2005

Development, evaluation and characterization of protein-isoflavone enriched soymilk

Janette Ethel-Pessi Saidu
Louisiana State University and Agricultural and Mechanical College, jsaidu@netscape.net

DEVELOPMENT, EVALUATION AND CHARACTERIZATION OF PROTEIN-ISOFLAVONE ENRICHED SOYMILK

A Dissertation

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

in

The Department of Food Science

by
Janette Ethel Pessi Saidu
B.S., Njala University College - USL, 1989
M.S., Louisiana State University, 1997
M.P.A., Louisiana State University, 1999
December 2005

DEDICATION

With a fresh look toward the harvest of prosperity and blessings ahead, I seize
each moment to reflect positively on my journey through
traditional education to this summit.
Therefore, it is with immense thanks and invaluable appreciation that I dedicate
This work to the Lord who by his abundant grace saw me through it all
And to the ones dear and close to my heart I also dedicate this work
to my lovely daughter Shirley that she shall work on through this path
to abundant success and better life,
to my parents Patrick and Rosaline Saidu whose immense love, teachings,
and parenting acumen is like a garland on my head
and a chain adorning my neck as a new beginning awaits me.
And to my host parents Rose and Felton Glasper,
whose spiritual, emotional acumen also thrusted a
deeper sense of inspiration, love and purpose in me.

ACKNOWLEDGEMENTS

First and foremost, I thank God, the Son, and the Holy Spirit for divine strength through the years in my studies, because without his divine guidance this would be impossible. Immense thanks and appreciation to my major and minor advisors Drs. Witoon Prinyawiwatkul and Maren Hegsted, for believing in me and affording me the much needed opportunity to work with them in completing my studies. Many blessings of thanks for your intellectual acumen, support and funding provided through out my studies. To my committee members Drs Charles Boneke, Zhimin Xu, Marlene Janes, and James Board, my gratitude for your willingness, patience, understanding and guidance also with my research. My appreciation and thanks to Drs Trappey and Wilson for the financial support they provided as well. A special thank you to Dr. Godber for his support and under whom I started my doctoral studies. Thanks to the SB&B (North Dakota) and Alcatris Company (Minnesota) for donating soybeans and soy germ samples, respectively for this research, and to the LSU agronomy department for running our nitrogen analysis.

If I am to choose a family in another world, I will choose the same family I have today. Thus, to my daughter – Shirley, the apple of my eyes, thanks for bringing laughter, sunshine and joy to my life amidst the ups and downs we have had and shared. Yet we both know it was worth it all. I therefore, pass the academic touch to you with prayers that you will stretch out your hands for God to hold and guide you through his chosen paths for you, and may his light forever shine upon you, and may he be gracious to you always, Amen. To my loving parents Patrick and Rosaline Saidu, without whom I would not have made it this far, I love and appreciate you two very much. Thank you both ever so much for everything in my life. To my siblings both at home

and here in Baton Rouge, thank you all for your emotional, physical, spiritual support and companionship throughout my studies.

To my host mom and dad, Rose and Rose Glasper and family, my immeasurable thanks for your unreserved love, friendship, support, encouragement, and prayers that made weathering the storms of my studies and my stay in the United States very bearable. Thanks for talking, listening, answering, sharing, giving, and caring over and above the call of your duties even as a host family. Aunty Rose and Uncle Felton, you have a special place in my heart and I love you two dearly. To Mohammed Sherrif, Vivian Ferdinad, Maxine Cormier, Clovier Torres, Paula Douglas, Agnes Farmer, Beatrice Njapau and family, Paulina Nabiu, thanks a million for loving, caring, and your friendship through the years.

This acknowledgement will be incomplete without saying thank you to Mayurachat (Kai) Natvaratat for her statistical assistance; to all the students who assisted me during the consumer studies; the faculty and staff of the food science department; the staff and students of the Dairy Science Department for all their assistance during my work; and Mr. Larry Butcher and Ms. Yvette Knight (Ms. T) at the Bursars office. Special thank you for every support given me in small and great ways that all contributed to this end result. Thank you all and may the grace and spirit of the Lord ever shine upon each one, and the fellowship of the Holy Spirit be with each one in all your trials and victory, in your visions and achievements, and at the start and end of each day in your lives, Amen.

TABLE OF CONTENTS

DEDICATION ……………………………………………………………………………..ii

ACKNOWLEDGEMENTS …………………………………………………………………iii

LIST OF TABLES …………………………………………………………………………...ix

LIST OF FIGURES ………………………………………………………………………xii

LIST OF ABBREVIATIONS …………………………………………………………...xiii

ABSTRACT ……………………………………………………………………………….xv

CHAPTER 1. INTRODUCTION...1
1.1 Research Prelude...1
1.2 Research Justification..4
1.3 Research Objectives...6

CHAPTER 2. LITERATURE REVIEW..7
2.1 Soybeans..7
2.1.1 Composition and Nutritional Value..7
2.1.2 Soy Germ Status and Applications...9
2.1.3 Soy Protein..11
2.1.4 Soy Isoflavones...12
2.1.5 Soybean Enzymes..15
2.1.6 Soy Products..16
2.2 Soymilk..17
2.2.1 Production...18
2.2.2 Comparison of Soymilk Composition to Cow Milk......................................21
2.2.3 Critical Factors Affecting Soybean and Soymilk Composition.....................22
2.2.4 Sensory Attributes of Soymilk...28
2.2.5 Sanitary State of Milk..31
2.3 Isoflavone Extraction and Analysis...33
2.3.1 Extraction..34
2.3.2 Analysis...35
2.4 Soybean and Health...36
2.4.1 Soy Isoflavone and Health...38
2.4.2 Soy Protein and Health..40
2.4.3 In-Vitro Mechanism of Soy Protein and Isoflavone......................................42
2.4.4 Controversy with Soy Protein and Isoflavone Effects on Health...................46
2.4.5 Other Bioactive Compounds in Soybeans..48

CHAPTER 3. DEVELOPMENT AND PROCESS OPTIMIZATION OF PROTEIN-ISOFLAVONE ENRICHED (PIE) SOYMILK...........................50
3.1 Introduction.. 50

3.2 Materials and Methods....52
3.2.1 Experimental Design....52
3.2.2 Soymilk Ingredients....55
3.2.3 Production of Soymilk....55
3.2.4 Physiochemical Analysis....56
3.2.5 Isoflavone Standards and Analysis....59
3.2.6 Statistical Analysis....62
3.3 Results and Discussions....62
3.3.1 Proximate Composition....62
3.3.2 Processing Effects on Protein and Isoflavone in Soymilk Beverages....64
3.3.3 Effect of Processing Conditions (Temperature and Time) on Protein and Isoflavone Content....66
3.3.4 Effects of Homogenizing Pressure on Soymilk Protein and Isoflavone Content....68
3.3.5 Processing Treatment Effects on Viscosity....70
3.3.6 pH as Determinant of Shelf-life and Beverage Quality....72
3.3.7 Processing Effects on Color of Soymilk Beverages....74
3.4 Conclusion....76

CHAPTER 4. FORMULATION OPTIMIZATION OF PROTEIN -ISOFLAVONE ENRICHED (PIE) SOYMILK: A COMPARISON OF BICARBONATE AND NO-BICARBONATE PROCESSING, AND THEIR EFFECT ON SOYMILK PHYSICOCHEMICAL PROPERTIES (FORMULATION OPTIMIZATION - PART I)....79
4.1 Introduction....79
4.2 Materials and Methods....81
4.2.1 Attaining Optimal Formulation....81
4.2.2 Production of Soymilk with or without $NaHCO_3$....81
4.2.3 Physicochemical Evaluation of $NaHCO_3$ and no- $NaHCO_3$ Soymilk....82
4.2.4 Isoflavone Standards, Extraction and Analysis....83
4.2.5 Statistical Analysis....86
4.3 Results and Discussions....86
4.3.1 Proximate Composition of $NaHCO_3$ and no- $NaHCO_3$ Soymilk Beverages....86
4.3.2 Isoflavone Composition of $NaHCO_3$ and no-$NaHCO_3$ PIE Soymilk....89
4.3.3 Storage Quality of $NaHCO_3$ and no- $NaHCO_3$ Soymilk Beverages (pH)....93
4.3.4 Color of $NaHCO_3$ and no- $NaHCO_3$ Soymilk Formulations....95
4.3.5 Optimal Formulation and Processing Treatment of Soymilk Beverages....96
4.5 Conclusion....98
.
CHAPTER 5. QUALITY AND MICROBIAL SHELF LIFE STUDY OF PROTEIN-ISOFLAVONE ENRICHED (PIE) SOYMILK BEVERAGES....100
5.1 Introduction....100
5.2 Materials and Methods....102
5.2.1 Preparation of Soymilk....102
5.2.2 Apparent Colloidal Stability....102

5.2.3 Protein Separation....103
5.2.4 pH....103
5.2.5 Microbial Assay....104
5.2.6 Statistical Analysis....105
5.3 Results and Discussions....105
5.3.1 Apparent Colloidal Stability....105
5.3.2 Protein Separation....106
5.3.3 pH of Refrigerated Soymilk Beverages....107
5.3.4 Aerobic / Total Plate Count of PIE Soymilk Beverages....108
5.4 Conclusion....111

CHPATER 6. SENSORY EVALUATION OF BLAND SOY GERM INCORPORATED PROTEIN-ISOFLAVONE ENRICHED (PIE) SOYMILK (FORMULATION OPTIMIZATION-PART II)....113
6.1 Introduction....113
6.2 Materials and Methods....114
6.2.1 Soymilk Processing....114
6.2.2 Attaining and Evaluating for Optimal Formulation....115
6.2.3 Consumer Preference and Acceptance Test....115
6.2.4 Statistical Analysis....116
6.3 Results and Discussions....118
6.3.1 Demographic Strata....118
6.3.2 Product Information....118
6.3.3 Mean Consumer Overall Liking....120
6.3.4 Mean Consumer Acceptance and Purchase Intent Responses....120
6.3.5 Mean Acceptance and Purchase Intent Scores by Gender....123
6.3.6 Overall Product Differences – Pooled within Canonical Structure's....126
6.3.7 Logistic Regression Analysis vs. Predictive Discriminant Analysis (PDA) for Acceptance and Purchase Intent....127
6.3.8 Proportional Odds Model of Predictors Relative to Like/Dislike of Products....130
6.3.9 The McNemar Test for Change in Probability of Purchase Intent....131
6.3.10 Principal Component Analysis of Bland Soymilk Product Attributes....132
6.3.11 Product Optimization....134
6.4 Conclusion....135

CHAPTER 7. SENSORY DISCRIMINANT TEST FOR FLAVORED SOYMILK....137
7.1 Introduction....137
7.2 Materials and Methods....139
7.2.1 Production of Soymilk....139
7.2.2 Spiking / Flavoring of Soymilk....139
7.2.3 Attaining and Evaluating for Optimal Flavor....140
7.2.4 Consumer Preference and Acceptance Test....140
7.2.5 Statistical Analysis....141
7.3 Results and Discussions....142

7.3.1 Product Information....................142
7.3.2 Mean Consumer Overall Liking and Acceptance Responses....................144
7.3.3 Mean Acceptance and Purchase Intent by Gender....................145
7.3.4 Overall Product Differences – Pooled Within Canonical Structures' r....................150
7.3.5 Logistic Regression Analysis and Predictive Discriminant Analysis (PDA), of Consumer Sensory Profile Critical to Product Acceptance and Purchase Decision....................151
7.3.6 Proportional Odds Model of Predictors Relative to Like/Dislike of Products....153
7.3.7 Principal Component Analysis of Flavored Soymilk Product Attributes……....154
7.3.8 Flavored Soymilk Optimization....................155
7.4 Conclusion....................156

CHAPTER 8. SUMMARY, CONCLUSION(S) AND RECOMMENDATION(S)....................158

REFERENCES....................162

APPENDIX A. CHARTS FOR CHAPTER 2....................184

APPENDIX B. CHARTS AND DATA FOR CHAPTER 3....................185

APPENDIX C. CHART FOR CHAPTER 5……………………....................201

APPENDIX D. RESEARCH CONSUMER CONSENT FORM....................202

APPENDIX E. CHARTS AND DATA FOR CHAPTER 6....................203

APPENDIX F. CHARTS AND DATA FOR CHAPTER 7....................209

VITA....................215

LIST OF TABLES

Table 2.1 Proximate Composition (%) of Soybeans and Seed Parts......................................9

Table 2.2 Anatomical Distribution of Soybean Isoflavones in Two Varieties of Soybeans (mg/100g)..15

Table 2.3 Proximate Composition (%) of Bovine Milk and Soymilk..................................21

Table 3.1a Processing Condition (Time, Temperature and Pressure) Chart...........................54

Table 3.1b Explanation of Processing Condition (Time, Temperature and Pressure) Chart…………………………………………………………………………...54

Table 3.2 Mean Proximate Composition (%) of Soymilk by Processing Method.................63

Table 3.3 Mean Protein and Isoflavone Contents by Treatment in Each Process.................65

Table 3.4 Effect of Heating Time and Temperature on Protein and Isoflavone contents by Processing Method 67

Table 3.5 Effect of Homogenizing Pressure on Protein and Isoflavone contents by Treatment in each Process 69

Table 3.6 Viscosity (cPs) of Soymilk Beverages by Treatment in each Process...................70

Table 3.7 pH as a Measure of Soymilk Beverage Quality..73

Table 3.8 Color Comparison of Soymilk Beverages by Processing Method.........................74

Table 4.1 Proximate Composition of $NaHCO_3$ and no-$NaHCO_3$ PIE Soymilk Beverages....87

Table 4.2 Within, and Between Treatment Comparisons of Isoflavone Content (mg/100g) and Composition of PIE soymilk Beverages......................................90

Table 4.3 Keeping quality of $NaHCO_3$ and no-$NaHCO_3$ PIE Soymilk Beverages as Determined by pH...93

Table 4.4 Color Comparison of $NaHCO_3$ and no-$NaHCO_3$ PIE Soymilk..............................96

Table 5.1 Apparent Colloidal Stability of Soymilk Beverages……...................................106

Table 5.2 Protein Separation of Soymilk Beverages...107

Table 5.3 Initial Aerobic / Total Plate Count of PIE Soymilk Beverages..........................109

Table 5.4 Final Aerobic / Total Plate Count of PIE Soymilk Beverages...........................111

Table 6.1 Consumer Demographic Frequency...118

Table 6.2 Overall Consumer Frequency Responses for all PIE Soymilk Beverages..........119

Table 6.3 Comparison of Mean Consumer Scores for all Attributes of Soymilk Beverages...121

Table 6.4 Overall Frequency Response of Bland Soymilk for Acceptance, Purchase Intent and Soy Health Benefit Purchase Intent Response...................................123

Table 6.5 Mean Scores for Acceptance, Purchase Intent and Soy Health Benefit Purchase Intent Responses by Gender……………………………………….124

Table 6.6 Multivariate Statistics and F Approximation (Wilks' < .0001) of Soymilk Beverages with all Attributes Considered Simultaneously.................................126

Table 6.7 Canonical Structures' r of Critical Soymilk Sensory Attributes Affecting Differences among Formulations (Based on Pooled within-group variance)......126

Table 6.8 Prob>X^2 and Odds Ratio Estimates for Consumer Acceptance and Purchase Intent of Soymilk Beverages...128

Table 6.9 % Hit Rate for Acceptance, Purchase Intent, and Soy Health Benefit Purchase Intent based on the Predictive Discriminate Analysis..........................129

Table 6.10 Proportional Odds Model of Predictors Relative to Like/Dislike of Products....131

Table 6.11 McNemar Test for Change in Probability of Purchase Intent of Soymilk Beverages... 132

Table 7.1 Frequency Responses of Consumers' Perception of the most Important Sensory Attributes of the Five Soymilk Beverages..143

Table 7.2 Frequency Responses of Consumers for Acceptance and Purchase Intents of Flavored and Plain Soymilk (5 Beverages)..144

Table 7.3 Overall Consumer Scores of Flavored and Plain Soymilk on all Attributes.......146

Table 7.4 Overall Responses for Acceptance and Purchase Intent....................................147

Table 7.5 Mean Scores for all Attributes including Acceptance and Purchase Intent of Flavored and Plain Soymilk by Gender...148

Table 7.6 Frequency Response for Overall Liking of Flavored Soymilk by Gender..........149

Table 7.7 Positive Response for Acceptance and Purchase Intent of Flavored Soymilk by Gender....149

Table 7.8 Multivariate Statistics and F Approximations (Wilks' <.0001 - Test Criteria and F Approximations for the Hypothesis of no Overall Form Effect)....150

Table 7.9 Canonical Structures' r of Critical Soymilk Sensory Attributes Responsible for Differences among Soy milk Beverages....151

Table 7.10 Prob>X^2 and Odds Ratio Estimates for Consumer Acceptance and Purchase Intent....151

Table 7.11 % Hit-Rate for Acceptance and Purchase Intent of Flavored Soymilk....153

Table 7.12 Proportional Odds Model Ratio Estimates of Sensory Attribute Predictors Relative to Like / Dislike of Flavored Soymilk Beverages....153

LIST OF FIGURES

Figure 2.1 Compositions of Whole Soybean Seed....................8

Figure 2.2 Skeletal Structures of Isoflavone Isomers....................14

Figure 3.1 Main Soymilk Production Stages…………….......53

Figure 3.2 Chromatogram of 6 mixed Isoflavone Standards....................61

Figure 4.1 pH during 21 days Storage of Soymilk Processed of $NaHCO_3$....................94

Figure 4.2 pH during 21 days Storage of Soymilk Processed with no-$NaHCO_3$....................95

Figure 5.1 pH of Optimized Process PIE Soymilk Beverages....................108

Figure 6.1 PCA bi-plot of Bland Soymilk Product attributes comprising Principal Component 1 and 2....................133

Figure 7.1 PCA bi-plot of Flavored Soymilk Product attributes comprising Principal Component 1 and 2....................154

LIST OF ABBREVIATIONS

6OAceGly -	6'-O-Acetyl Glucosides
6OMalGly -	6'-O-Malonyl Glucoside
α-GOS -	Alpha-galactooligossccharides (alpha – GOS)
α-GAL -	Alpha-galactosidase (alpha-Gal)
ACN -	Acetonitrile
AGC -	Aglycones
AGD -	Acetyl Glucosides
ANOVA -	Analysis of Variance
AOAC -	Association of Official Analytical Chemists
BGD -	Beta (β) Glucosides
CCK -	Cholecystokinin
DSHEA -	Dietary Supplement Health Education Act
DRI -	Dietary Reference Intake
Glc -	Glucosides
FDA -	United States Food and Drug Administration
HACCP -	Hazard Analysis and Critical Control Point
HBPI -	Health Benefit Purchase Intent
HDL -	High Density Lipoprotein
HPLC -	High Performance Liquid Chromatography
IFA -	Isoflavone Aglycones
IFG -	Isoflavone Glucosides

LAB - Lactic Acid Bacteria

LDL - Low Density Lipoprotein

LO - Lipoxygenase Enzyme

MGD - Malonyl Glucosides

MANOVA - Multivariate Analysis of Variance

MFV - Mouth Feel Viscosity

$NaHCO_3$ - Sodium Bicarbonate

PI - Purchase Intent

PIE - Protein-Isoflavone Enriched Soymilk

RSWT - Rated Sweetness

SFP - Soy Fortified Paneer

SG - Soy Germ

SPC - Standard Plate Count

SWT - Sweetness

TI - Trypsin Inhibitor

TKV - Thickness Viscosity

UHT - Ultra High Temperature

UV - Ultra Violet Detector

WSB - Whole soybean

WSB/SG25 - Whole soybean milk containing 25% soy germ

WSB/SG30 - Whole soybean milk containing 30% soy germ

WSB/SG35 - Whole soybean milk containing 35% soy germ

WSB/100 - Whole soybean milk

ABSTRACT

Adequate provision of soy isoflavone and protein in soybean products is essential in meeting the soy health claim and the promotion of a healthy product. Commercial soymilks contain inadequate amounts of protein and isoflavone to meet the FDA recommended 25g and 40-50mg/day level, respectively, in 1-2 servings. This research study set out to investigate the possibility of producing a protein-isoflavone enriched (PIE) soymilk by incorporating soy germ in soymilk to provide the recommended FDA level in 1-2 servings. Three PIE soymilks containing 25 (SG25), 30 (SG30), and 35% (SG35) germ were prepared and compared to 100% whole soybean milk (WSB-control) and a commercial soymilk. Product development comprised process and formulation optimization, accompanied by physicochemical analysis, shelf life and beverage quality evaluation, as well as consumer sensory evaluation.

The optimal processing method for incorporating soy germ into soymilk was at the starting stage (soaking) in soymilk production, and the WSB/SG25 was the optimal soymilk. The 25% soy germ beverage had the best composition profile closely followed by the WSB/SG30, WSB and WSB/SG35. The composition profile of the PIE beverages was better than the commercial soy milk. Shelf life and physicochemical quality of the PIE soymilk demonstrated stable shelf life, high beverage quality characterized by good colloidal stability, high degree of dispersion and less protein separation as germ amount increases.

Validation of the optimal formulation from consumer perspective identified the WSB/SG25 soymilk as the most liked product by both male and female consumers. This was clearly revealed in the acceptance and purchase intent of the beverages. Spiking of the optimal formulation (WSB/SG25) with green mango, orange, almond and chocolate flavors, also revealed that green mango and almond flavor were preferred based on the overall liking, acceptance and purchase intent responses. Aroma, sweetness, overall flavor, color and mouth feel viscosity were crucial attributes that determined overall liking and in turn influenced the underlying differences among the beverages. These results suggest that incorporation of soy germ into soymilk yielded sufficient isoflavone and protein to meet the FDA requirement in 1 serving for isoflavone and 3 servings for protein.

CHAPTER 1. INTRODUCTION

1.1 RESEARCH PRELUDE

Internationally, the functional foods industry has evolved at a rapid pace to meet consumer interest in healthy eating and self – medication. In affluent countries like the United States, Japan, the Netherlands, Sweden etc, functional foods and / or nutraceutical foods have transcended consumers regular diets as exemplified in the rush of energy bars, meal replacement beverages, fortified foods, supplements and herbal extracts. The functional foods market generates billions of dollars in developed countries as soy food products move out of small niche stores into mainstream markets. Manufacturers' sales of soy foods were projected to reach $6.9 billion in 2005. While soymilk sales alone reached $550 million in 2001, increased sales were projected to reach $1 billion by 2005. Even the soy ingredient market, including soy proteins and isoflavones, was projected to increase from approximately $524 million in 2000 to over $660 million in 2005 (Soyatech, 2002).

Amongst the many soy products, soymilk is one of the popular traditional products in China and other Asian countries (Shun-Tang et al., 1997) consumed as a nutritious and economical protein food (Matsuura et al., 1989). Consumers in western countries consume soymilk mainly as an important replacer of cow milk due to lactose intolerance or allergic reaction to cow's milk, and as a low cost source of good quality protein and energy (Rosenthal et al., 2003; Lui, 1997; Kwok and Niranjan, 1995; Kanthamani et al., 1978). In the United States, adoptation of soymilk as a cow milk substitute has now received widespread attention because soybeans and soybean products have gone mainstream. An interesting coalition of forces have converged on the health sector resulting in increasing use of soy as a good source of protein and healthful components. Most consumers now associate soy with a healthy lifestyle due to its potential health benefits.

Soy isoflavones, one of the biologically active components in soybeans is implicated as a potential alternative therapy for a range of hormone and age-dependent health conditions, including cancer, osteoporosis, cardiovascular disease, post menopausal symptoms, etc., (Coulston, 1999). These phytochemicals offer specific biological effects (Hazen, 2004); such as anticarcinogenicity (Xu et al., 1995), cholesterol and blood pressure lowering effects (Nagata et al., 1997; Sirtori et al., 2001); reduced mortality from sex hormone dependent cancer (Barnes and Messina, 1991); and prevention of heart disease, obesity, diabetes, kidney disease, and osteoporosis. These health benefits from soy isoflavones have been reported and confirmed in numerous studies (Anderson, 1997; Messina et al., 1994; Booth et al., 1999; Coward et al., 1993; Setchell 1998; Setchell et al., 1999, 2001, 2002, 2003; Caragay 1992; Garcia et al., 1997; Hassler, 1998; Lui, 1997; Raiz, 1999). Postmenopausal women are most aware of soy isoflavones health benefits since bone health and mid-life transition quality of life issues concerns them the most. In fact, this is reflected in the considerable number of healthy food items and dietary supplements containing isoflavone in the marketplace, aimed at these consumers.

In the U.S, soymilk has increased in popularity in the food market (Soyatech, 2002). The overall sales of soymilk beverages in the U.S. market grew nearly 50% in 2000. Refrigerated soymilk grew from 13% to 30% of the U.S. market. The surging popularity in soy products consumption has also transcended increasing utilization of other legumes and whole grains containing the healthful component - isoflavones. In spite of its good nutritional profile, soymilk suffers two main drawbacks - (1) limited acceptance by consumers due to the unpleasant beany flavor developed sometimes during processing (Rosenthal, 2002), and (2) the inadequate amounts of protein and isoflavone per serving to meet the recommended FDA requirements set at 6.25g of soy protein per serving for a total of at least 25g of soy protein daily, and 44 - 50mg of isoflavone per serving (USFDA, 1999; United Soybean Board, 2003). The FDA's determination that 25g of soy protein per day as part of a diet low in saturated fat and cholesterol may reduce the risk of heart disease by reducing blood cholesterol levels.

The first drawback has been overcome with the addition of different food flavors including vanilla, chocolate, banana, mango, strawberry etc., or a combination of these to enhance sensorial acceptance. But, the second problem still remains because the low protein and isoflavone concentrations found in commercial soymilk cannot meet the minimal FDA requirement in fewer than 4 - 5 and 3 - 3.5 servings daily for protein and isoflavone, respectively. In other words, consumers will have to consume three or more servings (254 ml = 1 cup = 1 serving) of soymilk to meet the recommended daily protein and isoflavone intake. Protein concentrations in soymilk range between 4 - 7g (7 - 18%) per (254g) serving; 2.7 - 3.7g (5-7%) per 100g; and 0.8 - 1.1g (2%) per one fluid ounce (United States Department of Agriculture (USDA), 2001b). It is highly unlikely that consumers will want to consume large amounts of soymilk just to meet the heart health recommendation. Reported, high protein contents above 7g per cup or 2.7g per 100g, and 0.8 g per ounce is usually obtained in fortified liquid or soy protein powdered milk. Similarly, the total isoflavone amount in liquid soymilk is very low ranging from 4.71 – 9.65 mg per serving in non-fortified soymilk, and from 10 - 43mg or less per serving in fortified soy liquids. The isoflavones, daidzein, genistein, and glycitein also have concentrations ranging from 1.90 - 4.45mg/100g (daidzein); 2.81 - 6.06mg/100g (genistein); and 0 -

0.56mg/100g (glycitein), respectively (USDA, 2001b; United Soybean Board, 2001; Tsangalis et al., 2004; USDA, 2004).

Soybeans are the only food source containing nutritionally significant amounts of isoflavones and having a protein profile similar to eggs or red meat. But, the fact that soybeans are rich in isoflavones does not necessarily imply that all soy products contain them. Isoflavones can be lost during processing because they are water soluble and can be washed away during alcohol extraction as well. Depending on how a product is processed, soy protein concentrate may or may not contain significant amounts of isoflavones. Alcohol-extracted soy protein and soy products contain less isoflavone content compared to water-washed concentrates. Typically, soy sauce and soy oil do not contain isoflavones, but soy flour has very high concentrations followed by soy milk, tofu, tempeh, and miso (Hazen, 2004). Base products like soy flour, water-washed soy protein isolate provide modest isoflavone amounts around 1.0 to 1.5 mg/g of isoflavone.

1.2 RESEARCH JUSTIFICATION

As noted above, soymilk contains inadequate amounts of protein and isoflavones per serving to adequately meet the FDA daily recommendation, yet very few studies have surveyed the potential of simultaneously increasing protein and isoflavone concentrations in soymilk to adequate levels for consumers to meet the FDA requirements in fewer than three or four servings per day and without additional dietary supplementation. Moreover, no studies have been reported using soy germ alone in soymilk as a potential alternative in enhancing protein and isoflavone concentrations in soymilk. Existing literature on soymilk and other soy-based foods makes ample reference of soy germ usage in isoflavone extraction for use in other soy-based products and pharmaceutical supplements, but not as a promising natural nutrient enhancing source in soymilk. Research in this area is challenging, needs more investigation, and is, therefore, the primary focus of this study. Some studies have attempted to enhance isoflavone or specifically the isoflavone isomers using various modern technologies, bacteria, hydrolyzing enzymes etc., to increase nutrient content and quality. Tsangalis et al., (2003) investigated the possibility of enhancing isoflavone and other nutrients in soymilk using bacteria fermentation and in another study used reconstituted soy protein isolate mix (Tsangalis et al., 2004) with a combination of different processing methods. These authors demonstrated that the development of a highly nutritious soymilk using, for instance, using selected lactic acid bacteria without heat treatment is possible. The process, however, produced undesirable secondary effects such as microbial translocation and weight loss when fed to animals. But the application of heat treatment to the same product in the presence of bacteria stimulated innate immune response in the animals fed soymilk without causing secondary effects. Meaning that, it is not always necessary for lactic acid bacteria to be alive to exert a beneficial physiological effect (LeBlanc et al., 2004).

No previous or known studies have investigated the possibility of enhancing isoflavone and protein concentrations in soymilk using only food grade soy germ added to whole beans and in the absence of other nutrient enhancing technologies. The proposed study was conducted to test the hypothesis that enhancing protein and isoflavone simultaneously in soymilk can be done through the process of incorporating soy germ in soymilk at different ratios and at different stages of the soymilk process without affecting sensory acceptability. Developing an acceptable protein-isoflavone-enriched (PIE) soymilk product with soy germ is our primary goal because soy germ which makes up only 2% of the soybean, is comparable in protein and other nutrient composition in comparison to the whole bean / cotyledons. The germ contains 41% protein, and has the highest concentration of isoflavone (Cowan, 1973; Weingartner, 1987; Liu, 1997;

Schryver, 2002; LeBlanc et al., 2004). Since incorporation of soy germ into soymilk is wholesome as a food - grade material, there are no concerns over chemical residues that may otherwise remove anti-nutritional compounds or impair biological activities *in vivo*.

1.3 RESEARCH OBJECTIVES

With whole soy bean containing reasonable amounts of isoflavone and protein, it was hoped that a careful blend of the two components in a formulation would increase the protein and isoflavone contents to meet the FDA soy intake recommendations in fewer than three servings. The study, therefore, aimed at developing an acceptable protein-isoflavone-enriched (PIE) soymilk product in which protein and isoflavone concentrations were maximized yet maintaining sensory acceptability. Specific objectives were to:

1) Develop and optimize a process by which soy germ can be incorporated into soymilk to maximize protein and isoflavone concentrations.
2) Develop and determine optimal soymilk formulation for enhancing protein-isoflavone contents.
3) Evaluate the physicochemical attributes of all soymilk formulations.
4) Determine shelf life, stability and quality of the soy beverage.
5) Characterize the sensorial attributes and acceptability of the formulations.

CHAPTER 2. LITERATURE REVIEW

2.1 SOYBEANS

Soybeans have played an integral part in most Asian societies both as food and medicine for centuries (Messina, 1995), and today they are best known in occidental cultures for their nutritional value. Most prominently, their bioactive role in disease treatment and prevention has increased their use. Hence, the inherent therapeutic and medicinal benefits of soybeans have gained widespread appeal because this "multi-use and functional food" has naturally occurring antioxidants that benefit human health (Anderson and Wolf, 1995).

2.1.1 Composition and Nutritional Value

Unlike other legumes, soybeans contain a variety of nutritional components that provides health promoting benefits (Schryver, 2002). They are also unique because all the parts of the plant including leaves, stalk, and seeds are utilized for food, medicine or forage for animals. Typically, whole soybeans or bean cotyledons separated from the germ are utilized for commercial soy and soy-products. Soybean composition includes varying amounts of protein content (38 - 40%), and fat (18%) of which 85% is unsaturated and high in linoleic and linolenic acids (a precursor to omega-3 fatty acids), 23% oleic acid and 16% palmitic acid (Figure 2.1). Most fatty acids in soybean and its derivatives are unsaturated, and, therefore, susceptible to oxidation (Penalvo et al., 2004). In addition, the bean contains 30% carbohydrates, of which 15% is insoluble and the other 15% soluble carbohydrates (Orthoefer, 1978; United Soybean Board, 2001). Other components include varying concentration of isoflavone, high levels of minerals, including iron, calcium, zinc; vitamins including α-tocopherol, niacin, pyridoxine, and folacin. Soybeans are also a good source of anti-nutrient factors such as saponins, phospholipids, protease inhibitors, phytates, and trypsin inhibitors.

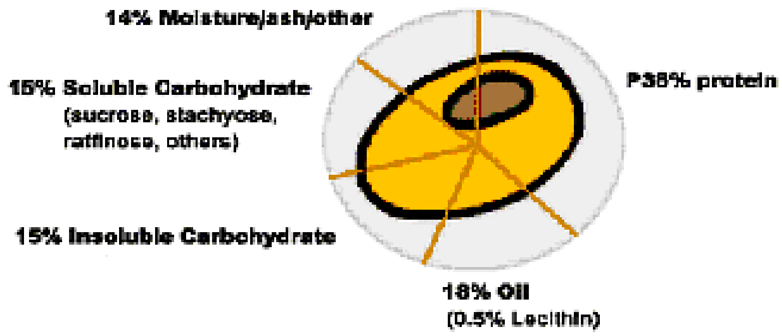


Figure 2.1 Composition of Whole Soybean Seed (Source: World Initiative for Soy in Human Health (http://www.wishh.org/whysoy/affects.html)

While the cotyledons make up 95% of the bean, the hull and germ comprises 3% and 2% of the whole bean, respectively. The germ is a small structure at the lower end of the bean from which sprouting begins and a new plant grows. The germ comprises about 2% of the total bean and is rich in fat, protein, ash, and vitamins (Table 2.1). In addition, the germ is the most potent source of isoflavone containing about five to six times the amount of isoflavones found in the cotyledon (embryo). The germ also contains the highest percentage of polyunsaturated fatty acids (Liu, 1997). Like the cotyledons, the germ is an excellent source of macro nutrients, containing approximately 38 - 42% protein, 11 - 22% fat, 43% carbohydrates, and about 4.4% ash. (Cowan, 1973; Weingartner, 1987). Isoflavone amounts could vary between 20 - 30 mg/g and about 80 - 90% of isoflavone is found in the germ. High amounts of saponins, phytoesterols, linolenic acid, iron, zinc, foliate, and vitamin E are also found in soy germ on a per gram basis, compared with soy flour containing only about 1 - 2 mg / g of isoflavone (Lui, 1999; USDA, 2001a; USDA, 2001b; Schryver, 2002).

In addition, soy germ also contains large amounts of oligosaccharides or alpha-galactooligosaccharides (alpha-GOS) that are generally indigestible because mammals lack pancreatic alpha-galactosidase (alpha-Gal) necessary for their hydrolysis. These sugars reach the large intestine causing gastrointestinal disorders in sensitive individuals (LeBlanc et al. 2004).

Removing the hypocotyls (germ) is a difficult process and requires mechanical cracking or removal of the soybean hull, then separation of the soybean cotyledon. Similar to wheat germ, soy germ is fairly compact, but once the germ has been isolated, functional nutrients can be extracted for food and supplements (Schryver, 2002).

Table 2.1 Proximate Composition (%) of Soybeans and Seed Parts

Soybean Parts	Whole Portions (%)	Protein (%)	Fat (%)	Carbohydrate (%)	Ash (%)
Whole beans	-	40	21	34	4.9
Cotyledon	90	43	23	29	5
Hull	3 to 8	9	1	86	4.3
Hypocotyl	2	41	11	43	4.4

Source: Wolf and Cowman (1971)

Present also in beans or bean fractions including the germ are other naturally occurring biologically active agents or anti-nutritional factors that may affect utilization and nutrient availability. These compounds include saponins, protease inhibitors, lectins, oligosaccharides, goitrogens, allergens, tannins, phytates, estrogens, antivitamins, hemagglutinins, trypsin inhibitors and phenolic compounds (Orthoefer, 1978; Carro-Panizzi and Mandarino, 1994; Schryver, 2002). Trypsin inhibitors are the principal antinutritional agents present in soybeans since they occur in large quantities. However, these compounds are typically destroyed by heat, cooking and other processing treatments that eliminate most of these anti-nutritional factors. Besides the presence of anti-nutritional factors, soy germ provides great potential as a bioactive and nutritional enrichment component for soy-based products including soymilk.

2.1.2 Soy Germ Status and Applications

The FDA recognizes soy germ as a food ingredient which offers food producers a clean label, suggesting to consumers a natural ingredient with health benefits (Schryver, 2002). Soy germ is regulated under the Dietary Supplement Health and Education Act of 1994 (DSHEA) with suggested use for products within the dietary supplement industry such as pills, tablets, bars, powdered beverages, etc. Supplement labels refer to the extract as a "standardized 10% soy extract," "standardized soy germ extract", "soy germ extract", or derivations of these options. Because of the relatively high proportion of isoflavones found within soy germ, inclusion rates are typically 1 - 2% of a product's total formulation. At such a low rate of inclusion, there is no negative impact on flavor. But, determining how much soy germ should be included in the formulation can be first done by determining how many milligrams of isoflavone per serving are desired. Although there are no standard dietary reference intakes (DRI) for soy isoflavones, researchers have established a recommended levels of 20-50mg/day based on a typical Asian diet (Setchell and Cassidy, 1999). Soy germ has been used in a variety of food applications, because isoflavones are water soluble, stable across all pH levels, and stable at temperatures up to 260^{o}C (500^{o}F).

Soy germ is widely used in cereal-based products, baked and extruded products to increase overall isoflavone levels. Depending on granular size, large granulations are used in heart healthy products such as whole-grain breads and granola, or sprinkled on top of grain-based foods. Medium granulation sizes are used in baked or extruded goods, with finer sizes best suited for beverages including juices and smoothies. Low inclusion rates also allow for soy germ use in a wide variety of dairy-based products, such as milk, frozen dairy desserts, smoothies, and yogurts. It has also been added to dinner meal formulations such as ravioli making the dough less likely to break and release its contents when added to boiling water. One last unexpected use of soy germ is in the cosmetics industry, where cosmeceutical products are becoming more common (Schryver, 2002).

2.1.3 Soy Protein

Soybeans protein or isolated soy protein is widely used in the food industry (Wang and Murphy, 1994), and in many food products including infant formulas, and other health foods (Eldridge and Kwolek, 1983), because soybean contains several biologically active components (Anderson, et al., 1979), of which proteins and isoflavones are of particular interest due to their estrogenic activities (Drane et al., 1975; Kitts et al., 1980). On a dry weight basis, the seeds contain 40 -45% protein that contributes to the nutritional value of foods and feeds, and is responsible for a number of functional characteristics in a variety of foods (Anderson and Wolf, 1995). Soybean meal or cake as it has been called is generally regarded as by-product that has little value (Smith and Circle, 1978). It was used as cattle feed or occasionally as fertilizer. The use of soy protein for poultry, swine, and other animal feeds was not developed until the late 1930's. The protein and lipids in soybeans are the principal parts of commercial interest, accounting for approximately 60% of the seeds (Orthoefer, 1978).

Based on sedimentation coefficients, soy protein resolves into four major fractions: 2, 7, 11, and 15 S fractions. The 2 S fractions predominates during early development and makes up about 20% of the seed protein. The 7 S fractions consists primarily of beta-conglycinin, and glycoprotein and represents slightly over one-third of the total soluble protein, with one-half being a glycoprotein consisting of twelve glucosamine and thirty-nine mannose residues per mole. The 11 S fractions make up about one-third of the total soy protein and has only one protein component, glycinin. Glycinin, is, therefore, the major storage protein of soybeans (Nielsen, 1985; Yagasaki et al., 1997) accounting for about 35% of total seed protein and consists of six sub units each made up of an acidic and a basic polypeptide component linked by a single disulphide bond (Staswick, et al., 1984). The 15 S fractions is only one-tenth of the total protein (Hou and Chang, 1998). The bulk of the protein resides in storage sites called protein bodies or aleurone grains. Protein bodies account for 60-70% of the total protein seed. Based on human requirement, the essential amino acids are equal to or exceed the levels found in egg protein except for the sulfur-containing amino acids, with methionine being the first limiting amino acid. Most of the soy proteins are globulins that are soluble at their isoelectric point but solubilizes upon addition of salt. The isoelectric point occurs at pH 4.2 – 4.6. Heating is necessary to attain maximum nutritional value and to modify functional properties of soybean proteins (Smith and Circle, 1978; Orthoefer, 1978).

Soybean proteins are denatured by heat, extreme pH, organic solvents, and detergents. At $100^{o}C$, soy protein approaches minimum solubility, after which solubility increases with continued heating because high molecular weight aggregates are formed during heating with gels appearing at protein concentrations near 8%. At 8-12% concentrations, the gels break down at $125^{o}C$. Pure organic solvents are less effective in denaturing soy proteins than their aqueous solutions. Alcohols denature the protein more effectively as hydrocarbon chain length increases. The 7 S fractions appear to be the most sensitive to alcohol and the 2 S the least sensitive. As with other grains or legumes, the proteins of soybeans are of relatively low biological value (69%). However, when utilized with other protein-containing foods that supply the lacking amino acids, the nutritive value is greatly enhanced (Bennion, 1995).

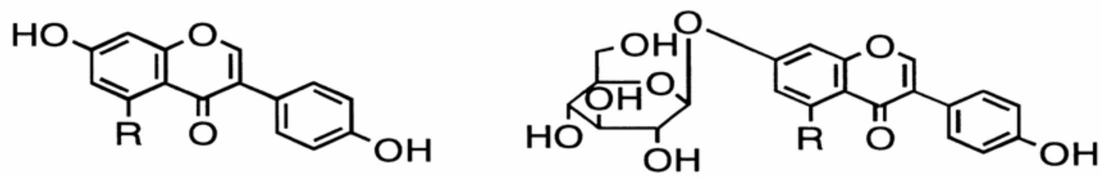
2.1.4 Soy Isoflavones

Similar to soy proteins, isoflavones are plant derived phytoestrogens and belong to a class of compounds known as flavonoids. They are phenolic compounds with structural homology to human estrogens (Tsangalis et al., 2002). Isoflavones are predominantly found in soybeans and non-fermented soy foods as biologically inactive glucoside conjugates comprising 80% to 95%

of the total isoflavone concentration (King and Bignell, 2000). They are well known to provide beneficial effects for the prevention and treatment of many aging diseases, as well as a protective role in a range of conditions including cardiovascular diseases, high cholesterol, osteoporosis, and breast, colon and prostate cancers.

Isoflavones are present mostly as β-glucoside conjugates which includes daidzin, genistin, and glycitin, and their malonyl and acetyl derivatives (Kodou et al., 1991; Xu et. al., 2002). In other words, they are present in four chemical forms with each chemical form consisting of three isomers making a total of twelve (Figure 2.2): the aglycones - daidzein, genistein, and glycitein; the glucosides - daidzin, genistin, and glycitin; the acetylglucosides (6OAceGlc) - 6"-O-acetyldaidzin, 6"-O-acetylgenistin, 6"-O-acetylglycitin; and the malonylglucosides (6OMalGlc) - 6"-O-malonyldaidzin, 6"-O-malonylgenistin, and 6"-O-malonylglycitin (Kodou et al., 1991; Xu et al., 2002).

Although for many years it was assumed that soybeans contained large amounts of isoflavones as their glucosides (Glc), the principal chemical forms of isoflavones in the soybean are 6"-O-malonyl-glucoside / β-glucosides (6OMalGlc) conjugates (Eldridge and Kwolek, 1983). From their chemical orientation, a portion of the glucoside is substituted on the C-6 hydroxyl of the glucose by a malonyl or acetyl group. In soy germ / hypocotyl, mostly, the malonyl group is substituted on the C-6 hydroxyl of the glucose. The acetyl derivatives are mostly considered as degradable products resulting from decarboxylation of the malonyl-isoflavones during processing and extraction. With a wide range of isoflavone concentration from <50 μg/g to >3500μg/g in the seeds and products, the glucoside forms are higher in concentration with approximately twice the molecular weight of the aglycones.



R = OH: Genistein and R=H: Daidzein
Aglycones (IFA)

R=OH: Genistin and R=H: Daidzin
Glucosides (IFG)

R_1	R_2	Aglycone
H	H	Daidzein
OH	H	Genistein
H	OCH_3	Glycitein

R_3	R_4	R_5	Glucosides
H	H	H	Daidzin
OH	H	H	Genistin
H	OCH_3	H	Glycitin
H	H	$COCH_3$	6'-O-Acetyldaidzin
OH	H	$COCH_3$	6'-O-Acetylgenistin
H	OCH_3	$COCH_3$	6'-O-Acetylglycitin
H	H	$COCH_3COOH$	6'-O-Malonyldaidzin
OH	H	$COCH_3COOH$	6'-O-Manolygenistin
H	OCH_3	$COCH_3COOH$	6'-O-Malonylglycitin

Figure 2.2 Skeletal Structure of Isoflavone Isomers (Source: Wang and Murphy, 1994 (J. Agric Food Chem: 42 (8), 1667-1673); Izumi et al. (*Journal of Nutrition.* 2000; 130:1695-1699.)

The aglycones - daidzein, genistein, and glycitein are not as abundant in soy foods (0.2 – 1.5mg/g) (Wang and Murphy, 1994), but are best known as the most bioactive phytochemicals with unique biological properties. These soy isomers are absorbed faster and in higher amounts than their glucoside counterparts *in vivo* (Izumi et al., 2000). Of the three aglycones, glycitein is present in smaller amounts (Hazen, 2004), accounting only for ≈ 5-10% of the total isoflavones in soy foods, but with stronger unique biological activities (Song et al., 1998) than daidzein and genistein. The forms and amounts of each isoflavone vary in different soy foods depending on the source of the beans and the processing method used. Typically, genistein occurs in higher concentrations than daidzein in soy foods. However, differences in chemical structure of different soy isoflavones may also relate to variable bioavailabities in the biological system. For instance, daidzein, in some studies, has been reported to have higher bioavailability than genistein in clinical trials in adult women (Xu et al., 1994).

The distributions of individual isoflavone differ in the hypocotyls and cotyledon (Eldridge and Kwolek, 1983) with isoflavone compounds occurring in greater concentrations in the hypocotyls of soybeans than in the cotyledons (Wang and Murphy, 1994). Primarily two glucosides are found in the hypocotyl, daidzin and glycitin 7-β-glucoside, whereas in the cotyledon, there is about 20 times as much genistin as in the hypocotyls. On a weight basis concentrations of isoflavones are highest in the hypocotyls (1400-1700 mg/100g) and lowest in the hull of the seed (10-20mg/100g), with the cotyledons containing roughly between 150-320mg/100g. Similarly, individual isoflavone concentrations have been reported to vary within the germ, hull and cotyledon of soybean seeds (Table 2.2).

Table 2.2 Anatomical Distribution of Soybean Isoflavones in Two Varieties of Soybeans (mg/100g)

	Hull		**Hypocotyl**		**Cotyledon**		
Isoflavone	Amsoy	Tiger	Amsoy	Tiger	Amsoy	Tiger	LSD (.05)
Daidzin	6.6	8.6	1031.5	759.9	37.5	102.8	539.2
Glycitin			664.1	588.8	1.7	1.6	127
Genistin	2.8	7.4	5.3	9.1	113.9	205.8	167.4
Daidzein	0.7	1	19	14	1.4	2.8	10.6
Glycitein		1.5	11.8	9.3			5.8
Genistein	0.5	1.5	24.7	24.2	2.8	5.9	5.8
Total	10.6	20	1756.2	1405.2	158.5	319.2	808.1

Source: Eldridge and Kwolek, 1983. J. Agric. Food Chem. 31 (2), 394-396

2.1.5 Soybean Enzymes

The beany flavor of soybean is primarily the result of an enzymatic complex – lipoxygenases (Carro-Panizzi and Mandarino, 1994). Commercially, lipoxygenases are considered of major importance because these catalyze the oxidation of lipids, forming fatty acid hydroperoxides. The hydroperoxides undergo scission and dismutation resulting in the development of off-flavors and aromas (Orthoefer, 1978). The lipoxigenases are specific for lipids containing a cis, cis,-1, 4-pentadiene system. Only the 13-hydroperoxide is formed with linoleic acid as the substrate. The 9 - hydroperoxide isomer is formed as a result of autoxidation.

Several chemical compounds are responsible for the unacceptable soybean flavor. But, for the reaction to occur, lipoxygenase, damaged seeds with exposed substrate (linoleic and linolenic acids) and water are necessary (Nelson et al., 1980). The reaction does not occur when damaged seeds are soaked in water. However, lipoxygenase enzymes are easily inactivated by boiling for three to five minutes, and it is the blanching process that prevents the formation of the characteristics beany flavor, making soybean products less or more acceptable. The use of hot water and sodium bicarbonate blanch before grinding is also known to reduce off-flavors (Golbitz, 1995), while modern dairy processing technologies deodorizes the milk during soymilk processing, and desired flavors are added.

As previously mentioned, the consumption of soy and soy-derived products was also hampered by concerns over the presence of alpha-galactooligosaccharides (alpha-GOS) because mammals lack pancreatic alpha-galactosidase (alpha-Gal) which is necessary for their hydrolysis. These sugars reach the large intestine causing gastrointestinal disorders in sensitive individuals (LeBlanc et al., 2004). But, the use of lactic acid bacteria (LAB) expressing alpha-Gal seems to be a promising solution for the degradation of alpha-GOS in soyamilk.

2.1.6 Soy Products

A wide variety of soy foods are available throughout the world today. Some are produced by modern processing methods in large scale plants, and others still in more traditional ways owing their history to oriental processing methods, thus are referred to as traditional soy foods

(Golbitz, 1995). Soy foods are typically divided into two categories: nonfermented and fermented. Traditional non-fermented foods include fresh green soybeans, whole dry beans, soy nuts, soy sprouts, whole-fat soy flour, soymilk and soymilk products, tofu, okara and yuba. Traditional fermented foods include tempeh, miso soy sauces, natto and fermented tofu and soymilk products. Westerners have adopted some of the foods from either group whole heartedly whereas others will undoubtedly take more time to accept. In the U.S, the most popular soy foods from either group include tofu, soymilk, soy sauce, miso, tempeh, and soy protein isolate primarily used in other food products.

2.2 SOYMILK

Soymilk is the aqueous extract of whole soybeans (dehulled or non-dehulled) (King and Bell, 2000), closely resembling dairy milk in physical appearance and composition (Penalvo et al., 2004). Also, called vegetable milk or Fu Chang in Chinese, it was reportedly developed in China before the Christian era (Piper and Morse, 1943) by the philosopher whi Nain Tze, who is also credited with the development of tofu that is closely associated with soymilk since the preparation of milk is the first step in its production. The traditional soymilk is a simple water extract of soybeans made from soaking the beans in water overnight, wet grind the beans (Appendix 2.1), steam the wet mash to improve flavor and nutritional value, and filter (Howell and Caldwell, 1978). More recently, modified modern flavored versions have hit the mainstream market as meal replacement beverages and cow milk replacer. Apart from its beverage form, soymilk is used as a base in a wide variety of products, including tofu, soy yogurts and soy-based cheeses (Golbitz, 1995).

2.2.1 Production

In general, soymilk can be processed from whole soybeans, degermed soybeans or full-fat soy flour. Present modern preparations in developed countries have evolved to utilize soy flour or other soy materials in combination with soy protein isolate in an attempt to simplify manufacturing process but also to enhance the nutritional and sensory profile (Tsangalis et al., 2002, King and Bignell, 2000). Aside from traditional production, soymilk production using non-conventional methods has shown varying physiological and antinutrient effects. Examples of familiar production applications include the use of soy protein isolate (Tsangalis et al., 2003) to enhance isoflavone levels, lactic acid bacteria (LAB) in fermented soymilk expressing α-gal as a promising solution for the degradation of α-GOS (LeBlanc et al., 2004; Garro et al., 2004), or the use of other bacteria strains to enhance beverage quality (Wang et al., 2004), stimulate or enhance immunomodulatory properties of soy bioactive compounds - isoflavones.

LeBlanc et al. (2004) investigated the use of the *Lactobacillus fermentum* CRL 722 on commercial soymilk that completely eliminated stachyose and raffinose during fermentation due to its high alpha-Gal activity. Rats fed with the soymilk had smaller caecums compared with rats fed with unfermented soymilk, implying that soyamilk fermentation by *Lactobacillus fermentum* CRL 722 resulted in the reduction of alpha-GOS concentrations in soymilk, thus eliminating possible undesirable physiological effects normally associated with its consumption. Hence, fermentation with *Lactobacillus Fermentum* CRL 722 could prevent gastrointestinal disorders in sensitive individuals normally associated with the consumption of soya-based products. In a similar study by LeBlanc et al. (2004) using different bacteria species, *Bifidobacteria longum* CRL 849 and *Lactobacillus fermentum* CRL 251 in a mixed culture were able to grow on soymilk and they showed slower growth and lower acid production in soymilk by reducing stachyose and α – galactosidase activity.

A recent study by Behrens et al. (2004) on soymilk inoculated with a mixture of *Streptococcus thermophillus, Bifidobacterium lactis and Lactobacillus acidophilus*, and supplemented with 2% sucrose, demonstrated the possibility of formulating highly acceptable soymilk beverages by way of lactic fermentation and addition of flavorings. The resulting beverages flavored with pineapple, strawberry, coconut, kiwi, guava and hazelnut, and submitted to a sensory acceptance test using the 9-point structured hedonic scale, were more acceptable and liked due to their enhanced sensory quality. Choi et al. (2002) used *Lactobacillus delbrueckii subsp. delbrueckii* KCTC 1047, grown in deMan, Rogosa and Sharpe (MRS) or soymilk media to completely hydrolyze the isoflavone glucosides, genistin and daidzin into their respective aglycones, genistein and daidzein within 30 min. Other lactic acid bacteria used did not produce beta-glucosidase, the enzyme responsible for the hydrolysis of isoflavone glucosides when cultured in MRS medium. However, glucoside-hydrolyzing activity was induced in some lactic acid bacteria when cultured in soymilk medium. These strains hydrolyzed 70-80% of genistin into genistein and 25-40% of daidzin into daidzein.

Generally, isoflavone glycosides are hydrolyzed by beta-glucosidase from gut microbes to the bioactive aglycones. However, the specific bacteria from the human intestinal tract that are involved in the metabolism of these compounds are not known (Jeon et al., 2002). In their study, Jeon et al. (2002) developed a fermented soymilk in which isoflavones were converted to the more bioactive aglycones form using a *Bifidobacterium* strain. The beta-glucosidase activity of 15 *Bifidobacterium* strains was measured during cell growth. Among the 15 strains, the *Bifidobacterium sp*. Int-57 (B-sp. Int-57) was selected for further study because it had the highest beta-glucosidase activity. Variables measured included growth, acid development, beta-glucosidase activity, and the hydrolysis of daidzin and genistin in four soymilks inoculated with B-sp. Int-57. After 12 h of fermentation, the counts of viable *Bifidobacterium sp*. Int-57 in all the soymilks reached a level of more than 8logcfu/ml, which was then maintained. While pH of the soymilks started to decrease rapidly after 6 h of fermentation but then leveled off after 18 h, titratable acidity of the soymilk increased. After 24 h of fermentation, beta-glucosidase activity also increased between 40.64 and 70.84, mU/ml. Isoflavone glycosides, daidzin and genistin in the soymilks were hydrolyzed completely in a relatively short fermentation time of 18 h. The study concluded that *Bifidobacterium sp.* Int-57 can be used as a potential starter culture for developing fermented soymilk which has completely hydrolyzed isoflavone glycosides.

With regards to nutrient changes, soymilk produced by *Bifidobacterium longum* B6 and *B. infantis* CCRC 14633 at different fermentation periods (Hou et al., 2000) showed changes in the contents of crude protein, sugars, B-vitamins, acetic and lactic acids. Crude protein and titratable acidity increased during fermentation, while soymilk fermented with *B. infantis* had higher titratable acidity than that fermented with *B. longum*. The degree of protein hydrolysis, thiamin and riboflavin contents also increased while niacin content decreased in soymilk fermented with either *B. infantis or B. longum.* Acetic and lactic acid contents were reported to increase while the molar ratio of acetic and lactic acid was decreased during fermentation. Stachyose, raffinose and sucrose contents decreased, with stachyose showing the largest magnitude of reduction. On the other hand, contents of fructose and glucose plus galactose contents were reported to increase during fermentation. However, such novel soy products have been reported to cause undesirable secondary effects such as microbial translocation and animal weight loss (LeBlanc et al., 2004).

2.2.2 Comparison of Soymilk Composition to Cow Milk

Proximate composition of whole soymilk contains 2.86 – 3.12% protein, 90 - 93.81% moisture, 1.53 - 2% fat, 0.27 – 0.48% ash, 1.53 – 3.90 % carbohydrate calculated as the difference from 100% (Rosenthal et al., 2003; Yadav et al., 2003). Typically, soymilk contains higher moisture than bovine milk, and their proximate constituents differs significantly between the two (Table 2.3) (Yadav et al., 2003). The protein furnished by cow milk consists largely of casein with few other minor proteins. Protein from soymilk consists mainly of glycinin with few minor other fractions. The proteins in soymilk have a well recognized deficiency of the essential sulphur amino acids – methionine and cysteine, but comparatively rich in lysine. Cow milk carbohydrate is exclusively in the form of lactose sugar and soymilk carbohydrate is oligosaccharides mainly as stachyose and raffinose. Though cow milk contains significantly high levels of calcium and phosphorus, it is extremely low in iron content. But fractions components could vary in soymilk depending on formulation, processing and solids contents of soymilk.

Table 2.3 Proximate Composition (%) of Bovine Milk and Soymilk

Sample	Moisture %	Protein %	Fat %	Ash %	CHO %	Calcium mg/100g	Phosphorus mg/100g	Iron mg/100g
Bovine Milk	84.8	3.18	4	0.79	7.23	122.2	76.3	0.08
Soymilk	90.5	3.12	2	0.48	3.9	21.2	30.94	0.87
SD at 5%	2.25	0.04	0.35	0.02	0.01	1.6	1.6	0.01
SEM±	0.57	0.01	0.09	0.005	0.004	0.41	0.41	0.004

Source: Yadav et al. 2003; SD = Standard Deviation and SEM = Standard Error Margin

When compared on weight basis (100g portions), cow's milk contains 61calories and soymilk 33 calories. Cow's milk has about 14 milligrams of cholesterol, lactose and no dietary fiber, whereas soymilk contains 1.3g of fiber and zero cholesterol, and no lactose. While both contain much protein and a full range of amino acids, soy milk contains greater amounts of the amino acids arginine, alanine, aspartic acid and glycine. Arginine slows the growth of cancers by strengthening the immune system, alanine aids in the metabolism of sugars, aspartic acid increases stamina and plays a vital role in metabolism by acting as an antioxidant, and glycine is necessary for brain and nervous system functioning and muscle / energy metabolism. With processing, pasteurization to a large extent destroys vitamins C in cow milk, and the same can be said of soymilk, except that soymilk contains over four times the amount of thiamin (vitamin B1) and nearly twice the amount of niacin than cow milk. Soymilk also contains 12 times the copper, 42 times the manganese and more magnesium than cow's milk (Cohen, 1998; Crouch, 1998). The high nutrient composition of soymilk over cow's milk certainly gives it numerous health advantages.

2.2.3 Critical Factors Affecting Soybean and Soymilk Composition

Environmental factors, varietal differences, processing methods and conditions result in varying composition and nutrient levels of soybean products. Environmental stress during reproductive growth has been shown to alter mineral composition of soybean seeds which in turn affects overall nutritional value, functional quality, and seed quality (Gibson and Mullen, 2001). Environmental factors and varietal differences affect isoflavone concentrations and have recently received extensive focus by many researchers.

a) Environment and Climate

While environment and climate play crucial roles in the growth and development of crops through maturity, unfavorable conditions also result in substantial effects on growth, nutrient composition, functionality and seed quality. Nutrient concentrations of phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) as well as several minerals in seeds increase during the reproductive growth period in high temperatures, but concentrations do not necessarily correlate with the seed size (Gibson and Mullen, 2001). The climate, mainly temperature and moisture, during seed development is the major factor determining isoflavone accumulation in the soybean grains (Kitamura et al., 1991; Tsukamoto et al., 1995)

Reports on the content of genistein and daidzein and their glycosides in a few soybean varieties and in some soy foods (Murphy, 1982; Farmakilidis and Murphy, 1985) as well as discussions on the effects of processing on these compounds (Wang and Murphy, 1994) have shown varying results. Eldridge and Kwolek (1983) reported that total isoflavone of soybeans varies from 116 to 309mg/g within variety and varies from 46 to 195 mg/g with the same variety in different locations. Isoflavone concentrations have also been reported to vary from year to year when soybeans are grown in the same location, suggesting that unknown climatic and environmental factors contribute to variation in isoflavone concentrations and perhaps as well to overall nutrient concentration in soybeans (Eldridge and Kwolek, 1983).

With regards to soybean variety effects on isoflavone and general nutrient content, some bean varieties may have high concentrations of nutrients while others have lower concentrations. Genetics significantly influences variation in bean variety. Choi et al. (2000) reported that wild soybeans tend to have more isoflavone content than cultivars or landraces. Thus, genetics, climate and environmental factors produce greater impacts on isoflavone and other nutrient contents in soybeans that, affect final composition of food products.

b) Processing

(i) Processing and nutrient content changes - The type of processing and ensuing processing conditions such as high or low temperatures, short or prolonged temperature and cooking time, ultra high temperature (UHT), processing treatment combinations with alkali or other chemicals etc., all affect the physicochemical and sensory properties of soymilk. Heat treatment effects on nutrient content do not seem to be significant, except when prolonged cooking at extreme temperature is applied. However, excessive heat can cause protein denaturation with concomitant loss of its functionality, and generates additional volatile organic compounds responsible for cooked or toasted off-flavors (Ha et al., 1992). It is also known that the denaturation of protein by heating increases hydrophobicity (Sorgentini et al., 1991).

The interaction between protein and lipids are important in achieving acceptable soy product characteristics. Soy lipids play an important role in physical characteristics such as texture and sensory quality of products made from soymilk (Catsimpoolas and Meyer, 1971; Yamano et al., 1981). The lipid and protein forms a complex of characteristic buoyant densities by the dissociation of native protein, providing an increased surface area and greater proportion of hydrophobic residues (Kamat et al., 1978). A lot of the soy neutral lipids are in particulate phase of unheated soybean milk. With heating, half of the phospholipids are retained in the particles and half move to the soluble phase (Ono et al., 1996). When heated at 75^{o}C, lipids in the soluble and particulate fractions are liberated and shift to the floating fractions (Shung-Tang et al., 1997). Almost all lipid shifts to the floating fraction at 90 ^{o}C, then proteins in the fractions are rearranged by heating (Ono et al., 1991). Almost all of the lipids in heated soymilk can be separated as a floating layer containing a few proteins (Shibasaki et al., 1972; Ono et al., 1996).

With respect to micronutrient changes, even at higher temperatures, Kwok et al. (1998) observed no significant changes in available lysine during a 3 h heating period at 95^{o}C. In fact at elevated temperatures of 120 and 140^{o}C optimum heat processed soymilk had higher levels of available lysine than did soymilk processed at 95^{o}C. But, prolonged heating at these temperatures (120 and 140^{o}C) caused a decline in available lysine content.

(ii) Processing and changes in Sensory Attribute - The sensory attributes of perceived color and flavor are the most important characteristics in soymilk because they are readily assessed by consumers. Soymilk when subjected to severe heating acquires a brown color and cooked flavor (Kwok et al., 2000). Kwok and Niranjan (1995) have demonstrated the effects of thermal processing on the quality of soymilk and concluded that the main chemical reaction that gives rise to heat-induced color and flavor changes is the maillard reaction.

(iii) Processing Effects on Anti-nutrient - Processing temperature and cooking time have been demonstrated to inactivate the enzyme lipoxigenase (LO) and other antinutrient factors such as trypsin inhibitors (TI) (Kwok et al., 2002). Lipoxigenase is mostly inactivated by heating with residual activity of 14% when 0.01M sodium carbonate is used compared to water (residual activity 46%). According to Tomoko and Kazuyoshi (2001), lipoxigenase destruction is completed following a short heating time from 70^{o}C to boiling temperature of 96^{o}C. But, trypsin inhibitor (TI) is not fully inactived at 95^{o}C for many minutes (Geranazzo, et al., 1998). Tripson inhibitor is only inactivated to acceptable levels in soymilk in the final UHT step at 135^{o}C for 2 minutes. Several studies have reported to have successfully destroyed TI within temperature ranges of 100^{o}C (Miyagi et al., 1997), to 121-154^{o}C and at time intervals of 10-90 sec (Kwok et al., 2002). TI in soymilk was satisfactorily destroyed to 10% of original concentration at 143 and 154^{o}C with 62 and 29 secs heating time, respectively (Kwok et al., 2002). Though not quite significant, but conventional heating other than the microwave heating also decreases trypsin inhibitor activity in soymilk, and activity is further decreased upon fermentation of soymilk with the lactic acid bacterium, *Streptococcus thermophillus*, to about 30% (Tomoko and Kazuyoshi, 2001). The destruction of antinutrient factors tends to improve soymilk attributes such as beany flavor, taste and overall preference.

(iv) Processing and Isoflavone Composition - The body of literature on nutritional studies has reported that, in commercial practices, defatted soybean meals will contain essentially all of the isoflavones or isoflavones glucosides present in the starting soybeans. However, chemical extraction is deemed to have significant effects on isoflavone concentration. Depending on the product, chemical extraction may have from little to serious effects on final product composition and nutrient concentration. Research reviews indicate that extracting soy bean / soy flour with ethanol to produce soy protein concentrate removes the anti-nutritional or anti-carcinogenic compounds (Bennink, 1996; Bourquin and Bennink, 1996; Kano et al., 2000). Reduced levels of isoflavone in products like protein isolate (600 - 1000µg) in comparison with soybeans and flour is a result of aqueous processing, mainly aqueous alcohol. Although alcohol extraction/processing is considered to produce the blandest products (Berry, 1989) and the process retains low isoflavone amounts (73µg/g) because isoflavone is soluble in aqueous alcohol and largely removed during processing. Conversely, Eldridge and Kwolek (1983) have reported that oil extraction from soybeans with hexane does not remove isoflavones or the isoflavone glucosides because they are not soluble in hexane.

The distribution of different forms of isoflavones represents a history of the processing of foods. Heat processing, enzymatic hydrolysis and fermentation have been shown to significantly alter the isomeric distribution of the three groups of isoflavones. In other words, a particular form such as the malonyl- which is very heat sensitive, will differ in raw and heat treated soybean or products (Song et al., 1998). Soymilk contains approximately 4 to 7 mg total isoflavones / 100 g with considerable variations both in composition and content (King and Bignell, 2000; Murphy et al., 1999). Interestingly, the isoflavone content of soymilk made from soy protein isolate (SPI) is much lower than that made from whole soybeans because the mild alkali extraction used in the production of SPI causes isoflavone losses of approximately 53% (Wang and Murphy, 1996). According to Murphy et al. (1999), aseptically processed (i.e. ultra high temperature - UHT) soymilk contains predominantly concentrations of β-glucoside isomers whereas pasteurized soymilk contains mainly isoflavone concentrations as malonyl-glucosides.

A detailed kinetic study of the rearrangement of the different forms of isoflavone during heat processing of soymilk (Murphy et al., 2002) indicates that in raw soymilk, the predominant forms are the malonylglucosides. If the soymilk is not heat processed, less than 1% isoflavone glucosides are hydrolyzed by native soybean glucosidases to the aglycones. Immediate heat processing of soymilk (after extraction from soybeans) to model aseptic heat processing, results in conversion of malonylglucosides to glucosides. Few acetylglucosides are produced until soymilk is exposed for longer than 60 min at 80°C. Yen and Kao (2002) investigated the effects of processing conditions on the changes in isoflavone content of black soymilk and soymilk film (yuba). When black soybeans were soaked in water at 30 and 50^{o}C for various periods of time, the contents of daidzein and genistein increased with increased soaking time while daidzin and genistin decreased. No significant differences ($P > 0.05$) were observed in the contents of isoflavones in black soybean under soaking conditions at 30^{o}C for 12 h and at 50^{o}C for 6 h. But, the amounts of isoflavones in black soybean changed markedly under the soaking conditions at 20-60^{o}C for 8 h. The change in the isoflavone contents in black soybean during soaking was attributed to its beta-glucosidases activity. The effect of soaking temperature on beta-glucosidases activity of black soybean was in the order of 50 > 40 > 60 > 30 > 20^{o}C. The contents of daidzein and genistein in yuba prepared by soaking soybeans at 50^{o}C for 6 h were about 2.5 times of that prepared by soaking soybeans at 30^{o}C for 12 h, which suggested that changing the processing conditions could increase the contents of isoflavone aglycones in soybean products.

2.2.4 Sensory Attributes of Soymilk

‘We are what we eat’ - in other words, what we eat determines our health status. For most people, the food choices they make and the development of their food habits are influenced by many interacting factors such as income, culture, health concerns, social and traditional values and religion. However, food must be palatable or appetizing for people to eat. A palatable food is one considered to be both acceptable and agreeable to ones' taste. Hence, various sensory impressions or sensations, including, odor (aroma/flavor), taste, appearance, mouth-feel, touch / texture, are all involved in our judgment of acceptability, palatability, and quality of food (Bennion, 1995). These attributes or characteristics are important to both the food developer or producer and the consumer.

Beany taste/flavor and color undoubtedly are the principal obstacles to the use of soybean as human food in the United States (Eldridge, 1978). But, flavor is by far the factor that most limits the acceptance of soybean products by persons not accustomed to consuming them (Carro-Panizzi and Mandarino, 1994). Astringency, mainly caused by anti-nutritional factors, is also an important attribute to the soymilk consumer. Studies on the effect of isoflavone (as an anti-nutrient factor) on soymilk and tofu astringency have shown no consistency to be found between an undesirable astringent taste and isoflavone contents. Likewise, isoflavone-enriched extract (similar to 39% isoflavones) showed no astringency effects (Mahfuz et al., 2004), and soybean foods having high amounts of isoflavones showed less astringency. About 80% of isoflavones exist freely in both soymilk and tofu, but 55% of phytates (which plays an important role in the formation of the tofu curd network) exists freely in the soymilk, and 6-13%, on the basis of coagulation, exists freely in the tofu curds. A 1% potassium phytate solution at pH 7 showed the very same astringency as soymilk; however, calcium phytate at the same concentration and pH showed no undesirable sensation. Thus, it was assumed that the astringent characteristics caused by phytic ions in soy milk are lost upon conversion of phytic ions to their insoluble salt forms during soy milk coagulation (Mahfuz et al., 2004).

Soymilk incorporation into numerous foods has been shown to enhance sensory qualities in dairy foods such as yogurt, milk, ice cream, sherbets, etc. Many studies have suggested that soymilk can not only be successfully applied or incorporated into other food products, but also tremendously enhances sensory qualities. Soymilk application of 6% and 9% solid content into bulgur production showed improved color and sensory properties of pilav and fine bulgur (Hayta et al., 2003). Bulgur cooked in soymilk of the 6 and 9% solid content had significantly ($P < 0.05$) higher bulk density compared with bulgur cooked in soymilk of 3% solid content and the control bulgur cooked in water. As the soymilk solid content increased, pilav bulgur yield increased whereas bulgur yield decreased. The SDS-extractability of bulgur proteins increased with increased soymilk solid content. Water absorption capacity of bulgur samples was affected by soymilk incorporation, and variations in absorption capacity were not significant ($P > 0.05$).

Ara, et al. (2003) demonstrated that the application of different enzymes (CGTase, pullulanase, α-glucosidase, α-glucosidase, pectinase, naringinase, hesperidinase, and beta-glucuronidase) in soymilk production not only increases isoflavone components and the formation of isoflavone aglycones, but the CGTase-treated soymilk, for instance, showed a higher taste preference than control soymilk. Several hydrolytic enzymes-Celluclast 1.5L, Pectinex ultra sp, Rohalases (SEP, F, 7069 e 7118) and filtration with different pore size tissues (20; 30; 85; 100 mum) independently studied, regarding their effect on the sensory quality of whole soymilk, indicated further that enzymatically treated soymilk resulted in a higher overall impression of flavor than the filtered product and products submitted to one and two homogenizations (Rosenthal, et al., 2003).

Uprit and Mishra (2004) conducted studies to observe the effect of varying fat content in milk (0-6%) and varying proportion of soymilk (7.5^{o}C, 0-40%) in the blend on the textural properties of soy fortified paneer (parboiled bulgur). Samples prepared using a blend (15:85 v/v) of soy milk and buffalo milk (3.12%) was found to be suitable for producing textural characteristics similar to that of control paneer.

While undesirable flavors and objectionable bitter and astringent tastes are associated with soy products (Tsukamoto et al., 1995), many attempts to improve the unfavorable characteristics of soybean seeds by genetic means have been made. The elimination of lipoxigenase from seeds has been successfully achieved (Kitamura et al., 1983, 1985; Kitamura, 1984; Davies and Nielsen, 1986; Hajika et al., 1991) and contributes to the improvement of bean flavor (Matoba et al., 1985; Davies et al., 1987; Kitamura, 1993), other factors that impart bitter and astringent flavors to soymilk still remained. These undesirable characteristics are likely due to saponins, phenolic acids, oxidized phospholipids, oxidized fatty acids and most of all isoflavones (Arai et al., 1966; Sessa et al., 1976; Okubo et al., 1992).

Sensory analysis (quantitative and / or descriptive) is often used to assess the flavor, appearance, texture and other attributes of food products as a function of processing parameters (Kwok et al., 2000). When sensory analysis is used, two difficulties arise: 1) sensory data are difficult to quantify, and 2) sensory data based on hedonic responses are not always linearly correlated to the intensity of the sensory attribute (Trant et al., 1981; Lund, 1982). The hedonic 9-unit scale is commonly used in sensory analysis, and the data analyzed parametrically. These procedures can lead to serious methodological problems, as the ratings on this arbitrary scale are scored as though they are separated by equal intervals. Basker (1989) has demonstrated that this is approximately true for 13 and 15 unit scales, but certainly not for the 5 unit scale. In fact, there are no adequate published data for a 9-unit scale. Nonetheless, a wide array of lexicon has been developed for soy milk and other soy food products. N'Kouka et al. (2004) recently developed a lexicon for descriptive analysis of soymilk consisting of a developed descriptive vocabulary to fully describe the sensory characteristics of soymilk. Principal component analysis was conducted on the resulting data to eliminate redundant terms and differentiate between samples. Thirty-one terms were identified to describe soymilks. The lexicon could be used as a starting place to describe any product containing soy; therefore, it will be beneficial to soy food processors in research and development and quality assurance.

2.2.5 Sanitary State of Milk

Microbiological examinations are carried out to check whether proper hygienic procedures have been followed, and whether the finished product is safe to keep and eat (Kordylas, 1990). Most foods acquire a variety of contaminating micro-organisms from their surroundings. However, only a fraction of these diverse, initial microbial contaminants ever develop on the food to any significant numbers. The microbial population associated with a food is generally specific depending on the type of food and particular storage conditions. In cow milk and cow milk products, the dominating genera of micro-organisms when spoilage occurs during standard conditions of storage include *Streptococcus, Lactobacillus, Microbactrium,* and the gram positive rods like *Bacillus* (Ihekoronye and Ngoddy, 1986). At the same time, numerous methods including sterilization, pasteurization, fermentation, refrigeration and freezing, dehydration, ultra pasteurization, etc., are being used to improve sanitary quality by killing harmful or food spoilage micro-organisms. To be considered as safe, specific allowable numbers of micro-organism have been set as a standard number expressed as a log coliform unit per milliliter (log CFU/ml). Most foods have standards or limits for total counts and this is especially true for milk. Thus, examination of soy milk's sanitary state is based on standards for cow milk.

In adopting microbiological standards to milk, the first concern is product safety, followed by shelf-life. In this regards, total counts of microorganisms are an indication of the sanitary quality of a food product. Referred to as the Standard Plate Count (SPC) by the U.S. Public Health Service, the total count of viable microbes reflects the handling history, state of decomposition or degree of freshness of the food (Murphy, 1997). Total counts and coliform counts provide an indication of unsanitary production practices (Murphy, 1997) or may be taken to indicate the type of sanitary control exercised in the production, transport, and storage of the food. A Grade 'A' raw milk for pasteurization should not exceed 100,000 bacteria per milliliter (ml) prior to commingling with other producer milk; and should not exceed 300,000 per milliliter as commingled milk prior to pasteurization. In addition, a grade 'A' pasteurized milk and milk products (except cultured products) should not exceed over 20,000 aerobic bacteria count per milliliter, and not over 10 coliform per milliliter post pasteurization. These standard specifications apply to all dairy products. It must be remembered that a low SPC does not always represent a safe product because it is possible to have low SPC count foods in which toxin-producing organisms have grown. These organisms produce toxins that remain stable under conditions that may not favor the survival of the microbial cell. Therefore, enforcement of adequate microbiological control through HACCP concept in milk processing operations is strongly emphasized (Efiuvwevwere and Nwanebu, 1998).

Jeon, et al (2002) reported counts of viable *Bifidobacterium sp*. Int-57 in soymilks to have reached a level of more than 8 log CFU/ml, which was then maintained over the fermentation period. Efiuvwevwere and Nwanebu (1998) also reported substantially hazardous high levels (> 6 log CFU/ml) occurring in soymilk samples inoculated with mixtures of *Aspergilus flavus* + *Escherichia coli* (E.coli) + *Staphilococcus aureus* or *Aspergilus flavus* + *Staphilococcus aureus* and those singly-inoculated with *Escherichia coli* after about 30 h of contamination. Samples inoculated with *Staphilococcus aureus* were acceptable organoleptically (based on sensory evaluation) until 48 h, although being unacceptable microbiologically. It was considered that the concept of metabiosis was probably responsible for the changes. In addition, *Lactococcus lactis strain* (LL3) isolated from mothers' milk and used to produce fermented soymilk, showed, the strain survived at levels of over 7 log CFU/ml for 3 weeks in the fermented soymilk (Beasley et al., 2003).

2.3 ISOFLAVONE EXTRACTION AND ANALYSIS

Measuring isoflavone concentrations is time consuming and challenging with considerable variations among measurement techniques used (Schryver, 2002). However, there is good reliability between duplicate analyses for intra-lab measurements as opposed to greater variations more than two standard deviations (Verbruggen, 2002) with inter-lab measurements. Reasons for variability include differences in extraction conditions, molar extinction coefficients and unknown purity of standards used. According to Verbruggen, (2002), the AOAC international in 2001 developed a method for measuring isoflavone levels within foods using HPLC and UV detector. The overall resulting isoflavone level from this method is a little less than for most HPLC and UV methods because isoflavone calculations do not account for the malonyl and acetyl isoflavone forms.

HPLC is the popular instrumentation used for identification of soy isoflavones in soybeans and soy food products or matrices. Other instrumentation used in the analysis of volatile compounds from soymilk includes electrospray ionization mass spectrometry, gas chromatography-mass spectrometry to detect daidzin and genistin after solid-phase extraction and to confirm isoflavone conjugates in biological samples.

2.3.1 Extraction

Extraction procedures vary depending on the type of soy foods, and recovery, in turn, depends on extraction conditions. Isoflavones are generally extracted with glacial organic solvents or common organic solvents such as ethanol, methanol, acetonitrile, or acetone in aqueous medium usually distilled water (Murphy et al., 1997; Song et al., 1998). Common conditions used during isoflavone extraction include agitation and hot and cold temperatures to enhance efficiency. Because of the inherent chemical instability of some isoflavone conjugates (Acetyl and Malonyl group), Coward et al.(1998), investigated (1) the best conditions for extraction and (2) the effects of commercial processing procedures and cooking on isoflavone concentrations and composition. Results showed elevated temperatures to enhance isoflavone recovery and to de-esterify 60MalGlc conjugates. Although room temperature extraction slowed the conversion of one form to another, extraction at 4 degrees C for 2-4 h resulted in the highest yield of 6OMalGlc conjugates and the lowest proportion of beta-glucoside conjugates. Non-heat treatment of soy product consisted mostly of 6OMalGlc conjugates, whereas heat treated products contained large amounts of 6"-O-acetyl-beta-glucoside conjugates formed by heat-induced decarboxylation of the malonate group to acetate. Further results showed that soymilk and tofu consisted almost entirely of beta-glucoside conjugates and low-fat versions of these products are markedly depleted in isoflavones.

Certainly, alcohol-washed soy-protein concentrates contained few isoflavones, whereas isolated soy protein and textured vegetable protein consist of a mixture of all 3 types of isoflavone conjugates. Thus, optimization of isoflavone extraction conditions and times is critical to extracting all isoflavone forms. Baking or frying of textured vegetable protein at 375^{o}F for example did not alter the total isoflavone content, but there was a steady increase in Glc conjugates at the expense of 6OMalGlc conjugates. Similar, effects were observed in the cooking of soy flour in soy cookies. Under the most extreme cooking conditions (leading to hardly edible foods), unconjugated isoflavones became the predominant form in which the total amount of extractable isoflavones decreased (Coward et al., 1998).

Carro-Panizzi et al. (2002) investigated the optimization of extraction procedures for isoflavones determination in soybean by HPLC. Isoflavones were extracted from 100 mg samples of full fat soybean flour "Kinako" (milled toasted grains). Recovery of the average total isoflavones was higher when extraction was performed with agitation (217.2 mg/100g), than without agitation (191.0 mg/100g). Isoflavone extraction without agitation for 1, 4, 20 and 24 hours were equally efficient for total and individual compounds, suggesting that an efficient isoflavone recovery could also be achieved with extraction and agitation for one hour (Carro-Panizzi et al., 2002).

2.3.2 Analysis

Separation of the 12 isoflavone isomers can be done using a gradient elution at a wavelength between 254 and 260 nanometer (nm) near each of their UV absorption maxima. Equol, a daidzein metabolite is only detected at the UV absorption maximum of 280nm and eluted after genistein (Tsangalis et al., 2002). While the wavelength at which isoflavones are detected ranges between 254 and 260nm, different polar solvents still offer good detection within these ranges. Amongst the different solvents used in the analysis of isoflavones, acetonitrile has been reported to be superior to acetone, ethanol and methanol in the extraction and analysis of the 12 phytoestrogenic soy isoflavone forms in foods (Murphy et al., 2002). At 53% organic solvent in water, various soy food products extracted for isoflavone with acetonitrile, demonstrated extraction efficiency over ethanol and methanol.

According to Tsangalis et al. (2002), isoflavone isomers are eluted in order of their polarity and hydrophobic interaction in the reversed - phase HPLC column. The order of elution can also differ depending on the type of product, the mobile phase used, and HPLC column type. Tsangalis et al. (2002) studied the transformation pathway of isoflavones to biologically potent and bioavailable forms in soy foods using five strains of bifidobacteria. Elution of the isoflavone forms was in order of malonyl glucosides (MGD), beta-glucosides (BGD), acetyl glucosides (AGD) and aglycones (AGC). MGD was of the highest polarity and AGC the lowest in regards to their chemical structure and number of hydroxyl groups. In another study by Coward et al. (1998) and King and Bignell (2000), BGD isomers were first eluted followed by MGD, AGD, and AGC when a mobile phase of acetonitrile (ACN) and 0.1 % trifluoroacetic acid was used. But within each chemical form (that is AGC, MGD, AGD, and BGD), daidzein seems to consistently elute first, followed by glycetien and genistein (Coward et al., 1998; King and Bignell, 2000; Tsangalis, et al., 2002).

2.4 SOYBEAN AND HEALTH

Soybeans have grown in popularity in recent years due to their versatility and health beneficial attributes (Rinaldi et al., 2000). Although soy foods have been consumed for more than 1000 years, only in the past two decades have they made an in road into Western cultures and diets (Golbitz, 1995). As though making a case for soybeans, ongoing supporting scientific evidence indicates that replacement of cow milk with soymilk has received wide spread adoptation and acceptability in Western cultures because soymilk in many ways resembles cow milk in physical appearance, containing lower amounts of fat, no cholesterol and higher amounts of iron in comparison to cow milk (Yadav et al., 2003). In fact, home made preparations of soymilk have grown in popularity (Nsofor, 1992) as well as the sky rocketing consumption of numerous soybean products. In under developed countries, production and utilization of soybean had been suggested for consideration as a promising means of alleviating protein shortage (Aworth et al., 1997), because soybeans provide an excellent source of protein and an inexpensive supply of calories (Maity and Paul, 1991). Today, the most notable attributes of soybeans is their health benefits linked to the prevention and treatment of many chronic diseases owing to their protein and isoflavone activities. In particular, soy isoflavones, originating from soybeans have become the subject of intense scientific scrutiny because of their influence on many human physiological processes both at the systemic and cellular level. They are food factors that influence the physiological state in animals and in human beings (Choi et al., 2000).

Given the numerous concerns over the use of hormone replacement therapy (HRT), women especially are seeking natural alternatives to cope with the symptoms and effects of menopause. The bone sparing effects of soy protein and its isoflavones are well established in animal studies as well as in human subjects but with variable outcomes due in part to short study durations and other factors (Lydeking-Olsen et al., 2004). Numerous published studies from clinicians and health researchers continue to show great interest in the potential role of soybean-based foods in lieu of the broad range of effects which support hormone and age - dependent diseases such as breast cancer, kidney and prostate health, healthy cholesterol levels (Takatsuka et al., 2000; Setchell et al., 2002, 2003; Chen et al., 2004), strong bone tissues and prevention of osteoporosis, healthy cardiovascular function and especially healthy immune function (Coulston, 1999; FDA, 1999; Frankfeld, 2003).

2.4.1 Soy Isoflavone and Health

As discussed earlier, isoflavones are plant compounds with mild estrogenic activity and are found in high concentrations in soybeans and in most soy foods. It has been known for sometime that Asian populations with high intakes of isoflavone-rich products have high concentrations of these compounds in blood and concomitantly a lower incidence of cardiovascular disease, several types of cancer, osteoporosis, and menopausal symptoms. This key finding has since led to the notion that a diet containing soybeans may play a role in the prevention or treatment of hormone dependent diseases due to the presence of isoflavone and other bioactive compounds (Setchell et al., 1981; Adlercreutz 1984; Setchell et al., 1984). Of the four chemical forms of isoflavone, the aglycone isomers (genistein, daidzein, and glycitein) are the most readily absorbed in the intestinal system and reported to be more consistently beneficial to health. Though they are biologically weak estrogens (Setchell and Cassidy, 1999), their weak estrogenic activity and suggested dietary roles are now powerful allies in supporting the body's fight against cancer and other degenerative diseases. Thus, isoflavone are the center of ongoing research interest and scrutiny (Fletcher, 2003).

Research studies have demonstrated that soybean isoflavones may serve as good biomarkers of health behaviors (Frankenfeld et al., 2003; Lu et al., 2000) when intake correlates with reduced coronary heart disease Chen et al., 2004), prevention of bone loss in postmenopausal women (Scallet et al., 2003; Lydeking-Olsen et al., 2004) and, reduced mortality from cancer and other diseases. Isoflavones have further been implicated in having roles in inhibiting angiogenesis, inducing cell differentiation, triggering tumor cell breakdown (apoptosis), and increasing neurosecretary non-apeptides (Scallet et al., 2003). While the scientific community of researchers agree on the role of isoflavone in the reduction of many diseases to some or a greater extent, a striking concern over isoflavone is in progress in the medical, nutrition and pharmaceutical literature. The question being debated is whether health benefits observed with soybean consumption is a result of isoflavones alone or a synergistic effect with other bioactive compounds such as soy protein. The health functions of soy protein and soy isoflavones from epidemiological studies suggest that both compounds lend protection and prevention against cardiovascular disease.

Epidemiological reviews (Cassidy 1996; Setchell and Cassidy, 1999; Setchell 1995) and human clinical trials (Anderson et al., 1995; Kurzer, 2000, Anthony, 2000) do support the association between isoflavone intake and prevention or reduced risks of many hormone-associated disorders prevalent in occidental societies. *In vivo*, Messina and Barnes (1991) showed that soybean chips, soy protein isolate, and soy molasses, all of which are rich in isoflavones, inhibit induced mammary tumorgenesis in animals. The degree of mammary tumor estrogen receptors from feeding soy chips parallels the inhibition of tumorgenesis. Seemingly, the binding property of isoflavones to estrogen receptors may be how soy possibly reduces breast cancer, which is estrogen dependent (Henderson et al., 1982; Verdeal et al., 1980). Similar reviews have indicated lower incidence of osteoporosis, menopausal symptoms, cardiovascular and cancer incidence, and mortality in oriental populations with reported high intakes of 50 – 70 mg/day of soy-derived isoflavones (Nagata et al., 1998).

Further reports of isoflavone's antioxidant and antifungal activity, also lend credibility to their health benefits. According to Niam et al. (1976), isoflavones inhibit lipoxygenase action and prevent peroxidative hemolysis of sheep erythrocytes *in vitro*. However, the extent of this effect depends on the structure of the isoflavones. Pratt and Birac (1979) also reported that soybeans, defatted soy flour, soy protein concentrates, and soy isolates have appreciable antioxidant activity detected by the rate of β-carotene bleaching in a lipid – aqueous system, which was due to phenolic compounds. Their report suggests that isoflavones have a protective effect but not as significantly as radical scavengers like BHA and BHT. Because the soy isoflavones are antioxidants, their antioxidant activity contributes to the possible anti-atherogenic effect of soy protein. Fleury et al. (1992) also showed that malonyl isoflavones are good antioxidants in a storage test carried out at $37^{o}C$ and in UV light-induced oxidation of a β-carotene / linoleic acid system. In general, the isoflavones showed no antioxidant properties in the Rancimat test performed at $100^{o}C$ or in the heat-induced oxidation of a β-carotene / linoleic acid system in comparison with BHA and BHT. The rapid decomposition of malonyl isoflavones at $100^{o}C$ rendered them inactive.

2.4.2 Soy Protein and Health

From a nutritional perspective, soy protein holds many advantages over animal proteins above and beyond the fact that soybeans are low in saturated fat and cholesterol free (Messina et al., 1995). As with isoflavones, diets rich in soy protein but low in isoflavone levels have also been implicated in renal function (Nakamura et al., 1989; Kontessis et al., 1990); prevention or treatment of osteoporosis (Arjmandi et al., 1998) and promotion of bone health (Breslau et al., 1988); reduction of total and LDL-cholesterol, triglycerides and apolipoprotein B (apo B) level (D'Amico and Gentile, 1993; Anderson et al., 1998); diabetic nephropathy (Barsotti et al., 1988; Jibani et al., 1991); reduction of risks for various cancers (Messins et al., 1994), and other diseases.

Of utmost importance is the hypocholesterolimic effects adequately documented and extensively reported in the literature. As little as 25 g of soy protein intake per day is required to lower cholesterol in hypercholesterolemic subjects (Bakhit et al., 1994), hence soy protein represents a safe, viable and practical non-pharmacological approach to lowering cholesterol. Obviously, the FDA recommended intake for soy protein set at 25g per day referenced as the soy-heart health claim, stems from numerous animal, epidemiological and human studies that have demonstrated that diets high in soy protein and low in animal protein results in decreased levels of total cholesterol, low-density lipoprotein (LDL) cholesterol and triglycerides.

While many studies suggest that 25 grams of soy protein per day may reduce cholesterol, no definitive research has actually demonstrated that consuming soy protein daily in non-study scenario in the proposed FDA amount is really effective in lowering cholesterol (U.S.FDA, 1999). A plethora of studies have indicated that study subjects or people who drink milk shake containing 25g of soy protein for a prolonged period of time (up to 9 weeks) experienced on average a range between 5 – 14%, reduction in LDL cholesterol (Tang et al., 1998; Kerckhoffs et al., 2002). Also, subjects with very high LDL cholesterol experienced up to 11% drop in LDL levels, implying that practically, for each 10 - 15% drop in LDL level, the risk of a heart disease, or heart attack decreases by 20 - 25% in persons with abnormal cholesterol levels. But, for individuals with normal cholesterol levels, they need larger amounts of soy intake to produce the same or similar reduction.

In the vast literature, reports from human studies on dietary soy protein point to marked variability in the lipedemic response, which has not been adequately explained. While many studies have reported very large decreases in cholesterol in response to soy protein; some have also reported only minor or insignificant effects (Holmes et al., 1980; Calvert et al., 1981;

Fumagalli et al., 1982; Mercer et al., 1987; Laurin et al., 1991). It therefore seems that all the variations in response to soy protein can possibly be attributed to the different cholesterol levels of the subjects at the beginning of these studies. Meta-analysis of human studies has more recently established that soy consumption is significantly associated with reduction in plasma cholesterol levels in humans. Nonetheless, most of these reports do not stand alone, but somewhat agree that the non-protein constituent - isoflavone (Anthony et al., 1996) does contribute to beneficial effects exerted by soy protein. Soy protein seems effective primarily in people with elevated blood cholesterol than individuals with normal levels. Though the mechanism of the lipid-lowering effect of soy protein alone remains unclear, all soybeans and soy foods currently available for human consumption contain, in addition to high proteins, significant amounts of isoflavones, either as glycoside conjugates or the unconjugated aglycone form (Setchell, 1998). As much as soy protein isolates have become popular items in the nutritional supplement marketplace however, most of these supplements contain some if not most or all of the bioactive soy isoflavones.

2.4.3 In-Vitro Mechanism of Soy Protein and Isoflavone

Despite the surrounding controversies on isoflavone, soy protein and health, various mechanisms of bioactive activity have been proposed. *In vitro*, genistein has been demonstrated to specifically inhibit epidermal-growth-factor-receptor-tyrosine-kinase activity (Akiyama et al., 1987) and protein histidine kinase from yeast cell extracts (Huang et al., 1992). Genistein is a unique topoisomerase inhibitor in selectively suppressing the growth of oncogene *ras*-transformed NIH 3T3 cells but not normal NIH 3T3 cells (Okura et al., 1988). Sariaslani and Kunz (1986) had observed that soybean flour genistein induced cytochrome P-450 in *Streptomyces griseus*. Peterson and Bernes (1991) also observed that genistein was a potent inhibitor of the growth of human breast carcinoma cell lines while daidzein and biochanin A were weaker inhibitors. In a short term animal study, hepatic cumene hydroperoxidase activity was increased by feeding soy isoflavone extract (Hendrich et al., 1994). These findings may be alternative mechanisms of inhibition of non-hormone related tumorgenesis by soy isoflavone.

In hormone-related tumorgenesis, the isoflavone aglycone isomers bind to estrogen receptor sites and mimic the functions of estradiol in the human body (Setchel and Cassidy, 1999), since they are the most biologically available, and are absorbed faster in larger amounts than their respective glucosides in the gastrointestinal tract (Izumi et al., 2000; and Setchell et al., 2000). Hendrich et al. (1994) estimated the amount of soy isoflavones required to be consumed by humans to provide an anticarcinogenic dose at 1.5 – 2.0 mg (Kg of body weight)$^{-1}$ day^{-1}. One proposed theory is that intestinal microflora plays a key role in the metabolism and bioavailability of isoflavones, as they hydrolyze the glucoside component via β- glucosidase in the jejunum, releasing the bioavailable, bioactive aglycone form (Setchell, 2000). Intestinal bacteria chemically alter daidzein to equol (an intestinal by-product) (Setchell and Cassidy, 1999). Results with animal studies have reported that sheep, for instance, metabolized clover-derived formononetin (methoxylated precursor of daidzein) into equol, which was found to be more estrogenic than its precursors (Shutt and Cox, 1972). Equol exhibits similar estrogenic activities to genistein. However, the efficacy of microbial biotransformation and types of intestinal bacterial involved in isoflavone conversion to bioactive forms is not entirely known. Some strains of *bifidobacteria* are known to produce β-glucosidase required to deconjugate isoflavone glucosides to bioactive aglycones (Tochikura et al., 1986).

Some authors (Setchell, 1998; Setchell and Cassidy, 1999; Setchell, 2000; Setchell et al., 2001; Setchell et al., 2002; and Setchell and Cole, 2003) have summarized the mechanism of soy isoflavone on health as the two acting as antioxidant to counteract damaging effects of free radicals in tissues and block formation of new blood vessels via anti-angiogenic or anti-atherogenic activities. However, the mechanism of action as to their possible independent effects still remains unclear. Several other theories on the effect of soy isoflavone on blood cholesterol profiles have been proposed. These include increased thyroxin levels, decreased insulin-glucagon ratio and increased hepatic LDL-receptor activity in response to soy protein ingestion. Isoflavone globulin is postulated as helping in cholesterol excretion from the body as another possible theory (Setchell, 1998; Setchell and Cassidy, 1999). Other studies suggest that phytoestrogen helps increase HDL levels and genistein acts as a tyrosine kinase inhibitor that decreases thrombosis (blood clotting), protecting against cardiovascular diseases such as heart disease and stroke (Akiyama, et al, 1987; Setchell, 1998). In addition, genistein inhibits the growth of smooth muscle cells, one of the primary cell types that form fibrous plaques (Akiyama, et al, 1987; Setchell, 1998).

Isoflavones in soy also act as antioxidants that help in preventing LDL cholesterol oxidation, protecting against atherosclerosis (plaque build up in the arteries) (Gregory et al., 1964; Jha, et al., 1985; Raines and Ross, 1995; Wei, et al., 1995; Wei, et al., 1996; Setchell, 1998). Atherosclerosis plaque is considered to be the primary cause of cardiovascular disease and stroke. In one study, when subjects consumed soy daily for 6 months, cholesterol oxidation was about 50% lower in comparison to the control group (Raines and Ross, 1995; Setchell, 1998). This lends credence to the theory that, when daidzein and genistein exist in the diet as free isoflavones, they are absorbed in the upper portion of the intestine, and the colon cells are only exposed to blood concentrations of the isoflavone (Setchell, 1998; Setchell and Cassidy, 1999). In contrast, some studies have demonstrated otherwise that when the diet contains either the glucosyl or the malonyl-glucosyl form, the isoflavones are not absorbed in the upper intestine but travel to the colon where bacteria hydrolyze them into free isoflavones. The colon cells are then exposed to the isoflavones as the isoflavones are absorbed and as they circulate in the blood they carry out anti-angiogenic or anti-antherogenic activities (Schweigerer, et al., 1992; Fotsis, et al., 1993; Setchell, 1998).

The mechanism of the lipid-lowering activity of soy protein may have other possible explanations. One of these theories states that because soy protein is much richer in L-arginine than is animal protein, which is richer in L-lysine, dietary increases in L-arginine is accompanied by decreases in cholesterol levels (Setchell, 1998; Setchell and Cassidy, 1999). High intakes of L-arginine could enhance endothelial-dependent vasodilation and nitric oxide (NO) production. This could contribute to the possible anti-atherogenic activity of soy protein on cholesterol. As phytochemicals, soy protein and isoflavones have two important effects on reducing heart disease: first, soy protein and its isoflavone reduce the risk of heart disease by lowering total blood cholesterol and, in turn, lower low density lipoprotein (LDL), and increase or have no effect on high density lipoprotein (HDL). Second, they synergistically inhibit the oxidation of LDL cholesterol which is the first step in the accumulation of artery-clogging plaque. Some laboratory studies have shown how genistein in soy helps prevent blood clots and vessels from forming (Setchell, 1998).

Oral estrogens have been shown to decrease total cholesterol and LDL-cholesterol. Thus, the soy isoflavones may have similar actions. Interestingly, a few studies have shown that when the isoflavones are removed from the soy protein, the protein itself has little hypocholesterolemic activity (Setchell, 1998; Barnes, et al., 1990). Soy isoflavones themselves do not have the same hypocholesterolemic activity as the combination of soy protein and soy isoflavones (Setchell, 1998). In conclusion, there are probably synergistic effects of these substances that are not well understood at this time but hopefully research will continue to throw light on these issues.

2.4.4 Controversy with Soy Protein and Isoflavone Effects on Health

The scientific literature is not without inconsistencies or controversies, and soy is no exception. It is quite evident that soy products do reduce the risks of developing various age-related chronic diseases, and epidemiologic data strongly suggest that populations that regularly consume soy products have reduced incidence and prevalence of the aforementioned age-related conditions and diseases than populations that eat very little soy. The subject of what specific components is responsible for the plethora of reported health benefits of soybean remains a strong controversial issue, as the scientific community continues to understand what component(s) in soy is / are responsible for its health benefits.

Ongoing controversy about soybean and soy product effects on health focuses on the extent of bioavailability of isoflavone glycosides, the biological activity of the individual glycosidic conjugates of isoflavone (Setchell, 1998), mechanism of intestinal absorption of isoflavones in humans, and what component exerts most of the above discussed effects. Evidence from intestinal perfusion and *in vitro* cell culture studies indicates that isoflavone glycosides are poorly absorbed, yet isoflavones are bioavailable and appear in high concentrations in plasma, irrespective of whether they are ingested as aglycones or glycoside conjugates (Setchell et al., 2002). Disagreeing reports on the health effects of soy consumption due to the estrogenicity of isoflavone constituents have been reported (Tsangalis et al., 2002).

Further controversy is fueled by concerns regarding adverse effects on cognitive function (White et al., 2000), reproductive abilities (Cassidy et al., 1999; Martini et al., 1999), breast cancer risks (Wrensch et al., 1993; Petrakis et al., 1996; Hargreaves et al., 1999; Allred et al., 2001), and increase in sodium preference by genistein (Scallet et al., 2003) have all been examined. Setchell and his colleagues (2002) found that specific and sensitive electrospray mass spectrometry failed to detect even traces of daidzin or genistin in plasma collected 1, 2, and 8 h after their ingestion as pure compounds in a soy food matrix. However, when a soy food matrix was combined with enzyme preparations, plasma was enriched in isoflavones that were hydrolysable, implying that isoflavone glycosides are not absorbed intact across the enterocyte of healthy adults, and their bioavailability requires initial hydrolysis of the sugar moiety by intestinal beta-glucosidases for uptake to the peripheral circulation.

Xu et al. (2000) counteracts the bioavailability concept in his study that isoflavone bioavailability may not be affected by choice of background diet or food sources of isoflavone. Coward et al. (1998) emphasized that the chemical form of isoflavones in foods should be taken into consideration when evaluating their availability for absorption from the diet, because total isoflavone content is not altered by cooking method or temperatures around 190^{o}C. However, a steady increase in beta-glucoside conjugates at the expense of 6OMalGlc conjugates occurs.

Not only is bioavailability an issue but metabolism and disposition of isoflavones are also affected by duration of soy ingestions and gender (Lu and Anderson, 1998). According to Lu and Anderson, (1998), elimination rates for genistein, daidzein, and equol (a daidzein metabolite) differ in women and men. Thus, the excretion half-life values of genistein is longer in women (7 - 9 h) than in men (4 - 5 h) after first soy ingestion, but then shortens progressively in women and lengthens in men.

In Asian countries, where prevalence of heart disease or tumor disorders is relatively low, people consume soy foods in amounts that provide an estimated 20 to 200mg of soy isoflavone daily. The recommended 25g of soy a day proposed to have blood cholesterol lowering effects contains approximately 43 - 45mg of isoflavones. Hence, it can be assumed that, this equivalent amount of isoflavone in 25g of soy protein seem to be adequate in providing some amounts of soy isoflavone intake per day. Because isoflavones are weak estrogens, eating too much also more than 100mg a day is believed to be very dangerous and could increase the risk of cancer. Therefore, the suggested safe upper limit is 100mg/day. While, the current recommendation inadequately addresses the potential causative effect of soy protein alone on various biological factors including heart disease, there is limited research evidence to establish a strong relationship of isoflavone alone with these health benefits. Recent studies now point to a synergistic cholesterol lowering effect of protein and isoflavone, because these and any other biological effect require the soy protein and its isoflavone to act synergistically to support the proposed health claims (Setchell et al., 2001; Setchell et al., 2002; and Setchell and Cole, 2003).

2.4.5 Other Bioactive Compounds in Soybeans

Apart from isoflavone and protein, there are also other substances associated with soy protein and its lipid lowering activity. These include soy saponins, hemaglutinins, trypsin inhibitor, phytic acid, phenolic compounds and other bioactive peptides.

Though some studies have reported saponins to be responsible for an undesirable bitter and astringent taste (Kitagawa et al., 1988; Taniyama et al., 1988; Okubo et al., 1992), they are, however, associated with hypercholesterolemic and anti-carcinogenic effects when ingested. Saponins are reported to affect blood cholesterol by elevating bile excretion. Thus, speculations are that soy saponins in soy protein based diets mediates cholesterol lowering due to the rapid interaction with protein (casein) resulting in the formation of an insoluble complex, unlike the slow interaction of soy saponins with casein. But, the hypocholesterolemic effect of soy protein as a result of saponin interaction in inconclusive (Tsukamoto et al., 1995).

Phytic acid known for its mineral binding capabilities in the gut (Messina and Barnes, 1991) chelates both divalent and trivalent metals including iron, calcium, zinc, and magnesium, thus decreasing their absorption. By modulations of the zinc to copper ratio in the blood, phytic acid is speculated to play a cholesterol lowering effect associated with soy protein. Copper deficiency or high zinc to copper ratio in humans is associated with elevated blood cholesterol and in the gut, zinc and copper share the same carrier (Messina and Barnes, 1991). Since diets containing soy are rich in both phytic acid and copper, when ingested, zinc is chelated by phytic acid causing more copper to get absorbed resulting in decreased zinc to copper ratio in blood, and thereby mediates low plasma cholesterol concentration (Messina and Barnes, 1991). Little is really known about dietary phytic acid effects on human blood lipid profiles. But some research studies have indicated that in addition to the possible lipidemic effect of phytic acids, chelation of iron in the gut results in decreased absorption, thereby suppressing oxidative damage to lipids and protein in blood and decreasing initiation of atherosclerotic lesion formation (Messina and Barnes, 1991).

Trypsin inhibitors also display anticarcinogenic properties (Messina and Barnes, 1991), and stimulate gastrointestinal secretions including cholecystokinin (CCK) that, in turn, stimulates contraction of the gall bladder and bile secretion into the intestinal tract. Topical applications of soybean trypsin inhibitors or Bowman-Birk inhibitor from non-denatured soymilk have also been implicated in inhibiting the formation and growth of skin tumors (Huang et al., 2004). One scientific postulation is that trypsin inhibitors may play a role in cholesterol lowering because adequate amounts of relatively heat-stable Bowman-Birk inhibitor, a serine protease inhibitor with anticarcinogenic activity (Wang et al., 2002) are present in soy products. However, some studies have also indicated otherwise as not being responsible for a hypocholesterolemic effect.

CHAPTER 3. DEVELOPMENT AND PROCESS OPTIMIZATION OF PROTEIN-ISOFLAVONE ENRICHED (PIE) SOYMILK

3.1 INTRODUCTION

Soymilk is produced from either non-genetically modified (N-GMO) or transgenic raw materials. The soybean seeds are soaked, ground, heated and filtered to produce fluid milk that is a good substitute for cow's milk. According to the FDA (2003), plain, unfortified soymilk is an excellent source of high quality protein and B vitamins. Protein (approximately 40%) in soybeans contributes to the largest portion of total dry matter (Liu, 1997). However, sulfur-containing amino acids including L-cysteine are deficient in soybeans (Liu, 1997). In the diets of affluent societies, animal protein sources rich in the limiting amino acids from soybean complement protein quality. In developing countries, sources rich in amino acids from other cereal grain groups complement the protein quality.

The germ or hypocotyl makes up approximately 2% of the whole soy bean, yet contains the highest concentration of isoflavone (kodou et al., 1991), and is similar in protein content (38-42%) to the whole bean (Cowan, 1973; Weingartner, 1987). According to Schryver (2002), soy germ is not used in soymilk production due to its insoluble carbohydrate content and low proportion in the whole beans. However, some reports indicated that variable small amounts of soy germ in conjunction with isoflavone extract from soy germ or other technologies have been utilized in the production of soymilk (Richelle et al., 2000). Prior work by Tsangalis et al. (2002, 2003, 2004*a*) utilized bacteria (*Streptococcus sp., Lactobacillus sp., bifidobacteria sp.)* in the production of isoflavone rich soymilk via fermentation to hydrolyze isoflavone glucosides into bioactive and bioavailable aglycones. Sherkat et al. (2001) also reported that enzyme-hydrolyzed or fermented soymilk had higher isoflavone content than commercial soymilks / beverages. But overheating (greater than or equal to 100^{o}C) could lower isoflavone levels in soymilks prepared according to traditional methods that involve boiling the aqueous extract for 20-30 min.

Researchers have reported different and, in some cases, similar results on the effects of processing on quality, composition, and other factors in soymilk (Kwok et al., 1998; Kwok et al., 2002). Color and flavor are affected by extreme heat resulting from maillard reaction decreasing the quality of the soymilk (Kwok et al., 2002). Changes in available nutrients such as amino acids may or may not be affected depending on temperature and heating time (Kwok et al., 1998; Kwok et al., 2000, Kwok et al., 2002).

Iwuoha and Umunnakwe (1997) demonstrated that processing method, storage temperature and storage duration have significant combined effects on the proximate chemical composition, physicochemical and sensory attributes of soymilk. According to these authors, soymilk produced from flour produces better nutritional profile and more desirable physicochemical properties than milk produced from wet blanched beans. However, liquid soymilk produced from the traditional wet methods, are most stable in sensory attributes when stored at very low temperatures. In general, the most affected parameters of soymilk include protein, fat, fiber, viscosity and flavor, while the least affected are moisture, carbohydrates, specific gravity and mouthfeel. In addition, enzymatically treated soymilks with hydrolytic enzyme (Rosenthal et al 2003), have been shown to have enhanced soymilk flavor. In some instances, the development of novel fermented soymilk with probiotic bacteria does alter proximate chemical composition, physicochemical and sensory attributes of soymilk. But, such novel fermented products sometimes caused undesired secondary effects such as microbial translocation and animal weight loss (LeBlanc et al., 2004).

The antinutrient factors, trypsin inhibitor, lipoxigenase, saponins and phytic acids, are destroyed or inactivated at an elevated temperature (Geronazzo et al., 1998; Kwok et al., 2002). The inactivation of antinutrient factors to the acceptable levels for soymilk in the ultra high temperature (UHT) processing at 135^{o}C for 2 seconds, makes it possible to also simultaneous destroy harmful microorganisms (Geronazzo et al., 1998). No previous or known studies have investigated the incorporation of soy germ alone in the production of unfermented soymilk. Since no known study has actually investigated the possibility of simultaneously enhancing protein and isoflavone in soymilk using food grade soy germ in the absence of fermentation, use of hydrolytic enzymes, extracts or the combination of these, the present study was, therefore, undertaken to specifically investigate an optimal production process for developing a novel protein-isoflavone enriched (PIE) soymilk using the traditional production approach with soy germ incorporated into soymilk.

3.2 MATERIALS AND METHODS

3.2.1 Experimental Design

The study consisted of a 12x4 experimental processing conditions with two soymilk formulations. One formulation was prepared with 75% whole beans and 25% soy germ, and the second (control) with 100% whole soybeans. The first factor - processing method, consisted of four processes by which germ was either incorporated or not incorporated into soymilk. These included the control (WSB/100), process 1 (P1) with soy germ (SG) added at the soaking step; Process 2 (P2) in which germ was incorporated at the grinding step, and process 3 (P3) had soy germ added at the homogenizing and pasteurizing step (Figure 3.1). Using P1, P2, and P3 processes allowed soy germ to simultaneously undergo processing along with the whole beans.

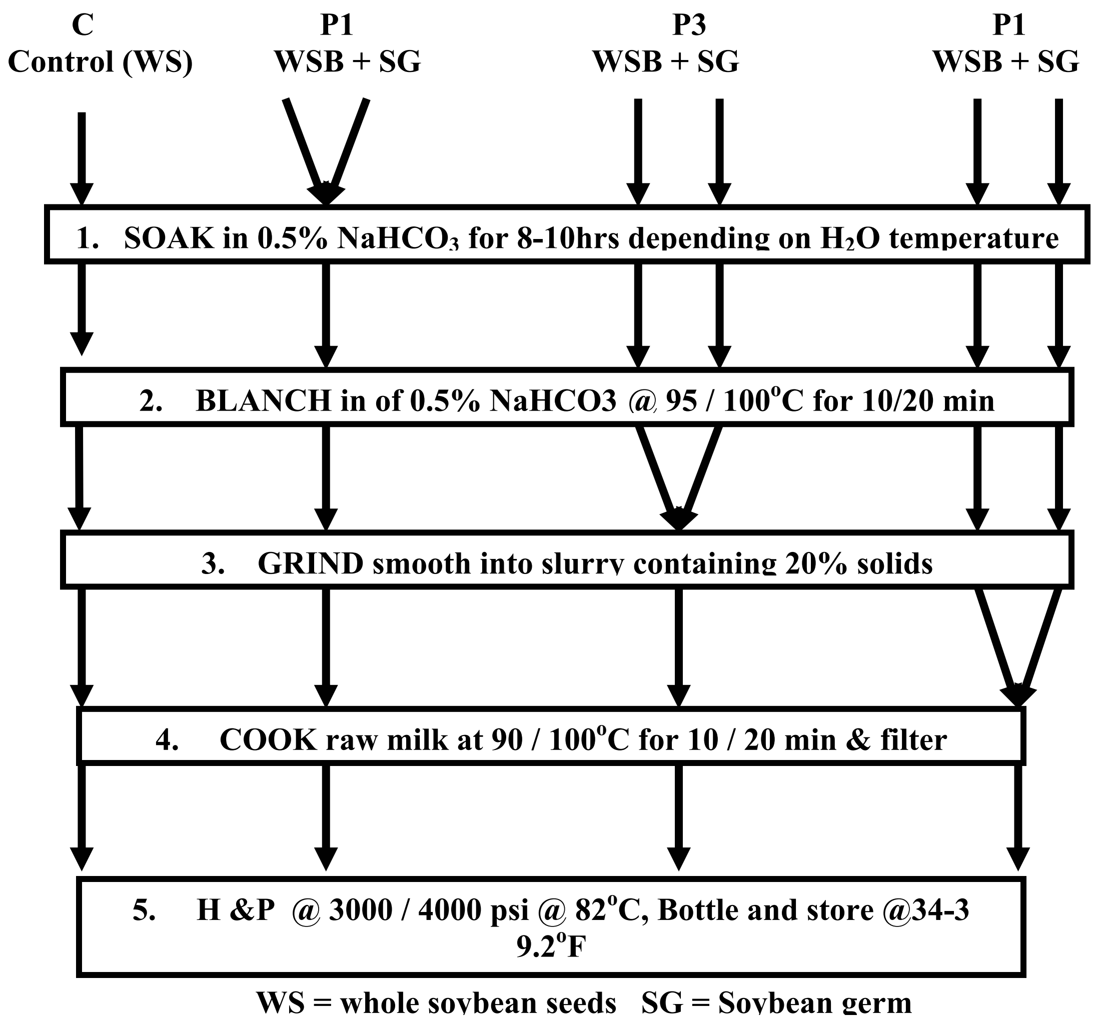


Figure 3.1 Main Soymilk Production Stages

The second factor - the twelve processing treatments (T1-T12), comprised of a combination of two levels of blanching, cooking and pasteurizing temperatures (90°C and 100°C), two levels of heating/blanching time (10 and 20 minutes), and two levels of homogenizing pressures (3000 and 4000 psi) (Table 3.1 and 3.2). Processing conditions of 90°C, 10 minutes cooking, 3000 psi was referenced as 'low processing conditions', 100°C, 20 minutes, and 4000 psi as high processing conditions, and a combination of low and high processing condition as medium processing conditions (Appendix 3.1b). Commercial silk soymilk (Silk Soy

Table 3.1a Processing Condition (Time, Temperature and Pressure) Chart

Trt #	Blanching Time (min)	Temp °C	Cooking Time (min)	Temp °C	Pasteurizing Pressure (psi)	Temp °C
Block 1						
T1	10	100°C	10	100	3000	100°C
T2	20	90°C	20	90°C	3000	90°C
T3	10	100°C	10	100°C	4000	100°C
T4	20	90°C	20	90°C	4000	90°C
Block 2						
T5	10	90°C	10	90°C	3000	90°C
T6	20	100°C	20	100°C	3000	100°C
T7	10	90°C	10	90°C	4000	90°C
T8	20	100°C	20	100°C	4000	100°C
Block 3						
T9	10	100°C	20	90°C	3000	90°C
T10	20	90°C	10	100°C	3000	90°C
T11	10	100°C	20	90°C	4000	90°C
T12	20	90°C	10	100°C	4000	90°C

Table 3.1b. Explanation of Processing Condition (Time, Temperature and Pressure) Chart

TRT	Blanch Time (min)	Blanch Temp °C	Cook Time (min)	Cook Temp (°C)	H&P Temp (°C)	H&P Pressure (psi)
Block 1						
T1	Short	High	Short	High	High	Low (SHL_p)
T2	Long	Low	Long	Low	Low	Low (LLL_p)
T3	Short	High	Short	High	High	High (SHH_p)
T4	Long	Low	Long	Low	Low	High (LLH_p)
Block 2						
T5	Short	Low	Short	Low	Low	Low (SLL_p)
T6	Long	High	Long	High	High	Low (LHL_p)
T7	Short	Low	Short	Low	Low	High (SLH_p)
T8	Long	High	Long	High	High	High (LHH_p)
Block 3						
T9	Short	High	Long	Low	High	Low ($SHLLHL_p$)
T10	Long	Low	Short	High	Low	Low ($LLSHLL_p$)
T11	Short	High	Long	Low	High	High ($SHLLHH_p$)
T12	Long	Low	Short	High	Low	High ($LLSHLH_p$)

soymilk, Boulder, Colorado, USA) commonly sold in supermarkets in the U.S. was used as a reference against our samples for analytical evaluation of physicochemical components except for processing effects on these components. This commercial soymilk was, therefore, referenced as T13. T -1, 2, 5, 6, 9, 10 were subjected to 3000psi homogenizing pressure using a lab scale homogenizer (Model 31M, Gaulin Corporation, Everett, MA, U.S.A) and T – 3, 4, 7, 8, 11, and 12 to 4000psi pressure. T 9, 10, 11, and 12 utilized medium processing conditions of low and high temperature-heat combinations.

Two experimental replicates / batches were done, and duplicate samples from each batch of soymilk collected for physiochemical quantification of protein, isoflavone, fat, moisture, total solids, ash, soluble sugars / ^{o}brix, color, and viscosity. Selection of optimal process or method of incorporating soy germ in soymilk was primarily based on isoflavone and protein contents as well as viscosity data. The selected process or production condition was further utilized in subsequent studies (Chapter 4).

3.2.2 Soymilk Ingredients

Initial 30kg of non-GMO whole soybeans was donated by SB & B Foods, Inc (Casselton, North Dakota, USA) and, additional soybeans of the same variety were obtained from the same company later. The macronutrient composition of the dry soybean seed was approximately 40g protein, 16g fat, 25g carbohydrate, moisture 11% and other 8% per 100g. Soy germ (SG) was donated by Acatris / Schouten USA Company (Minnesota, USA).

3.2.3 Production of Soymilk

Soymilk containing 75:25% whole bean to germ and 100% whole bean seeds were produced by **soaking** dried material in 0.5% $NaHCO_3$ (Sodium bicarbonate, USP Grade 1, Church and Dwight Co., Inc. NJ, U.S.A) solution followed by **blanching** in 0.5% $NaHCO_3$ solution in a steam jacket kettle (Model F 20 40 WP, Groen Manufacturing company, Chicago, U.S.A) with stirring for either 10 or 20 minutes at 100^{o}C or 90^{o}C, then g**round** into slurry with approximately 17% solids content using a warren blender (Model CB, Warring Products Co., Winsted, Connecticut, U.S.A) till finely ground. Thereafter, raw soymilk was **cooked / heated** for 10 or 20 minutes at 100°C or 90°C (See Appendix B, 3.1.a and 3.1.b). The resulting fluid milk was first **sieved** through a 425µm (0.0165 inches) mesh sieve (U.S.A Standard Test Sieve, No. 40, ASTM E - 11 Specification, Fisher Scientific Company, Ohio, U.S.A), then a second time using the same sieve mesh size but lined with cheese cloth (Grade BSC 1153, 140µm, 28x24 threads/square inch, Nelson Jameson, Inc. Marshfield, WI, U.S.A). The filtrate was **homogenized** using lab scale homogenizer (Model 31M, Gaulin Corporation, Everett, MA, U.S.A) **and pasteurized** using plate heat exchanger (APV pasteurizer, Model JR S\S, APV company, Tonawanda, NY, U.S.A), then rapidly cooled to 4.4°C in a cooling tank constructed with cold wall that chills milk as it enters the tank through a pipeline directly from the pasteurizer. The pasteurized cold soymilk was dispensed / collected in sterile half gallon bottles. Ninety six bottles of soymilk were stored at 34°F for pH readings and other analytical quantification.

3.2.4 Physicochemical Analysis

Approximately 250ml aliquots from each bottle of milk obtained under aseptic conditions was freeze dried using a VirTis freeze drier (Model - Genesis 35XL, Virtis an SP Industries Company, Gardiner, NY,USA) for protein quantification (Nitrogen amount X 6.25) and isoflavone analysis. Moisture, fat, ash, total solids and pH were analyzed using acceptable standard methods and equipment, and protein (nitrogen content x 6.25) was done using AOAC method no. 992.23. Freeze dried samples were used only for nitrogen, ash and isoflavone analyses while liquid sample was used for all other analysis. Results obtained on a dry basis were calculated back to wet weight as mg/100g for isoflavone and g/100g for protein. Therefore, final results are presented as wet weight for protein and isoflavone contents.

a) Proximate analysis - Nitrogen analysis was performed commercially by the Agronomy lab at Louisiana State University using the PF-428 Nitrogen Analyzer (Model No. FP-428, Leco Corporation, St. Joseph, MI, U.S.A.) and AOAC method No. 992.23. Nitrogen results were multiplied by 6.25 to give protein equivalent.

b) Moisture and total solids - were determined using the Smart System 5 CEM Moisture/Solids Analyzer (LabWave 9000™ or AVC-80, CEM Corporation, Mathews, NC). An analytical method for soymilk was developed based on suggested Moisture/Fat – Dilution protocol from CEM Corporation. Based on solid contents of milk samples, five to seven grams of liquid sample was smeared on a round pad, and then microwave dried in the Moisture/Solids Analyzer for 3 – 5 minutes. Dried sample pads were automatically re-weighed and results provided either as % moisture or total solids.

c) Fat - was determined using the CEM Fat Analysis System (FAS-9001 (CEM Corporation, Mathews, NC) in conjunction with the CEM Moisture/Solids Analyzer (LabWave 9000™ or AVC-80). Fat extraction and analysis utilized methylene Chloride in the FAS-9001 system. Upon completion of the moisture analysis, pads containing dried samples were removed and transferred to the FAS-9001 system where fat extraction with methylene Chloride was done. Extraction, washing and vacuuming lasted 7 minutes. At the completion of fat extraction, the pad with the extracted sample was removed and returned to the Moisture/Solids Analyzer where it is re-dried (removal of any moisture / solvents), re-weighed and percent fat calculated by difference.

d) Total solid - was determined by difference after moisture analysis by the CEM Moisture/Solids Analyzer and values expressed as g/100g or %.

e) Soluble solids / ^{o}brix - was measured by specific gravity using the lab bench top digital refractometer (Model RFM 80, Kerno Instruments Co. Inc., Elpaso, Texas, USA). With samples equilibrated at 25^{o}C prior to taking measurement, 1 ml of each sample was poured on refractometer prism and readings taken. Values were expressed as ^{o}brix.

f) Color - was measured using the CIE L*, a*, b*, c*, h* and ΔE values with a 5100 LabScan (Hunter Color Lab, Fairfax, VA) and Minolta CM-508d color spectrophotometer (Minolta Co., Ltd. New Jersey, USA) attached to a computer containing the Wingather color software. Color evaluation was done on all products equilibrated to room temperature (25^{o}C). Milk samples were thoroughly shaken, poured out into a 2-oz (60 X 15 mm / 59ml) multi-use translucent petri dish and placed on the surface of the spectrophotometer. Six data points representing one sample replication were taken. Measurement of CIE L*, a*, b*, c*, h* and ΔE* at 10^{o} observer angle with D65 illumination in the wavelength of 400-700nm was taken with spectral components excluded. Port size was ¼ inch. Degree of whiteness was calculated as:

$$\text{Whiteness} = 100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{1/2}.$$

g) Viscosity - was measured in centipoise (cP) using a Brookfield DV II+ viscometer (Brookfield Engineering Lab Inc, Stoughton, MA) and a #5 disc spindle. 1000ml of each sample was poured into 6.0 inches depth sterile cups and spindle submerged into the milk. A helipath bar lowered the spindle up and down at a speed of 50 rpm taking measurements at various depths. All samples were equilibrated to 35^{o}F prior to measurement, implying that viscosity of the soymilk is measured at that temperature. With the rotating spindle submerged into the milk, an average reading of 25 data points representative of one sample replication was collected.

h) pH measurement - was performed to predict quality and shelf life stability and possibly microbial activity of the products. Measurements were done using a hand held pH/mV/Temperature meter (Model IQ240. IQ 150 / 240 Scientific Instruments, Inc. San Diego, CA, USA) attached to a stainless steel pH/Temperature probe at 1, 14, 28 and 60 days post pasteurization. Prior to taking measurements, a three point calibration with three buffers - pH7.0, pH4.0, and pH10.0, was performed.

3.2.5 Isoflavone Standards and Analysis

Aglycone standards of genistein, daidzein and glycitein, and β-Glucoside isomers of genistin, daidzin and glycitin (all synthetic) were purchased from Indofine Chemical Co. (Somerville, NJ, USA) for use in soymilk isoflavone analyses. Acetyl- and malonyl- forms unavailable as pure standards, therefore calculation of their concentrations assumed unity responses in molar absorption with the respective β-glucosides. Identification by comparison of UV absorption and retention time patterns with published results was used to verify the acetyl- and malonyl- forms. Mixed and single isoflavone standards used in the analysis of isoflavones in soymilk were prepared as described by Tsangalis et al. (2002) with some modifications. Six mixed standards containing daidzin, genistin, glycitin, daidzein, genistein and glycitein (0.32mg/L or 0.32ppm) were used for the quantification of isoflavones. Single standards were prepared for peak identification and the retention times (min) of isoflavone isomers.

(a) Reversed-phase HPLC Apparatus and Reagents

Chromatographic analyses was carried out on the Waters Alliance 7000 series high performance liquid chromatograph (Waters Corporation, Milford, MA, USA) with auto-sampler 2690, solvent delivery system (9010), the dual absorbance photodiode array ultraviolet-visible (UV/VIS) detector (2487), all attached to computer with millennium 32 software. A Hypersil

5μm C18-ODS - 250 x 4.6 mm diameter, (Thermo Electron Corp, Bellefonte, PA, USA) reversed-phase column was used to separate isoflavone isomers. HPLC grade methanol and acetonitrile were purchased from Sigma-Aldrich (Sigma-Aldrich Corporation, St. Louis, MO, USA). All reagents used for isoflavone extraction and HPLC analyses were filtered through a 0.5 μm membrane (Millipore, Bedford, MA, USA) and mobile phases sparged with helium.

b) Extraction and HPLC Analysis of Isoflavones in Soymilk

A gram of freeze dried milk samples was used for the extraction of the isoflavone isomers malonyl-, acetyl-, β-glucoside and aglycone forms, as described by Xu et al. (2001); Tsangalis *et al*. (2002); and Wang and Murphy, (1994), with modifications. Isoflavone calculations are based on 1.0g freeze dried sample weight. Samples were extracted in 80% methanol heated at 80^{o}C and simultaneously shaken at 100u/min r.p.m in water bath (Polystat Water bath – Model 12050-00, Cole-Palmer Instrument Company, Chicago, Illinois) for 60 min. All samples were immediately filtered through a No. 1 Whatman filter paper (Qualitative circles 150mm Dia, Whatman International Ltd, England) into disposable tubes, then centrifuged (Hermle Labnet model Z383K. Hermle Labotechnik, Wehingen, Germany) at 3000 x g for 20 min, and the mobile phase evaporated under a stream of nitrogen at 60^{o}C to complete dryness using a Pierce nine-needle evaporating unit (CentriVap Console Labcocono - Pierce Biotechnology Inc., Rockford, Ill., U.S.A.). Milk samples were extracted and analyzed in batches over consecutive days with all samples from the same batch ran together.

Prior to injecting into column, sample extracts were re-suspended in 1 ml of methanol, vortex-mixed (Model type 37600 mixer series, Thermolyne Corporation, Dubuque, Iowa, USA) and centrifuged (Hermle Labnet model Z383K, Hermle Labotechnik, Wehingen, Germany) at 3000 x g for 10 min. Approximately, 300μL of supernatant of each of the 96 milk samples was transferred into a clean HPLC vial and analyzed for their aglycone and glucoside concentrations using Reverse Phase-HPLC. HPLC gradient elution consisted of glacial acetonitrile (solvent C) and water containing 10% acetonitrile (solvent D), set at a flow rate of 0.8ml/min. After 25 µL injection of sample into the column (25 °C), solvent D (water containing 10% acetonitrile) was set at 100% for 2 min, then, reduced to 80% over 20 min, followed by 50% for 10 min, and finally increased to 100% over 5 min prior to the next injection. Total analysis time was 37 min.

The diode array UV/VIS detector was set at 254 nm and the retention times (min) of the 6 mixed isoflavone isomers were as follows: daidzin, 14.06; glycitin, 14.65; genistin, 18.77; daidzein, 26.3; glycitein, 26.94; and genistein, 30.4 (Figure 3.2). Retention times for aglycone

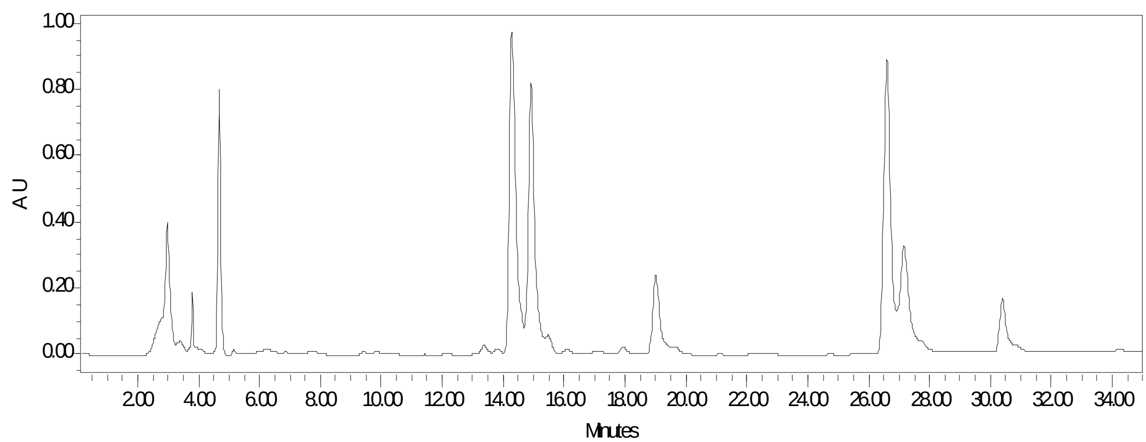


Figure 3.2 Chromatogram of 6 mixed Isoflavone Standards

and β-glucoside isoflavone isomers were determined using mixed standards, and those of malonyl- and acetyl-glucoside isomers were based on the retention times reported by Xu et al. (2002) under similar HPLC conditions. Quantification of isoflavone isomers in soymilk was as per Xu *et al*. (2002) based on retention times of the glucosides and aglycones matched with reference standards. Malonyl and acetyl conjugates were calculated based on the unity responses in molar absorption with the respective β-glucosides or identified by comparison of UV absorption and retention time patterns with published results. Isoflavone concentrations are expressed as mg / 100g of soymilk calculated back to wet basis as per Tsangalis et al. (2002).

3.2.6 Statistical Analysis

Treatments and processes were analyzed in a complete block design procedure. Data for proximate analysis were analyzed using ANOVA at 95% confidence intervals. Before testing of treatments by ANOVA, the data were tested for normal distribution and homogeneity of variance. Results were reported as mean ± standard deviation of duplicate samples from two replications. Mean value differences were determined by the Tukey's Studentized range test at $P \leq 0.05$. Differences among treatments were analyzed using proc mixed procedure and comparison between processes calculated using paired t-test method. All data were analyzed using the SAS (SAS Institute Inc., Cary, NC).

3.3 RESULTS AND DISCUSSIONS

3.3.1 Proximate Composition

The mean (± standard deviation) proximate composition of P1, P2, P3, and Control (n = 96) in Table 3.2, showed no significant differences ($P>0.05$) between processes for soluble sugars / ^{o}brix (P=0.39), total solids (*P=0.46)*, ^{o}Brix (*P=0.39)*, moisture (P=0.46), ash (*P=0.9)*, viscosity (P=0.68), protein (P=0.46), and isoflavone (*P=0.5)*, except fat (*P=0.01*). P1 contained the highest isoflavone amount (21.7 mg/100g) which was 1.2 times and 1.6 times higher than control (19.4mg/100g) and commercial silk soymilk (13.49mg/100g), respectively. Similarity in protein content of the control (3.06g/100g) and the three processes was observed. Protein content in the control was followed by P1 (3.0g/100g), P3 (3.02g/100g), P2 (2.99g/100g) and commercial soymilk with 2.06g. Nonetheless, it seemed that the effect of soy germ incorporated into soymilk was more pronounced with isoflavone content, but not necessarily with protein content. It also seemed that regardless of soy germ being added, protein content in particular declined with increase in moisture content. Thus and overall, it did not appear that processing significantly influenced composition and isoflavone contents in soymilk.

With regards to the reason for this investigation, i.e. increasing the protein and isoflavone content so consumers can drink less amount of soymilk to meet the FDA recommendation of at least 6.25 g of protein per serving for a total of 25g per day, and 40- 50mg of isoflavone per day to meet the recommended heart health claim, these soymilk beverages do meet the above recommendations in fewer servings (254g = 1 serving). Consumption of the commercial silk soymilk containing 2.06g protein and 13.5mg of isoflavone will require consumers to consume 4 servings of soymilk to meet protein requirement and 1.5 serving to meet isoflavone claim.

Table 3.2 Mean Proximate Composition (%) of Soymilk by Processing Methods

Process Methods	Fat %	Total Solid %	°Brix	Moisture %	Ash %	Viscosity cP	Wet Protein g/100g	Total ISF mg/100g
P1	1.01ab (0.09)	***4.63a*** (1.18)	***4.58a*** (1.78)	***95.37a*** (1.18)	***0.15a*** (0.04)	***86.33a*** (71.19)	3.00a (0.15)	***21.68a*** (5.78)
P2	***0.99b*** (0.14)	4.72a (1.15)	5.05a (1.36)	95.28a (1.15)	0.15a (0.03)	102.90a (83.52)	***2.99a*** (0.17)	21.09a (7.46)
P3	1.00ab (0.11)	4.88a (1.06)	5.08a (1.63)	95.13a (1.06)	***0.15a*** (0.03)	***107.96a*** (71.85)	3.02a (0.15)	20.67a (4.31)
C	***1.08a*** (0.11)	***5.10a*** (1.01)	***5.29a*** (1.31)	***94.90a*** (1.01)	0.15a (0.04)	91.90a (56.51)	***3.07a*** (0.18)	***19.45a*** (5.08)
Silk	1.12a (0.18)	8.32a (0.45)	9.50a (0.00)	91.69c (0.44)	0.26a (0.00)	***40.00***c (0.23)	2.06b (0.49)	13.49a (4.79)
Range	0.09	0.46	0.71	0.46	0.005	21.63	0.07	2.23

ISF = Isoflavone; Numbers in parentheses represent standard deviation; Range = the highest score minus the lowest score. [a, b, c] Means (SD) within the same column followed by different letters are significantly different (P<0.05). P1 = germ added at the soaking stage; P2 = germ added at grinding stage; P3=germ added at homogenizing and pasteurizing stage; C=no germ added; and Silk = commercial soymilk.

Amounts from either three processes will meet isoflavone needs in one serving and protein between 3 and 3¼ servings. Although the number of serving achieved was not exceptionally low, but they certainly make a difference in the amount of milk to consume without getting tired of drinking soymilk.

Mean fat values ranged from 0.99 – 1.12g/100g, total solids from 4.63 – 8.32g/100g, moisture between 91.69 – 95.37 %, ^{o}Brix from 4.58 – 9.50, and ash, 0.15 – 0.26g/100g. The low fat content meets the FDA requirement which stipulates that "products should be low in fat, saturated fat and cholesterol to be eligible for this claim". The low amount of fat in the beverages technically qualifies them for the soy health claim and also classifies them as skim milk.

At 5 and 8 ^{o}brix, similar protein content between 1.97 and 3.65% have been reported by Prawiradjaja and Wilson (2002), and high amounts between 5.17 and 5.75% reported in soymilk with 12 ^{o}brix. It seems quite obvious that protein content tends to increase only with increase in total solids / ^{o}brix and vice versa. Other proximate component values are in agreement with most published data on soymilk composition. The high total solids observed in commercial soymilk but not synonymous with increased protein and isoflavone contents, can be attributed to nutritional enhancement regime such as fortification with vitamins, calcium, the addition of stabililizers, emulsifiers and liquid thinners or ingredients that resulted in the high total solids / ^{o}brix content. The addition of such components must have affected the viscosity of the commercial soymilk, since retention of more solids in soymilk beverages does correlate with increased solid / ^{o}brix content as observed in the commercial soymilk in this study.

3.3.2 Processing Effects on Protein and Isoflavone in Soymilk Beverages

The effect of variable temperature, cooking time and homogenizing pressure on soymilk composition were significant among treatments within each process, except for protein in P1, P2, P3, and control; fat in P1 and control; and isoflavone for P2 and control (Appendix B - 3.2-3.6). Results in Table 3.3 show that T4 - (LLH_p) Long cooking time (20min) in combination with low temperature (90°C) and high homogenizing pressure (4000psi) yielded the most protein content for P1 (3.12g), P3 (3.14), control (3.27), and T5 - short cooking time (10min) combined with

Table 3.3 Mean Protein and Isoflavone Contents by Treatment in each Process

TRT	P1 Protein g/100g	P1 ISF mg/100g	P2 Protein g/100g	P2 ISF mg/100g	P3 Protein g/100g	P3 ISF mg/100g	C Protein g/100g	C ISF mg/100g
T1	3.05a (0.05)	16.75bc (0.20)	3.09a (0.00)	17.55a (1.15)	3.05a (0.04)	21.02ab (2.16)	3.09a (0.00)	18.40a (1.11)
T2	3.08a (0.03)	26.40a (1.20)	3.11a (0.10)	18.41a (4.76)	3.07a (0.01)	19.75ab (0.28)	3.18a (0.11)	15.38a (2.35)
T3	3.08a (0.01)	27.34a (2.75)	3.08a (0.02)	18.23a (2.79)	3.03a (0.02)	19.74ab (4.69)	3.26a (0.15)	18.42a (0.59)
T4	***3.12a*** (0.04)	26.02a (0.29)	2.98a (0.06)	21.64a (2.66)	***3.14a*** (0.08)	***27.09a*** (1.90)	***3.27a*** (0.01)	23.65a (6.11)
T5	3.03a (0.02)	23.15ab (1.10)	***3.18a*** (0.05)	***33.97a*** (1.00)	3.12a (0.02)	20.78ab (0.99)	3.09a (0.01)	***13.58a*** (0.03)
T6	3.02a (0.00)	17.58bc (2.69)	3.10a (0.02)	28.25a (0.41)	3.11a (0.04)	20.29ab (1.97)	3.13a (0.02)	16.28a (7.42)
T7	3.03a (0.01)	13.89c (1.82)	2.86a (0.05)	31.68a (1.53)	***2.95a*** (0.01)	23.20ab (2.08)	2.97a (0.06)	17.95a (1.27)
T8	2.99a (0.01)	***13.80c*** (1.95)	***2.84a*** (0.02)	23.17a (0.11)	***2.95a*** (0.03)	24.10a (1.93)	***2.95a*** (0.01)	16.41a (2.16)
T9	***2.96a*** (0.08)	23.94ab (0.40)	2.98a (0.06)	18.69a (3.30)	3.08a (0.07)	18.50ab (2.74)	3.04a (0.04)	23.03a (1.24)
T10	***2.95a*** (0.07)	***28.64a*** (2.10)	3.00a (0.01)	16.61a (2.18)	3.00a (0.01)	***13.85b*** (0.95)	3.01a (0.08)	24.84a (1.47)
T11	3.05a (0.07)	24.60ab (0.73)	3.05a (0.01)	***13.05a*** (6.48)	3.08a (0.04)	20.94ab (0.40)	3.12a (0.04)	***26.47a*** (2.94)
T12	3.08a (0.05)	26.29a (1.99)	3.06a (0.02)	19.52a (1.96)	3.06a (0.05)	25.95a (2.43)	3.08a (0.01)	24.95a (2.10)

Numbers in parentheses represent standard deviation. [a, b, c] Means (SD) within the same column followed by different letters are significantly different (P<0.05). P1 = germ added at the soaking stage; P2 = germ added at grinding stage; P3=germ added at homogenizing and pasteurizing stage; C=no germ added.

high temperature (100°C) and low pressure (3000psi) resulted in protein yield of 3.18g for P2. The lowest protein yields were observed in P2, P3 and control from T7 and T8, and in P1 from T9 and T10. Though variations in protein content were not significantly different, but subtle differences in protein among the processes were observed. Protein value in each process was high over the commercial soymilk T13 (2.06g/100g).

According to Kwok et al. (1998), heating at 95°C over 3 hrs does not cause any changes in available amino acids. Similarly, elevated temperatures of 120 and 140°C at a short heating time give high measured values of some available amino acids, which could mean that total protein content is not affected by high temperature treatment, except in the case of temperatures at 120 and 140°C over prolonged heating time that can cause decline in available nutrients.

Mean values for the total isoflavone content by treatments within each process showed that T5 had the highest isoflavone yield in P2 (34mg), followed by T10 in P1 (28.64mg), T4 in P3 (27.09mg), and T11 in control (26.47mg). The least isoflavone contents were observed in T10, T8, T5 and T11 for P3 (13.85mg), P1 (13.80mg), control (13.58mg) and P2 (13.05mg), respectively. The lower end of the isoflavone and protein content across treatment in each process ranked similar to the concentration in commercial silk soymilk (2.06g/100g protein and 13.49mg/100g isoflavone). But, overall, isoflavone content in experimental soy beverages was about 1.2 – 2.5 times higher than in the commercial soymilk which points to process efficiency in enhancing isoflavone content in soymilk.

3.3.3 Effect of Processing Conditions (Time and Temperature) on Protein and Isoflavone Content.

For processing condition (time and temperature) effects on protein and isoflavone contents, the twelve treatments were pooled into six groups, combining the 3000 and 4000psi pressure treatments together. Results are shown in Table 3.4. Variations in protein content as influenced by heating time, and temperature was not as apparent as with isoflavone concentrations. The isoflavone yield from 10 min heating (blanching and cooking) at 90°C and 90°C pasteurizing temperature in P2 (33mg) was higher compared to yield from 20 min heating (blanching and cooking) at 90°C followed by 100°C pasteurization in P1 (27.5mg), C (25mg), and 20 min heating (blanching and cooking) at 90°C and 90°C pasteurizing temperature in P3 (23.4) that were also high but of low isoflavone content than P2. The lowest isoflavone yield was observed with 20 min heating (blanching and cooking) at 100°C and pasteurization at 100°C in P1 (15.69mg), 10 min heating (blanching and cooking) at 100, pasteurization at 90°C in P2 (15.87mg) and P3 (19.72mg), and heating (blanching and cooking) for 10 min at 90, with pasteurization at 90°C in C (15.77) that were also high but of lower isoflavone content than P2.

Table 3.4 Effect of Heating Time and Temperature on Protein and Isoflavone contents by Processing Method

Treatment	P1	P1	P2	P2	P3	P3	C	C
	Prot g/100g	ISF mg/100g	Prot g/100g	ISF mg/100g	Prot g/100g	ISF mg/100g	Prot g/100g	ISF mg/10g
10min -100 -100°C	3.07 (0.03)	22.04 (1.47)	***3.09*** (0.01)	17.89 (1.97)	3.04 (0.03)	20.38 (3.42)	3.18 (0.07)	18.41 (0.85)
20min -90 -90°C	***3.1*** (0.03)	26.21 (0.74)	3.04 (0.08)	20.02 (3.71)	***3.10*** (0.04)	***23.42*** (1.09)	***3.225*** (0.06)	19.51 (4.23)
10min -90 -90°C	3.03 (0.01)	18.52 (1.46)	3.02 (0.05)	***32.82*** (8.26)	***3.03*** (0.01)	21.99 (1.53)	***3.03*** (0.03)	***15.77*** (0.65)
20min -100 -100°C	***3.005*** (0.00)	***15.69*** (2.32)	***2.97*** (0.02)	25.71 (0.26)	***3.03*** (0.03)	22.195 (1.95)	3.04 (0.01)	16.34 (4.79)
10min -100 -90°C	***3.005*** (0.07)	24.27 (0.56)	3.015 (0.03)	***15.87*** (4.89)	3.08 (0.05)	***19.72*** (1.57)	3.08 (0.04)	24.75 (2.09)
20min -90 -100oC	3.01 (0.06)	***27.47*** (2.04)	3.03 (0.01)	18.065 (2.07)	***3.03*** (0.03)	19.9 (1.69)	3.04 (0.04)	***24.89*** (1.78)

ISF = Isoflavone; Prot = Protein; Numbers in parentheses represent standard deviation. P1 = germ added at the soaking stage; P2 = germ added at grinding stage; P3=germ added at homogenizing and pasteurizing stage; C=no germ added.
10 or 20 min = blanching time of soaked material or cooking time of raw soymilk;
90 or 100°C = Temperature used at blanching, cooking and pasteurizing steps

The apparent variations in total isoflavone content as influenced by heating time and temperatures agrees with similar reports in the literature (Wang and Murphy, 1994; Carro-Panizzi et al., 1999; Xu et al., 2002). Likewise notable effects of modern processing technologies such as enzyme-hydrolyzed or enzyme fermented soymilk, on isoflavone content have also been reported (Sherkat et al., 2001). However, overheating greater than or equal to 100^{o}C could lower isoflavone levels in soymilk prepared according to traditional methods that involve boiling the aqueous extract around 20 – 30 min (Sherkat et al., 2001).

In terms of isoflavone composition, the β-glucosides were predominantly (> 85%) present in the soymilk beverages. This is not surprising as numerous research reports have indicated that isoflavone in soymilk and tofu in particular consist almost entirely of beta-glucoside conjugates, and low-fat versions of these products are markedly depleted in isoflavones (Murphy et al., 1982; Coward et al., 1998; Murphy et al., 1999). Lower concentrations of isoflavone between 5 and 7 mg/100g in soymilk have been reported with considerable variations both in composition and isoflavone content (King and Bignell, 2000; Murphy et al. 1999). These concentrations (5 - 7 mg/100g) are even lower than the levels observed in the commercial silk soymilk. Starting materials often contain the glucosidic conjugates (Malonyl- and Acetyl- isomers) as main isoflavone forms found in soybean grains (Barnes et al., 1994), which become structurally unstable during routine processing by multiple factors including enzymes in raw soy material, additives, heating temperature and time resulting in quantitative conversion and amounts of β-glucosides (Barnes et al., 1994; Xu et al., 2002).

3.3.4 Effects of Homogenizing Pressure on Soymilk Protein and Isoflavone Content

Homogenization is a process in which milk is forced through a small opening or aperture under such a high pressure that fat globules subdivide until their diameter averages to about one tenth of the original droplet diameter. Newly formed fat globules are immediately covered with layers of milk protein which prevents them from re-forming fat globules (Kordylas, 1989). Homogenization has been reported to enhance sensory qualities in soymilk (Nelson, 1976). According to the results in this study based on homogenizing pressure (Table 3.5) used in conjunction with heating time and temperature, homogenizing pressure did not significantly affect protein content. But significant effects of homogenizing pressure were observed on isoflavone content, and the extent depended on the process used. Nonetheless, these results again revealed higher values for protein and isoflavone content than amounts in the commercial silk soymilk or protein amounts (6 - 7g/serving) claimed in labels of other brands in stores.

Table 3.5 Effect of Homogenizing Pressure on Protein and Isoflavone Contents by Treatment in each Process

TRT	P1	P1	P2	P2	P3	P3	C	C
	Protein g/100g	**ISF mg/100g**	**Protein g/100g**	**ISF Mg/100g**	**Protein g/100g**	**ISF mg/100g**	**Protein g/100g**	**ISF mg/100g**
1 + 2 **3000psi**	3.06 (0.04)	21.58 (0.7)	3.1 (0.05)	17.98 (2.96)	3.06 (0.02)	20.39 (1.22)	3.13 (0.06)	16.89 (1.73)
3 + 4 **4000psi**	***3.1*** (0.02)	***26.68*** (1.52)	3.03 (0.04)	19.93 (2.72)	3.08 (0.05)	23.41 (3.29)	***3.27*** (0.08)	21.03 (3.35)
5 + 6 **3000psi**	3.02 (0.01)	20.37 (1.89)	***3.14*** (0.03)	***31.11*** (0.70)	***3.11*** (0.03)	20.53 (1.48)	3.11 (0.01)	***14.93*** (3.72)
7 + 8 **4000psi**	3.01 (0.01)	***13.84*** (1.89)	2.85 (0.03)	27.42 (7.82)	***2.95*** (0.02)	***23.65*** (2.00)	***2.96*** (0.03)	17.18 (1.71)
9 +10 **3000psi**	***2.96*** (0.07)	26.29 (1.25)	***2.99*** (0.03)	17.65 (2.74)	3.04 (0.04)	***16.18*** (1.84)	3.02 (0.06)	23.93 (1.36)
11 + 12 **4000psi**	3.07 (0.06)	25.44 (1.36)	3.055 (0.01)	***16.29*** (4.22)	3.07 (0.04)	23.44 (1.41)	3.1 (0.02)	***25.71*** (2.52)
3000psi	3.01 (0.04)	***22.74*** (1.28)	***3.08*** (0.04)	***22.25*** (2.13)	***3.07*** (0.03)	19.03 (1.51)	3.09 (0.04)	18.59 (2.27)
4000psi	***3.06*** (0.03)	21.99 (1.59)	2.99 (0.03)	21.21 (4.92)	3.03 (0.04)	***23.50*** (2.24)	***3.10*** (0.05)	***21.30*** (2.53)

Numbers in parentheses represent standard deviation. P1 = germ added at the soaking stage; P2 = germ added at grinding stage; P3 = germ added at homogenizing and pasteurizing stage; C = no germ added.

3.3.5 Processing Treatment Effects on Viscosity

Viscosity is a critical attribute to Newtonian liquids / fluid beverages which should be just about right (not thick or watery) to be classified as fluid beverage. Cow milk has a viscosity of 18 -20 centipoise (cPs), and commercial soymilk about twice that of cow milk (40cPs – Table 3.2 and 3.6). Mean viscosity of all prepared soymilk beverages were either 2 - 3 times thicker

Table 3.6 Viscosity (cPs) of Soymilk Beverages by Treatment in each Process

TRT	P1 Viscosity	P2 Viscosity	P3 Viscosity	C Viscosity
T1	83.36bc (1.36)	96.84bc (17.14)	***180.88abc*** (43.33)	79.64c (0.85)
T2	79.68bc (9.28)	98.20b (39.99)	101.76cde (20.14)	77.84c (16.18)
T3	34.92e (1.64)	42.12bc (0.62)	63.00de (4.47)	46.00c (1.24)
T4	43.04de (3.39)	51.24bc (4.02)	***142.00bcd*** (18.33)	***183.16ab*** (30.04)
T5	31.20e (0.68)	46.52bc (0.85)	45.20e (1.24)	52.88c (2.83)
T6	28.76e (2.55)	50.64bc (1.70)	45.12e (1.36)	50.64c (7.58)
T7	***253.16)a*** (1.64	***281.64a*** (10.13)	***230.72ab*** (55.44)	***143.60b*** (23.99)
T8	***230.08a*** (16.07)	***292.84a*** (25.96)	***251.2a*** (20.93)	***224.04a*** (14.31)
T9	96.16b (2.94)	89.04bc (4.19)	76.96de (2.04)	84.80c (1.70)
T10	64.64cd (16.52)	87.68bc (4.98)	59.12de (1.70)	77.36c (7.13)
T11	57.44cde (2.04)	74.16bc (2.60)	88.04de (0.74)	50.40c (2.26)
T12	79.80bc (5.71)	86.8bc (2.83)	79.44de (23.08)	84.28c (2.43)
T13	40.00de (0.23)	40.00c (0.23)	40.00e (0.23)	40.00c (0.23)
Range	224.4	252.84	211.2	184.04

Numbers in parentheses represent standard deviation. Range = the highest score minus the lowest score. [a, b, c] Means (SD) within the same column followed by different letters are significantly different (P<0.05) than commercial silk soymilk (Table 3.6). But, the low viscosity seen in supermarket brands can be attributed to ingredients incorporated in the milk that affect the fluidity of the end product.

Very high viscosity was observed in T7 across processes that ranged between 143 and 281cp, and the same for T8 with high values between 224 and 293cp. High viscosity was also observed in T1 with P3, and in T4 with P3 and C. However, the viscosities in T1 and T4 were much lower than the viscosity observed in T7 and T8 in each process. Since P1, P2 and P3 had germ added in the soymilk, presumably resulting in increased carbohydrate content which could invariably affect viscosity or thickening of fluids, yet the ^{o}brix or total solids among these treatments (T7, T8, T1, T2, and T4) in each process (P1, P2, P3, and C) were not quite different from the other. In which case, the high viscosity observed in the beverages with these treatment regimes could be attributed to other unknown factors other than the presence of germ in soymilk, or the presence of hulls or total solid content.

Other than the high viscosity values, general fluidity was grouped as (1) <55cPs, (2) between 50 and 105cPs, and (3) > 105cPs with small overlaps. Overall, T3, T5, T6 and T4 in PI and P2, exhibited fluid viscosity <55cPs; while T2, T9, T10, T11, T12, and T1 in P1 and P2 exhibited viscosities between 55 and 105cPs, and T7 and T8 exhibited fluidity above 140cPs. Observations from other results in this study also revealed that the high viscosity in T7, T8 bore no relationship to pH, total solids or fat content in the soymilk which's value were quite similar to the other treatment conditions (Appendices 3.4 – 3.12). In cow milk, agitation at high temperatures increases fat dispersion and lower temperatures enhance clumping of fat as with whipped cream. Thus, higher fat content increases viscosity and furnishes more fat globules for clumping, while product aging increases viscosity (Bennion, 1995).

Obviously, the low fat content in all the soymilk could not have influenced the high viscosities observed in the treatments, in particular T7, T8, T1, T2, and T4. That notwithstanding, the pH results (Appendix B - 3.7 - 3.11) of the beverages evidently showed that, T7 and T8 like the other treatments, had high initial pH >7.84 and ending pH between 6.12 and 7.37 through out storage in all the processes except for T8 in P1 with an ending pH of 5.78. It is well known that pH correlates with acid production primarily caused by lactic acid bacteria that in turn causes protein to coagulate and milk to curdle. This was not observed in any of the beverages irrespective of their high viscosity values. Hence, one of two things could explain the high viscosity in beverages from T7, T8, T1, T2, and T4. One explanation could perhaps be due to the presence and growth of putrefactive bacteria such as some species of *Streptococcus and Lactobacillus genera* could have caused thickening of the beverages without influencing pH (Ihekoronye and Ngoddy, 1986), because refrigeration retarded further bacteria action and exponential growth that would have resulted in increased lactic acid production. The second explanation could be attributed to the degree of dispersion in fluid in which smaller particles show higher viscosity than one of the same concentration of larger particles.

3.3.6 pH as Determinant of Shelf-life and Beverage Quality

With regards to shelf life measured by pH, there were no significant differences (P>0.05) in the PIE processed soymilk throughout the 60day storage period (Table 3.7). Initial pH of the PIE soymilk were well above neutral (pH of 7.0) and ranged from 7.40 in control to 7.69 in P3 and 8.45 in silk soymilk. Final pH at 60 days was 6.61 for silk soymilk and between 6.0 and 6.07 for the processed soymilk and control. Throughout the 60 day period pH was high in silk soymilk as opposed to control and the three processes. The initial pH through day 28 exceeded levels considered for fresh cow milk (between 6.6 – 6.8) and even higher than that (6.8) reported in the literature for soymilk by some authors (Matsuura et al., 1989; Tsangalis et al., 2004).

Processing condition / treatment effects on pH within processes demonstrated high initial pH values generally above pH 6.28 and in some cases above neutral (pH 7.0), with varied ending pH at 60 days between pH 7.37 and pH 5.35 (Appendices 3.7-3.11). As with cow milk, the immediate cooling of milk and holding of the milk at 7°C (45°F) or below considerably slows down the rapid multiplication of any types of bacteria (Kordylas, 1989), especially pathogenic bacteria such as *Escherichia Coli*, *Staphylococcus, brucella*, bacteria causing diphtheria, typhoid fever, scarlet fever, etc. Presumably, the use of sodium bicarbonate (an alkaline compound with pH 8.3, 0.1M @ 25°C) during production was responsible for the high pH in the beverages, which invariably compensated for product quality and shelf life and retarded bacterial growth for several days.

Table 3.7 pH as a Measure of Soymilk Beverage Quality

Process Method	0-24h	14d	28d	60d
P2	7.61ab (0.82)	***7.38ab*** (0.75)	***6.92ab*** (0.72)	***6.00a*** (0.52)
P3	***7.69ab*** (0.72)	7.36ab (0.78)	6.91ab (0.87)	6.07a (0.66)
P1	7.51ab (0.70)	7.27ab (0.64)	6.89ab (0.58)	6.02a (0.49)
C	***7.40ab*** (0.64)	***7.12ab*** (0.57)	***6.85ab*** (0.53)	6.05a (0.46)
Silk	***8.54a*** (0.02)	***8.22a*** (0.01)	***7.03ab*** (0.18)	***6.61a*** (0.14)
Range	0.21	0.26	0.07	0.07

Numbers in parentheses represent standard deviation. Range = the highest score minus the lowest score. [a, b, c] Means (SD) within the same column followed by different letters are significantly different ($P<0.05$). P1 = germ added at the soaking stage; P2 = germ added at grinding stage; P3=germ added at homogenizing and pasteurizing stage; C=no germ added.

The most reduction in pH as seen in Table 3.7 occurred between 28 and 60 days in storage. But prior to the sharp decline between day 28 and 60, the drop in pH between day 14 and 28 could be explained as perhaps the growth phase where favorable conditions caused lactic acid bacteria to increase acid production with increased growth of viable cells.

3.3.7 Processing Effects on Color of Soymilk Beverages

Color is very important in foods because it imparts eye appeal, satisfies people more than flavor and the only attribute on which people can base a decision to purchase or consume (Meilgaard, et al, 1999). The CIE L*, a*, b*, C*, h*, ΔE colors and whiteness are summarized in Table 3.8. Differences in color between processes were not significantly (P<0.05) except for a*. However, significant differences were found between treatments (T1 - T12) within each process (Appendices 3.12- 3.17). Overall, color in P1, P2, and P3 compared fairly well with control and silk soymilk (Table 3.8). Mean values of each illuminant revealed high L*, and h* values above 81. CIE b* and C* values were in close range with control having higher b* and C* values over P2, P1, and P3, but lower than silk soymilk.

Table 3.8 Color Comparison of Soymilk Beverages by Processing Method

Process	L*	a*	b*	C*	H*	ΔE	Whiteness
P2	81.90a (1.58)	0.40b (0.62)	14.77a (1.20)	14.79a (1.19)	88.55a (2.60)	2.93a (1.18)	***76.61***a (1.75)
P3	***81.87a*** (1.26)	0.44b (0.66)	***14.61***a (1.66)	***14.64***a (1.65)	***88.45***a (2.99)	2.93a (1.62)	76.66a (1.51)
P1	82.14a (1.56)	***0.48***b (0.78)	14.66a (1.88)	14.69a (1.86)	88.49a (3.61)	***2.99***a (1.77)	***76.84***a (2.13)
C	***82.39***a (1.27)	***-0.03*** a (0.88)	***15.10***a (1.92)	***15.13***a (1.89)	***90.29***a (3.80)	***2.64***a (1.58)	76.76a (2.02)
Silk	***83.28***a (0.05)	***0.26***b (0.03)	***16.73***a (0.08)	***16.73***a (0.08)	***89.10***a (0.11)	***0.00***bc (0.00)	76.35a (0.10)
Range	0.52	0.51	0.49	0.49	1.84	0.35	0.23

Numbers in parentheses represent standard deviation; Range = the highest score minus the lowest score. [a, b, c] Means (SD) within the same column followed by different letters are significantly different (P<0.05). P1 = germ added at the soaking stage; P2 = germ added at grinding stage; P3=germ added at homogenizing and pasteurizing stage; C=no germ added

The color of beverages as indicated by the high h* values between 88.45 and 90.29 reflected more of a light - bright yellow color across the border. Although overall color was not significantly different among the beverages, yet L* values for the commercial soymilk was slightly higher, implying lighter color than control and the PIE soymilk beverages.

Color is one of the major detrimental factors for soymilk as affected by extreme heat resulting from maillard reaction (Yazici et al. 1997). The light deep yellow hue in P1, P2, and P3 can, therefore, be attributed to heat damage of sugars in beverages caused by a maillard reaction particularly with the incorporation of soy germ containing substantial amounts of carbohydrates and protein in addition to that from the whole beans. In addition, and unlike bisulfites and acids that inhibit enzymatic browning (browning induced by polyphenol oxidase) and non-enzymatic (maillard) reactions, the use of 0.5% sodium bicarbonate during blanching and cooking could have contributed to the slightly deep cream / yellow color of the PIE soymilk. According to Hawthorn (1981), reduction in maillard reaction can be achieved by lowering the pH of the beverages. To that end, the use of the 0.5% sodium bicarbonate imparted a high pH, which in turn must have precipitated more maillard reaction.

In modern color measurement the CIE tristimulus values a*, b*, and C* are the most important values. The CIE (Commission Internationale de l'Eclairage which is the French title of the International Commission on Light / Illumination) 1976 L*a*b* which is directly based on CIE XYZ is an attempt to linearize the perceptibility of color differences. The non-linear relations for L*, a*, and b* are intended to mimic the logarithmic response of the eye. CIE primaries are not real colors, but convenient mathematical constructs. As such, these tristimulus values uniquely represent a perceivable hue, and different combinations of light wavelengths. But, the same set of tristimulus values are indistinguishable in chromaticity to the human eye.

Therefore, coloring information is referred to the color of the white point of the system. The measurement of whiteness was very important in determining how far apart were the tristimulus values L*, a*, b*, C* since whiteness is a complex perceptual phenomenon that depends not only on the luminance of a sample but also on the chromaticity (CIE, 1995). For uniformity, the evaluation of whiteness was calculated as: $\text{Whiteness} = 100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{1/2}$.

The higher the value of whiteness, the closer to whiteness is the product. Despite the less bright yellow hue in P1, P2, and P3 compared to control and the silk soymilk, calculation of whiteness as a measure of how close the beverage colors were to white milk color indicated no significant difference ($P>0.05$) in whiteness among all the soymilk beverages and the three processes. Values for whiteness ranged between 76.35 and 76.84. Values for whiteness (Table 3.8) were in high order of 76.84 for P1, followed by control (76.76), P3 (76.66), P2 (76.61), and commercial silk (76.35), respectively.

Intra-treatment variation (**treatment effect on color*)*** in color is shown in appendices 3.12 - 3.16. Though subtle, but significant ($P<0.05$) differences in the L*, a*, b*, C, h, ΔE, and whiteness were in close range of each other that is also reflected in the overall mean values between processes. While soaking and blanching in 0.5% sodium bicarbonate ($NaHCO_3$) may have inhibited anti-nutritional factors or destroyed enzymic activity, heat treatment in the presence of this alkaline compound ($NaHCO_3$) seemed to have further compounded maillard reaction resulting in heat damage on color.

3.4 CONCLUSION

Soy germ is a natural ingredient successfully used throughout the industry as a source for high quality isoflavone extract for use in a variety of soy-based food application. The addition of soy germ at either the soaking, grinding or homogenizing stages enhanced isoflavone concentrations over whole soybean milk and commercial silk soymilk, although protein did not increase as much as expected. Protein amount was 1.4 - 1.5 times higher than that in the commercial soy milk. Most importantly, soymilk from the three processes was able to adequately meet the FDA recommendations on soy protein and isoflavone at reduced servings per day. Based on isoflavone amounts, 1 serving of soymilk a day would meet the 40 – 50mg recommended daily intake compared to 1.5 or more servings with commercial soymilk. Protein amounts based on our PIE soymilk beverages would take 3 - 3¼ servings to meet the 25g/day of soy protein recommendation compared to 4 servings with commercial soymilk.

From a processing perspective, the results evidently suggested that processing conditions exhibited varied influence on isoflavone and protein contents as well as on other physicochemical components. Nonetheless, the incorporation of 25% soy germ enriched isoflavone and protein concentration in comparison to commercial silk soymilk, but did not necessarily demonstrate increased protein when compared to the control whole soybean milk. Differences in components between processes were negligible and not significant in most comparisons except to commercial silk soymilk. The low viscosity of P1 which was close to the viscosity of commercial soymilk also made this process a better approach than P2 and P3. In light of these findings, the optimal process seemed to be P1. Thus and based on the viscosity, isoflavone and protein concentrations, P1 with germ incorporated at the start of the soymilk production showed greater potential for incorporating soy germ into soymilk and, therefore, the need for further studies to develop optimal formulation for the enrichment of protein and isoflavone was subsequently done. Subsequent studies to determine optimal formulation for enhancing protein and isoflavone in soy milk by increasing the soy germ amounts, and characterizing the products based on shelf life and consumer perception was investigated.

Inclusion of the varied processing conditions may simply be overwhelming. But, in view of the process / method by which germ can best be incorporated in soymilk for maximal nutrient enhancement and retention of sensory quality, finding the best fit depended on what needed to be achieved in the finished product - the nutrition, cost, flavor, and functionality. For industry, small scale or even home processing, direct incorporation of soy germ into soymilk is a cost-effective solution compared to the use of extract or enzyme technologies for enhanced nutrition. The results in this study point to the application flexibility of soy germ which can deliver nutrition advantages that can capture market opportunities in increased isoflavone and protein with less calories / carbs.

CHAPTER 4. FORMULATION OPTIMIZATION OF PROTEIN-ISOFLAVONE ENRICHED (PIE) SOYMILK: A COMPARISON OF BICARBONATE AND NO-BICARBONATE PROCESSING, AND THEIR EFFECT ON SOYMILK PHYSIOCHEMICAL PROPERTIES (FORMULATION OPTIMIZATION - PART I)

4.1 INTRODUCTION

Soymilk, the aqueous extract of whole soybeans or reconstituted soy-protein isolate, is a nutritious beverage similar to cow's milk in nutrition and appearance. However, the presence of antinutritional factors or endogenous volatile compounds, responsible for off-flavors (Blase, 1990), limits its utilization. Such compounds are rapidly formed following processing treatment that initiate lipoxigenase-hydroperoxide lyase pathways for converting linoleic acid to hexanal and linolenic to cis-3-hexanal (Matoba et al., 1985; Gardner et al., 1990; Morr and Ha, 1991) in the presence of moisture. In soy products, the undesirable 'beany' off-flavor interacts with protein and remains in the food product making it difficult to remove due to their high affinities (Aspelund and Wilson, 1983; Arai et al., 1970a, b; Damodaran and Kinsella 1981a, 1981b).

The development of off-flavors in soy products is largely due to lipoxygenase enzyme complexes, and therefore, inactivation or removal of these enzymes and other antinutritional compounds has received much research attention. Inactivation or removal with carbon and anionic exchange (How and Morr, 1982; Seo and Morr, 1985), and sodium bicarbonate or similar alkaline treatments (Nelson, 1976) greatly enhances color and flavor of soymilk. Heat processing alone or in conjunction with alkaline treatment has been widely used to inactivate lipoxigenase enzymes, and trypsin inhibitors. However, heat treatment also causes protein denaturation with concomitant loss of functionality and generates additional volatile organic compounds responsible for cooked or toasted off-flavors (Ewan et al., 1991). Several other processing treatments such as the use of pH (Asbi et al., 1989; Che Man et al., 1989; Kon et al., 1970; Ediriweera et al., 1989) and other methods have been developed for inactivating lipoxigenase and other soybean enzymes (Hand et al., 1964; Nelson et al., 1976; Ashraf and Snyder, 1981; Ewan et al., 1991).

Modern methods of soymilk production often utilize defatted soy material instead of whole soybeans (Tsangalis et al., 2004). According to Shurtleff and Aoyagi (1984), soymilk made from soy protein isolate (SPI) has no undesirable aftertaste generally referred to as ‘beany’. SPI is prepared from defatted soy meal using aqueous or mild alkali extraction of proteins and soluble carbohydrates (Liu, 1997). Though high in protein, soymilk prepared from SPI has reduced levels of the biologically active isoflavones – aglycones due to losses during protein isolation (Wang and Murphy, 1996). During processing, the aglycones are readily conjugated to form malonyl-, acetyl-, and β-glucoside configurations.

Traditional fermented soy foods such as tempeh, natto and soy sauce are richer in aglycone isoflavones (Wang and Murphy, 1994) and total isoflavones (Kwok et al., 1998; 2001; 2002; Sherkat et al., 2001) than unfermented soy. Research reports have indicated that isoflavones from fermented products are more bio-available to humans (Hutchins et al., 1995; Slavin et al., 1998; Izumi et al., 2000; Tsangalis et al., 2002, 2003, 2004) than those from unfermented products or alcohol-washed soy-protein concentrates containing high protein but fewer isoflavone content. Protein in soymilk also varies between 1.90 and 5.0g/100g depending on soy material used, form of product (dry or wet) in addition to other factors in milk production. Of the total concentration in soymilk, more than 90% isoflavone exist in glucosidic forms (Murphy et al., 1999; Tsangalis et al., 2002).

Soymilk treatments with probiotic, hydrolytic or other enzymes have been investigated and reports have suggested they may increase both aglycone and other isoflavone formation (Ara et al., 2003; Tsangalis et al., 2002, 2003, 2004*a*). However, the use of soy germ in soymilk production in the absence of enzymes, fermentation and other nutrient enhancing technologies have not been well studied with reference to protein and isoflavone enhancement. The purpose of this study, therefore, was two fold: (1) to develop optimal formulation of a PIE soymilk on the notion that increasing soy germ amounts in soymilk may result in concomitant increase in protein and isoflavone, and (2) based on optimal process and conditions identified in the previous study this study set out to further determine the physiochemical properties of soymilk as affected by the processing treatment of bicarbonate and no-bicarbonate in soymilk production. The ultimate goal in this study was to identify the optimal formulation using optimal processing conditions for subsequent studying on product shelf life, colloidal stability and quality, as well as the sensory and consumer perception of the soymilk beverages.

4.2 MATERIALS AND METHODS

4.2.1 Attaining Optimal Formulation

Assessment of beverage optimization was done using a two-component mixture in three formulations, and a single component beverage. Each formulation in the two-component mixture contained a different ratio of whole soybeans (WSB) to soy germ (SG) labeled as follows: WSB/SG25 = WSB:SG was 75:25, WSB/SG30 = WSB:SG was 70:30, and WSB/SG35 = WSB:SG was 65:35. The single component soymilk, 100% whole soybean (WSB/100) was used as control for comparison purposes. Formulation with the highest protein and isoflavone and lowest viscosity was considered the optimal formulation.

4.2.2 Production of Soymilk with or without $NaHCO_3$

U.S. No. 1 grade clean soybean seeds (SB & B Foods, Inc; Casselton, North Dakota, USA 58012), soy germ (Acatris Company, Minnesota, USA) and tap water were used for soymilk production. Soymilk was produced as reported by Nelson et al. (1976) with modifications. Based on study results from the previous study (chapter 3), optimized processing conditions consisted of short heating (blanching) time at low temperature (10min at 90^{o}C), in combination with long heating time of raw milk at low temperature (20 min at 90^{o}C), and high temperature with high homogenizing pressure (100^{o}C at 4000psi). Soybeans were soaked in either 0.5% $NaHCO_3$ (Sodium bicarbonate, USP Grade 1, Church and Dwight Co., Inc. NJ, U.S.A) solution or no - $NaHCO_3$ (bean: water ratio was 1:3) for 8 hr after which the beans was steam blanched in 0.5% $NaHCO_3$ solution or no- $NaHCO_3$ with constant stirring for 10 minutes at 100^{o}C, then ground into slurry with approximately 17% solids using a warren blender (Model CB, Warring Products Co., Winsted, Connecticut, U.S.A). The raw slurry was cooked for 20 min at 90^{o}C, then sieved through a 425µm (0.0165 inches) mesh sieve (U.S.A Standard Test Sieve, No. 40, ASTM E - 11 Specification, Fisher Scientific Company, Ohio, U.S.A), and a second time using the same sieve mesh size lined with cheese cloth (Grade BSC 1153, 140µm, 28x24 threads/square inch, Nelson Jameson, Inc. Marshfield, WI, U.S.A). The cooked soymilks were each homogenized using a lab scale homogenizer (Model F 20 40 WP, Groen Manufacturing company, Chicago, U.S.A) and pasteurized at 4000psi at 90^{o}C using plate heat exchanger (APV pasteurizer, Model JR S\S, APV company, Tonawanda, NY, U.S.A). Pasteurized milk was rapidly cooled to 4.4°C in a cooling tank that chills milk as it enters the tank through a pipeline directly from the pasteurizer, then collected in sterile half gallon containers and refrigerated at 4°C (34^{o}F).

4.2.3 Physicochemical Evaluation of $NaHCO_3$ and no-$NaHCO_3$ Soymilk

Proximate analysis was done as follows: Nitrogen analysis was carried out by the Agronomy lab at LSU using the PF-428 Nitrogen Analyzer (Model No. FP-428, Leco Corporation, St. Joseph, MI, U.S.A.) and AOAC method No. 992.23. Nitrogen results were multiplied by 6.25 to give protein equivalent, and protein content was then calculated back to wet basis. Moisture and total solids were determined using the Smart System 5 CEM moisture/Solids Analyzer, and the Smart system in conjunction with the FAS-9001 (CEM Corporation, Mathews, NC) was used for fat extracted with methylene chloride. Color was measured using the CIE L*, a*, b*, h*, C* and ΔE* values with a 5100 LabScan (Hunter Color Lab, Fairfax, VA) and Minolta CM-508d color spectrophotometer (Minolta Co., Ltd. New Jersey, USA) attached to a computer with a wingather software.

Color evaluation was done on all product equilibrated to room temperature (25°C). Viscosity was measured in centipoise (cP) using a Brookfield DV II+ viscometer (Brookfield Engineering Lab Inc, Stoughton, MA). An average of 25 data points was collected as a rotating #5 disc spindle submerged into the milk by a helipath stand, moved up and down at a speed of 50 rpm, taking measurements at various depths. Total solids was calculated by difference and soluble sugars / °brix measured by a bench top refractometer. pH was measured using a pH/mV/Temperature meter (Model IQ240. IQ Scientific Instruments, Inc. San Diego, CA, USA), attached to a stainless steel probe at 1, 7, 14, 21, and 28 days post pasteurization.

4.2.4 Isoflavone Standards, Extraction and Analysis

Aglycone standards of genistein, daidzein and glycitein, and β-Glucoside isomers of genistin, daidzin and glycitin (all synthetic) were purchased from Indofine Chemical Co. (Somerville, NJ, USA) for use in soymilk isoflavone analyses. Acetyl- and malonyl- forms were unavailable as pure standards, therefore calculation of their concentrations assumed unity responses in molar absorption with the respective β-glucosides. Identification by comparison of UV absorption and retention time patterns with published results was used to verify the acetyl- and malonyl- forms. Mixed and single isoflavone standards used in the analysis of isoflavones in soymilk were prepared as described by Tsangalis et al. (2002) with some modifications. Six mixed standards containing daidzin, genistin, glycitin, daidzein, genistein and glycitein 0.32mg/L or 0.32ppm) were used for the quantification of isoflavones. Single standards were prepared for peak identification and the retention times (min) of isoflavone isomers.

(a) Reversed-phase HPLC Apparatus and Reagents

Chromatographic analyses was carried out on the Waters Alliance 7000 series high performance liquid chromatograph (Waters Corporation, Milford, MA, USA) with auto-sampler 2690, solvent delivery system (9010), the dual absorbance photodiode array ultraviolet-visible (UV/VIS) detector (2487), all attached to computer with millennium 32 software. A Hypersil 5μm C18-ODS - 250 x 4.6 mm diameter, (Thermo Electron Corp, Bellefonte, PA, USA) reversed-phase column was used to separate isoflavone isomers. HPLC grade methanol and acetonitrile were purchased from Sigma-Aldrich (Sigma-Aldrich Corporation, St. Louis, MO, USA). All reagents used for isoflavone extraction and HPLC analyses were filtered through a 0.5 μm membrane (Millipore, Bedford, MA, USA) and mobile phases sparged with helium.

b) Extraction and HPLC Analysis of Isoflavones in Soymilk

A gram of freeze dried milk samples was used in the extraction of the isoflavone isomers malonyl-, acetyl-, β-glucoside and aglycone forms, as described by Xu et al. (2001); Tsangalis *et al*. (2002); and Wang and Murphy, (1994), with modifications. All isoflavone calculations are based on 1.0g by weight. Samples were extracted in 80% methanol heated at 80^{o}C and simultaneously shaken at 100u/min r.p.m in water bath (Polystat Water bath – Model 12050-00, Cole-Palmer Instrument Company, Chicago, Illinois) for 60 min. All samples were immediately filtered through a No. 1 Whatman filter paper (Qualitative circles 150mm Dia, Whatman International Ltd, England) into disposable tubes, then centrifuged (Hermle Labnet model

Z383K. Hermle Labotechnik, Wehingen, Germany) at 3000 x g for 20 min, and the mobile phase evaporated under a stream of nitrogen at 60°C to complete dryness using a Pierce nine-needle evaporating unit (CentriVap Console Labcocono - Pierce Biotechnology Inc., Rockford, Ill., U.S.A.). Milk samples were extracted and analyzed in batches over consecutive days with all samples from the same batch ran together.

Prior to injecting into column, sample extracts were re-suspended in 1 ml of methanol, vortex-mixed (Model type 37600 mixer series, Thermolyne Corporation, Dubuque, Iowa, USA) and centrifuged (Hermle Labnet model Z383K, Hermle Labotechnik, Wehingen, Germany) at 3000 x g for 10 min. Approximately, 300µL of supernatant of each of the 96 milk samples was transferred into a clean HPLC vial and analyzed for their aglycone and glucoside concentrations using Reverse Phase-HPLC. HPLC gradient elution consisted of glacial acetonitrile (solvent C) and water containing 10% acetonitrile (solvent D), set at a flow rate of 0.8ml/min. After 25 µL injection of sample into the column (25 °C), solvent D (water containing 10% acetonitrile) was set at 100% for 2 min, then, reduced to 80% over 20 min, followed by 50% for 10 min, and finally increased to 100% over 5 min prior to the next injection. Total analysis time was 37 min. The diode array UV/VIS detector was set at 254 nm and the retention times (min) of 6 isoflavone isomers were as follows: daidzin, 14.06; glycitin, 14.65; genistin, 18.77; daidzein, 26.3; glycitein, 26.94; and genistein, 30.4. Retention times for aglycone and β-glucoside isoflavone isomers were determined using mixed standards, and those of malonyl- and acetyl-glucoside isomers were based on the retention times reported by Xu et al. (2002) under similar HPLC conditions. Quantification of isoflavone isomers in soymilk was as per Xu *et al*. (2002) based on retention times of the glucosides and aglycones matched with reference standards. Malonyl and acetyl conjugates were calculated based on the unity responses in molar absorption with the respective β-glucosides or identified by comparison of UV absorption and retention time patterns with published results. Isoflavone concentrations are expressed mg / 100g of soymilk calculated back to wet basis as per Tsangalis et al. (2002).

4.2.5 Statistical Analysis

Soymilk beverages were produced in batches on different days. Duplicate samples were collected for all analysis and results were reported as mean ± standard deviation of 3 experimental replicates. Analysis of variance (ANOVA) utilized the general linear model (GLM), and differences between sample means was analyzed by Fisher's least significant difference (lsd) test at $\alpha = 0.05$. Hence, ANOVA data with a $P<0.05$ was classified as statistically significant (two-sided test). All ANOVA outputs at 95% confidence intervals utilized the Statistical Analysis System (SAS - 8.02, SAS Institute Inc., Cary, NC).

4.3 RESULTS AND DISCUSSIONS

4.3.1 Proximate Composition of $NaHCO_3$ and no-$NaHCO_3$ Soymilk Beverages

The mean (± standard deviation) proximate analysis data presented in Table 4.1 show no significant differences amongst variables and soymilk type for the no-$NaHCO_3$ group. Similarly, no significant differences were found in the $NaHCO_3$ group except for ash and viscosity. Based on processing treatment with $NaHCO_3$ and no-$NaHCO_3$, comparison of total proximate composition between treatment groups exhibited significant differences between soymilk types mainly for ash content and ^{o}brix. Other differences were more sporadic within treatment. Overall, PIE soymilks compared well with the commercial soymilk, except for the high ^{o}brix (9.24), and total solids (8.32%), and low protein (2.06g) and viscosity (40cP) in the commercial silk soymilk. The high ^{o}brix and total solids can be attributed to the nutrient and quality enhancing components such as calcium, vitamins, stabilizer and emulsifiers. The PIE soymilks exhibited high viscosity (≈ 50cP), moisture (≈ 90%), fat (≈ 1.08%), and protein (≈ 3.14g). Protein content in both treatment groups was about 1.4 times higher compared to the commercial soymilk. Both $NaHCO_3$ and non-$NaHCO_3$ soymilks had similar protein content, ranging from 3.04 - 3.22g/100g, irrespective of the whole bean to soy germ ratio.

Table 4.1 Proximate Composition of $NaHCO_3$ and no-$NaHCO_3$ PIE Soymilk Beverages

Milk	TRT	(°Brix)	Moist %	Fat %	Ash %	Vis Cp	Total Solids %	Protein Wet wt g/100g	ISF mg/100g
WSB/100	B	5.45a	***94.67a***	1.19a	0.16a	62.32a	5.33b	3.08a	***37.14a***
	NB	4.27b	95.17a	1.04a	***0.12b***	65.50a	4.83b	3.22a	30.24b
WSB/SG25	B	5.50a	***95.08b***	1.17a	0.14bc	61.76a	4.92b	3.12a	***36.83a***
	NB	***3.97b***	95.37a	***0.94a***	***0.12b***	58.00a	***4.63b***	3.19a	34.92b
WSB/SG30	B	5.35a	95.20a	1.15a	0.14c	***52.16ab***	4.79b	3.09a	34.65b
	NB	4.15b	95.19a	1.03a	***0.11b***	65.50a	4.81b	3.19a	***36.52a***
WSB/SG35	B	5.35a	95.18a	1.17a	0.13c	56.96ab	4.82b	***3.04a***	***35.79a***
	NB	4.45b	95.37a	0.96a	***0.11b***	64.50a	4.63b	3.26a	28.11b
Silk	N/A	9.24a	91.69c	1.12a	0.26a	40.00b	8.32a	2.06b	13.49c
T-100	NB+B	4.86	***94.92***	1.115	0.14	63.91	***5.08***	3.15	33.69
T-25	NB+B	4.73	95.22	1.05	0.13	***59.88***	4.77	***3.16***	***35.88***
T-30	NB+B	4.75	95.19	1.09	0.12	***58.83***	4.8	3.14	35.59
T-35	NB+B	4.9	95.27	1.06	0.12	60.73	4.72	3.15	31.95
T-B	B	5.41	95.03	1.17	0.14	58.3	4.97	3.09	***36.10***
T-NB	NB	4.21	95.27	0.99	0.11	63.37	4.72	***3.21***	32.45

[a, b] Means (SD) within the same column followed by different letters are significantly different ($p<0.05$). NB = no-bicarbonate; B = bicarbonate; Moist = moisture; Vis = viscosity; ISF = isoflavone. T-100 (WSB/100), T-25 (WSB/SG25), T-30 (WSB/SG30) and T-35 (WSB/SG35) = average for both $NaHCO_3$ and no-$NaHCO_3$ / NB+B. T-B = (average of all $NaHCO_3$ Soymilks - i.e. WSB/100, WSB/SG25, WSB/SG30 and WSB/SG35); T-NB = (average of all no-$NaHCO_3$ Soymilks - i.e. WSB/100, WSB/SG25, WSB/SG30 and WSB/SG35).

The $NaHCO_3$ treated soymilks contained high concentrations of isoflavone than that of the no- $NaHCO_3$ treated soymilk, except for WSB/SG30 (Table 4.1). The no-$NaHCO_3$ soymilks comprised 30.24, 34.92, 36.52, and 28.11 mg of isoflavone per 100ml serving for WSB/100, WSB/SG25, WSB/SG30, and WSB/SG35 respectively. The $NaHCO_3$ beverages contained 37.14, 36.83, 34.66, and 35.79g/100g of isoflavone for WSB/100, WSB/SG25, WSB/SG30, and WSB/SG35, respectively. Mean total of isoflavone for no-$NaHCO_3$ group was 32.43 mg/100ml and that for $NaHCO_3$ was 36.1mg/100ml serving. Similar studies that have examined other soy products prepared with calcium sulfate to enhance total isoflavone (Kao et al. 2004) had very low isoflavone concentration in tofu made with 0.3% calcium sulfate (a protein coagulant), but reported their observation as containing very high yield of total isoflavones 2.272 mg/g (2272.3ug/g), and lower yield with increased level (0.7%) of calcium sulfate 1.950 (1956.6ug/g).

While the ash content in PIE soymilk for both treatments was lower than that in silk milk, the $NaHCO_3$ group had high values over the no-$NaHCO_3$ group, except WSB/SG25. Viscosity was not statistically different in the soymilk between treatment groups.

The commercial soymilk had lower viscosity of 40cp where as the experimental products were approximately 1.3 to 1.6 times greater than that of commercial silk soymilk. The lowest viscosity occurred in the $NaHCO_3$ WSB/SG30 (52.16cp). In general, the no-$NaHCO_3$ had slightly high viscosity than the $NaHCO_3$ group, except WSB/SG25. ^{o}Brix in soymilk samples ranged between 3.97 and 5.45, and 10.35 ^{o} in silk soymilk. Although with similar total solids contents, but the no-$NaHCO_3$ group had lower ^{o}brix (3.97- 4.45) compared to the $NaHCO_3$ (5.35-5.55). Within each group, ^{o}brix was not significantly different among soymilk samples.

Combining both $NaHCO_3$ and no-$NaHCO_3$ groups, the isoflavone concentration was 35.87, 35.58, 33.69 and 31.95 mg/100g for WSB/SG25, WSB/SG30, WSB/100 and WSB/SG35, respectively, whereas the protein content was 3.16, 3.14, 3.15g and 3.15g/100g, respectively. These results point to the fact that, regardless of processing treatment, the addition of 25% soy germ does enhance protein and isoflavone contents over commercial soymilk. However, the incorporation of soy germ above 25% seemed to have very subtle to no effect on protein and isoflavone contents in comparison to whole soybean milk, but significant was observed when compared to commercial soymilk.

4.3.2 Isoflavone Composition of $NaHCO_3$ and no-$NaHCO_3$ PIE Soymilk

The composition of isoflavone isomers are shown in Table 4.2. Mean distribution of isoflavones did not significantly differ between formulations in each group ($NaHCO_3$ and no-$NaHCO_3$), except for glycitin in the no-$NaHCO_3$ group and daidzein in the $NaHCO_3$. In comparison, distribution between the no-$NaHCO_3$ and the $NaHCO_3$ varied significantly among the formulations. The amount of β-glucosides for daidzin varied between 6.08 (no-$NaHCO_3$) and 12.60 ($NaHCO_3$), glycitin between 0.64 (no-$NaHCO_3$) and 2.98 (no-$NaHCO_3$), and genistin was between 16.51 (non-$NaHCO_3$) and 21.81 (no-$NaHCO_3$). Aglycone composition also varied between 0.54 ($NaHCO_3$) and 3.54 (no-$NaHCO_3$) for glycitein, between 0.16 and 0.88 for daidzein, and between 0.23 ($NaHCO_3$) and 1.26 (no-$NaHCO_3$) for genistein. Notably, the β-glucosides and aglycones made up the total isoflavone concentrations.

Principal isoflavone isomers, identified from their retention indices and confirmed by photodiode array detection were the β-glucoside. On average genistin was higher (15.89 mg/100ml serving) than daidzin (8.55mg/100ml), and glycitin (2.07mg/100ml). Percent composition in the beverages within each group was approximately 89.22% in the WSB/SG35, 88.14% for WSB/SG30, 75.3% in WSB/100 and 74.48% in WSB/SG25 for the $NaHCO_3$ group. In stark contrast to the $NaHCO_3$ group, total β-glucoside isomers were highest in the WSB/100 (98.8%), closely followed by WSB/SG25 (95.3%), WSB/SG30 (93.74%) and WSB/SG35

(92.68%). Silk soymilk also contained 98.37% of the β-glucoside isomers. The differences in either group accounted for the aglycone isomer. As evident, the no-$NaHCO_3$ group contained

Table 4.2 Within, and Between Treatment Comparisons of Isoflavone Content (mg/100g) and Composition of PIE Soymilk Beverages

Soymilk	TrT	Daidzin	Glycitin	Genestin	Daidzein	Glycitein	Genestein	ISF Total
WSB/100	NB	6.08a (0.80)	0.64bc (0.06)	18.93a (1.75)	2.66a (1.32)	0.30a (0.32)	1.26a (0.54)	30.24 (4.79)
WSB/SG25	NB	10.4a (0.65)	2.25ab (0.11)	17.92a (0.65)	3.54a (0.66)	0.62a (0.13)	1.14a (0.28)	34.92 (2.48)
WSB/SG30	NB	11.44a (0.54)	3.2a (0.16)	17.68a (0.71)	1.95a (0.44)	0.67a (0.33)	0.63a (0.06)	36.52 (2.24)
WSB/SG35	NB	9.90a (1.05)	2.7a (0.27)	16.51a (1.25)	1.85a (0.12)	0.47a (0.18)	0.49a (0.05)	28.11 (2.92)
WSB/100	B	12.60a (1.00)	***1.76ab*** (0.19)	21.81a (0.28)	***0.54b*** (0.25)	0.16a (0.09)	0.27a (0.17)	37.14 (1.98)
WSB/SG25	B	12.42a (0.04)	2.78a (0.11)	19.89a (0.06)	***1.19ab*** (0.46)	0.30a (0.33)	0.25a (0.08)	36.83 (1.08)
WSB/SG30	B	11.45a (1.77)	2.94a (0.89)	18.10a (0.90)	***1.06ab*** (0.08)	0.88a (0.05)	0.23a (0.10)	34.66 (3.79)
WSB/SG35	B	11.46a (1.69)	2.98a (0.21)	18.73a (1.48)	1.770a (0.02)	0.62a (0.49)	0.23a (0.20)	35.79 (4.09)
Silk		2.60d (3.22)	***2.56b*** (0.18)	8.11a (5.61)	***0.12b*** (0.06)	0.09a (0.00)	0.00a (0.04)	13.49 (1.82)
			Between	**TRT**				
WSB/100	NB	6.08a	0.64b	16.05b	4.77a	0.44a	2.246a	30.24
	B	12.60b	***1.76a***	21.81a	***0.54b***	0.16a	0.27b	37.14
WSB/SG25	NB	8.34b	1.72b	15.95b	5.90a	0.95a	2.03b	34.92
	B	12.42a	2.78a	19.89a	***1.19b***	0.30a	0.25a	36.83
WSB/SG30	NB	11.43a	3.50a	17.26a	2.85a	0.45a	1.04b	36.52
	B	11.45a	2.94a	18.09a	***1.06b***	0.88a	0.23a	34.65
WSB/SG35	NB	8.36a	2.42a	14.30a	1.93a	0.32a	0.76a	28.11
	B	11.46a	2.98a	18.73a	1.770a	0.62a	0.23a	35.79
MEAN	NB	8.55	2.07	15.89	***3.86***	***0.54***	***1.52***	32.43
	B	11.99	2.61	19.63	1.14	0.49	0.24	36.10

Numbers in parentheses represent standard deviation; [a, b] Means (SD) within the same column followed by different letters are significantly different ($P<0.05$).

lower amounts of β-glucosides isomers and slightly more of the aglycones than the $NaHCO_3$ treated soymilk. These data suggest that 74.48% to 98.8% of total isoflavones in soymilk was in esterified form.

Isoflavones have been implicated as an anticancer compound which has increased interest of using soybeans as part of the human diet (Adlercreutz, 1991; Coward et al., 1993; Persky and Horn, 1995). Numerous research reports have indicated that the amount of isoflavone varies among soybean cultivars due to genetic and environmental factors in particular (Eldrige & Kwolek, 1083; Wang & Murphy, 1994, Panizzi et al., 1999). More importantly, processing techniques affect the type and level of isoflavones remaining in the final product (Panizzi et al.,1999). During soaking, β-glucosidase hydrolyzes isoflavone glucosides (daidzin and genistin) to aglycones (daidzein and genistein) (Matsuura et al., 1989). But, also, fermentation has been shown to hydrolyze isoflavone glucosides resulting in increased levels of aglycones, whereas non fermented soybean products contain lower levels of aglycones (Coward et al., 1993; Wang & Murphy, 1994).

Though present in small quantities, the aglycones were much higher in the no-$NaHCO_3$ soymilks than in the $NaHCO_3$ soymilks. Of the total isoflavone content, the aglycones comprised 10.78 – 24.7% in the no-$NaHCO_3$ and between 1.16 and 7.32% in the $NaHCO_3$ group (Table 4.2). Mean aglycone amounts were 3.86 for glycitein, 1.52 for daidzein, and 0.54 for genistein. The approximate percent aglycone amounts in the no-$NaHCO_3$ group was highest in WSB/100 (24.7%), followed by WSB/SG25 (25.5%), WSB/SG30 (11.46%), and WSB/SG35 (10.78%). In contrasting order, aglycone amount in the $NaHCO_3$ was highest in WSB/SG35 (7.32%), followed by WSB/SG30 (6.26%), WSB/SG25 (4.7%), and WSB/100 (1.16%). These striking results have implications for consumer acceptance of soymilk. First, research results have indicated that increased astringency in soymilk is associated with high levels or presence of the isoflavone aglycones (Panizzi et al., 1999) resulting in increased beany flavor and subsequently decreased consumer acceptance of soy milk. In contrast, Al Mahfuz et al. (2004) did not find any relationship between the undesirable astringent taste and isoflavone contents in soymilk and tofu using isoflavone-enriched extracts. In conclusion, these authors state that since 55% of phytates (which play an important role in the formation of tofu curds in particular) exists freely in soymilk, and 6-13% exists freely in tofu on the bases of coagulation, it can be assumed that the astringent characteristics caused by phytic ions in soymilk are lost upon conversion of phytic ions to their stable insoluble salt forms during soymilk coagulation.

Second, isoflavones in the biological system are primarily metabolized and absorbed better in the aglycone forms over their glucoside counterparts (Xu et al., 1994; Izumi et al. 2000). These points suggests that the $NaHCO_3$ beverages have a higher propensity of being more acceptable to the consumers, but, lacks the vital isoflavone components required to efficiently provide the acclaimed soy health benefit in the biological system. On the other hand, the no-$NaHCO_3$ beverages are less likely to be accepted by consumers due to its high aglycone content that imparts strong astringency and beany flavor, but contains more bioavailable forms of isoflavone to efficiently impart more health benefits.

Negligible levels of the malonyl- and acetyl- (results not included) isomers was detected in the beverages. These compounds are more commonly formed during toasting of soy flour or extrusion of textured soy protein (Coward et al., 1998). In soymilk, isoflavones are present almost entirely as their β-glucosidic conjugate (daidzin and genistin), while the average amounts of aglycones, daidzein and genistein are very small with values ranging between 1.1μg/g and 3.6μg/g (Barnes et al., 1994; Panizzi et al., 1999).When soybeans are processed at temperatures

>80°C, malonylated isoflavone glucosides which are thermally unstable are converted to their corresponding conjugated glucosides, daidzin and genistin (Panizzi et al., 1999).

4.3.3 Storage Quality of $NaHCO_3$ and no-$NaHCO_3$ Soymilk Beverages (pH)

Table 4.3 shows the mean values of pH in four different soymilks prepared with or without $NaHCO_3$. Initial pH at <24hr through 14days storage was significantly different between soymilk beverages as well as between the $NaHCO_3$ and no-$NaHCO_3$ treatments. While the $NaHCO_3$ treatment contained higher pH that ranged between 8.12 and 8.44, the no-$NaHCO_3$ group showed lower pH between 7.72 and 7.94. The $NaHCO_3$ treated group maintained pH

Table 4.3 Keeping Quality of $NaHCO_3$ and no-$NaHCO_3$ PIE Soymilk Beverages as determined by pH

Sample	TRT	24hrs	7d	14d	21d	28d
WSB100	B	8.16b	8.10b	8.09b	7.34a	6.45b
	NB	7.72c	7.65c	7.61d	7.56b	7.38a
WSB/SG25	B	8.25ab	8.29ab	8.23ab	7.58a	6.26b
	NB	7.78c	7.71bc	7.65cd	7.63b	7.63a
WSB/SG30	B	8.37ab	8.40ab	8.35a	7.77a	6.30b
	NB	7.94b	7.84b	7.81b	7.70b	7.75a
WSB/SG35	B	8.44ab	8.40ab	8.36a	7.57a	6.35b
	NB	7.88b	7.77bc	7.74bc	7.69b	7.66a
Silk	N/A	8.52a	8.49a	8.24a	8.13a	7.72a
T – 100	NB+B	7.94	7.875	7.85	7.45	6.915
T – 25	NB+B	8.015	8	7.94	7.605	6.945
T – 30	NB+B	8.155	***8.12***	***8.08***	***7.735***	***7.025***
T – 35	NB+B	***8.16***	8.085	8.05	7.63	7.005
T – B	B	***8.305***	***8.2975***	***8.2575***	7.565	6.34
T – NB	NB	7.83	7.7425	7.7025	***7.645***	***7.605***

[a, b, c] Means (SD) within the same column followed by different letters are significantly different ($p<0.05$); T = Overall Total. T-100 (WSB/100), T-25 (WSB/SG25), T-30 (WSB/SG30) and T-35 (WSB/SG35) = average for both $NaHCO_3$ and no-$NaHCO_3$ / NB+B. T-B = (average of all $NaHCO_3$ Soymilks - i.e. WSB/100, WSB/SG25, WSB/SG30 and WSB/SG35); T-NB = (average of all no-$NaHCO_3$ Soymilks - i.e. WSB/100, WSB/SG25, WSB/SG30 and WSB/SG35).

above neutral throughout the 21 days storage, but pH dropped to between 6.26 and 6.45 by day 28 (Figure 4.1). Seemingly, the no-$NaHCO_3$ treated group maintained pH above neutral throughout the 28days storage period (Figure 4.2). In comparison, silk soymilk retained pH above 8.0 for 21 days, and then dropped slightly to pH 7.72 by the end of day 28. Thus, pH values for commercial silk soymilk and the $NaHCO_3$ group (T-B) were much similar compared to the no-$NaHCO_3$ (T-NB). Both groups stored very well with ending pH similar to initial pH (6.45) in cow's milk.

Regardless of the formulations, the $NaHCO_3$ group showed a higher pH throughout storage but pH dropped by day 28. The no-$NaHCO_3$ showed a consistent pH drop over the 28 days storage period. Bacteria seemed to have survived better in the no-$NaHCO_3$ group than the $NaHCO_3$ group.

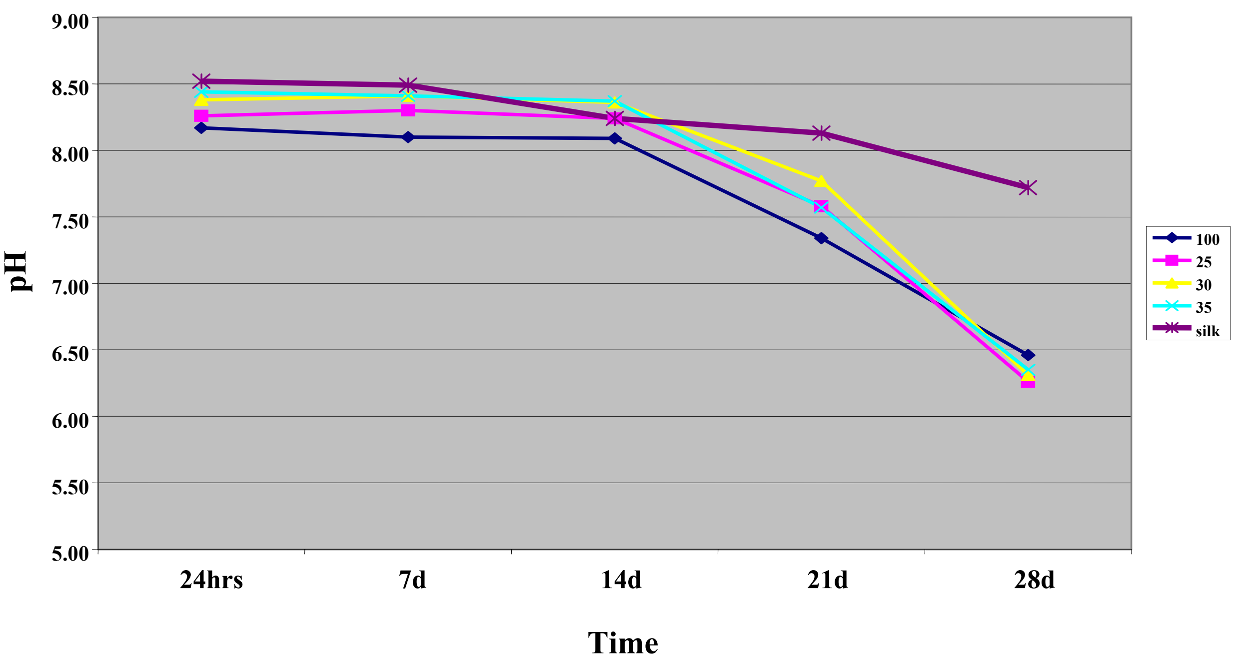


Figure 4.1 pH during 28-days Storage of Soymilk Processed with $NaHCO_3$

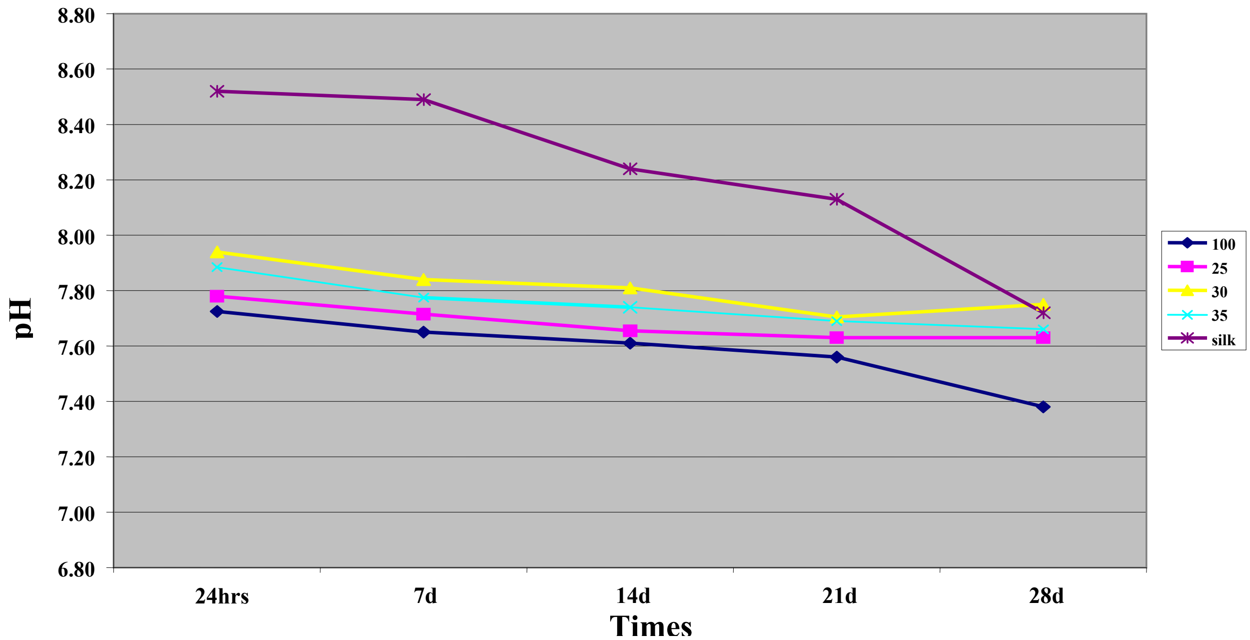


Figure 4.2 pH during 28-days Storage of Soymilk Processed with no-$NaHCO_3$

4.3.4 Color of $NaHCO_3$ and no-$NaHCO_3$ Soymilk Formulations

Soymilk color showed no significant difference for CIE L*, a*, b*, C, h, ΔE, and whiteness (Table 4.4) in the $NaHCO_3$ group. No differences were shown either for L* in the no-$NaHCO_3$ group, but obvious differences were observed for a*, b*, c*, H*, ΔE and whiteness. H* values were higher in the no-$NaHCO_3$ group than in the $NaHCO_3$ soymilks except WSB/SG30. The high h* values correlate with increased whiteness. Furthermore, the b* and C values tended to decline with increase in germ amounts in the no- $NaHCO_3$ group. In addition, ΔE tended to increase with increased soy germ for the no- $NaHCO_3$ group.

ΔE which indicates the size of the color difference but not in what way the colors are different indicated large color differences between the two groups. The mean color difference in $NaHCO_3$ soymilks was smaller (T-B = 2.99) compared to the no-$NaHCO_3$ group (T-NB = 5.37).

Table 4.4 Color Comparison of $NaHCO_3$ and no-$NaHCO_3$ PIE Soymilk

Sample	TRT	L*	a*	b*	C	H	ΔE	Whiteness
WSB100	B	81.66a	0.23a	16.08a	16.08a	89.24c	2.46d	75.60bc
	NB	83.68a	***-0.17b***	***12.44b***	12.44b	90.80a	***4.35c***	79.48b
WSB/ SG25	B	81.09a	0.59a	15.24a	15.26a	87.80c	2.74d	75.70bc
	NB	83.58a	***-0.28b***	***11.03cd***	***11.03cd***	91.45a	***5.75ab***	***80.21a***
WSB SG30	B	80.84a	0.39a	14.70a	14.71a	88.46c	3.23d	75.84bc
	NB	83.02a	0.28a	***11.57c***	***11.58c***	***88.60c***	***5.19b***	79.44b
WSB/ SG35	B	80.53a	0.90a	14.62a	14.65a	86.46c	3.53d	75.63bc
	NB	83.71a	***-0.14b***	***10.58d***	***10.58d***	***90.75ab***	6.18a	***80.57a***
Silk	B	83.28a	0.26a	16.73a	16.73a	89.10a	0.00	76.34a
T – 100	NB+B	82.67	0.03	14.26	14.26	***90.02***	3.405	77.54
T – 25	NB+B	***82.33***	0.15	13.13	13.14	***89.62***	4.24	***77.96***
T – 30	NB+B	81.93	0.33	13.13	13.14	88.53	4.21	77.64
T – 35	NB+B	82.12	0.38	12.6	12.61	88.60	4.85	***78.1***
T – B	B	81.03	0.52	15.16	15.18	87.99	2.99	75.75
T – NB	NB	***83.58***	-0.08	11.40	11.40	***90.4***	5.37	***79.92***

[a, b, c] Means within the same column followed by different letters are significantly different ($p<0.05$); T = Overall Total

4.3.5 Optimal Formulation and Processing Treatment of Soymilk Beverages

From a health perspective, isoflavones found in soybeans have been associated with greater health benefit to humans. Since these biologically active metabolites are found most abundantly in soybeans, it is important that the amounts consumed meet the recommended FDA intake. Although soy protein was generally low in the soymilk, added amounts are readily consumed from other soy-based sources high in protein that may not be concentrated sources of isoflavone. Therefore, it is imperative that concentrated amounts of isoflavones be provided in soymilk over soy protein content. As such the incorporation of 25% soy germ into soymilk meets the optimal formulation target in providing high protein and isoflavone contents.

As shown in this study, the influence of $NaHCO_3$ and no-$NaHCO_3$ on soymilk composition, pH and color showed significant differences ($P<0.05$) in some variables but not in others. The use of $NaHCO_3$ and no-$NaHCO_3$ also showed no significant effect on protein of the soymilk beverages within and between the two groups. Significant differences were more pronounced in the total isoflavone content and composition in the $NaHCO_3$ group over the no-$NaHCO_3$. This effect seen makes it obvious that soymilk processing with $NaHCO_3$ is the optimal way to go in soymilk production. To date no studies have been done investigating $NaHCO_3$ and no-$NaHCO_3$ treatment on proximate composition and isoflavone content. Nevertheless, similar studies have mostly focused on the effect of the use of $NaHCO_3$ and no-$NaHCO_3$ on bean texture during soaking or blanching, enzyme inactivation, destruction of antinutrient factors such as trypsin (Nelson et al. 1976).

The destruction of trypsin inhibitor after various combinations of soaking and blanching with or without $NaHCO_3$ was easily attained when 0.5% $NaHCO_3$ was added to blanch water regardless of prior soaking with or without $NaHCO_3$ (Albrecht et al., 1966). Furthermore, beans soaked in either 0.5% $NaHCO_3$ or plain water and then blanched for 5 min in 0.5% $NaHCO_3$ or water tested negative for trypsin inhibitor. Likewise, a 30 min blanching in 0.5% $NaHCO_3$ following an overnight soaking resulted in a beverage completely free of any trypsin inhibitor (Nelson et al., 1976). In addition, soy bean treatment with 0.5% $NaHCO_3$ also inactivates the lipoxigenase enzyme known to produce a highly objectionable beany or oxidized odor and flavor acceptable only to the oriental culture that have developed tolerance for it (Nelson et al., 1971), but not the occidental cultures. The use of other alkaline medium such as 0.01M sodium carbonate in soymilk processing appreciably inactivates lipoxigenase to 14% residual activity instead of water with residual activity of 46% (Geronazzo et al. 1998). The positive correlation between $NaHCO_3$ use in soybean/soymilk and reduction in antinutrient factors as well as enzyme inactivation over no-$NaHCO_3$ is an added advantage in the crucial consideration for optimal processing treatment in our study.

With regards to their isoflavone bioavailability which would be a critical point of concern, it is obvious that the no-$NaHCO_3$ processed beverage will provide better health benefits than the $NaHCO_3$ beverages. However, research reports have indicated that the use of hydrolyzing enzymes / bacteria using various processing technologies results in the hydrolysis of the non-bioavailable isoflavone isomers to more of the bioavailable isomers. With that in mind, it was concluded that for the purpose of this study, the $NaHCO_3$ processed soymilk is a better option over the no-$NaHCO_3$.

4.4 CONCLUSION

There is no doubt that incorporating soybean germ into soymilk yields a product with distinct advantages. From a standpoint of application, although offering low protein content and affording a lower yield of color profile, the $NaHCO_3$ group would offer better advantage of easy processing of soybeans due to its effectiveness in inducing soft texture of the beans, reducing / destroying anti-nutritional factors such as the lipoxigenase enzyme. Significant treatment interactions were observed for total isoflavone and composition, soluble sugar, moisture, ash, total solids and viscosity. Differences between beverages in the $NaHCO_3$ group was not the same for the no-$NaHCO_3$ group. Total isoflavone (unconjugated and conjugated forms) in $NaHCO_3$ soymilk was higher than in the no-$NaHCO_3$ group. Of the individual isomers, the percentage of β-glucosides (daidzin, glycitin, and genistin) was higher in the $NaHCO_3$ group than in the no-$NaHCO_3$.

Both treatment groups had pH above neutral post pasteurization through 21 days in storage after which pH declined below 6.5 for NaHCO3 group, but remained above pH 7.0 for the no-NaHCO3 group. The no-$NaHCO_3$ also had a better color profile in terms of color whiteness and hue than in the $NaHCO_3$ group. In general, the 25% germ provided a better color profile over WSB/100, WSB/SG30, WSB/SG35 and commercial silk soymilk. In addition, the WSB/SG25 provided a better protein, isoflavone and color profiles, with pH similar to the other beverages. The use of sodium bicarbonate ($NaHCO_3$) resulting in high pH in soymilk processing could deter rapid bacteria, yeast, mold or other bacterial spoilage, and extend product shelf life under proper storage conditions.

In conclusion, the effects of processing on the chemical and physical quality of the beverages in the two groups could be useful in improving traditional soymilk processing to give the product consistent uniformity in quality. Based on the above results, follow-up studies investigating beverage quality in relation to colloidal stability, and protein separation as important determinants of beverage quality were done in conjunction with microbial total plate count to validate beverage shelf life.

CHAPTER 5. QUALITY AND MICROBIAL SHELF LIFE STUDY OF PROTEIN ISOFLAVONE ENRICHED (PIE) SOYMILK BEVERAGES

5.1 INTRODUCTION

Soymilk products are valued for their high protein content. However, the inclusion of soybean products in the human diet is hampered by their flatus-inducing effect upon sensitive individuals, an effect due to the presence of indigestible galactosaccharides such as stachyose and raffinose (Mital and Steinkraus, 1975; Pinthong et al., 1980). Mainly, consumption of soy-derived products is limited by the presence of alpha-galactooligosaccharides (alpha-GOS) because mammals lack pancreatic alpha-galactosidase (alpha-Gal) which is necessary for their hydrolysis. These sugars reach the large intestine causing gastrointestinal disorders in sensitive individuals (LeBlanc, et al., 2004). Processes including fermentation of these carbohydrates by lactic acid and other types of bacteria have been surveyed to minimize or rid of such problems and to enhance sensorial acceptability as well as nutritional content

In previous studies, LeBlanc et al., (2004) reported that lactic acid bacteria expressing alpha-Gal was a promising solution for the degradation of alpha-GOS in soy milk. The authors found that lactic acid bacteria were able to grow in commercial soymilk and completely eliminated stachyose and raffinose during fermentation because of its high alpha-Gal activity. Rats fed with soymilk fermented by lactic acid bacteria had smaller caecums compared with those fed with unfermented soymilk. These results give promise to the possibility of eliminating possible undesirable physiological effects normally associated with soymilk consumption, thus preventing gastrointestinal disorders in sensitive individuals normally associated with the consumption of soy-based products (LeBlanc et al., 2004).

The use of some species of the bacteria genera *Streptococcus* and *Bifidobacteria*, has been studied by many researchers for their roles in enhancing soymilk quality (Garro et al., 1998;

1999; Hou-Jen-Wan et al., 2000; Tsangalis et al., 2002, 2003, 2004*a*), improving organoleptic qualities (Tsangalis et al., 2002, 2003), and hydrolysing sugars in the production of isoflavone isomers (Tsangalis et al., 2002, 2003).

In another study by Beasley et al (2003), *Lactococcus lactis strain* (LL3) isolated from human milk was used to produce fermented soymilk. Survival levels of the *Lactococcus lactis strain* (LL3) was over 7 log CFU/ml in the fermented soymilk. Beasley et al (2003) further observed that comparison of a similar product made with another *Lactococcus lactis* strain originally isolated from cow's milk, was rated equally attractive by consumers. While processing has many faces to its purpose in food production, destruction of food pathogens is the primary goal in food processing to enhance quality, safety and prolong shelf-life. Efiuvwevwere and Nwanebu (1998) have reported substantially hazardous high levels (>6 log CFU/ml) of pathogens in soymilk.

Li and Zhang, (2004) subjected soymilk enriched with dairy proteins to pulsed electric fields (PEF) to evaluate the inactivation of *Escherichia coli* and the extension of microbial shelf-life. Maximum thermal exposure level of samples was 60^{o}C for 1.6 sec during a PEF treatment. These authors found that a 5.7 log reduction was achieved using PEF at 41.1 kV/cm for 54 min. This level significantly extended the microbial shelf-life of soymilk. No significant change in brightness and viscosity of PEF-treated samples was observed during a 30 days storage at 4^{o}C. Just as with high heat temperature treatment, PEF was found effective in inactivation of *E. coli* and in extension of microbial shelf-life of enriched soymilk. In general, emphasis must be placed on adequate microbiological control in milk processing operations. The purpose of this study was to evaluate the quality and shelf life of refrigerated protein-isoflavone enriched soymilk produced with varying amounts of whole soybean (WSB) to soy germ (SG).

5.2 MATERIALS AND METHODS

5.2.1 Preparation of Soymilk

Three soymilk formulations comprising soy germ (SG) and whole soybeans (WSB) in the ratio of 25:75; 30:70 and 35:65, respectively; control samples contained 100% whole soy beans (WSB) and a commercial reference brand (Silk soymilk, Nestle) were utilized in the 28 days shelf life study. Soymilk production was done as described in chapter 3. Basically, the process involved soaking in a 1:3 ratio of bean + germ to water in 0.5% $NaHCO_3$ solution, followed by blanching in 0.5% $NaHCO_3$ solution, then grinding into slurry with 17% solids, cooking of raw milk, sieving, homogenizing and pasteurization, followed by rapid cooling to 4.4^{o}C, and bottling in sterile half gallon bottles. All samples were stored at 4°C (39.2°F) prior to microbial analysis.

5.2.2 Apparent Colloidal Stability

Apparent colloidal stability was measured to establish homogeneity of liquid mixtures. Colloidal stability as defined by Nelson et al. (1975) is the maintenance of a homogenous liquid system, implying the absence of the setting of solids within the liquid. However, the system is not regarded as true colloid because the average particle size of the solids far exceeds the range normally considered as being within the colloidal size range. Hence, apparent colloidal stability is indicated by separation at the top of a five inch total height. Soymilk samples were collected into a clear / transparent 6.0 inches height cup and left undisturbed for 14 days. The PIE soymilks were evaluated after standing quiescent at 4°C (39.2^{o}F) for 14 days, then visually assessed by a group of 50 consumers on a 9 – point hedonic scale for color and overall appearance where 1 = extremely disliked and 9 = extremely liked, and on a 5-point hedonic scale where 5 = extremely separated and 0 = none. Objective evaluation was done by determining any visible separation or line of demarcation on a 5-point scale where 1 = any separation at all, 2 =

separation at the top of a 5.5 inch beverage height, and 3 = separation at bottom of the container with visible thickness or sediment. Since commercial soymilk beverages are produced with stabilizers to uphold suspended particles in solution and enhance quality, the silk soymilk was not part of this study.

5.2.3 Protein Separation

Protein separation is another measure of quality determined by nitrogen analysis and the results multiplied by 6.25 to give approximate protein content expressed as grams per 100 gram wet weight. The objective quantitation gives a precise measure of the amount of protein suspended in liquid between 0 hour post-pasteurization and 14 days. The PIE soymilks were poured into 6 inch tall glass tubes and evaluated after standing undisturbed at 4°C (39^{o}F) for 14 days. Duplicate samples of 15ml were obtained using clean disposable pipettes inserted into beverages at approximately 1.5 inches (1) below the top surface of beverage, (2) above the bottom of the beverage and (3) after thoroughly mixing the beverages to obtain composite reading. Due to untimely production and other factors, commercial silk milk was not included in this aspect of the study.

5.2.4 pH

In conjunction with microbial studies, pH measurement was done to further predict quality and shelf life stability, microbial activity, chemical reactivity and physical properties. Measurements were done at 1 (24 h), 7, 14, 21, and 28 days post pasteurization using a hand held three point calibrated pH meter (Model IQ240. IQ 150 / 240 Scientific Instruments, Inc. San Diego, CA, U.S.A).

5.2.5 Microbial Assay

a) Total Plate Count - Microbiological assay of the soy beverages was done using aerobic count petrifilm^{TM} (3M, St. Paul, MN, USA) plates at 1, 7, 14, and 21 days post pasteurization. At day 1, soymilk samples for bacteria count was collected at three critical points (i.e. cooked milk (point #1 = 24hr-0), beverage from tank (point #2 = 24hr-1) and beverage from bottled containers (point #3 = 24hr-2), to determine possible areas of contamination in the production path. Serial dilutions of each sample were plated in duplicates and incubated at 37^{o}C for 48 hours. The results were expressed as log colony-forming unit per milliliter (log CFU/ml).

b) Plating – Samples were prepared using 1:10 dilutions in sterile phosphate buffer saline (20X (0.2M) PBS, pH 7.0) solution. Phosphate Buffer Saline (PBS) solution, is a general or multi-purpose buffer used for routine washes and dilutions. The buffer comprised of sodium phosphate monobasic (Na_2HPO_4 – 7.2g), sodium phosphate dibasic (NaH_2PO_4 – 8.52g), sodium chloride (NaCl – 25.5g), which were dissolved in 3 L of distilled water, then autoclaved (Vacamatic autoclave, Model 3023 Eagle Series, AMSCO, U.S.A), cooled and stored at room temperature. Five serial dilutions, 10^{-0} – 10^{-4} per sample were made. The aerobic count petrifilm^{TM} was placed on a leveled surface under a hood (Model - Class II A/B3 Biological Safety Cabinet, Forma Scientific Inc., Marjetta, OH, U.S.A) with top film lifted, 1 ml soymilk sample was dispensed in the center of the petrifilm^{TM}. With the top film gently dropped on to the sample and the recessed side of the spreader faced down, the sample was evenly distributed under the petrifilm^{TM} by pushing gently downward on the center of the plastic spreader. The spreader was lifted and the petrifilm^{TM} left undisturbed for at least one minute to permit the gel to solidify.

c) Incubation - The solidified gel plates were incubated with clear sides up and in stacks of 20 plates, at 37^{o}C (98.6^{o}F - relative humidity) for 48 h.

d) Total Bacteria Count and Interpretation - After 48 hrs of incubation, petrifilm™ plates were removed from incubator and colonies counted on a standard colony counter with magnified light source. All colonies were counted, regardless of size or intensity.

5.2.6 Statistical Analysis

Soymilk beverages were produced in batches on different days. Duplicate samples were collected for all analysis and results are reported as mean ± standard deviation of 3 replicates. Analysis of variance utilized the general linear model (GLM), and differences between sample means were analyzed by Fisher's least significant difference (lsd) test at $\alpha = 0.05$. Hence, ANOVA data with $P<0.05$ were classified as statistically significant (two-sided test). All ANOVA outputs at 95% confidence intervals utilized the Statistical Analysis System (SAS - 8.02, SAS Institute Inc., Cary, NC).

5.3 RESULTS AND DISCUSSIONS

5.3.1 Apparent Colloidal Stability

According to Nelson et al. (1976), an unstable beverage system is a severe problem particularly in soy and soy-based beverages. Table 5.1 shows that color and overall appearance of the beverages were slightly liked by consumers. The results further indicated that standing time at 34^{o}F for 14 days had no significant (P>0.05) effect on colloidal stability of the four beverages. There were no significant (P>0.05) effect among the beverages in colloidal separation at the bottom, top or in overall separation, except that overall separation and separation of colloid at the bottom was less apparent compared colloidal separation at the top. Since particle size seems to contribute to good stability in homogenized milk, variations in size inadvertently affect stability. This implies that particles in the colloidal system were more suspended in liquid about one inch beneath the top and slightly above the bottom. According to Nelson et al. (1976), tenderization of the soybeans in combination with homogenization of the slurry results in the formation of more hydrophilic protein-lipid complexes which are responsible for beverage stability.

Table 5.1 Apparent Colloidal Stability of Soymilk Beverages

PIE Soymilk	OAppear	Color	OSep	TopSep	Bottom
A WSB/SG 25	6.08a (1.35)	6.28a (1.30)	0.58a (0.81)	0.83a (0.96)	0.20a (0.46)
B WSB/SG30	***6.33a*** (1.51)	***6.43a*** (1.38)	0.55a (0.81)	***0.80a*** (1.02)	***0.15a*** (0.43)
C WSB/SG35	6.20a (1.38)	6.20a (1.51)	***0.50a*** (0.91)	0.83a (1.26)	0.23a (0.48)
D WSB/100	6.10a (1.45)	6.00a (1.41)	0.70a (0.82)	***0.80a*** (0.97)	0.33a (0.66)
Range	0.25	0.43	0.2	0.03	0.18

Oappear = overall appearance, Osep = Overall separation; TopSep = Top Separation.
The higher the value, the more the separation and vice versa; Numbers in parentheses represent standard deviation of 50 consumer responses; Range = the highest score minus the lowest score; [a, b, c] Means (SD) within the same column followed by different letters are significantly different ($p<0.05$)

5.3.2 Protein Separation

According to the results in Table 5.2, no significant differences ($P>0.05$) was found among the beverages in the top, center or bottom protein separation and content. In other words, protein separation was not significantly different ($P > 0.05$) among the beverages. However, the mean top protein content of the beverages ($n = 4$) was in order of 2.83g, 2.80g, 2.71g, 2.46g, 2.45g for WSB/SG30, Silk, WSB/100, WSB/SG35 and WSB/SG25, respectively. The center protein content was 2.90g, 2.88g, 2.69g, 2.47g, and 2.26 for WSB/100, Silk, WSB/SG30, WSB/SG35 and WSB/SG25, respectively; and the bottom content was in order of 3.0g, 2.52g, 2.49g, 2.47g and 2.42g for Silk, WSB/100, WSB/SG25, WSB/SG35, and WSB/SG30, respectively. WSB/SG35 and the commercial silk soymilk showed consistent protein suspended in solution from top to bottom, followed by WSB/SG25, WSB/SG30 and WSB/100. Protein was evenly suspended in liquid which relates to very good colloidal stability of the soymilk beverages.

Table 5.2 Protein Separation of Soymilk Beverages

Sample	Top	Center	Bottom	Total Across
WSB/100	2.71a	2.90a	2.52ab	**2.71**
WSB/SG25	***2.45a***	***2.26a***	2.49ab	**2.4**
WSB/SG30	2.83a	2.69a	***2.40b***	***2.64***
WSB/SG35	***2.46a***	***2.47a***	2.47ab	**2.46**
Total	**2.61**	**2.58**	**2.47**	**2.53**
Silk	**2.80a**	**2.88a**	**3.00a**	**2.89**

[a, b, c] Means (SD) within the same column followed by different letters are significantly different ($p<0.05$).

5.3.3 pH of Refrigerated Soymilk Beverages

Figure 5.1 shows pH of the different soymilks including the commercial silk soymilk. Starting pH for all the beverages was between 8.0 and 8.5, and pH remained within this range over a 14 day period, except for silk milk that retained pH > 8.0. Except for the commercial soymilk, pH of the beverages declined below 8.0 by day 21, and further declined below 6.50 by day 28. The steep decline in pH after 14 days in storage could be due to souring of the soy milk caused by lactic acid producing bacteria.

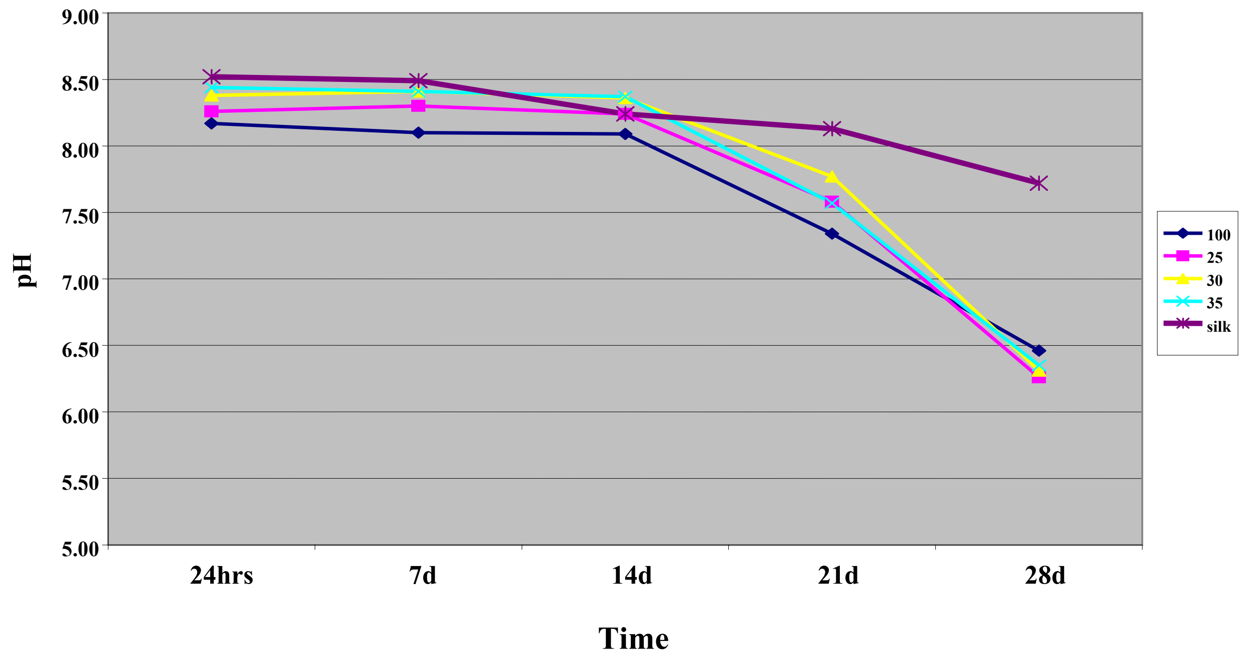


Figure 5.1 pH of Optimized Process PIE Soymilk Beverages

5.3.4 Aerobic / Total Plate Count of PIE Soymilk Beverages

There were no significant differences in bacteria populations found in PIE soymilks and control samples of the initial bacteria count throughout the 21 days storage (Table 5.3). Mean bacteria counts during storage expressed as CFU/ml were within the allowable 20,000 AC/ml. According to the results, the soymilk beverages at 24hr-0 (before pasteurization) were free of any viable bacteria. Therefore, the bacteria count observed during the 21 days storage period was a result of contamination between the homogenizing and pasteurizing step as evident from the 24hr-1 bacteria count. In general, there was no significant differences ($P>0.05$) in total bacteria count among the protein-isoflavone enriched beverages and control (i.e. WSB/SG25, WSB/SG30, WSB/SG35 and control – WSB/100). But, significant difference ($P<0.05$) was found in bacteria counts between the commercial silk soymilk and the other soymilk beverages between 24hr1 and 14days in storage. The low bacteria count found in the commercial silk soymilk can be attributed to the ultra high temperature (UHT) process of the commercial soymilk. UHT processing in combination with a high homogenizing pressure used in commercial soymilk processing have been reported to enhance beverage stability and quality (Nelson, et al., 1976). Also, the addition of antifungal and antibacterial preservatives such as sodium, calcium or potassium sorbate inhibits bacteria growth under normal refrigeration temperatures and prolongs shelf life. Even under ambient temperatures, commercial soymilk beverages stores well past the 14 day standard storage period for pasteurized cow milk.

Table 5.3 Initial Aerobic/Total Plate Count of PIE Soymilk Beverages

Sample	24hr0	24hr1	24hr2	7d	14d	21d
WSB/100	0.00a	3.76ab	3.24a	3.05ab	3.00a	3.22a
WSB/SG25	0.00a	***4.43ab***	***3.73a***	***3.65ab***	***3.76a***	***3.67a***
WSB/SG30	0.00a	3.93ab	4.16a	4.01ab	3.91a	4.21a
WSB/SG35	0.00a	5.25a	4.44a	4.36a	4.39a	3.31a
Silk	0.00a	0.00b	0.00b	2.38b	0.93b	0.32a

24hr0 = pre-pasteurized milk, 24hr1 = pasteurized milk from tank, 24hr2 – pasteurized milk in bottles; [a, b, c] Means (SD) within the same column followed by different letters are significantly different ($p<0.05$).

Starting bacteria count in pasteurized soymilk at 24hr – 1 (i.e. samples directly collected from cooling tank) ranged between 3.76 log CFU/ml in WSB/SG100 and 5.25 log CFU/ml in WSB/SG35. The starting bacteria counts were very similar to bacteria counts at 24hr - 2 (i.e. samples from sterile bottles) in the soymilk beverages, implying that contamination could have occurred between homogenizing and pasteurization and not necessarily from the sterile bottles. The presence of viable bacteria starting at 24hr-1 (i.e. pasteurized samples from the tank), and 24hr-2 (sterilized samples from the bottles) is crucial reasons for concern of the cleanliness of equipment during food processing. The homogenizing tank, the cooling pipelines and cooling tank can be associated with retention of bacteria because this part of the system is exposed to air through which bacteria were transmitted. End of storage count was between 3.22 log CFU/ml in

WSB/100 and 4.12 log CFU/ml in WSB/SG30. Among the beverages, bacteria count was more stable in the WSB/SG35, which showed a steady decline in total bacteria count over the 21 days storage period, unlike the other soymilks that showed inconsistency in bacteria count within the same storage period.

The presence of high bacteria populations has also been reported in fermented soymilks (Tsangalis et al., 2004) but these are mostly nutrient hydrolyzing bacteria such as the *Bifidobacterium sp.* Viable populations of *B. animalis* Bb-12 in fermented mixtures of soy protein isolate and soy germ soymilk ranged from 7.60 to 8.87 log CFU/ml (Tsangalis et al., 2004), and similar counts of 8 log CFU/ml have been reported in fermented soymilk hydrolyzed with *Bifidobacteruim sp* (Joen et al. 2002).

At termination of storage, all the soymilk beverages showed decline in total bacteria count except for WSB/SG30 that had a slightly higher bacteria count compared to its starting bacteria count at 24hr1. Irrespective of the inconsistent bacteria counts in the beverages, bacteria count was relatively stationary throughout storage, and decreased by day 21. The seemingly stationary bacteria count or decrease in bacteria count in the soymilks can be attributed to the inhibition of further growth upon immediate refrigeration at $4^{o}C$ ($39.2^{o}F$) or changes in conditions that permitted growth. Overall, the sanitary and hygienic conditions of the production process indicated by bacteria count, was fairly good.

However, final bacteria count after thorough cleaning and sanitizing of the homogenizing and pasteurizing equipment indicated the absence of bacteria to extremely low counts in the soymilk beverages (Table 5.4). Again, there were no significant differences among the soymilk beverages and the commercial silk milk, except for bacteria count at day 7 with WSB/SG25 and

Silk milk having significantly different bacteria counts from the other soymilks. Over all, bacteria count was less than 1 log CFU/ml in the beverages.

Table 5.4 Final Aerobic/Total Plate Count of PIE Soymilk Beverages

Sample	24hr0	24hr1	24hr2	7d	14d	21d
WSB/100	0.00a	0.00a	0.00a	0.00b	0.00a	0.00a
WSB/SG25	0.00a	0.00a	0.74a	0.72a	0.15a	0.00a
WSB/SG30	0.00a	0.00a	0.00a	0.00b	0.00a	0.00a
WSB/SG35	0.00a	0.00a	0.00a	0.00b	0.00a	0.00a
Silk	0.00a	0.00a	0.00a	0.81a	0.00a	0.30a

24hr0 = pre-pasteurized milk, 24hr1 = pasteurized milk from tank, 24hr2 – pasteurized milk in bottles; [a,] Means within the same column followed by different letters are significantly different ($p<0.05$).

With regards to the presence of pathogenic bacteria, evaluation was not done to specifically determine what pathogens if any could have been present in the soymilks. However, preliminary testing found no detectable Escherichia coli counts in all soymilks at 1 and 7 days (data not shown).

5.4 CONCLUSION

The results indicated that no significant differences ($P>0.05$) in colloidal stability, protein separation and bacteria counts were found among the protein-isoflavone enriched formulation, except for the difference in bacteria count between silk milk and the protein-isoflavone soymilks at 24hr1 and day 14, and at day 7 of the initial and final counts, respectively. Overall appearance and color of the beverages post 14 days standing showed no significant differences ($P>0.05$) either but all the beverages were scored at 6 (i.e. being liked slightly). Top liquid separation was rated high by consumer, low for bottom separation, and stable for overall separation, implying the beverages had good stable colloidal system. Colloidal stability and protein separation results certainly justify the caution on commercial packages that says ‘shake thoroughly before pouring out'. Thoroughly mixing the beverage would allow the particles and protein to disperse quite well before consumption.

Granted that low pH is necessary for preservation and stability, but low pH resulting in sour aftertaste in beverages can often overpower an intended flavor of a product. Thus, the high pH in the soymilk beverages was positively correlated with sustained bacterial count between 1 and 14 days. WSB/SG35 and commercial soymilk maintained a steady decline in pH as well as in bacteria count. On the whole, the WSB/100 and WSB/SG25 had lower bacteria counts than WSB/SG30 and WSB/SG35. Quality of beverage and colloidal stability may well be affected by viable bacteria presence and pH as well as other processing factors. The question arose as to what extent pH and bacteria population affect viscosity of soymilk beverages. Although this study did not investigate that, it is however, possible that significantly low pH or acidity would cause coagulation and eventual thickening of the beverage. In which case, the product would no longer be the same, and will be characterized by spoilage.

In spite of the nutritional, quality and shelf life distinctions made thus far amongst the PIE soymilk beverages and the control (WSB/100), it is obvious that their physicochemical properties had provided varied distinctions in the beverages. But, of the three PIE soymilks, WSB/SG25 demonstrated very good bacteria count, protein separation, and colloidal stability profile in addition to its isoflavone, protein, color and pH profiles, all of which point to this formulation as the optimal means for incorporating soy germ into soymilk. In view of the above conclusion, further studies determining the consumer perception of the beverages and characterizing them was undertaken to identify the final optimal formulation.

CHAPTER 6. SENSORY EVALUATION OF BLAND SOY GERM INCORPORATED PROTEIN-ISOFLAVONE ENRICHED (PIE) SOYMILK (FORMULATION OPTIMIZATION - PART II)

6.1 INTRODUCTION

Although soybeans are important food sources, high in protein and high in oil, undesirable flavors and objectionable bitter and astringent tastes are associated with soy products (Tsukamoto et a., 1995). Many attempts to improve the unfavorable characteristics of soybean seeds by genetic means have been made. Although the elimination of lipoxigenase from seeds has been successfully achieved, (Kitamura et al., 1983, 1985; Kitamura, 1984; Davies and Nielsen, 1986; Hajika et al., 1991) and contributes to the improvement of bean flavor (Matoba et al., 1985; Davies et al., 1987; Kitamura, 1993), factors that impart bitter and astringent flavors have remained. These undesirable characteristics are considered to be due to saponins, phenolic acids, oxidized phospholipids, oxidized fatty acids and even isoflavones (Arai et al., 1966; Sessa et al., 1976; Okubo et al., 1992).

Soymilk is conventionally prepared from non-transgenic whole beans by soaking, blanching / steaming, grinding, cooking, and filtering. The fluid milk is now being incorporated into numerous products to enhance sensory qualities in such foods as dairy foods (yogurt, milk, ice cream, and sherbet). Many studies suggest that soymilk can not only be successfully applied or incorporated into other food products, but also tremendously enhances sensory qualities and nutritional components (Hayta, et al., 2003). Soymilk with 6% and 9% solid content incorporated into bulgur showed improved color and sensory properties of pilav and fine bulgur, as well as enhanced bulk density. Increased soymilk solid content is synonymous with increased bulgur yield (Hayta et al., 2003). Even, enzyme treatment (CGTase and pullunase) of soymilk enhances sensorial characteristics as well as increases physicochemical components, suggesting addition of sugar to the isoflavone skeleton (Ara et al., 2003). Taste preference assessment with enzyme treated soymilk also shows a higher taste preference than non-enzyme treated soymilk.

Heat treatment of soymilk at varying temperatures and different length of time significantly affects color and flavor of the soymilk samples (Kwok et al., 2000). The SDS-extractability of bulgur proteins increased with increased soymilk solid content. Hence, water absorption capacity of fine bulgur samples was affected by soymilk incorporation, and variations in water absorption capacity were not significant (Hayta et al (2003).

As an anti-nutritional factor, isoflavones have been implicated in a role of causing bitter taste in soymilk, but isoflavone bears no consistency between undesirable astringent taste and its contents in soybean products. Isoflavone-enriched extracts (similar to 39% isoflavones) show no astringency either (Al Mahfuz et al 2004). Soybean foods having high amounts of isoflavones also show less astringency. Thus, it can only be assumed that the astringent characteristics caused by phytic ions in soy milk are lost upon conversion of phytic ions to their insoluble salt forms during soy milk coagulation. The aim of this study was to characterize the sensorial properties of optimized bland PIE soymilk and determine consumer acceptability.

6.2 MATERIALS AND METHODS

6.2.1 Soymilk Processing

U.S. No. 1 grade whole soybeans were purchased from SB & B Foods, Inc (Casselton, North Dakota, USA 58012) and soy germ (SG) was donated by Acatris / Schouten USA Company (Minnesota, USA). Soymilk production was done as in chapter 3. The process consisted of soaking in 0.5% NaHCO3, blanching, grinding, cooking, homogenization and pasteurization. A mixture of soybeans and soy germ in variable ratio of 25:75; 30:70; 35:65 SG to WSB was made for consumer to determine the best formulation for further study. Commercial soymilk was purchased from local area stores and served as external control.

6.2.2 Attaining and Evaluating for Optimal Formulation

Beverage optimization was done using a two-component mixture in three formulations, with each formulation containing a different ratio of whole soybeans (WSB) to soy germ (SG). WSB and SG at ratios of 75:25 (WSB/SG25), 70:30 (WSB/SG30), and 65:35 (WSB/SG35) were used in the manufacture of soymilk. 100% WSB and the commercial soymilk were used as control and reference sample, respectively. The decisive factors of the beverage for which the highest percent of consumer overall liking, acceptability and intent of purchase were identified was selected as the optimal formulation.

6.2.3 Consumer Preference and Acceptance Test

The consumer study protocol was approved by the Internal Review Board (IRB) of Louisiana State University (Baton Rouge, Louisiana, USA) and consisted of five bland soymilk samples. 500 consumers randomly selected participated in this study. Recruiting criteria consisted of: (1) all persons are at least 18 years of age, (2) not allergic to soymilk, sugar or fructose, and (3) available for complete evaluation of all five samples at one time. Sensory evaluation questionnaire (Appendix D-6.1) consisted of 13 questions, including a demographic question on gender. Consent to testing the beverages was signed (Appendix C) and collected from each participant prior to the tasting test.

Soymilk was evaluated the day it was prepared, and served daily for three days. The taste panel comprised of students, faculty, staff, lactose intolerant persons, technicians, post menopausal women at or around the university campus and its surrounding. Consumers were presented with WSB/SG25, WSB/SG30, WSB/SG35, a control (WSB/100), and a commercial reference sample – silk soymilk (Silk soymilk, Boulder Colorado), following a complete randomized design presented in a random order of A through E. Samples were served at 4.4°C (39.2°F) in 2 - oz (88.7ml) cups. Each assessor assigned scores and ratings by comparing samples to the reference sample and the control. The design used was selected because there were only few samples for testing and differences between samples are readily distinguished. Soymilk attributes evaluated included overall appearance, color, overall aroma, soy aroma, taste/beany flavor, sweetness, mouthfeel/viscosity, overall liking, using the 9-point hedonic scale where 1 = dislike extremely; 5 = neither like nor dislike; 9 = like extremely. Aroma and sweetness ratings were scored on a JAR 3-point scale: 1 = too weak; 2 = just about right; 3 = too strong (Appendix D-6.1). Acceptability and purchase intent were rated on a non-parametric scale where 'Yes' = 1 and 'No'= 2. This scale is useful in consumer testing because it defines psychological states of 'like' and 'dislike' on a linear scale. It is important to note that this scale is bipolar, which means that the descriptive adjectives at either end of the scale may not be opposite in sensory meaning (Gacula and Singh, 1984).

6.2.4 Statistical Analysis

a) ANOVA - Soymilk was produced in a single batch and served post 24 hr. Results of consumer evaluation were reported as a mean ± standard deviation. ANOVA data with $P<0.05$ were classified as statistically significant (two-sided test). All data were analyzed using the Statistical Analysis System (SAS - 8.02, SAS Institute Inc., Cary, NC). The analysis of variance test (ANOVA) was used in order to determine consumers' perceptions and acceptability of each sensory attribute and in overall liking of each beverage formulation. ANOVA was also used for determining formulation differences by gender for all variables. Post-hoc comparisons were done using Tukey's honestly significant difference (HSD).

b) MANOVA - The multivariate analysis of variance technique which is an extension of ANOVA (Pavon, 2003) was used wherein more than one variable were tested simultaneously to detect differences in groups across multiple dependent variables. Its application was used to examine relationships among the attribute descriptors and determine the underlying perceptual dimensions involved in explaining sensory quality. Descriptive discriminant analysis (DDA), proportional odds ratio model (PODDS), McNemar Test, % Hit Rate, Canonical Structure were all used to distinctly discriminate and determine significant differences between samples. DDA was used to determine the most discriminating attributes in terms of consumer perceptions. Predictive discriminative analysis (PDA) was used to determine both product acceptance and purchase decision with prediction intervals based on individual attributes according to consumers. PCA, a variable reduction technique, was used to identify the smallest number of latent variables that explained the greatest amount of variability. Both logistic regression and PDA were used to determine both product acceptance and purchase decision. Logistic regression was used to predict both acceptance and purchase decision using the odds ratio estimate. The odds are a nonnegative number with a value that is greater than 1.0 when a success is more likely to occur than a failure (Agresti, 1996). In this case, a "success" was either acceptable product or intent to purchase product. When values of θ (odds ratio) are above 1.0 in any given direction, this represents stronger levels of association. In order to determine if a change in the probability of the purchase intent of consumers before and after they tasted the milk occurred, the McNemar test was performed. Proportion odds model (PODM) was used to determine those attributes that were most or least liked or in between. In other words, the two ends of the scale represent consumers who either like or dislike the product, while the middle represents consumers who are undecided.

6.3 RESULTS AND DISCUSSIONS

6.3.1 Demographic Strata

The demographic information presented in Table 6.1 indicated that of the 500 untrained consumers who participated in the study, approximately 224 were male (48 %) and 247 were female (52%). The proportion of women participants was relatively equally to the number of men who evaluated the soy products. This strongly implies that interest in health benefit food today is not just a passing fad and is not only limited to women having interest in healthy eating or life styles. Increasing awareness of the various health benefits of soybean (Frankenfeld, 2003) gives both men and women or the general public another reason to eat soybean products, one of the fastest growing segments in the food industry (Soyatech, 1999). Therefore, the high numbers of male consumers with interest in healthy food consumption as seen in this study is no surprise.

Table 6.1 Consumer Demographic Frequency

Gender	Freq	Freq/5	%	Cumulative Frequency	CF/5	Cumulative (%)
Male	1121	224.2	47.6	1121	224.2	47.6
Female	1234	246.8	52.4	2355	471	100

Freq = Total Frequency of 5 samples; Freq/5 = Average frequency of 5 samples; CF/5 = Average cumulative frequency of 5 samples

6.3.2 Product Information

A composite view at consumer perception of soymilk indicated that soy aroma and overall soy aroma were the most important attributes as shown by 39 and 33% of respondents, respectively (Table 6.2 and Appendices D-6.2 - 6.4). Soy aroma was rated highest as ‘Just About Right’ in the products by 60.2% of consumers. Both attributes were scored at 5 being neither liked nor disliked. Color was another important attribute scored at 5 by 25.21% of respondents

Table 6.2 **Overall Consumer Frequency Responses for all PIE Soymilk Beverages**

Product Attributes	Score	Freq	%	Cumulative Frequency	CF/5	Cumulative (%)
Overall Appearance	6	500	20.03	1779	355.8	71.27
Color	5	629	25.21	1271	254.2	50.94
Overall Aroma	5	843	33.91	1532	306.4	61.63
Soy Aroma	5	967	38.98	1661	332.2	66.95
Rated Soy Aroma	2	1461	**60.22**	2025	405	83.47
Taste Beany flavor	6	471	18.92	2009	401.8	80.85
Sweetness	6	547	21.91	1976	395.2	79.13
Rated Sweetness	2	1125	**45.82**	2013	402.6	82
Mouth Feel Viscosity	6	504	20.19	1954	390.8	78.29
Overall Liking	6	479	19.25	1998	399.6	80.21
Acceptability	1	1386	55.89	1386	277.2	58.89
Purchase Intent	1	654	26.25	654	130.8	26.25
Health Benefit PI	1	990	39.73	990	198	39.73

Following color in descending order was sweetness scored at 5 by 21.19%, mouth feel viscosity, overall appearance and overall liking scored 6 by 20.19, 20.03, and 19.23%, respectively, and taste / beany flavor rated at 5 by 18.95%.

Similar to aroma, sweetness of the beverages was rated 2 as 'Just About Right' by 45.82% of consumers. With regards to product quality evaluated by the acceptability criteria, over half of the entire participants regarded soymilk as edible and, therefore, acceptable for consumption. However, less than 26.5% felt that they may purchase soymilk if commercially available. This low response could be due to the fact that typically, most of the consumer do not consume soymilk, but unknowingly consumer other soy enriched products. Hence, such a response points to four facts: (1) bland / unflavored soymilk is a strong deterrent to soymilk acceptance and consumption, (2) consumers are still struggling to accept soymilk as a substitute for cow's milk replacement in their diets, (3) they have limited knowledge of its health benefits, and (4) a combination of these 3 facts. Conversely, consumer response to purchase soymilk increased 13.5% from 26.5% to 39.73% when told about the health benefits of soymilk and soybeans in general. Such a change in response clearly supports the fact on limited knowledge of the health benefits of soymilk or other soy products.

6.3.3 Mean Consumer Overall Liking

The mean (± standard deviation) consumer acceptance scores are presented in Table 6.3. Mean overall liking was 5.09, 4.95, 4.42, 4.36 and 4.26 for silk soymilk, WSB/SG25, WSB/100 (control), WSB/SG35 and WSB/SG30, respectively. Acceptance was higher for silk and the 25% soy germ milk than control, WSB/SG30 and WSB/SG35 PIE soymilks. Hence, for the three PIE soymilks mean overall liking scores indicated consumer preference for formulation WSB/SG25 over the other two with a score of 4.95. Statistically, there were no significant differences ($P>0.05$) between control and PIE soymilks for overall appearance, color, soy aroma, and JAR rated soy aroma. However, silk soymilk was statistically different from the PIE soymilks and control for all attributes except for soy aroma and JAR rated soy aroma. The presence of emulsifiers and stabilizer in soymilk gives better effect of sensory attributes like appearance and color, body and texture, flavor, overall acceptability (Kumar and Mishra, 2004).

In general, scores for all attributes on the 9-point hedonic scale average 5.0 – 5.5 implying that all the soymilk were neither liked nor disliked. Similarly, ratings for soy aroma and sweetness on the JAR 3-point scale averaged about 1.5 – 2.0, also implying that the soy aroma and sweetness were neither weak nor strong but just about right.

6.3.4 Mean Consumer Acceptance and Purchase Intent Responses

Table 6.4 provides information on overall acceptability and purchase intents of each soymilk formulation versus the commercial silk soymilk. Each of the formulation was evaluated separately on a 2-point hedonic scale where 1 = yes and 2 = no for all three attributes.

Table 6.3 Comparison of Mean Consumer Scores for all Attributes of Soymilk Beverages

Sample ID	Overall Appear	Color	Overall Aroma	Soy Aroma	JAR Rated Soy Aroma	Taste Beany Flavor	Sweetness	Aroma JAR Rated Soymilk	Mouth Feel Viscosity	Overall Liking
WSB/ SG35	5.34b (1.73)	5.41b (1.74)	5.22ab (1.53)	5.17a (1.46)	1.92a (0.62)	4.19b (2.03)	4.75b (2.03)	1.71b (0.73)	4.73bc (1.87)	4.36b (1.94)
WSB/ SG30	5.35b (1.59)	5.32b (1.62)	5.14ab (1.57)	5.03a (1.45)	1.92a (0.65)	4.11b (1.96)	4.65b (1.94)	1.75b (0.71)	4.55c (1.86)	4.26b (1.95)
SILK	5.96a (1.73)	6.04a (1.66)	5.36a (1.70)	5.18a (1.67)	1.91a (0.59)	5.01a (2.11)	4.81ab (2.08)	1.52c (0.57)	5.40a (2.05)	5.09a (2.12)
WSB/ 100	5.41b (1.63)	5.34b (1.62)	5.06b (1.62)	4.92a (1.52)	2.00a (0.66)	4.36b (2.06)	4.65b (2.08)	2.10a (0.75)	4.69c (1.99)	4.42b (2.04)
WSB/ 25	5.45b (1.68)	5.51b (1.66)	5.26ab (1.69)	5.12a (1.61)	1.93a (0.61)	4.98a (2.04)	5.14a (1.97)	2.01a (0.65)	5.05b (1.99)	***4.95a*** (2.06)
Range	0.62	0.72	0.3	0.26	0.09	0.9	0.49	0.58	0.85	0.83

Numbers in parentheses represent standard deviation of 500 consumer responses.
Range = the highest score minus the lowest score.
[a, b, c] Means (SD) within the same column followed by different letters are significantly different ($p<0.05$)

Silk soymilk received the highest percentage of positive responses (63.43%), followed by the PIE soymilk WSB/SG25 with 63.38%, then WSB/100 (52.22%), WSB/SG35 (51.41%) and WSB/SG30 (48.99%). A resembling pattern is that consumer rated purchase intent of silk as the most likely to be purchased in stores, succeeded by WSB/SG25, WSB/100, WSB/SG30 and WSB/SG35. The preference for silk soymilk over experimental may not necessarily have to do with its protein and isoflavone concentrations, which in fact are very low compared to our soymilk beverages. Rather the driving preference can be attributed much more to its sensorial attributes as a result of the added components such as nutrient enhancers (calcium and vitamins, stabilizers etc), not added in any of our products. All four experimental soymilk were composed of either whole beans or soybeans and germ, with added sucrose for sweetening. Nevertheless, consumers seemed to maintain preference for soymilk with less amount of germ (WSB/SG25) compared to those with high amounts of germ incorporated.

From Table 6.4, purchase intent based on knowledge of soy protein and isoflavone health benefits, demonstrated commercial silk milk in the lead by 48.80% of consumers, followed by WSB/SG25 (45%), WSB/SG35 (36.75%), WSB/100 (35.67%), and WSB/SG30 (32.39%). The focal point in purchase intent based on consumer knowledge of soy health benefit is how much more are consumers willing to drink more soymilk to enhance their health. In that regard, the percentage change in purchase intent on average went up by 13.47%. The highest percent change for purchase intent based on knowledge of soymilk health benefits was seen in WSB/35 with 19.68% from 17.07 to 36.75%, followed by WSB/SG30 with 12.31% from 20.08 to 32.39%, WSB/SG25 with 11.6% from 33.40 to 45.0%, and WSB/100 (control) with 10.37% from 25.30 to 35.67%. Change in purchase intent for commercial soymilk was 13.39% from 35.41 to 48.80%. As expected, purchase intents increased after being informed of the health benefits of

Table 6.4 Overall Frequency Responses of Bland Soymilk for Acceptance, Purchase Intent and Soy Health Benefit Purchase Intent

Soymilk Type	Acceptability		Purchase Intent		Health Benefit Purchase Intent		P I + HBPI (Total)	% Change
	Freq	%	Freq	%	Freq	%	%	%
WSB/SG35	255.0	${}_{b}51.41^{4}$	85.0	${}_{c}17.07^{5}$	183.0	${}_{a}36.75^{3}$	26.91	19.68
WSB/SG30	243.0	${}_{c}48.99^{5}$	100.0	${}_{b}20.08^{4}$	161.0	${}_{c}32.39^{5}$	26.23	12.31
SILK	314.0	63.43^{1}	176.0	35.41^{1}	243.0	48.80^{1}	42.1	13.39
WSB/100	259.0	52.22^{3}	126.0	${}_{a}25.30^{3}$	178.0	${}_{b}35.67^{4}$	30.48	10.37
WSB/SG25	315.0	${}_{a}63.38^{2}$	167.0	33.40^{2}	225.0	45.00^{2}	39.2	11.60
Overall Average	1386.0	55.89	654.0	26.25	990.0	39.72	32.98	13.47

$^{a,\ b,\ and\ c}$ denotes comparison only between PIE soy germ milk, and $^{1,\ 2,\ 3,\ 4,\ 5}$ denotes comparison between all samples (PIE soymilks, control and commercial silk soy milk).

soy protein and isoflavone. The percent changes corresponded with soy germ content in the soymilks from high to no germ present in the milk. This implied that the general public's awareness of soy health benefits influences their food choices for enhanced health. Acceptance pattern of purchase was consistent with the trend in purchase intent prior to being informed of the health benefits of soymilk. These results correspond directly to the pooled sensory attributes with WSB/SG25 soymilk having higher ratings/scores for most of the attributes over WSB/SG35 and WSB/SG30, respectively. Overall, pooled purchase intent (PI+HBPI) had commercial silk milk with the highest score, closely followed by WSB/SG25, WSB/100, WSB/SG35 and WSB/SG30.

6.3.5 Mean Acceptance and Purchase Intent Scores by Gender

According to the mean (± standard deviation) gender scores on all sensory attributes, there were no significant differences between the five soymilk samples for overall appearance, color, overall aroma and soy aroma (Table 6.5). Specifically, no significant differences between men and women were observed for taste/beany flavor in silk milk, WSB/100 and WSB/SG25; neither for

Table 6.5 Mean Scores for Acceptance, Purchase Intent and Soy Health Benefit Purchase Intent Responses by Gender

Sample ID	Gender	OApp	Color	Overall Aroma	Soy Aroma	Taste Beany Flavor	SWT	MFV	Overall Liking	ACP Score	PI Score	HBPI Score
WSB/ SG35	Male	5.31	5.28	5.23	5.11	***4.44a***	***5.07a***	4.82	***4.58a***	1.45	1.81	1.62
	Female	5.43	5.55	5.21	5.20	***3.93b***	***4.44b***	4.60	***4.14b***	1.52	1.86	1.65
WSB/ SG30	Male	5.32	5.21	5.15	5.05	***4.37a***	***4.92a***	4.68	***4.49a***	1.49	1.77	1.65
	Female	5.39	5.43	5.13	5.02	***3.85b***	***4.42b***	4.39	***4.04b***	1.55	1.84	1.72
SILK	Male	5.90	5.99	5.39	5.20	5.05	4.83	5.34	5.18	***1.39***	***1.67***	***1.51***
	Female	6.05	6.13	5.34	5.17	4.94	4.77	5.40	4.97	***1.36***	***1.63***	***1.53***
WSB/ 100	Male	5.36	5.28	5.07	5.00	4.63	***5.04a***	***4.84a***	***4.70a***	1.43	1.71	1.62
	Female	5.45	5.41	5.05	4.83	4.10	***4.27b***	***4.48b***	***4.15b***	1.54	1.78	1.68
WSB/ 25	Male	5.45	5.48	5.22	5.11	5.07	5.28	5.16	***5.15a***	***1.33***	***1.65***	***1.53***
	Female	5.47	5.55	5.30	5.13	4.90	5.03	4.94	***4.75b***	***1.39***	***1.68***	***1.57***

[a, b, c] Means (SD) within the same column followed by different letters are significantly different ($p<0.05$)
OApp = Overall Appearance
SWT = Sweetness
MFV = Mouth Feel Viscosity
ACP = Acceptance; 1 = Yes and 2 = No
PI = Purchase Intent; 1 = Yes and 2 = No
HBPI = Health Benefit Purchase Intent; 1 = Yes and 2 = N

sweetness in silk soy milk and WSB/25; nor for mouthfeel viscosity in all samples except WSB/100 and for overall liking in silk soymilk. But, for these same attributes, significant differences were observed between men and women in the other soymilk. Men had higher preference/liking than women for taste/beany flavor and sweetness in WSB/SG35 and WSB/SG30; mouthfeel viscosity in WSB/SG100; overall liking in WSB/100, WSB/SG25, WSB/SG35 and WSB/SG30, respectively.

Although there were no significant differences, the results from the Table 6.5 further indicated in general, women (1.36) rated the commercial silk milk as more acceptable (1=yes and 2=no) than the men (1.39) did, and all the other soymilks were rated as more acceptable by men than women. The likelihood of either men or women purchasing the soymilk beverages was high for men than women for all the beverages, except the commercial soymilk. Change in purchase intent based on knowledge of soy health benefits was consistent with purchase intent based on availability or prior to knowledge of soy health benefits. However, it was observed that percent change in purchase intent for both men and women more willingness to buy more of the health enhancing soymilk (WSB/SG35 – 20%, WSB/SG30 – 12%, WSB/SG25 – 11.5%) containing germ than the WSB/100 (9.5%) containing no germ. Change in purchase intent for WSB/SG30 was same for both men and women (12%). Percent change in purchase intent of the beverages for men was higher for WSB/SG35 (19%), followed by commercial silk milk (16%), WSB/SG25 (12%), and WSB/100 (9%). Percent change in purchasing intent for women was highest for WSB/SG35 (21%) than for men, but followed by WSB/SG30 (12%), WSB/SG25 by 11%, and commercial soymilk and WSB/100 both by 10% only. These results could mean that on the one hand, men are increasingly becoming health conscious like women, and therefore, more apt to go for the enhanced health benefiting soymilk containing soy germ than one offering less health benefit. On the other hand, and with increasing knowledge of the health benefits of soy protein and isoflavone, women tend to buy products offering more soy health value as seen with percent change in response to health benefit purchase intent, and the soymilk containing more soy germ

6.3.6 Overall Product Differences – Pooled Within Canonical Structure's

Further analysis (Table 6.6 and 6.7) of the four soymilk formulations to determine inter-differences considering all of the sensory attributes simultaneously was done by multivariate analysis of variance (MANOVA). The Wilks's lambda p-value was used in assessing the

Table 6.6 Multivariate Statistics and F Approximations (Wilks' < .0001) of Soymilk Beverages with all Attributes Considered Simultaneously

Statistic	Value	F Value	Numerator DF	Denominator DF	Pr > F
Wilks' Lambda	***0.892***	***8.81***	***32***	***8955.6***	***<0.0001***
Pillai's Trace	0.110	8.61	32	9724	<0.0001
Hotelling-Lawley	0.118	8.99	32	6339.5	<0.0001
Roy's Greatest Roots	0.094	28.6	8	2431	<0.0001

MANOVA - Test Criteria and F Approximations for the Hypothesis of No Overall Form Effect
H = Type III SSCP Matrix for Forms; E = Error SSCP Matrix; S = 2 M = 2 N = 143.5

Table 6.7 Canonical Structures' r of Critical Soymilk Sensory Attributes Affecting Differences among Formulations (Based on Pooled within-group variances)

Sensory Attribute	can1	can2
Overall Appearance	0.4356	0.3609
Color	**0.4996**	0.3374
Overall Aroma	0.1611	0.0480
Soy Aroma	0.0856	0.0678
Taste / Beany Flavor (TBF)	**0.5677**	**0.6018**
Sweetness	0.1142	**0.5507**
MouthFeel Viscosity (MFV)	**0.4975**	0.0693
Overall Liking	**0.5092**	0.3653
Cumulative Variance (%)	79.37	93.09

influence of all sensory attributes at the same time. The P-value of <0.0001 presented in Table 6.6, indicated that all five soymilks were significantly different when all eight sensory attributes (6.7) were simultaneously considered. Since, multivariate techniques reveal whether significant differences exist between treatments when all attributes are compared simultaneously (Lawless and Heymann, 1998), this technique was extremely useful in light of the aforementioned objective. According to Koeferli et al. (1998), the use of this technique has greatly expanded the field of sensory analysis, where in it is used to correlate, reveal patterns, and classify data collected from consumers.

Descriptive discriminant analysis (DDA) was also used to determine which attributes were responsible for the underlying differences among the five soymilk beverages. According to the pooled within canonical structure in the first dimension (Can 1), TBF (0.568), overall liking (0.51), color (0.499), and MFV (0.497) significantly contributed to overall differences among the beverages resulting in 79.4% cumulative variance explained (Table 6.7). Sweetness in the second dimension (Can2) also contributed to the overall differences in the beverages.

6.3.7 Logistic Regression Analysis vs. Predictive Discriminant Analysis (PDA) for Acceptance and Purchase Intent

Logistic regression analysis for consumer acceptance, purchase intent and health benefit purchase intent are presented in Table 6.8 and 6.9. Results indicated that overall liking was the most critical attribute for acceptance, purchase intent, and health benefit purchase intent with odds ratio of 2.973, 3.66 and 2.69 (based on the single – variable model), respectively. This implies that for every 1 point increase in overall liking on the hedonic scale, acceptance, purchase intent and health benefit purchase intent will increase by 197%, 266% and 169%, respectively. In addition to overall liking, TBF, sweetness and MFV were also critical attributes

Table 6.8 **Prob>X^2 and Odds Ratio Estimates for Consumer Acceptance and Purchase Intent of Soymilk Beverages**

Acceptance			
Independent Variable	**Prob> X^2 (Full Model)**	**Odds Ratio Estimate (Full Model)**	**Odds Ratio Estimate (Single)**
Overall Appearance	0.7758	0.981	1.426
Color	0.4011	1.06	1.435
Overall Aroma	0.2262	0.92	1.544
Soy Aroma	0.7262	0.975	1.631
Taste / Beany Flavor (TBF)	0.0027	1.17	***2.349***
Sweetness	<.0001	1.231	***2.26***
Mouth Feel Viscosity (MFV)	<.0001	1.196	***2.11***
Overall Liking	<.0001	2.108	***2.973***
Purchase Intent (Buy)			
Independent Variable	**Prob> X^2 (Full Model)**	**Odds Ratio Estimate (Full Model)**	**Odds Ratio Estimate (Single)**
Overall Appearance	0.6165	1.045	1.634
Color	0.9884	0.999	1.617
Overall Aroma	0.7882	1.022	1.859
Soy Aroma	0.032	1.202	2.011
Taste / Beany Flavor (TBF)	<.0001	1.342	***2.738***
Sweetness	0.1609	1.088	***2.513***
Mouth Feel Viscosity (MFV)	0.1069	1.098	***2.289***
Overall Liking	<.0001	2.366	***3.656***
Health Benefit Purchase Intent (HBPI)			
Independent Variable	**Prob> X^2 (Full Model)**	**Odds Ratio Estimate (Full Model)**	**Odds Ratio Estimate (Single)**
Overall Appearance	0.2014	1.094	1.569
Color	0.4637	1.053	1.574
Overall Aroma	0.1469	0.904	1.693
Soy Aroma	0.0007	1.283	1.892
Taste / Beany Flavor (TBF)	0.0004	1.202	***2.244***
Sweetness	0.0009	1.176	***2.172***
Mouth Feel Viscosity (MFV)	0.2024	1.061	***1.982***
Overall Liking	<.0001	1.852	***2.685***

Table 6.9 % Hit Rate for Acceptance, Purchase Intent, and Soy Health Benefit Purchase Intent based on the Predictive Discriminant Analysis

Attributes	Acceptance	Purchase Intent	Health Benefit PI
All combined attributes	**82.57**	**81.42**	**79.84**
Overall Appearance	59.01	62.18	64.02
Color	60.12	62.12	64.33
Overall Aroma	58.81	68.66	66.89
Soy Aroma	59.37	72.19	70.05
Taste / Beany Flavor (TBF)	***76.94***	***79.40***	***76.46***
Sweetness	***76.17***	***73.85***	***75.49***
Mouth Feel Viscosity (MFV)	***74.28***	***74.24***	***73.16***
Overall Liking	***82.30***	***81.65***	***78.48***
Rated Soy Aroma	58.45	59.23	52.14
Rated Sweetness	66.56	54.62	59.10

for product acceptance. The odds ratio estimates for these three attributes were 2.35, 2.26, 2.11 and 2.35 (based on the single – variable model) indicating that for every 1 point increase in TBF, sweetness and MFV on the hedonic scale, the acceptance will increase by 126%, 111% and 135%, respectively for product acceptance.

Overall liking, TBF, and soy aroma were critical to both purchase intent and health benefit purchase intents (in addition to sweetness). For initial purchase intent of products, odds ratio estimate (Prob>χ^2 less than 0.05) for TBF, soy aroma, and overall liking were 2.74, 2.01, and 3.66, respectively. This also implies that for every one point increase in these attributes on the 9-point hedonic scale, overall purchase intent would likely increase by 2.74, 2.01, 2.3, and 3.66 times. For health benefit purchase intent, the attributes (TBF, soy aroma, sweetness and overall liking), were the defining critical attributes with odds ratio estimate of 2.24, 1.89, 2.17 and 1.98 (Prob>χ^2 less than 0.05), respectively. Again, for every one point increase in these attributes on the hedonic scale, purchase intent based on these attributes will increase 2.24, 1.89, 2.17 and 1.98 times, respectively. These results agrees with those from Table 6.7 based on Can1 and Can2 that identified overall liking, sweetness, TBF and MFV as discriminating attributes responsible for the underlying differences among the beverages.

Based on % hit ratio from predictive discriminate analysis (PDA) of the soymilks', overall liking of the beverages was the best single predictor for acceptance with 82.30% accuracy, purchase intents with 81.65% accuracy, and health benefit purchase intent 78.48% accuracy (Table 6.9). For purchase intent and health benefit purchase intent, the same attributes overall liking, TBF, sweetness and MFV were important predictors. Again, these results validated to a great extent, the logistic regression results.

6.3.8 Proportional Odds Models of Predictors Relative to Like / Dislike of Products

Results of the odds of accepting/liking (on a 9-point hedonic scale) a formulation is presented in (Table 6.10). Unlike the full odds model in Table 6.8 that took all 8 predictors (overall appearance, color, overall aroma, soy aroma, TBF, sweetness, MFV, and overall liking), the short model (Table 6.10) using proportional odds model which is a backward stepwise selection procedure takes into account only the critical predictors that included, overall appearance, overall aroma, TBF, sweetness, and MFV. All non significant predictors were eliminated leaving only the main predictors from which the odds ratios were calculated.

The odds ratio estimates for the reduced model in Table 6.10 showed that the chance that the product will be liked will be increased 2.47 times (147%) as the score for TBF increases by 1 point, conditioning that all other factors are held constant. Likewise, the odds of liking the product increases 1.91 times (91%), 1.75 for times (75%) for mouthfeel viscosity and sweetness, respectively, when other variables are held constant. Basically, these results imply that all of

Table 6.10 Proportional Odds Model of Predictors Relative to Like / Dislike of Products

Attribute	Pr > X^2	Odds Ratio Estimate
Overall Appearance	0.0013	1.164
Overall Aroma	0.0088	1.145
Taste / Beany Flavor (TBF)	<.0001	2.473
Sweetness	<.0001	1.753
Mouth Feel Viscosity (MFV)	<.0001	1.914

these predictors (overall appearance, overall aroma, TBF, sweetness, MFV) are important predictors for overall liking. With the exception of overall appearance, these results agree with earlier results that identified some or all of these predictors as critical product acceptance, consumer purchase intent and consumer health purchase intent based on knowledge of soy health benefits.

6.3.9 The McNemar Test for Change in Probability of Purchase Intent

In order to determine if a change in the probability of the purchase intent of consumers without knowledge of soybeans health benefit and after having been told about the health benefits of soy protein and isoflavone, the McNemar test was performed. The null hypothesis (H_0: $\pi_{1+} = \pi_{+1}$) states that the probability of the purchase intent is the same before and after consumers knowledge of the product health benefits. There is no significant difference in the probability of purchase intent before and after knowledge of the product health benefits. Therefore, whether the probability of consumer knowledge of the products health benefits is significantly different was tested.

Examination of purchase intent based on knowledge of soy health benefit response distinguished WSB/SG35 soymilk from the other beverages (Table 6.11). Thus, we could predict with 95% confidence that purchase intent after the health benefits of soy protein and isoflavone was given to consumers will increase between 16.13 to 23.3% for WSB/SG35. However also, the

Table 6.11 McNemar Test for Change in Probability of Purchase Intent of Soymilk Beverages

Formulation	χ^2	p-value	95% Confidence Interval	
WSB/SG35	94.16	<.0001	0.161	0.233_{1}
WSB/SG30	55.54	<.0001	0.092	0.153_{2}
SILK	54.45	<.0001	0.099	0.166
WSB/100	43.21	<.0001	0.076	0.137
WSB/SG25	49.47	<.0001	0.085	0.150_{3}

probability of purchase intent changes based upon knowledge of the health benefits from soy protein and isoflavone was significantly different at $\alpha = 0.05$ for the other beverages. Hence, with confidence, the probability of purchase intent increased by approximately: 10 and 16% for commercial silk soymilk, 9 and 15% for WSB/SG30, 8 and 15% for control (WSB/SG25), and by 8 and 14% for WSB/100. For the PIE soymilk beverages, it can be deduced that consumer's knowledge of the health benefits of soy protein and isoflavone influences their purchase decision to dramatically change with increased willingness to buy more soymilk with soy germ incorporated. This effect was clearly demonstrated in the gender response to health benefit purchase intent in which both men and women would buy more of the WSB/SG35 soymilk, followed by WSB/SG30, WSB/SG25 and WSB/100.

6.3.10 Principal Component Analysis of Bland Soymilk Product Attributes

According to the bi-plot (product – attribute) space using principal components 1 and 2 (Figure 6.1), it is evident that the attributes discriminating the WSB/SG25 (E) from the other beverages (A-D) consist of sweetness, TBF, overall liking, and MFV. The quadrant with the discriminating factors contains mainly the PIE soymilk (E) formulation. This is a verification of the descriptive discriminative analysis (DDA) result, where the pooled within canonical structure in the first dimension identified TBF (0.577), overall liking (0.509), MFV (0.497), and color (0.499), and the second dimension sweetness (0.550) as critical attributes that did contribute significantly to overall differences among the beverages (Table 6.5).

Similarly, the plots comparing principal components 1 and 3 and principal components 2 and 3 determined the same specific discriminating factors (See Appendix D-6.5 & 6.6). This could mean consumers preferred the commercial formulation over the PIE soymilk. According to

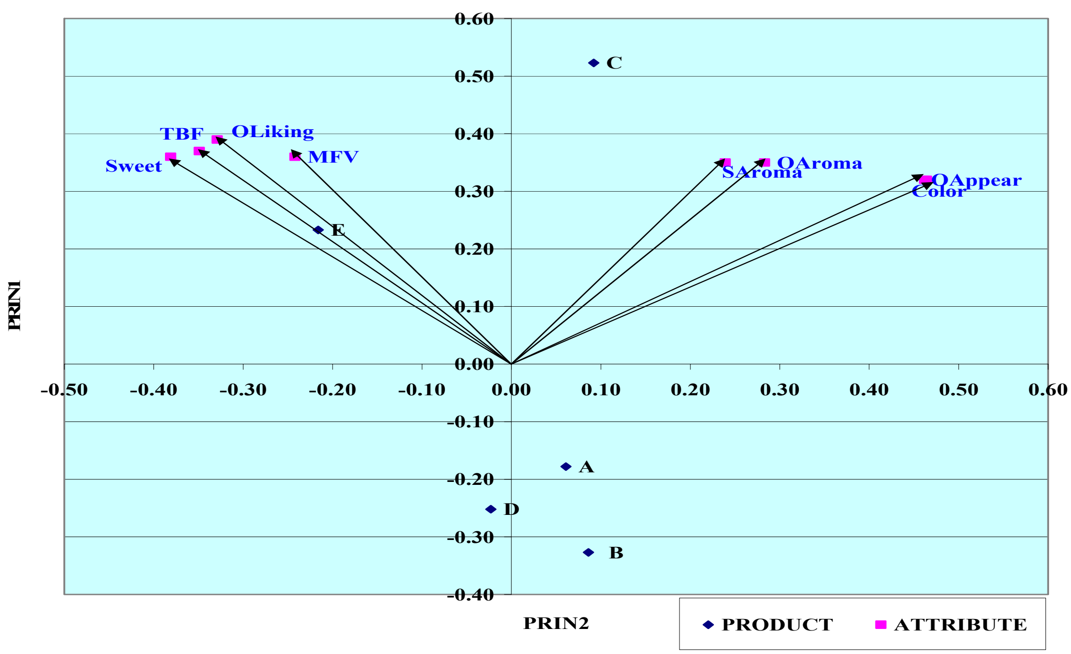


Figure 6.1 PCA bi-plot of Bland Soymilk Product attributes comprising Principal Component 1 and 2 (A = WSB/SG35; B = WSB/SG30; C = Commercial Silk Soymilk; D = WSB100; and E = WSB/SG25)

the descriptive discriminative analysis, logistic regression analysis, and principal component analysis, the attributes that separated the soymilk beverages from the other were overall liking, MFV, TBF, sweetness, color and soy aroma.

6.3.11 Product Optimization

In this study, product formulation optimization was performed using a two-component formulation in conjunction first with ANOVA and then logistic regression. ANOVA which was used to determine the most preferred formulation as depicted by overall liking, validated WSB/SG25 as most liked of the four formulation and WSB/SG30 the least liked (Table 6.4). Overall results and by gender for acceptance and purchase intent strongly revealed that WSB/SG25 was the most acceptable and, therefore, was much likely to be purchased, over the other beverages, except for commercial soymilk. The percent change in purchase intent based on knowledge of soy protein and isoflavone presented a reverse but expected turn with WSB/SG35 most likely to be purchased followed by WSB/SG30, WSB/SG25 and WSB/100. At this junction, one would be tempted to make a decision about the optimal formulation based on the latter results. But, further considerations from previous studies on the optimal composition, in particular protein and isoflavone contents of the beverages, gives credit to WSB/SG25 as containing more isoflavone in comparison to the other soymilks.

Predictive models were obtained using a logistic regression analysis for product acceptance, purchase intent and health benefit purchase intent. Change in purchase intent was obtained from the McNemar test. In any case, optimal formulation was determined based on overall liking in conjunction with these three factors (i.e. TBF, MFV, and sweetness) mentioned above considering all of the attributes. It was quite evident that the overall liking, TBF, MFV, and sweetness amongst other attributes influenced consumer acceptance, purchase intent and health benefit purchase intent. Logistic regression analysis also used in order to determine the most critical attributes in terms of consumer acceptance, purchase intent and health benefit purchase intent eliminated the non-critical attributes by Prob > χ^2 value. If the probability greater than Chi-square (χ^2) (Prob > χ^2) for a particular attributes was less than 0.1 (α = 1%), that attribute was considered significant in relation to consumer acceptance and purchase intents. As shown in Table 7.7 the Prob > χ^2 for consumer acceptance implicated overall liking, sweetness, TBF and MFV with Prob > χ^2 = 0.0001 for overall liking, MFV and sweetness, and Prob > χ^2 = 0.0027 for TBF as the most critical attributes. These same attributes and including soy aroma were also significant with respect to consumer purchase intent and health benefit purchase intent. Overall liking, TBF, MFV, sweetness and soy aroma were the underlying predicting attributes for acceptance, purchase intent and health benefit purchase intents that influenced the differences among the beverages. In light of these results and other considerations WSB/SG25 was selected as optimal formulation for further studies to evaluate its marketing and purchasing potential in flavored forms.

6.4 CONCLUSION

The objective of study was to determine optimal formulation for enhancing PIE soymilks. The data thus far suggested that WSB/SG25 was much liked compared to WSB/SG30 and WSB/SG35. Although consumers were more than willing to buy the other soy germ soymilk beverages after their awareness about the health benefits of soy isoflavone and protein, initial purchasing intent based on availability of the different soymilk beverages still pointed to WSB/SG25 as the optimal potential formulation for enhancing PIE soymilk. Overall and by gender, the WSB/SG35, WSB/SG30 and WSB/100 all received overall liking/acceptance below 5.0 except for WSB/SG25 and silk soymilk that obtained acceptance above 5.0. However also, the fact that the WSB/SG35 and WSB/SG30 were in most attributes and analysis rated over the WSB/100 could mean that they can also be improved upon in further testing studies.

The practicality and implications of these results point to the fact that a finished bland product is not quite appealing to consumers. Yet, the addition of soy germ either in liquid or powder form and not just in nutrient extract form opens a whole new business category for retail market. It also enhances the value margin growth by offering consumers soymilk that delivers added health benefits. The fact that the study demonstrated that it was possible to formulate acceptable PIE soymilk beverages by way of incorporating soy germ into soymilk, presents a new avenue for small and large companies to make this type of soymilk taste better, and increase consumption.

CHAPTER 7. SENSORY DISCRIMINANT TEST FOR FLAVORED SOYMILK

7.1 INTRODUCTION

Flavor is the main limiting factor affecting soybean acceptability in the Occidental countries (Carrao-Panizzi et al. 1999). Western populations, who are accustomed to the taste of dairy milk, generally dislike the flavor profile of traditional soymilk because of its aftertaste, often referred to as 'beany' or 'bitter' (Tsangalis et al., 2004). The predominant beany flavor is due to the presence of hexanal and pental aldehydes mainly formed as a result of the hydroperoxidation of polyunsaturated fatty acids, catalyzed by the enzyme lipoxigenase (Wilkens et al., 1967; Wilken and Lin, 1979; Tsangalis et al., 2004). Generally, the hydroperoxides undergo scission and dismutation resulting in the development of off-flavors and aromas (Orthoefer, 1978). In soymilk production, this oxidation usually occurs at the soaking and grinding stage. But, for the reaction to occur, lipoxigenase, damaged seeds with exposed substrate (linoleic and linolenic acids) and water are necessary (Nelson et al., 1980). The reaction does not occur when damaged seeds are just soaked in water. However, lipoxigenase enzymes are easily inactivated by boiling for three to five minutes, and it is the blanching process that prevents the formation of the characteristics beany flavor, making soybean products less or more acceptable. Today, commercial production implements methods that either prevent the formation of objectionable volatiles such as the use of hot water and sodium bicarbonate blanch before grinding to reduce off-flavors (Golbitz, 1995), or the use of modern dairy deodorizing processing technologies to remove residual off-flavors. The addition of a range of desired flavors also masks off-flavors (Liu, 1997).

Soymilk subjected to severe heating acquires a brown color and cooked flavor. Many researchers have studied and reviewed the effects of thermal processing on the quality of soymilk

(Kwok and Niranjan, 1995). Primary chemical reactions that give rise to heat-induced color and flavor changes are the maillard reactions. According to kwok et al. (2000), sensory attributes of perceived color and flavor are the most important characteristics because they are readily assessed by consumers. If thermal processes are designed on the basis of consumer appeal, it is important to know the effect of processing parameters on these sensory attributes using good quantified data. Sensory analysis is often used to assess flavor, appearance and texture of food products as a function of processing parameters. The 9-point hedonic scale is commonly used in sensory analysis, and the data analyzed parametrically. Alternately, non-parametric statistical analysis can be employed for consumer data analysis.

Despite their biological importance to human health, isoflavone remains to be associated with the undesirable characteristics of bitter and astringent flavor in soy products (Huang et al., 1981; Matsuura et al., 1989; Kudou et al., 1991; Okubo et al., 1992). According to Carrao-Panizzi, et al (1999), pre-soaking of grains intensifies beany flavor in the soymilk, reducing the perception of astringency, which is caused by the aglucones that are developed in reduced amounts. Their observations further indicated that the whole soybean grains cooked under pressure (1.5 kgf /cm(2) at 127^{o}C) presented reduced levels of isoflavones malonyl-glucosides. Due to thermal instability, these compounds were converted to conjugated glucosides, genistin and daidzin. In the cooked whole soybean grains, no aglucones were formed and consequently it was not possible to detect differences in astringency. These results suggest that pre-heating of grains promote better flavor in soybean products. Iwuoha, and Umunnakwe, (1997) also demonstrated that major factors such as processing method, storage temperature and duration and their interaction have significant impact on composition, physicochemical and sensory characteristics of soymilk. The most affected parameters were protein, fat, fiber, viscosity and flavor, while the least affected included moisture, carbohydrates, specific gravity and mouthfeel. In this present work, attention is directed to taste panel assessment based on hedonic response to characterize the optimized flavored PIE soymilk

7.2 MATERIALS AND METHODS

7.2.1 Production of Soymilk

After concluding the first study of bland soymilk, optimal formulation based on chemical evaluations of protein and isoflavone contents, and consumer study results for overall liking, acceptability, and purchase intent, the WSB/SG25 was selected as the optimal formulation for food grade PIE soymilk. U.S. No. 1 grade whole soybeans were purchased from SB & B Foods, Inc (Casselton, North Dakota, USA 58012) and soy germ (SG) was donated by Acatris / Schouten USA Company (Minnesota, USA). Using optimal processing conditions identified from previous section (chapter 3 and 4) of this research, soymilk production followed the procedure of Nelson et al. (1976) with modifications in a single batch. 1362g (3lbs) of soybeans were soaked in 4086 ml of 0.5% NaHCO3 solution for 8 hr. The remaining production process consisted of blanching, grinding with water to 17% solids, cooking, homogenization and pasteurization, then flavoring prior to consumer tasting. 10% sugar per every 1 liter was added to cooked filtrate, stirred thoroughly to dissolve, then homogenized at 4000psi and bottled.

7.2.2 Spiking / Favoring of Soymilk

Food flavor extracts (Mango, Almond, Orange all in propylene glycol) were purchased from Flavors of North America, Inc (Illinois, U.S.A). Chocolate powder was purchased from Forbes Chocolate Company (Cleveland, Ohio, USA). 10% Sugar was added to lightly sweeten the beverage, while liquid flavors were added at 1.0% for orange (40mL / 4 L), 0.5% for green mango and almond (20mL / 4L), and 3% of chocolate powder added.

7.2.3 Attaining and Evaluating for Optimal Flavor

No limits were set as boundaries for the optimization of flavored soymilk from the four flavors (orange, green mango, almond, and chocolate) used. A bland sample of the same formulation was included in the design as the control sample for comparison. As with the previous consumer study of bland soymilk, the decisive factors of the beverage here for which the highest percent of consumer overall liking, acceptability and intent of purchase were identified yielded the optimal flavored soymilk drink.

7.2.4 Consumer Preference and Acceptance Test

The consumer study protocol was approved by the Internal Review Board (IRB) of Louisiana State University (Baton Rouge, Louisiana, USA) and consisted of four flavored soymilk and a bland milk sample. 202 consumers randomly selected participated in this study. Recruiting criteria consisted of: (1) all persons are over 18 years of age, (2) not allergic to soymilk, sugar or fructose a, and (3) is available for complete evaluation of all five samples at one time. Sensory evaluation questionnaire (Appendix E-7.1) also consisted of 13 questions, including a demographic question on gender. Consent to testing the beverages was signed (Appendix C) and collected from each participant prior to start of the tasting test.

Soymilk was evaluated the day it was prepared, and served daily for three days. The taste panel comprised of students, faculty, staff, lactose intolerant persons, technicians, post menopausal women at or around the university campus and its surrounding. Consumers were presented with four flavored soymilk drinks and a bland sample following a complete randomized design presented in a random order of A through E. Samples were served at cool temperatures between $4^{o}C$ / $39.2^{o}F$ - $10^{o}C$ / $50^{o}F$ in 2 oz (88.7ml) cups. Each assessor assigned scores and ratings by comparing among samples. The design used was selected because there were only few samples for testing and differences between samples are readily distinguished. Soymilk attributes evaluated included overall appearance, color, overall flavor (taste & aroma), flavor aroma, sweetness, mouthfeel/viscosity, and overall liking using the 9-point hedonic scale where 1 = dislike extremely; 5 = neither like nor dislike; 9 = like extremely. Aroma, sweetness, thickness/viscosity were rated on a JAR 3-point scale: 1 = too weak; 2 = just about right; 3 = too strong (Appendix E-7.1). Acceptability and purchase intent were rated on a non-parametric scale where 'Yes' = 1 and 'No'= 2. This scale is useful in consumer testing because it defines psychological states of 'like' and 'dislike' on a linear scale. It is important to note that this scale is bipolar, which means that the descriptive adjectives at either end of the scale may not be opposite in sensory meaning (Gacula and Singh, 1984).

7.2.5 Statistical Analysis

a) ANOVA - Soymilk was produced in a single batch and served post 24 hr. Results of consumer evaluation were reported as a mean ± standard deviation. ANOVA data with $P<0.05$ were classified as statistically significant (two-sided test). All data were analyzed using the Statistical Analysis System (SAS - 8.02, SAS Institute Inc., Cary, NC). The analysis of variance test (ANOVA) was used in order to determine consumers' perceptions and acceptability of each sensory attribute and in overall liking of each beverage formulation. ANOVA was also used for determining formulation differences by gender for all variables. Post-hoc comparisons were done using Tukey's honestly significant difference (HSD).

b) MANOVA - The multivariate analysis of variance technique which is an extension of ANOVA (Pavon, 2003) was used wherein more than one variable was tested simultaneously to detect differences in groups across multiple dependent variables. Its application was used to examine relationships among the attribute descriptors and determine the underlying perceptual dimensions involved in explaining sensory quality. Descriptive discriminant analysis (DDA), proportional odds ratio model (PODDS), McNemar Test, % Hit Rate, Canonical Structure were all used to distinctly discriminate and determine significant differences between samples. DDA was used to determine the most discriminating attributes in terms of consumer perceptions. Predictive discriminative analysis (PDA) was used to determine both product acceptance and purchase decision with prediction intervals based on individual attributes according to consumers. PCA, a variable reduction technique, was used to identify the smallest number of latent variables that explained the greatest amount of variability. Both logistic regression and PDA were used to determine both product acceptance and purchase decision. Logistic regression was used to predict both acceptance and purchase decision using the odds ratio estimate. The odds are a nonnegative number with a value that is greater than 1.0 when a success is more likely to occur than a failure (Agresti, 1996). A "success" was either acceptable product or intent to purchase product. When values of θ (odds ratio) are above 1.0 in any given direction, this represents stronger levels of association. In order to determine if a change in the probability of the purchase intent of consumers before and after they tasted the milk occurred, the McNemar test was performed. Proportion odds model (PODM) was used to determine those attributes that were most or least liked or in between. In other words, the two ends of the scale represent consumers who either like or dislike the product, while the middle represents consumers who are undecided.

7.3 RESULTS AND DISCUSSIONS

7.3.1 Product Information

The mean sensory scores for all attributes evaluated on the 9-point hedonic scale were between 5 and 6 representing the mid-range of the scale. Overall appearance and color were both scored at 5 (neither like nor dislike) while the others were scored at 6 (like slightly - Table 7.1). Rated sweetness of the soymilks as well as the aroma, and thickness or viscosity were all rated 2 (just about right) which is the mid range of the JAR 3-point hedonic scale. The frequency of consumers who evaluated the flavored soymilks is also presented in Table 7.1. Over 70% of consumers reported liking the overall flavor (70.69%), aroma (66.73%), sweetness and mouthfeel viscosity (74.7%), with less than 45% that expressed likeness for overall appearance and color. It can, be deduced that sensory attributes with frequency percent of over 60% were most important to consumers and, therefore, more than likely influenced decisions on overall

Table 7.1 Frequency Responses of Consumers' Perception of the most Important Sensory Attributes of the Five Soymilk Beverages

Attributes	Score	Frequency	%	Cumulative Frequency	Cumulative %
Overall Appearance	5	50.6	25.1	91	45.14
Color	5	47	23.27	85.6	42.38
Overall Flavor	6	39.6	19.74	141.8	70.69
Aroma	6	45	22.48	133.6	66.73
Sweetness	6	54.4	26.93	151	74.75
Mouth Feel Viscosity	6	45	22.32	150.6	74.7
Overall liking	6	41.6	20.74	144.4	71.98
Thickness Viscosity	2	124.4	63.99	172.6	88.79
Rated Aroma	2	110	56.82	157.6	81.4
Rated Sweetness	2	115.4	59.12	169.4	86.78

liking, acceptance, or purchase intent of the soymilks'. Overall liking was rated "6" by 72% of the consumers while ratings for thickness/viscosity, aroma, and sweetness perceived as 'Just About Right' (JAR) by 89%, 81.4% and 87%, respectively.

According to the overall consumer responses for acceptance and purchase intent shown in Table 7.2, all the beverages were judged as acceptable by 63.35% of the participants. Approximately, 47% were willing to purchase the beverages if available in stores.

Table 7.2 Frequency Responses of Consumers for Acceptance and Purchase Intents of Flavored and Plain Soymilk (5 Beverages)

Attributes	Score	Frequency	%	Cumulative Frequency	Cumulative %
Acceptable	1 (Yes)	127.2	**63.35**	127.2	63.35
	2 (No)	73.6	36.65	200.8	100
Purchase Intent	1 (Yes)	94.6	**46.88**	94.6	46.88
	2 (No)	107.2	53.12	201.8	100

7.3.2 Mean Consumer Overall Liking and Acceptance Responses

The mean overall sensory scores of all attributes are presented in Table 7.3 for each of four flavored and plain soymilk. Overall response of the attributes indicated no significant differences ($P<0.05$) between the different flavors for six out of the ten attributes that included overall appearance, color, rated aroma, rated sweetness, and rated thickness/viscosity. For overall flavor, green mango, chocolate, and almond were statistically ($P<0.05$) not different from each other but were more acceptable than orange flavor. While green mango was slightly different from all the other flavors in aroma and mouth feel viscosity (MFV), chocolate and almond did not differ from each other, but were different from orange for aroma. Across the sensory spectrum evaluated, green mango had the highest overall liking ratings (although not statistically significant), followed by chocolate, almond, plain milk and orange.

Consumer evaluation indicated that three flavors (green mango, almond, and chocolate) were judged similarly in terms of overall liking. Green mango, chocolate and almond each scored at 5.37, 5.18, and 5.13, respectively were not significantly different from each other. The plain soymilk was not statistically different from the green mango, chocolate and almond flavors. Flavored soymilk drinks are becoming very popular as researchers continue to test for ways of enriching soymilk sensorial attributes for its nutritional enhancement. Behrens et al. (2004) reported increased acceptance of soymilk beverages flavored with pineapple, guava, strawberry, kiwi, and coconut flavors. Pineapple and guava flavors were most liked, while hazelnut was rejected with acceptance below 5.0 score.

With regards to acceptability and purchase intents scored on a 2 – point hedonic scale where 1 = yes and 2 = no, the percent (%) of positive responses is shown in Table 7.4. The flavors with the highest acceptability were green mango, chocolate and almond with responses of 67.33%, 67%, and 64%, respectively. These flavors were closely followed by 63.0% and 55.45% for the plain and orange flavor, respectively. These results correspond directly to the mean consumer overall liking, where the three of the four flavors had the highest overall liking. In terms of purchase intents, the percent of consumers who would buy the flavored milk ranged between 43.35 and 48.02%. The results seen with acceptance changed with consumer purchase intents responses. Almond (49.75%), plain soymilk (48.02%), and green mango (47.52%) were more likely to be purchased than chocolate (45.54%) and orange (43.56%) flavored soymilk.

7.3.3 Mean Acceptance and Purchase Intent by Gender

Demographic distribution of the study indicated that of the 202 untrained consumers, 182 identified their gender as either male or female while 18 did not (Table 7.5). The gender demographics showed that about 53% of the participants were males and 47% were females. Mean (± standard deviation) values of sensory attributes showed no statistical difference between men and women on all attributes, except for acceptance with almond flavored soymilk, as well as for overall appearance, aroma, sweetness and acceptance for plain soymilk. The men differed

Table 7.3 Overall Consumer Scores of Flavored and Plain Soymilk on all Attributes

Soymilk Flavors	OAppr	Color	OFlavor	Aroma	RAroma	SWT	RSWT	MFV	TKV	Oliking
Orange	5.72a (1.68)	5.89a (1.62)	4.49b (2.20)	4.93c (1.96)	2.05 (0.69)	4.92b (1.94)	1.87 (0.74)	4.95b (1.93)	1.94 (0.64)	4.53b (2.01)
Green Mango	5.68a (1.57)	5.82a (1.53)	5.30a (2.04)	5.97a (1.88)	2.05 (0.58)	5.71a (1.71)	1.95 (0.54)	5.45a (1.80)	1.84 (0.56)	5.37a (1.96)
Almond	5.67a (1.63)	5.83a (1.53)	5.15a (2.21)	5.59ab (2.12)	2.14 (0.59)	5.38ab (1.84)	1.91 (0.60)	5.28ab (1.76)	1.85 (0.58)	5.13a (2.18)
Chocolate	5.84a (1.59)	5.89a (1.49)	5.19a (2.12)	5.77ab (2.00)	1.99 (0.61)	5.53a (1.78)	1.92 (0.57)	5.38ab (1.72)	1.89 (0.53)	5.18a (2.06)
Plain	5.91a (1.82)	5.97a (1.86)	5.00ab (2.09)	5.31bc (1.89)	1.46 (0.56)	4.99b (1.88)	1.63 (0.60)	5.19ab (1.79)	1.80 (0.60)	4.96ab (2.10)
Range	0.24	0.15	0.81	1.04	0.68	0.79	0.32	0.5	0.14	0.84

Numbers in parentheses represent standard deviation of 202 consumer responses; Range = the highest score minus the lowest score.
[a, b, c] Means (SD) within the same column followed by different letters are significantly different ($p<0.05$)
OAppr = Overall Appearance; OFlavor = Overall Flavor; RAroma = JAR Rated Aroma; SWT = Sweetness; RSWT = Rated Sweetness; MFV = MouthFeel Viscosity; TKV = Thickness Viscosity; Oliking = Overall Liking.

Table 7.4 **Overall Responses for Acceptance and Purchase Intent**

Flavored Milk	Acceptance		Purchase Intent	
	Freq	**%**	**Freq**	**%**
Orange	112.00	55.45	88.00	43.56
Green Mango	136.00	***67.33***	96.00	***47.52***
Almond	128.00	***64.00***	100.00	***49.75***
Chocolate	134.00	***67.00***	92.00	45.54
Plain	126.00	63.00	97.00	***48.02***
Overall	636.00	63.36	473.00	46.88

from females on overall appearance, aroma, and sweetness in which they scored plain soymilk higher, and more apt to like it better. The same was seen with the green mango flavor which was more liked by the men than the women. For acceptance and purchase intent, the lower the score below 1.5 on the scale the higher the likelihood or tendency to accept and buy the soymilk, and the higher the score above 1.5 the stronger the tendency not to accept or buy the product.

Table 7.6 indicated overall liking of the flavored beverages to be relatively higher for men than women. With the exception of the green mango flavor, men liked all the other flavors and the plain soymilk better than women. This difference could be attributed to overall flavor and perhaps less sweetness that was slightly disliked by the women (Table 7.5). Chocolate flavored soymilk was much liked by men than women (Table 7.6). Suggestions from women indicated their preference for very strong flavored soymilk drink. This was noteworthy in that the flavors were slightly weak according to women, and considering U.S flavored drinks/beverages, chocolate in particular was quite weak for them.

Table 7.5 Mean Scores for all Attributes including Acceptance and Purchase Intent of Flavored and Plain Soymilk by Gender

Soymilk Flavors	Gender	Overall Appear	Color	Overall Flavor	Aroma	Rated Aroma	SWT	RSWT	MFV	TKV	Overall Liking	Accept.	PI	HBPI
Orange	Male	5.67a	5.84a	4.64a	4.92a	2.02a	5.06a	1.75a	4.88a	1.95a	4.54a	1.43a	1.77a	1.56a
	(≈ 53%)	(1.59)	(1.54)	(2.05)	(1.81)	(0.73)	(1.79)	(0.68)	(1.89)	(0.65)	(1.89)	(0.5)	(0.42)	(0.5)
	Female	5.83a	5.93a	4.32a	4.9a	2.09a	4.83a	1.95a	5.03a	1.93a	4.56a	1.44a	1.65a	1.56a
	(≈ 47%)	(1.7)	(1.7)	(2.34)	(2.11)	(0.65)	(2.03)	(0.77)	(2.04)	(0.64)	(2.18)	(0.5)	(0.48)	(0.5)
Green Mango	Male	5.79a	5.7a	5.39a	6.07a	2.02a	5.85a	1.99a	5.46a	1.85a	5.5a	***1.24b***	1.66a	1.51a
	(≈ 53%)	(1.44)	(1.47)	(2.0)	(1.69)	(0.58)	(1.51)	(0.5)	(1.64)	(0.54)	(1.75)	(0.43)	(0.48)	(0.5)
	Female	5.49a	5.88a	5.12a	5.85a	2.11a	5.37a	1.9a	5.33a	1.83a	5.16a	1.41a	1.69a	1.56a
	(≈ 47%)	(1.75)	(1.66)	(2.19)	(2.13)	(0.56)	(1.96)	(0.59)	(1.98)	(0.58)	(2.18)	(0.49)	(0.47)	(0.5)
Almond	Male	5.69a	5.81a	5.31a	5.68a	2.15a	5.48a	1.93a	5.36a	1.87a	5.36a	1.32a	1.61a	1.44a
	(≈ 53%)	(1.54)	(1.44)	(2.03)	(2.0)	(0.55)	(1.67)	(0.59)	(1.56)	(0.55)	(2.02)	(0.47)	(0.49)	(0.5)
	Female	5.66a	5.78a	4.98a	5.58a	2.16a	5.19a	1.87a	5.15a	1.86a	4.87a	1.39a	1.67a	1.53a
	(≈ 47%)	(1.71)	(1.68)	(2.43)	(2.28)	(0.59)	(2.04)	(0.62)	(1.98)	(0.59)	(2.34)	(0.49)	(0.47)	(0.5)
Chocolate	Male	5.79a	5.79a	5.39a	5.75a	1.98a	5.61a	1.91a	5.4a	1.93a	5.29a	1.33a	1.71a	1.54a
	(≈ 53%)	(1.6)	(1.39)	(1.96)	(1.98)	(0.63)	(1.57)	(0.57)	(1.51)	(0.51)	(1.81)	(0.47)	(0.46)	(0.5)
	Female	5.93a	5.99a	4.87a	5.76a	2.06a	5.35a	1.93a	5.3a	1.87a	4.94a	1.35a	1.7a	1.58a
	(≈ 47%)	(1.49)	(1.51)	(2.3)	(2.07)	(0.59)	(2.0)	(0.58)	(1.96)	(0.55)	(2.33)	(0.48)	(0.46)	(0.5)
Plain	Male	6.01a	6.1a	5.33a	5.59a	1.45a	5.28a	1.64a	5.21a	1.84a	5.27a	***1.26b***	1.64a	1.48a
	(≈ 53%)	(1.53)	(1.66)	(1.81)	(1.65)	(0.54)	(1.57)	(0.58)	(1.67)	(0.58)	(1.85)	(0.44)	(0.48)	(0.5)
	Female	5.7a	5.71a	***4.62b***	***4.86b***	1.46a	***4.58b***	1.64a	5.02a	1.75a	***4.5b***	***1.49a***	1.66a	1.58a
	(≈ 47%)	(2.11)	(2.13)	(2.35)	(2.13)	(0.57)	(2.12)	(0.63)	(1.95)	(0.62)	(2.34)	(0.5)	(0.48)	(0.5)
Total	**Male**	**5.79**	**5.85**	**5.21**	**5.60**	**1.92**	**5.46**	**1.84**	**5.26**	**1.89**	**5.19**	**1.31**	**1.68**	**1.50**
Total	**Female**	**5.72**	**5.86**	***4.78***	**5.39**	**1.98**	**5.07**	**1.86**	**5.17**	**1.85**	**4.80**	**1.41**	**1.67**	**1.562**

Numbers in parentheses represent standard deviation of 202 consumer responses; Range = the highest score minus the lowest score.
[a, b, c] Means (SD) within the same column followed by different letters are significantly different ($p<0.05$)
SWT = Sweetness; RSWT = Rated Sweetness; MFV = Mouth Feel Viscosity
TKV = Thickness Viscosity; Accept = Acceptance; PI = Purchase Intent
HBPI = Health Benefit Purchase Intent

Table 7.6 Frequency Response for Overall Liking of Flavored Soymilk by Gender

Flavored Soymilk	Gender	Score	Frequency	%	Cumulative Frequency	Cumulative %
Orange	Male	6	19	***20.00***	80	84.21
	Female	6	14	16.47	67	78.82
Green Mango	Male	6	25	26.04	65	67.71
	Female	6	24	***27.91***	61	70.93
Almond	Male	6	21	***21.88***	64	66.67
	Female	7	17	19.77	76	88.37
Chocolate	Male	6	28	***29.40***	71	74.74
	Female	7	22	26.51	74	89.16
Plain	Male	6	21	***22.11***	69	72.63
	Female	6	13	15.12	65	75.58

The overall acceptability of the flavored soymilk suggested that again men accepted the soymilk's better than women (Table 7.7). Unlike the mean overall pattern for acceptance discussed earlier, gender comparison pointed to almond flavor with the highest acceptance, followed by plain/bland soymilk, green mango, chocolate and orange. The high acceptability for almond and green mango flavors of the four flavored beverages suggests a potential for the U.S.

Table 7.7 Positive Responses for Acceptance and Purchase Intent of Flavored Soymilk by Gender

Flavored Soymilk	Gender	Acceptance		Purchase Intent	
	%	Freq	%	Freq	%
Orange	Male (≈ 53%)	55	57.29	42	43.75
	Female (≈ 47%)	48	55.81	38	***44.19***
Green Mango	Male (≈ 53%)	***73***	***76.04***	***47***	***48.96***
	Female (≈ 47%)	51	59.30	38	44.19
Almond	Male (≈ 53%)	***65***	***67.71***	***53***	***55.79***
	Female (≈ 47%)	51	60.71	40	46.51
Chocolate	Male (≈ 53%)	***63***	***67.02***	***44***	***45.83***
	Female (≈ 47%)	56	65.12	36	41.86
Plain	Male (≈ 53%)	***71***	***73.96***	***50***	***52.02***
	Female (≈ 47%)	43	51.19	36	41.86

market. The 4 flavored soymilk frequency response from the consumer purchase intent revealed higher purchase intent of the flavored beverages by men than women. General purchase intent was high for almond by both men and women. Gender separation showed purchase intent change with women having more preference to buy green mango and orange flavored soymilk over chocolate and plain soymilk. Men had purchasing preference for plain soymilk after almond flavor, closely followed by green mango, chocolate and orange flavors.

7.3.4 Overall Product Differences – Pooled within Canonical Structures' r

Examination of inter-differences of the soymilk flavors considering all sensory attributes simultaneously employed the multivariate analysis of variance (MANOVA) method. The Wilks' Lambda p-value of less than 0.0001 (Table 7.8) indicated that all four flavors and bland soymilk were significantly different. In addition, descriptive discriminant analysis (DDA) was used for determining attributes underlying differences amongst the soymilk beverages. The pooled within canonical structure in the first and second dimension (Can1 and Can2 - Table 7.9), indicated that aroma (0.741118), sweetness (0.709349), overall liking (0.530677) in Can1, and overall flavor (0.681689) in Can2 significantly contributed to the overall differences among the five beverages.

Table 7.8 Multivariate Statistics and F Approximations (Wilks' <.0001 - Test Criteria and F Approximations for the Hypothesis of no Overall Form Effect)

Statistic	Value	F -Value	Numerator DF	Denominator DF	Pr > F
Wilks'	***0.930***	2.54	28	3506	<.0001
Pillai's	0.070	2.51	28	3900	<.0001
Hotelling	0.074	2.57	28	2420.3	<.0001
Roy's	0.058	8.09	7	975	<.0001

H = Type III SSCP Matrix for Forms
E = Error SSCP Matrix
S = 7 M = 0.5 N = 437

Table 7.9 Canonical Structures' r of Critical Soymilk Sensory Attributes Responsible for Differences among Soymilk Beverages

Attributes	can1	can2
Overall Appearance	-0.095722	0.397088
Color	-0.080777	0.174686
Overall Flavor	0.493953	***0.681689***
Aroma	***0.741118***	0.522511
Sweetness	***0.709349***	0.118908
Mouth Feel Viscosity	0.393166	0.477803
Overall Liking	***0.530677***	0.621096
Cumulative%	78.48	94.01

The pooled within canonical structure in the first and second dimensions

7.3.5 Logistic Regression Analysis and Predictive Discriminant Analysis (PDA) of Consumer Sensory Profile Critical to Product Acceptance and Purchase Decision

The use of logistic regression analysis for consumer acceptance of the soymilk's is presented in Table 7.10. Overall liking is the most important attribute for both acceptance and purchase intent

Table 7.10 Prob>X^2 and Odds Ratio Estimates for Consumer Acceptance and Purchase Intent

Acceptance			
Independent Variable	**Prob> X^2 (Full)**	**Odds Ratio Estimate (Full)**	**Odds Ratio Estimate (Single)**
Overall Appearance	0.4478	0.932	1.455
Color	0.5448	1.056	1.376
Overall Flavor	0.0502	1.182	***2.209***
Aroma	0.0206	1.169	***2.011***
Sweetness	0.0674	1.15	***2.148***
Mouthfeel Viscosity	0.6344	1.033	1.791
Overall liking	<.0001	1.814	***2.55***
Purchase Intent			
Overall Appearance	0.0953	1.175	1.66
Color	0.3767	1.086	1.552
Overall Flavor	0.0007	1.339	***2.211***
Aroma	0.4596	0.948	1.839
Sweetness	0.4592	1.059	***2.081***
Mouthfeel Viscosity	0.7499	1.022	1.801
Overall liking	<.0001	1.775	***2.462***

Table 7.11 % Hit-Rate for Acceptance and Purchase Intent of Flavored Soymilk

Attributes	Acceptable	Purchase Intent
All 7 attributes combined	80.87	78.54
Overall Appearance	61.01	68.75
Color	61.49	62.57
Overall Flavor	***78.86***	***77.27***
Aroma	74.53	69.53
Sweetness	73.86	72.48
Mouthfeel Viscosity	64.38	69.25
Overall Liking	***81.46***	***78.96***
Rated Aroma	63.33	48.24
Rated Sweetness	66.60	54.92
Thickness/Viscosity	64.92	49.38

with odds ratio estimate of 2.55 (Prob>χ^2 less than 0.05) for acceptance and 2.5 for purchase intent. These results imply that for every one point increase in overall liking on the 9-point hedonic scale, overall product acceptance and purchase intent would both increase by about 155%. For acceptance, the next most important attributes with odds ratio estimates of 2.209, 2.15 and 2.01 were overall flavor, sweetness, and aroma, respectively. Again, for every one point increase in these attributes on the hedonic scale, overall product acceptance increases by 129%, 115% and 101%, respectively. Extensive analysis using predictive discriminative analysis (PDA) / % hit rate, enhanced prediction for beverage acceptance with 81.5% and 79% accuracy based on overall liking and overall flavor, respectively (Table 7.11). These results agrees in part with the discriminating attributes in Table 7.9 (overall flavor, aroma, and sweetness and mouthfeel viscosity) based on their canonical correlation in the first and second dimensions.

Purchase intent of soymilk's as determined from odds ratio estimates further revealed that not only was overall liking critical to purchase intent, but overall flavor was significantly critical to purchase decision (Prob>χ^2 less than 0.05) based on the logistic regression analysis data in

Table 7.10. Overall flavor had an odds ratio of 2.21, implying that purchase intent would have increased by 121%. The flavor will increase for every one point increase in flavor score on the 9-point hedonic scale. Therefore, purchase decision was predicted by overall liking and overall flavor using PDA % hit ratio with 79% and 77.3% accuracy.

7.3.6 Proportional Odds Model of Predictors Relative to Like / Dislike of Products

The full model using the proportional odds model takes all predictors into account, including overall flavor, aroma, sweetness, and MFV. The odds ratio estimate for the reduced model is presented in Table 7.12. The odds of liking (based on a 9-point hedonic scale) the soymilks relative to neither like or dislike increases about 3.0 times as x increases to x + 1 for overall flavor while all other factors are being held constant. Also, the odds of liking the product relative to neither like or dislike increases 1.5 times as x increases to x + 1 for MFV, as all other factors remain the same. The odds of liking the product relative to neither like or dislike increases 1.63 times as x increases to x + 1 in terms of sweetness, with all other factors held constant. Similarly, the odds of liking the soymilks relative to neither like or dislike increases 1.34 times as x increases to x + 1 for aroma

Table 7.12 Proportional Odds Model Ratio Estimates of Sensory Attribute Predictors Relative to Like / Dislike of Flavored Soymilk Beverages

Attribute	Pr > X2	Odds Ratio Estimate
Overall Flavor	<.0001	2.974
Aroma	0.0001	1.344
Sweetness	<.0001	1.63
Mouth Feel Viscosity	<.0001	1.502

with other factors being held constant. A reasonable deduction in view of these results is that all of these predictors are critical to the overall liking of soymilk. Examination of relationships among the soymilks from the various analysis suggests that these results closely relates to results from logistic regression analysis which concluded that overall flavor, aroma and sweetness were critical factors for consumer acceptance in particular.

7.3.7 Principal Component Analysis of Flavored Soymilk Product Attributes

The bi-plot (product – attribute) of the flavored soymilk using principal components 1 and 2 is presented in Figure 7.1. It is quite evident that in this study, the attributes discriminating the green mango flavored soy milk from the other beverages comprise of sweetness, overall liking, aroma, and overall flavor. This confirms results of the descriptive discriminative analysis (DDA) result, where the pooled within canonical structure in the first dimension identified aroma (0.741), sweetness (0.71), overall liking (0.53), and overall flavor (0.68) in the second dimension as crucial attributes that did significantly contribute to overall differences among the flavored soymilk formulations (Table7.9).

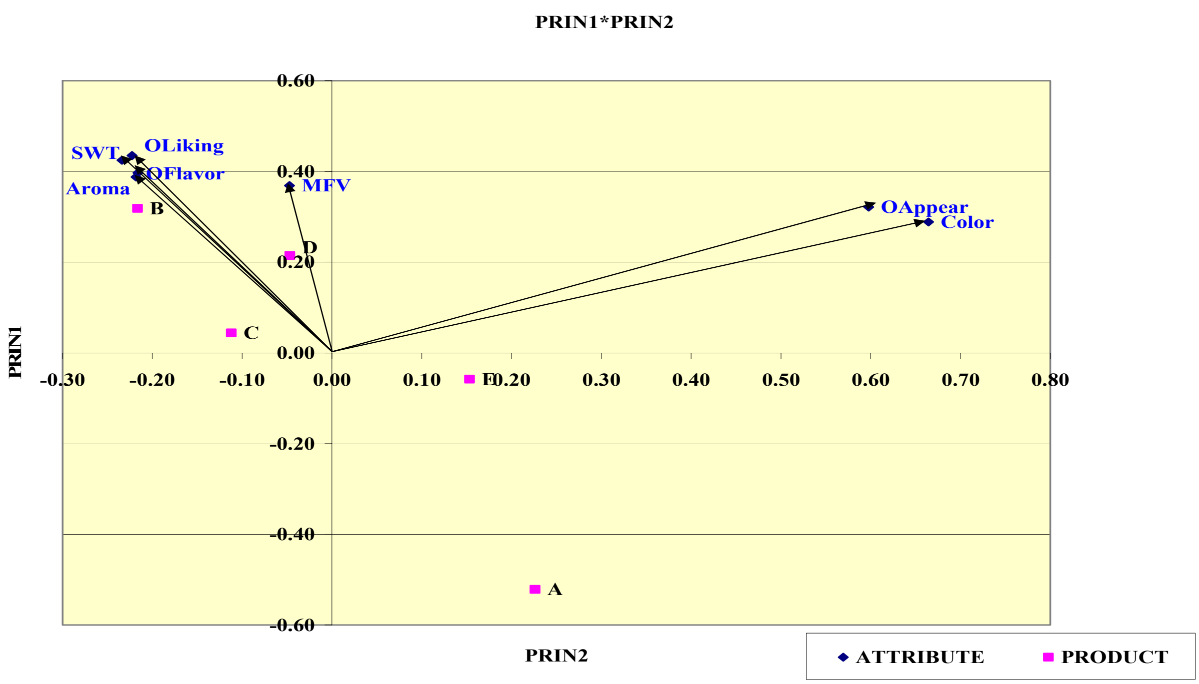


Figure 7.1 PCA bi-plot of Flavored Soymilk Product attributes comprising Principal Component 1 and 2 (A = Orange; B = Green Mango; C = Almond; D = Chocolate; and E = Plain

However, the plots comparing principal components 1 and 3 and principal components 2 and 3 also identified almond and chocolate flavored soymilks in addition to green mango with these same specific discriminating factors including color (See Appendix E-7.5 & 7.6). In this regards, it can be deduced that consumers had a higher preference for the green mango flavor soymilk, followed by almond and chocolate flavors. Overall, the descriptive discriminative analysis, logistic regression analysis, and principal component analysis, pointed to overall liking, aroma, overall flavor, and sweetness as significant attributes that distinguished green mango flavor in particular from the other soy milk beverages.

7.3.8 Flavored Soymilk Optimization

Product optimization was performed using a two-component formulation spiked with four different flavors. Optimal flavored soymilk was attained in conjunction with logistic regression, PDA / % hit rate and the proportional odds ratio estimate in which each of the sensory attributes in question were evaluated in relation to all the other attributes. The optimal flavored soymilk was the green mango as determined by all analysis against all attributes. Overall consumer liking and overall liking by gender rated green mango flavor the best, followed by almond and chocolate excluding the plain soymilk. The elimination of unimportant attributes critical to both consumer acceptance and purchase intent by logistic regression analysis further validated green mango flavor as the optimal soymilk flavor. The green mango may have provided the complementary masking effect for the beany flavor in the soymilk.

The probability greater than Chi-square (χ^2) was looked at in order to determine these critical attributes in which, if the Prob > χ^2 for a particular attributes was less than 0.1 (α = 1%), then that attribute was considered significant in terms of either consumer acceptance, purchase intent, or both. The results in this case showed overall liking, aroma, overall flavor and sweetness with Prob > χ^2 = <0.0001, 0.0206, 0.0502, and 0.0674, respectively, to be significant attributes for acceptance. Overall liking, overall flavor, and overall appearance with Prob > χ^2 = <0.0001, 0.007, and 0.0953, respectively, were significantly critical for consumer purchasing intent. In the final analysis, overall liking, aroma, overall flavor and sweetness were the critical attributes used in determining the optimal flavored beverage.

7.4 CONCLUSION

The main purpose of this study was to determine the optimal flavored beverage of the four flavors. Results in this study indicated that the addition of flavor is desirable in improving the sensory characteristics, particularly the flavor on the product. Most of the sensory attributes including the overall liking, acceptance and purchase intent were improved considerably by the addition of green mango and almond flavors in particular. Of the four flavors, overall liking as perceived by consumers was high for green mango, almond, and chocolate. Contributing attributes to overall liking included overall flavor, aroma, sweetness and mouthfeel viscosity. Acceptance and purchasing decisions were most influenced by overall flavor, aroma, overall liking and sweetness.

More importantly, green mango flavor, chocolate, and almond flavors were more accepted, implying that these flavors have potential for introduction into the current soymilk market. Chocolate soymilk is now on the market. But, the application of green mango and almond as uniquely versatile flavors can be used in traditional and non-traditional soymilk to promote flavors that are rounder, fuller and more pronounced. The high preference purchase intent seen for green mango and almond in particular validates marketing potential of these flavored soymilks. Based on the above results (from - overall acceptance and purchase intent, gender difference in acceptance and purchase intent, pooled within canonical structures' r, % hit rate and proportional odds ratio), it can be stated that the aroma and overall flavor of green mango and almond flavors can strongly alter or minimize unwanted after taste, make mouthfeel more satisfying, increase acceptance and purchase intent, as well as alter other valuable attributes or sensory profile for soymilk. The application of the two can perhaps be synergistic with a wide array of flavors, and would be more valuable in flavor blend. In conclusion, green mango and almond could create new opportunities in flavor manipulation, new product development and product improvement with soymilk.

8. SUMMARY, CONCLUSION(S) AND RECOMMENDATION(S)

The study investigated the possibility of producing protein-isoflavone enriched soymilk by traditional technologies, but with acceptable sensory qualities based on the hypothesis that protein and isoflavone can be simultaneously increased in soymilk using only food grade soy germ incorporated into soymilk. A conclusion of great importance that can be drawn from this study is that food grade soy germ incorporated into soymilk can successfully enhance isoflavone contents in soymilk, but may or may not necessarily enhance the protein content. The above investigations notwithstanding the extent and application of the results went beyond what may be attributed to processing and product optimization of the PIE soymilk beverages. The results thus far have made a definite demonstration that, incorporating soy germ into soymilk adequately meets the recommended FDA soy protein and isoflavone intake per day in approximately 1 serving down from 1.5 or 2.0 servings for isoflavone, and in about 3 servings give or take, down from 3.5 or 4 servings from commercial products.

It was evident therefore that since, traditional processing was applied in a partial industrial production setting; processing conditions as observed do have significant but varied effect on final composition and quality of soymilk. Far more than processing conditions is the composition of the starting material that may dictate in part the final composition of the product. Incorporating soy germ into soymilk at unconventional stages in the production cycle yielded lower isoflavone and protein content compared to a soy germ incorporated milk particularly at the start (soaking) of the production cycle. Thus, this route is the optimal process method for incorporating soy germ into soy milk. Processing conditions with this route would comprise short heating time (10 min), high temperature (100^{o}C), with long cooking time of the raw slurry (20 min) at same temperature, then homogenize and pasteurize at same temperature at 4000psi.

Granted that the incorporation of soy germ in soymilk does enhance soymilk nutrient content, but an enhancing effect was not seen with increase in soy germ amount. While no known study has specifically investigated this area, it was apparent that the production of soymilk with $NaHCO_3$ or no-$NaHCO_3$ (plain water), did have varied effect on soymilk composition, pH, and color. No significant differences were found in the soymilk beverages within each treatment group for the variables evaluated. But, significant differences were found between the treatment groups with the $NaHCO_3$ soymilks having better nutrient composition overall over the no-$NaHCO_3$ (plain water). Shelf life also appears to be much better in the $NaHCO_3$ treated soymilk than the no-$NaHCO_3$ (plain water) soymilk. The overall appearance or color of no-$NaHCO_3$ (plain water) soymilks was bright cream color and closer to whiteness than the $NaHCO_3$ that was more yellow. The yellow color of the $NaHCO_3$ treated soymilk is typical of sodium bicarbonate action at elevated temperature and cannot be avoided. The brighter color in the no-$NaHCO_3$ (plain water), perhaps makes the no-$NaHCO_3$ (plain water) treated product a better candidate for consumer acceptance, but not necessarily a strong selection considering the presence of anti-nutrient factors responsible for stomach upsets and the enzyme lipoxigenase responsible for the objectionable painty of-flavor in soymilk.

It is quite evident from the study that soymilk beverages homogenized under low pressures of 3500 - 4000 psi provide a homogenous liquid system that is stable with components fairly dispersed in solution. In addition, the shelf life of soymilk as demonstrated in the 28 days study points to stable and safe product for consumption with longer shelf life post-pasteurization than cow's milk. The low coliform / total plate counts < 10 log cfu/ml coupled with high pH above neutral ensures product safety beyond the recommended 14 days shelf life for cow's milk.

Sensory evaluation of bland soymilk as part of determining an optimal formulation of the four products either containing no soy germ, or 25%, 30% and 35% focused on overall liking acceptance, purchase intents and health benefit purchase intents as deciding factors in this study. Overall liking as indicated was much strong for the WSB/SG25 soymilk formulation, followed by WSB/100, WSB/SG35 and WSB/SG30. This pattern was the same for overall liking for both men and women. A similar pattern was also revealed for acceptance and initial purchase intent that was based on product availability. In reverse, the WSB/SG35 was most preferred for purchasing based on knowledge of the health benefits of soy protein and isoflavone. This was followed by WSB/SG30, WSB/SG25 and WSB/100, respectively showing an order of purchase intent preference with decrease in soy germ. Regardless of the results on change in purchase intent based on knowledge of soy health benefits, WSB/SG25 was the optimal formulation based also on other considerations of the entire study thus far. In any case, critical attributes that influenced the decisive factors which contributed to the final selection overall and by gender included the soy aroma, TBF, sweetness, and MFV.

Additional sensory testing with the WSB/SG25 when spiked with four different flavors showed preference for the product in flavored version over the plain / bland form. Overall, preference for green mango flavor, almond and chocolate were higher than for orange flavor. But the bland milk was actually preferred over the chocolate flavor and orange as well. Gender differences showed preference green mango, then chocolate, almond, plain/bland and orange. In general, men rated the beverages as more acceptable and were more willing to purchase them than the women. Contributing attributes that underscored the differences noted among the flavored soymilk and influenced acceptance and purchase intent of the beverages were overall liking, overall flavor, aroma and sweetness for acceptance, overall liking and overall flavor for purchase intent.

It can be concluded that the study did achieve its goals and objectives. However, the following suggestions are put forward to potential users of these research findings especially the scientific community and industry users as a whole. The process to develop a PIE soymilk can be improved upon perhaps with mild enzyme activity or fermentation process to enhance protein in soymilk, because it seems to be difficult to do so by conventional process. In order to bring about more and better sensorial quality, increased acceptance and accelerated purchasing intentions, the soymilk should be prepared as in industry technology consisting of added nutrients, stabilizers and all the elements that go into commercial soymilk beverages. This could tremendously change acceptance and purchasing intent of the soy germ soymilk versions. Flavoring of the beverages as done in this study, should be surveyed again, perhaps looking at varying strengths / concentrations of spiking which may entirely influence overall liking, acceptance and purchasing decisions.

The predominant carbohydrates in soymilk are oligosaccharides, raffinose, and stachyose which are mostly indigestible and commonly associated with stomach discomfort (Rackis, et al., 1970) because of the absence of α – galatosidase in the small intestine. These components are also high in soy germ suggesting that the incorporation of soy germ into soymilk may increase amounts in beverages. Therefore, the need to quantify residual carbohydrates and their composition in PIE soymilk beverages should be investigated. The study findings can be improved upon by using a full industrial production system to reduce hand work during production.

REFERENCES

Adlercreutz, H. 1984. Does fiber-rich food containing animal lignan precursors protect against both colon and breast cancer. An extension of the "fiber hypothesis". Gastroenterology. 86: 761

Adlercreutz H., Honjo H., Higashi A., Fotsis T., Hamalainen E., Hasegawa T., Okada H. 1991. Urinary excretion of lignans and isoflavonoid phytoestrogens in Japanese men and women consuming a traditional Japanese diet. Am. J. Clin. Nutr. 54:1093

Agresti, A. 1996. An Introduction to Categorical Data Analysis. Wiley and Sons. New York. 226pp.

Albrecht, W J., Mustakas, G C., and McGhee, J E. 1966. Rate studies on atmospheric steaming and immersion cooking of soybeans. Cereal Chem. 43: 400

Allred, C. D., Allred, K. F., Ju, Y. H., Virant, S. M., Helferich, W. G. 2001a. Soy diets containing varying amounts of genistein stimulates growth of estrogen-dependent (MCF-7) tumors in a dose-dependent manner. Cancer Res. 61: 5045

Allred, C. D., Ju, Y. H., Allred, K. F., Chang, J., Helferich, W. G. 2001b. Dietary genistin Stimulates growth of estrogen-dependent breast cancer tumors to that observed with genistein.Carcinogenesis. 22: 1667

Akiyama T., Ishida J., Nakagawa S., Ogawara, H., Watanabe S., Itoh N., Shibuya M., and Fukami Y. 1987. Genistein, a specific inhibitor of tyrosine protein kinase. J. Biol. Chem. 262: 5592

Anderson, R. L and Wolf, W.J. 1995. Compositional changes in trypsin inhibitors, phytic acid, saponins and isoflavones related to soybean processing. Biopolymer Research, National Center for Agricultural Utilization Research, ARS, USDA, Peoria, IL. The Journal of nutrition, Mar 1995. v. 125 (35): 581S

Anderson, J. J., Ambrose, W. W., Garner, S. C. 1995. Orally dosed genistein from soy and prevention of canellous bone loss in two overectomized rat models. J Nutr. 125: 799S

Anderson, J., Garner, S. 1997. Phytoestrogens and human function. Nutr. Today 32: 232

Anderson, J. J. B., and Garner, S. C. (1997) The effects of phytoestrogens and bone. Nutr. Res. 17:1617

Anderson, J. W., Blake, J. E., Turner, J., Smith, B. M. 1998. Effects of soy protein on renal function and proteinuria inpatients with type 2 diabetes. Am J Clin Nutr. 68:1347S

Anderson, J. J. B., Anthony, M., Messina, M., Garner, S. C. 1999. Effects of phyto-oestrogens on tissues. Nutr. Research Reviews. 12 (1): 75

Anthony, M S. 2000. Soy and cardiovascular disease: cholesterol and beyond. J Nutr. 130 (suppl): 662S

Anthony, M. S., Clarkson, T. B., Hughes, C. L., Moregan, T. M., Burke, G. L. 1996. Soybean isoflavones improve cardiovascular risks factors without affecting the reproductive system of peripubertal rhesus monkeys. J Nutr. 126: 43

AOAC, 1990. Official Methods of Analysis, 15th ed.; Association of Official Analytical Chemists: Washington, DC

AOAC, 2000. Official Methods of Analysis, 17th ed.; Association of Official Analytical Chemists: Washington, DC

Ara, K; Yoshimatsu, T; Ojima, M; Kawai, S; Okubo, K. 2003. Effect of enzymic treatment on sensory taste of soymilk. Nippon Shokuhin Kagaku Kogaku Kaishi, 50(1), 13-21.

Arai, S., Suzuki, H., Fujimaki, M., Sakurai Y. 1966. Studies on flavor components of soybean. Part II. Phenolic acids in defatted soybean flour. Agric. Biol. Chem. 30: 364

Arai, S., Noguchi, M., Kaji, M., Kato, H., and Hujimaki, M. 1970a. n-Hexanal and some volatile alcohols, their distribution in raw soybean tissues and formation in crude soy protein concentrate by lipoxigenase. Agric. Biol. Chem. 32: 1420

Arai, S., Noguchi, M., Yamashita, M., Kato, H., and Hujimaki, M. 1970b. Studies on flavor components in soybean. Part VI. Some evidence for occurrence of protein-flavor binding.

Arjmandi, B., Getlinger, M., Gotal, N., Alekel, L., Hasler, C., Joma, S., Drum, M., Hollis, B., Kukreja, S. 1998. Role of soy protein with normal or reduced isoflavone content in reversing bone loss induced by ovarian hormone deficiency in rats. Am. J of Clin Nutr. 68 (Suppl): 1358S

Arjmandi, B. H; Khan, D. A; Juma, S. S; Svanborg, A. 1997. The ovarian hormone deficiency-induced hypercholesterolemia is reversed by soy protein and the synthetic isoflavone, ipriflavone. Nutrition Research. 17(5): 885

Arjmandi, B. H; Getlinger, M. J; Goyal, N. V; Alekel, L; Hasler, Clare M; Juma, S; Drum, M. L; Hollis, B. W; Kukreja, S. C. 1998. Role of soy protein with normal or reduced isoflavone content in reversing bone loss induced by ovarian hormone deficiency in rats. American Journal of Clinical Nutrition. 68 (6, Suppl.): 1358S

Asbi, B. A., Wei, L. S., and Steinburg, M. P 1989. Effect of pH on the kinetic of soybean lipoxigenase-1. J Food Sci. 54:1594

Ashraf, H.R Lee and Snyder, H.E. 1981. Infleunce of ethanolic soaking of soybeans on flavor and lipoxygenase activity of soymilk. J Food Sci. 46: 1201

Aspelund, T. G and Wilson, L. A. 1983. Adsorption of off-flavor compounds onto soy protein: A thermodynamic study. J. Agric Food Chem. 31:539

Aworth, O. C; Adedji, A; Nwanekezi, P. C. 1997. Effect of partial substitution of cow's milk with soymilk on yield and quality attributes of West American salt cheese. Int. J Food Sci Technol. 22 (2): 53

Axelson, M; Kirk DN; Farrant RD; Cooley G; Lawson AM; Setchell KDR. 1982. The identification of the weak estrogen equol in human urine. Biochemical Journal. 201: 353

Axelson, M; Sjovall, J; Gustafsson, B. E; Setchell, K. D. R. 1982. Origin of lignans in mammals and identification of a precursor from plants. Nature. 298: 659

Axelson, M; Sjovall, J; Gustafsson, B. E; Setchell, K. D. R. 1984. Soya-a dietary sources of non-steroidal estrogen in man and animals. J Endocrinol. 102: 49

Ayres, D. C; Loike, J. D. 1990. Lignans. Chemical, biological and clinical properties. Cambridge, U K. Cambridge University Press.

Bakhit, R. M; klein, B. P; Essex-Sorlie, D; Ham, J. O; Erdman, J. W, Jr; Potter, S. M. 1994. Intake of 25 g of soybean protein with or without soybean fiber alters plasma lipids in men with elevated cholesterol concentrations. J Nutr. 124: 213

Blasé, R.J. 1990. Factors influencing endogenous flavor compounds in soybeans. Ph.D. Dissertation. Clemson University., Clemson, SC.

Barsotti, G; Navelesi, R; Giampietro, O; et al., 1988. Effects of a vegetarian, supplemented diet on renal function, proteinuria, and glucose metabolism in patients with 'overt' diabetic nephropathy and renal insufficiency. Clin Nephrol. 65: 87

Barnes S., Peterson G., Grubbs C., Setchell K.D.R. 1994. Potential role of dietary isoflavones in the prevention of cancer. Adv. Exp. Med. Biol.354:135

Barnes S. Effect of genistein on in vitro and in vivo models of cancer. J. Nutr. 125: 777S

Barnes, S., Messina, M. 1991. The role of soy products in reducing cancer risk. J. Natl. Cancer Inst. 83: 541

Basker, D A. 1989. A useful hedonic 'Smiley' scale. J Test Eval. 17: 307

Beasley, S.; Tuorila, H.; Saris, P. E. J. 2003. Fermented soymilk with a monoculture of Lactococcus lactis. International Journal of Food Microbiology. 81(2): 159

Behrens, JH; Roig, SM; Da Silva, MAAP. 2004. Fermentation of soymilk by commercial lactic cultures: Development of a product with market potential. Acta Alimentaria. 33 (2): 101

Bennink, M.R. 1996. Biological basis for dietary modulation of colon carcinogenesis. In 'Hypernutritious Foods'; Eds: J.W. Finley et al., Agscience, Inc., 103-122pp.

Bennion, M. 1995. Introductory Foods. Tenth Edition (Chpt. 20 and 28). Princeton Hall, Inc. New Jersey, USA.

Beery, K. E. 1989. Preparation of soy protein concentrate products and their application in food system. In: proceedings of the World Congress on Vegetable Protein Utilization in Human Foods and animal Feedstuffs (Applewhite, T. H., ed.) pp.62 – 65. American Oil Chemist' Society, Champaign, IL

Boor, K.J., Brown, D.P, Murphy, S.C, Kozlowski, S.M and Bandler, D. K. 1998. Microbial and chemical quality of raw milk in New York State. J. Dairy Sci. 81:1743

Booth, C., Hargreaves, D. F., Hadfield, J. A., McGown A. T., Potten, C. S. 1999. Isoflavones inhibit intestinal epithelial cell proliferation and induce apoptosis in vitro. Br. J. Cancer. 80:1550

Bourquin, L.D. and Bennink, M.R. 1996. Differential effects of genistein and daidzein on growth of human colon cancer cell lines. Presented at the Second International Symposium on the Role of Soy in Preventing and Treating Chronic Disease held in Brussels, Belgium, September 19, 1996. Am J Clin Nutr. 68: 1527S (Abst)

Breslau, N. A; Brinkley, L; Hill, K. D; Pak, C. Y. C. 1988. Relationship of animal protein-rich diet to kidney stone formation and calcium metabolism. J Clin Endocrinol Metab. 66: 140

Calvert, G. D; Blight, L; Illman, R. J; Topping, D. L; Potter, J. D. 1981. A trail of the effects of soya-bean flour and soya-bean saponins on plasma lipids, fecal bile acids and neutral sterols in hypercholesterolemic men. Br J Nutr. 45: 277

Caragay, A. 1992. Cancer-preventive foods and ingredients. Food Technol. 46: 65

Carrao-Panizzi, M C, Beleia, A D P., Prudencio-Ferreira, S H., Oliveira, M C N., Kitamura, K. 1999/2000. Effects of isoflavones on beany flavor and astringency of soymilk and cooked whole soybean grains. Pesq. Agropec. Bras., Brasillia. 34 (6): 1045

Carrao-Panizzi, M C, Favoni S P G, Kikuchi A. 2002. Extraction time for soybean isoflavone determination. Brazilian Archives of Biology and Technology. 45 (4): 515

Cassidy, A. 1996. Physiological effects of phyto-oestrogens in relation to cancer and other human health risks. Proc. Nutr. Soc. 55: 399

Cassidy A; Faughnan M; Hughes R; Fraser C; Cathcart, A; Taylor, N; Setchell, K D R, and Bingham, S. 1998. Hormonal effects of phytoestrogen in postmenopausal women and middle aged men. Am J Clin. Nutr. 68: 1531S (abst).

Catsimpoolas, N and Meyer, W. E. 1971. Gelation phenomena of soybean globulins. Protein-lipid interactions. Cereal Chem. 48: 159

Che Man, Y. B., Wes, L. S., and Nelson, A. L. 1989. Acid inactivation of soybean lipoxigenase with retention of protein solubility. J. Food Sci. 54: 963

Chen, X.; Anderson, J. J. B. 2002. Isoflavones and bone: animal and human evidence of efficacy. Journal of Musculoskeletal & Neuronal Interactions. 2 (4): 352

Chen, JR; Liu, SM; Yang, SC; Suetsuna, K. 2004. Soymilk intake is associated with plasma liver lipid profiles in rats fed a high-cholesterol diet. Nutr. 20 (10): 929

Choi, Yong-Soon; Lee, Bung-Hoon; Kim, Jong-Hwa; Kim, Nam-Soo. 2000. Concentration of phytoestrogens in soybeans and soybean products in Korea. Journal of the Science of Food and Agriculture. 80 (12): 1709

Choi, YB; Kim, KS; Rhee, JS. 2002. Hydrolysis of soybean isoflavone glucosides by lactic acid bacteria. Biotechnol Letters. 24 (24): 2113

CIE. 1995. Industrial color-difference evaluation. CIE Pub. 116, Commission International de L'Eclairage, Vienna.

Circle S J, Smith A K. 1978. Processing soy flour, protein concentrates, and protein isolates. In: Liu, K. 1997. Soybeans: Chemistry, Technology and Utilization. Chapman and Hall, New York.

Cohen, R. 1998. Soymilk vs. Cow's Milk.http://www.notmilk.com. Accessed August, 2004.

Coulston, A. N. (ADA President) 1999. FDA Health Claim Proposal for Soy Protein and Coronary Heart Disease, http://www.eatright.org/Public/GovernmentAffairs/

Cowan, J. C. 1973. Processing and Products. In: Soybeans: Improvement, Production, and Uses. (Caldwell, B. E., Howell, R. W., Judd, R. W., Johnson, H. W. eds.) American Society of Agronomy, Inc. Wisconsin, USA

Coward, L., Barnes, N., Setchell, K D R., and Barnes, S. 1993. Genistein, Daidzein, and their β-glycoside conjugates: Antitumor isoflavones in soybean foods from American and Asian diets. J. Agric. Food Chem. 41: 1961

Coward L; Smith M; Kirk M; Barnes S. 1998. Chemical modification of isoflavones in soy foods during cooking and processing. Amer. J. of Clinical Nutr. 68(6 Suppl): 1486S

Crouch, T. H. 1998/1999. Soy vs. Cow milk. Vegan Views. Winter, v.81 http://www.

D'Amico, G and Gentile, M. G. 1993. Influence of diet on lipid abnormalities in human renal disease. Am J Kidney Dis. 22: 151

Dairy Practices Council. 1991. Guidelines for raw milk quality tests, Publication DPC 21, Barre, VT.

Dairy Practices Council. 1997. Guidelines for troubleshooting on-farm bacteria counts in raw milk, Publication 24, Barre, VT.

Damodaran, S and Kinsella, J. E. 1981a. Interaction of carbonyls with soy protein: Thermodynamic effects. J. Agric. Food Chem. 29.1249

Damodaran, S and Kinsella, J. E. 1981b. Interaction of carbonyls with soy protein: Conformational effects. J. Agric. Food Chem. 29.1253

Davis, J.G. 1981. Dairy Microbiology. Vol.II pp. 42.

Davies, C. S and Nielsen, N. C. 1986. Genetic analysis of a null-allele for lipoxigenase-2 in soybeans. Crop Sci. 26: 460

Davies, C. S; Nielsen, S. S; Nielsen, N. C. 1987. Flavor improvement of soybean preparations by genetic removal of lipoxigenase-2. J. Am. Oil Chem. Soc. 64: 1428

Davis, B.D., Dulbecco, R., Eisen, H.N., Ginsberg, H.S. and Wood, W.B. 1973. Microbiology. 2nd ed. London. New York Harper and Row

Drane H; Patterson D; Roberts B; Saba N. 1975. The chance discovery of estrogenic activity in laboratory rat cake. Food Cosmet. Toxicol. 13: 491

Ebel, J; Grisebach, H; Bonhoff, A. 1986. Phytoalexin synthesis in soybean following infection of roots with fungal elicitor. In: Lugtenberg, B. (Ed). Recognition in microbe-plant symbiotic and pathogenic interactions: NATO Advanced Research Workshop. New York: Springer Verlag. 345 – 361. [NATO ASI (Advanced Sci.Inst.) Series H,Vol. 4]

Ediriweera, N., Akiyama, Y., and Saio, K. 1987. Inactivation of lipoxigenase in soybeans with retention of protein solubility. J. Food Sci. 52: 685

Efiuvwevwere, BJO and Nwanebu, FC. 1998. Contamination of sterilized soymilk with different microorganisms. Milchwissenschaft – Milk Science International. 53 (7): 370

Eldridge, A. C. 1982. determination of isoflavones in soybean flours, protein concentrates and isolates. J. Agric. Food Chem. 30: 353

Eldridge, A. C, Kwolek, W. F. 1983. Soybean isoflavones: effect of environment and variety on composition. Journal of Agricultural and Food Chemistry (1983), 31(2), 394-396.

Farmakalidis, E., and Murphy, P. A. 1985. Isolation of 6"-*O*-Acetylgenistin and 6"-*O*-Acetyldaidzin from toasted defatted soy flakes. J. Agric. Food Chem. 33: 385

Fletcher, R. J. 2003. Food sources of phyto-oestrogens and their precursors in Europe. British J of Nutr. 89(Suppl.1): S39

Fleury, Y; Welti, D. H; Phillippossian, G; Magnolato, D. 1992. Soybean (malonyl) isoflavones characterization and anti-oxidant properties. In: phenolic compounds in food and their effects on health. Eds. Huang, M. T; Ho, C. T; Lee, C. Y. American Chemical Society: Washington, DC. Vol. II, pp 98 -113.

Fotsis T, Pepper M, Aldlercreutz H, Fleischmann, G; Hase, T; Montesano, R and Schweigerer, L. 1993. Genistein, a dietary-derived inhibitor of Angiogenesis. Proc Natl Acad Sci. U.S.A. 90: 2690

Franke, A. A., Custer, L. J., Cerna, C. M., and Narala, K. 1995. Rapid HPLC analysis of dietary phytoestrogens from legumes and from human urine. Proc. Soc. Exp. Biol. Med., 208:18

Frankenfeld Cara L; Patterson Ruth E; Horner Neilann K; Neuhouser Marian L; Skor Heather E; Kalhorn Thomas F; Howald William N; Lampe Johanna W. 2003. Validation of a soy food-frequency questionnaire and evaluation of correlates of plasma isoflavone concentrations in postmenopausal women. Am. J of Clin. Nutr. 77(3): 674

Frankenfeld Cara L; Patterson Ruth E; Horner Neilann K; Neuhouser Marian L; Skor Heather E; Kalhorn Thomas F; Howald William N; Lampe Johanna W. 2003. Validation of a soy food-frequency questionnaire and evaluation of correlates of plasma isoflavone concentrations in postmenopausal women. Am. J. of Clin. Nutr. 77(3): 674

Frankenfeld Cara L; Patterson Ruth E; Kalhorn Thomas F; Skor Heather E; Howald William N; Lampe Johanna W. 2003. Validation of a soy food frequency questionnaire with plasma concentrations of isoflavones in US adults. J of the Am Diet. Assoc. 102(10): 1407

Frazier, W.C. and Westhoff, D.C. 1988. Food Microbiology. 3rd edn. Tata McGraw-Hill Publishing Company Ltd., New Delhi.

Fumagalli, R; Soleri, L; Farina, R et al., 1982. Fecal cholesterol excretion studies type II hyper - cholesterolemic patients treated with the soybean protein diet. Atherosclerosis. 43: 341

Garcia, M. C., Torre, M., Marina, M. L., Laborda, F. 1997. Composition and characterization of soybean and related products. Critical Reviews in Food Sci. and Nutr. 37 (4):361

Gardner, H. W., Dornbos Jr., D. L., and Desjardins, A. E. 1990. Hexanal trans-2-hexanal, and trans-2-nonenal inhibits soybean. J. Agric. Food. Chem. 38:1316

Garro, M S G; Oliver, F V G; Savoy de Giori, G. 1998. Growth characteristics and fermentation products of *Streptococcus salivarius subsp. Thermophillus, lactobacillus casei, and L. fermentum* in soymilk. Z. Lebensm. Unters. Forsch. A. 206: 72

Garro, M S G; Oliver, F V G; Savoy de Giori, G. 1999. Hydrolysis of soya milk oligosaccharides by *Bifidobacterium longum* CRL 849. Z. Lebensm. Unters. Forsch. A. 208: 57

Garro, MS; de Valdez, GF; de Giori, GS; 2004. Temperature effect on the biological activity of Bifidobacterium longum CRL 849 and Lactobacillus fermentum CRL 251 in pure and mixed cultures grown in soymilk. Food Microbiology. 21 (5): 511

Geronazzo, H; Macoritto, A; Mercado, A; Toro, MA; Cuevas, CM. 1998. Lipoxygenases and trypsin inhibitors inactivation in a soymilk processing by direct milling and ultra high temperature (DM-UHT). Archivos Latino Americanos De Nutricion. 48 (1): 52

Gibson, LR and Mullen, RE. 2001. Mineral concentrations in soybean seed produced under high day and night temperature. Canadian J of Plant Science. 81 (4): 595

Golbitz, Peter. 1995. Traditional soy foods: processing and products. Soyatech, Inc., Bar Harbor, ME, USA. J of Nutr. 125 (3S): 570S

Gomes AMP & Malcata FX (1999) *Bifidobacterium* spp. and *Lactobacillus acidophilus*: biological, biochemical, technological and therapeutical properties relevant for use as probiotics. Trends Food Sci Technol. 10: 139

Gyorgy P, Murata K, Ikehata H. 1964. Antioxidants isolated from fermented soybeans (Tempeh). Nature. 203: 870

Ha, E. Y. W; Morr, C. v; Seo, A. 1992. Isoflavone aglucones and volatile organic compound in soybeans: Effects of soaking treatment. J Food Science. 57: 414

Hahlbrock, K. 1981. Flavonoids. In: Conn, E. E. ed. The biochemistry of plants: a comprehensive treatise – secondary plant products. New York: Academic Press, Chpt. 7: 425 – 456.

Hajika, M; Igita, K; Kitamura, K. 1991. A line lacking all the lipoxigenase isozymes in soybean (*Glycine max (L.*) *Merill*) induced by gamma-ray irradiation. Jpn. J. Breed. 41: 507

Hand, D. B., Steinkraus, K. H., Van Buren, J. P., Hackler, L. R., Rawis, I., and Pallesen, H. R. 1964. Pilot-plant studies on soymilk. Food Technol. 18: 1963

Hargreaves DF; Potten CS; Harding C; Shaw LE; Morton MS; Roberts SA; Howell A; Bundred NJ. 1999. Two week dietary soy supplementation has an estrogenic effect on normal premenopausal breast. J Clin. Endocrinol. Metab. 84: 4017

Harrigan, W.F. 1976. Laboratory Methods in Food and Dairy Microbiology, Revised edn. Academic Press, London, New York San Francisco.

Hassler, C. M. 1998. Functional foods: Their role in disease prevention and health promotion. Food Technol. 52 (11): 63

Hayta, M.; Alpaslan, M.; Cakmakli, U. 2003. Physicochemical and Sensory Properties of Soymilk-Incorporated Bulgur. J Food Science. 68 (9): 2800

Hawthorn, J. 1981. Foundations of Food Science. W.H. Freeman and Company Ltd. U. K

Henderson, B. E; Ross, R. K; Pike, M. C; Casagrande, J. T. 1982. Endogenous hormones as a major factor in human cancer. Cancer Res. 42: 3232

Hendrich S; Lee K W; Xu X; Wang H J; Murphy P A. 1994. Defining food components as new nutrients. J. of Nutr. 124 (9 Suppl): 1789S

Holmes, W. L; Rubel, G. B; Hood, S. S. 1980. Comparison of the effect of dietary meat versus soybean protein on plasma lipids of hyperlipidemic individuals. Atherosclerosis. 36: 379

Hou, H J and Chang, K C. 1998. Yield and quality of soft tofu as affected by soybean physical damage and storage. J Agric. Food Chem. 46: 4798

Hou, J. W; Roch, C. Y; Cheng, C. C. 2000. Changes in some components of soymilk during fermentation with bifodobacteria. Food Res. Int. 33: 393

How, J. S. L. and Mor, C. V. 1982. Removal of phenolic compounds from soy protein extracts using activated carbon. J Food Sci. 47: 933

Howell R W and Caldwell, B E. 1978. Genetic and other biological characteristics. In: Liu, K. 1997. Soybeans: Chemistry, Technology and Utilization. Chapman and Hall, New York.

Huang, A., Hsieh, O A L., Chang, S. S. 1981. Characterization of the non-volatile minor constituents responsible for the objectionable taste of defatted soybean flour. J Food Sci. 47: 19

Huang, J; Nasr, M; Kim, Y; Matthews, R. 1992. Genistein inhibits protein histidine kinase. J. Biol. Chem. 267: 15511

Huang, MT; Xie, JG; Lin, CB; Kizoulis, M; Seiberg, M; Shapiro, S; Conney, AH. 2004. Inhibitory effect of topical applications of nondenatured soymilk on the formation and growth of UVB-induced skin tumors. Oncology Res. 14 (7-8): 387

Hutchins AM, Slavin JL & Lampe JW (1995) Urinary isoflavonoid phytoestrogen and lignan 13 excretion after consumption of fermented and unfermented soy products. J Am Diet Assoc. 95: 545

Ihekoronye, A.I and Ngoddy, P.O. 1986. Integrated food science and technology for the tropics. Macmillan International Publishers, College Edition,U.K

Iijima, M; Okubo, K; Yamauchi, F; Hirono, H; Yoshikoshi, M. 1987. Effects of glycosides like saponin on vegetable food processing. Part II. Undesirable taste of glycosides like saponin. *Proceedings Paper,* International symposium on New Technology of Vegetable Protein, Oils and Starch Processing, Beijing, China; Chinese Cereals and Oil Association, China International Conference Center for Science and Technology, Technical University of Berlin, American Oil Chemists' Society. Pp2:109-2:123

Izumi, T; Piskula, MK; Osawa, S; Obata, A; Tobe, K; Saito, M; Kataoka S; Kubota, Y; Kikuchi, M. 2000. Soy isoflavones aglycones are absorbed faster and in higher amounts than their glucosides in human. J Nutr. 130: 1695

Jeon, Ki Suk; Ji, Geun Eog; Hwang, In Kyeong. 2002. Assay of □-glucosidase activity of bifidobacteria and the hydrolysis of isoflavone glycosides by Bifidobacterium sp. Int-57 in soymilk fermentation. J of Micro. and Biotechnol, 12(1): 8.

Jha H C, Von Recklinghausen G, Zilliken F. 1985. Inhibition of in vitro microsomal lipid peroxidation by isoflavonoids. Biochem Pharmacol. 34: 1367

Jibani, M. M; Bloodworth, L. L; Foden, E et al., 1991. Predominantly vegetarian diet in patients with incipient and early clinical diabetic nephropathy: early effects on albumin excretion rate and nutritional status. Diabetes Med. 8: 949

Kamat, V. B; Graham, G. E; Davis, M. A. F. 1978. Vegetable protein: lipid interactions. Cereal Chem. 55: 295

Kano, M.; Ishikawa, F.; Matsubara, S.; Kikuchi-Hayakawa, H.; Shimakawa, Y. 2002. Soy milk products affect ethanol absorption and metabolism in rats during acute and chronic ethanol intake. J of Nutr. 132(2): 238

Kanthamani, S., Nelson, A. I., Steinberg, M. P. 1978. Home preparation of soymilk: a new concept. Whole soybean foods for home and village use. Intsoy. 14 (5): 5-9

Kao, T. H; Nan-Wei Su, and Min-Hsiung Lee. 2004. Effect of Water-to-Bean Ratio on the Contents and Compositions of Isoflavones in Tofu. J. Agric. Food Chem.; *52*(8):2277

Kerckhoffs, D. A. J. M; Brouns F; Hornstra G; Mensink, RP. 2002. Effects on the human serum lipoprotein profile of β-glucan, soy protein and isoflavones, plant sterols and stanols, garlic and tocotrienols. Am J of Clinical Nutr. 2494

King RA; Bignell CM. 2000. Concentrations of isoflavone phytoestrogens and their glycosides in Australian soya beans and soya foods. Aust. J. Nutr. Diet. 57: 70

Kitamura, K; Davies, C. S; Kaizuma, N; Nielsen, N. C. 1983. Genetic analysis of a Null-Allele for lipoxigenase-3 in soybean seeds. Crop Sci. 23: 924

Kitamura, 1984. Biochemical characterization of lipoxigenase lacking mutants, L-1-less, L-2-less, and L-3-less soybeans. Agric. Biol. Chem. 48: 2339

Kitamura K; Kumagai, T; Kikuchi, A. 1985. Inheritance of lipoxigenase-2 and genetic relationships among genes for lipoxigenase-1, -2, and -3 isozymes in soybean seeds. Jpn. J. Breed. 35: 413

Kitamura, K.1993. Breeding trials for improving the food processing quality of soybeans. Trends Food Sci. Technol. 4: 64

Kitts DD.; Krishnamurti CR.; Kitts WD. 1980. Uterine weight changes and 3H-uridine uptake in rats treated with phytoestrogen. Can. J. Anim. Sci. 60: 531

Kodou, S; Shimoyamada, M; Imura, T; Uchida, T; Okubo, K. 1991a. A new isoflavone glycoside in soybean seeds (*Glycine max Merrill*), Glycitein. 7-O-β-D(6”-O-acetyl)-glucopyranoside. Agric. Biol. Chem. 55:859

Kodou, S; Fleury, Y; Welti, D; Magnolato, D; Ushida, T; Kitamura, K; Okubo, K. 1991b. Malonyl isoflavone glycosides in soybean seeds (*Glycine max Merrill*). Agric. Biol. Chem. 55: 2227

Koeferli, C. S., Schwegler, P. P., and Hong-Chen, D. 1998. Application of classical and novel sensory techniques in product optimization. Lebensmittel-Technologie. 31: 407

Kon, S., Wagner, J. R., Guadagni, D. G., and Hovart, R. J. 1970. pH adjustment control of oxidative off-flavors during grinding of raw legume seeds. J Food Sci. 35: 343

Kon, S.K. 1972. Milk and Milk Products in Human Nutrition. 2nd edn. FAO Rome.

Kontessis, P; Jones, S; Dodds, R; Trevisan, R; Nosadini, R; Fioretto, P; Borsato, M; Sacerdoti, D; Viberti, G. 1990. Renal metabolic and hormonal response to ingestion of animal and vegetable proteins. Kid Inter. 38: 136

Kordylas, J. M. 1990. Processing and preservation of tropical and subtropical foods. (Chpt. 8, 20, and 24). Macmillan Publishers, Hong Kong

Kumur, P and Mishra, H. N. 2004. Effect of stabilizer addition on physiochemical, sensory, textural properties and starter culture counts of mango soymilk fortified yoghurt (MSFY). Food Chem. 87 (4): 501

Kurzer MS (2000) Hormonal effects of soy isoflavones: studies in premenopausal and postmenopausal women. J Nutr. 130: 660S

Kurzer, MS. 2003. Phytoestrogen supplement use by women. J Nutr. 133: 1983S

Kwok, K and Niranjan, K. 1995. Review: Effect of thermal processing on soymilk. International J Food Sci. and Technol. 30: 263

Kwok, KC; Shiu, YW; Yeung, CH; Niranjan, K. 1998. Effect of thermal processing on available lysine, thiamine and riboflavin content in soymilk. J Sci. Food and Agric. 77 (4): 473

Kwok, Kin-Chor; Basker, Dov; Niranjan, Keshavan. 2000. Kinetics of sensory quality changes in soymilk during thermal processing, by parametric and non-parametric data analyses. J Sci. Food and Agric. 80(5): 595

Kwok,-Kin-Chor, Liang,-Han-hua, Niranjan,-Keshavan. 2002. Mathematical modeling of the heat inactivation of trypsin inhibitors in soymilk at 121-154^{o}C. J Sci. Food Agric. 82(3): 243

Kwon, Bin; Kim, Young Bae; Lee, Jong-Hoon; Lee, Hyong Joo; Chung, Dae Kyun; Ji, Geun Eog. 2002. Analysis of sugars and □-galactosidase activity during soymilk fermentation by Bifidobacteria. Food Sci. Biotechnol. 11(4): 389

Laurin, D; Jacques, H; Moorjani, S et al. 1991. Effects of a soy-protein beverage on plasma lipoproteins in children with familial hypercholesterolemia. Am J Clin Nutr. 54: 98

Lawless, H. T., and Heyman, H. Sensory Evaluation of Food: Principles and Practices. Chapman & Hall/International Thomson Pub. New York. 608pp.

LeBlanc, JG; Garro, MS, De Giori, GS, De Valdez, GF. 2004. A novel functional soy-based food fermented by lactic acid bacteria: Effect of heat treatment. J of Food Sci. 69 (8): 246

LeBlanc, JG; Garro, MS; Silvestroni, A; Connes, C; Piard, JC; Sesma, F; de Giori, GS. 2004. Reduction of alpha-galactooligosaccharides in soyamilk by Lactobacillus fermentum CRL 722: in vitro and in vivo evaluation of fermented soyamilk. J of Applied Micro. 97 (4): 876

Leiner, I.E. 1981. Factors affecting the nutritional quality of soya product. J. Am. Oil Chemist Soc. 58: 406

Lu L J; Anderson K E. 1998. Sex and long-term soy diets affect the metabolism and excretion of soy isoflavones in humans. American Journal of Clinical Nutrition. 68 (6 Suppl): 1500S

Lu L J; Anderson K E; Grady J. J; Kohen F; Nagamani M. 2000. Decreased ovarian hormones during a soya diet: implications for breast cancer prevention. Cancer Res. 60(15): 4112

Lu L J; Cree M; Josyula S; Nagamani M; Grady J. J; Anderson K E. 2000. Increased urinary excretion of 2-hydroxyestrone but not 16alpha-hydroxyestrone in premenopausal women during a soya diet containing isoflavones. Cancer Res. 60(5): 1299

Liu, K. 1997. Soybeans: Chemistry, Technology and Utilization. Chapman and Hall, New York.

Lund, D B. 1982. Quantifying reactions influencing quality of foods: texture, flavor and appearance. J Food Proc Pres. 6: 133

Lydeking-Olsen, E; Beck-Jensen, JE; Setchell, KDR; Holm-Jensen, T. 2004. Soymilk or progesterone for prevention of bone loss - A 2 year randomized, placebo-controlled trial. European J of Nutr. 43 (4): 246

Maity, T. K and Paul, S. C. 1991. Low oligosaccharides soymilk: application of alpha-galactosidase for hydrolyzing soy-oligosaccharides. Indian Dairyman, 43: 443

Macedo, R F; Freitas, R J S; Pandey, A; Soccol, C R. 1999. Production and shelf-life studies of low cost beverage with soymilk, buffalo cheese whey and cow milk fermented by mixed culture cultures of *Lactobacillus casei ssp. shirota* and *Bifidobacterium adolescentis*. J Basic Microbiol. 39 (4): 243

Mahfuz, A., Tsukamoto, C., Kudou, S., Ono, T. 2004. Changes of astringent sensation of soy milk during tofu curd formation . J of Agric. And Food Chem. 52 (23): 7070

Martini M. C, Dancisak B. B, Haggans C. J, Thomas W, Slavin J L. 1999. Effect of soy intake on sex hormone metabolism in premenopausal women. Nutr. Cancer. 34: 133

Matoba, T; Hidak, H; Narita, H; Kitamura, K; Kaizuma, N; Kito, M. 1985. Lipoxigenase -2 isozyme is responsible for generation of n-hexanal in soybean homogenate. J. Agric. Food Chem. 33: 852

Matsuura,M. Obata, A., Fukushima, D. 1989. Objectionable flavor of soymilk developed during the soaking of soybeans and its control. J. Food Sci. 54: 602

Meilgaard, M., Civille, G. V and Thomas Carr, B. 1999. Sensory evaluation techniques. 3rd Edition. Chapter 1, p.8. CRC Press, Washington, D.C. U.S.A.

Mercer, N. J. H; Carroll, K. K; Giovannetti, P. M; Steinke, F. H; Wolfe, B. M. 1987. Effects on human plasma lipids of substituting soybean protein isolate for milk protein in the diet. Nutr Rep Int. 35: 279

Messina, M and Barnes, S. 1991. The role of soy products in reducing cancer risk. J. Natl. Cancer Inst. 83: 541

Messina, Mark J.; Persky, Victoria; Setchell, Kenneth D. R.; Barnes, Stephen. 1994. Soy intake and cancer risk: a review of the in vitro and in vivo data. Nutr and Cancer. 21(2): 113

Messina, M. 1995a. Modern applications for an ancient bean: soybeans and the prevention and treatment of chronic disease Port Townsend, WA, USA. J of Nutr. 125(3S): 567S

Messina, M. 1995b. Isoflavone intakes by Japanese were overestimated. Am. J Clin. Nutr. 62: 645 (Letter)

Mital, B K; and Steinkraus, K H. 1975. Utilization of oligosaccharides by lactic acid bacteria during fermentation. J Food Sci. 40: 114

Miyagi, Yuko; Shinjo, Sumie; Nishida, Ryoko; Miyagi, Chika; Takamatsu, Kiyoharu; Yamamoto, Takashi; Yamamoto, Shigeru. 1997. Trypsin inhibitor activity in commercial soybean products in Japan. J of Nutr. Sci. and Vitaminology. 43(5): 575

Morr, C. V and Ha, E. Y. W. 1991. Processing to preserve formation of off-flavors in soy products. Comments in Agric. Food Chem. 2: 247

Murphy, P. A. 1982. Phytoestrogen content of processed soybean products. Food Tech. 36: 60

Murphy, S.C. 1997. Raw milk bacteria tests: Standard plate count, preliminary incubation count, lab pasteurization count and coliform count - What do they mean for your farm? p34-42 in Proc. National Mastitis Council Regional Meeting, Syracuse, NY

Murphy, P. A.; Song, T; Buseman, G; Barua, K; Beecher, G. R.; Trainer, D; Holden, J. 1999. Isoflavones in Retail and Institutional Soy Foods. J Agric. Food Chem. 47(7): 2697

Murphy, Patricia A.; Barua, Kobita; Hauck, Catherine C. 2002. Solvent extraction selection in the determination of isoflavones in soy foods. Journal of Chromatography, B: Analytical Technologies in the Biomedical and Life Sciences. 777(1-2): 129

Nagata C; Takatsuka N; Inaba S; Kawakami N; Shimizu H. 1998a. Effect of soymilk consumption on serum estrogen concentrations in premenopausal Japanese women. J of The National Cancer Institute. 90(23): 1830

Nagata, C., Takatsuka, N., Kurisu, Y., Shimizu, H. 1998b. Decreased serum total cholesterol concentration is associated with high intake of soy products in Japanese men and women. J. Nutr. 128: 209

Naim, M., Gestetner, B., Kirson, I; Birk, Y., and Bondi, A. 1973. A new isoflavones from soya beans. Phytochemistry. 12: 169

Naim, M., Gestetner, B., Zilkah, S; Birk, Y., and Bondi, A. 1974. Soybeans isoflavones. Characterization, determination and antifungal activity. J. Agric. Food Chem. 24: 1174

Naim, M., Gestetner, B., Zilkah, S., Birk, Y., and Bondi, A. 1976. Soybean isoflavones, characterization, determination, and antifungal activity. J. Agric. Food Chem., 22: 806

Nakamura, H; Takasawa, M; Kasahara, S; et al., 1989. Effects of acute protein loads of different sources on renal function of patients with diabetic nephropathy. Tohoku J Exp Med. 159: 153

Nelson, A. I., Wei, L. S., Steinberg, M. P. 1971. Food products from whole soybeans. Soybean Digest. 31 (3): 32

Nelson, A. I; Steinberg, M. P; Wei, L. S; 1975. Soybean beverage base. U.S. Patent 3,901, 978

Nelson, A. I; Steinberg, M. P; Wei, L. S; 1976. Illinois process for preparation of soymilk. J Food Sci. 41: 57

Nielsen, S. S; Liener I. E. 1984. Degradation of the major storage protein of *Phaseolus vulgaris* during germination. Role of endogenous proteases and protease inhibitors. Plant Physiol. 74: 494

N'Kouka, KD; Klein, BP; Lee, SY. 2004. Developing a lexicon for descriptive analysis of soymilks. J of Food Sci. 69 (7): S259

Nsofor, Leslie M. 2002. Beverage prepared from hydrolyzed soybean slurry. (Soy Ultima, LLC, USA). PCT Int. Appl. (2002), 52 pp. Patent: Application: WO 2001-US24267 20010803. Priority: US 2000-634933 20000808.

Nsofor, L. M; Anyanwu, K. B. 1992. Development and evaluation of concentrated soymilk beverage. J Food Sci. Technol. 29: 331

Okubo, K; Iijima, M; Kobayashi, Y; Yoshikoshi, M; Uchida, T; Kudou, S. 1992. Components responsible for the undesirable taste of soybean seeds. Biosc. Biotech. Biochem. 56: 99

Okura, A; Arakawa, H; Oka, H; Yoshinari, T; Monden, Y. 1988. Effect of genistein on topoisomerase activity and on the growth of [Val12] Ha-*ras*-transformed NIH 3T3 cells. BioChem.Biophys. res. Commun. 157: 183

Olson, J.C. Jnr., and Mocquot, J. 1980. Milk and Milk Products. In: Microbial Ecology of Foods. Vol.2 edn. International Commission on Microbiological Specifications for Foods. Academic Press, London pp. 470-520.

Ono, T; Coi, M. R; Ikeda, A; Odagiri, S. 1991. Changes in the composition and size distribution of soymilk protein particles by heating. Agric. Biol. Chem. 55: 2291

Ono T; Takeda, M; Guo, S. T. 1996. Interaction of protein particles with lipids in soybean milk. Boisci. Biotechnol. Biochem. 60: 1165

Orthoefer, F. T. 1978. Processing and utilization. In: Soybean physiology, agronomy, and utilization. Ed. Norman, G. A. Academic Press. New York, U.S.A

Pavon, N. 2003. Sensory Characteristics of Flavored Milk Candies. Thesis. Louisiana State University. Baton Rouge, LA. http://etd.lsu.edu/docs/available/etd-0703103-120331/.

Penalvo, JL; Matallana, MC; Torija, ME. 2004. Chemical composition and nutritional value of traditional soymilk. J of Nutr. 134 (5): 1254S

Penalvo, JL; Castilho, MC; Silveira, MIN; Matallana, MC; Torija, ME. 2004. Fatty acid profile of traditional soymilk. European Food Res. And Technol. 219 (3): 251

Peterson, G and Barnes, S. 1991. Genistein inhibition of the growth of human breast cancer cells: independence from estrogen receptors and the multi-drug resistance gene. Biochem. Biophys. Res. Commun. 179: 661

Petrakis NL; Barnes S; King EB; Lowenstein J; Wiencke J; Lee MM; Mike R; Kirk M; Coward L. 1996. Stimulatory influence of soy protein isolate on breast secretion in pre- and postmenopausal women. Cancer Epidemiol. Biomarkers Prev. 5: 785

Pinthong, R; Macrae, R; and Dick, J. 1980. The development of soya-based yoghurt. III. Analysis of oligosaccharides. J Food Technol. 15: 661

Pratt, D. E., and Birac, P. M. 1979. Source of antioxidant activity of soybeans and soy products. J. Food Sci., 44: 1720

Prawiradjaja S, and Wilson, L. A. 2002. Efficiency in lipid removal from soymilk made from full fat soy flake or whole soybeans at three solid levels. (Abst).

Prinyawiwatkul, W., Beuchat, L. R., and Resurreccion, A. V. A. 1993. Optimization of sensory qualities of an extruded snack based on cornstarch and peanut flour. Lebensmittel-Technologie. 26: 393

Prinyawiwatkul, W., Mcwatters, K. H., Beuchat, L. R, and Phillips, R. D. 1997. Optimizing acceptability of chicken nuggets containing fermented cowpea and peanut flours. Journal of Food Science. 64 (4): 889

Raiz, M. N. 1999. Soybeans as functional foods. Cereal Food World. 44 (2): 88

Raines E. W, and Ross R. 1995. Biology of atherosclerotic plaque formation: possible role of growth factors in lesion development and the impact of soy. J Nutr. 125: 624S

Reinemann, D.J., G.A. Mein, D.R. Bray, D. Reid, and J.S. Britt. 1997. Troubleshooting high bacteria counts in farm milk. p. 65-79 in Proc. 36th Annual Meeting of National Mastitis Council, Madison, WI.

Richelle M, Pridmore-Merten S, Bodenstab S, Enslen M & Offord EA. 2002. Hydrolysis of isoflavone glycosides to aglycones by β-glycosidase does not alter plasma and urine isoflavone pharmacokinetics in postmenopausal women. J Nutr. 132: 2587

Rosenthal, A., Pyle, D. L., Niranjan, K., Gilmour, S., Trinca, L. 2001. Combined effect of operational variables and enzyme activity on aqueous enzymatic extraction of oil and protein from soybean. Enzyme and Microbial Technol. 28: 499

Rosenthal, Amauri; Deliza, Rosires; Cabral, Lourdes M. C.; Cabral, Lair C.; Farias, Carlos A. A.; Domingues, Aline M. 2003. Effect of enzymatic treatment and filtration on sensory characteristics and physical stability of soymilk. Food Control. 14(3): 187

Sariaslani FS.; kunz, DA. 1986. Induction of cytochrome P-450 in *Streptomyces griseus* by soybean flour. Biochem. Biophys. Res. Commun. 141: 405

SAS Institute Inc. 2002. SAS Proprietary Software Release 8.0., Trade mark of SAS Institute Inc., Cary, NC

SAS Institute (2004), SAS Proprietary Software Release 9.1. Trade mark of SAS Institute Inc., Cary, NC

Scallet, AC; Wofford, M; Meredith, JC; Allaben, WT; Ferguson, SA. 2003. Dietary exposure to genistein increases vasopressin but does not alter beta-endorphin in the rat hypothalamus. Toxicological Sci. 72 (2): 296

Schryver, T. 2002. Increasing health benefits using soy germ. Cereal Foods World. v. 47 (5): 185

Schweigerer L, Christeleit K, Fleischmann G, et al. 1992. Identification in human urine of a natural growth inhibitor for cells derived from solid paediatric tumors. Eur J Clin Invest. 22: 260

Seo, A and Morr, C. V. 1985. Activated carbon and ion exchange treatments for removing phenolics and phytate from peanut protein products. J Agric. Food Chem. 32: 530

Sessa DJ; Rackis JJ. 1977. Lipid-derived flavors of legume protein products. J Am Oil Chem. Soc. 54: 468

Sessa D J, Warner, K; Rackis, J. J. 1976. Oxidized phosphatidylcholines from defatted soybean flakes taste bitter. J. Agric. Food Chem. 24:16

Setchell KDR (1995) Non-steroidal estrogens of dietary origin: possible roles in health and disease, metabolism and physiological effects. Proc Nutr Soc. 20: 1-21.

Setchell K.D.R. 2000a. Absorption and metabolism of soy isoflavones-from food to dietary supplements and adults to infants. J. Nutr. 130:654S

Setchell KDR. 2000b. Soy isoflavones – benefits and risks from nature's selective estrogen receptor modulators (SERMs). J. Am. Coll. Nutr. 20: 345S

Setchel, KDR and Cassidy, A. 1999. Dietary isoflavones: Biological effects and relevance to human health. J. Nutr. 129: 758S

Setchell, KDR 1995. Discovery and potential clinical importance of mammalian lignans. In: Cunnane S. S, Thompson, L. U (Eds). Flaxseed in human nutrition. Champaign, IL: AOCS Press, p 82 - 98

Setchell, KDR; Lawson, AM; Conway, E et al. 1981a. The definitive identification of the lignans *trans* -2,3 bis (3 – hydroxybenzyl)-g-butyrolactone and 2,3 bis (3-hydroxybenzyl) butane-1,4-diol in human and animal urine. Biochem J. 197: 447

Setchell, K. D. R; Lawson, A. M; Borriello, SP; Harkness, R; Gordon H; Morgan, DM; Kirk DN; Adlercreutz H; Anderson LC; Axelson M. 1981b. Lignan formation in man – microbial involvement and possible roles in relation to cancer. Lancet. 2: 4 – 7

Setchell, K. D; Borriello, S. P; Hulme, P; Kirk, D. N; Axelson, M. 1984. Non-steroidal estrogens of dietary origin: possible roles in hormone dependent diseases. Am J Clin Nutr. 40: 569

Setchell, KDR; Zimmer-Nechemias, Linda; Cai, Jinnan; Heubi, James E. 1998. Isoflavone content of infant formulas and the metabolic fate of these phytoestrogens in early life. Am J of Clin. Nutr. 68(6Supp): 1453S

Setchel, K. D. R and Cassidy, A. 1999. Dietary Isoflavones: biological effects and relevance to human health. J Nutr. 129: 758S

Setchel, KDR and Radd, S. 2000. Soy and other legumes: bean around a long time but they are the "superfood" of the millennium and what are the safety issues for their constituent phytoestrogens. Asai Pac. J. Clin. Nutr. 9: S13

Setchell, Kenneth D. R.; Brown, Nadine M.; Desai, Pankaj; Zimmer-Nechemias, Linda; Wolfe, Brian E.; Brashear, Wayne T.; Kirschner, Abby S.; Cassidy, Aedin; Heubi, James E. 2001. Bioavailability of pure isoflavones in healthy humans and analysis of commercial soy isoflavone supplements. J of Nutr. 131(4Supp): 1362S

Setchell, KDR; Brown, NM; Zimmer-Nechemias, L; Brashear, WT; Wolfe, BE; Kirschner, AS; Heubi, JE. 2002a. Evidence for lack of absorption of soy isoflavone glycosides in humans, supporting the crucial role of intestinal metabolism for bioavailability. Am J. of Clin. Nutr. 76 (2): 447

Setchell, KDR; Brown, NM; Lydeking-Olsen, E. 2002b. The clinical importance of the metabolite equol – A clue to the effectiveness of soy and its isoflavones. J Nutr. 132: 3577

Setchel, K. D. R., Brown, N. M., Desai, P. B., Zimmer-Nechemias L, Wolfe, B., Jakate, A. S., Creutzinger, V., Heubi, J. E.2003. Bioavailability, disposition, and dose-response effects of soy isoflavones when consumed by healthy women at physiologically typical dietary intakes. J Nutr. 133: 1207

Setchell, Kenneth D. R.; Cole, Sidney J. 2003. Variations in Isoflavone Levels in Soy Foods and Soy Protein Isolates and Issues Related to Isoflavone Databases and Food Labeling. J Agric. Food Chem. 51(14): 4146

Sherkat et al. 2001. Food Australia. 53 (97): 264

Shibasaki, K; Okubo, K; Sata, T. 1972. Food chemical studies on soybean proteins part X. Shifting of protein to cream layer of soybean milk and the emulsifying capacity. Nippon Shokuhin Kougyo Gakkaishi. 19: 580

Shung-Tang, G; Ono, T; Mikami, M. 1997. Incorporation of soymilk lipid into protein coagulum by addition of calcium chloride. J Agric. Food Chem. 47: 901

Shun-Tang, G, Ono, T and Mikami, M. 1997. Interaction between protein and lipid in soybean milk at elevated temperatures. J. Agric. Food Chem. 45: 4601 - 4605

Shurtleff, W and Aoyagi, A. 1984. Soymilk industry and market. Soy foods Center, Lafayette, Cal. CA

Shutt, D. A; Cox, R. L. 1972. Steroid and phyto-estrogens binding to sheep uterine receptors in vitro. J Endocrinol. 52: 299

Sirtori C. R, Bosisio R, Pazzucconi F, Bondioli A, Gatti E, Lovati M. R., Murphy P. 2002. Soy milk with high glycitein content does not reduce low-density lipoprotein cholesterolemia in type II hypercholesterolemic patients. Ann Nutr Metab. 46: 88

Slavin JL, Karr SC, Hutchins AM & Lampe JW (1998) Influence of soybean processing, habitual diet, and soy dose on urinary isoflavonoid excretion. Amer J Clin Nutr 68: 1492S

Smith A K, Circle, S J. 1978. Protein products as food ingredients. In: Liu, K. 1997. Soybeans: Chemistry, Technology and Utilization. Chapman and Hall, New York.

Song T; Barua K; Buseman G; Murphy P A. 1998. Soy isoflavone analysis: quality control and a new internal standard. Amer. J. of Clin. Nutr. 68(6 Suppl): 1474S

Song, T.; Lee, S.-O.; Murphy, P. A.; Hendrich, S.. 2003. Soy Protein With or Without isoflavones, Soy Germ and Soy Germ Extract, and Daidzein Lessen Plasma Cholesterol Levels in Golden Syrian Hamsters. Experimental Biol. Med. 228 (9): 1063

Soyatech, 1999. Soy Protein Health Claim is Announced by the FDA. Bluebook Update. Soyatech Inc. Vol. 6 (4), 1 – 7. www.soyatech.com.

Soyatech, 2002. Soya Foods: The U.S. Market 2002. Bar Habour, Maine: SoyaTech Inv. and Senechal, Jorgenson and Hale Co., www.soyatech.com.

Staswick, P. E; Hermodson, M. A; Nielsen, N. C. 1984. Identification of the cystines which link the acidic and basic components of glycinin subunit. J Biol. Chem. 259: 13431

Sydner, I. S; Johnson, W; Zoltala. 1978. Significant pathogen in dairy product (Marth, E.H. ad.) Public Health Association, Washington, D.C. pp. 11-32.

Takatsuka N; Nagata C; Kurisu Y; Inaba S; Kawakami N; Shimizu H. 2000. Hypo-cholesterolemic effect of soymilk supplementation with usual diet in premenopausal normolipidemic Japanese women. Preventive Med. 31(4): 308

Tang JL; Armitage JM; Lancaster T; Silagy CA; Fowler GH; Neil HA. 1998. Systematic review of dietary intervention trials to lower blood total cholesterol in free-living subjects. Br Med. J. 316: 1213

Taniyama, T; Yoshikawa, M; and Kitagawa, I. 1988. Saponin and sapogenol. XLIV. Soya saponin composition in soybeans of various origins and soya saponin content in various organs of soybeans. Structure of soya saponin V from soybean hypocotyl. Yakugaku Zasshi. 108: 562.

The Institute of Food Technologists. http//www.ift.org

Tochikura T, Sakai K, Fujiyoshi T, Tachiki T, Kumagai H. 1986. p-Nitrophenyl glycoside-hydrolysing activities in *bifidobacteria* and characterization of β-D-galactosidase of *Bifidobacterium longum* 401. Agric Biol. Chem. 50 (9): 2279

Trant, A S., Pangborn, R M., Little, A. 1981. Potential fallacy of correlating hedonic scale responses with physical and chemical measurements. J Food Sci. 46: 836

Tsangalis, D; Ashton, J. F. McGill, A. E. J, Shah, N. P. 2002. Enzymic transformation of isoflavone phytoestrogens in soymilk by beta-glucosidase-producing bifidobacteria. J. of Food Science. 67 (8): 3104

Tsangalis, D; Ashton, J.F, Mcgill,A. E. J, Shah, N. P. 2003. Biotransformation of isoflavones by bifidobacteria in fermented soymilk supplemented with D-glucose and L-cysteine. J. of Food Sci. 32: 4

Tsangalis, D.; Ashton, J. F.; Stojanovska, L.; Wilcox, G.; Shah, N. P. 2004a. Development of an isoflavone aglycone-enriched soymilk using soy germ, soy protein isolate and bifidobacteria. Food Res. Internat. 37 (4): 301

Tsangalis, D.; Shah, N. P. 2004b. Metabolism of oligosaccharides and aldehydes and production of organic acids in soymilk by probiotic bifidobacteria. Internat. J Food Sci. and Technol. 39 (5): 541

Tsangalis, D., Wilcox, G., Shah, N. P., Stojanovska, L. 2004. Bioavailability of isoflavone phytoestrogens in postmenopausal women consuming soymilk fermented with probiotic bifidobacteria. British J. Nutr. 93 (6): 867

Tsukamoto, C.; Shimada, S.; Igita, K.; Kudou, S.; Kokubun, M.; Okubo, K.; Kitamura, K. 1995. Factors affecting isoflavone content in soybean seeds: changes in isoflavones, saponins, and composition of fatty acids at different temperatures during seed development. MAFF, Ibaraki, Japan. J of Agric. and Food Chem. 43 (5): 1184

United States Food and Drug Administration, 1999. Food labeling: Health claims. Soy protein and coronary heart disease (CHD). 21 CFR part 101.82. Fed Regist. 64: 57699

United States Department of Agriculture, Iowa State University, 2001. Database on isoflavone content of foods, release 12/13 -2000. Nutrient Data Laboratory Research Service, Riverdale, MD, 2001. Published online at www.nal.usds.gov/fnic/foodcomp Accessed, 2004.

United States Food and Drug Administration, 2002. Health claims. Soy protein and risk of coronary heart disease. 21 CFR part 101. FDA Docket No. 98P-0683, p.57,700. FDA Dockets Management Branch, Rockville, MD

United States Department of Agriculture. 2004. SR16 Nutrition Database published at http://www.Nutrition Data.com/. or www.nal.usds.gov/fnic/foods/Data/index.html. Nutrient Data Laboratory Research Service, Riverdale, MD, 2001. Accessed, 2004.

United Soybean Board, 2001. Soy and Health. The healthful balances nutrient. Published online at http://www.talksoy.com. USB, Chesterfield, MO. Accessed, 2004

United Soybean Board. 2003. Soy Foods Guide. Helpful Tips and Information for using Soy Food. Published online at http://www.talksoy.com. USB, Chesterfield, MO. Accessed, 2004

Uprit, S and Mishra, HN. 2004. Instrumental textural profile analysis of soy fortified pressed chilled acid coagulated curd (Paneer). Intern. J of Food Properties. 7 (3): 367

Verbruggen, M.A and Olieman, K. 2002. Glycitein for clinical trials. J. nutr. 132: 601S

Verbruggen, M.A; van Rooijen, J. J. M; and van der Vat, B. J. C. 2002. Isoflavone analysis in soy and soy products – results of a ring test. J. nutr. 132: 595S

Verdeal, K; Brown, R. R; Richardson, T; Ryan, D. S. 1980. Affinity of phytoestrogen for estradiol-binding proteins and effect of coumestrol on growth of 7, 12-dimethylbenz [a] anthracene-induced rat mammary tumors. J Natl Cancer Inst. 64: 285

Walter, E.D. 1941. Genistin (an isoflavone glucoside) and its aglucone, genistein from soybean. J. Am. Oil Chem. Soc. 63: 3274

Walz, E. 1931. Isoflavone- and saponin-glucoside in soja hispida. Ann. Chem. 489: 118

Wang, H. J and Murphy, P.A. 1994. Isoflavone composition of American and Japanese soybeans in Iowa: Effects of variety, crop year, and location. J. Agric. Food Chem. 42: 1674

Wang, H-J., and Murphy, P. A. 1994. Isoflavone content in commercial soybean foods. J. Agric. Food Chem., 42 :1666

Wang, H-J., and Murphy, P. A. 1996. Mass balance study of isoflavones during soybean processing. J. Agric. Food Chem. 44(8): 2377

Wang, YC; Yu, RC; Chou, CC. 2004. Viability of lactic acid bacteria and bifidobacteria in fermented soymilk after drying, subsequent rehydration and storage. Int. J of Food Microbiol. 93 (2): 209

Wei H, Bowen R, Cai Q, Barnes S, Wang Y. 1995. Antioxidant and antipromotional effects of the soybean isoflavone genistein. Proc Soc Exp Biol Med. 208: 124

Wei H, Bowen R, Cai Q, Barnes S, Wang Y. 1996. Inhibition of UV light- and Fenton reaction-induced oxidative DNA damage by the soybean isoflavone genistein. Carcinogen 17: 73

Weingartner, K. E. 1987. Processing, Nutrition and Utilization of Soybeans. In: Soybeans for the Tropics. Research Production and Utilization (Singh, S. R; Rachie, K. O., Dasheil, K. E. eds). International Institute of Tropical Agriculture, IITA. John Wiley & Sons. UK. USA, Singapore. Canadá. A Wiley – Interscience Production

White L R, Petrovitch H, Ross G W, Masaki K, Hardman J, Nelson J, Davis D, Markesbury W. 2000. Brain aging and midlife tofu consumption. J Am Coll Nutr. 19: 242

Wilkens, W F., and Lin, L M. 1970. Gas chromatographic and mass spectral analysis of soybean milk volatiles. J Agric. And Food Chem. 18: 333

Wilkens, W F., Mattick, L R., and Hand, D B. 1967. Effect of processing method on oxidative off-flavors of soybean milk. Food Technol. 21: 1630

Wolf, W. J and Cowman, J. C. 1971. Soybeans as a food source. CRC Crit. Rev. Food Technol. 2: 81

Wrensch M, Petrakis N L, King E B, Lee M. M, Mike R. 1993. Breast cancer risk associated with abnormal cytology in nipple aspirates of breast fluid and prior history of breast biopsy. Am J Epidemiol. 137: 828

Xu, X., Wang, H. –J., Murphy, P. A., Cook, L., Hendrich, S. 1994. Daidzein is a more Bioavailable soymilk isoflavone than is genistein in adult women. J. Nutr. 124: 825

Xu, X., Harris, K. S., Wang, H. –J., Murphy, P. A., Hendrich, S. 1995. Bioavailability of soybean isoflavones depends upon gut microflora in women. J. Nutr. 125: 2307

Xu X; Wang H J; Murphy P A; Hendrich S. 2000. Neither background diet nor type of soy food affects short-term isoflavone bioavailability in women. J. of Nutr. 130 (4): 798

Xu, Z., Wu, Q and Godber, S. J. 2002. Stabilities of Daidzin, glycitin, genistin and generation of derivatives during heating. J. Agric. Food Chem. 50: 7402

Yadav, D. N.; Chauhan, G. S.; Chauhan, O. P.; Sharma, P.; Bajpai, A. 2003. Quality Evaluation of Curd Prepared from Milk-Soymilk Blends. J Food Sci. Technol. 40 (4): 403

Yagasaki, K; Takagi, T; Sakai, M; Kitamura, K. 1997. Biochemical characterization of soybean protein consisting of different subunits of glycinin. J Agric. Food Chem. 45: 656

Yamano, Y; Miki, E; Fukui, Y. 1981. Incorporation of lipid into soybean protein gel and the role of 11S and 7S protein. Nippon Shyokuhin Kogyo Gakkaishi. 28: 136

Yen, Gow-Chin; Kao, Chia-Horn. 2002. Influence of processing conditions on isoflavones content of soybean products. ACS Symposium Series, 816 (Bioactive Compounds in Foods), 73-84.

Zhang Y; Wang G J; Song T.T; Murphy P A; Hendrich S. 2001. Urinary disposition of the soybean isoflavones daidzein, genistein and glycitein differs among humans with moderate fecal isoflavone degradation activity. Erratum in: J of Nutr. 131(1):147 or J of Nutr. 129(5): 957

APPENDIX A. CHARTS FOR CHAPTER 2

Main Soymilk Production Stages

Whole Soybeans

↓

1. **SOAK in $NaHCO_3$ for 8-10hrs depending on H_2O temperature**

↓

2. **BLANCH in NaHCO3 @ 95 / 100^oC for 10/20 min**

↓

3. **GRIND smooth into slurry containing 20% solids**

↓

4. **COOK raw milk at 90 / 100^oC for 10 / 20 min & filter**

↓

5. **H &P @ 3000 / 4000 psi @ 82^oC, Bottle and store @34-**

WS = whole soybean seeds SG = Soybean germ

APPENDIX B. CHARTS AND DATA FOR CHAPTER 3

3.2 ANOVA of Bland Soymilk between treatments and by Process

Sample	Dependent variable	DF	SS	MS	F-value	Pr > F
P1	Sugar	12	45.42	3.78	78.53	<.0001
	Fat	12	0.42	0.03	6.49	0.0010
	Total Solid	12	31.02	2.59	15.56	<.0001
	Actual TS	12	28.44	2.37	16.73	<.0001
	Moisture	12	30.98	2.58	15.63	<.0001
	Ash	12	0.03	0.00	28.84	<.0001
	Viscosity	12	171644.27	14303.69	67.68	<.0001
	Wet Protein	12	0.42	0.04	1.69	0.1796
	Isoflavone	12	1025.19	85.43	3.04	0.0288
P2	Sugar	12	65.46	5.46	77.57	<.0001
	Fat	12	0.22	0.02	4.13	0.0084
	Total Solid	12	27.37	2.28	45.61	<.0001
	Actual TS	12	25.55	2.13	59.50	<.0001
	Moisture	12	27.36	2.28	46.52	<.0001
	Ash	12	0.03	0.00	79.50	<.0001
	Viscosity	12	122372.78	10197.73	19.80	<.0001
	Wet Protein	12	0.31	0.03	1.27	0.3384
	Isoflavone	12	384.34	32.03	5.16	0.0031
P3	Sugar	12	78.41	6.53	98.04	<.0001
	Fat	12	0.13	0.01	2.47	0.0595
	Total Solid	12	33.41	2.78	26.37	<.0001
	Actual TS	12	30.77	2.56	29.60	<.0001
	Moisture	12	33.41	2.78	26.52	<.0001
	Ash	12	0.03	0.00	21.93	<.0001
	Viscosity	12	126021.63	10501.80	198.38	<.0001
	Wet Protein	12	0.26	0.02	1.06	0.4553
	Isoflavone	12	778.79	64.90	14.88	<.0001
C	Sugar	12	42.51	3.54	93.11	<.0001
	Fat	12	0.19	0.02	2.21	0.0849
	Total Solid	12	25.23	2.10	71.28	<.0001
	Actual TS	12	24.55	2.05	124.94	<.0001
	Moisture	12	25.21	2.10	72.30	<.0001
	Ash	12	0.04	0.00	23.88	<.0001
	Viscosity	12	77755.50	6479.63	40.56	<.0001
	Wet Protein	12	0.52	0.04	1.93	0.1274
	Isoflavone	12	498.54	41.54	3.71	0.0131

3.3 Mean & SD of Treatment for Process 2 (P2)

Trt	°Brix	Fat	Total Solid	Actual TS	Moisture	Ash	Viscosity	Wet Protein	Isoflavone
T1	5.12b 0.12	0.95ab 0.03	4.15bc 0.06	3.20b 0.04	95.86ab 0.06	0.135bc 0.01	96.84bc 17.14	3.09a 0.00	17.55a 1.15
T2	4.25bc 0.54	1.01ab 0.00	4.52bc 0.05	3.51b 0.05	95.49ab 0.04	0.150bc 0.00	98.20b 39.99	3.11a 0.10	18.41a 4.76
T3	4.15c 0.03	0.66c 0.15	4.35bc 0.04	3.70b 0.11	95.66ab 0.04	0.140bc 0.00	42.12bc 0.62	3.08a 0.02	18.23a 2.79
T4	4.25bc 0.11	0.76bc 0.07	3.70c 0.54	2.95b 0.47	96.30a 0.54	0.145bc 0.01	51.24bc 4.02	2.98a 0.06	21.64a 2.66
T5	4.45bc 0.07	1.03ab 0.04	4.50bc 0.04	3.48b 0.01	95.50ab 0.04	0.130bc 0.00	46.52bc 0.85	3.18a 0.05	33.97a 1.00
T6	4.92bc 0.30	1.06a 0.02	4.38bc 0.20	3.33b 0.17	95.62ab 0.20	0.115c 0.01	50.64bc 1.70	3.10a 0.02	28.25a 0.41
T7	5.02bc 0.21	1.05ab 0.04	5.38b 1.22	4.33b 1.18	94.63b 1.22	0.130bc 0.00	281.64a 10.13	2.86a 0.05	31.68a 15.53
T8	5.07b 0.33	1.05ab 0.01	4.49bc 0.16	3.44b 0.18	95.52ab 0.16	0.160b 0.03	292.84a 25.96	2.84a 0.02	23.17a 0.11
T9	4.58bc 0.07	1.02ab 0.03	4.41bc 0.20	3.40b 0.16	95.59ab 0.20	0.155b 0.01	89.04bc 4.19	2.98a 0.06	18.69a 3.30
T10	4.85bc 0.07	1.06a 0.01	4.46bc 0.12	3.41b 0.12	95.55ab 0.11	0.145bc 0.01	87.68bc 4.98	3.00a 0.01	16.61a 2.18
T11	4.72bc 0.21	1.07a 0.06	4.44bc 0.05	3.37b 0.11	95.57ab 0.06	0.145bc 0.01	74.16bc 2.60	3.05a 0.01	13.05a 6.48
T12	4.80bc 0.04	1.04ab 0.05	4.35bc 0.21	3.32b 0.16	95.66ab 0.21	0.150bc 0.00	86.8bc 2.83	3.06a 0.02	19.52a 1.96
T13	9.50a 0.00	1.12a 0.18	8.32a 0.45	7.21a 0.26	91.69c 0.44	0.260a 0.00	40.00c 0.23	2.70a 0.49	13.49a 4.79
Range	5.35	0.46	4.62	4.26	4.61	0.14	252.84	0.48	20.92

3.4 Mean & SD of Treatment for Process 3 (P3)

TRT	°Brix	Fat	T-Solid	Actual TS	Moisture	Ash	Viscosity	Protein	Isoflavone
T1	5.89b 0.16	0.95ab 0.04	4.48bc 0.30	3.53bc 0.25	95.53ab 0.29	0.130bc 0.00	180.88abc 43.33	3.05a 0.04	21.02ab 2.16
T2	5.15bc 0.03	1.09a 0.05	5.35b 0.21	4.27b 0.25	94.66b 0.20	0.125c 0.01	101.76cde 20.14	3.07a 0.01	19.75ab 0.28
T3	4.92bc 0.16	0.79b 0.04	4.52bc 0.23	3.73bc 0.28	95.49ab 0.23	0.140bc 0.00	63.00de 4.47	3.03a 0.02	19.74ab 4.69
T4	1.65d 0.31	0.99ab 0.00	4.65bc 0.17	3.66bc 0.17	95.35ab 0.17	0.140bc 0.00	142.00bcd 18.33	3.14a 0.08	27.09a 1.90
T5	4.75c 0.07	0.98ab 0.08	4.23c 0.13	3.25c 0.06	95.78a 0.14	0.130bc 0.01	45.20e 1.24	3.12a 0.02	20.78ab 0.99
T6	4.97bc 0.09	1.00ab 0.01	4.25c 0.10	3.25c 0.08	95.76a 0.10	0.125c 0.01	45.12e 1.36	3.11a 0.04	20.29ab 1.97
T7	4.88bc 0.49	0.88ab 0.01	4.51bc 0.26	3.63bc 0.25	95.50ab 0.26	0.135bc 0.01	230.72ab 55.44	2.95a 0.01	23.20ab 2.08
T8	5.34bc 0.33	1.10a 0.08	4.69bc 0.35	3.60bc 0.26	95.32ab 0.35	0.140bc 0.00	251.2a 20.93	2.95a 0.03	24.10a 1.93
T9	5.03bc 0.14	1.07a 0.03	4.68bc 0.05	3.61bc 0.08	95.33ab 0.05	0.150b 0.00	76.96de 2.04	3.08a 0.07	18.50ab 2.74
T10	4.88bc 0.21	1.00ab 0.02	4.48bc 0.06	3.49c 0.07	95.53ab 0.05	0.150b 0.00	59.12de 1.70	3.00a 0.01	13.85b 0.95
T11	4.70c 0.10	1.06ab 0.02	4.65bc 0.12	3.59bc 0.10	95.36ab 0.13	0.145bc 0.01	88.04de 0.74	3.08a 0.04	20.94ab 0.40
T12	4.50c 0.57	1.11a 0.08	4.63bc 0.04	3.53bc 0.04	95.38ab 0.05	0.140bc 0.00	79.44de 23.08	3.06a 0.05	25.95a 2.43
T13	9.50a 0.00	1.12a 0.18	8.32a 0.45	7.21a 0.26	91.69c 0.44	0.260a 0.00	40.00e 0.23	2.70a 0.49	13.49b 4.79
Range	7.85	0.33	4.09	3.96	4.09	0.13	211.2	0.44	13.6

3.5 Mean & SD of Treatment for Process 1 (P1)

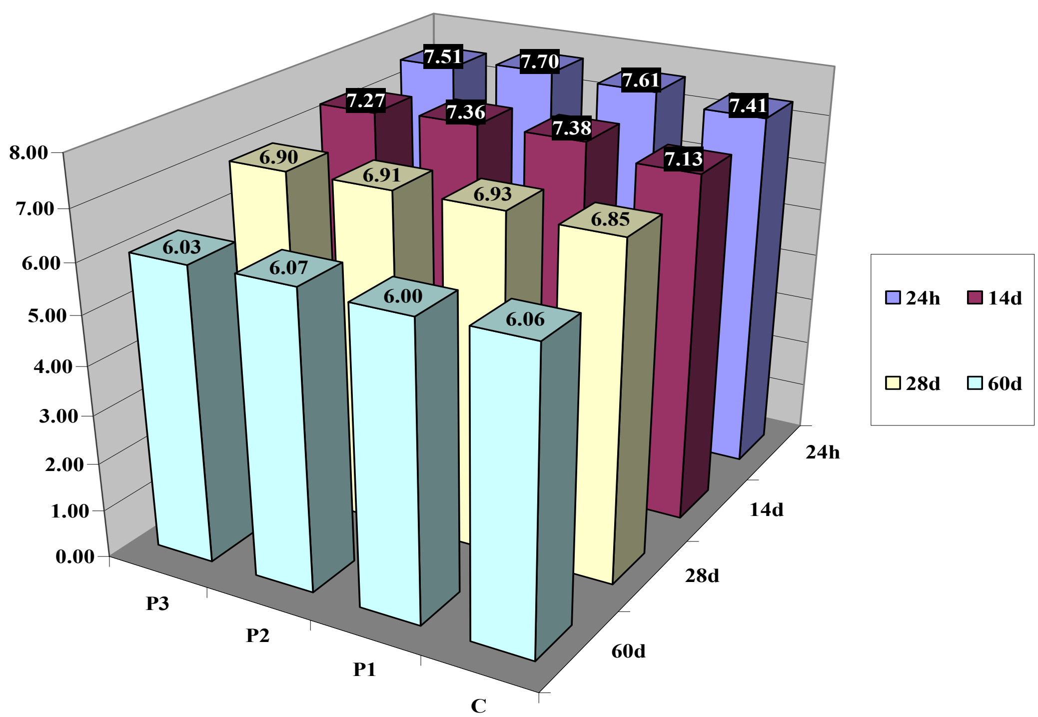
TRT	°Brix	Fat	T-Solid	Actual TS	Moisture	Ash	Viscosity	Protein	Isoflavone
T1	5.03b 0.14	0.94a 0.04	3.72bc 0.13	2.78bc 0.08	96.29ab 0.12	0.140bc 0.00	83.36bc 1.36	3.05a 0.05	16.75bc 0.20
T2	4.89b 0.02	1.02a 0.00	4.38bc 0.11	3.36bc 0.10	95.63ab 0.11	0.135bc 0.01	79.68bc 9.28	3.08a 0.03	26.40a 1.20
T3	2.58c 0.21	1.03a 0.06	4.41bc 0.08	3.38bc 0.14	95.60ab 0.09	0.140bc 0.00	34.92e 1.64	3.08a 0.01	27.34a 2.75
T4	2.50c 0.10	1.00a 0.03	4.10bc 0.23	3.10bc 0.26	95.92ab 0.23	0.145bc 0.01	43.04de 3.39	3.12a 0.04	26.02a 0.29
T5	2.74c 0.33	0.89a 0.05	3.48c 0.03	2.60c 0.08	96.53a 0.04	0.175b 0.04	31.20e 0.68	3.03a 0.02	23.15ab 1.10
T6	3.12c 0.16	0.91a 0.10	3.96bc 0.90	3.05bc 0.81	96.05ab 0.90	0.135bc 0.01	28.76e 2.55	3.02a 0.00	17.58bc 2.69
T7	4.77b 0.33	0.98a 0.04	4.44bc 0.40	3.46bc 0.44	95.57ab 0.39	0.130c 0.00	253.16a 1.64	3.03a 0.01	13.89c 1.82
T8	4.93b 0.28	1.06a 0.02	4.64bc 0.05	3.59bc 0.08	95.37ab 0.05	0.120c 0.00	230.08a 16.07	2.99a 0.01	13.80c 1.95
T9	4.94b 0.19	1.01a 0.03	4.60bc 0.08	3.59bc 0.11	95.42ab 0.08	0.145bc 0.01	96.16b 2.94	2.96a 0.08	23.94ab 0.40
T10	4.52b 0.59	1.07a 0.04	4.71bc 0.16	3.65bc 0.11	95.29ab 0.16	0.150bc 0.00	64.64cd 16.52	2.95a 0.07	28.64a 2.10
T11	4.79b 0.26	1.06a 0.03	4.64bc 0.30	3.58bc 0.27	95.37ab 0.30	0.150bc 0.00	57.44cde 2.04	3.05a 0.07	24.60ab 0.73
T12	5.29b 0.12	1.13a 0.04	4.91b 0.00	3.79b 0.03	95.1b 0.01	0.150bc 0.00	79.80bc 5.71	3.08a 0.05	26.29a 1.99
T13	9.50a 0.00	1.12a 0.18	8.32a 0.45	7.21a 0.26	91.69c 0.44	0.260a 0.00	40.00de 0.23	2.70a 0.49	13.49c 4.79
Range	7	0.24	4.84	4.61	4.84	0.14	224.4	0.42	15.15

3.6 Mean & SD of Treatment for Control

TRT	°Brix	Fat	T-Solid	Actual TS	Moisture	Ash	Viscosity	Protein	Isoflavone
T1	5.29b 0.12	0.99a 0.02	3.96d 0.02	2.97d 0.00	96.05a 0.02	0.130bc 0.00	79.64c 0.85	3.09a 0.00	18.40a 1.11
T2	5.20b 0.14	1.07a 0.10	4.77c 0.03	3.7c 0.07	95.24b 0.03	0.125bc 0.02	77.84c 16.18	3.18a 0.11	15.38a 2.35
T3	4.13c 0.00	1.04a 0.05	5.53b 0.23	4.5b 0.18	94.48c 0.23	0.095c 0.02	46.00c 1.24	3.26a 0.15	18.42a 0.59
T4	4.14c 0.09	1.04a 0.01	5.00bc 0.04	3.96c 0.06	95.01bc 0.04	0.155b 0.01	183.16ab 30.04	3.27a 0.01	23.65a 6.11
T5	5.22b 0.21	0.95a 0.07	4.75c 0.12	3.80c 0.04	95.26b 0.12	0.135bc 0.01	52.88c 2.83	3.09a 0.01	13.58a 0.03
T6	5.24b 0.37	1.01a 0.10	4.63cd 0.13	3.62c 0.23	95.38ab 0.13	0.135bc 0.02	50.64c 7.58	3.13a 0.02	16.28a 7.42
T7	4.63bc 0.14	1.25a 0.16	4.94bc 0.16	3.70c 0.01	95.07bc 0.16	0.125bc 0.01	143.60b 23.99	2.97a 0.06	17.95a 1.27
T8	5.30b 0.00	1.24a 0.06	4.87bc 0.04	3.65c 0.02	95.13bc 0.04	0.135bc 0.01	224.04a 14.31	2.95a 0.01	16.41a 2.16
T9	4.97b 0.19	1.07a 0.03	4.83c 0.16	3.77c 0.13	95.17b 0.16	0.160b 0.00	84.80c 1.70	3.04a 0.04	23.03a 1.24
T10	4.90bc 0.42	1.05a 0.03	4.74c 0.20	3.69c 0.17	95.27b 0.19	0.150b 0.00	77.36c 7.13	3.01a 0.08	24.84a 1.47
T11	4.93b 0.14	1.14a 0.03	4.96bc 0.08	3.82c 0.05	95.05bc 0.08	0.160b 0.00	50.40c 2.26	3.12a 0.04	26.47a 2.94
T12	5.40b 0.10	1.13a 0.01	4.98bc 0.05	3.85c 0.06	95.03bc 0.04	0.155b 0.01	84.28c 2.43	3.08a 0.01	24.95a 2.10
T13	9.5a 0.00	1.12a 0.18	8.32a 0.45	7.21a 0.26	91.69d 0.44	0.260a 0.00	40.00c 0.23	2.70a 0.49	13.49a 4.79
Range	5.37	0.3	4.36	4.24	4.36	0.16	184.04	0.57	12.98

3.7a Total Mean pH ANOVA between Treatment

Sample	Dependent variable	DF	SS	MS	F-value	Pr > F
P2	24h	12	16.88	1.41	544.23	<.0001
	14d	12	13.81	1.15	45.06	<.0001
	28d	12	11.47	0.96	8.08	0.0003
	60d	12	4.91	0.41	3.02	0.0293
P3	24h	12	13.06	1.09	276.27	<.0001
	14d	12	12.66	1.06	5.76	0.0018
	28d	12	16.83	1.40	8.07	0.0003
	60d	12	10.48	0.87	43.89	<.0001
P1	24h	12	12.28	1.02	499.98	<.0001
	14d	12	10.04	0.84	69.19	<.0001
	28d	12	7.59	0.63	10.29	<.0001
	60d	12	4.28	0.36	2.54	0.0547
C	24h	12	10.28	0.86	225.91	<.0001
	14d	12	8.19	0.68	152.78	<.0001
	28d	12	6.21	0.52	7.53	0.0005
	60d	12	3.83	0.32	3.04	0.0288



3.7b pH of Different Processed Soymilk Beverages during 60 days Storage Period

3.8 Mean pH ANOVA by Treatment for Process 1 (P1)

Treatment	Mean & SD for Process One (P1) 24h	14d	28d	60d
T1	8.33a 0.00	7.81a 0.28	7.04ab 0.40	6.14a 0.13
T2	8.43a 0.08	8.08a 0.02	7.27ab 0.08	6.17a 0.98
T3	6.86e 0.01	6.56cd 0.02	5.80c 0.69	6.26a 0.47
T4	6.98e 0.02	6.64cd 0.04	6.47bc 0.00	6.83a 0.55
T5	6.54f 0.04	6.47d 0.09	6.40bc 0.08	6.21a 0.04
T6	6.55f 0.00	6.51d 0.01	6.36bc 0.00	5.92a 0.35
T7	8.06b 0.01	7.87a 0.04	7.74a 0.04	6.12a 0.28
T8	8.07b 0.01	7.94a 0.00	7.79a 0.00	5.78a 0.23
T9	7.79c 0.01	7.29b 0.08	7.17ab 0.11	5.87a 0.23
T10	6.85e 0.13	6.87bcd 0.11	6.61bc 0.25	5.41a 0.01
T11	7.38d 0.02	7.28b 0.07	7.10ab 0.03	5.62a 0.04
T12	7.36d 0.04	6.99bc 0.21	6.87ab 0.19	5.42a 0.08
T13	8.45a 0.02	8.22a 0.01	7.03ab 0.18	6.61a 0.14
Range	1.91	1.75	1.99	1.42

3.9 Mean pH ANOVA by Treatment for Process 2 (P1)

Treatment	Mean & SD 24h	14d	28d	60d
T1	8.42a 0.02	8.18a 0.02	7.68abc 0.11	6.59a 0.51
T2	8.46a 0.02	7.87ab 0.11	6.74bcd 1.22	6.08a 0.30
T3	7.20c 0.02	6.91cd 0.02	6.76abcd 0.01	5.74a 0.03
T4	7.06c 0.04	6.74d 0.04	6.49cd 0.03	5.67a 0.14
T5	6.28d 0.01	6.27d 0.04	6.29d 0.02	6.00a 0.04
T6	6.44d 0.13	6.34d 0.06	6.28d 0.00	5.92a 0.22
T7	8.42a 0.01	8.13ab 0.01	8.12a 0.01	6.37a 0.01
T8	8.44a 0.01	8.10ab 0.00	8.09ab 0.01	6.61a 0.03
T9	7.80b 0.01	7.59ab 0.08	6.90abcd 0.06	6.18a 1.14
T10	6.46d 0.08	6.33d 0.53	5.78d 0.10	5.39a 0.10
T11	7.83b 0.05	7.49bc 0.16	6.94abcd 0.03	5.54a 0.10
T12	7.65b 0.04	7.74ab 0.01	6.94abcd 0.02	5.35a 0.01
T13	8.45a 0.02	8.22a 0.01	7.03abcd 0.18	6.61a 0.14
Range	2.18	1.95	2.34	1.26

3.10 Mean pH ANOVA by Treatment for Process 3 (P3)

Treatment	Mean & SD 24h	14d	28d	60d
T1	8.36a 0.03	7.74ab 0.50	6.56abc 1.47	5.57d 0.06
T2	8.29a 0.11	6.74abc 1.29	5.69c 0.01	5.67d 0.03
T3	7.80b 0.00	7.04abc 0.65	5.77c 0.15	5.68d 0.02
T4	6.39d 0.05	6.03c 0.02	5.96bc 0.02	5.85cd 0.07
T5	7.74bc 0.01	7.67abc 0.01	7.61ab 0.02	6.44b 0.18
T6	7.78b 0.02	7.67abc 0.12	7.60ab 0.04	6.39bc 0.16
T7	8.36a 0.14	8.26a 0.01	8.07a 0.01	7.18a 0.23
T8	8.36a 0.11	8.22a 0.04	8.06a 0.01	7.37a 0.27
T9	7.50c 0.02	7.32abc 0.09	7.17abc 0.13	5.47d 0.10
T10	6.56d 0.01	6.45bc 0.13	6.30bc 0.03	5.40d 0.08
T11	7.85b 0.02	7.70abc 0.06	7.51ab 0.15	5.69d 0.18
T12	6.61d 0.02	6.65abc 0.03	6.52abc 0.01	5.61d 0.01
T13	8.45a 0.02	8.22a 0.01	7.03abc 0.18	6.61b 0.14
Range	2.06	2.23	2.38	1.97

3.11 Mean pH ANOVA by Treatment for Control (C)

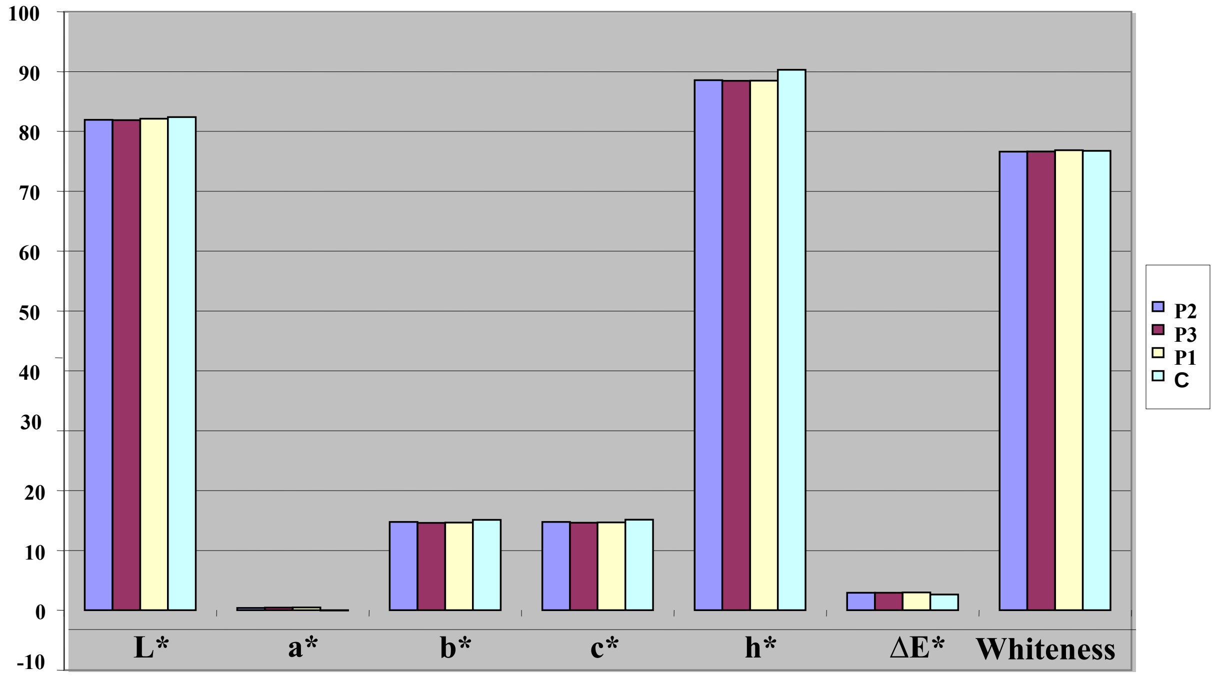
Treatment	24h	14d	28d	60d
	Mean & SD			
T1	8.17b 0.02	7.52cd 0.19	6.9ab 0.91	5.58a 0.29
T2	8.23ab 0.09	7.21ef 0.04	6.77ab 0.11	5.91a 0.13
T3	6.94ef 0.00	6.65h 0.01	6.37b 0.06	5.83a 0.03
T4	6.86ef 0.02	6.58hi 0.05	6.27b 0.06	6.08a 0.05
T5	6.77fg 0.06	6.49hi 0.02	6.45b 0.03	6.29a 0.18
T6	6.76fg 0.15	6.65h 0.07	6.44b 0.00	6.23a 0.13
T7	7.92c 0.01	7.87b 0.04	7.78a 0.01	6.27a 0.21
T8	7.84cd 0.06	7.73bc 0.07	7.77a 0.01	6.69a 0.16
T9	7.67d 0.01	7.35de 0.06	7.20ab 0.00	5.88a 0.88
T10	6.58g 0.08	6.38i 0.01	6.29b 0.15	5.46a 0.13
T11	7.09e 0.04	7.05fg 0.01	6.99ab 0.05	6.38a 0.08
T12	7.02e 0.04	6.94g 0.04	6.85ab 0.00	5.53a 0.57
T13	8.45a 0.02	8.22a 0.01	7.03ab 0.18	6.61a 0.14
Range	1.87	1.84	1.51	1.23

3.12 Total Mean - Color ANOVA by Treatment for all Processes

		ANOVA ; Between TRT*				
Sample	Dependent variable	DF	SS	MS	F-value	Pr > F
P2	L	12	59.50	4.96	24.09	<.0001
	A	12	9.29	0.77	30.88	<.0001
	B	12	34.90	2.91	43.60	<.0001
	C	12	34.41	2.87	42.56	<.0001
	H	12	163.48	13.62	34.61	<.0001
	ΔE	12	33.79	2.82	42.12	<.0001
	whiteness	12	73.86	6.16	33.41	<.0001
P3	L	12	38.24	3.19	24.13	<.0001
	A	12	10.47	0.87	25.71	<.0001
	B	12	65.87	5.49	24.35	<.0001
	C	12	64.89	5.41	25.02	<.0001
	H	12	210.70	17.56	17.17	<.0001
	ΔE	12	62.91	5.24	24.76	<.0001
	whiteness	12	55.22	4.60	28.20	<.0001
P1	L	12	57.38	4.78	19.99	<.0001
	A	12	14.90	1.24	36.09	<.0001
	B	12	86.64	7.22	52.57	<.0001
	C	12	85.05	7.09	51.51	<.0001
	H	12	317.33	26.44	42.13	<.0001
	ΔE	12	77.27	6.44	59.82	<.0001
	whiteness	12	110.19	9.18	35.21	<.0001
C	L	12	39.29	3.27	40.35	<.0001
	A	12	19.44	1.62	164.09	<.0001
	B	12	90.37	7.53	58.64	<.0001
	C	12	88.02	7.34	60.03	<.0001
	h	12	358.08	29.84	105.87	<.0001
	ΔE	12	60.87	5.07	49.03	<.0001
	whiteness	12	99.99	8.33	70.29	<.0001

3.13 Mean Color ANOVA by Treatment for Process 1

Trt	L	a	B	C	h	ΔE	whiteness
			Mean & SD : Sample P1				
T1	81.48cdef 0.21	0.61ab 0.06	14.59c 0.02	14.60cd 0.02	87.62bc 0.24	2.82bc 0.11	76.42bc 0.18
T2	81.85cdef 0.56	1.07a 0.09	14.57c 0.94	14.61cd 0.93	85.79c 0.62	2.80bc 0.47	76.69bc 1.02
T3	83.08bcd 0.01	0.77ab 0.11	14.75bc 0.13	14.78bcd 0.12	87.00bc 0.43	2.05c 0.15	77.54b 0.08
T4	81.89cdef 0.15	1.10a 0.04	16.16ab 0.18	16.2ab 0.19	86.11bc 0.11	1.73c 0.08	75.70bc 0.23
T5	84.48ab 1.23	-1.38c 0.27	10.58d 0.12	10.66e 0.08	97.19a 1.51	6.53a 0.05	81.16a 0.96
T6	85.32a 0.62	-1.04c 0.13	11.02d 0.22	11.07e 0.21	95.41a 0.81	6.21a 0.43	81.62a 0.62
T7	80.09ef 0.09	0.78ab 0.25	14.88bc 0.33	14.90bcd 0.35	87.01bc 0.86	3.74b 0.05	75.13c 0.28
T8	80.03f 0.64	0.49ab 0.48	14.49c 0.50	14.51d 0.51	88.12bc 1.85	3.97b 0.77	75.31c 0.21
T9	81.48cdef 0.30	0.79ab 0.09	15.96abc 0.22	15.98abc 0.22	87.16bc 0.29	2.04c 0.20	75.54bc 0.37
T10	81.54cdef 0.53	0.50ab 0.06	16.85a 0.47	16.86a 0.47	88.29bc 0.16	1.79c 0.55	75.00c 0.70
T11	81.23def 0.14	1.09a 0.12	15.16bc 0.36	15.20bcd 0.37	85.90c 0.37	2.73bc 0.06	75.85bc 0.34
T12	82.02cde 0.21	1.12a 0.10	14.86bc 0.16	14.90bcd 0.16	85.67c 0.35	2.42c 0.02	76.65bc 0.26
T13	83.28bc 0.05	0.26b 0.03	16.73a 0.08	16.73a 0.08	89.10b 0.11	0.00d 0.00	76.35bc 0.10
Range	5.29	2.5	6.27	6.2	11.52	6.53	6.62



3.14 Color profile of Soymilk Beverages from Four Process Methods

3.15 Mean Color ANOVA by Treatment for Process 2

Mean & SD : Sample P2

Trt	**L**	**A**	**B**	**C**	**h**	**ΔE**	**Whitenes s**
T1	80.88def 0.90	1.00a 0.05	14.85cd 0.36	14.88cd 0.36	86.14c 0.11	3.19bc 0.47	75.78cde 0.93
T2	81.49cdef 0.42	0.56abc 0.36	14.22de 0.32	14.23de 0.33	87.78bc 1.41	3.11bcd 0.46	76.65cd 0.13
T3	83.17abc 0.16	0.91ab 0.10	12.70f 0.10	12.73f 0.11	85.92c 0.40	4.09ab 0.08	78.90ab 0.19
T4	82.21bcde 0.23	0.79abc 0.04	15.95ab 0.15	15.97ab 0.15	87.15bc 0.16	1.44e 0.08	76.09cde 0.27
T5	83.93ab 0.97	-0.87d 0.35	13.58ef 0.29	13.62ef 0.27	93.74a 1.47	3.49bc 0.19	78.93a 0.57
T6	84.88a 0.20	-0.94d 0.00	12.64f 0.13	12.68f 0.13	94.32a 0.01	4.55a 0.19	80.27a 0.24
T7	80.00f 0.51	0.46abc 0.16	15.45bc 0.62	15.46bc 0.62	88.31bc 0.52	3.57abc 0.26	74.72e 0.79
T8	79.85f 0.57	0.52abc 0.14	15.35bc 0.15	15.36bc 0.14	88.10bc 0.53	3.72abc 0.47	74.66e 0.54
T9	81.22def 0.01	0.60abc 0.06	14.99bcd 0.18	15.00bcd 0.19	87.71bc 0.19	2.72cd 0.10	75.96cde 0.12
T10	82.57bcd 0.04	0.29bc 0.11	14.70cd 0.21	14.71cd 0.21	88.88b 0.40	2.15de 0.19	77.20bc 0.17
T11	80.58ef 0.20	0.85abc 0.04	15.43bc 0.12	15.46bc 0.12	86.85bc 0.11	3.06bcd 0.14	75.18de 0.24
T12	80.70ef 0.02	0.76abc 0.02	15.37bc 0.06	15.39bc 0.06	87.15bc 0.05	2.96cd 0.00	75.31de 0.05
T13	83.28abc 0.05	0.26c 0.03	16.73a 0.08	16.73a 0.08	89.10b 0.11	0.00f 0.00	76.35cde 0.10
Range	5.03	1.94	4.09	4.05	8.4	4.55	5.61

3.16 Mean Color ANOVA by Treatment for Process 3

Trt	L	A	b	C	H	ΔE	whiteness
	Mean & SD : Sample P3						
T1	81.89ab 0.01	0.81ab 0.08	15.42abc 0.29	15.44abc 0.30	86.98cde 0.26	2.00def 0.16	76.20def 0.20
T2	82.41ab 0.03	1.22a 0.03	14.00cde 0.03	14.05cde 0.04	85.03e 0.13	3.02bcde 0.03	77.48bcd 0.00
T3	82.81a 0.19	0.99ab 0.01	12.66def 0.07	12.70def 0.07	85.55de 0.01	4.16abc 0.09	78.63ab 0.11
T4	83.23a 0.19	0.90ab 0.03	14.45bcd 0.03	14.47bcd 0.03	86.43cde 0.11	2.38cdef 0.02	77.84abc 0.13
T5	82.28ab 0.56	-0.55de 0.56	12.41ef 1.33	12.44ef 1.28	93.01ab 3.30	4.52ab 1.50	78.34ab 0.28
T6	82.60a 0.66	-1.04e 0.20	11.27f 0.22	11.32f 0.20	95.33a 1.18	5.67a 0.34	79.24a 0.44
T7	79.18d 0.37	0.01cd 0.09	14.47bcd 0.07	14.47bcd 0.06	89.99bc 0.34	4.70ab 0.30	74.64f 0.35
T8	80.44cd 0.07	0.44bc 0.23	14.63bc 0.37	14.64bc 0.38	88.32cde 0.83	3.55bcd 0.15	75.77ef 0.28
T9	82.01ab 0.05	0.59abc 0.03	16.04ab 0.28	16.05ab 0.28	87.88cde 0.07	1.50efg 0.08	75.89def 0.23
T10	82.84a 0.34	0.37bc 0.04	16.05ab 0.24	16.06ab 0.24	88.67cde 0.16	0.87fg 0.02	76.50cde 0.41
T11	80.31cd 0.19	1.17a 0.06	15.80abc 0.27	15.85abc 0.27	85.76de 0.15	3.25bcde 0.12	74.72f 0.32
T12	81.03bc 0.77	0.60abc 0.10	16.02ab 0.82	16.04ab 0.82	87.85cde 0.24	2.49cdef 0.48	75.16ef 1.12
T13	83.28a 0.05	0.26bc 0.03	16.73a 0.08	16.73a 0.08	89.10bcd 0.11	0.00g 0.00	76.35cde 0.10
Range	4.1	2.26	5.46	5.41	10.3	5.67	4.6

3.17 Mean Color ANOVA by Treatment for Control

Mean & SD : Sample C							
Trt	L	A	b	C	h	ΔE	whiteness
T1	82.65cde 0.15	0.45b 0.05	13.94de 0.11	13.95de 0.11	88.14cd 0.20	2.87cde 0.14	77.74c 0.05
T2	82.18de 0.06	0.50b 0.05	15.53bc 0.22	15.54bc 0.22	88.14cd 0.17	1.65ef 0.11	76.36de 0.19
T3	84.48a 0.16	0.62b 0.02	11.22g 0.09	11.23f 0.09	86.85de 0.06	5.65a 0.12	80.84a 0.19
T4	81.58ef 0.10	1.15a 0.07	16.04bc 0.06	16.09bc 0.06	85.91e 0.26	2.04def 0.03	75.55ef 0.11
T5	83.49abc 0.43	-1.81d 0.18	12.46fg 0.54	12.60ef 0.52	98.43a 0.95	4.76ab 0.54	79.23b 0.04
T6	83.96ab 0.14	-1.66d 0.20	12.91ef 0.77	13.03e 0.74	97.45a 1.43	4.33b 0.80	79.33b 0.58
T7	79.88g 0.55	-0.53c 0.17	16.45bc 0.57	16.47bc 0.56	91.92b 0.61	3.54bc 0.45	74.00g 0.78
T8	80.85fg 0.37	-0.79c 0.05	15.39bc 0.15	15.41bc 0.15	92.95b 0.21	2.98cd 0.22	75.42ef 0.39
T9	81.80ef 0.00	0.41b 0.08	15.86bc 0.22	15.87bc 0.23	88.51cd 0.28	1.73def 0.11	75.85e 0.15
T10	83.02bcd 0.37	0.34b 0.04	15.10cd 0.19	15.10cd 0.19	88.71cd 0.17	1.68ef 0.13	77.28cd 0.40
T11	81.60ef 0.29	0.37b 0.07	18.01a 0.47	18.02a 0.47	88.82cd 0.19	2.15def 0.06	74.25fg 0.12
T12	82.32de 0.33	0.34b 0.01	16.61ab 0.21	16.62b 0.21	88.82cd 0.05	0.99fg 0.30	75.74e 0.38
T13	83.28bcd 0.05	0.26b 0.03	16.73ab 0.08	16.73ab 0.08	89.10c 0.11	0.00g 0.00	76.35de 0.10
Range	4.6	2.96	6.79	6.79	12.52	5.65	6.84

APPENDIX C. CHART FOR CHAPTER 5

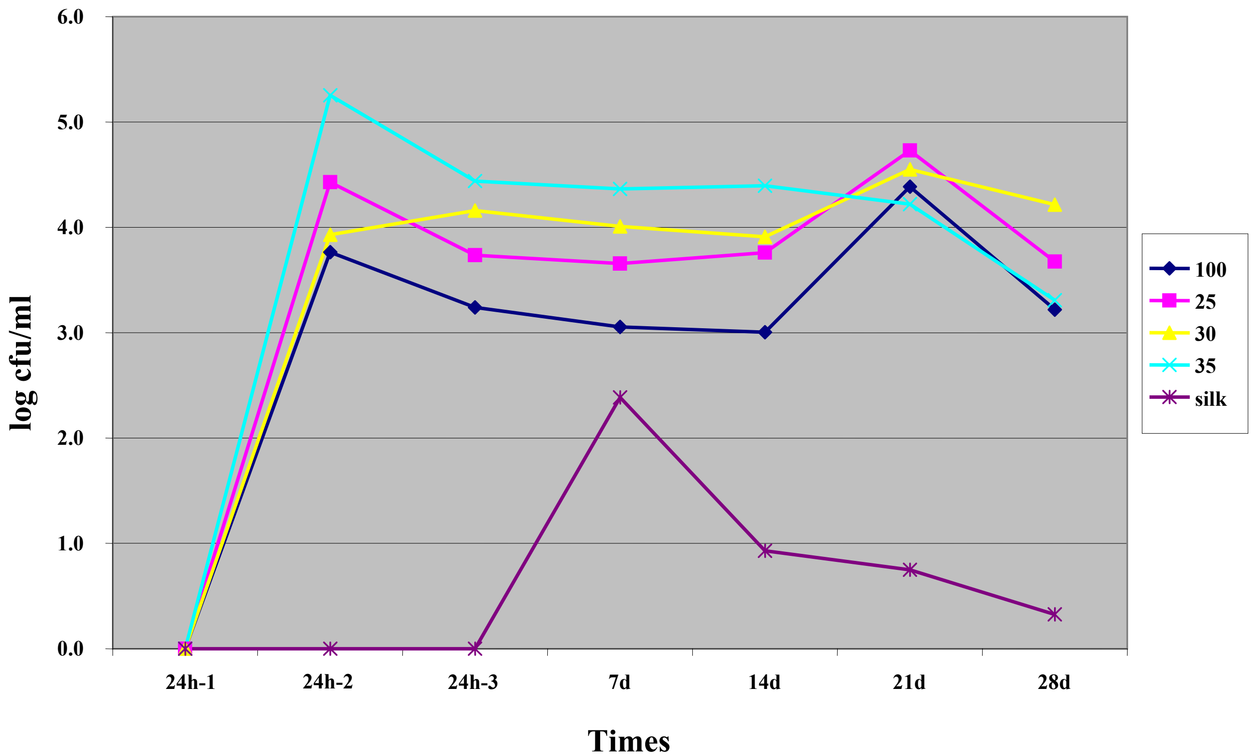


Figure 5.2 TPC / Coliform Count of PIE Soymilk Beverages during 28 – day Storage (24h-1 = pre-pasteurization; 24h-2 = pasteurized milk from tank; 24h-3 = post-pasteurized milk in bottles.

APPENDIX D. RESEARCH CONSUMER CONSENT FORM

Research Consent Form for Consumer Testing

Research Consent Form

I, ______________________, agree to participate in the research entitled "Consumer Acceptance of Soymilk," which is being conducted by Janette Saidu (Ph.D Student) under the supervision of Dr. Witoon Prinyawiwatkul of the Department of Food Science at Louisiana State University, phone number (225)578-5188. This research is part of her doctoral research in Food Science.

I understand that participation is entirely voluntary and whether or not I participate will not affect how I am treated on my job. I can withdraw my consent at any time without penalty or loss of benefits to which I am otherwise entitled and have the results of the participation returned to me, removed from the experimental records, or destroyed. One hundred and fifty consumers will participate in this research. For this particular research, about 10-15 minute participation will be required for each consumer.

The following points have been explained to me:

1. In any case, it is my responsibility to report prior participation to the investigator any allergies I may have.
2. The reason for the research is to gather information on consumer sensory acceptability of functional drinkable soymilk beverage. The benefit that I may expect from it is a satisfaction that I have contributed to solution and evaluation of problems relating to such health problems.
3. The procedures are as follows: Five coded samples will be placed in front of me, and I will evaluate them by normal standard methods and indicate my evaluation on score sheets. All procedures are standard methods as published by the American Society for Testing and Materials and the Sensory Evaluation Division of the Institute of Food Technologists.
4. Participation entails minimal to no risk: The only risk which can be envisioned is that of an allergic reaction to soymilk, Splenda® (sucralose), sugar or fructose, vanilla extract, chocolate and peach flavor. However, because it is known to me beforehand that the food to be tested contains common food ingredients, the situation can normally be avoided.
5. The results of this study will not be released in any individual identifiable form without my prior consent unless required by law.
6. The investigator will answer any further questions about the research, either now or during the course of the project.

The study has been discussed with me, and all of my questions have been answered. I understand that additional questions regarding the study should be directed to the investigator listed above. In addition, I understand the research at Louisiana State University AgCenter that involves human participation is carried out under the oversight of the Institutional Review Board. Questions or problems regarding these activities should be addressed to Dr. David Morrison, Associate Vice Chancellor of LSU AgCenter at 578-8236.

____________________________ ____________________________________

Signature of Investigator Signature of Participant

Witness: ___________________ Date: ______________________________

APPENDIX E. CHARTS AND DATA FOR CHAPTER 6

6.1 Questionnaire for Bland Soymilk Testing

What is your gender? Male______ Female_______ Sample – A

1. How would you rate the **OVERALL APPEARANCE** of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐
1	2	3	4	5	6	7	8	9

2. How would you rate the **COLOR** of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐
1	2	3	4	5	6	7	8	9

3. How would you rate the **OVERALL AROMA** of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐
1	2	3	4	5	6	7	8	9

4. How would you rate the **SOY AROMA** of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐
1	2	3	4	5	6	7	8	9

5. Please rate the **SOY AROMA** of this product based on your preference.

Weak	Just About Right	Strong
[ ]	[ ]	[ ]

6. How would you rate the **TASTE/ BEANY FLAVOR** of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐
1	2	3	4	5	6	7	8	9

7. How would you rate the **SWEETNESS** of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐
1	2	3	4	5	6	7	8	9

8. Please rate the **SWEETNESS** of this product based on your preference.

Weak	Just About Right	Strong
[ ]	[ ]	[ ]

9. How would you rate the **MOUTHFEEL/VISCOSITY** of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐
1	2	3	4	5	6	7	8	9

10. Please rate your **OVERALL LIKING** of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐
1	2	3	4	5	6	7	8	9

11. Is this product **ACCEPTABLE**? Yes [ ] No [ ]

12. Would you **BUY** this product if it were commercially available? Yes [ ] No [ ]

13. Would you buy this product after been told about the health benefit from soy protein and isoflavone in this product? Yes [ ] No [ ]

6.2 Frequency for Overall Appearance, Color, Overall Aroma of Bland Soymilk

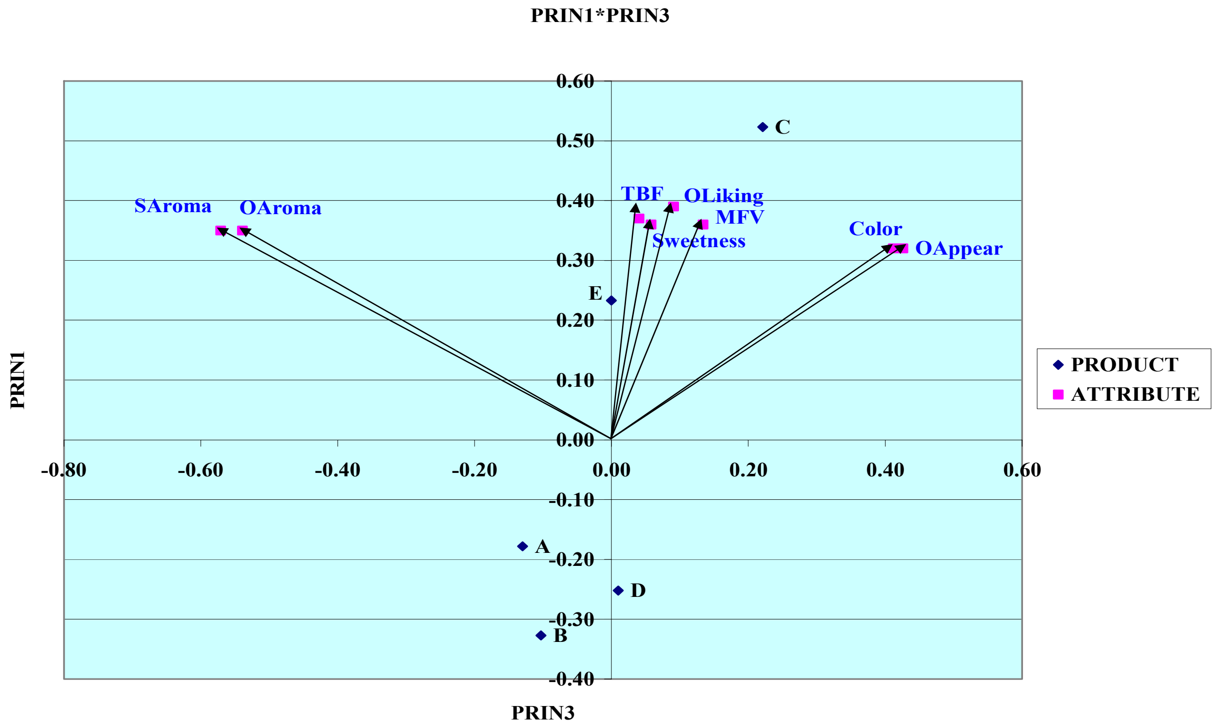
Overall Appearance	**Frequency**	**%**	**Cumulative Freq**	**Cumulative %**
Dislike Extremely	10	2	10	2
Dislike Very Much	12.6	2.52	22.6	4.53
Dislike Moderately	30.4	6.09	53	10.62
Dislike Slightly	73	14.62	126	25.24
Neither Like/Dislike	129.8	26	255.8	51.24
Like Slightly	100	20.03	355.8	71.27
Like Moderately	83.8	16.79	439.6	88.06
Like very much	44.8	8.97	484.4	97.04
Like Extremely	14.8	2.96	499.2	100
Color	**Frequency**	**%**	**Cumulative Freq**	**Cumulative %**
Dislike Extremely	6.8	1.36	6.8	1.36
Dislike Very Much	14.8	2.97	21.6	4.33
Dislike Moderately	29	5.81	50.6	10.14
Dislike Slightly	77.8	15.59	128.4	25.73
Neither Like/Dislike	125.8	25.21	254.2	50.94
Like Slightly	100.4	20.12	354.6	71.06
Like Moderately	81.6	16.35	436.2	87.41
Like very much	46	9.22	482.2	96.63
Like Extremely	16.8	3.37	499	100
Overall Aroma	**Frequency**	**%**	**Cumulative Freq**	**Cumulative %**
Dislike Extremely	11.8	2.37	11.8	2.37
Dislike Very Much	16.6	3.34	28.4	5.71
Dislike Moderately	34.2	6.88	62.6	12.59
Dislike Slightly	75.2	15.12	137.8	27.72
Neither Like/Dislike	168.6	33.91	306.4	61.63
Like Slightly	89.8	18.06	396.2	79.69
Like Moderately	59.4	11.95	455.6	91.63
Like very much	30.6	6.15	486.2	97.79
Like Extremely	11	2.21	497.2	100

6.3 Frequency for Soy Aroma, Rated Soy Aroma, TBF and Sweetness of Bland Soymilk

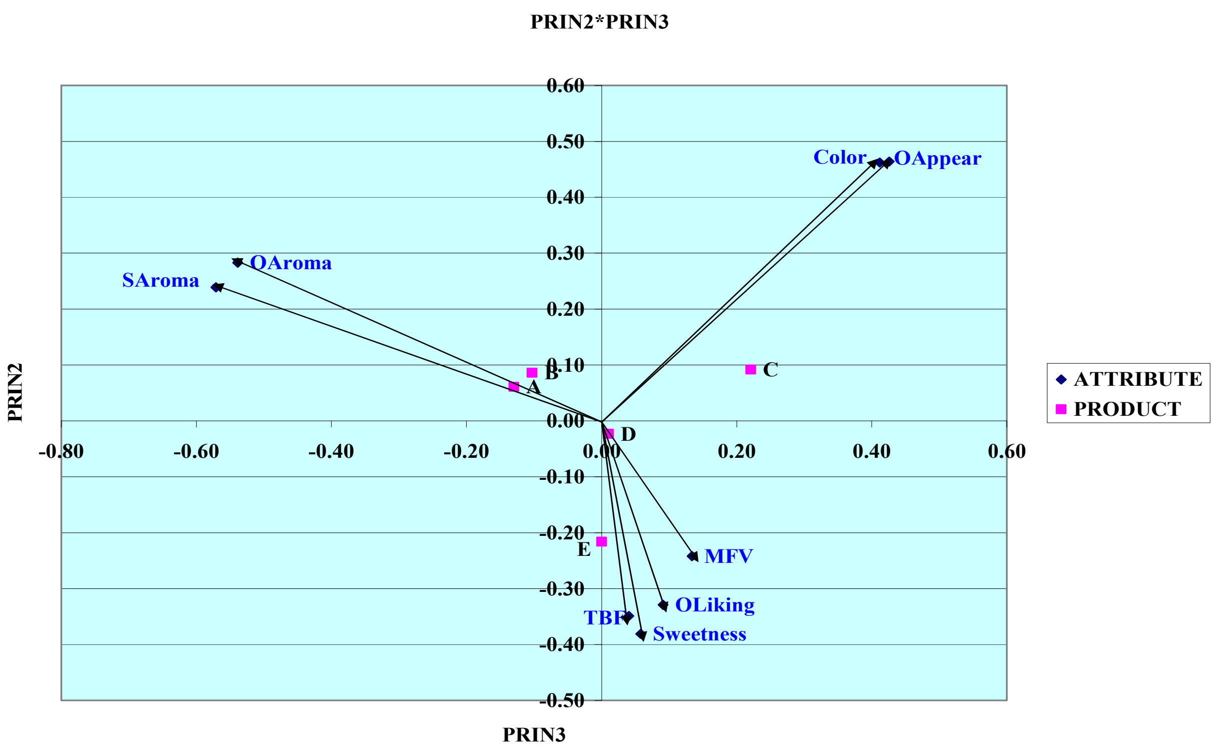
Soy Aroma	**Frequency**	**%**	**Cumulative Freq**	**Cumulative %**
Dislike Extremely	12	2.42	12	2.42
Dislike Very Much	16.6	3.35	28.6	5.76
Dislike Moderately	36.2	7.3	64.8	13.06
Dislike Slightly	74	14.91	138.8	27.97
Neither Like/Dislike	193.4	38.98	332.2	66.95
Like Slightly	84.6	17.05	416.8	84
Like Moderately	46	9.27	462.8	93.27
Like very much	24.6	4.96	487.4	98.23
Like Extremely	8.8	1.77	496.2	100
Rated Soy aroma	**Frequency**	**%**	**Cumulative Freq**	**Cumulative %**
Weak	112.8	23.25	112.8	23.25
Just About Right	292.2	60.22	405	83.47
Strong	80.2	16.53	485.2	100
Taste Beany Flavor	**Frequency**	**%**	**Cumulative Freq**	**Cumulative %**
Dislike Extremely	40.2	8.09	40.2	8.09
Dislike Very Much	57.4	11.55	97.6	19.64
Dislike Moderately	70.4	14.16	168	33.8
Dislike Slightly	89.6	18.03	257.6	51.83
Neither Like/Dislike	50	10.06	307.6	61.89
Like Slightly	94.2	18.95	401.8	80.85
Like Moderately	58.6	11.79	460.4	92.64
Like very much	28	5.63	488.4	98.27
Like Extremely	8.6	1.73	497	100
Sweetness	**Frequency**	**%**	**Cumulative Freq**	**Cumulative %**
Dislike Extremely	33.8	6.77	33.8	6.77
Dislike Very Much	45	9.01	78.8	15.78
Dislike Moderately	55.4	11.09	134.2	26.87
Dislike Slightly	90.8	18.18	225	45.05
Neither Like/Dislike	60.8	12.17	285.8	57.23
Like Slightly	109.4	21.91	395.2	79.13
Like Moderately	58.6	11.73	453.8	90.87
Like very much	36.8	7.37	490.6	98.24
Like Extremely	8.8	1.76	499.4	100

6.4 Frequency for Rated Sweetness, MFV, Overall Liking, Acceptability, Purchase Intent (Buy), Health Benefit Purchase Intent (Buy) of Bland Milk

Rated Sweetness	Frequency	%	Cumulative Freq	Cumulative %
Weak	177.6	36.17	177.6	36.17
Just About Right	225	45.82	402.6	82
Strong	88.4	18	491	100
Mouth Feel Viscosity	**Frequency**	**%**	**Cumulative Freq**	**Cumulative %**
Dislike Extremely	26.4	5.29	26.4	5.29
Dislike Very Much	39.6	7.93	66	13.22
Dislike Moderately	56.6	11.34	122.6	24.56
Dislike Slightly	97	19.43	219.6	43.99
Neither Like/Dislike	70.4	14.1	290	58.09
Like Slightly	100.8	20.19	390.8	78.29
Like Moderately	61.6	12.34	452.4	90.63
Like very much	34.8	6.97	487.2	97.6
Like Extremely	12	2.4	499.2	100
Overall Liking	**Frequency**	**%**	**Cumulative Freq**	**Cumulative %**
Dislike Extremely	33.4	6.7	33.4	6.7
Dislike Very Much	57.4	11.52	90.8	18.23
Dislike Moderately	68.6	13.77	159.4	32
Dislike Slightly	89.2	17.9	248.6	49.9
Neither Like/Dislike	55.2	11.08	303.8	60.98
Like Slightly	95.8	19.23	399.6	80.21
Like Moderately	61.6	12.36	461.2	92.57
Like very much	26.8	5.38	488	97.95
Like Extremely	10.2	2.05	498.2	100
Acceptability	**Frequency**	**%**	**Cumulative Freq**	**Cumulative %**
Yes	277.2	55.89	277.2	55.89
No	218.8	44.11	496	100
Purchase Intent	**Frequency**	**%**	**Cumulative Freq**	**Cumulative %**
Yes	130.8	26.25	130.8	26.25
No	367.4	73.75	498.2	100
Health Benefit Purchase Intent	**Frequency**	**%**	**Cumulative Freq**	**Cumulative %**
Yes	198	39.73	198	39.73
No	300.4	60.27	498.4	100



6.5 Principal Component 1 and 3 of Bland Soymilk Beverages



6.6 Principal Component 2 and 3 of Bland Soymilk Beverages

APPENDIX F. CHARTS AND DATA FOR CHAPTER 7

7.1 Questionnaire for Flavored Soymilk Testing

What is your gender? Male______ Female_______ Sample – A

1. How would you rate the **OVERALL APPEARANCE** of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐
1	2	3	4	5	6	7	8	9

2. How would you rate the **COLOR** of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐
1	2	3	4	5	6	7	8	9

3. How would you rate the **OVERALL FLAVOR (Taste & Aroma)** of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐
1	2	3	4	5	6	7	8	9

4. How would you rate the **ORANGE AROMA** of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐
1	2	3	4	5	6	7	8	9

5. Please rate the **ORANGE AROMA** of this product based on your preference.

Weak	**Just About Right**	**Strong**
[ ]	[ ]	[ ]

6. How would you rate the **SWEETNESS** of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐
1	2	3	4	5	6	7	8	9

7. Please rate the **SWEETNESS** of this product based on your preference.

Weak	**Just About Right**	**Strong**
[ ]	[ ]	[ ]

8. How would you rate the **MOUTHFEEL/VISCOSITY** of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐
1	2	3	4	5	6	7	8	9

9. Please rate your **THICKNESS OR VISCOSITY** of this product?

Weak	**Just About Right**	**Strong**
[ ]	[ ]	[ ]

10. Please rate your **OVERALL LIKING** of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐
1	2	3	4	5	6	7	8	9

11. Is this product **ACCEPTABLE**? Yes [ ] No [ ]

12. Would you **BUY** this product if it were commercially available? Yes [ ] No [ ]

13. Would you buy this product after been told about the **HEALTH BENEFITS** from soy protein and isoflavone in this product? Yes [ ] No [ ]

7.2 Frequency for Gender, Overall Appearance, Color, Overall Aroma of Flavored soymilk

Gender	Frequency	%	Cumulative Freq	Cumulative %
Male	96	52.75	96	52.75
Female	86	47.25	182	100

Overall Appearance	**Frequency**	**%**	**Cumulative Freq**	**Cumulative %**
Dislike Extremely	2.6	1.29	2.6	1.29
Dislike Very Much	3.4	1.69	6	2.98
Dislike Moderately	10.2	5.06	16.2	8.04
Dislike Slightly	24.2	12	40.4	20.04
Neither Like/Dislike	50.6	25.1	91	45.14
Like Slightly	41.2	20.44	132.2	65.58
Like Moderately	40	19.84	172.2	85.42
Like very much	19.6	9.72	191.8	95.14
Like Extremely	9.8	4.86	201.6	100

Color	**Frequency**	**%**	**Cumulative Freq**	**Cumulative %**
Dislike Extremely	1.4	0.69	1.4	0.69
Dislike Very Much	2.2	1.09	3.6	1.78
Dislike Moderately	8.8	4.36	12.4	6.14
Dislike Slightly	26.2	12.97	38.6	19.11
Neither Like/Dislike	47	23.27	85.6	42.38
Like Slightly	43.6	21.58	129.2	63.96
Like Moderately	37.8	18.71	167	82.67
Like very much	25.8	12.77	192.8	95.45
Like Extremely	9.2	4.55	202	100

Oflavor	**Frequency**	**%**	**Cumulative Freq**	**Cumulative %**
Dislike Extremely	12	5.98	12	5.98
Dislike Very Much	19.2	9.57	31.2	15.55
Dislike Moderately	22	10.97	53.2	26.52
Dislike Slightly	33.2	16.55	86.4	43.07
Neither Like/Dislike	15.8	7.88	102.2	50.95
Like Slightly	39.6	19.74	141.8	70.69
Like Moderately	33.8	16.85	175.6	87.54
Like very much	19.4	9.67	195	97.21
Like Extremely	5.6	2.79	200.6	100

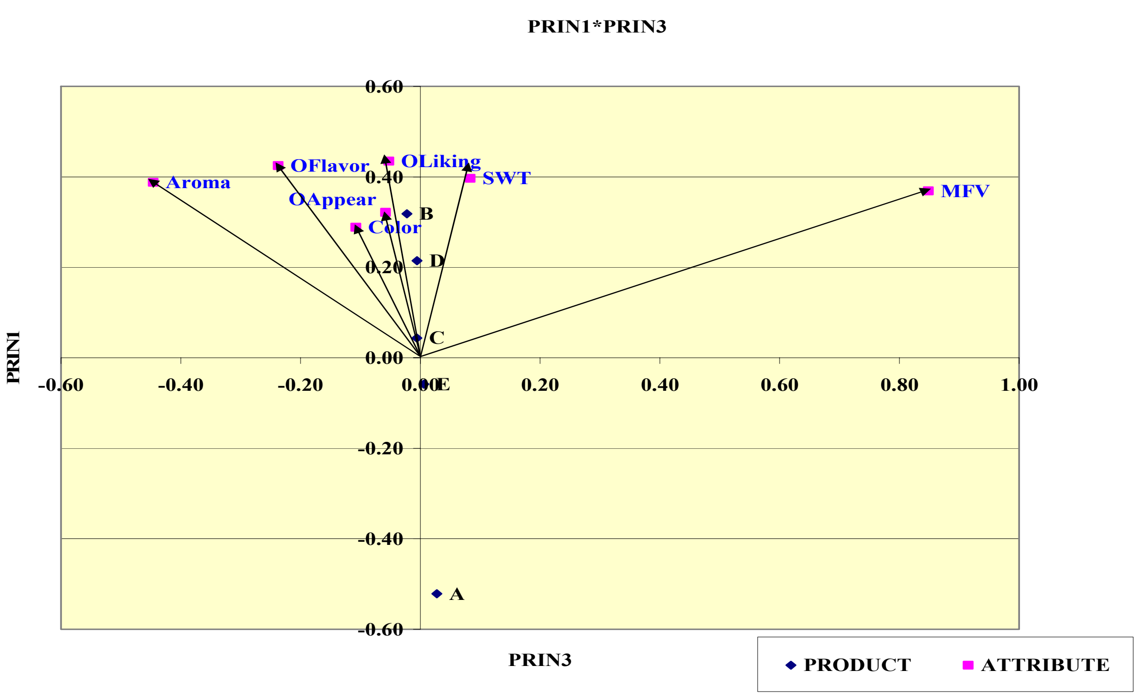
Aroma	**Frequency**	**%**	**Cumulative Freq**	**Cumulative %**
Dislike Extremely	8.6	4.3	8.6	4.3
Dislike Very Much	10	5	18.6	9.29
Dislike Moderately	14.2	7.09	32.8	16.38
Dislike Slightly	25.4	12.69	58.2	29.07
Neither Like/Dislike	30.4	15.18	88.6	44.26
Like Slightly	45	22.48	133.6	66.73
Like Moderately	31.6	15.78	165.2	82.52
Like very much	27	13.49	192.2	96
Like Extremely	8	4	200.2	100

7.3 Frequency for Rated Aroma, Sweetness, Rated Sweetness, Mouth Feel Viscosity, Thickness Viscosity, Overall Liking

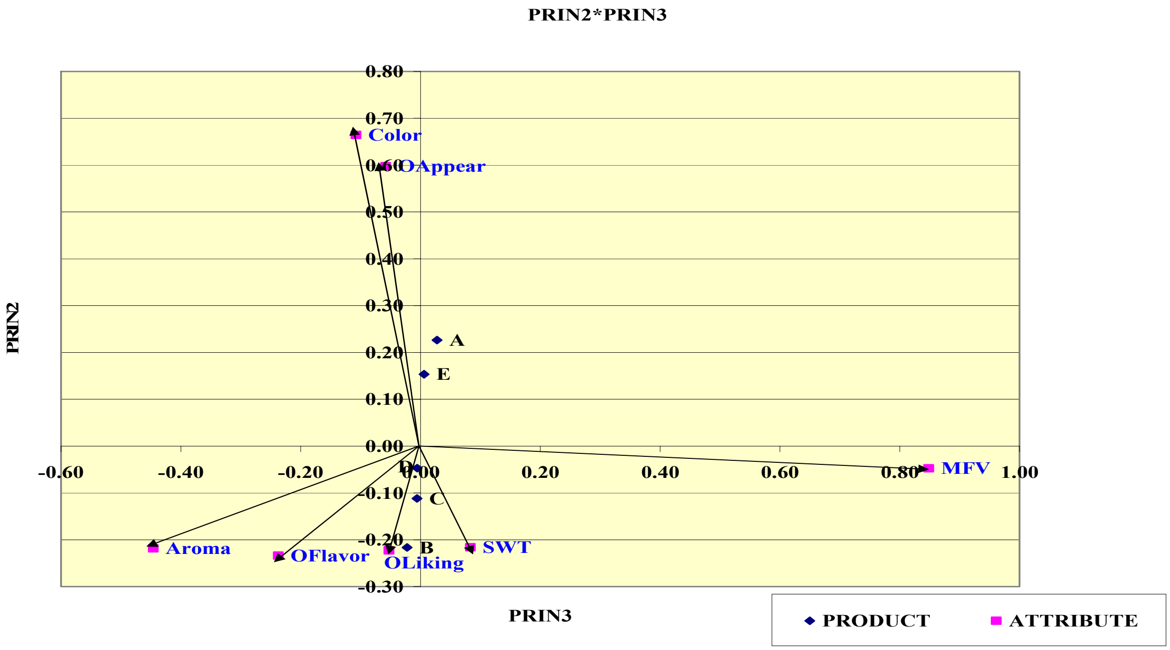
RatedA	Frequency	%	Cumulative Freq	Cumulative %
Weak	47.6	24.59	47.6	24.59
Just About Right	110	56.82	157.6	81.4
Strong	36	18.6	193.6	100
Sweetness	**Frequency**	**%**	**Cumulative Freq**	**Cumulative %**
Dislike Extremely	6.4	3.17	6.4	3.17
Dislike Very Much	11.8	5.84	18.2	9.01
Dislike Moderately	15	7.43	33.2	16.44
Dislike Slightly	32.2	15.94	65.4	32.38
Neither Like/Dislike	31.2	15.45	96.6	47.82
Like Slightly	54.4	26.93	151	74.75
Like Moderately	27	13.37	178	88.12
Like very much	19.4	9.6	197.4	97.72
Like Extremely	4.6	2.28	202	100
Rated Sweetness	**Frequency**	**%**	**Cumulative Freq**	**Cumulative %**
Weak	54	27.66	54	27.66
Just About Right	115.4	59.12	169.4	86.78
Strong	25.8	13.22	195.2	100
Mouth Feel Viscosity	**Frequency**	**%**	**Cumulative Freq**	**Cumulative %**
Dislike Extremely	5.8	2.88	5.8	2.88
Dislike Very Much	9.4	4.66	15.2	7.54
Dislike Moderately	17	8.43	32.2	15.97
Dislike Slightly	36.6	18.15	68.8	34.13
Neither Like/Dislike	36.8	18.25	105.6	52.38
Like Slightly	45	22.32	150.6	74.7
Like Moderately	30.4	15.08	181	89.78
Like very much	15.6	7.74	196.6	97.52
Like Extremely	5	2.48	201.6	100
Thickness / Viscosity	**Frequency**	**%**	**Cumulative Freq**	**Cumulative %**
Weak	48.2	24.79	48.2	24.79
Just About Right	124.4	63.99	172.6	88.79
Strong	21.8	11.21	194.4	100
Overall liking	**Frequency**	**%**	**Cumulative Freq**	**Cumulative %**
Dislike Extremely	9.8	4.89	9.8	4.89
Dislike Very Much	18.4	9.17	28.2	14.06
Dislike Moderately	26.4	13.16	54.6	27.22
Dislike Slightly	27.6	13.76	82.2	40.98
Neither Like/Dislike	20.6	10.27	102.8	51.25
Like Slightly	41.6	20.74	144.4	71.98
Like Moderately	34.6	17.25	179	89.23
Like very much	16.2	8.08	195.2	97.31
Like Extremely	5.4	2.69	200.6	100

7.4 Frequency for Acceptance, Purchase Intent, and Health Benefit Purchase Intent

Gender	Frequency	%	Cumulative Freq	Cumulative %
Yes	96	52.75	96	52.75
No	86	47.25	182	100
Acceptance	**Frequency**	**%**	**Cumulative Freq**	**Cumulative %**
Yes	127.2	63.35	127.2	63.35
No	73.6	36.65	200.8	100
Purchase Intent	**Frequency**	**%**	**Cumulative Freq**	**Cumulative %**
Yes	65.8	32.8	65.8	32.8
No	134.8	67.2	200.6	100
Health Benefit P I	**Frequency**	**%**	**Cumulative Freq**	**Cumulative %**
Yes	94.6	46.88	94.6	46.88
No	107.2	53.12	201.8	100



7.5 Principal Component 1 and 3 of Flavored Soymilk Beverages



7.6 Principal Component 2 and 3 of Flavored Soymilk Beverages

VITA

The author was born in Freetown, Sierra Leone, on February 27, 1968. Shortly after her birth, her parents who were of rural stock returned to the country side where they worked for the University of Sierra Leone at Njala. She went through primary, secondary school and university (undergraduate) in this rural setting, and graduated from the department of Home Economics majoring in foods and nutrition in 1989. Upon leaving college in 1989 she taught Foods and Nutrition and Home Economics for one year to both the junior high school classes and to the graduating class at the St. Andrews Secondary School, Bo. After that she took up appointment as Research Assistant in the Nutrition Unit at the Institute of Agricultural research (IAR) in Sierra Leone. The Institute has mandate to carry out research on all food crops except rice, in Sierra Leone. As a research assistant attached to the Nutrition Unit of the Institute, she took part in research and extensive extension services for the adoption of the Institute's improved crop varieties by farmers and rural women in the country. Under her supervision were 13 nutrition instructors in six operational zones of the Institute in the country.

Soon after joining the IAR and with her growing expertise with the Institute, she was nominated for a short-term training program sponsored by the International Institute of Tropical Agriculture (IITA) at Ibadan, Nigeria, in 1991, on Root Crops Research and Technology Transfer. The following year, she completed another training course in Post-Harvest Research Technology at Ibadan, Nigeria, conducted by the IITA. While working at the IAR she contributed to two reports presented at the Institute's fourth and fifth Annual Work Program Conference at Njala.

As training facilities in Nutrition, Food Science and related areas were not available beyond the baccalaureate level in Sierra Leone, she sought out for scholarships and appropriate graduate programs abroad. In 1994, she was awarded the Netherlands Fellowship award for post-graduate studies in Food Science and Nutrition in Wageningen - The Netherlands. That same year, she was also nominated for the Fulbright Scholarship Award to pursue graduate studies (M.S) in Food Science at Louisiana State University. She joined the Department of Food Science at Louisiana State University in fall 1994 where she completed her master's in 1997. Due to a gruesome war in her country that forbade her to return home, she went on to complete another master's in public administration with a concentration in healthcare administration and management in 1999. With lingering hope of her country ever returning to normalcy and civilian rule, she returned to school to her doctoral studies under the supervision of Dr. Witoon Prinyawiwatkul and is a candidate for a Doctor of Philosophy degree in food science. Her degree will be conferred in December at the Fall 2005, commencement.

Through out her life, her parents have been the most influential in guiding her both academically and socially. The influence of her academic mentors who shared their wisdom and knowledge provided her, the much needed determination to seek for new knowledge and strive for her dreams. Her work would not be completed without the immense support of her daughter, siblings, host parents, friends and other family members all of whom have in ways contributed to her success thus far. Even in her absence and in the wake of the appalling and devastating situations in her home country, the voracious support and undying love of her family, friends and professors is the spiritual, physical strength and main stay of her perseverance for her goals. It is hoped that amongst all things, she will be able to make a contribution to the possible solutions and complexities of food and nutritional problems in her country and around the world.