



Fermented Foods and Beverages Series

Handbook of Indigenous Foods Involving Alkaline Fermentation

Edited by
Prabir K. Sarkar • M.J. Robert Nout



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CRC Press

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Boca Raton London New York

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CRC Press
Taylor & Francis Group
6000 Broken Sound Parkway NW, Suite 300
Boca Raton, FL 33487-2742

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Version Date: 20140320

International Standard Book Number-13: 978-1-4665-6530-2 (eBook - PDF)

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Contents

SERIES PREFACE	XIX
PREFACE	XXI
EDITORS	XXIII
CONTRIBUTORS	XXV
CHAPTER 1 INTRODUCTION	1
<i>Prabir K. Sarkar and M. J. Robert Nout</i>	
References	6
CHAPTER 2 DIVERSITY OF PLANT-BASED FOOD PRODUCTS INVOLVING ALKALINE FERMENTATION	7
2.1 Legume Products	7
2.1.1 Soybean Products	8
2.1.1.1 Natto	8
<i>Toshirou Nagai</i>	
2.1.1.1.1 History	10
2.1.1.1.2 Starter Culture	12
2.1.1.1.3 Fermentation	13
2.1.1.1.4 Nutritional and Chemical Components	14
2.1.1.1.5 Consumption	17
2.1.1.1.6 Conclusion	18

2.1.1.2	Douchi	18
	<i>Li-Te Li and Yan-Li Ma</i>	
2.1.1.2.1	Origin of Douchi	18
2.1.1.2.2	Classification	20
2.1.1.2.3	Production Methods	22
	2.1.1.2.3.1 Pre-Treatment	22
	2.1.1.2.3.2 Pre-Fermentation	24
	2.1.1.2.3.3 Post-Fermentation	27
2.1.1.2.4	Chemical and Nutritional Components	28
2.1.1.2.5	Functional Properties	31
2.1.1.2.6	Consumption and Perspectives	31
2.1.1.3	Kinema and Similar Products	33
	<i>Prabir K. Sarkar and M. J. Robert Nout</i>	
2.1.1.3.1	Origin and Culture	33
2.1.1.3.2	Indigenous Preparation of Kinema and Its Socio-Economic Impact	36
2.1.1.3.3	Changes in the Substrate during Kinema Production	38
	2.1.1.3.3.1 Stage 1: Soaking	38
	2.1.1.3.3.2 Stage 2: Cooking	38
	2.1.1.3.3.3 Stage 3: Fermentation	39
2.1.1.3.4	Safety of Kinema	50
2.1.1.3.5	Plasmid for γ -Polyglutamate Production	50
2.1.1.3.6	Process/Product Improvisation	51
2.1.1.3.7	Development of Kinema Starter	52
2.1.1.3.8	Development of Kinema-Fortified Cookies	52
2.1.1.3.9	Conclusion	53

CONTENTS**VII**

2.1.1.4	Doenjang	53
	<i>Kun-Young Park and Ji-Kang Jeong</i>	
2.1.1.4.1	Manufacturing Procedures	54
2.1.1.4.2	Microorganisms in Doenjang	56
2.1.1.4.3	Functional Properties	58
2.1.1.4.4	Conclusion	63
2.1.1.5	Chongkukjang	63
	<i>Jang-Eun Lee, Ggot-Im Lee, and Cherl-Ho Lee</i>	
2.1.1.5.1	Origin of Chongkukjang	63
2.1.1.5.2	Methods of Preparation	64
2.1.1.5.3	Biochemical Composition	65
2.1.1.5.4	Usage and Function	68
2.1.1.5.5	Safety Aspects	69
2.1.1.6	Thua Nao	71
	<i>Ekachai Chukeatirote</i>	
2.1.1.6.1	Conventional Preparation of Thua Nao	72
2.1.1.6.2	Biochemistry and Microbiology	73
2.1.1.6.3	Nutritional Quality	74
2.1.1.6.4	Conclusion	75
2.1.1.7	Meitauza	76
	<i>Li-Te Li and Yan-Li Ma</i>	
2.1.1.7.1	Okara: The Raw Material of Meitauza Production	76
2.1.1.7.2	Production Methods of Meitauza	77
2.1.1.7.3	Microbiology of Meitauza Production	79
2.1.1.7.4	Chemical Changes Accompanying Meitauza Production	79
2.1.1.7.5	Nutrition and Functionality of Meitauza	82
2.1.1.7.6	Problems about Meitauza Production	85
2.1.1.7.7	Consumption and Prospects of Meitauza	86

2.1.1.8	Yandou	87
	<i>Hongjiang Yang</i>	
2.1.1.8.1	History	87
2.1.1.8.2	Manufacturing Procedure	88
2.1.1.8.3	Starter Culture	89
2.1.1.8.4	Nutritional and Physicochemical Characteristics	91
2.1.2	Non-Soybean Products	92
2.1.2.1	Dawadawa (Iru) and Similar Products	93
	<i>Olusola Bandele Oyewole, Adewale Olusegun Obadina, and Paulin Azokpota</i>	
2.1.2.1.1	Antiquity	93
2.1.2.1.2	Traditional Method of Production	94
2.1.2.1.3	Starters	96
2.1.2.1.4	Microbial Succession	98
2.1.2.1.5	Chemical Changes	100
2.1.2.1.6	Volatile Compounds Profile	101
2.1.2.1.7	Culinary Practice and Economics	102
2.1.2.2	Ugba	102
	<i>Abiodun Isiaka Sanni and Folarin Anthony Oguntinyinbo</i>	
2.1.2.3	Kawal	107
	<i>Abiodun Isiaka Sanni and Folarin Anthony Oguntinyinbo</i>	
2.1.2.4	Okpehe	110
	<i>Abiodun Isiaka Sanni and Folarin Anthony Oguntinyinbo</i>	
2.1.2.4.1	Traditional Method of Preparation	110
2.1.2.4.2	Microbiology	111
2.1.2.4.3	Nutritional Value	113
2.1.2.5	Otiru	114
	<i>Yemisi Adefunke Jeff-Agboola</i>	
2.1.2.5.1	Traditional Process of Otiru Production	115
2.1.2.5.2	Microorganisms	116
2.1.2.5.3	Changes in Proximate Composition	116

CONTENTS		IX
2.1.2.6	Oso	118
	<i>Olusola Bandele Oyewole and Adewale Olusegun Obadina</i>	
2.1.2.6.1	Traditional Method of Production	118
2.1.2.6.2	Chemical and Microbial Succession and Composition	119
2.2	Non-Legume Products	121
2.2.1	Ogiri	122
	<i>Maureen-Theodore Chinwe Ojinnaka</i>	
2.2.1.1	Microorganisms Involved in Traditional Fermentation	124
2.2.1.2	Nutritional Value	125
2.2.1.3	Enzymes Involved during Fermentation	126
2.2.1.4	Ammonia and Ricin Contents in Ogiri	126
2.2.1.5	Organic Acid Contents and Volatile Compounds in Fermenting Mash	127
2.2.1.6	Conclusion	128
2.2.2	Bikalga/Furundu/Mbuja	129
	<i>Labia Irène Ivette Ouoba</i>	
2.2.2.1	Traditional Technology for Production and Product Utilization	130
2.2.2.2	Physicochemical and Nutritional Characteristics	131
2.2.2.3	Microbiology of Fermentation	134
2.2.2.4	Safety	135
2.2.2.5	Functional Properties	136
2.2.2.6	Conclusion	136
2.2.3	Maari/Dikouanyouri/Tayohounta	136
	<i>Abiodun Isiaka Sanni and Folarin Anthony Oguntoyinbo</i>	
2.2.4	Ntoba Mbodi	140
	<i>Abiodun Isiaka Sanni and Folarin Anthony Oguntoyinbo</i>	
2.2.5	Cabuk	143
	<i>Kris Herawan Timotius</i>	
2.2.5.1	Substrates of Cabuk	144
2.2.5.2	Preparation of Cabuk	145
2.2.5.3	Microorganisms Involved	146

2.2.5.4	Changes Accompanying Cabuk Production	147
2.2.5.5	Prospects of Cabuk	147
2.2.6	Semayi	148
	<i>Kris Herawan Timotius</i>	
2.2.6.1	Ampas Kelapa – The Substrate of Semayi	148
2.2.6.2	How to Make Semayi	149
2.2.6.3	Microbiology of Semayi	150
2.2.6.4	Changes in Proximate Composition during Fermentation	150
2.2.7	Owoh	151
	<i>Abiodun Isiaka Sanni and Folarin Anthony Oguntoyinbo</i>	
2.2.8	Salt Rising Bread	154
	<i>Susan Ray Brown and Genevieve Bardwell</i>	
2.2.8.1	History	155
2.2.8.2	Science	157
2.2.8.3	Baking Methods	159
2.2.8.4	Conclusion	161
	References	162
CHAPTER 3	DIVERSITY OF ANIMAL-BASED FOOD PRODUCTS INVOLVING ALKALINE FERMENTATION	189
3.1	Fish Sauces and Pastes	189
	<i>Wonnop Visessanguan and Siriporn Chaikaew</i>	
3.1.1	Garum (Italy and Greece)	191
3.1.2	Terkin (Sudan and Central Africa)	192
3.1.3	Mahyaveh (Iran)	193
3.1.4	Ngari (India)	194
3.1.5	Hentak (India)	194
3.1.6	Tungtap (India)	195
3.1.7	Jaadi (Sri Lanka)	195
3.1.8	Ngapi and Ngan-Byar-Yay (Myanmar)	195
3.1.9	Nam Pla (Thailand)	197
3.1.10	Kapi Plaa (Thailand)	199
3.1.11	Tai Plaa (Thailand)	199
3.1.12	Budu (Thailand and Malaysia)	200
3.1.13	Prahok (Cambodia)	202
3.1.14	Nuoc-Mam (Vietnam)	202
3.1.15	Bakasang (Indonesia)	203
3.1.16	Terasi Ikan (Indonesia)	204
3.1.17	Wadi Betok (Indonesia)	205
3.1.18	Kecap Ikan (Indonesia)	205
3.1.19	Pedah (Indonesia)	206

CONTENTS**XI**

3.1.20	Bagoong and Patis (Philippines)	206
3.1.21	Bagoong Isda (Philippines)	207
3.1.22	Yu-Lu (China)	208
3.1.23	Shottsuru (Japan)	209
3.1.24	Ikanago Shoyu (Japan)	210
3.1.25	Konago (Japan)	210
3.1.26	Jeotkal (Korea)	210
3.2	Shellfish Products	211
	<i>Wonnop Visessanguan and Siriporn Chaikaew</i>	
3.2.1	Shrimp/Krill Paste	212
3.2.1.1	Bagoong (Philippines)	212
3.2.1.2	Belacan (Malaysia)	213
3.2.1.3	Kapi (Thailand and Cambodia)	215
3.2.1.4	Mam Ruoc and Mam Tom (Vietnam)	216
3.2.1.5	Nappi (Bangladesh)	217
3.2.1.6	Ngapi (Myanmar)	217
3.2.1.7	Terasi Udang (Indonesia)	218
3.2.2	Shrimp/Krill Sauce	219
3.2.2.1	Nam-Poo (Thailand)	219
3.2.2.2	Nan Ji Xia Jiang Zhi (Taiwan)	220
3.2.2.3	Ngan Pya Ye (Myanmar)	220
3.2.2.4	Xia You (Taiwan)	221
3.2.3	Other Shellfish Products	221
3.2.3.1	Ogiri-Nsiko (Nigeria)	221
3.2.3.2	Jeotgal (Korea)	221
3.2.3.3	Shiokara (Japan)	223
3.3	Hongeoehoe	224
	<i>Ggot-Im Lee and Cherl-Ho Lee</i>	
3.3.1	Manufacturing Process of Hongeoehoe	225
3.3.2	Chemical Composition and Physiological Function	226
3.3.3	Microbial and Chemical Changes	227
3.3.4	Safety Aspects and HACCP	227
3.3.5	Conclusion	228
3.4	Pidan	228
	<i>Soottawat Benjakul and Palanivel Ganesan</i>	
3.4.1	Manufacturing of PIDAN	229
3.4.1.1	Traditional Processing	230
3.4.1.1.1	Rolling Powder Method	230
3.4.1.1.2	Coating Method	231
3.4.1.1.3	Immersion Method	232
3.4.1.2	Commercial and Novel Processing of PIDAN	232
3.4.1.2.1	Production of PIDAN	232
3.4.1.2.2	Coating of PIDAN	234

3.4.2	Characteristics of Pidan	234
3.4.2.1	Textural Property	234
3.4.2.2	Color	238
3.4.2.3	Odor	242
3.4.2.4	Pine-Floral Crystal	242
3.4.3	Lysinoalanine Formation in Pidan	242
3.4.4	Nutritive Value of Pidan	244
3.4.4.1	Amino Acids	244
3.4.4.2	Minerals	245
3.4.4.3	Lipid and Cholesterol	246
	References	247
CHAPTER 4	MICROORGANISMS PREDOMINATING IN ALKALINE-FERMENTED FOODS	259
4.1	<i>Bacillus</i> and Related Genera	259
	<i>Charles Parkouda, Bréhima Diawara, and Kwaku Tano-Debrah</i>	
4.1.1	Biodiversity	260
4.1.2	Taxonomic Characteristics	260
4.1.2.1	Phenotypic Characteristics	262
4.1.2.1.1	Morphological Characteristics	263
4.1.2.1.2	Biochemical, Physiological, and Metabolic Characteristics	263
4.1.2.2	Molecular Characteristics	264
4.1.2.2.1	Genotypic Grouping Methods	265
4.1.2.2.2	Gene Sequencing	266
4.1.2.2.3	Culture-Independent Method	266
4.1.3	Ecology	267
4.1.4	Metabolism	268
4.2	Lactic Acid Bacteria	270
	<i>Donatien Kaboré and Charles Parkouda</i>	
4.2.1	Biodiversity	271
4.2.2	Taxonomic Characteristics	271
4.2.3	Ecology	274
4.2.4	Metabolism	275
4.2.5	Genomics	278
4.3	Yeasts	279
	<i>Lene Thorsen, Elmer Nayra Kpikpi, and Lene Jespersen</i>	
4.3.1	Taxonomy, Ecology, and Functionality of Yeasts	279
4.3.2	Yeasts in Legume-Based Alkaline-Fermented Foods	282

CONTENTS**XIII**

4.3.2.1	Soybean Products	282
4.3.2.2	Non-Soybean Legume Products	289
4.3.3	Yeasts in Non-Legume Plant-Based Food Products	291
4.3.4	Yeasts in Animal-Based Alkaline Food Products	292
4.3.5	Current Use and Perspectives for Application of Yeasts in Alkaline-Fermented Foods	294
	References	295
CHAPTER 5	QUALITY ASPECTS OF ALKALINE-FERMENTED FOODS	315
5.1	Sensory Characteristics <i>Paulin Azokpota</i>	315
5.2	Nutritional Value <i>Shawn Mark Somerset</i>	321
5.2.1	Asian Foods	324
5.2.2	African Foods	326
5.2.3	Other Putative Health Benefits	329
5.2.4	Reliability of Alkaline-Fermented Food Compositional Data	330
5.2.5	Conclusion	333
5.3	Health-Promoting Effects	334
5.3.1	Reduction of Anti-Nutritional Factors <i>Paulin Azokpota</i>	334
5.3.2	Production of Vitamins <i>Paulin Azokpota</i>	337
5.3.3	Increase in Digestibility <i>Paulin Azokpota</i>	338
5.3.4	Medicinal Benefits	342
5.3.4.1	Antioxidative Activity <i>Toshirou Nagai</i>	342
5.3.4.2	Probiotic Activity <i>Toshirou Nagai</i>	346
5.3.4.2.1	Antibacterial Activity of <i>Bacillus subtilis (natto)</i>	346
5.3.4.2.2	Probiotic Activities of <i>Bacillus subtilis (natto)</i>	347
5.3.4.2.3	Behavior of <i>Bacillus subtilis (natto)</i> in Digestive System	349
5.3.4.2.4	Conclusion	351

5.3.4.3	Immunomodulating Activity	351
	<i>Toshirou Nagai</i>	
5.3.4.3.1	Nonspecific Immunity Conferred by <i>Bacillus subtilis (natto)</i>	351
5.3.4.3.2	Mechanism of Immunomodulation	352
5.3.4.3.3	Anti-Tumor Activity	354
5.3.4.3.4	Conclusion	355
5.3.4.4	Fibrinolytic Activity	355
	<i>Toshirou Nagai</i>	
5.3.4.5	Anti-α-Glucosidase (Antidiabetic) Activity	359
	<i>Li-Te Li and Yan-Li Ma</i>	
5.3.4.5.1	Diabetes Mellitus	359
5.3.4.5.2	α -Glucosidase Inhibitors and Diabetes Mellitus	360
5.3.4.5.3	Main Source of α -Glucosidase Inhibitors	362
5.3.4.5.4	α -Glucosidase Inhibitory Activity of Alkaline-Fermented Foods	363
5.3.4.5.5	Prospects	365
5.3.4.6	Antihypertensive Activity	365
	<i>Li-Te Li and Yan-Li Ma</i>	
5.3.4.6.1	Hypertension	365
5.3.4.6.2	Angiotensin- Converting Enzyme Inhibitors and Hypertension	366
5.3.4.6.3	ACE Inhibitory Peptides Derived from Fermented Foods	368
5.3.4.6.4	ACE Inhibitory Activity of Alkaline- Fermented Foods	370
5.3.4.6.5	Prospects	372
5.3.4.7	Tooth Enamel Hardness	373
	<i>Chanya Chuenarrom</i>	
	References	379

CONTENTS

XV

CHAPTER 6	SAFETY ASPECTS OF ALKALINE-FERMENTED FOODS	399
	<i>Ggot-Im Lee and Cherl-Ho Lee</i>	
6.1	Alkaline-Fermented Foods Are Generally Safe	399
6.1.1	Initial Thermal Treatment	400
6.1.2	Growth of <i>Bacillus subtilis</i>	401
6.1.3	pH Change	404
6.1.4	Cooking Process	405
6.2	Hazards in Alkaline-Fermented Foods	405
6.2.1	Microbiological Hazards	405
6.2.1.1	<i>Bacillus cereus</i>	405
6.2.1.2	Other Potential Pathogens	406
6.2.1.3	Mycotoxins	407
6.2.2	Chemical Hazards: Biogenic Amines	410
6.3	Control of Hazards	415
6.3.1	GAP	415
6.3.2	HACCP	415
6.4	Conclusion	418
	References	419
 CHAPTER 7	 CHALLENGES ASSOCIATED WITH TECHNOLOGICAL ASPECTS FOR MODERNIZATION OF ALKALINE-FERMENTED FOODS	 425
7.1	Modulation of Product Chains	425
	<i>Anita R. Linnemann</i>	
7.1.1	Key Determinants of Successful Modernization of Traditional Foods	425
7.1.2	How to Determine Consumer Preferences	426
7.1.3	Applying a Chain Approach to Ensure Constant Product Quality	429
7.1.4	Benchmarking	431
7.1.5	Conclusion	432
7.2	Development of Starter Cultures	432
	<i>Egon Bech Hansen</i>	
7.2.1	Food Fermentation and Food Cultures	432
7.2.1.1	Microorganisms Used in Food Fermentations	433
7.2.1.2	Inoculation for Food Fermentations	434
7.2.1.3	Requirements for a Starter Culture	436
7.2.1.4	Suppliers of Starter Cultures	438
7.2.2	Starters for Alkaline-Fermented Foods	439
7.2.2.1	Potential Improvements of Inoculation Practice	439

	7.2.2.2	Development and Production of Starter Cultures for Alkaline-Fermented Foods	440
	7.2.3	Conclusion	441
7.3		Hygienic Design Aspects of Alkaline Fermentation Processes	441
		<i>Huub L. M. Lelieveld</i>	
	7.3.1	Chemical Contamination	442
	7.3.2	Physical Contamination	443
	7.3.3	Microbial Contamination	444
	7.3.4	Managing Food Safety	444
	7.3.5	Hygienic Processing and Plant Design	445
	7.3.5.1	Construction Materials	446
	7.3.5.2	Surface Roughness	447
	7.3.5.3	Drainability	447
	7.3.5.4	Dead Spaces	447
	7.3.5.5	Crevices and Corners	448
	7.3.5.6	Pipe Connections	449
	7.3.5.7	Preventing Ingress of Contamination	449
	7.3.5.8	Inoculation and Sampling	449
	7.3.5.9	Continuous Fermentation	450
	7.3.5.10	Conveyor Belts for Solid-State Fermentations	450
	7.3.5.11	Process Control	451
	7.3.5.12	Equipment Installation	451
	7.3.6	Further Reading	452
7.4		Food Packaging	452
		<i>Jenneke K. Heising and Matthijs Dekker</i>	
	7.4.1	Challenges	453
	7.4.2	Basic Functions of Food Packaging	454
	7.4.2.1	Containment	454
	7.4.2.2	Protection	455
	7.4.2.3	Convenience	456
	7.4.2.4	Communication	456
	7.4.3	Interactions between Food and Package: Flavor Scalping and Migration	457
	7.4.4	Interactions between Packaging and Environment	458
	7.4.5	Packaging Materials	459
	7.4.6	Fermentation in Package	462
	7.4.7	Case: Application of a Package on the Product Kinema	462
	7.4.7.1	Product Description	462
	7.4.7.2	Traditional Package	463
	7.4.7.3	Package Requirements	463
	7.4.8	Conclusion	464
		References	464

CHAPTER 8	VALUE-ADDED PRODUCTS FROM ALKALINE-FERMENTED FOODS OR FROM MICROORGANISMS PREDOMINATING THEREIN	469
8.1	Alkaline Proteases	469
	<i>C. Ganesh Kumar</i>	
8.1.1	Alkalophilic Microorganisms and Their Habitats	470
8.1.2	Isolation and Screening of Alkalophiles	474
8.1.3	Production of Alkaline Proteases	475
8.1.3.1	Improvement in Alkaline Protease Yields	475
8.1.3.2	Regulation and Medium Optimization for Protease Biosynthesis	478
8.1.3.2.1	Nitrogen Source	480
8.1.3.2.2	Carbon Source	481
8.1.3.2.3	Trace Salt Requirement	481
8.1.3.2.4	pH and Temperature	482
8.1.3.2.5	Statistical Design-Based Optimization	482
8.1.4	Purification and Characterization of Alkaline Proteases	483
8.1.5	Applications of Alkaline Proteases	486
8.2	Poly-γ-Glutamic Acid	498
	<i>Toshirou Nagai</i>	
8.2.1	PGA Produced on <i>Natto</i>	499
8.2.2	Role of Natto Plasmid and γ -Glutamyl Transferase in PGA Production	499
8.2.3	Genes Related to the Production of PGA	502
8.2.4	PGA Resin	504
8.2.5	PGA-Decomposing Enzymes	504
8.2.5.1	<i>Bacillus subtilis (natto)</i> Bacteriophages and Their PGA-Depolymerizing Enzymes	504
8.2.5.2	YwtD, γ -DL-Glutamyl Hydrolase of <i>Bacillus subtilis (natto)</i>	506
8.2.6	PGA-Decomposing Enzymes from Other Sources	507
8.2.7	Conclusion	507
8.3	Lipopeptides	508
	<i>Philippe Jacques and Aly Savadogo</i>	
8.3.1	Microbial Lipopeptides	508
8.3.2	Lipopeptides and Fermented Food Products	510
8.3.3	Structures of Lipopeptides Produced by Strains Isolated from Fermented Products	511

8.3.4	Biological Activities of Lipopeptides	513
8.3.5	Biosynthesis	514
8.3.6	Regulation and Influence of Environmental Factors on Lipopeptide Production	515
8.3.7	Potential Role of Lipopeptide in Fermented Products	517
8.4	Short-Chain Volatile Organic Acids and Various Other Organic Compounds	518
	<i>Maureen-Theodore Chinwe Ojinnaka</i>	
8.5	Flavor Additives	522
	<i>Maureen-Theodore Chinwe Ojinnaka</i>	
8.5.1	Esters	524
8.5.2	Acids	525
8.5.3	Pyrazines	526
8.5.4	Aldehydes	527
8.5.5	Furan	527
8.5.6	Ketones	527
8.5.7	Sulfur-Containing Compounds	528
	References	528
CHAPTER 9	FUTURE OF ALKALINE-FERMENTED FOODS FOR TRADITIONAL MARKETS	559
	<i>M. J. Robert Nout and Prabir K. Sarkar</i>	
	References	561
CHAPTER 10	OUTLOOK	563
	<i>M. J. Robert Nout and Prabir K. Sarkar</i>	
	References	565

in fortified red colored biscuits, which had greater organoleptic acceptability compared with full-fat soy-flour-supplemented biscuits. Protein content of the product was more than 17%.

2.1.1.3.9 Conclusion Traditional knowledge of tribal women in preparing kinema has relevance to the sustainable food and nutritional security and subsistence economy of the kinema-practicing societies. To promote commercialization of kinema, the product must maintain superior quality. It is necessary to increase efforts to introduce consumer preferences in terms of taste and produce high-quality products, packaging, and design as well as safety management. Given the concerted efforts of academia, industry, and government and private organizations, kinema has a potential to become an enjoyable part of the menu.

2.1.1.4 Doenjang

Kun-Young Park and Ji-Kang Jeong

Korea has a long history of eating fermented soybean foods, and doenjang is one of the representative Korean traditional fermented soybean foods. Historically, soybean and processed soybean foods have been one of the most important protein sources in the Korean diet because Koreans mainly eat vegetables. Doenjang has also been used as a seasoning for enhancing the taste of foods (Park and Jung, 2005). As a traditional Korean fermented soybean food, doenjang has unique qualities and characteristics in terms of its flavor and taste compared with those of other similar products from Asian countries. This is due to the fermentation process and Korea's unique natural environment (Park et al., 2000; Kim, 2002).

Doenjang is recognized as a nutritious food that provides essential amino acids that are lacking in cereal and vegetable diets as well as fatty acids, organic acids, minerals, and vitamins, which are highly related to its quality and diverse nutritional benefits (Lim et al., 1999). Proximal composition of doenjang is 54.7% water, 13.8% crude protein, 8.0% crude lipid, 14.4% titratable acidity, and 11.8% salt. Free amino acids are comprised 3.8% of the nitrogen content, of which glutamic acid content (25%) is the highest, but leucine, alanine, histidine, lysine, proline, and valine are also present in relatively high quantity. Lactic acid was the most abundant organic acid, but acetic,

malic, citric, and oxalic acids were also detected. Linoleic acid (52.2%), oleic acid (20.7%), and linolenic acid (8.7%) are the major unsaturated fatty acids, comprising 81.6% of total fatty acids. Doenjang also contains oligosaccharides and 3.1% dietary fiber. The digestibility of raw soybean is approximately 55% and that for the cooked soybean is 65%. Digestibility of doenjang increased up to 85% (Park et al., 2000). Doenjang had been used as a folk medicine. It has various health functionalities of antioxidative, anticancer, antimutagenic, antiatherosclerosis, and antiobesity (Park and Jung, 2005).

2.1.1.4.1 Manufacturing Procedures Doenjang (Figure 2.19a) has been traditionally made from meju, which is a fermented rectangular block of cooked and crushed soybeans (Figure 2.19b). Other Korean traditional fermented soybean foods such as ganjang and kochujang are also made from meju. The standardized process to prepare traditional doenjang is shown in Figure 2.20. To prepare traditional meju, which is the main ingredient and starter of doenjang, raw soybeans are soaked, cooked, crushed, and then molded into a rectangular block. Next, it is dried for 3 days in the air, tied up with rice straw and hung at the edge of an eave for its natural fermentation (Figure 2.19b). In general, traditional meju is prepared only with cooked soybeans and fermented with various beneficial microorganisms, typically *Bacillus subtilis* and *Aspergillus oryzae*, which are naturally harbored in rice straw. The duration for traditional meju making generally requires 1 or 2 months (Park and Kim, 1970; Kim et al., 1993, 2011).

After 1 or 2 months, the mejus are washed with water to remove any grown molds, straw, and dirt from the surface. Then, those are placed in an earthen jar, and brine is added at the ratio of meju, salt, and water of approx. 1:1:4 w/w/w (Kim et al., 2010). Some dried red

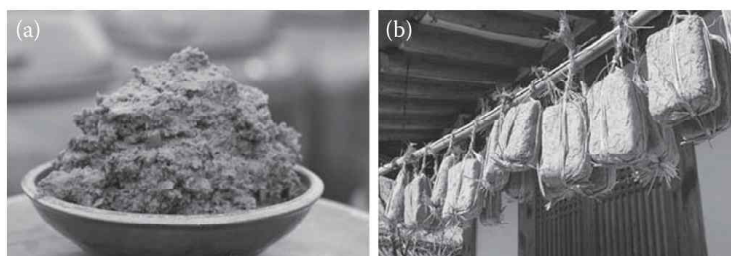


Figure 2.19 Appearance of traditional doenjang (a) and meju (b).

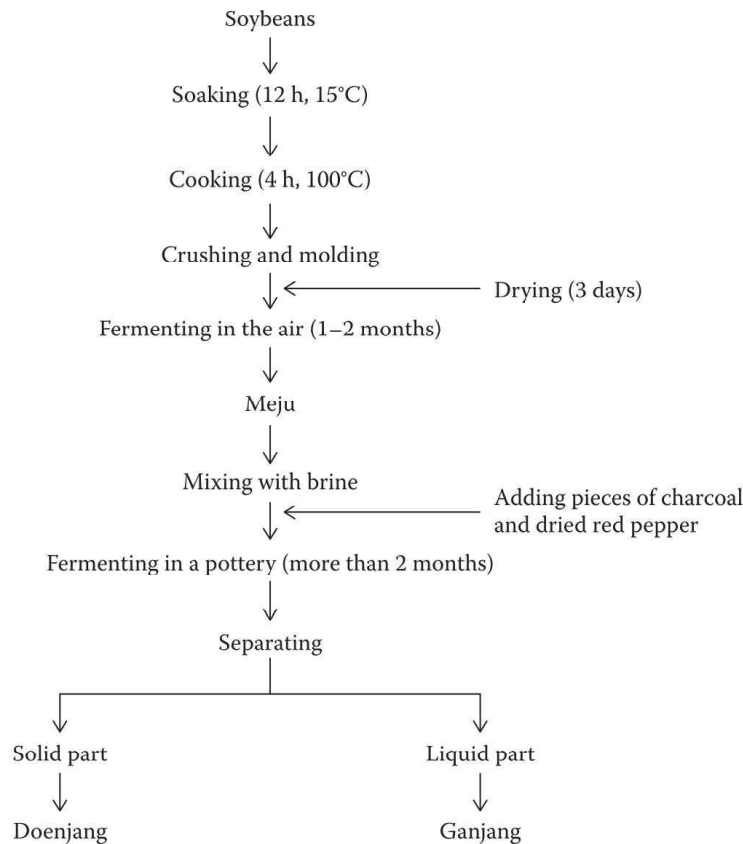


Figure 2.20 Traditional manufacturing method for meju and doenjang.

peppers and pieces of charcoal are placed on top of the mixture and fermented for 2 to 3 months in a jar. After fermentation, the meju-brine mixture is filtered. The solid part of the mixture becomes doenjang, and the crushed solid part is put into another earthen jar. Then, the doenjang undergoes an additional 2 to 6 months of fermentation. The long fermentation time of more than 1 year gives high quality and health functionality to doenjang. The liquid part of the mixture is ganjang (soy sauce), which can be boiled to destroy harmful microorganisms and remove off-flavors.

Recently, traditional doenjang has been commercially produced by several Korean manufacturing companies, and the traditional method of making doenjang has to be adapted for mass production (Kim et al., 2010). Compared with traditional meju fermented with many natural microorganisms, only *A. oryzae* is generally used as fermentation starter when meju is manufactured industrially. Koji, a modified

meju, is made of wheat flour or rice by inoculating with only *A. oryzae*. Koji is widely used to produce doenjang, especially commercial products. Because using the koji is more convenient than the traditional method, it can be easily applied to mass production and it has been widely used (Kim et al., 2006; Choi et al., 2009). However, it is known that the taste and quality of traditional doenjang are generally higher than those of industrially manufactured doenjang, and one of its reasons is the microorganisms related to fermentation. These microorganisms are more diverse in traditional doenjang than in commercial one. Therefore, to develop the fermented soybean products that have reduced fermentation time and are convenient to prepare, as well as, improve the quality and functionalities, various methods have been tried.

Attempts have been made to develop doenjang manufacturing methods that take advantage of both traditional and industrial methods (Kim et al., 2000; Kim, 2002; Ko et al., 2010), and one of such methods is the use of effective and beneficial microorganisms as starter cultures. In the most recently conducted study, Jeong (2012) used mixed starter cultures to manufacture doenjang. Numerous microorganisms were isolated from traditional meju; then three microorganisms, *A. oryzae*, *B. subtilis*, and *Lactococcus lactis*, which showed the highest enzyme activity and functionality in the isolates were selected as starters. Grain-type meju (industrial method) was prepared with the inoculation of this mixed starter cultures, and doenjang was manufactured using this meju. This doenjang showed excellent fermentation qualities, organoleptic qualities, and health functionalities compared with industrial doenjang and was comparable to traditional one. Therefore, the doenjang manufacturing method of using beneficial mixed starter cultures can improve the qualities and functionalities of doenjang. In addition, it is expected to be applicable to the fermented soybean product industry due to its great food safety, health functionality, and sensorial characteristics.

2.1.1.4.2 Microorganisms in Doenjang Microorganism is the most important factor in the fermentation of doenjang, and various kinds of microorganisms participate in the fermentation. The traditionally manufactured meju has various fermenting microbiota depending on the geographical location. Commercially manufactured mejus, where

Aspergillus spp. are generally used as a starter, also have different microbiota by fermentation condition (Lee et al., 2010). Many studies have reported that *Aspergillus*, *Mucor*, and *Rhizopus* species were detected in meju and doenjang, and *B. subtilis* and *Bacillus licheniformis* are the dominant microorganisms in them (Yoo et al., 1999; Kang et al., 2000; Cho and Seo, 2007).

These earlier studies were based on culture media and methods and were limited to identifying the strains that grow on specific media. Recently, several molecular biological methods such as denaturing gradient gel electrophoresis (DGGE) have been used to investigate, identify, and quantify microorganisms, and it has also been applied to analyze the microbial community in doenjang (Kim et al., 2009). As a result, some microorganisms such as *Staphylococcus equorum* and some lactic acid bacteria (LAB), which have been thought to be unrelated to doenjang production, are newly considered as important microorganisms in doenjang production (Yoo et al., 1999; Kang et al., 2000; Cho and Seo, 2007).

Recent studies have shown that the strains of bacteria and fungi existed in meju and doenjang are quite different by the region and manufacturing method. Lee et al. (2010) reported that a variety of fungi including *Absidia corymbifera*, *Aspergillus* spp., and *Candida rugosa* were the main fungi in meju; besides, *Mucor* spp., *Pichia farinosa*, and *Penicillium* spp. were also detected in meju. *Bacillus subtilis* is the major *Bacillus* spp. present in meju, and *B. licheniformis* is detected in lower amounts. LAB are the major fermenting microorganisms in meju. *Enterococcus durans* is a very common LAB species, and *Pediococcus* spp., *Lactobacillus* spp., *Leuconostoc* spp., and *Lactococcus* spp. are also detected in meju. They also reported that LAB including *Enterococcus faecium*, *Leuconostoc mesenteroides*, and *Tetragenococcus halophilus* and fungi and yeasts including *A. oryzae*, *Mucor plumbeus*, and *Debaryomyces hansenii* were generally present in doenjang. *Aspergillus oryzae* was the most representative fungus in meju and is used generally as a starter in commercially manufactured doenjang (Kim et al., 2009).

Although traditionally manufactured fermented soybean products including doenjang have high qualities and health functionalities, they can also produce toxic substances that are harmful to human health. Doenjang once had been suspected of being contaminated with mycotoxin, especially aflatoxins from the growth of *Aspergillus flavus* and

Aspergillus paprasiticus, and the contamination by these strains occurred during the procedures of meju fermentation. Aflatoxin is known as a potent carcinogen linked to liver cancer. However, it was demonstrated that the aflatoxins can be destroyed by ammonia, sunlight, mixed culture conditions, melanoidin formation, addition of charcoal, etc. during the fermentation period. In addition, attempts to reduce contamination possibilities have been made by controlling the microorganisms related to meju fermentation and using bacteriocin-producing microorganisms. Thus, doenjang may not have any significant harmful effect, despite the possible contamination by aflatoxins (Park and Lee, 1985, 1989; Park and Kweon, 1987; Park et al., 1988, 1989).

2.1.1.4.3 Functional Properties Many epidemiological studies suggest that the consumption of doenjang provides various health benefits in human. Doenjang is rich in flavonoids, vitamins, and unsaturated fatty acids and especially, one of the most important active compounds is isoflavone. Genistein and daidzein are the two major soy isoflavones, and they principally occur in nature as their glycosylated or methoxylated derivatives, which are cleaved in the intestine to yield free aglycones and further metabolites. It has been demonstrated that isoflavone glucosides in soybean foods are decomposed to aglycone by microorganisms during fermentation. Genistein and daidzein are absorbed better than their glucosides in human, indicating that isoflavone aglycone-rich foods may be more effective than glucoside-rich foods in preventing chronic diseases such as cancer and cardiovascular disease (Grun et al., 2001; Park et al., 2008). Other active compounds in doenjang are linoleic acid, trypsin inhibitor, saponins, etc., and it seems that the fermented soybean paste contained more bioactive compounds than the soybean itself (Choi et al., 2001).

It has been reported that eating traditional doenjang demonstrated strong antimutagenic activities against various carcinogens and mutagens including aflatoxin B₁. Park et al. (1996) reported that strong antimutagenic activity toward aflatoxin B₁ (AFB₁) was observed by treatment with a methanol extract of doenjang. The AFB₁-mediated mutagenesis was completely inhibited at the level of 25 mg/plate of the traditional doenjang extract, and it showed the highest antimutagenic activity compared with other fermented soybean food such as miso and chongkukjang (Figure 2.21).

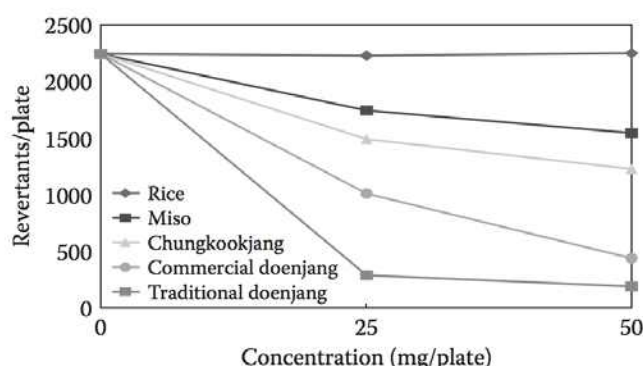


Figure 2.21 Effect of methanol extract of various fermented soybean foods on the mutagenesis of aflatoxin B₁ in *Salmonella typhimurium* TA100.

The antimutagenic effects of doenjang were not limited to AFB₁, but were also exhibited toward other mutagens and carcinogens such as the indirect mutagens, benzo(*a*)pyrene (BaP) and *N,N*-dinethylnitrosamine (DMN), and the direct mutagens, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) and 4-nitroquinoline 1-oxide (4-NQO) (Park et al., 1996). Trypsin inhibitor, genistein, soya saponin, α -tocopherol, β -sitosterol, and linolenic acid are believed to be active compounds in doenjang that possess antimutagenic effects. All active compounds exerted a strong antimutagenic effect against AFB₁. Genistein and linolenic acid were identified as the most active antimutagenic compounds in doenjang (Jung et al., 2006) (Table 2.6).

Table 2.6 Effect of Various Active Compounds (1.25 mg/plate) on the Mutagenicity Induced by Aflatoxin B₁ in *Salmonella typhimurium* TA100

SAMPLE	AFB ₁	
	REVERTANT/PLATE	INHIBITION RATE (%)
Spontaneous	104 ± 2	
Control (AFB ₁)	1218a ± 9	
Genistein	307c ± 31	82
Genistin	579b ± 37	57
Linoleic acid	324c ± 28	80
α -Tocopherol	786b ± 104	39
β -Sitosterol glucoside	549b ± 99	60
Soya saponin	621b ± 142	54
Trypsin inhibitor	646b ± 87	51

Note: Means (\pm SD), with the different letters beside data, are significantly ($p < 0.01$) different by Duncan's multiple range test.

Doenjang is also known to exhibit anticancer activities. The growth of several human cancer cells such as AGS gastric adenocarcinoma cells, HT-29 colon cancer cells, and MCF-7 human breast cancer cells was significantly reduced by treatment with the methanol extract of doenjang. Choi et al. (1999) also indicated that the hexane fraction from methanol extract of doenjang suppressed various tumor cells. The doenjang hexane fraction (DHF) was evaluated for its effects on the cell cycle progression in MCF-7 human breast cancer cells. DHF induced a G1-phase arrest of the cell cycle in MCF-7 cells, which correlated with the accumulation of hypophosphorylated form of retinoblastoma protein (pRB) and enhanced association of pRB with the transcription factor E2F1. As shown in Figure 2.22, the expression of D-type cyclins was decreased by DHF in a time-dependent manner, but DHF did not affect the level of cyclins E and A. However, the activity of Cdk2 and cyclin E-associated kinase was decreased in a time-dependent manner. The tumor suppressor, p53, and Cdk inhibitor, p21, which is a known downstream effector of p53, and association of p21 with Cdk2 were markedly induced in DHF-treated cells (Figure 2.23). Thus, DHF inhibited cancer cell growth by inducing an

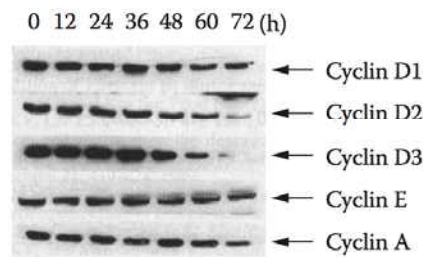


Figure 2.22 Western blot analysis of cyclins from MCF-7 cells after increasing period of exposure to doenjang hexane fraction.

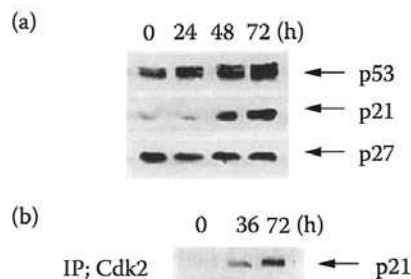


Figure 2.23 Induction of Cdk inhibitor p21 (a) and association of p21 with Cdk2 (b) by doenjang hexane fraction in MCF-7 cells.

inhibition of pRB phosphorylation, decreasing expression of D-type cyclins, and increasing of Cdk inhibitor p21 that appears to be responsible for the observed G1 arrest (Choi et al., 2001).

Anticancer activity of doenjang was also demonstrated in an animal study by inhibiting the solid tumor growth from transplanted sarcoma 180 tumor cells (Park et al., 1999). In a recent study, doenjang exerted the inhibitory effect on chemically induced colitis associated colon cancer in mice (Jeong, 2012). In this study, two kinds of doenjang were used to investigate and compare their functionalities. One was existing commercial doenjang and the other was newly developed doenjang, which was prepared using mixed beneficial starter cultures. Mice were given azoxymethane (AOM) and dextran sodium sulfate (DSS) to induce colitis-associated colon cancer, and doenjang were orally administered during a certain period. As a result, doenjang especially mixed starter used doenjang treatment decreased the loss of crypt in the colonic mucosa and relieved the inflammation of colonic mucosa. It also significantly reduced total number of colonic neoplasia. Doenjang administration also prevented significant increases in serum pro-inflammatory cytokine levels such as tumor necrosis factor (TNF)- α and interferon (IFN)- γ (Figure 2.24). These cytokines are important mediators of inflammation and its related cancer and elevated production of pro-inflammatory cytokines is found in the inflamed colons. This finding suggests that doenjang-treated mice were protected from inflammation and its

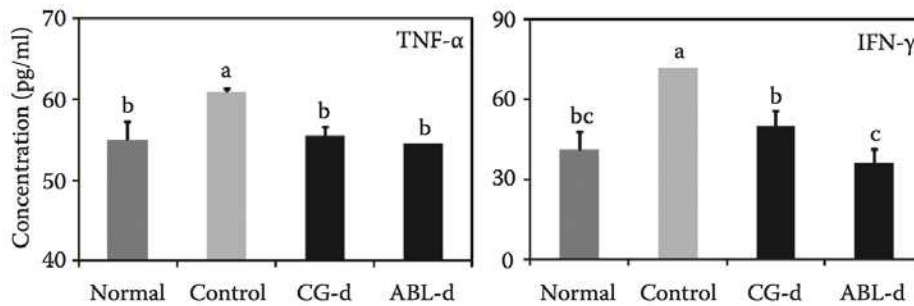


Figure 2.24 Effects of doenjang treatment on serum pro-inflammatory cytokines in AOM and DSS-induced colitis associated cancer (CAC) mice. Normal, group received PBS without AOM and DSS; control, group received PBS and induced CAC by AOM and DSS; CG-d, group received commercial doenjang and induced CAC by AOM and DSS; ABL-d, group received mixed starter used doenjang and induced CAC by AOM and DSS; a–c, means with the different letters on bars representing SD are significantly different ($p < 0.05$) by Duncan's multiple range test.

associated cancer. Treatment with doenjang caused a marked decrease in the expression of iNOS and COX-2, which are important factors related to inflammation and its associated cancer, in colonic tissue of the mice. In addition, pro-apoptotic Bax significantly decreased and anti-apoptotic Bcl-2 increased in DSS and AOM induced CAC mice and they showed a contrary tendency in doenjang-treated group. The expression of p53 tumor suppressor gene and p21 Cdk inhibitor increased in doenjang-fed group compared with the control group (Figure 2.25). Collectively, these results confirm that doenjang has protective effect in the progression of colitis-associated cancer, and it is correlated with a reduction of iNOS and COX-2 expression in colon tissues and an induction of cancer cell apoptosis by regulating Bax, Bcl-2, p21, and p53.

Doenjang has been documented as having antioxidative properties. Many studies have reported that doenjang inhibited lipid oxidation, lipoxygenase activity, and increased metal chelation and free radical scavenging activities in *in vitro* model system (Cheigh et al., 1990; Lee and Cheigh, 1997). Doenjang can also reduce cardiovascular disease by having fibrinolytic, antihypertensive, and serum cholesterol lowering effects (Park and Jung, 2005). Recently, anti-obesity effect of doenjang and genistein has also been observed *in vitro* and *in vivo* (Kwak et al., 2012).

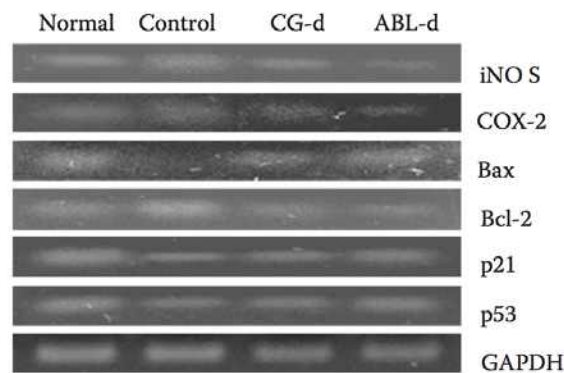


Figure 2.25 Effects of doenjang treatment on mRNA expressions of iNOS, COX-2, Bax, Bcl-2, p21, and p53 in the colon tissue of AOM- and DSS-induced CAC mice. Normal, group received PBS without AOM and DSS; control, group received PBS and induced CAC by AOM and DSS; CG-d, group received commercial doenjang and induced CAC by AOM and DSS; ABL-d, group received mixed starter used doenjang and induced CAC by AOM and DSS.

2.1.1.4.4 Conclusion Doenjang has long been traditionally consumed and manufactured in Korea. Recently, traditional manufacturing method has been replaced by a more convenient and hygienic method. However, maintaining the consistent quality, and manipulating increased quality and functionality were still difficult problems in Korean soybean paste industry. Therefore, many attempts have been made and continue be made to develop the additional standardized manufacturing, and one of such methods is the use of effective and beneficial microorganisms as starter cultures.

2.1.1.5 Chongkukjang

Jang-Eun Lee, Ggot-Im Lee, and Cherl-Ho Lee

2.1.1.5.1 Origin of Chongkukjang Chongkukjang is a type of Korean soybean-fermented product. It is a bacterial-fermented soybean food, similar to natto of Japan, but different in the usage. It is made by easy and rapid fermentation process using cooked soybeans. It can be eaten within 2–3 days, unlike other soybean products with a long fermentation period. It is also used as one of the fermented soybean condiments stemmed from the long history of soybean fermentation in Korea, as classified in Figure 2.26.

Shi is prepared by fermenting cooked soybean whole grain with bacteria, mainly *Bacillus subtilis*, while maljang is made by meju, a fermented soybean loaf where molds and bacteria are grown. *Aspergillus oryzae* is the major mold outside of meju, and *Bacillus* spp. are the main bacteria inside of meju. The term “shi,” the Chinese word indicating fermented soybean, first appeared in *Jijiupian*, written in the Han period (206 BC to 208 AD) of China. *Bowuzhi* of Jin (265–420 AD) of China described that shi originated in a foreign country as the word “shi” is a dialect. *Xintangshu* (618–807 AD) of Tang Dynasty



Figure 2.26 The position of chongkukjang in Korean fermented soybean products. (Modified from Lee, C.H., *Fermentation Technology in Korea*, Korea University Press, Seoul, 2001.)

of China named shi as a special product of Balhai or Bohai (688–826 AD), a nation founded by the refugees from defeated Kokuryo Dynasty, the ancient Korea (37 BC–668 AD). *Chi-Min-Yao-Shu*, written by Jia-Si-Xie of Late-Wei in the 6th century, described several methods of shi preparation (Lee, 2001).

It is generally recognized that Koreans are the first people who conducted soybean fermentation, sparking the soy sauce culture in the world. The history book of Wei Dynasty praised the fermentation skill of Korean people. Their techniques were transferred to Japan during 200–300 AD from Kokuryo. The Japanese term of soybean paste, “miso,” was originated from the Korean language maljang, an old term referring to fermented soybean products (Lee, 1990). *Masakurainbunsho*, written in Japan in 752, described miso as a dialect from Koryo and called as Koryojang (Lee, 1990). In the classification of Figure 2.26, “jang” is the soybean product fermented with other cereals such as rice, wheat, and barley to make koji, mostly developed in Japan, although the term “jang” is generally used for the fermented condiments in Asia. Chongkukjang is called as yumshi in the early Korean literatures like *Sam-kuk-sa-ki*, written by Kim Bu-Shik (1075–1151 AD). The 18th century literatures, like *San-lim-kyung-je* (1715), described it as “jeonkukjang,” whose name implies the need for rapid processing of soybean paste during the war time of Byungjahoran in 1636, as “jeon” means war and “kukjang” means soup in Korean. Food historians believe that the phonetic change from “jeonkuk” to “chongkuk” took place at that time. Another story is that the Qing army used to carry the instant fermented soybean as military ration, and it was called chongkukjang (Chong/Qing – kukjang). What all these names imply is that this product was made for extraordinary situation such as war or famine conditions for the urgent supply of nutritious savory food ingredient.

2.1.1.5.2 Methods of Preparation The manufacturing process of chongkukjang is unique compared with the other Korean soybean foods, doenjang and gochujang, which are mainly prepared by mixing and fermenting meju with salt and water or red pepper powder, glutinous rice, salt, and water, respectively. While the other two pastes need at least 6 months of fermentation time for ripening, chongkukjang is made by fermenting microorganisms naturally transferred from the air or rice straw on cooked soybeans.

The first written record on the preparation of jeonkukjang appeared in *Jeung-bo-san-lim-kyung-je* written by Jung-Im Yoo in 1765. Newly harvested soybean is cooked and covered with straw mat, and placed on the warm “ondol” (typical Korean stone floor heated by fire underneath) for 3 days until the mucous string (Figure 2.27) is formed with a strong fermented smell. It is mixed with dehulled and roasted soybean powder, pounded in a stone mortar with the addition of salt, and sun-dried. The mixing ratio of fermented soybean and roasted soybean powder is 2 to 1, and the amount of salt addition is determined by its taste. The product appears to be dried form, easy for storage and transport, and suitable for the military use. This process is slightly different from the current method.

The current process is very simple as shown in Figure 2.28. Soybean is cooked and covered with straw mat or cloth and placed on the warm ondol for 3–4 days until the mucous string is formed. It is mixed with chopped ginger, chopped garlic, and salt, and pounded slightly until the bean kernels are divided into halves, and stored in an earthen jar (Figure 2.29a). Industrial fermentation is made by adding pure culture of *B. subtilis* or placing pieces of rice straw to cooked soybean. Rice straw is the source of *B. subtilis*, which grows fast at 40°C and becomes naturally dominating flora in the system (Lee, 1995). Chongkukjang has its characteristic flavor and smell that are partially masked by ginger and garlic. The spicy seasoning is thus prepared in 3–4 days, and nowadays, the product is marketed in attractive packages (Figure 2.29b).

2.1.1.5.3 Biochemical Composition During the production of chongkukjang, proteases and lipases produced by microorganisms can generate amino acids, organic acids, and fatty acids. Analysis of the



Figure 2.27 Chongkukjang with mucous strings.

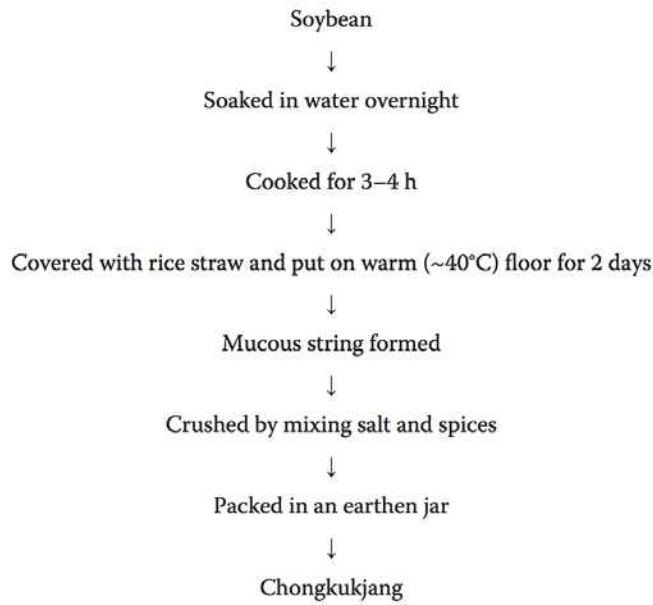


Figure 2.28 Chongkukjang making process. (From Lee, C.H., *Fermentation Technology in Korea*, Korea University Press, Seoul, 2001.)



Figure 2.29 Chongkukjang packages: traditional earthen jars (a) and a modern package (b).

chemical composition of samples of chongkukjang collected from various regions in Korea revealed that chongkukjang contains many biochemical components including fatty acids, amino acids, carbohydrates, and organic acids. Through chongkukjang analysis, various amino acids such as alanine, glycine, valine, leucine, isoleucine, γ -aminobutyric acid, serine, threonine, methionine, proline, aspartic acid, arginine, phenylalanine, glutamic acid, asparagine, lysine, histidine, tyrosine, tryptophan, and β -alanine are found. Also, lactic acid, oxalic acid, malonic acid, succinic acid, fumaric acid, malic acid,

tartaric acid, citric acid, itaconic acid, 2-hydroxyglutaric acid, gluconic acid, and galactaric acid are the major organic acids of chongkukjang. For fatty acids, myristic acid (14:0), palmitic acid (16:0), palmitoleic acid (16:1), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2), linolenic acid (18:3), arachidic acid (20:0), and behenic acid (22:0) are detected in chongkukjang. Especially, tryptophan, citric acid, β -alanine, itaconic acid, 2-hydroxyglutaric acid, γ -aminobutyric acid, leucine, malic acid, and tartaric acid are the major components of chongkukjang (Park et al., 2010). The biochemical compositions of chongkukjang and other soybean products are shown in Table 2.7.

The pH value of chongkukjang is increased from about pH 6.0 to 8.0 during fermentation (Baek et al., 2008; Jeong et al., 2009). Figure 2.30 shows the pH change of chongkukjang during fermentation. The initial pH was 6.3, which gradually increased as fermentation proceeded, reaching 8.1 in chongkukjang produced by *B. subtilis* at 40°C for 72 h. Different pH values reflected different capabilities of strains to produce metabolites such as ammonia, responsible for the

Table 2.7 Chemical Composition of Soybean and Chongkukjang

PARAMETER	SOYBEAN (YELLOW)	CHONGKUKJANG
Proximate (g/100 g)		
Energy (kcal/100 g)	420	108
Water	9.7	70.7
Protein	36.2	10.2
Fat	17.8	0.8
Ash	5.6	3.4
Carbohydrate	30.7	14.9
Mineral (mg/100 g)		
Calcium	245	96
Phosphorus	620	177
Iron	6.5	3.8
Potassium	1340	602
Sodium	2	961
Vitamin (mg/100 g)		
Vitamin B ₁	0.53	0.15
Vitamin B ₂	0.28	0.29
Niacin	2.2	1.5
Vitamin C	0	0

Source: RDA (Rural Development Administration), *The Korean Food Composition Table*. 8th revision, Rural Development Administration (National Academy of Agricultural Science), Suwon, 2011.

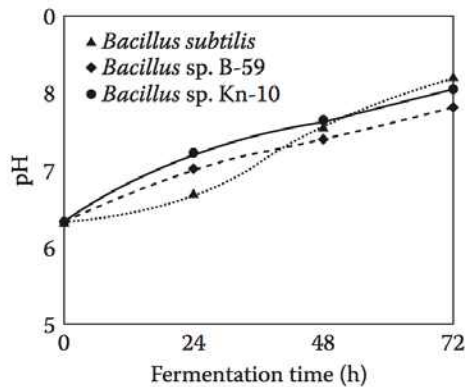


Figure 2.30 Changes in pH during chongkukjang preparation using different strains of *Bacillus* for 72 h at 40°C. (From Baek, L.M. et al., *Korean Journal of Food Science and Technology*, 40, 400–405, 2008.)

pH increase in chongkukjang. The pH values of chongkukjang are in the same range as reported by others (Baek et al., 2008).

The maximum proteolytic activity reaches after 12–24 h, and it remains fairly constant during the remaining period of fermentation. Degradation of carbohydrate to low-molecular-weight compounds also occurs due to the fermentation. The major metabolic activity of *B. subtilis* is the proteolysis of proteins and utilization of released amino acids, and thus, ammonia is produced. It is responsible for the pH increase and the unpleasant strong ammonia flavor. The production of ammonia is a consequence of the utilization of amino acids by the bacteria as sources of carbon and energy. The typical smell can be controlled as regulating ammonia production by bacteria (Jeong et al., 2009).

2.1.1.5.4 Usage and Function *Bacillus subtilis* produces strong proteolytic enzymes, and soybean proteins rapidly undergo partial hydrolysis into peptides and even to amino acids and ammonia during the short fermentation period. This process produces the typical chongkukjang flavor, and a strong meaty and sharp smell. It is used for making chigae, a stew of vegetables, meats, fish, and shellfish, same as doenjang. Chongkukjang chigae is one of the most favorite menus in Korea, especially for the aged group. The amount of chongkukjang added to the stew is large enough to supplement protein to the diet. The strong desire for chongkukjang of elderly Koreans may be not only the sensory nostalgia, but also the physiological demands for their better health.

Recently, scientists have discovered the beneficial physiological functions of some components produced in chongkukjang. The soybean peptides are inhibiting angiotensin converting enzyme (ACE), and thus expected to suppress the incidence of high blood pressure. The mucous substance in chongkukjang is peptido-polysaccharides produced by *B. subtilis* and contains enzyme kinase, which has fibrinolytic and immunomodulating activities. The isoflavonoids in soybean support estrogen hormone function and relieve the malfunction of human menopause (Lee, 2004).

It is not surprising to find that fermented soybean products have been used as medicine in many Asian medicine subscriptions, such as *Dong-eui-bo-gam*, a classic of eastern medicine written by Hur Jun in 1611. Recently, the dried forms of the bacterial fermented soybean, either in granules, pills, or powder form, are sold as a healthy food in Korea.

2.1.1.5.5 Safety Aspects Chongkukjang is generally considered as a safe food, which includes sufficient thermal processing for cooking soybean and relatively high temperature (40°C) fermentation. During the production of chongkukjang the content of free amino acids increases, and it has the potential to produce biogenic amines. For biogenic amines to be produced, free amino acids and bacteria containing decarboxylase enzymes need to be present together with suitable temperature and pH. As the microbial spoilage of food may be accompanied by the increased production of decarboxylases, the presence of biogenic amines might serve as a useful indicator of food spoilage. For these reasons, it is important to monitor biogenic amine levels in foods. Biogenic amines in commercial chongkukjang sold in Korea were analyzed, and the results are given in Table 2.8. Currently, histamine is the only biogenic amine for which maximum limits have been set in the European Union and the United States. The U.S. Food and Drug Administration (FDA) considers histamine level of ≥ 500 mg/kg in food to be a danger to health (Shukla et al., 2011). The levels found in the commercial products do not seem to be hazardous for human health.

The possible biological and chemical hazards in chongkukjang manufacturing process are presented in Table 2.9. Ten samples of commercial products were analyzed to detect pathogenic bacteria (*Escherichia coli*, *Bacillus cereus*, *Salmonella* spp., *Staphylococcus aureus*, *Yersinia enterocolitica*, and *Listeria monocytogenes*) and aflatoxin B₁ (Lee, 2010). Potential

Table 2.8 Levels of Biogenic Amines in Commercial Chongkukjang in Korea

SAMPLE	BIOGENIC AMINE (mg/kg)												
	PUT	CAD	SPD	SPM	TRY	HIS	TYR	PHE	SER	NOR	DOP		
1	8.3 ± 1.2	4.7 ± 0.3	54.3 ± 5.7	9.4 ± 101	76.9 ± 15.2	3.6 ± 0.9	483.1 ± 28.4	32.6 ± 5.4	8.7 ± 2.7	ND	1.5 ± 0.2		
2	121.3 ± 8.4	14.6 ± 2.9	44.5 ± 8.9	13.5 ± 5.2	19.2 ± 1.9	54.3 ± 2.7	164.9 ± 23.4	12.2 ± 3.3	5.2 ± 0.9	1.4 ± 0.2	2.5 ± 0.6		
3	8.0 ± 1.2	9.3 ± 4.8	67.7 ± 9.2	22.8 ± 4.4	31.1 ± 6.3	0.4 ± 0.1	92.7 ± 18.5	17.1 ± 1.9	8.9 ± 1.0	ND	1.5 ± 0.2		
4	4.8 ± 0.9	4.6 ± 0.5	65.9 ± 11.7	23.1 ± 5.4	22.4 ± 3.8	0.2 ± 0.1	5.9 ± 1.5	8.4 ± 1.1	9.8 ± 2.8	2.2 ± 0.4	16.5 ± 3.4		
5	4.7 ± 1.1	5.3 ± 1.9	57.5 ± 3.7	12.5 ± 2.4	18.0 ± 5.1	1.3 ± 0.1	4.2 ± 0.9	6.4 ± 0.1	5.7 ± 1.9	ND	6.2 ± 1.3		
6	112.4 ± 9.2	9.6 ± 2.2	60.7 ± 3.6	22.4 ± 5.6	6.7 ± 0.9	1.6 ± 0.2	354.2 ± 18.9	0.3 ± 0.1	10.1 ± 2.7	1.9 ± 0.5	0.8 ± 0.2		
7	21.9 ± 4.2	13.3 ± 0.9	39.6 ± 7.6	8.4 ± 0.7	30.8 ± 7.4	5.7 ± 0.5	22.7 ± 6.4	26.3 ± 4.4	7.4 ± 2.2	1.2 ± 0.1	2.0 ± 0.1		
8	78.8 ± 6.9	7.0 ± 0.4	40.4 ± 7.5	14.2 ± 1.5	17.6 ± 4.9	38.2 ± 8.7	153.1 ± 16.4	ND	3.2 ± 0.6	0.4 ± 0.1	1.2 ± 0.4		
9	148.5 ± 0.7	70.6 ± 4.4	33.4 ± 0.8	15.7 ± 6.8	66.3 ± 9.7	70.3 ± 12.6	457.4 ± 15.2	23.8 ± 9.2	11.3 ± 0.6	ND	11.3 ± 1.3		
10	5.4 ± 1.2	8.9 ± 1.8	57.4 ± 4.9	7.2 ± 1.9	16.8 ± 0.5	1.0 ± 0.1	105.0 ± 3.4	9.0 ± 0.2	2.7 ± 0.5	0.4 ± 0.1	ND		

Source: Han, G.H. et al., *Korean Journal of Food Science and Technology*, 39, 541–545, 2007.

Note: PUT, putrescine; CAD, cadaverine; SPD, spermidine; SPM, spermine; TRY, tryptamine; HIS, histamine; TYR, tyramine; PHE, 2-phenylethylamine; SER, serotonin; NOR, noradrenaline; DOP, dopamine; ND, not detected.

Table 2.9 Possible Hazard Components in Chongkukjang

COMPONENT	HAZARD
Biological hazard	Pathogenic bacteria (<i>Escherichia coli</i> , <i>Bacillus cereus</i> , <i>Bacillus anthracis</i> , etc.), yeasts, molds
Chemical hazard	Aflatoxin, Pesticide residue
Physical hazard	Extraneous material (weed, seed, stem, piece of wood, straw, etc.)

Source: Lee, S.O., A study on the hazard analysis and HACCP system for Chongkookjang manufacturing, M.Sc. thesis, Hoseo University, Asan, Korea, 2010.

hazards associated with raw materials, manufacturing process, and work environment were also analyzed to establish HACCP model. The results showed that the pathogenic bacteria, except for *B. cereus*, *S. aureus*, and aflatoxin B₁, were not detected in the samples. Both pathogenic bacteria and aflatoxin B₁ were not detected in raw materials, manufacturing facility, and work environment; however, general bacteria levels were highly detected. Therefore, three CCPs (boiling, sterilization, and metal detection) were selected to secure the safety. As chongkukjang is mainly eaten boiled rather than raw in Korea, it is likely free of microbial hazards.

2.1.1.6 *Thua Nao*

Ekachai Chukeatirote

Thua nao (in Thai, “thua” means soybean and “nao” means spoiled) is an ethnic, fermented soy product of Thailand. In general, thua nao has been produced on a small-scale basis using traditional method widely practiced among local communities of Northern Thailand (i.e., Chiang Rai, Chiang Mai, and Mae Hong Son). This product, exhibiting a distinct smell of ammonia, can be eaten directly or used as an important ingredient in various local dishes. Thua nao (Figure 2.31a)

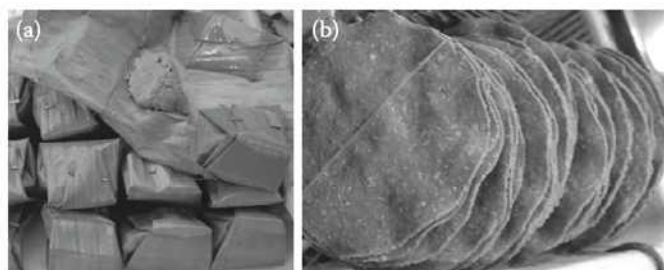


Figure 2.31 Appearance of roasted thua nao covered in banana leaves (a) and its dried form, called thua nao kab (b), as marketed in Chiang Rai. (Courtesy of Pichitchai Nantararat.)

and *Staphylococcus wurneri* were found during fermentation (Okuzumi and Fujii, 2000). Whereas *Micrococcus* sp. was responsible for the ripening, halophilic *Vibrio* spp. and *Achromobacter* spp. were responsible for spoilage (Zenitani, 1955). Many food poisoning and putrefying bacteria are unable to grow in shiokara due to its high salt content. For example, *Vibrio parahaemolyticus*, the halophilic bacterium grows well in the conditions of 2%–5% salt, but cannot grow if salt concentration is more than 10%. Of food poisoning bacteria, many staphylococci are present in shiokara but *S. aureus* is not found at all. In traditional shiokara, a salt concentration of 10% or more prevents putrefaction, and the functions of autolytic enzymes proceed to digest the raw material. At the same time, its characteristic flavor is created. However, because the growth of putrefying bacteria is not inhibited in low-salt shiokara, it cannot be laid in for a long period and umami cannot be produced by ripening. Therefore, the product has to be flavored by seasonings and its preservability has to be maintained by means other than adding salt. To this end, it is treated with a combination of low-temperature storage, adjustment of pH and water activity, improved preservability through the use of additives, and so on. The final products are closer to dressed dishes than fermented food (Okuzumi and Fujii, 2000).

3.3 Hongeohoe

Ggot-Im Lee and Cherl-Ho Lee

Hongeohoe is a fermented raw skate that is traditionally eaten in the southern area of South Korea. Hongoeo is a generic name of skate in Korea. Skates are cartilaginous fish belonging to the family Rajidae in the superorder Batoidea of rays. Skates live in oceans all over the world from fresh water to the depth of 3000 m. The history of hongeohoe is traced back to the 14th century in Korea. A few documents of the late Koryo Dynasty recorded that the residents of Heuk-San Do, an island located far away in the southeastern part of Korea moved into the mainland (a town named Naju) due to the frequent foreign invasion. They, however, kept on going out fishing to the sea near Heuk-San Do because they knew very well about the currents of the sea and had lots of information of fisheries of that region. Heuk-San Do was so far away that it took several days for them to sail back home, and this caused hongoeo to be fermented on board. They learned

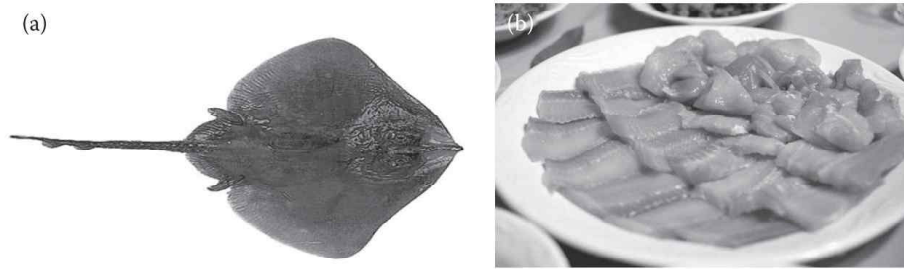


Figure 3.2 Skate (hongeο) (a) and hongeοhoe (b).

that eating fermented hongeο not only did no harm to their stomachs even in hot summer but also its taste was even better than fresh one. Fermented hongeο (called hongeοhoe) has usually been served uncooked (without any further preparation) with other side dishes like kimchi or rice wine, called makgeolli (Figure 3.2). Hongeοhoe is a widely loved food and especially essential for the banquets of wedding and mourning nowadays.

3.3.1 Manufacturing Process of Hongeοhoe

The traditional making of hongeοhoe differs depending on the climate (Figure 3.3). It is not easy to have hongeο fermented because of extreme cold weather in winter. Therefore, the people keep hongeο in a

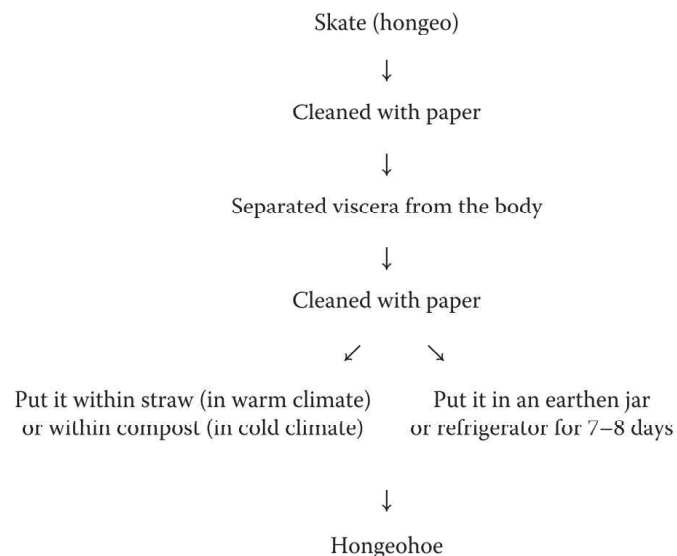


Figure 3.3 Manufacturing process of hongeοhoe.

compost pile to make it fermented. They, on the other hand, make hongeohoe fermented in a stack of straws, which maintains warmth. However, for the sake of safety control and constant quality, refrigerators or low-temperature chambers are presently used for the fermentation process.

3.3.2 Chemical Composition and Physiological Function

Proximate composition of hongeohoe according to their fermentation temperature (4°C, 10°C, and 20°C) and fermentation period was analyzed (Choi et al., 2003). The crude protein, crude lipid, and crude ash content using freeze-dried samples were 86%–88%, 2.3%–2.7%, and 9.6%–11.3%, respectively, when it was fermented at 20°C for 6 days, and they did not show any big difference between fermentation period (days). The protein and lipid contents did not show significant change in proximate composition according to fermentation temperature and period. However, ash content was increased from 9.6% up to 11.3% over time, resulting from the loss of extracts (Table 3.1). These results are consistent with those from skate skin. The moisture, protein, lipid, and ash content of fresh and fermented skate skin were studied (Cho et al., 2004). Fresh and fermented skate skin contained moisture content of each 76.0% and 74.5%, protein content of 22.7% and 21.8%, lipid content of 0.5% and 0.7%, and ash content of 0.6% and 0.9%, respectively. The predominant minerals were potassium and phosphorus (53.5 and 33.0 mg/100 g in fresh skin and 10.46 and 10.5 mg/100 g fermented skin, respectively). Amino acid concentrations were lower in the fermented skin compared with the fresh skin. Histidine, glycine, alanine, and glutamic acid were the major free amino acids in both skins. Palmitic acid was the major fatty acid in both fresh (16.68%) and fermented (20.38%) skate skin.

Table 3.1 Changes in Proximate Composition during Fermentation of Hongeohoe

PARAMETER	BEFORE FERMENTATION	AFTER FERMENTATION (AT 20°C FOR 6 DAYS)
Crude protein (g/100 g)	88.09 ± 2.68	86.04 ± 6.11
Crude lipid (g/100 g)	2.29 ± 0.03	2.72 ± 1.06
Crude ash (g/100 g)	9.55 ± 0.24	11.31 ± 0.80
pH	6.30	8.93

Source: Choi, M.R. et al., *Korean Journal of Life Science* 13: 675–683, 2003.

Note: Values are mean ± SD.

Hongeo has plenty of functional peptides that affect antihypertension (Lim, 2003). Antimicrobial property of the peptide, kenojeinin I, has been proven against *Bacillus subtilis*, *Escherichia coli*, and *Saccharomyces cerevisiae* (Cho et al., 2005). The increased taurine content during fermentation was known to prevent the accumulation of cholesterol. Liver oil, especially, contains a large amount of ω -3 fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which reduce low-density lipoproteins (LDL)-cholesterol and increase high-density lipoproteins (HDL)-cholesterol (Nam and Lee, 1999). Furthermore, anticancer and antibacterial activities increased with the progress of fermentation (Choi et al., 2003).

3.3.3 Microbial and Chemical Changes

Microbial and chemical properties of hongeo change significantly during fermentation. Especially, hongeo, which has finished fermentation, has a strong ammoniacal odor. The unique taste and odor of hongeo come from the nitrogenous compounds such as ammonia, urea, and trimethylamine, which are essential for osmoregulation in the deep sea. Ammonium concentration of fermented skate collected from Korean market ranges from 4.4 to 14.1 mg/g (Lee et al., 2008). Another study showed a similar result that ammonia concentration in skate extracting solutions was not detected before fermentation, but increased to 14.2 mg/ml on day 8 at 10°C (Choi et al., 2003). Due to the ammonia, the pH value of hongeo, which is less than 7 before fermentation goes up to 9.4 after fermentation, which shows that hongeo is one of the AFFs (Lee et al., 2008). The ammonia that is produced during the fermentation process neutralizes the stomach acid and inhibits bacterial growth (Cho and Kim, 2008). The major microflora in the both fresh and fermented skin includes *Photobacterium* sp. (2.4×10^4 cfu/g) and *Vibrio* sp. (7.7×10^7 cfu/g). These bacteria inhabit on the skate skin are known to promote fermentation (Cho et al., 2004).

3.3.4 Safety Aspects and HACCP

The research on safety aspects including a risk factor was carried as an R&D project under Korea Food and Drug Administration in

2006 (Kim, 2006). From the fields such as manufacturing process, on-site supervision, and shelf-life study to laboratory tests, all different aspects using 17 fermented skate samples were tested. *Escherichia coli* and *Staphylococcus aureus* were detected in one and two samples, respectively, out of 17 samples. It occurred in the sample contaminated with *S. aureus* when the catering cook used the same gloves in various jobs. To examine shelf life, samples were stored at 4°C and 10°C, and then changes of total plate counts, volatile basic nitrogen (VBN), pH, and ammonia concentration were examined. The pH ranged from 6.72 to 9.43, and VBN values were significantly different between samples (12.61–621.91 mg/100 g). The ammonia concentration was 5.2–14.8 mg/g. During the storage period, VBN values showed severe changes depending on the temperature. The VBN value increased to 500 mg/100 g at 10°C after 7 days of fermentation, but it increased to 200 mg/100 g at 4°C during the same period. Most of all, storage temperature plays a key role in above the safety factor, and thus it is suggested to be kept below 4°C.

3.3.5 Conclusion

Critical control point (CCP) at which controls can be applied might exist in the storage and distribution process of fermented skates. As hongeohoe is eaten raw without heating process, pathogens can be a major control point in the defrosting, washing, caring for fish offal, fermentation process (container and temperature), packaging, and storage temperature. However, as long as hongeohoe is properly fermented, it is generally as safe as other AFFs.

3.4 Pidan

Sootawat Benjakul and Palanivel Ganesan

Pidan, or “century egg,” also known as “alkaline egg,” “preserved egg,” “hundred-year egg,” and “thousand-year-old egg,” is a Chinese cuisine ingredient made by preserving duck, chicken, or quail eggs in a mixture of clay, ash, salt, lime, and rice straw for several weeks to several months depending on the method of processing (Wang and Fung, 1996). The formation of pidan is mainly caused by the penetration of alkali through the egg shell and membrane, leading to several

6

SAFETY ASPECTS OF ALKALINE- FERMENTED FOODS

Ggot-Im Lee and Cheryl-Ho Lee

6.1 Alkaline-Fermented Foods Are Generally Safe

Alkaline-fermented foods (AFFs) are less known and studied than alcohol fermentation and acid fermentation even though they are widely consumed in various regions of the world. These foods have been good diet sources for many people who have difficulty in accessing to enough food and proper storage facilities.

AFF refers to the food containing alkaline substances that are formed during the fermentation process. For instance, fermented legume seeds and fish, which contain abundant amount of protein, are AFFs. Korean chongkukjang and doenjang, Japanese natto, Chinese douchi, Thai thua nao, and Nepalese and Indian kinema, which are all made from soybean, belong to this class of fermented foods. Nigerian dawadawa, Ivory Coast soumbara (alternatively soubala), and Beninese iru, sonru, and afitin are made from locust bean (*Parkia biglobosa* (Jacq.) G.Don), and they are also AFFs. Other AFFs include Nigerian ogiri made by fermenting melon (*Citrullus lanatis* (Thunb.) Matsum. & Nakai) seeds, ogiri-ugbo from castor bean (*Ricinus communis* L.) seeds, ogiri-nwan from fluted pumpkin (*Telfairia occidentalis* Hook.f.) seeds, and ugba made from African oil bean (*Pentaclethra macrophylla* Benth.), Burkina Faso maari from baobab (*Adansonia digitata* L.) seeds, and Sierra Leone ogiri-saro from the sesame seed (*Sesamum indicum* L.). A typical alkaline fermentation using fish is Korean hongeohe made from flat fish, the skate (Steinkraus, 1997; Lee et al., 2008).

The essential and main microorganisms responsible for making AFFs are *Bacillus subtilis* and related bacilli. The organisms are

highly proteolytic, so that the proteins are hydrolyzed to peptides and amino acids. For example, the concentration of free amino acids of dawadawa indicates around 2.4 mg/g before fermentation; however, it surprisingly goes up to around 84.3 mg/g after 72 h of fermentation (Omafuybe et al., 2000). Ammonia concentration of thua nao is 0.5 g/kg before fermentation, but it increases to 5.0 g/kg at 72 h of fermentation (Leejeerajumnean, 2000). The released ammonia triggers the rapid pH increase of as high as 8.0 or higher. This makes the products keep quite stable and well-preserved even though they are often manufactured in an unhygienic environment.

These foods contain large amounts of ammonia and amino acids, which are formed by the strong proteolytic activity of the indigenous enzymes and microorganisms. The released ammonia causes a strong smell and contributes to the typical taste of the AFF. Unlike other fermented foods, such as acid and alcoholic fermented foods that contain strong antimicrobial substances formed during the fermentation, AFFs do not have enough protective agents against harmful microorganisms. Furthermore, alkaline fermentation of protein-rich substrates can produce biogenic amines and other hazardous compounds. However, well-made traditional AFFs are safe and have been widely consumed, especially in Asia and Africa, as condiments and favorite traditional foods.

A number of researches support that AFFs are safe, although they are usually produced and consumed in developing countries where hygienic measures are not properly practiced. It indicates that AFFs use their own protective systems during the fermentation process. Several mechanisms of how they work will be explained below.

6.1.1 Initial Thermal Treatment

Most of the AFFs are made from beans and seeds, which have hard seed coats. Therefore, raw materials first need to be soaked in water and boiled for a long time so that the seed coat can be softened before fermentation. This process may take more than 12 h depending on the condition and the kind of the material. Soaking and boiling steps prior to the fermentation help removing the anti-nutritional factors of plant materials such as trypsin inhibitor, hemagglutinin, phytic acid, and also other harmful microorganisms (Khokhar and Chauhan,

1986; Egounlety and Aworh, 2003). For instance, locust beans contain highly toxic substances such as oxalic acid, phytic acid, and cyanhydric acid. However, they disappear during the fermentation process (Parkouda et al., 2009).

The boiling process is quite important for setting up a good environment for fermentation. It makes the fermentation environment favorable to useful microorganisms while keeping biological hazards away from the whole fermentation step. Most of non-spore-forming pathogenic bacteria are killed during the boiling process, although some spore-forming bacteria, mainly *Bacillus* spp., survive. Table 6.1 shows an example of bacterial occurrence through all steps of iru production. Most microorganisms, except *Staphylococcus epidermidis*, are present in the raw locust beans. However, pathogenic microorganisms, except *Fusarium* sp., are all gone after initial boiling step (Olayinka and Omobayonle, 2006). After dehulling with the boiled beans, potential pathogens, except *Clostridium* sp., disappear during the second boiling step. In the case of hongoehoe, which does not have a cooking process, raw fish is mixed with ash or buried in the ash pile, by which alkaline environment is made at the initial stage of fermentation process (Kim, 2006). The pH reaches the degree of up to 9.4 when the fermentation is completed (Lee et al., 2008).

6.1.2 Growth of *Bacillus subtilis*

The surviving microorganisms after cooking, mainly *Bacillus* spp., grow rapidly in the system because of their thermophilic nature, of which the optimal growth temperature is around 40°C, and become the dominant flora. Among the *Bacillus* spp., *B. subtilis* is known to be the most dominant organism for AFFs such as chongkukjang, natto, kinema, soumbara, dawadawa, iru, and sonru (Sarkar et al., 2002; Azokpota et al., 2006). *Bacillus subtilis* grows significantly during the fermentation process (Sarkar and Tamang, 1995; Parkouda et al., 2010).

Several reports demonstrated the ability of *B. subtilis* to produce metabolites that inhibit the growth of pathogenic bacteria (N'dir et al., 1994; Guo et al., 2006). During kinema production, *B. subtilis* appeared to have the ability to reduce the growth of *Bacillus cereus*

Table 6.1 Distribution of Isolated Microorganisms in Samples Collected at Different Stages during Traditional Processing of Locust Beans to Iru

ISOLATE	WHOLE BEANS		COTYLEDONS					COTYLEDONS FERMENTED AT DIFFERENT TIME PERIOD				PRODUCT (IRU)
	RAW	BOILED	FRESHLY DEHULLED	WASHED	BOILED	DRAINED	12 h	24 h	36 h			
<i>Bacillus subtilis</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>Bacillus pumilus</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>Bacillus licheniformis</i>	+	-	-	+	+	+	+	+	+	+	+	+
<i>Clostridium</i> sp.	+	-	-	+	+	+	-	-	-	-	-	+
<i>Micrococcus</i> sp.	+	-	+	+	-	-	-	-	-	-	-	+
<i>Staphylococcus aureus</i>	+	-	+	+	-	-	-	-	-	-	-	+
<i>Staphylococcus epidermidis</i>	-	-	+	+	-	-	-	-	-	-	-	+
<i>Aspergillus niger</i>	+	-	+	+	-	+	-	-	-	-	-	-
<i>Saccharomyces saccharolyticus</i>	+	-	+	-	-	+	+	-	-	-	-	-
<i>Fusarium</i> sp.	+	+	+	+	-	+	-	-	-	-	-	-
<i>Neurospora</i> sp.	+	-	-	+	-	-	-	-	-	-	-	-
<i>Escherichia coli</i>	+	-	+	+	-	+	-	-	-	-	-	-
<i>Lactobacillus amylovorus</i>	+	-	+	+	-	+	+	-	-	+	+	+
<i>Leuconostoc mesenteroides</i>	+	+	+	+	-	+	+	+	+	+	+	+
<i>Rhizopus oligosporus</i>	+	+	+	+	-	+	-	-	-	-	-	+

Source: Olayinka, E.M., and Omobayonle, F., *Research Journal of Microbiology*, 1, 503–511, 2006.

and to prevent it from producing enterotoxin (Nout et al., 1998). Therefore, the strains of *B. subtilis* have been used as a good starter for the controlled production of natto and kinema (Sarkar et al., 1993; Owens et al., 1997).

Table 6.2 shows that *B. subtilis* and *Bacillus pumilus*, isolated from soumbala, inhibit both Gram-positive and Gram-negative bacteria including *Micrococcus luteus*, *Staphylococcus aureus*, *B. cereus*, *Enterococcus faecium*, *Listeria monocytogenes*, *Escherichia coli*, *Salmonella typhimurium*, *Shigella dysenteriae*, *Yersinia enterocolitica*, as well as ochratoxigenic molds such as *Aspergillus ochraceus* (Ouoba et al., 2007).

The *Bacillus* species not only have an antibiotic effect, especially *B. subtilis*, but they also produce strong proteolytic enzymes that hydrolyze the protein in the beans and seeds into peptides and amino acids and finally into ammonia. The combination of high pH and high concentration of free ammonia, along with rapid growth of *B. subtilis* in the high temperature condition (above 40°C), makes it very difficult for other pathogenic microorganisms to grow (Steinkraus, 1997).

Table 6.2 Inhibition of Pathogenic Bacteria after 24 h by *Bacillus* spp. Isolated from Soumbala

TARGET BACTERIUM	<i>B. subtilis</i> STRAIN			<i>B. pumilus</i> STRAIN	
	B7	B9	B15	B6	B10
<i>Micrococcus luteus</i> A1	++	+	+	+	+
<i>Bacillus cereus</i> A4	+	–	+	–	–
<i>Bacillus cereus</i> A10	++	++	+	++	++
<i>Staphylococcus aureus</i> A11	–	–	+	+	+
<i>Staphylococcus aureus</i> A12	++(p)	–	+++	+	+
<i>Enterococcus faecium</i> A19	–	–	–	+	+
<i>Listeria monocytogenes</i> A18	++	+	+	+	+
<i>Escherichia coli</i> A13	++	–	+(p)	–	++(p)
<i>Salmonella enteritidis</i> A14	+	–	+	–	–
<i>Salmonella typhimurium</i> A15	++	–	+	–	+
<i>Shigella flexneri</i> A16	+	–	–	–	–
<i>Shigella dysenteriae</i> A17	++	–	+	–	++
<i>Yersinia enterocolitica</i> A21	++(p)	–	++	–	+

Source: Ouoba, L.I.I. et al., *Journal of Applied Microbiology*, 102, 963–970, 2007.

Note: –, No inhibition (<1 mm); +, inhibition 1–<3 mm; ++, inhibition 3–5 mm; +++, inhibition ≥5 mm; p, partial (inhibition zone not totally clear).

404 FOODS INVOLVING ALKALINE FERMENTATION

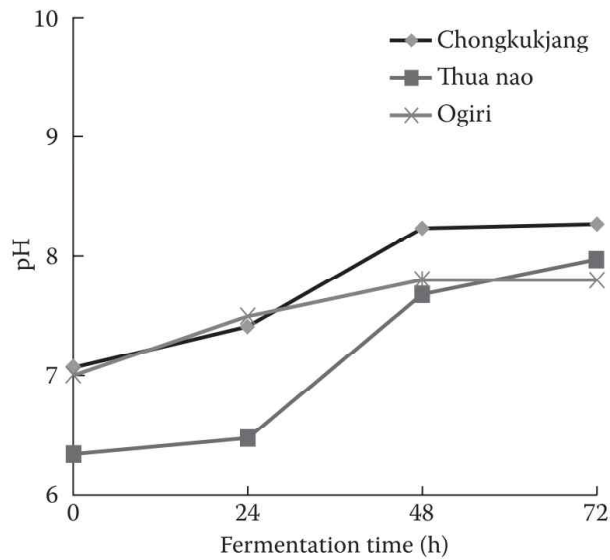


Figure 6.1 The pH change of several alkaline-fermented foods. (From Odufa, S.A., *Journal of Food Science*, 50, 1758–1759, 1985; Chukeatirote, E. et al., *Research Journal of Microbiology*, 1, 38–44, 2006; Ann, Y.G., *Korean Journal of Food and Nutrition*, 24, 124–131, 2011.)

6.1.3 pH Change

The increase in pH during fermentation plays a key role in the safety aspects of AFFs. The pH before fermentation begins at 6.0–7.0, and it reaches 7.5–9.0 at the final stage of fermentation. Most AFFs such as chongkukjang, thua nao, kinema, and ogiri show rapid pH changes until 48 h of fermentation, and then they become stable around 8.0 (Figure 6.1). This is a quite fast and vigorous fermentation that it can

Table 6.3 Approximate pH Tolerance of Some Microorganisms

ORGANISMS	pH TOLERANCE	
	MINIMUM	MAXIMUM
<i>Escherichia coli</i>	4.4	9.0
<i>Salmonella typhi</i>	4.5	8.0
<i>Campylobacter jejuni</i>	2.3 and 5.8	–
<i>Shigella</i> sp.	4.5	8.0
<i>Streptococcus lactis</i>	4.3–4.8	–
<i>Lactobacillus</i> sp.	3.0	7.2
Yeasts	1.5	8.0–8.5
Molds	1.5–2.0	11.0

Source: Blaser, M.J. et al., *Journal of Clinical Microbiology*, 11, 309–313, 1980; Gill, C.O., and Harris, L.M., *Applied and Environmental Microbiology*, 44, 259–263, 1982; Jay, J.M., *Modern Food Microbiology*, 3rd ed., Van Nostrand Reinhold, New York, 1986.

be completed in 2–3 days (Odunfa, 1985; Chukeatirote et al., 2006; Ann, 2011).

Many of the pathogenic and spoilage microorganisms are inhibited by the higher pH over 8.0. Table 6.3 shows that *Salmonella typhi* and *Shigella* sp. cannot grow at the degree of pH over 8.0 and *E. coli* at over 9.0 (Mensah, 1997).

6.1.4 Cooking Process

Most of the AFFs are consumed as forms of soups or condiments through the cooking process. Boiling or stewing is normally applied for the preparation of these kinds of meal. Thanks to this heat treatment; the pathogenic microorganisms that possibly contaminated during and after fermentation or storage can be removed. This is how indigenous AFFs have been consumed relatively safely in Asia and Africa.

6.2 Hazards in Alkaline-Fermented Foods

AFFs have beneficial effects and can be considered safe as mentioned above. However, safety issues can be raised, although it appears to be not only the problem of AFFs but also that of all types of traditional fermented foods. The hazards occurring in AFFs can be divided into two groups, namely microbiological and chemical. The possible microbiological hazards include *B. cereus* contamination and mycotoxin in raw materials. The chemical hazards include the formation of biogenic amines during the fermentation and contamination of pesticides in raw materials. These safety issues are discussed in more detail below.

6.2.1 Microbiological Hazards

6.2.1.1 Bacillus cereus Although the initial step of alkaline fermentation includes a long cooking process, some hazardous spore-forming bacteria like *B. cereus* can survive if the raw material is heavily contaminated with these bacteria. It has been reported that *B. cereus* exists in various AFFs like iru, ogiri (10^{6-8} cfu/g; Oguntoyinbo and Oni, 2004), and chongkukjang (10^{5-6} cfu/g; Oguntoyinbo and Oni,

2004; Lee, 2010). Pathogens found in fermented foods mainly come from the raw materials, handlers, or poor kitchen utensils. This bacterium is known to produce emetic toxin (cereulide) and enterotoxins that cause vomiting and diarrhea (Granum, 2001; Ehling-Schulz et al., 2004; Ouoba et al., 2008). Therefore, it is important to avoid cereulide production in foods because cereulide, unlike enterotoxins, is heat-stable in a wide range of pH (2–11), and is not degraded by the digestive enzymes like pepsin and trypsin (Shinagawa et al., 1995, 1996). However, it was reported that cereulide production of an emetic *B. cereus* isolated from sonru appeared to be dependent on the fermentation temperature as being strongly inhibited at 40°C (Parkouda et al., 2009).

Bacillus subtilis has been regarded as a dominant microorganism for ugba production. However, *B. cereus*, rather than *B. subtilis*, has been recently reported to be the dominant strain in the production of this food (Sanni et al., 2002; Ahaotu et al., 2013). All the *B. cereus* strains isolated from ugba-expressed enterotoxin genes, but no genes encoding cereulide were detected (Ahaotu et al., 2013). Consumption of foods contaminated with *B. cereus* does not always cause diarrhea or vomiting. However, it should be well cooked for a suitable period of time to prevent the risk of enterotoxins from ugba. In addition, an investigation regarding toxin production by *Bacillus* spp., other than *B. cereus*, noticed that some strains of *B. subtilis*, *B. pumilus*, and *Bacillus fusiformis* can produce either cytotoxins or ring-formed emetic toxins. Therefore, special care is also required to select strains for starter culture even though *Bacillus* spp. related to AFFs does not show pathogenic activities (From et al., 2005).

6.2.1.2 Other Potential Pathogens Apart from *B. cereus*, the presence of other potential pathogenic microorganisms like *Staphylococcus* spp., *E. coli*, *Salmonella* spp., *Shigella* spp., *Clostridium* spp., and *Staphylococcus* spp. were found in iru and chongkukjang (Azokpota et al., 2006; Lee, 2010). *Bacillus cereus*, Enterobacteriaceae, and coliform bacteria were detected over 10⁴ cfu/g in kinema. *Salmonella* was detected in papad, legume-based popular fermented foods in India (Roy et al., 2007). It was reported that *Staphylococcus sciuri* involves in the fermentation of baobab seeds to produce maari (Parkouda et al., 2010). *Staphylococcus sciuri* is well known as a highly pathogenic bacterial

species responsible for endocarditis, peritonitis, septic shock, urinary tract infection, endophthalmitis, pelvic inflammatory disease, and most wound infections (Dakić et al., 2005). Recently, people are more interested in the capacity of this species to carry antimicrobial resistance determinants. Further investigation is required to illuminate the exact role of pathogenic microorganisms mentioned above, because the presence of microorganisms itself does not mean expression of toxin substances. AFFs, especially, have several inhibitory systems to suppress gene-encoding toxins.

6.2.1.3 Mycotoxins Mycotoxins are toxic secondary metabolites produced by fungal contamination, which is a very hazardous factor causing diseases including cancer. Most of the AFFs are made in warm and humid areas such as Africa and Asia using seeds and beans, which are high-protein foods. These conditions may cause molds to grow quickly and amplify the chance of mold contamination, and the handling and storage of fermented foods accordingly should be processed with care. Contamination of mold on grains during storage and transportation is liable to be migrated rapidly to another country due to the increasing world trade. For this reason, many international agencies are trying to set up universal standard limits for mycotoxins.

There are two possible sources of mycotoxin contamination: (1) toxin from the contaminated cereals and beans themselves and (2) toxin produced by the mold contaminated during fermentation (Lee and Lee, 2002). Table 6.4 summarizes mycotoxins, origins of fungi, and related symptoms (Bullerman, 1986).

Aflatoxins are one of the representative mycotoxins produced by *Aspergillus flavus* and *Aspergillus parasiticus*, and they have four types called B₁, B₂, G₁, and G₂. *Aspergillus flavus* is the most common contaminant in agricultural products. *Fusarium*, *Alternaria*, and *Acremonium* species are typical parasites that exist on roots and stems of plants and produce zearalenone, alternaria, and tremorgenic toxins (Betina et al., 1984).

The traditional fermentation starters used in Asia and Africa have a high risk of mycotoxin contamination because natural fermentation environment of cereals and beans often leaves foods exposed to numerous kinds of molds. *Penicillium* and *Aspergillus* are the major fungi related to the toxin production on various foods, which often

408 FOODS INVOLVING ALKALINE FERMENTATION

Table 6.4 Mycotoxins Produced by Fungi

TOXIN	PRODUCER FUNGI	SYMPTOMS
Aflatoxin	<i>Aspergillus flavus</i> , <i>Aspergillus parasiticus</i> , <i>Aspergillus nomius</i>	Hepatotoxic, hepatocarcinogenic, mutagenic, teratogenic
Zearalenones	<i>Fusarium roseum</i> , Other <i>Fusarium</i> spp.	Estrogenic, infertility in cattle/swine/poultry
Vomitoxin	<i>Fusarium</i> spp.	Weight loss in most livestock, feed refusal, vomiting, immunosuppression
T-2 toxin	Pink <i>Fusarium</i> spp.	Feed refusal, vomiting, nervous system dysfunction, immunosuppression
Trichothecenes	<i>Fusarium</i> spp.	Gastrointestinal tract hemorrhaging, alimentary toxic aleukia, feed rejection, vomiting, immunotoxicity
Fumonisin	<i>Fusarium moniliforme</i>	Esophageal carcinogenic in human (ELEM) in equines, hepatocarcinogenic in rats
Orchatoxins	<i>Aspergillus</i> spp., <i>Penicillium</i> spp.	Nephrotoxic, teratogenic, immunotoxic, suspected carcinogen
Sterigmatocystin	<i>Aspergillus versicolor</i> , Other <i>Aspergillus</i> spp.	Hepatotoxic, hepatocarcinogenic
Citrinin	<i>Aspergillus</i> spp., <i>Penicillium</i> spp.	Nephrotoxic
Rubratoxin	<i>Penicillium</i> spp.	Hepatotoxic

Source: Bullerman, L.B., *Food Technology*, 40, 59–66, 1986.

grow during storage and natural fermentation. *Penicillium glandicola* produces ochratoxin A on cereals containing over 19% moisture, *Penicillium expansum* produces patulin on apples and pear, and *Penicillium roqueforti* produces citrinin on blue cheese (Lee and Lee, 2002). *Aspergillus flavus* and *A. parasiticus* produce aflatoxin B and G on corn, peanut, and rice, and *A. ochraceus* produces ochratoxin. *Aspergillus flavus* can also produce mycotoxin cyclopiazonic acid.

It is an unavoidable problem that AFFs are contaminated with mycotoxin. The aflatoxin was detected at the level of 0.20 µg/g in melon seeds sold in the markets of Nigeria (Ekundayo and Idzi, 1990). Three mold species, *Rhizopus stolonifer*, *Aspergillus fumigatus*, and *Triscelophorus monosporus*, were found in ogiri sold in the local markets (Ijabadeniyi, 2007). A report showed that *R. stolonifer* and other *Rhizopus* isolates have powerful (>95%) detoxification activity of ochratoxin A in 16 days, assuming that *Rhizopus* sp. have a role of suppressing mycotoxins during fermentation (Varga et al., 2005). However, *A. fumigatus* is well known to produce cytotoxic mycotoxin

and gliotoxin in marine conditions and to cause more than 80% of aspergillosis (Gareis and Wernery, 1994). *Triscelophorus monosporus* is known to be related to iru production (Evans et al., 2013).

The incidence of aflatoxin B₁ in ogiri was examined using TLC (thin layer chromatography) in Nigeria in 2005 and 2006. The results showed that 25% of the total samples were contaminated with aflatoxin B₁. The mean values of aflatoxin B₁ indicated 8.9 and 9.0 µg/kg in 2005 and 2006, respectively (Bankole et al., 2010). The range of contamination varied from 2 to 13 µg/kg in 2005 and from 4.1 to 14.3 µg/kg in 2006. Another result reported in 2004 showed a small increase in aflatoxin levels in melon seeds: 14.8 µg/kg in the forest and 11.3 µg/kg in the savanna regions (Bankole et al., 2004). This difference may be due to different processing manuals. None of the samples had more than 20 µg/kg, which is within the Nigerian foods standard, but daily total dose should be considered.

Numerous studies have been carried out to control the mycotoxin contamination in fermented soybean products in Korea. It has been proven that aflatoxins (B₁ and G₁) decreased to 80%–90% after 2 months of fermentation and to 100% after 3 months of fermentation when the target mold was introduced into the meju, a fermented soybean loaf, which is used for doenjang preparation (Park et al., 2003). Indeed, the doenjang samples were collected from various markets to detect aflatoxin levels by ELISA (enzyme-linked immunosorbent assay)/HPLC (high-performance liquid chromatography). The results showed that aflatoxin levels were not detected in any of the samples. Another analysis was conducted using 10 commercial samples of chongkukjang (Lee, 2010). There also aflatoxin could not be detected in any of the samples. Previous report performed in 2004–2005 showed that the aflatoxin B₁ was detected (0.05–0.17 µg/kg) in 13 of 56 samples of doenjang (Chun et al., 2006). They were under the permitted level (10 µg/kg) of Korea Foods Standards (Table 6.5). Although those are within the safe levels, well-controlled manufacturing process and proper management practices (including cultivating and storage technology) are important to minimize contamination of mycotoxin, including aflatoxin and ochratoxin, in every stage of production (Lee et al., 2012). Table 6.5 shows the different standards limits for mycotoxin. According to foods consumed frequently in each country, the food standards for mycotoxins are determined.

410 FOODS INVOLVING ALKALINE FERMENTATION

Table 6.5 Regulation Levels of Mycotoxin Applied in Different Countries ($\mu\text{g}/\text{kg}$)

MYCOTOXINS	CODEX	USA	CANADA	JAPAN	AUSTRALIA AND NEW ZEALAND	EU	KOREA
Aflatoxin B1	—	—	—	10	—	0.1–8	10
Aflatoxins	10–15	20	15	—	15	4–15	15
Aflatoxin M1	0.5	0.5	—	—	—	0.025–0.05	0.5
Ochratoxin A	5	—	—	—	—	0.5–10	5–20
Deoxynivalenol	—	1000	2000	1100	—	200–1750	1000–2000
Zearalenone	—	—	—	—	—	20–400	200–1000
Patulin	50	—	—	50	50	10–50	50
Fumonisin	—	2000–3000	—	—	—	200–4000	1000– 4000
Ergot	—	—	—	—	500,000	—	—

Source: Oh, S.S., Strategy for the advancement of risk evaluation on food contaminants. *National Institute of Food and Drug Safety Evaluation (Korea NIFDS) research report*, 2011.

Several factors can be considered in degrading aflatoxins during fermentation. It was reported that NH_3 produced during the fermentation, light, microbial competitions with *Bacillus* spp., and addition of charcoal or vitamin C contribute to reducing aflatoxins from soybean products. Furthermore, it was found that increasing concentrations of CO_2 or N_2 suppress the formation and growth of aflatoxin producers, and it is because aflatoxin-producing molds are aerobic organisms, requiring O_2 . The most important is to suppress aflatoxin producers during the fermentation process because it is hard to remove aflatoxin even by heating. Therefore, it was suggested that storage temperature should be kept at 0°C – 7.5°C , as optimum temperature for aflatoxin production is 25°C – 30°C . For this reason, food industries in Korea are launching the refrigerated soybean produce system to enhance safety. Current refrigerated soybean paste takes about 16% of the entire brand. It was also reported that this method removes off-flavor and improves taste.

6.2.2 Chemical Hazards: Biogenic Amines

Biogenic amines are biologically active amines that are formed by microbial decarboxylation of amino acids. They are frequently found in fish and fish products, alcohols, dairy products, and fermented foods (BIOHAZ, 2011). Biogenic amines are naturally produced in

the human body and are related to various biological activities such as synaptic transmission, blood pressure control, allergy response, and cellular growth control. Nonetheless, biogenic amines are regarded as toxic substances that cause diseases and food poisoning when their levels in the body reach a critical threshold (Ladero et al., 2010). Common symptoms of intoxication of biogenic amines are headache, sweating, burning nasal secretion, facial flushing, red rashes, nausea, dizziness, itching, diarrhea, blood pressure disorders, neurological disorders, vomiting, and carcinogenic effects (Rice et al., 1976).

Amino acid decarboxylases convert amino acids to amines, and microorganisms that have amino acid decarboxylases are responsible for the formation of biogenic amines in foods (Beutling, 1996). It has been reported that decarboxylase activity is found in some microbial groups: *Bacillus*, *Citrobacter*, *Clostridium*, *Klebsiella*, *Escherichia*, *Proteus*, *Pseudomonas*, *Salmonella*, *Shigella*, *Photobacterium*, *Lactobacillus*, *Pediococcus*, and *Streptococcus* (Rice et al., 1976; Ten Brink et al., 1990). Amino acid decarboxylation takes place by removing a carboxyl group from amino acid, forming the corresponding amine. The important biogenic amines found frequently in food are histamine (2-(1*H*-imidazol-4-yl)ethanamine), tyramine (4-(2-aminoethyl)phenol), putrescine (butane-1,4-diamine), cadaverine (1,5-diaminopentane), and phenylethylamine (2-phenyl-2-ethanamine), which are products of the decarboxylation of histidine, tyrosine, ornithine, lysine, and phenylalanine, respectively. Putrescine can also be formed by the deimination of agmatine (1-(4-aminobutyl)guanidine – decarboxylation product of arginine). The precursors of the main biogenic amines are described in Table 6.6.

Occurrences of biogenic amines have been reported in AFFs such as natto, chongkukjang, and doenjang (Cho et al., 2006; Tsai et al., 2007a, 2007b; Kim et al., 2012). Contents of biogenic amines in different fermented soybean products are listed in Table 6.7.

Shalaby (1996) reported that miso contained high levels of histamine (4620 mg/kg), putrescine (12,340 mg/kg), cadverine (6340 mg/kg), and tyramine (35,680 mg/kg). Cho et al. (2006) reported that the levels of histamine, tyramine, and putrescine in traditional Korean doenjang were as high as 596.4, 669.5, and 462.6 mg/kg, respectively. Another study examined using four traditional doenjang and six commercial doenjang showed similar results that the content of putrescine

412 FOODS INVOLVING ALKALINE FERMENTATION

Table 6.6 Main Biogenic Amines and Their Precursors

COMPOUND NAME	PRECURSOR	STRUCTURE	MOLECULAR WEIGHT
Agmatine			130.2
Tryptamine			160.2
2-Phenylethyl amine			121.2
Putrescine			88.2
Cadaverine			202.2
Histamine			111.0
Tyramine			137.3
Spermidine			145.3
Spermine			348.1

Source: Shukla, S. et al., *Soybean and Health*, ed. H. El-Shemy, pp. 181–206, InTech, Rijeka, Croatia, 2011.

Table 6.7 Contents of Biogenic Amines in Different Fermented Soybean Products (mg/kg)

FOOD	TYRAMINE	TRYPTAMINE	HISTAMINE	PUTRESCINE	CADAVERINE	PHENYLETHYLAMINE	SPERMINE	SPERMIDINE	REFERENCE
Miso (Japanese soybean paste)	35,680	—	4620	12,340	6340	—	—	—	Shalaby (1996)
Doenjang (Korean – traditional type)	669.5	105.5	596.4	462.6	23.5	244.7	3.8	15.6	Cho et al. (2006)
Doenjang (Korean – modern type)	133.0	22.4	83.6	46.4	3.2	6.5	2.4	7.4	
Miso	48.6	22.6	0.9	19.8	3.0	4.4	2.2	15.7	
Chongkukjang	133.8	69.9	10.1	26.4	9.7	22.0	10.7	52.0	
Chongkukjang powder	68.1	35.0	1.0	10.2	12.1	17.0	15.5	54.6	
Natto (Japanese)	1.2	9.1	35.4	17.1	21.9	ND	8.6	45.0	Tsai et al. (2007a)
Natto (Taiwan)	ND	ND	45.1	1.6	0.5	ND	ND	25.0	Kim et al. (2012)
Natto (Japanese)	47.1	19.2	2.6	8.0	10.3	22.8	45.5	339.7	

Source: Modified from Shukla, S. et al., *Soybean and Health*, ed. H. El-Shemy, pp. 181–206, Intech, Rijeka, Croatia, 2011.

Note: ND, not detected.

(29–1077 mg/kg) and tyramine (13–968 mg/kg) exhibited high values compared with those of other biogenic amines (Lee et al., 2009). According to the results of a comparison between traditional doenjang and commercial doenjang, the levels of biogenic amines in commercial doenjang were significantly reduced to 83.6 mg/kg (histamine), 133.0 mg/kg (tyramine), and 46.4 mg/kg (putrescine) (Cho et al., 2006). The differences in the contents of biogenic amines between the traditional doenjang and the commercial doenjang could be attributed to the variation of target starter and fermentation conditions. Tsai et al. (2007b) tested the biogenic amine levels in seven samples of soybean and 19 samples of black bean douchi. The Chinese ferment black beans used for condiments, which are sold in Taiwan. The test found that four of the soybean douchi samples had high histamine levels (563–808 mg/kg). They are greater than 500 mg/kg, a toxic level to humans. Natto showed particularly high levels of spermidine (339.7 mg/kg) among other biogenic amines (Kim et al., 2012).

The variability and high deviation of biogenic amine levels in the same/similar products are characteristic of all kinds of fermented foods. This means that it is not easy to control fermentation environment such as NaCl concentration, temperature, moisture content, additives, microorganisms including starters, and the environment related to the production of biogenic amines. Nonetheless, there is no doubt that it is important to reduce biogenic amine contents in fermented foods. Several studies suggested that addition of garlic and glycine can suppress amino acid decarboxylase activity in fish sauce. For example, the contents of cadaverine and tyramine were reduced by up to 18.4% and 30.9%, respectively, in the culture treated by garlic extract (Mah et al., 2009). The glycine has the greatest inhibitory activities on biogenic amine production. The contents of putrescine, cadaverine, histamine, tyramine, and spermidine were reduced by 32.6%, 78.4%, 93.2%, 100%, and 100%, respectively, compared with control (Mah and Hwang, 2009).

There are different regulatory limits for biogenic amines by food type. The upper limits of histamine and tyramine in foods were set at 100 and 100–800 mg/kg, respectively. The 30 mg/kg of β -phenylethylamine has been reported to be a toxic dose in food. Total biogenic amine levels of 1000 mg/kg in food are considered harmful to human health (Taylor and Eitenmiller, 1986; Ten Brink et al., 1990).

6.3 Control of Hazards

The AFFs in Korea and Japan are highly commercialized, and industrial quality control systems are well established. On the other hand, many of the African AFFs are still produced at the cottage or household level. Contamination of mycotoxin and/or pathogenic microorganisms in raw materials, especially maize, beans, and seeds are often found in this region. This proves that it is needed to control the harvesting and storage processes by applying good agricultural practices (GAP) and hazard analysis and critical control points (HACCP). In the case of African countries that do not have enough facilities, small-scale industrial systems could be an alternative option.

6.3.1 GAP

Numerous regulations, standards, codes, manuals, and practices have been recently made for the purpose of providing safe foods. The objective of GAP is to ensure safety and quality of produce in the food chain, to capture new market advantages by modifying supply chain governance, to improve natural resources use, workers' health and working conditions, and to create new market opportunities for farmers and exporters in developing countries (FAO GAP principles). These principles can be applied not only to local production, harvest, postharvest processes, and storage but also to soil, water, seed, pesticide, and fertilizer. There are potential benefits when GAP is applied to farming systems because it helps reduce chemical, microbiological, and physical contamination hazards. Applying GAP into the African environment may require a lot of business to control. However, HACCP can be implemented with minimal cost and some changes (e.g., techniques, utensils).

6.3.2 HACCP

The hygienic conditions of cottage level production of fermented foods are generally poor in most of the developing countries, and people in these regions are not well-educated to control their processing procedure in hygienic ways. It is critical to equip clean utensils, working place, clean water, and clean rooms to make safe foods. Most of all, it

is important to educate people who are involved in food processing in the developing countries.

The HACCP system has been well-recognized as an approach to eliminate, minimize, or prevent hazards from farm to fork. This control system includes seven principles designed to prevent problems before they occur and to correct deviations as soon as they are found. The critical control points (CCPs) are placed in the flowchart of operational steps, where each practice can be applied to its corresponding steps. Many countries have put effort into developing HACCP models for improving safety and reducing hazards of their fermented foods. The potential hazard lists of fermented products can be summarized as follows: biological hazards (pathogens like *B. cereus* and mycotoxins), chemical hazards (biogenic amines), and physical hazards (foreign substances like grass, seed, metal, string, stick, and wood waste). In the following models, chongkukjang and iru are provided as examples (Figures 6.2 and 6.3).

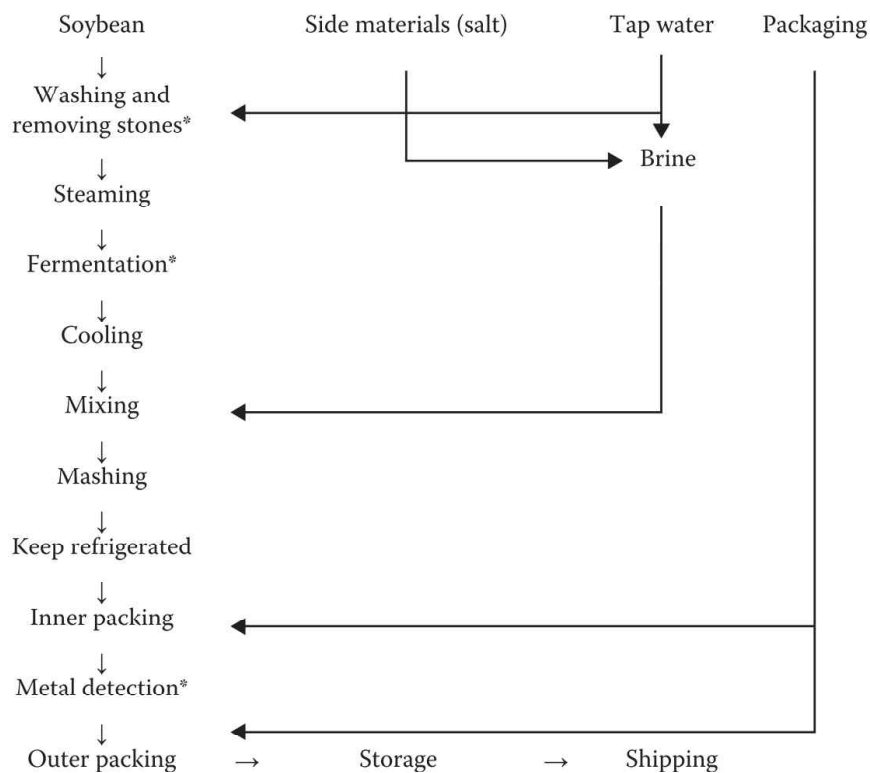


Figure 6.2 Chongkukjang production process marked with CCPs (*). (From Shin, D.H., Research for safety improve and quality improvements of small business and traditional fermented soybean products, *National Institute of Food and Drug Safety Evaluation [Korea NIFDS] research report*, 2011.)

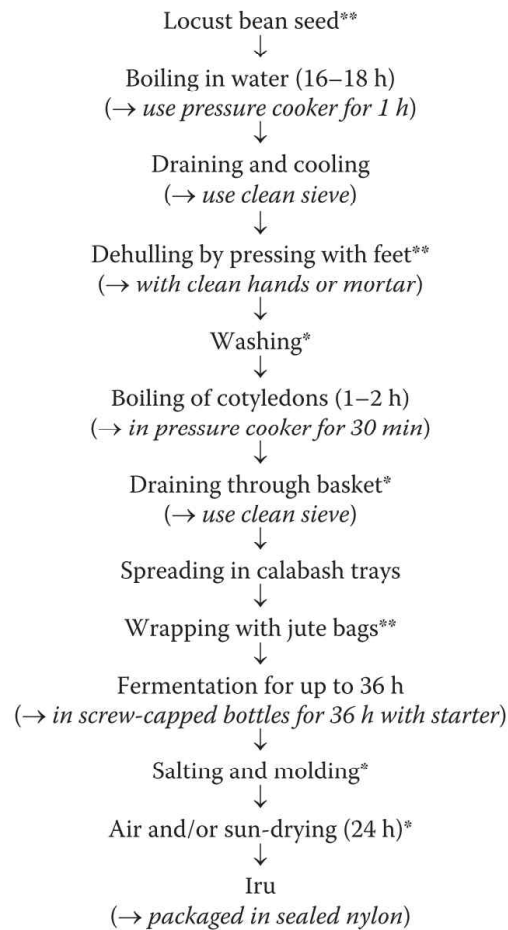


Figure 6.3 Traditional processing of locust beans into iru showing the CCPs (*). **CCP and food safety risk. The *italic* phrase indicates recommendation for safety process. (From Olayinka, E.M., and Omobayonle, F., *Research Journal of Microbiology*, 1, 503–511, 2006.)

According to the chongkukjang HACCP model suggested by Shin (2011), three CCPs were noticed in their procedures (Figure 6.2). First, he suggests that foreign substances like stones, string, and insects should be removed in the steps of washing and removing stones, with keeping washing speed of 6 rpm. Second, in the fermentation step, proper time (44 ± 1 h) is required to avoid the possibility of contamination with *B. cereus* ($<10,000$ cfu/g). Lastly, he indicates the second metal detection step just before packaging as another critical control point.

The traditional steps of iru were examined to identify CCPs and then optimize the process (Olayinka and Omobayonle, 2006). The CCPs they identified were the process of including locust beans, dehulling by pressing with feet, and wrapping in the bag (Figure 6.3).

To reduce the chance of contamination, a pressure cooker, dehulling with hands or mortar, and defined culture with known starter were applied. It is evident that these simple changes can reduce microbial contamination and improve nutritional values. As for starters, the mixed culture of *Bacillus* sp. and lactic acid bacteria is recommended. Several studies have found that GAP, GMP (good manufacturing practices), and HACCP are effective as a quality management system for assuring the safety of traditional local foods in Africa (Amoa-Awua et al., 2007).

HACCP systems introduced above are one of the models to verify safety of traditional fermented foods. To ensure both validity and security of the HACCP system, other food safety assurance systems like GAP, GMP, GHP (good hygiene practices), and ISO (International Organization for Standardization) standards can be applied in the food supply chain. These certifications should be encouraged and supported by government authorities. Personal training in terms of sanitation principles and food safety control is definitely required. In addition, universal standards like Codex are required for the global trade of traditional foods because more and more countries are signing FTAs (free trade agreements). Having Codex standards for more traditional foods will facilitate exports since the standards have been applied primarily in international trade and commerce.

6.4 Conclusion

AFFs are very popular in Africa and Asia as a protein source and condiments. Their own indigenous techniques for making fermented foods were defined and succeeded as a food culture. These foods were produced according to traditional methods transferred from their ancestors, and the safety of these foods has been confirmed over the centuries. Recently, fermented foods like natto, doenjang, and chong-kukjang, have been produced industrially using their own target starters and exported to other countries.

A number of studies have been performed to identify toxic substances and to find ways to reduce toxin production. The goal for securing safety can be achieved by various actions. First, the whole system from the stage of raw materials (beans and seeds) to storage should be inspected by applying GAP and HACCP. Personal training based on

guidelines of GAP, GMP, and HACCP is critical. When looking at the HACCP in detail, each step can be checked whether there are factors to contaminate. Microbial starter cultures should be selected and controlled so they may not produce biogenic amines, mycotoxins, and pathogenic microorganisms during the fermentation process. It needs to be defined which target starter or combination of starters would be better. Biogenic amines can occur during the food production and storage process under unsanitary conditions. Therefore, poor quality of raw materials and inappropriate conditions during food production process result in microbial contamination. Finally, changes to better equipment like mortar, pressure cooker, and appropriate packaging make a big difference to safety.

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422 FOODS INVOLVING ALKALINE FERMENTATION

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