C with holding time 3 min during storage.
Fig. IV-18. Total vitamin E vitamers in rice bran extruded at 110, 120, 130, and 140°C with holding time 6 min during storage.
study. TBA value in rice bran extruded at 140°C evidenced a lag phase until 165 day storage, then TBA number increased sharply during the remainder of storage period. With an extrusion temperature of 130°C, the TBA values increased relatively slowly during 165 day and increased similarly to rice bran extruded 140°C. Those tendencies were highly related to the decomposition of E vitamers in rice bran extruded at 120, 130, and 140°C. While TBA values were low, the decomposition rates of E vitamers were high. These might indicate that low TBA values corresponding to the induction period of TBA was due to E vitamers’ protective role. TBA value or peroxide values were low when decomposition rate of E vitamers or antioxidants was high (Thafvelin and Oksanen, 1966).

Artz and Rao (1991) reported that corn starch-soybean oil extruded at 115 and 135°C had no significant difference in peroxide values during 16 days of storage. In this study, lipid stability in rice bran extruded at 120, 130, and 140°C with holding time 3 and 6 min was considered to be similar. Retention of total E vitamers and oryzanol in those rice bran samples was not significantly different at the end of storage period although initial total E vitamers were significantly different.

Retention of vitamin E vitamer in rice bran extruded at 120, 130, and 140°C was lower than that of raw rice bran, although extruded rice bran
contained less free fatty acids and and lower TBA values (Setlhako-Tlhomelang, 1993) than those of raw bran.

Rice bran extruded at 110°C contained significantly higher vitamin E vitamers and oryzanol from 105 day through 375 day storage even though it contained significantly higher free fatty acid (following experiment) than rice bran extruded at 120, 130, and 140°C. One of the factors affecting the content of total vitamin E vitamers in extruded bran might be trace mineral from imparted from the extruder. Oxidation of E vitamers is accelerated by the presence of certain trace minerals, such as ferric iron and cupric copper. Although ferrous and cuprous ions, and ground-state copper, do not react with E vitamers (Ball, 1995). Rao and Artz (1989) reported that a substantial increase in transition metal concentration, particularly iron, was observed with an increase in extrusion temperature. An increase in iron concentration for the samples extruded at 135 and 175°C was nearly 3 and 6 times that of the unextruded samples, respectively (Rao and Artz, 1989). This iron was considered to contribute to lipid instability in extruded samples at higher temperature. Also, Rao and Artz (1989) noted that some of the increase in oxidation may be due to the increase in surface area associated with an increase in expansion ratio. Another possible factor was that physical force of extruder damaged cell membrane where vitamin E vitamer and oryzanol are present. Tocopherols and tocotrienols were destroyed more
rapidly in cracked beans (Lhu and Lin, 1993) and rice seeds with intact hull (Ramarathnam et al., 1989) than those in normal condition. It is suggested that damaged grains are more readily subjected to oxidation conditions. In raw bran and rice bran extruded at 110°C, total E vitamers and oryzanol remained higher at the end of storage. Also the high pressure of extrusion might extract oil components with antioxidants from bran cell membrane to surface so as to facilitated antioxidant’s own oxidation of antioxidant compounds rather than as a secondary effect of fatty acid oxidation.

6. Kinetics of Vitamin E and Oryzanol Degradation During Post Extrusion Processing

The order of degradation of vitamin E and oryzanol in rice bran during extrusion processing was explored graphically by plotting different functions of the concentration against post extrusion holding time at a given extrusion temperature. A plot of \( \log_e(C_0/C) \) versus time generated a straight line, thus indicating first order kinetics for degradation of vitamin E and oryzanol during post extrusion processing. Such first order reactions are defined as:

\[
\log_e(C_0/C) = kt
\]

where \( C_0 \) is the amount of vitamin E isomer or oryzanol at 0 holding time at a given extrusion temperature and \( C \) is the amount of vitamin E isomer or oryzanol at 3 and 6 min holding time at that extrusion temperature.
The reaction rate constant was determined from the slope of the line (calculated by regression analysis with all the lines forced through zero as instantaneous heating and cooling was assumed during and after post extrusion processing). The activation energy and the frequency factor were derived from the Arrhenius equation defined as:

\[ k = A \exp \left( - \frac{E_a}{RT} \right) \]

where \( k \) = rate constant (min\(^{-1}\))
\( A \) = frequency factor (min\(^{-1}\))
\( E_a \) = activation energy (kcal/mole\(^{-1}\))
\( R \) = gas constant (1.986 x 10\(^{-3}\) kcal/mole)
\( T \) = absolute temperature (K).

To determine the activation energy, \( \ln(k) \) was plotted against the inverse of absolute temperature and \( E_a \) deduced from the slope of the line.

The degradation rate constants calculated from linear regression analysis are shown in Table IV-12. The high correlation coefficients indicated that over the extrusion temperature and holding time range studied the kinetics of degradation of vitamin E isomers and oryzanol were consistent with first order kinetics. The degradation rate constants increased 1.3 - 2.1 fold with an increase in extrusion temperature from 110 to 140°C. Activation energies (\( E_a \)) in Table IV-13 were determined by regression
Table IV-12. First order reaction rate constants (k) for retention of vitamin E and oryzanol during post extrusion holding time

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Compound</th>
<th>k</th>
<th>SEC(^a)</th>
<th>R(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(°C)</td>
<td>(min(^{-1}))</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>α-T</td>
<td>9.76 x 10(^{-3})</td>
<td>1.02 x 10(^{-3})</td>
<td>0.989</td>
</tr>
<tr>
<td></td>
<td>α-T3</td>
<td>8.15 x 10(^{-3})</td>
<td>1.06 x 10(^{-3})</td>
<td>0.983</td>
</tr>
<tr>
<td></td>
<td>β-T</td>
<td>8.24 x 10(^{-3})</td>
<td>1.19 x 10(^{-3})</td>
<td>0.979</td>
</tr>
<tr>
<td></td>
<td>γ-T</td>
<td>8.68 x 10(^{-3})</td>
<td>1.16 x 10(^{-3})</td>
<td>0.964</td>
</tr>
<tr>
<td></td>
<td>γ-T3</td>
<td>9.70 x 10(^{-3})</td>
<td>7.31 x 10(^{-4})</td>
<td>0.994</td>
</tr>
<tr>
<td>110</td>
<td>δ-T</td>
<td>4.79 x 10(^{-3})</td>
<td>3.01 x 10(^{-3})</td>
<td>0.995</td>
</tr>
<tr>
<td></td>
<td>δ-T3</td>
<td>8.02 x 10(^{-3})</td>
<td>7.52 x 10(^{-4})</td>
<td>0.991</td>
</tr>
<tr>
<td></td>
<td>Oryzanol</td>
<td>2.54 x 10(^{-3})</td>
<td>9.13 x 10(^{-4})</td>
<td>0.867</td>
</tr>
<tr>
<td></td>
<td>Tocopherols</td>
<td>9.12 x 10(^{-3})</td>
<td>1.23 x 10(^{-3})</td>
<td>0.981</td>
</tr>
<tr>
<td></td>
<td>Tocotrienols</td>
<td>9.16 x 10(^{-3})</td>
<td>6.52 x 10(^{-3})</td>
<td>0.994</td>
</tr>
<tr>
<td></td>
<td>Total E isomers</td>
<td>9.15 x 10(^{-3})</td>
<td>8.60 x 10(^{-4})</td>
<td>0.991</td>
</tr>
<tr>
<td>120</td>
<td>α-T</td>
<td>1.06 x 10(^{-2})</td>
<td>8.61 x 10(^{-4})</td>
<td>0.993</td>
</tr>
<tr>
<td></td>
<td>α-T3</td>
<td>9.65 x 10(^{-3})</td>
<td>6.72 x 10(^{-4})</td>
<td>0.995</td>
</tr>
<tr>
<td></td>
<td>β-T</td>
<td>9.05 x 10(^{-3})</td>
<td>6.60 x 10(^{-4})</td>
<td>0.994</td>
</tr>
<tr>
<td></td>
<td>γ-T</td>
<td>1.08 x 10(^{-2})</td>
<td>1.42 x 10(^{-3})</td>
<td>0.983</td>
</tr>
<tr>
<td></td>
<td>γ-T3</td>
<td>1.19 x 10(^{-2})</td>
<td>4.01 x 10(^{-4})</td>
<td>0.998</td>
</tr>
<tr>
<td></td>
<td>δ-T</td>
<td>4.96 x 10(^{-3})</td>
<td>6.90 x 10(^{-4})</td>
<td>0.985</td>
</tr>
<tr>
<td></td>
<td>δ-T3</td>
<td>1.08 x 10(^{-2})</td>
<td>5.00 x 10(^{-3})</td>
<td>0.999</td>
</tr>
<tr>
<td></td>
<td>Oryzanol</td>
<td>4.64 x 10(^{-3})</td>
<td>8.01 x 10(^{-4})</td>
<td>0.954</td>
</tr>
<tr>
<td></td>
<td>Tocopherols</td>
<td>1.04 x 10(^{-2})</td>
<td>1.04 x 10(^{-3})</td>
<td>0.990</td>
</tr>
<tr>
<td></td>
<td>Tocotrienols</td>
<td>1.12 x 10(^{-2})</td>
<td>4.10 x 10(^{-4})</td>
<td>0.998</td>
</tr>
<tr>
<td></td>
<td>Total E isomers</td>
<td>1.09 x 10(^{-2})</td>
<td>6.41 x 10(^{-4})</td>
<td>0.996</td>
</tr>
</tbody>
</table>

(Table con'd.)
<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Compound</th>
<th>k (min⁻¹)</th>
<th>SEC*</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>α-T</td>
<td>1.22 x 10⁻²</td>
<td>1.34 x 10⁻³</td>
<td>0.978</td>
</tr>
<tr>
<td></td>
<td>α-T3</td>
<td>1.33 x 10⁻²</td>
<td>7.15 x 10⁻³</td>
<td>0.982</td>
</tr>
<tr>
<td></td>
<td>β-T</td>
<td>1.23 x 10⁻²</td>
<td>2.40 x 10⁻³</td>
<td>0.999</td>
</tr>
<tr>
<td></td>
<td>γ-T</td>
<td>1.06 x 10⁻²</td>
<td>1.47 x 10⁻³</td>
<td>0.999</td>
</tr>
<tr>
<td></td>
<td>γ-T3</td>
<td>1.28 x 10⁻²</td>
<td>1.90 x 10⁻⁴</td>
<td>0.999</td>
</tr>
<tr>
<td>130</td>
<td>δ-T</td>
<td>7.72 x 10⁻³</td>
<td>5.01 x 10⁻⁵</td>
<td>0.997</td>
</tr>
<tr>
<td></td>
<td>δ-T3</td>
<td>6.17 x 10⁻³</td>
<td>1.22 x 10⁻³</td>
<td>0.996</td>
</tr>
<tr>
<td></td>
<td>Oryzanol</td>
<td>5.21 x 10⁻³</td>
<td>2.01 x 10⁻⁴</td>
<td>0.998</td>
</tr>
<tr>
<td></td>
<td>Tocopherols</td>
<td>1.15 x 10⁻²</td>
<td>3.70 x 10⁻⁴</td>
<td>0.994</td>
</tr>
<tr>
<td></td>
<td>Tocotrienols</td>
<td>1.22 x 10⁻²</td>
<td>4.21 x 10⁻⁴</td>
<td>0.998</td>
</tr>
<tr>
<td></td>
<td>Total E isomers</td>
<td>1.20 x 10⁻²</td>
<td>4.01 x 10⁻³</td>
<td>0.997</td>
</tr>
<tr>
<td></td>
<td>α-T</td>
<td>1.30 x 10⁻²</td>
<td>8.61 x 10⁻³</td>
<td>0.989</td>
</tr>
<tr>
<td></td>
<td>α-T3</td>
<td>1.19 x 10⁻²</td>
<td>6.72 x 10⁻⁴</td>
<td>0.996</td>
</tr>
<tr>
<td></td>
<td>β-T</td>
<td>1.62 x 10⁻²</td>
<td>6.61 x 10⁻⁴</td>
<td>0.978</td>
</tr>
<tr>
<td></td>
<td>γ-T</td>
<td>1.22 x 10⁻²</td>
<td>1.42 x 10⁻³</td>
<td>0.985</td>
</tr>
<tr>
<td></td>
<td>γ-T3</td>
<td>1.42 x 10⁻²</td>
<td>4.02 x 10⁻³</td>
<td>0.999</td>
</tr>
<tr>
<td>140</td>
<td>δ-T</td>
<td>8.60 x 10⁻³</td>
<td>6.91 x 10⁻⁴</td>
<td>0.999</td>
</tr>
<tr>
<td></td>
<td>δ-T3</td>
<td>1.27 x 10⁻²</td>
<td>5.01 x 10⁻⁴</td>
<td>0.990</td>
</tr>
<tr>
<td></td>
<td>Oryzanol</td>
<td>5.61 x 10⁻³</td>
<td>8.02 x 10⁻⁴</td>
<td>0.998</td>
</tr>
<tr>
<td></td>
<td>Tocopherols</td>
<td>1.28 x 10⁻²</td>
<td>1.04 x 10⁻³</td>
<td>0.999</td>
</tr>
<tr>
<td></td>
<td>Tocotrienols</td>
<td>1.35 x 10⁻²</td>
<td>4.11 x 10⁻⁴</td>
<td>0.999</td>
</tr>
<tr>
<td></td>
<td>Total E isomers</td>
<td>1.32 x 10⁻²</td>
<td>6.40 x 10⁻⁴</td>
<td>0.999</td>
</tr>
</tbody>
</table>

* Standard error of coefficient
Table IV-13. Calculated $E_a$ and $Q_{10}$ values for vitamin E isomers and oryzanol.

<table>
<thead>
<tr>
<th>Compound</th>
<th>$k$ (min$^{-1}$)$^a$</th>
<th>$E_a$ (kcal/mole)</th>
<th>$Q_{10}$ ($110 - 120^\circ$C)</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha$-T</td>
<td>-1.592</td>
<td>3.164</td>
<td>1.117</td>
<td>0.984</td>
</tr>
<tr>
<td>$\alpha$-T3</td>
<td>-1.798</td>
<td>3.571</td>
<td>1.134</td>
<td>0.986</td>
</tr>
<tr>
<td>$\gamma$-T</td>
<td>-3.709</td>
<td>7.370</td>
<td>1.296</td>
<td>0.951</td>
</tr>
<tr>
<td>$\gamma$-T3</td>
<td>-2.999</td>
<td>5.959</td>
<td>1.233</td>
<td>0.992</td>
</tr>
<tr>
<td>$\delta$-T</td>
<td>-1.735</td>
<td>3.448</td>
<td>1.129</td>
<td>0.977</td>
</tr>
<tr>
<td>$\delta$-T3</td>
<td>-3.471</td>
<td>6.897</td>
<td>1.275</td>
<td>0.889</td>
</tr>
<tr>
<td>Oryzanol</td>
<td>-3.982</td>
<td>7.913</td>
<td>1.321</td>
<td>0.813</td>
</tr>
<tr>
<td>Tocopherols</td>
<td>-1.783</td>
<td>3.543</td>
<td>1.132</td>
<td>0.997</td>
</tr>
<tr>
<td>Tocotrienols</td>
<td>-1.997</td>
<td>3.967</td>
<td>1.150</td>
<td>0.960</td>
</tr>
<tr>
<td>Total E isomers</td>
<td>-1.921</td>
<td>3.817</td>
<td>1.143</td>
<td>0.977</td>
</tr>
</tbody>
</table>

$^a$ mg of vitamin E loss/kg rice bran per min.

$^b$ $Q_{10}$ value = The increase in the rate of change for each 10°C rise in temperature.
analysis of reaction rate constant and the resultant Arrhenius plot (Fig.IV-19 - Fig.IV-21). The higher the value of activation energy \( (E_a) \) the greater was that substance's sensitivity to an increase in temperature (Labuza, 1972; Saguy and Karel, 1980). The order of \( E_a \) value of vitamin E vitamers during post extrusion processing was \( \beta-T > \delta-T > \gamma-T > \alpha-T3 > \delta-T3 > \gamma-T3 > \alpha-T \) in Table IV-13. This order was nearly the reverse of the order of degradation of vitamin E. The order of degradation of vitamin E vitamers in extruded rice bran for all extrusion temperature was \( \gamma-T3 > \alpha-T > \alpha-T3 > \gamma-T > \delta-T3 > \beta-T > \delta-T \) in Table IV-10. The vitamin E isomers with high activation energy were more stable to post extrusion temperature.

Tocotrienols had higher \( E_a \) value than that of tocopherols. This indicated that tocopherols were less stable than tocotrienols during post extrusion holding. As oryzanol was relatively stable to heat processing it had the highest \( E_a \) value of the antioxidants. However, oryzanol had a lower linear correlation coefficient. The range of Activation energies \( (E_a) \) of vitamin E vitamers was 3.1 to 7.3 kcal/mole. Those values were relatively low compared to ascorbic acid in canned orange juice (21 - 41 kcal/mole) (Nagy and Smoot, 1977), vitamin A in beef liver (26 kcal/mole) (Wilkinson et al., 1981), and thiamine in beef puree (18 - 30 kcal/mole) (Mulley et al., 1975). This might suggest that extrusion processing improve degradation of vitamin E vitamers in rice
Fig. IV-19. Arrhenius plots for degradation of α-, β-, and γ-tocopherol and α-tocotrienol in rice bran on post extrusion processing (- - 95% confidence interval).
Fig. IV-20. Arrhenius plots for degradation of δ-tocopherol, γ- and δ-tocotrienol, and oryzanol in rice bran on post extrusion processing (----95% confidence interval).
Fig. IV-21. Arrhenius plots for degradation of total tocopherol and total tocotrienol in rice bran on post extrusion processing (----- 95% confidence interval).

A
- Total tocopherol
- $r = 0.997$

B
- Total tocotrienol
- $r = 0.962$
Lee et al. (1977) reported that $E_a$ value for anaerobic destruction of ascorbic acid in tomato juice was 3.3 kcal/mole. The loss of tocopherol has been reported by Jensen (1969) in seaweed dehydrated at 4 - 25°C. The activation energy was about 10 kcal/mole. Differences in activation energy ($E_a$) may be due to compositional factors such as water activity, moisture content, solids concentration, pH, and others (Hendel et al., 1955; Jensen, 1969; Karel and Nickerson, 1964; Labuza, 1972; Lee et al., 1977; Saguy et al., 1980). Furthermore, when the reaction mechanism changes with temperature, the activation energy may vary substantially. Therefore, all equations, including the Arrhenius relation, have limited applicability (Tannenbaum, 1975). However, activation energy for vitamin E vitamers in post extrusion processing was lower. This may be due to two different degradative pathways, extrusion and post extrusion processing.

The increase in the rate of change for each 10°C rise in temperature, also known as $Q_{10}$ value, was calculated from activation energy ($E_a$) values and is shown in Table IV-13. The $Q_{10}$ values were calculated for the temperature change between 110 and 120°C. For reactions such as enzymatically induced color or flavor change in foods, degradation of natural pigments, nonenzymatic browning, and microbial growth rate, $Q_{10}$ is usually around 2. $Q_{10}$ values of vitamin E vitamers in this study ranged from 1.1 to 1.3. This is due to the low activation energy value.
D. EXPERIMENT 4: Quality of Rice Bran Oil Extracted from Extruded Rice Bran

1. Composition of Crude Rice Bran Oil

Total crude rice bran lipids accounted for 22.1% of the bran (dry basis) and consisted of 88.8% neutral lipid (NL), 7.3% glycolipid (GL) and 3.9% phospholipid (PL) (Table IV-14). Fatty acid analysis showed that oleic (C18:1) was the predominant fatty acid, followed by linoleic (18:2) and palmitic (C16:0) acids in all lipid fractions. The fatty acid profile of NL reflected largely that of TL. Arachidic (C20:0) acid was not detected in GL and PL fractions. Palmitoleic (C16:1) acid was present in some rice bran (Hemavathy and Prabhakar, 1986; Lugay and Juliano, 1964; Miyazawa et al., 1977). The NL fraction contained free fatty acids (FFA). The term "neutral lipid" is used normally to define tri-, di-, and mono-glycerides, waxes, and FFA (Christie, 1982). This is less precise and may occasionally be ambiguous because FFA are frequently classed as neutral lipids despite the presence of the free carboxyl group. However, FFA is relatively less polar than GL and PL compounds. Column chromatography using chloroform, acetone, and methanol could not separate FFA from NL. In this study, most samples were analyzed with a liquid-liquid fractionation method that can fractionate into free fatty acid (FFA), NL, and PL. Column chromatography was used for limited samples to monitor fatty acid composition of GL fraction during rice bran storage.
<table>
<thead>
<tr>
<th>Lipid class</th>
<th>Wt. (%)</th>
<th>14:0</th>
<th>16:0</th>
<th>18:0</th>
<th>18:1</th>
<th>18:2</th>
<th>18:3</th>
<th>20:0</th>
<th>Saturated</th>
<th>Unsaturated</th>
</tr>
</thead>
<tbody>
<tr>
<td>TL</td>
<td>21.2</td>
<td>0.30</td>
<td>21.13</td>
<td>1.68</td>
<td>39.89</td>
<td>36.18</td>
<td>0.33</td>
<td>0.42</td>
<td>23.53</td>
<td>76.47</td>
</tr>
<tr>
<td>NL</td>
<td>88.8</td>
<td>0.32</td>
<td>20.08</td>
<td>1.71</td>
<td>41.55</td>
<td>35.25</td>
<td>0.62</td>
<td>0.46</td>
<td>22.58</td>
<td>77.42</td>
</tr>
<tr>
<td>GL</td>
<td>7.3</td>
<td>0.06</td>
<td>25.05</td>
<td>0.13</td>
<td>40.16</td>
<td>34.51</td>
<td>0.11</td>
<td>0.00</td>
<td>25.23</td>
<td>74.77</td>
</tr>
<tr>
<td>PL</td>
<td>3.9</td>
<td>0.13</td>
<td>19.45</td>
<td>0.14</td>
<td>41.52</td>
<td>38.60</td>
<td>0.16</td>
<td>0.00</td>
<td>19.72</td>
<td>80.28</td>
</tr>
</tbody>
</table>

Values are means of three analyses.

TL, Total lipids in crude rice bran oil; NL, neutral lipids; GL, glycolipids; PL, phospholipids.

Fractionated column chromatography.
The solvent used for oil extraction can cause differences in fatty acid composition in oil. Table IV-15 showed fatty acid composition in crude rice bran oil extracted with petroleum ether. Total lipid extracted with chloroform/methanol contained more linoleic acid and less palmitic and oleic acid than oil extracted with petroleum ether. Similar differences in linoleic and oleic acids were found in milled rice oil (Resurreccion and Juliano, 1975) and rice bran oil (Lee et al., 1965) extracted with the same solvent systems as in this study. Their explanation was that the polar solvent chloroform/methanol extract more lipid, particularly polar oil such as phospholipids, than non-polar solvents such as petroleum ether and hexane. This is a well recognized mechanism in oil extraction (Poling et al., 1955).

As seen in Tables IV-14 and IV-15, PL in oil extracted with chloroform/methanol contained more linoleic acid and less palmitic and oleic acid than those of PL in oil extracted petroleum ether.

There are three major phospholipids in rice bran oil, phosphatidylcholine (35-38%), phosphatidylethanolamine (27-29%), and phosphatidylinositol (22-23%) (Sayre, 1988; Hemavathy and Prabhakar, 1986; Miyazawa et al., 1977). These compounds have different solubilities in aqueous ethanol or ethanol. Phosphatidylcholine (PC) is alcohol soluble, phosphatidylethanolamin (PE) is distributed between alcohol soluble and insoluble and phosphatidylinositol (PI) is insoluble (Sonntag, 1985).
Table IV-15 Major lipid classes of crude bran oil extracted with petroleum ether and their fatty acid composition

<table>
<thead>
<tr>
<th>Lipid class</th>
<th>Wt. %</th>
<th>14:0</th>
<th>16:0</th>
<th>18:0</th>
<th>18:1</th>
<th>18:2</th>
<th>18:3</th>
<th>20:0</th>
<th>Sat. rated</th>
<th>Uns. rated</th>
</tr>
</thead>
<tbody>
<tr>
<td>TL</td>
<td>21.4</td>
<td>0.22</td>
<td>23.07</td>
<td>1.43</td>
<td>42.21</td>
<td>31.33</td>
<td>0.31</td>
<td>0.43</td>
<td>25.25</td>
<td>75.75</td>
</tr>
<tr>
<td>NL</td>
<td>89.7</td>
<td>0.33</td>
<td>21.12</td>
<td>1.73</td>
<td>41.49</td>
<td>34.22</td>
<td>0.63</td>
<td>0.48</td>
<td>23.18</td>
<td>76.82</td>
</tr>
<tr>
<td>GL</td>
<td>7.1</td>
<td>0.08</td>
<td>26.21</td>
<td>0.17</td>
<td>40.06</td>
<td>33.36</td>
<td>0.14</td>
<td>0.00</td>
<td>26.46</td>
<td>73.54</td>
</tr>
<tr>
<td>PL</td>
<td>3.2</td>
<td>0.14</td>
<td>25.84</td>
<td>0.26</td>
<td>43.24</td>
<td>30.25</td>
<td>0.18</td>
<td>0.00</td>
<td>26.24</td>
<td>73.66</td>
</tr>
</tbody>
</table>

* Values are means of three analyses.

** TL, Total lipids in crude rice bran oil; NL, neutral lipids; GL, glycolipids; PL, phospholipids.

---

* Fractionated column chromatography.
According to thin-layer chromatography in a chloroform:methanol:ammonia (65:35:5) solvent system (Miyazawa et al., 1977; Christie, 1982), PE might have higher solubility than the others in chloroform/methanol solvent. The fatty acid profile of PE reflected mainly fatty acid composition of PL. The PE contained more linoleic acid (46-50%) than that of PC (34-39%) and that of PI (28-32%) in rice bran oil (Miyazawa et al., 1977). The linoleic acid in PE was concentrated in position $sn-2$ (61-70%) and palmitic acid in position $sn-1$ (49-54%). The PL fraction in oil extracted petroleum contained higher palmitic acid than that of oil extracted chloroform/methanol. The PI constituents normally are soluble in non-polar solvents and contained more palmitic acid (37%) than that of PE (24-27%) and PC (21-20%) in rice bran oil. PE and PI solubilities changed composition of fatty acid in oil extracted with different solvents. The mixture of chloroform and methanol is preferred for analysis of phospholipid or linoleic acid in lipid and oil.

2. Free Fatty Acid in Extruded Rice Bran

Extrusion Holding Time: Table IV-16 shows the effect of post extrusion holding time for all extrusion temperatures on the free fatty acid content of extruded rice bran during 375 days of storage. Statistical analysis indicated that there was no significant effect of post extrusion holding time on free fatty acid content of rice bran. Randall et al., (1985) studied the effect of post extrusion holding time (0, 3, 5, and 10 min) at 105, 120, 130, and 140°C extrusion temperature on lipase activity. They reported that
Table IV-16. Effect of post extrusion holding time on free fatty acid during storage

<table>
<thead>
<tr>
<th>Holding time (minute)</th>
<th>Mean value of free fatty acid (g/100g oil)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.78&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>2.71&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>6</td>
<td>2.64&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Total FFA calculated by sum of individual free fatty acid.

<sup>b</sup> Means with the same letter are not significantly (p < 0.05) different.
holding time after extrusion under 10.5 and 13.4% moisture conditions did not appear to affect free fatty acid content. Also, Saunders and Heltved (1985) reported that holding time had no beneficial effect on reducing lipase activity.

Storage Stability: Since there was no significant difference between holding time treatments, holding time values were pooled. Fig.IV-22 shows changes of total free fatty acid content in raw (A) and stabilized rice bran (B). Free fatty acid contents in extruded rice bran for all temperatures were slightly higher than that of raw rice bran at 0 day storage. Yoshida and Kajimoto (1986) reported that heat treated soybean lost triglycerides and increased free fatty acid. In the initial period of storage, free fatty acid content of raw rice bran rapidly increased and reached 42% by day 35. The rate of FFA formation after day 35 decreased. The formation of free fatty acid in raw rice bran in the present experiment was lower compared with published data (Loeb et al., 1949; Randall et al., 1985; Roseman et al., 1963). In these studies, free fatty acids in raw rice bran was found to be over 60% at 40 days storage. FFA formation in rice bran during storage is dependent on moisture content, microbial contamination, and humidity. Moisture content of the bran was a limiting factor in growth of microorganisms and reaction of enzymes (Loeb and Mayne, 1952; Dirks et al., 1955).
Fig. IV-22. Total free fatty acid in raw rice bran and stabilized rice bran stored for one year.
The FFA level in rice bran for all extrusion temperatures was not significantly (p > 0.05) different for 7 day storage (Fig. IV-22(b)). Beyond 105 day of storage, the FFA level was significantly higher (p < 0.05) for extrusion temperature 110°C than for the other three extrusion temperatures. There was no difference (p > 0.05) in FFA content until 165 days storage among extrusion temperature of 120, 130, and 140°C. The FFA level for extrusion temperature 120°C at 210 days was higher (p < 0.05) than that for the extrusion temperatures 130 and 140°C, while there was no significant difference in FFA level between 130 and 140°C. However, there were differences (p < 0.05) in FFA content among extrusion temperature 120, 130, and 140°C at 375 day storage. The increase in FFA in rice bran extruded at 110°C might be related to residual lipolytic enzymes initially. Residual peroxidase activity in rice bran (12.2% moisture) extruded at 105°C was about 22% (Randall et al., 1985) and residual lipase activity in rice bran extruded at 110°C was 2.3% (Saunders and Heltved, 1985). These activities were higher than those of 120, 130, and 140°C (residual peroxidase, below 4%; lipase activity, below 0.4). The moisture content in rice bran extruded at 120, 130, and 140°C was 6.3-4.8% (Sayre et al., 1985). Loeb and Mayne (1952) reported that counts of yeast and bacteria did not increase in rice bran with less than 10.7% moisture content during 45 day storage. They found a relationship among moisture content of rice bran, microflora, and FFA formation in rice bran. During long-term storage, the increase in moisture due to humidity and heat resulted in growth of microorganisms. The increase
in FFA contents in rice bran extruded at 120, 130, and 140°C after 165 day storage might be due to microbial contamination and thus action of microbial enzymes. Also, the significant difference (p < 0.05) in FFA content among these three extrusion temperatures at 375 day storage might be relative to initial moisture content and/or residual lipolytic enzymes. Loeb et al., (1949) reported that an increase in moisture in rice bran during storage was dependent on initial moisture content as well as humidity and heat. The moisture in predried rice bran increased from 3.52, 3.05, and 2.92 to 6.02, 4.41, and 3.91 (increases of 71, 44.5, and 33.5%) during 7 days storage, respectively.

Composition of Free Fatty Acid: Table IV-17 shows the composition of free fatty acid in raw bran and stabilized rice bran during storage. Although most of the FFA was hydrolyzed from neutral lipid, the fatty acid composition differed slightly in oleic and linoleic acid between FFA and NL composition (Table IV-18). Myristic (C14:0) and arachidic (C20:0) acids were not detected in raw rice bran at 0 day; however, these FFA were liberated upon storage, presumably from the neutral lipid. Since, these two FFA were not detected in rice bran extruded at 140°C during one year storage, these results indicate that a relatively lower amount of neutral was hydrolyzed in well stabilized bran. As expected, linoleic acid decreased with storage time. The decomposition of linoleic acid was higher in raw rice bran and rice bran extruded to 110°C than at the other extrusion temperatures.
Table IV-17. Free fatty acid composition of raw bran and stabilized rice bran during storage*  

<table>
<thead>
<tr>
<th>Extrusion temperature (°C)</th>
<th>Storage day</th>
<th>Fatty acid composition (%)</th>
<th>Saturated</th>
<th>Unsaturated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>14:0</td>
<td>16:0</td>
<td>18:0</td>
<td>18:1</td>
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<tr>
<td>0</td>
<td>-</td>
<td>19.12</td>
<td>1.42</td>
<td>39.91</td>
</tr>
<tr>
<td>7</td>
<td>0.50</td>
<td>20.29</td>
<td>1.49</td>
<td>39.56</td>
</tr>
<tr>
<td>Raw</td>
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<td>0.79</td>
<td>20.93</td>
<td>1.58</td>
</tr>
<tr>
<td></td>
<td>165</td>
<td>0.83</td>
<td>21.15</td>
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</tr>
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<td></td>
<td>210</td>
<td>1.19</td>
<td>22.80</td>
<td>1.87</td>
</tr>
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<td>1.48</td>
<td>22.82</td>
<td>2.16</td>
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<td>1.65</td>
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<tr>
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<td>-</td>
<td>19.83</td>
<td>1.70</td>
</tr>
<tr>
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<td>-</td>
<td>20.77</td>
<td>1.65</td>
</tr>
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<td>165</td>
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<td>21.30</td>
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<td>20.83</td>
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<td>-</td>
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<td>1.78</td>
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</table>

* Values are means of 18 analyses.
Table IV-18. Composition of neutral lipid in raw bran and stabilized rice bran during storage

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<tr>
<th>Extrusion Storage</th>
<th>Fatty acid composition (%)</th>
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<th>Unsaturated</th>
</tr>
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<td></td>
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<tr>
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<td>76.63</td>
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<tr>
<td>105</td>
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<tr>
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<td>77.27</td>
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<td>77.09</td>
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<td>77.42</td>
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<tr>
<td>7</td>
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<td>22.67</td>
<td>77.33</td>
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<tr>
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<td>76.77</td>
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</tr>
<tr>
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<td>77.13</td>
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<tr>
<td>7</td>
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<td>22.73</td>
<td>77.27</td>
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<tr>
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<td>77.27</td>
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<tr>
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<td>77.60</td>
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<td>76.29</td>
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<tr>
<td>375</td>
<td>0.46 22.66 1.92 41.26 32.51 0.70 0.49</td>
<td>25.53</td>
<td>74.47</td>
</tr>
</tbody>
</table>

* Values are means of 18 analyses.
Oleic acid composition did not change much during storage. Linolenic acid (C18:3) decreased markedly in raw rice bran compared with extruded rice bran. Total unsaturated acid content was dependent on linoleic acid. Total saturated acid content was dependent on palmitic acid. Lee et al., (1965) found that oleic acid increased and linoleic acid decreased in free fatty acid fraction of rice oil with storage time (24 weeks).

3. Phospholipid in Rice Bran during Storage

Fig.IV-23 shows the effect of stabilization temperature on phospholipid in rice bran during storage. Phospholipid in raw rice bran decreased steadily with 81% reduction by 375 days storage (Fig.IV-23A). The % phospholipid destroyed initially due to extrusion was 38.4, 31.1, 27.7, and 26.1% for extrusion temperature of 110, 120, 130, and 140°C, respectively. The trend in phospholipid reduction was related to an increase in FFA. Secondary antioxidant effects of phospholipids have been observed on frequent occasions. This has been ascribed to their metal chelating character (Brandt et al., 1973; Linow and Mieth, 1976). In contrast, Hudson and Lewis (1983) observed that the antioxidant properties of dipalmitoyl phosphatidyl ethanolamine in combination with α-tocopherol increased with phospholipid concentration up to 0.6% which was well above the level required for complexing with metal ions, and it was therefore concluded that metal complexing was not an important mechanism for this effect. Hildebrand (1984) reported that additions of tocopherols and all phospholipids increased the stability of soybean oil. Phosphatidylinositol
Fig. IV-23. Phospholipid in raw rice bran and stabilized rice bran stored for one year.
(PI) and phosphatidylethanolamine (PE) appear to be more effective than phosphatidylcholine (PC) in increasing oil stability. The report suggested that the antioxidant effect of phospholipids was caused by a synergism with tocopherol rather than by a special ability of the pyrophosphate to bind traces of mineral. Phospholipids may act by releasing protons and thus cause the rapid decomposition of hydroperoxides without the formation of free radical (Tai et al., 1974; Pokorny, et al., 1981). Chu and Lin (1993) showed that the content of PE in soybean oil decreased gradually during storage at 60°C, whereas the contents of PC, PI and phosphatidic acid fluctuated during storage. They claimed that PE clearly acts synergistically in concert with tocopherols and some of their derivatives as antioxidants, likely as a result of the strong proton-donating ability of PE as compared to the other phospholipids. Many studies found similar results in wheat flour lipid (Watt et al., 1949), in perilla oil (Kashima et al., 1991), in butter fat (Bhatia, 1978) and in a lard model system (Ishikawa et al., 1984). The decomposition rates of phospholipid during storage was significantly higher than those of neutral lipid or the formation rate of free fatty acid. However, the rates were less than those of total vitamin E vitamers. The correlation coefficients between retention of total E vitamers and that of phospholipid, between FFA formation and retention of phospholipid, and between retention of total E vitamers and FFA formation are shown in Fig.IV-24, Fig.IV-25, and Fig.IV-26, respectively.
Fig. IV-24. Regression of total vitamin E vitamer content on phospholipid content. The correlation is significant (p < 0.01); r=Correlation coefficient.
Fig. IV-25. Regression of total FFA content on phospholipid content. The correlation is significant ($p < 0.01$); $r$=Correlation coefficient.
Fig. IV-26. Regression of total vitamin E vitamer content on total FFA content. The correlation is significant (p < 0.01); r = Correlation coefficient.
Table IV-19 shows composition of phospholipid in raw rice bran and stabilized rice bran during storage. Oleic acid increased slightly and linoleic acid decreased in raw rice bran with storage time as was previously found by Lee et al. (1965). However, changes of oleic acid and linolenic acid in extruded rice bran were not consistent with storage. Linoleic acid in extruded rice bran decreased with storage time. Arachidic acid, interestingly, was not detected in extruded rice bran.

4. Neutral Lipid in Rice Bran during Storage

Table IV-20 shows neutral lipid (NL) content in raw and extruded rice bran during storage. The neutral lipid fraction contained glycolipid at a level of 7.3 g/100 g rice bran oil. Raw rice bran lost 71.6% of its NL content during storage. NL content decreased slightly in all extrusion temperature as FFA increased in Fig.IV-22. NL content was not significantly (p < 0.05) different in rice bran extruded to 120, 130, and 140°C.

Fatty acid composition of NL in raw and extruded rice bran was stable compared with that of FFA and NL (Table IV-20). Only linoleic acid decreased in raw and extruded rice bran during storage. Hove and Harris (1951) showed that there was a correlation between vitamin E vitamer content and total content of linoleic acid in high quality fats of vegetable origin. Thafvelin and Oksanen (1965) reported that a strong correlation (correlation coefficient=0.72) existed between the tocopherol and the linolenic acid content in dried hay. In contrast, the linolenic acid in this
Table IV-19. Composition of phospholipid in raw bran and stabilized rice bran during storage*  

<table>
<thead>
<tr>
<th>Extrusion storage temperature (°C)</th>
<th>Fatty acid composition (%)</th>
<th>Saturated</th>
<th>Unsaturated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>14:0</td>
<td>16:0</td>
<td>18:0</td>
</tr>
<tr>
<td>0</td>
<td>0.12</td>
<td>19.42</td>
<td>0.13</td>
</tr>
<tr>
<td>7</td>
<td>0.13</td>
<td>20.38</td>
<td>0.13</td>
</tr>
<tr>
<td>105</td>
<td>0.13</td>
<td>21.26</td>
<td>0.15</td>
</tr>
<tr>
<td>165</td>
<td>0.14</td>
<td>21.88</td>
<td>0.15</td>
</tr>
<tr>
<td>210</td>
<td>0.15</td>
<td>22.36</td>
<td>0.17</td>
</tr>
<tr>
<td>375</td>
<td>0.16</td>
<td>22.68</td>
<td>0.18</td>
</tr>
</tbody>
</table>

*Values are means of 18 analyses.
Table IV-20. Neutral lipid contents in raw and stabilized rice bran during storage

<table>
<thead>
<tr>
<th>Extrusion temperature (°C)</th>
<th>Storage (day)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>7</td>
<td>105</td>
<td>165</td>
<td>210</td>
<td>367</td>
</tr>
<tr>
<td>Raw bran</td>
<td>92.4</td>
<td>82.9</td>
<td>51.5</td>
<td>44.2</td>
<td>35.7</td>
<td>26.2</td>
</tr>
<tr>
<td>110</td>
<td>92.2</td>
<td>91.5</td>
<td>90.2</td>
<td>88.4</td>
<td>86.1</td>
<td>84.6</td>
</tr>
<tr>
<td>120</td>
<td>92.1</td>
<td>91.6</td>
<td>90.4</td>
<td>89.5</td>
<td>88.3</td>
<td>86.8</td>
</tr>
<tr>
<td>130</td>
<td>91.8</td>
<td>91.8</td>
<td>91.2</td>
<td>89.9</td>
<td>88.7</td>
<td>87.4</td>
</tr>
<tr>
<td>140</td>
<td>91.7</td>
<td>91.7</td>
<td>90.7</td>
<td>89.4</td>
<td>88.8</td>
<td>87.5</td>
</tr>
</tbody>
</table>

* Neutral fraction included glycolipid.
* Mean of 18 replicate analyses.
* Dewaxed and degummed.
experiment was not related to decomposition of total E vitamers due to the low content of linolenic acid in rice bran compared with 49% linolenic acid in hay lipid. Also, the researchers suggested that when fats are subject to autoxidation, tocopherol destruction takes place either by a spontaneous oxidation by atmospheric oxygen or by reaction with reactive hydroperoxides in the fat. The change of fatty acid composition during heating and storage is dependent on the amount of individual fatty acid, especially polyunsaturated fatty acid, and antioxidants in oil and fat. Marero et al. (1986) reported that composition of linoleic and linolenic acid with a higher amount of tocopherols in spices was unaffected, but oil in spices that had a small amount of tocopherols was subjected to degradation. This suggested that vitamin E in the presence of a large amount of polyunsaturated fatty acid in oil is destroyed much faster than in oils with a small amount of polyunsaturated fatty acid.

5. Color Change in Extruded Rice Bran during Storage

Color change was strongly related to temperature of heating and moisture content during extrusion (Bailey et al., 1994). Fig.IV-27 show color changes in extruded rice bran during storage. Rice bran extruded at higher temperature had higher "L", "a", and "b" values than the rice bran extruded at lower temperature. The latter samples were darker (lower lightness) in color, more red (higher "a" value) in color, and more yellow (higher "b" value) in color.
Fig. IV-27. Hunter color values of stabilized rice bran stored at ambient temperature for one year.
E. EXPERIMENT 5: Effect of Microwave Heating on Vitamin E, Oryzanol, and Fatty Acids in Rice Bran

1. Composition of Crude Rice Bran Oil

Rice bran (bran II) used in this and the following experiment (i.e., experiment 5 and 6) was different from the rice bran (bran I) used in previous studies (i.e., experiment 3 and 4). The contents of major lipid class and composition of fatty acid in crude rice bran oil (Table IV-21) were markedly different from those of the previous rice bran oil (Table IV-14). Fatty acids detected in both oils were the same. The TL, NL, GL, and PL from bran II contained 38.8, 37.2, 36.6, and 37.9% oleic acid (C18:1), and 34.6, 35.3, 35.8, and 39.5% linoleic acid (C18:2) compared to 39.8, 41.6, 40.2, and 41.5% oleic acid, and 36.2, 35.2, 34.5, and 38.6% linoleic acid, respectively. Bran II contained higher total saturated fatty acids (22.3 - 27.6%) than bran I (19.7 - 25.2%). The GL fraction of bran II was 6.8% compared to against 7.3% in bran I. Hemavathy and Prabhakar (1987) reported lipid composition of rice bran from three varieties of rice. Lipids amounted to 21.9-23.0% of the bran dry weight and consisted of 88.1-89% neutral lipids, 6.3-7.0% glycolipids and 4.5-4.9% phospholipids.

2. Free Fatty Acids in Rice Bran

Microwave Treatment: The temperature of the rice bran, determined after each treatment, increased from 25°C to 138°C, and moisture content decreased from 10.5% to 4.9% as the time of microwave treatment increased
Table IV-21. Major lipid classes of crude bran oil extracted with a mixture of chloroform and methanol, and their fatty acid composition.

<table>
<thead>
<tr>
<th>Lipid class</th>
<th>Wt. (%)</th>
<th>14:0</th>
<th>16:0</th>
<th>18:0</th>
<th>18:1</th>
<th>18:2</th>
<th>18:3</th>
<th>20:0</th>
<th>Saturated (%)</th>
<th>Unsaturated (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TL</td>
<td>20.1</td>
<td>0.40</td>
<td>22.21</td>
<td>2.21</td>
<td>38.85</td>
<td>34.58</td>
<td>1.14</td>
<td>0.61</td>
<td>25.43</td>
<td>74.57</td>
</tr>
<tr>
<td>NL</td>
<td>89.2</td>
<td>0.43</td>
<td>23.41</td>
<td>1.88</td>
<td>37.24</td>
<td>35.29</td>
<td>1.07</td>
<td>0.68</td>
<td>26.40</td>
<td>73.60</td>
</tr>
<tr>
<td>GL</td>
<td>6.8</td>
<td>0.09</td>
<td>27.34</td>
<td>0.18</td>
<td>36.45</td>
<td>35.76</td>
<td>0.18</td>
<td>0.00</td>
<td>27.61</td>
<td>72.39</td>
</tr>
<tr>
<td>PL</td>
<td>4.0</td>
<td>0.12</td>
<td>22.04</td>
<td>0.17</td>
<td>37.96</td>
<td>39.55</td>
<td>0.16</td>
<td>0.00</td>
<td>22.33</td>
<td>77.67</td>
</tr>
</tbody>
</table>

* Values are means of three replicate analyses.

* TL, Total lipids in crude rice bran oil; NL, neutral lipids; GL, glycolipids; PL, phospholipids.

* Crude rice bran oil extracted with chloroform and methanol as described in text.

* Fractionated by column chromatography.
to 12 min (Table IV-22). A small reduction in moisture content was observed in the initial stage of heating, i.e. by 1% at 4 min. After 4 min of heating, there was a linear relationship between moisture and exposure-time. Where the also was observed in soybean during microwave heating (Pour-El et al. 1981; Yoshida and Kajimoto, 1986; Yoshida and Kajimoto, 1988). The loss of weight depended upon total volatile substances, but it was considered to be mostly moisture.

Bran temperature varied dependent on position of revolving carousel. Bran temperature was the highest in the middle of the carousel followed by center, and outside of carousel. The temperature in samples was raised and moisture in sample was lost as a function of exposure time, initial moisture contents, amount of sample loaded in microwave oven, and power levels (Pour-El et al., 1981; Rhee and Yoon, 1984; Pohle et al., 1964). The higher the moisture of soybeans, the lower the internal temperature of the soybeans over the same heating time after 4 min exposure time (Yoshida and Kajimoto, 1988). At the 8 min heating, a dark brownish color in bran was observed and burnt odor became apparent. At 12 min heating, bran was partially burned in the middle of the carousel.

**Free Fatty Acid**: Fig.IV-28(A) shows formation of total free fatty acid in raw (A) and microwave treated rice bran. FFA contents of raw rice bran increased rapidly up to 63% after 49 days storage. Similar results were
Table IV-22. Moisture and free fatty acid, and temperature of rice bran as affected by microwave treatment

<table>
<thead>
<tr>
<th>Microwave Time (min)</th>
<th>Moisture content (%)</th>
<th>Bran temperature (°C)</th>
<th>FFA content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>10.5</td>
<td>-</td>
<td>3.6</td>
</tr>
<tr>
<td>4</td>
<td>9.5</td>
<td>65 - 95</td>
<td>3.5</td>
</tr>
<tr>
<td>8</td>
<td>8.2</td>
<td>88 - 121</td>
<td>3.7</td>
</tr>
<tr>
<td>12</td>
<td>4.8</td>
<td>118 - 138</td>
<td>3.9</td>
</tr>
</tbody>
</table>

* 450 watts with frequency of 2,450 MHz.
Fig. IV-28 Total free fatty acid in raw rice bran and microwave treated rice bran during 52 weeks storage.
reported in raw rice bran during storage (Loeb and Mayne, 1952; Dirks et al., 1955). This bran had a more rapid rise in FFA than did bran I (Fig. IV-22). Initial FFA content (3.6%) in bran II was higher than bran I (2.04%). Small differences, not statistically significant, were observed in FFA in rice bran oil during microwave heating (Table IV-22). A significant increase in FFA was reported in beef tallow, palm oil and lard after 8 to 20 min microwave heating (Charkey et al., 1961). Yoshida et al. (1991) reported that the FFA levels in oils increased slowly in the first 8 min of microwave heating (500 watt, 2450 MHz) and rapidly thereafter, in the following order: safflower > soybean > rapeseed > palm > coconut. There were no significant differences (p > 0.05) in the FFA levels for all oils until 8 min of heating. After 12 min of heating there were substantially greater differences (p < 0.01) with time for coconut and palm oils, which contain shorter fatty acyl chains such as caprylic, capric, lauric and myristic acids. Yoshida and Kajimoto (1986) reported that FFA level in soybean (moisture, 8.5%) increased 3-fold within 8 min of microwave heating at 500 watt, 2450 KHz. Triglycerides gradually decreased by 3.0% within 5 min and 17% in 8 min. Conversely, total lipids increased during the microwave heating process. Heat caused a certain amount of protein denaturation, which could have improved lipid extractability. Yoshida and Kajimoto (1988) observed that the higher the moisture content in soybean, the greater the increase in
FFA level. In contrast, Pour-El et al. (1981) reported that FFA level in soybean remained between 0.6 to 0.8% during microwave heating.

As seen in Fig. IV-28(B), FFA content in microwave treated rice bran was significantly ($p < 0.05$) different among the heating times after 7 weeks storage. FFA level in rice bran with microwave treatment for 4 and 8 min remained below 5.1% during 3 weeks storage and increased significantly after 7 weeks storage. There was no significant increase ($p > 0.05$) in FFA level by 24 weeks with microwave treatment for 12 min. During 3 weeks storage, the low FFA level in rice bran treated microwave for 4 and 8 min might be due to moisture content and partial inactivation of lipolytic enzymes. Lobe et al. (1949) reported that pre-dried rice bran (70°C for 1, 2, and 3 hr) with 7.87, 5.42, and 4.16% moisture content, respectively, contained about 32, 14, and 10% of FFA after 122 day storage. Also, they demonstrated that the rate of formation of free fatty acids is dependent upon moisture that heating and drying stabilized the bran, that bran will absorb moisture at the higher relative humidities, and that free fatty acids will not be formed in bran that had been sterilized and inactivated by autoclaving and maintained in that condition. Young et al. (1975) reported that the FFA levels of high moisture corn (25% moisture) increased from 5.4% to 27.0% at 48 days storage while that of dried corn remained relatively constant (5.3%) throughout storage (230 day). Rhee and Yoon (1984) studied the
effect of microwave heating (at 480 watt, 2450 MHz) on residual lipolytic enzyme activity in rice bran (10.4% moisture content). This study showed that residual peroxidase activity was 83% and residual lipase activity was 45% after 90 seconds microwave treatment. The results also indicated that peroxidase was more resistant to heat than lipase. Wang and Toledo (1987) reported that complete inactivation of lipoxygenase occurred when soybean temperature was around 100°C during microwave heating and lipoxygenase inactivation was favored in the samples that contained higher moisture contents (over 26.9%). The report suggested that not only is the temperature of the sample important for enzyme inactivation, but the moisture content of the samples also plays an important role, since it will result in higher energy absorption. Pour-El et al. (1981) reported that lipoxygenase and peroxidase activity in soybean retained relatively high levels compared with trypsin inhibitor activity after microwave heating. It is difficult to obtain uniform temperature in samples during heating with domestic microwave ovens even with the sample carousel rotating.

The changes of FFA composition in raw rice bran (Table IV-23) was similar to that of a previous study (Table IV-17). Typically, oxidation caused a decrease in the percentage of linoleic and linolenic acid and an increase in the percentage of saturated fatty acids. During 52 weeks storage, the fatty acid composition in raw rice bran reflected this. The higher the FFA levels,
Table IV-23 Free fatty acid composition of raw and microwave treated rice bran during 52 weeks storage

<table>
<thead>
<tr>
<th>Heating time (min)</th>
<th>Storage (weeks)</th>
<th>Fatty acid composition (%)</th>
<th>Saturated</th>
<th>Unsaturated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>14:0</td>
<td>16:0</td>
<td>18:0</td>
</tr>
<tr>
<td>0</td>
<td>0.37</td>
<td>20.11</td>
<td>1.30</td>
<td>40.86</td>
</tr>
<tr>
<td>1</td>
<td>0.58</td>
<td>11.42</td>
<td>1.36</td>
<td>40.34</td>
</tr>
<tr>
<td>Raw</td>
<td>0.91</td>
<td>23.07</td>
<td>1.44</td>
<td>40.70</td>
</tr>
<tr>
<td>3</td>
<td>0.95</td>
<td>23.37</td>
<td>1.71</td>
<td>41.85</td>
</tr>
<tr>
<td>7</td>
<td>1.35</td>
<td>23.86</td>
<td>1.69</td>
<td>41.86</td>
</tr>
<tr>
<td>24</td>
<td>1.67</td>
<td>24.75</td>
<td>1.93</td>
<td>42.03</td>
</tr>
</tbody>
</table>

|                  |                | 0.37 | 11.42 | 40.34 | 33.82 | 0.61 | 0.88 | 25.24 | 74.77 |
|                  | 0.91           | 23.07 | 1.44 | 40.70 | 32.26 | 0.55 | 1.07 | 26.49 | 73.51 |
|                  | 0.95           | 23.37 | 1.71 | 41.85 | 30.39 | 0.55 | 1.17 | 27.21 | 72.79 |
|                  | 1.35           | 23.86 | 1.69 | 41.86 | 29.70 | 0.42 | 1.12 | 28.02 | 71.98 |
|                  | 1.67           | 24.75 | 1.93 | 42.03 | 27.90 | 0.38 | 1.35 | 29.70 | 70.30 |

| 0                 | 20.85          | 1.52 | 40.80 | 36.17 | 0.66 | -     | 22.37 | 77.63 |
| 1                 | 21.53          | 1.50 | 41.35 | 35.07 | 0.55 | -     | 23.03 | 76.97 |
| 4                 | 0.08           | 21.76 | 1.51 | 41.30 | 34.77 | 0.58 | -     | 23.35 | 76.65 |
| 3                 | 0.12           | 22.04 | 1.50 | 41.15 | 34.51 | 0.57 | 0.11 | 23.77 | 76.23 |
| 7                 | 0.17           | 22.74 | 1.64 | 41.26 | 33.32 | 0.54 | 0.33 | 24.88 | 75.31 |
| 24                | 0.22           | 23.06 | 1.68 | 41.54 | 32.50 | 0.49 | 0.52 | 25.47 | 74.53 |
| 52                | 0.23           | 22.75 | 1.76 | 41.28 | 33.19 | 0.42 | 0.37 | 25.11 | 74.89 |

| 0                 | 21.05          | 1.55 | 40.69 | 36.09 | 0.62 | -     | 22.60 | 77.40 |
| 1                 | 21.31          | 1.49 | 41.32 | 35.29 | 0.59 | -     | 22.80 | 77.20 |
| 8                 | 21.86          | 1.63 | 41.35 | 34.61 | 0.55 | -     | 23.48 | 76.52 |
| 7                 | 22.13          | 1.64 | 41.29 | 34.41 | 0.53 | -     | 23.77 | 76.23 |
| 24                | 22.18          | 1.65 | 41.59 | 34.14 | 0.53 | -     | 23.82 | 76.18 |
| 52                | 0.37           | 20.11 | 1.30 | 40.86 | 35.90 | 0.62 | 0.85 | 22.62 | 77.38 |

* Values are means of 4 analyses.
the greater the changes of fatty acid composition because free fatty acids were susceptible to autooxidation. The ratios of rates of oxidation of oleic to linoleic to linolenic have been reported to be 1:10:20 (Wang and Hammond, 1977; Fatemi and Hammond, 1980). However, this was not observed in rice bran. The FFA composition in rice bran oil was dependent on the change of amount of polyunsaturated fatty acids (C18:2). During storage, fatty acid composition of FFA in rice bran heated for 4 min was similar to that for 8 min. Rice bran heated for 12 min maintained relatively stable fatty acid composition during 52 weeks storage. The results indicate that a higher level of FFA increased rate of oxidation.

3. Vitamin E Vitamers and Oryzanol in Microwave Stabilized Rice Bran

Rice bran II used in this experiment contained lower total E vitamers (271.5 mg/kg rice bran) than that (339.9 mg/kg rice bran) of the rice bran I used in previous experiments. The composition of tocopherols in rice bran II (Table IV-24) was higher (39.2%) compared with rice bran I (35.8%). Fig. IV-29 shows the decomposition of vitamin E vitamers and oryzanol in raw rice bran during storage. Rice bran II lost 11.6% and 5.5% of its total vitamin E vitamers and oryzanol at 7 days storage, respectively. After 49 days storage, rice bran II lost 55.1% and 39.38% of its total E vitamer and oryzanol, respectively. The order of losses of vitamin E vitamers and
Table IV-24 Tocopherol, tocotrienol, and oryzanol content of raw rice bran and rice bran after microwave treatment\textsuperscript{a,b,c}

<table>
<thead>
<tr>
<th></th>
<th>(\alpha)-T</th>
<th>(\alpha)-T(_3)</th>
<th>(\beta)-T</th>
<th>(\gamma)-T</th>
<th>(\gamma)-T(_3)</th>
<th>(\delta)-T</th>
<th>(\delta)-T(_3)</th>
<th>Ory</th>
<th>Tot-T</th>
<th>Tot-T(_3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bran I\textsuperscript{d}</td>
<td>68.26</td>
<td>50.71</td>
<td>7.72</td>
<td>42.05</td>
<td>145.39</td>
<td>3.86</td>
<td>21.96</td>
<td>3284.29</td>
<td>121.9</td>
<td>218.71</td>
</tr>
<tr>
<td>Bran II\textsuperscript{d}</td>
<td>63.16</td>
<td>38.19</td>
<td>8.99</td>
<td>32.25</td>
<td>119.71</td>
<td>1.99</td>
<td>7.26</td>
<td>3101.52</td>
<td>106.4</td>
<td>165.17</td>
</tr>
<tr>
<td>Heating time</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>4</td>
<td>58.91</td>
<td>36.11</td>
<td>8.86</td>
<td>30.58</td>
<td>111.74</td>
<td>1.92</td>
<td>6.92</td>
<td>2955.73</td>
<td>100.1</td>
<td>154.79</td>
</tr>
<tr>
<td>8</td>
<td>51.58</td>
<td>32.31</td>
<td>7.87</td>
<td>27.91</td>
<td>98.95</td>
<td>1.76</td>
<td>6.07</td>
<td>2881.29</td>
<td>89.1</td>
<td>137.33</td>
</tr>
<tr>
<td>12</td>
<td>38.94</td>
<td>24.62</td>
<td>6.44</td>
<td>21.09</td>
<td>76.21</td>
<td>1.48</td>
<td>4.77</td>
<td>2744.82</td>
<td>67.9</td>
<td>105.61</td>
</tr>
</tbody>
</table>

\textsuperscript{a} mg/kg rice bran.  
\textsuperscript{b} Based on mean of four analyses.  
\textsuperscript{c} \(\alpha\)-tocopherol; \(\alpha\)-tocotrienol; ory, oryzanol; Tot-T, total tocopherol; Tot-T\(_3\), total tocotrienol.  
\textsuperscript{d} Used in experiment 3 and 4.  
\textsuperscript{e} Used in current experiment.
Fig. IV-29. Tocopherols and tocotrienols in raw rice bran during 52 weeks storage.
oryzanol in raw rice bran II was similar to that of raw rice bran I. Also, the decomposition pattern of total E vitamers and oryzanol in rice bran II were similar to those of rice bran I (Fig. IV-13). After one year storage, FFA contents in rice bran oil was 63%. The decomposition rate of total E vitamers was coincided with FFA production. Yoshida et al. (1992) reported that the shorter the chain length and the higher the level of fatty acids, the greater was the reduction of tocopherols in oils. A trend similar to decomposition of tocopherols in rice bran was found in corn during storage (Young et al., 1975). During FFA formation period in corn, about 50% of its $\alpha$-tocopherol was lost.

Table IV-24 shows progressive deterioration of the E vitamers during microwave treatment. There were no significant ($p > 0.05$) decreases in individual vitamin E vitamers until 4 min microwave heating. After 8 min heating, individual E vitamers decreased ($p < 0.05$) with exposure time. The order of decomposition of vitamin E vitamers and oryzanol in rice bran during heating was the same as that found for extruded rice bran. $\alpha$-Tocopherol and $\alpha$-tocotrienol were the least stable of the E vitamers. This indicates that tocopherol and tocotrienol are effective antioxidants in rice bran during heating. The predominance of $\gamma$-tocotrienol in rice bran is significant in relation to the stability of the lipids to oxidative damage during heating and storage. Approximately 6% of the total vitamin E vitamers present in rice bran were destroyed at 4 min heating, 16% at 8 min heating,
and 36% at 12 min heating. While oryzanol in rice bran was destroyed 4.7% at 4 min heating, 7.1% at 8 min heating, and 11.5% at 12 min heating. Oryzanol, which is a relatively weaker antioxidant than α-tocopherol (Ramarathnam et al., 1986), was found to be stable to heat. With an increase in microwave heating, γ-tocopherol concentrations in linseed oil decreased with about a 54% reduction after 8 min heating, 72% after 12 min, and 80% after 20 min (Yoshida et al., 1990). The reduction in tocopherols in soybean and corn oils was much less than that in linseed oil; 8% after 12 min, 20% at 20 min. The reduction in olive and palm oil was 62% and 40% after 8 min, 83% and 60% after 12 min, and 100% and 78% at 20 min, respectively. This study reported that the decomposition of tocopherols in oil during microwave heating was related to fatty acid composition such as polyunsaturated fatty acids in oil. However, the reduction in amounts of tocopherols in oils in not necessarily directly related to other chemical properties. Yuki and Ishikawa (1976) reported that the ratio of tocopherol decomposition in vegetable frying oil, treated under simulated deep-fat frying conditions, was much greater in saturated oils than in unsaturated oils. The loss of E vitamers after 12 min heating was higher than that (24.7%) of extrusion at 140°C with holding time 6 min.

Fig. IV-30 shows changes of tocopherols, tocotrienols and oryzanol in raw rice bran and microwave treated rice bran during storage. At 7 day storage, rice bran heated for 12 min lost 25% and 8% of its total E vitamers
Fig. IV-30. Tocopherols, tocotrienols, and oryzanol in raw rice bran and microwave treated rice bran during 52 weeks storage.
and oryzanol. Rice bran treated for 4 min and 8 min lost 20% and 18% of its total E vitamers, and 6% and 4% of its oryzanol, respectively. The retention of total E vitamers in rice bran heated for 12 min was lower (p <0.05) than that of other rice brans during storage. Total E vitamer in rice bran for 4 min heating was similar to that of rice bran for 8 min heating from 7 to 21 days storage, and then became closer to that of raw rice bran. The difference in retention of total E vitamer among raw rice bran and microwave treated rice bran became smaller with storage time. This was also observed for oryzanol. The retention of oryzanol was similar to that of total E vitamers in rice bran. Total E vitamers in rice bran was reduced by 49-66% during 24 weeks storage. Thereafter, the loss of E vitamers was relatively lower. As discussed previously, it seems that a certain amount of E vitamers are stable from oxidation or not available as a radical scavenger although oxidation of lipid occurs. Esterified forms of E vitamers are more stable than free E vitamers. Rice bran oil extracted from rice bran used in this study contained about 4 - 5% esterified tocopherols and tocotrienols, although it is difficult to precisely determine esterified tocopherols and tocotrienols in rice bran. The decomposition rate of oryzanol was relatively higher than that of total E vitamers after 49 day storage and throughout the remainder of storage. Oryzanol was retained in rice bran for longer periods and may play a role as antioxidatnts during long term storage.
Fig. IV-31. Phospholipid in raw rice bran and microwave treated rice bran during 52 weeks storage.
4. Phospholipid in Rice Bran

Although only a minor component of total lipids in food, phospholipids are an important class of lipids due to their high content of unsaturated fatty acids. The tendency of phospholipids to oxidize very rapidly is of great importance for the development of off-flavor in food. Fig. IV-31 shows phospholipid in raw rice bran and microwave treated rice bran during storage. Phospholipid contents in rice bran during 8 min heating did not decreased significantly. At 12 min heating, phospholipid decreased (p < 0.05). In contrast, Yoshida and Kajimoto (1986) reported that the glycolipid component increased considerably, and the phospholipid component also increased slightly. They noted that browning substances may be attributed to the increase of glycolipid because their sugar moiety could produce browning substances. Tomioka and Kaneda (1974) reported that browning products formed in heated lecithin retained the structure of original lecithin, but were polymerized in the fatty acid portions. Hafez et al. (1989a) reported that phospholipids and phospholipid phosphorus were significantly (p < 0.05) reduced when the soybean seeds were subjected to microwave heating treatment for 9 or 12 min. No further reduction was observed in the phospholipids content when the treatment time was increased. The reduction in phospholipids following microwave heat treatment may be due to the decomposition of phospholipids and/or formation of complexes with protein or carbohydrate, which probably could not be extracted with chloroform. In
earlier reports, Hafez et al. (1983) observed that an increase in microwave heating time was accompanied by increasing browning substances in soybean. In the Maillard reaction, phospholipids are particularly reactive as they contain both polyunsaturated fatty acid and amines (Pokorny, 1981). Tomioka and Kaneda (1976) studied the different mechanisms of the Maillard reaction and found that browning proceeded as a base-catalyzed aldol condensation where the phosphorylcholine group acted as the main condensing agent.

During 21 day storage, phospholipid decreased significantly ($p < 0.05$) in raw and microwave treated rice bran. Thereafter, phospholipids in rice bran heated with microwave decreased slightly. Phospholipid in raw rice bran were decreased by 78% during one year storage. This decrease might be due to hydrolysis of lipolytic enzyme. The phospholipid in rice bran heated for 4 min was significantly different from raw and microwave treated rice bran at the end of storage. This may be associated with lipid oxidation and hydrolysis of lipolytic enzyme.

Table IV-25 shows the composition of phospholipid in raw rice bran and stabilized rice bran during storage. The palmitic acid increased in raw rice bran oil with storage time and linoleic acid decreased. The linoleic acid composition in phospholipid of rice bran oil extracted from bran treated with microwave for 4, 8, and 12 min was not significantly ($p > 0.05$) different during storage.
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*Values are means of 18 analyses.
F. EXPERIMENT 6: Effect of Gamma-Irradiation on Vitamin E, Oryzanol, and Fatty Acids in Rice Bran

Radiation processing of food may be used to achieve a wide variety of technological objectives and to serve many valuable purposes. Low doses of gamma-radiation were applied for sprout inhibition (20-150 Gy) in potatoes, onion, garlic, etc., ripening delay and stimulation of fruits (0.1-1 KGY), to control insect proliferation (0.3-1 KGY) and other pests in grains, cereal products, dried fruit, spices, and the practical elimination of pathogenic organisms and micro-organisms (0.1-1 kGY) other than viruses and tape worm and non-spore-forming micro-organisms (2-8 kGY) (Katta et al., 1991). High doses of radiation were used to enhance the keeping quality of fresh meat and seafood as well as of vegetables and fruits (0.4-10 kGY) and to reduce the number and/or the activity of viable microorganisms to such an extent that very few, if any, were detectable (10-50 kGY, radappertization) (Katta et al., 1991). In this study, rice bran was irradiated with gamma rays at 5, 10, and 15 kGY, levels similar to what has been applied to rice hulls (Ramarathnam et al., 1987; Ramarathnam et al., 1989) and soybean (Hafez et al., 1989b).

1. Free Fatty Acids in Irradiated Rice Bran

Gamma Irradiation: The effects of gamma-irradiation on quantitative changes in FFA levels in rice bran is shown in Table IV-26. FFA level in
Table IV-26. Changes of free fatty acids and phospholipids in rice bran immediately following gamma irradiation$^a$

<table>
<thead>
<tr>
<th>Irradiation (kGy)</th>
<th>Total FFA(%)$^c$</th>
<th>Phospholipids$^d$</th>
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<tr>
<td>15</td>
<td>6.05$^f$</td>
<td>3.66$^e$</td>
</tr>
</tbody>
</table>

$^a$ Based on means of four analyses.

$^b$ Means with the same letter are not significantly (p > 0.05) different.

$^c$ % in rice bran oil.

$^d$ g/100 g rice bran oil.
rice bran irradiated at 5 kGy was not significantly different from raw rice bran. An increase in irradiation at doses of 10 and 15 kGy resulted in a significant (p < 0.05) increase in FFA levels. The increase in FFA levels at both doses resulted from radiolysis of glycerides. Also, enzymatic hydrolysis of glycerides might contribute an increase in FFA level during gamma-irradiation because rice bran were irradiated at 20°C for about 10 and 15 hr for 10 and 15 kGy, respectively. Parameters that affect the chemical reactions resulting from the irradiation of lipids include irradiation conditions such as dose, dose-rate, temperature, and presence of oxygen (Delincee, 1983a). Bhushan et al. (1981) found that total FFA contents were slightly decreased in lamb liver irradiated at 5, 15, and 20 kGy. When rice was irradiated at 0.1, 0.2, 0.3, and 0.5 kGy there was a slight decrease in fat acidity in rice with an increase in dose (Ismail et al., 1977). In contrast, FFA level was increased by over 86% in beef irradiated at high dose, 500 kGy (Vajdi and Nawar, 1979).

Free fatty acids were more susceptible to irradiation change than esterified acids (Tipples and Norris, 1965). Generally, the major radiolytic products of fatty acids are carbon dioxide, hydrogen, carbon monoxide, a series of hydrocarbons and the Cn aldehydes (Delincee, 1983a). Radiolytic products with a higher molecular weight than the parent fatty acid are also formed, such as dimeric alkane and symmetric ketone (Delincee, 1983a). Quantitative analyses indicated a greater yield of various radiolytic
compounds from free fatty acids than from the corresponding triglycerides in model systems (Vajdi and Nawar, 1978). These studies indicated that free fatty acids readily decompose to form shorter chain compounds and/or condense into higher molecular weight compounds. At lower dosages, the rate of decomposition of FFA was higher than that of formation of FFA. In contrast, the rate of decomposition of FFA was lower than that of formation of FFA at higher dosages.

Tipple and Norris (1965) reported that silicic acid fractionation results showed a reduction in triglyceride and a corresponding increase in mono- and diglycerides in wheat irradiated at 100 kGy. This could be due to a splitting-off of one or two fatty acids from triglyceride molecules. However, there was no increase in the amount of free fatty acid recovered. This could mean that the splitting of the ester linkage produced not free fatty acid but a degraded molecule or free radical (Tipple and Norris, 1965). In rice bran, the rate of formation of FFA was higher even though doses of irradiation were relatively low. This might be due to lipolytic enzymes in rice bran active during the irradiation process.

The effect of storage on total FFA in rice bran oil extracted from irradiated rice bran is shown in Fig. IV-32. Irradiated and raw rice bran showed similar increases in FFA content. Rice bran irradiated at 5 kGy was not different (p > 0.05) from raw rice bran during storage. FFA contents in irradiated rice bran increased significantly (p < 0.05) during 24 weeks
Fig. IV-32. Total free fatty acid in rice bran oil extracted from raw and irradiated rice bran during 52 weeks storage.
storage when the doses were increased. FFA content in rice bran irradiated at 15 kGy decrease significantly (p < 0.05) after 24 weeks storage. FFA contents in raw and rice bran irradiated at 5 and 10 kGy increased slightly. Unlike rice bran, FFA content in irradiated cereal grain, and flour usually increased during irradiation (10-100 kGy) and was similar to control during storage. Ismail et al. (1977) reported that fat acidity in brown rice irradiated at 0.1, 0.2, 0.3, and 0.5 kGy decreased slightly (N.S., p > 0.05) compared to control brown rice during five months' storage. The development of FFA in the lipid of wheat flour irradiated at 50 and 100 kGy did not appear to be impaired by irradiation (Tipples and Norris, 1965). After 680 day storage of the whole-meal flour, the FFA content had risen to about 40%.

Gamma irradiation in rice bran seems to not inactivate lipolytic enzyme in the range used in this study. Delincee (1983b) reported that enzymes causing autolysis during the storage of high protein foods generally cannot be inactivated at radiation dose levels for radurization (2-5 kGy) or radappertization (~50 kGy), thus long-term stability of radiation-sterilized meats is achieved by enzyme inactivation through heating. An increase in enzyme activity was observed in irradiated fruits and vegetables, while a decrease was observed for others. In banana fruits subjected to gamma irradiation (0.15-2 kGy), skin browning was observed to be due to an activation of polyphenol oxidase (Thomas and Nair, 1971). Delincee (1983b) demonstrated that gamma irradiation induced tissue browning of potato tubers
and was accompanied by a marked increase in peroxidase activity and a transient increase in o-diphenol oxidase activity. At low radiation doses of 0.1-3 kGy used for fruits and vegetables, the effect on enzymes seems to be due to the metabolic changes induced by irradiation. Bhushan et al. (1981) found that lamb liver irradiated at 5, 10, and 20 kGy had similar lipolytic enzyme activities to control during post-irradiation autolyses at 37°C. Hafez et al. (1985) reported that inhibition of 26 and 71% of lipoxygenase activities, 16 and 25% trypsin inhibitor activities, and 7 and 16.7% chymotrypsin inhibitor activities were observed when the soybean seeds were irradiated at 20 and 100 kGy, respectively. Losty et al. (1973) found that gamma irradiation with increasing doses from 20 to 60 kGy destroyed 75% of the proteolytic activity but was less effective than blanching, especially when the latter was carried out at 70°C. Patel et al. (1965) reported that lipase activity of groundnut irradiated at 0.1 to 1.2 kGy was the same as control at doses of 0.5 to 1.2 kGy and was higher than control at 0.1 to 0.3 kGy. However, lipolytic enzymes in rice bran seems to be not affected by gamma irradiation at 5, 10, and 15 kGy.

FFA Composition: The effect of gamma irradiation on FFA composition of rice bran oil extracted from irradiated rice bran during storage is shown in Table IV-27. Upon irradiation, the linoleic and linolenic acid in rice bran irradiated at 15 kGy were significantly (p < 0.05) different.
Table IV-27. Free fatty acid composition of raw and irradiated rice bran during 52 weeks storage

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<td>1.77</td>
<td>23.90</td>
<td>2.05</td>
</tr>
</tbody>
</table>

* Values are means of 4 analyses.
from those in raw rice bran. With longer storage of the rice bran, the FFA of unirradiated and irradiated samples showed a decrease in linoleic and linolenic acids, with a corresponding apparent increase in oleic and palmitic acids as found in raw rice bran in previous studies. Differences in FFA composition between raw and irradiated samples were slight during storage. Also, the changes of FFA composition among irradiated rice brans were not significantly (p > 0.05) different during storage. Tipples and Norris (1965) reported that the peroxide values of wheat flour lipids increased with increasing irradiation. Upon storage (185 day), the increase in peroxide value became less with increasing irradiation and the value was higher in control than in irradiated samples. Also, a decrease in linoleic and linolenic acid was observed to be less in irradiated samples than in control. The chemical reactions and changes resulting from the irradiation of lipids are affected by composition of lipid (saturated or unsaturated), presence of other substances (e.g. antioxidants), form of lipid (liquid or solid) and moisture content, as well as irradiation conditions (Delincee, 1983a). Post-irradiation treatment (storage atmosphere, storage temperature) is also of great importance.

2. Tocopherols and Tocotrienols in Rice Bran

**Gamma Irradiation:** Many studies have been reported on the effect of gamma irradiation on α-tocopherol or total vitamin E vitamers during irradiation. Only a few studies reported the effect of irradiation on
individual E vitamers and post-irradiation storage. The decomposition of tocopherols, tocotrienols, and oryzanol in rice bran during irradiation is shown in Table IV-28. The decomposition of individual E vitamers increased significantly (p < 0.05) with an increase in irradiation level. Oryzanol contents in rice bran irradiated at 5 kGy was lower (p < 0.05) than in raw bran. Decomposition of oryzanol increased (p < 0.05) with an increase from 5 to 10 kGy. However the decrease in oryzanol was not significant (p > 0.05) with an increase from 10 to 15 kGy. α-Tocotrienol in rice bran was the most sensitive to gamma irradiation. Rice bran irradiated at 5, 10, and 15 kGy lost 67, 82, and 89% of its α-tocotrienol. The order of losses of vitamin E vitamers in bran irradiated at 5 kGy was α-T3 > α-T > β-T = γ-T3 > δ-T > γ-T > δ-T3. The order of losses and rate of decomposition changed slightly with an increase in irradiation level. An increase in decomposition rate of total tocotrienol was higher than that of total tocopherols with increased irradiation level.

Available reports provide no information on the order of stability of vitamin E vitamers in either irradiated food samples or in model systems. The double bonds between certain carbon atoms in long chain fatty acids esterified with glycerol are selectively attacked by some of the free radicals produced by irradiation, particularly the superoxide and hydroxyl radicals (Murray, 1990). The tocotrienols with three double bonds in their sidechains were more susceptible to gamma irradiation than tocopherols with no
<table>
<thead>
<tr>
<th>Irradiation (kGy)</th>
<th>α-Τ</th>
<th>α-T3</th>
<th>δ-Τ</th>
<th>γ-Τ</th>
<th>γ-T3</th>
<th>δ-Τ</th>
<th>δ-T3</th>
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<th>Tot-T</th>
<th>Tot-T3</th>
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</tr>
<tr>
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<td>32.60</td>
<td>4.65</td>
<td>21.44</td>
<td>61.92</td>
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<td>(48.2)</td>
<td>(33.5)</td>
<td>(48.2)</td>
<td>(42.6)</td>
<td>(21.5)</td>
<td>(11.1)</td>
<td>(46.0)</td>
<td>(51.4)</td>
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<td>6.85</td>
<td>4.05</td>
<td>16.50</td>
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<td>(54.9)</td>
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</tr>
<tr>
<td></td>
<td>(79.3)</td>
<td>(89.2)</td>
<td>(44.1)</td>
<td>(67.7)</td>
<td>(86.7)</td>
<td>(69.0)</td>
<td>(42.6)</td>
<td>(22.4)</td>
<td>(74.2)</td>
<td>(85.4)</td>
</tr>
</tbody>
</table>

*a mg/kg rice bran.
*b Based on mean of four analyses.
*c T, tocopherol; T3, tocotrienol; ory, oryzanol; Tot-T, total tocopherol; Tot-T3, total tocotrienol.
*d Figures in parentheses refer to percentage lost of total E vitamers or oryzanol.
double bonds. However, δ-tocotrienol was the most stable to gamma irradiation.

Rice bran irradiated at 5, 10, and 15 kGy lost 11, 18, and 22% of its oryzanol, respectively. Oryzanol was found to be relatively stable to gamma irradiation. Ramarathnam et al. (1989) reported that α-tocopherol content decreased markedly in rice seeds irradiated with or without hull. At a dose of 15 kGy, only traces of α-tocopherol could be detected in rice seeds irradiated with and without intact hull. The oryzanol content ranged from 96 to 246 μg/g lipid in rice seed, while the decrease in oryzanol contents in rice seed irradiated at 5 to 15 kGy ranged from 7 to 42% of its oryzanol. In this study, the reduction of oryzanol in rice bran was less than that of rice seeds. This could be due to the higher amount of total E vitamers in rice bran. The most striking difference between lipids extracted from unirradiated and irradiated wheat was the increase in the amount of free sterol (Tipples and Norris, 1965). The research noted that the irradiation could have severed or weakened the linkage by which sterol was bound, thus making the sterol more completely available for solvent extraction. The results suggest that ester linkage in oryzanol could be affected by gamma irradiation, and thus oryzanol could be lost.

The destruction of total tocopherols in wheat was different from variety to variety (Tipples and Norris, 1965). Manitoba wheat irradiated at 1, 10, and 100 kGy lost 19, 39, and 79% of its total tocopherols while
Minister wheat lost 8, 13, and 39%, respectively. Knapp and Tappel (1961) studied comparative radiosensitivities of fat-soluble vitamins under controlled conditions. Vitamin E was by far the most sensitive, followed in order of decreasing sensitivity by carotene and vitamins A, D, and K. Irradiation of fluid whole milk with 4 kGy resulted in destruction of 40% of the carotenoids, 70% of vitamin A and 61% of vitamin E (Kung et al., 1953).

**Post-Gamma Irradiation Storage:** The effect of post-gamma irradiation storage on individual vitamin E vitamers is shown in Table IV-29. There was no apparent pattern in the order of decomposition of E vitamers in irradiated rice bran during storage, unlike that of rice bran extruded or heated by microwave. γ-Tocopherol and δ-tocopherol in irradiated rice bran had relatively higher retention during storage, and α-tocopherol and α-tocotrienol had relatively less retention. Increased irradiation dose did not significantly (p > 0.05) increased decomposition rate of E vitamers during storage (Fig.IV-33). The amount of retention of vitamin E vitamers during storage was dependent on the amount of retention during irradiation. During 7 weeks storage, the relative decomposition rate of total E vitamers in raw rice bran was higher than that of irradiated rice bran. This was considered to be due to the lower amount of vitamin E vitamers. Diehl (1981) found that irradiation (1 kGy) of hazel nuts to 1 kGy produced an 18% loss of α-tocopherol, while baking produced a 13% loss. When the nuts were irradiated and then cooked, the total loss of tocopherol was 67%. The researcher
**Table IV-27. Retention of vitamin E vitamers in irradiated rice bran during 52 weeks storage**

<table>
<thead>
<tr>
<th>Irradiation dose (KgY) (weeks)</th>
<th>Storage (weeks)</th>
<th>Vitamin E vitamers</th>
<th>Total</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>α-T</td>
<td>α-T3</td>
<td>β-T</td>
<td>γ-T</td>
</tr>
<tr>
<td>0</td>
<td>30.16</td>
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<td>1</td>
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<td>9.53</td>
<td>3.89</td>
<td>18.76</td>
</tr>
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<td>7.96</td>
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<td>17.65</td>
</tr>
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<td>2.79</td>
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</tr>
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</tr>
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</table>

* mg/kg rice bran.

* Values are means of four analyses.
Fig. IV-33. Total E vitamers and oryzanol in raw and irradiated rice bran during 52 weeks storage.
proposed that the explanation for the apparent synergistic loss was due to free radicals and secondary reaction products that may attack vitamin E during processing or subsequent storage. Murray (1990) reported that free radicals in irradiated food items decayed for over one month. Initial rates of decay were high, so that 50% loss had occurred in 4 days for seeds. The retention of free radical in irradiated food could contribute to chemical changes during post-gamma irradiation. Oryzanol content in rice bran irradiated at 5 kGy was lower than that of raw rice bran during 3 weeks storage. Thereafter, it was higher than that of raw rice bran. During 7 weeks storage, the loss of oryzanol in irradiated rice bran was about 60% of its loss during 52 weeks storage.

3. Phospholipid in Rice Bran

Phospholipids are an important part of biological and food systems, even though they are not a major constituent of these systems. No information has been reported concerning the effect of gamma irradiation and post-irradiation storage on phospholipid in rice bran.

Gamma Irradiation: Increasing gamma irradiation doses from 5 to 10 kGy did not significantly (p > 0.05) decreased the phospholipid content in rice bran. At 15 kGy, phospholipid did decrease (p < 0.05) (Table IV-26). There were slight changes of fatty acid composition of phospholipid in rice bran irradiated at 5 and 10 kGy (Table IV-30). The FFA of rice bran irradiated at 15 kGy showed a decrease in linoleic and linolenic acids, with
Table IV-30. Composition of phospholipid in raw and irradiated rice bran during 52 weeks storage*

<table>
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<tr>
<th>Irradiation dose (KGy)</th>
<th>Storage (week)</th>
<th>Fatty acid composition (%)</th>
<th>Saturated</th>
<th>Unsaturated</th>
</tr>
</thead>
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<td></td>
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<td>14:0</td>
<td>16:0</td>
<td>18:0</td>
</tr>
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<td>0.16</td>
</tr>
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<td>1</td>
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<td>22.52</td>
<td>0.16</td>
</tr>
<tr>
<td>3 (raw)</td>
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<td>23.44</td>
<td>0.18</td>
</tr>
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<td>0.13</td>
<td>23.99</td>
<td>0.18</td>
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<td>25.75</td>
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</table>

* Values are means of 4 analyses.
a corresponding apparent increase in oleic and palmitic acids. Hafez (1989) reported that gamma irradiation at high doses (20, 40, 60, 80, and 100 kGy) significantly \((p < 0.05)\) decreased the phospholipid content of soybean. Furthermore, an increase in the moisture content of soybean seeds and irradiation at doses of 56 and 65 kGy resulted in a significant \((p < 0.05)\) reduction in phospholipids. They noted that an increase in the amount of reactive species formed by radiolysis of water was high enough to cause severe damage to phospholipids. Nene et al. (1975) studied the effect of gamma irradiation on red gram \((Cajanus cajan)\) lipids. They observed a destruction of the polar lipids. Mironova et al. (1970) reported that changes in surface-active properties, saturation, and solubility in hexane, heptene and chloroform were observed when sunflower oil phospholipids were irradiated at a dosage rate of \(5.8 \times 10^3\) rad/hr. Phosphatidic acid and the lysophospholipids were the major radiolytic products observed for the phospholipids in model systems (Tinsley and Maerker, 1993). Nawar and Handel (1977) reported that the major radiolytic products from phospholipids were aldehydes, methyl esters, ethyl ester ketones, hexadecanal, and methyl fatty acids. Bancher et al. (1972) and Colbey (1959) observed decreases in phosphatidyl choline (lecithin) following irradiation of walnut and ground nut and simultaneous increases in phosphatidic acid. Phospholipids of walnuts were more sensitive to irradiation than phospholipids of ground nuts. This was explained on the basis of a greater degree of unsaturation of walnut
lipids compared to ground nuts. In contrast, the most likely explanation of the decrease in phospholipids extracted from irradiated wheat was that the phospholipid was "denatured" and was thus less available for solvent extraction (Tipples and Norris, 1965) rather than the effect of radiolysis on actual phospholipid by gamma irradiation.

Post-Gamma Irradiation Storage: Very little information is available regarding changes in phospholipid during post-gamma irradiation storage. Total phospholipid in raw and irradiated rice bran decreased significantly (p < 0.05) during storage (Fig. IV-34). The retention of phospholipid in raw rice bran and rice bran irradiated at 5 and 10 kGy was not significantly (p > 0.05) different during 52 weeks storage except between 3 and 7 weeks in raw rice bran. Gamma irradiation at 15 kGy resulted in significantly (p < 0.05) different retention of phospholipid in rice bran during storage. The fatty acids in rice bran oil extracted from irradiated rice bran contained a reduced proportion of linoleic and linolenic acids with increased palmitic and oleic acid composition during 52 weeks storage. The fatty acid profile in phospholipid was similar to the fatty acid composition of neutral lipid from irradiated rice bran (Table IV-31). The effect of gamma irradiation on fatty acid composition was slightly more marked with phospholipid than with neutral lipid. Quantitative analysis showed that the amounts of volatile products produced from phospholipids during irradiation were significantly less than those formed in glycerides (Delincee, 1983). Also, the contribution
Fig. IV-34. Changes of phospholipid in rice bran oil extracted from raw and irradiated rice bran during 52 weeks storage.
Table IV-31. Composition of neutral lipid from raw and irradiated rice bran during 52 weeks storage*

<table>
<thead>
<tr>
<th>Irradiation dose (KgY)</th>
<th>Storage week</th>
<th>Fatty acid composition (%)</th>
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<th>Unsaturated</th>
</tr>
</thead>
<tbody>
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<td>16:0</td>
<td>18:0</td>
</tr>
<tr>
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<td>24.48</td>
<td>1.68</td>
</tr>
<tr>
<td>raw</td>
<td>3</td>
<td>0.34</td>
<td>23.03</td>
<td>1.92</td>
</tr>
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<td>0.32</td>
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* Values are means of 4 analyses.
of free fatty acid to the total amount of radiolytic products is relatively small compared to the yield from triglycerides, which account for the majority of lipids present in food. Fat-containing foodstuffs usually contain only small amounts of FFA.
CHAPTER V
SUMMARY AND CONCLUSIONS

Rice bran contains valuable components such as oil, proteins, vitamins, oryzanol, and essential minerals. It also contains enzymes, microorganisms, insects, natural toxicant constituents, harmful contaminants and adulterants. Some components have to be preserved, others must be removed or their activity arrested.

Enzymes, microorganisms, and insects are major causes of deterioration of rice bran. Lipases and to a lesser extent oxidases, are responsible for the deteriorative changes. Lipases promote the hydrolysis of the bran oil into glycerol and free fatty acids. In the intact grain, lipases are dormant. The enzyme and the substrate are not together in the resting grain. Lipases are localized in the testa/cross layer of the rice while the oil is in the aleurone and subaleurone layers and germ. When bran is abraded during rice milling, the enzyme and substrate are brought together and oil deterioration begins.

Well stabilized bran has excellent keeping qualities with adequate protection from microbial, insect and other pest attacks. Most previous research and development work on stabilization has been focused only on lowering free fatty acid (FFA) level and inactivation of lipolytic enzymes during short term storage (less than three months). There is an urgent need
to develop an appropriate stabilization and storage technology to maximize retention of the valuable components, in addition to lowering FFA level, during stabilization and post-stabilization storage. Otherwise, the many valuable components and efforts made in developing a successful rice bran stabilization technology will be wasted.

Four vitamers of tocopherol and tocotrienol were isolated from a mixture of natural source by semi-preparative HPLC for use in analytical HPLC. Recovery was higher than by conventional TLC. The semi-preparative HPLC column is useful to obtain small amounts of pure tocopherols and tocotrienols as an alternative to conventional methods.

A method was developed for improving column stability and reproducibility for analysis of vitamin E vitamers on normal-phase HPLC column with isoctane/ethyl acetate/acetic acid/2,2-dimethoxypropane (DMP) (98.5:0.9:0.85:0.1) mobile phase. Acetic acid in the mobile phase reduced retention times of vitamin E vitamer and increased column stability. A small concentration of DMP, which reacts with water to form acetone and methanol, reduced the need for column regeneration and stabilized retention times.

The effects of extrusion temperature and post extrusion holding time on antioxidants and lipid composition were studied. Rice bran extruded at 110, 120, 130, and 140°C with post extrusion holding times of 0, 3, and 6 minutes and stored at ambient temperature for one year. Increased holding
times reduced (p < 0.05) total vitamin E. Oryzanol concentration was lower (p < 0.05) only after 6 min holding time. Oryzanol was relatively more stable to extrusion temperature than vitamin E. Free fatty acid levels were not affected by post extrusion holding times during storage. The highest retention of total vitamin E and oryzanol, and the highest free fatty acid content were found in raw rice bran during storage. Increased extrusion temperatures reduced the retention of vitamin E and oryzanol during storage. Inactivation of lipolytic enzymes obtained by extrusion temperatures of 120, 130, and 140°C. An extrusion temperature of 110°C provided the highest retention of vitamin E and oryzanol of the extrusion temperatures during storage although FFA level was higher (p < 0.05) than that of the other extrusion temperatures from 105 to 375 days. An extrusion temperature of 110°C is suitable for stabilization when stabilized rice bran is expected to be stored for less than five months storage and retention of vitamin E and oryzanol is a concern. An extrusion temperature of 120°C is recommended when rice bran is expected to have long term stability. This extrusion temperature would provide an adequate safety margin for unanticipated changes of operating condition and rice bran conditions (moisture contents, degree of contamination).

The effects of microwave heating (500 watt power, 2450 MHz for 4, 8, 12 min) on the vitamin E and oryzanol content of rice bran were studied in relation to chemical changes in the lipids. There were no significant (p >
0.05) decreases in individual vitamin E vitamers after 4 min microwave heating. After 8 min heating, individual E vitamers decreased significantly (p < 0.05) with exposure time. Small differences, not statistically significant, were observed in FFA in rice bran oil with increasing microwave heating. FFA content increased with a decrease in heating time after 7 weeks storage. There was no significant increase (p > 0.05) in FFA level by 24 weeks with microwave heating for 12 min. Microwave heating for 4, 8, and 12 min did not provide adequate inactivation of lipolytic enzymes. This may be due to non-uniform microwave heating. Total vitamin E and oryzanol losses occurred in the ranges of 6-36%; losses of 4-11% occurred during microwave heating, with a further 44-69% and 56-58% during storage, respectively. Stabilization with microwave heating was considered to be not suitable for long term storage.

The effects of gamma-irradiation (5, 10, and 15 kGy) on antioxidants and lipid composition of rice bran were studied. FFA level in rice bran irradiated at 5 kGy was not significantly different from raw rice bran. An increase in irradiation doses to 10 and 15 kGy resulted in an increase in FFA levels. Gamma irradiation in rice bran did not inactivate lipolytic enzymes in the range used in this study. Gamma irradiation had deleterious effects on lipid stability, E vitamers, and oryzanol in rice bran during irradiation and storage. The decomposition of individual E vitamers increased with an increase in irradiation level. Oryzanol contents in rice bran irradiated at 5
kGy were lower (p < 0.05) than raw bran. Decomposition of oryzanol increased (p < 0.05) with an increase from 5 to 10 kGy. However, the decrease in oryzanol was not significant (p > 0.05) with an increase from 10 to 15 kGy. The loss of total E vitamers and oryzanol occurred in two stages: 50-82% and 12-33% immediately following irradiation, and a further 10-35 and 39-42% during storage. The decomposition of total vitamin E was higher during irradiation than during 52 weeks storage. Oryzanol was more stable to gamma irradiation and heat than vitamin E. Gamma irradiation did not provide any beneficial effect during post irradiation storage.
LITERATURE CITED


APPENDIX 1

Appendix 1. Structures of tocopherols and tocotrienols

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Appendix 2. Total vitamin E vitamers and oryzanol in rice bran extruded at 110°C with holding time 0, 3, and 6 min during storage.
Appendix 3. Total vitamin E vitamers and oryzanol in rice bran extruded at 120°C with holding time 0, 3, and 6 min during storage.
Appendix 4. Total vitamin E vitamers and oryzanol in rice bran extruded at 130°C with holding time 0, 3, and 6 min during storage.
Appendix 5. Total vitamin E vitamers and oryzanol in rice bran extruded at 140°C with holding time 0, 3, and 6 min during storage.
Appendix 6. Gas chromatogram of fatty acid in rice bran oil. Chromatographic conditions are given in the text.
Appendix 7. Tocopherols and tocotrienols in rice bran treated with microwave for 4 min during 52 weeks storage.
Appendix 8. Tocopherols and tocotrienols in rice bran treated microwave for 8 min during 52 weeks storage.
Appendix 9. Tocopherols and tocotrienols in rice bran treated microwave for 12 min during 52 weeks storage.
Appendix 10. Tocopherols and tocotrienols in rice bran irradiated at 5 kGy during 52 weeks storage.
Appendix 11. Tocopherols and tocotrienols in rice bran irradiated at 10 kGy during 52 weeks storage.
Appendix 12. Tocopherols and tocotrienols in rice bran irradiated at 15 kGy during 52 weeks storage.
The author, Taisun Shin, the fifth son of Kabsuk Shin and keumsub Choi, was born on January 22, 1957, in Seoul, Republic of Korea. He entered King Sejong university in 1978. After his freshman year, he served in the army for 3 years. In February, 1987, he graduated from King Sejong university with a Bachelor of Engineering degree in Food Science and Technology. From January, 1987, he worked at Lotte Confectionery Inc. while he entered the Graduate School in Food Science and Technology.

The author was accepted to the Graduate School of Louisiana State University through the Department of Food Science in August, 1989. He had worked as a research assistant (1990-1993). He accepted a full-time Research Associate position at Department of Food Science on July, 1993. The author concurrently works as a Research Associate and is a candidate for the degree of Doctor of Philosophy in Food Science.
Candidate: Taisun Shin

Major Field: Food Science

Title of Dissertation: Changes of Antioxidants and Fatty Acid Composition in Stabilized Rice Bran During Storage

Date of Examination: May 5, 1995