DEVELOPMENT OF CHOCOLATE-FLAVORED, PEANUT-SOY BEVERAGE

by

RASHMI PRAKASH DESHPANDE

(Under the Direction of Manjeet Singh Chinnan)

ABSTRACT

A novel formulation and pilot-plant scale processing protocol was developed for a protein-based, nutritious beverage. Roasted peanut and chocolate flavors were utilized to enhance acceptability and soy flour or soy protein isolate to improve nutritional profile. Extreme vertices, constrained-mixture design for peanut ($X_1$:30.56%-58.70%), soy ($X_2$:28.26%-43.52%), and chocolate syrup ($X_3$:13.04%-25.93%) yielded 28 formulations for sensory evaluation. Commercial chocolate milk was used as a control. Optimization of sensory data was done using response surface methodology. Since control ratings were 6-7, the regions of maximum consumer acceptability for each sensory attribute were ratings $\geq$ 5.0. Optimum formulations for soy flour were all combinations of 34.1%-45.5% $X_1$, 31.2%-42.9% $X_2$, and 22.4%-24.1% $X_3$; and for soy protein isolate 35.8%-47.6% $X_1$, 31.2%-43.5% $X_2$, and 18.3%-23.6% $X_3$. A formulation with 43.9% peanut, 36.3% soy protein isolate, and 19.8% chocolate syrup had the highest consumer acceptability. Soy protein isolate performed better than soy flour and gave beverage characteristics closer to that of control.

INDEX WORDS: Chocolate-flavored, peanut-soy beverage, Soy flour, Soy protein isolate, Extreme vertices constrained mixture design, Sensory evaluation, Response surface methodology (RSM), Consumer acceptability

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# DEDICATION

To

Aai, Baba and Kanchu

Their encouragement at every tiny little moment led to this life-long achievement. Their love and support is my biggest asset and I am fortunate to have their blessings with me, always!

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# CHAPTER 1

# INTRODUCTION

## Beverage business and current market trends

Beverage business is taking a new shape and has tremendous potential for future developments. Beverage industry is focusing on consumer desires. Both consumers and industry are looking for alternative product options suitable for a changing marketplace. Recently, beverage products have created a new category, a whole new business through innovative new products. In addition to the traditional kinds like alcoholic beverages, carbonated soft drinks, ready-to-drink fruit or dairy based beverages, and smoothies, a wave of innovative terminologies such as flavored milks, energy beverages (*Xtreme Exquisite*™), liquid meal-replacements (*Nouriche*™), weight-directed products (*Slim-Fast*™), ready-to-drink meals (*Golean*™, a soy drink segment), and functional beverages are becoming popular in the beverage market. Almond, rice, and oat milks are helping to fill the milk and cereal void (Sloan 2003c). All these beverage categories are available in various distribution locations such as supermarkets, convenience stores, restaurants, wholesale clubs, retail stores, and fountain or vending machines. In short, there is a scope and market for the beverage products in a variety of ways.

A synopsis of the new beverage product categories and major areas of interest are presented in Table 1.1. It can be observed that there has been substantial decrease in hot beverages, carbonated soft drinks, RTD (ready-to-drink) iced tea/coffee, and beer/cider category, whereas the energy/sports drinks category shows significant growth. As per the survey done by BevNet.com, 2003 was an interesting year for a soft drink industry, since the sales of carbonated

soft drinks did not show much growth on the other hand the energy drinks sales boomed along with the continued emergence of many health-directed products (Anonymous 2004c). From the top 10 trends to be watched and worked on in year 2003 (Sloan 2003a), Figure 1.1, ready-to-eat, packaged for on-the-go, high protein, and anything that could replace a meal are some of the trends consumers are willing to try over the next few years.

As a part of convenience and single serving choices, "meals in motion" are becoming popular. The changing life-styles and eating preferences have inspired a new generation of portable products. One in ten meals was eaten "on-the-go" in 2002, and the liquid and bar meal-replacement products represented the ultimate in contemporary one-dish meals (Sloan 2003b). A new generation of great-tasting products (Sloan 2003b), such as Snapple's *Snapple-A-Day*™ and Yoplait's *Nouriche*™, has helped the liquid meal replacements skyrocket to a $2.5-billion market segment, up 11% compared to 2002. The "*Meal in a Bottle*", a combination of whey and soy protein with more than 20 vitamins and minerals, is the latest addition to the explosive soy and dairy based market segment (Sloan 2003b).

Next to the single-serve, health has been the most dramatic factor influencing beverage sales in the past few years with PepsiCo and Nestle focusing on the health for their future acquisitions (Sloan 2003b). The desire for good health and concern over carbonated soft drink consumption has driven consumers to juice and juice drinks (Sloan 2003b). While purchasing a food item for the first time, price and brand name are deciding factors but the impact of health claims, types of preservatives/additives, and organic claims have increased. In 2002, 72% shoppers which was up 6% over the previous year, said that they almost always/sometimes look at the health claims (Sloan 2003a). Annual soymilk sales have grown to $550 million with 8% of the households using it on a regular basis (Sloan 2003a). Healthier product alternatives

including juice/soy combinations, juice/fruit/dairy smoothies, fortified juices/drinks, single-serve lemonade, and organic drinks are among those enjoying the strongest growth (Sloan 2003b). Consumers are fascinated by functional beverages, showing increase in the sales from $10.35 billion in 2002 to projected $15.9 by 2010 (NBJ 2003). The growth of "Functional/Nutraceutical/Wellness Foods and Beverages" has been attributed to several factors such as consumers interest in maintaining better health; rise in available information regarding the link between diet and health; changes in food laws that have affected label and product claims; and an increasing sector of the public aging quickly and purchasing functional food products (Ohr and others 2003).

Overall, consumers are looking to lead more healthy lives, thus consuming more nutritious foods**.** Taking advantage of this fact, companies also are developing new functional beverage products. For example, Minute Maid Co. recently introduced *Minute Maid® Premium Heart Wise*™ Orange Juice, a cholesterol-reducing orange juice that contains 1 g of plant sterols per serving. The functional beverage market is expected to grow at an annual growth rate of 5.7% (Ohr and others 2003). "Better for you" products can help boost up the beverage sales and have great applications in the beverage industry.

**Why a new beverage product?**

One of the ways to increase chances of obtaining a successful product is to gather market information so that new market niches can be located for new product ideas prior to product development and to follow a consumer driven food product development process (Knox and Mitchell 2003). Looking at current market scenario and health driven food choices, the beverage category seems to receive consumer attention the most. Hence, a novel protein-based nutritious beverage from peanut and soy was developed. Both peanut and soy are two important protein-

rich oilseed sources which can complement each other in order to enhance the nutritional characteristics of the final product. Both of them have functional benefits which can result into an optimum blend in combination with a popular chocolate flavor.

New food products may be innovative, adaptive, imitative, line extensions, or new forms. New products and line extensions, typically representing 10-15% of category volume each year, are going to be a very meaningful percentage of total category volume over next 3-to 5-year period (Lord 2000). Brand and line extensions are still a good way to leverage consumer awareness and reduce risk and entry cost, but innovative new products potentially reap more rewards (Lord 2000). A new product is a product not previously marketed or produced by the organization for which it is developed or made available (Segall 2000). Also, the new product which is novel, unique, and distinctly untried, unfamiliar, or even previously nonexistent is called an invention, especially if it is innovation in technology, and is eligible for protection by patent (Segall 2000). As per the definition a chocolate-flavored, peanut-soy beverage being developed in this study was termed as the invention.

In a completely new product category, researcher develops a new process and a prototype product by optimum methods to test the acceptance. The prototype development and consumer acceptance study are therefore, essential parts of a new product development process. The consumer input in the form of sensory analysis of the new product is crucial in the development stages. Although commercially processed foods must attract consumer by appearance of the package, repeat purchases will depend on how pleasing a sensory experience is perceived when the product is eaten (Toledo and Brody 2000). Hence, analyzing consumer response in terms of sensory acceptability of the new product was considered important during this study.

**Role of consumers in the new product development**

The basic reason for any research and development is to increase current profits and ensure future sales. Product development has always been at the heart of food industry and rapid technological changes, accompanied by a steady increase in the standard of living, resulted in even greater opportunities for product development (Earle 1997). Globally a new product is launched every twenty minutes and speed is the fuel that drives the process (Robinson 2000). The process of new product development is at the best a delicate and tortuous path (Figure 1.2)

The consumer needs/desires are major driving force of the new food product development, and perceptions about the food form the basis of those perceived needs (Bursey 1983). According to Brody and others (2000), today's food consumers have adopted different patterns of food consumption suitable to their more mobile and more diversified lifestyles and hence the task of food system is changing from "bringing the consumer to the food" to "bringing the food to the consumer". Any single product on the supermarket shelf must compete with some 10000 others to get into a shopper's grocery cart and hence constant market appraisal is necessary (Desrosier and Desrosier 1971). A sequence of activities that leads to the introduction of a successful new product into today's highly competitive marketplace has its beginnings and foundation in the extensive and ongoing market research which elicits and defines the changing consumer desires (Bursey 1983). The consumers want access to novel and interesting foods that are fresh, convenient, and tasty. The changing scenario poses a challenge for the industry as well as an opportunity for the new product development.

The new food product development should be a systematic effort founded in a strategic plan to please-and even delight-consumers (Robinson 2000). Earlier, consumers wanted value for money, variety, convenience and foods that were attractive to their senses but now-a-days,

they are also aware of nutrition, food safety, as well as the social and environmental effects of food production (Earle 1997). The developers of new food products, or of more convenient forms of processed foods, should consider retention of nutritional quality as a primary factor in their measure of success (Anonymous 1973). The nutritional quality should be ranked alongside safety, acceptability and convenience in this modern, mechanized, and computer-run society, where most of the food eaten is processed and mass-distributed. The relation of diet to health is but one of the many factors that influence food purchase decisions and, thus, the stimulus for developing new food products (Bursey 1983).

**Development of a chocolate-flavored, peanut-soy beverage**

New food product innovation is necessary for survival in today's competitive global market and the innovation is important because unique product that fills the need/desire will succeed in the current market which is becoming rapidly overcrowded (Knox and Mitchell 2003). However, it is important and absolutely necessary to integrate technology, consumer understanding and marketing in order to maximize a product's potential (Robinson 2000).

The main objective of this study was to develop a new nutritional beverage which was accomplished by utilizing peanut and soy proteins. A market survey of flavored milk-type products, the literature search on similar products, beverage ingredients, and various processing methods, and the pilot-plant scale beverage preparation trials were crucial in order to get the possible new alternatives. Once the list of ingredients, ingredient levels, and the definite pilot-plant scale processing protocol were obtained, a three component mixture design approach was used to get the number of possible formulations required for a consumer sensory evaluation. Using the mixture design, a set of formulations were prepared and subjected to the sensory evaluation. The consumers responded to various sensory attributes using a 9-point hedonic

scale. The hedonic ratings were analyzed in comparison with a commercial chocolate milk (control) to obtain the most acceptable formulation. Also, the optimum formulation of the new product developed was obtained using a response surface methodology (RSM) applied to the consumer sensory data. Two optimum formulations, one with soy flour and another using soy protein isolate as a source of soy protein in the combination with peanut flour and chocolate syrup were achieved. These formulations had the maximum consumer acceptability, and physical and sensory properties close to the commercial chocolate milk. In this way, a new nutritionally enhanced product having the physical and sensory characteristics similar to that of the commercial chocolate milk was developed.

Table 1.1: Beverage product introductions in year 2000, 2001 and 2002
(Roberts 2003a)

| **Beverage Category** | **2002** | **2001** | **2000** |
|---|---|---|---|
| Hot beverages | 324 | 479 | 470 |
| RTD juices/juice drinks | 211 | 265 | 217 |
| Concentrates/mixes | 146 | 122 | 154 |
| Energy/sports drinks | 121 | 104 | 86 |
| Carbonated soft drinks | 59 | 82 | 68 |
| RTD iced tea/coffee | 61 | 43 | 102 |
| Beer/cider | 73 | 25 | 93 |
| Water | 76 | 50 | 65 |
| Flavored alcoholic beverages | 19 | 29 | 16 |
| Total | 1090 | 1199 | 1426 |

Table 1.2: What consumers say about soy foods? The United Soybean Board's 2003-04 annual study (Ohr 2003)

| Type of soy food | Soy products used regularly | Soy products tried at least once during the year |
|---|---|---|
| Soymilk | 17% | 39% |
| Tofu | 12% | 48% |
| Soy veggie burgers | 12% | 44% |
| Soy protein bars | 5% | 22% |
| Soy nuts | 4% | 26% |

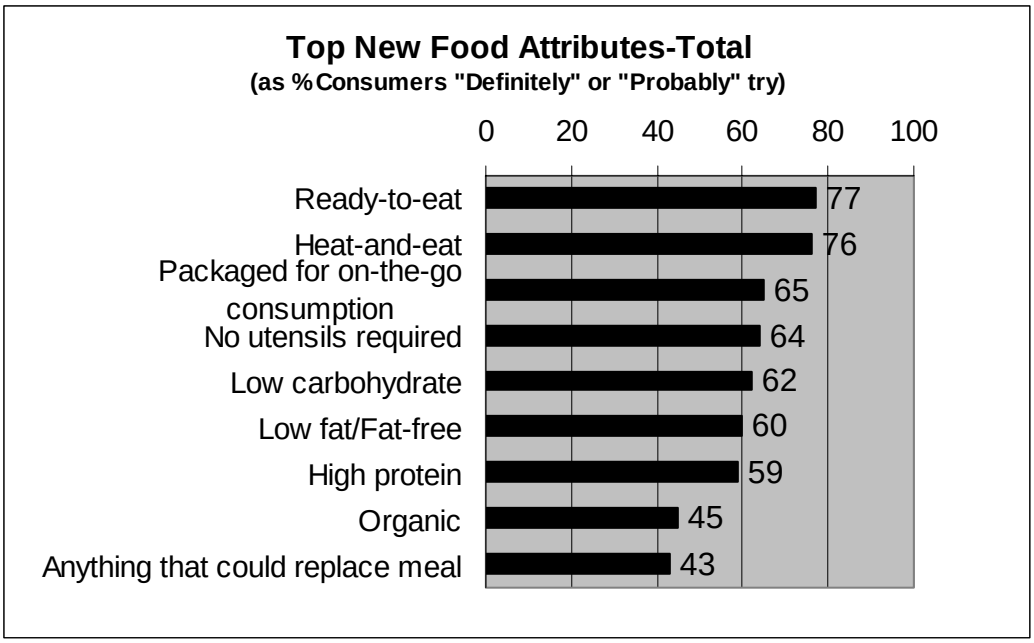


Figure 1.1: Consumers are open to variety of new food concepts, as long as they provide convenience first (Sloan 2003a)

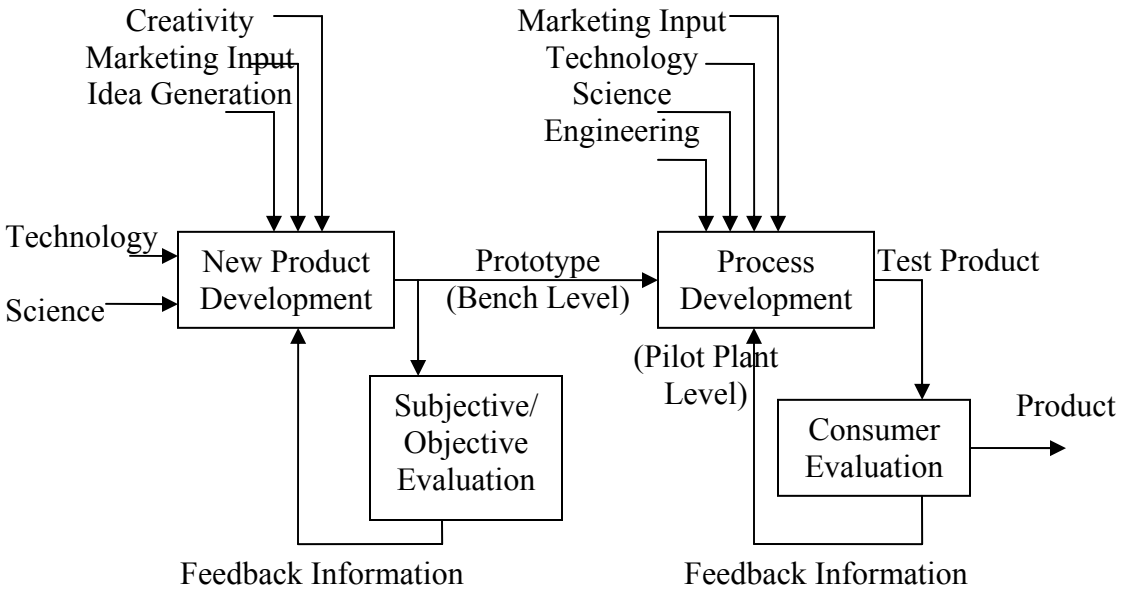


Figure 1.2: Interaction of key elements in new product development (Pyne 2000)

# CHAPTER 2

# LITERATURE REVIEW

## Sources of protein for a nutritional beverage

Proteins are added as ingredients to foods to achieve functional and nutritional goals (Giese 1994). Proteins are fundamental, integral, and essential food components. Proteins serve as a source of energy and amino acids essential for growth and maintenance. Proteins provide unique properties to foods such as emulsification, water and/or fat binding, foam or gel formation and alteration of flavor, texture and appearance.

There are two basic types: animal protein and plant protein. Milk is an important animal protein which has casein and whey proteins as two principle proteins. Whole milk is about 87.5% water, 4.5% lactose, 3.5% fat, 1.0% ash and the remainder -3.5%-is protein (Giese 1994). Plant seeds are the most utilized components of plants as possible source of protein. Other plant components being explored to some extent include leaves, stems and roots. Cereals especially wheat and corn are one of the important category of plant proteins having commercially available products. Oilseeds such as soybean, cottonseed, and peanut have also been studied to obtain protein ingredients. Soybeans are available in various processed forms like soy flour, grits, concentrates, isolates, and textured products (Giese 1994). A variety of textured products are made from soy protein by processing flours, concentrates and isolates. Hydrolyzed soy proteins are used in dairy products, confectionery, baked goods, and beverages (Roberts 2003b).

Milk, whey, and soy are main proteins having applications in infant formulas, protein enriched foods, and beverages. Nutrition, solubility, viscosity, and emulsification are some of the important functions of food proteins which play a significant role in beverage products. Protein additives are useful in the beverage applications (nutritional or sports drink) where the main functionality is imparting flavor and providing soluble proteins. Whey and soy proteins can be used in various fruit-based beverages that are high in protein for example, children's health drinks, weight management products or fruit-based sports drink, and products with pH ranging from 3 to 4. Once a desirable function is known such as enhancing nutritive value, emulsification, or modifying flavor and appearance, suitable form of protein can be selected to impart those characteristics to the food product.

**Peanut** (*Arachis hypogeae* L.) **as a source of protein**

Worldwide, about 67% of the peanut crop is processed for peanut oil, 20% is used in confectionary, and remainder for other peanut-based products. Georgia produces almost half of the total U.S. peanut crop. In the United States, more than 50% of the crop goes to peanut butter production (Anonymous 2004d). Although peanuts come in many varieties, there are four basic market types: Runner, Virginia, Spanish and Valencia. Within four basic types of peanuts, there are several "varieties" for seed and production purposes. Each variety contains distinct characteristics which allow a producer to select the peanut that is best suited for its region and market (Anonymous 2004a).

Among the major oil seed crops, peanut has specific advantages as it can be used in many food forms. From consumption point of view, pleasant aroma, nutty flavor and desirable textured raw and roasted nuts are the unique features of peanuts that place them above all other edible grain legume (Singh and Singh 1991; Table 2.1).

Other Peanut Products:

- Specially processed, defatted peanuts are available as roasted snack peanuts; they are ground into flour, which can be used to make high protein drinks and snacks (Anonymous 2004b)
- Peanut in the form of flour, protein isolates, and meal in a mixed product are desirable from a sensory quality point of view (Singh and Singh 1991)
- Peanuts are utilized to make imitation milk, cheese and ice cream.
- A chocolate-flavored shake-type beverage containing 84% whey and 8% peanuts has been developed by soaking peanuts in sodium bicarbonate solution overnight to develop desirable flavor before being incorporated into a whey slurry (Nolan 1983)
- Peanut meal (made from the by-product of peanuts pressed for oil) is an important high protein animal feed (Anonymous 2004b)

In recent years, several cereal and legume-based foods using peanuts as the protein supplements have been developed to alleviate a protein calories-malnutrition problem (Singh and Singh 1991). Millions of people in the developing countries like Asia and Africa depend on vegetable products for protein source. About 80% of the proteins consumed by humans are supplied by plants especially cereals and legumes taking larger share as sources of dietary proteins. Dietary deficiencies of protein and calories particularly among preschool children and lactating women in such countries is one of the essential factors causing food scientists and nutritionists to think in the direction of development of nutritionally balanced protein based foods. Oilseeds and grain legumes are principle raw materials utilized to manufacture and market high protein foods at reasonably low prices. Defatted and full fat soy flour, non-fat dry milk, dry whey, dry butter milk, processed corn germ, wheat concentrates, several peanut and

soy fortified food blends have been extensively used as the sources of concentrated proteins for various cereal-based fortified formulations.

The research shows that regular peanut consumption helps to reduce the risk of cardiovascular disease without weight gain (Alper and Mattes 2003; Kirkmeyer and Mattes 2000). Peanuts also contain powerful plant chemicals such as phytosterols which can inhibit cancer growth. Rick Mattes from Purdue University in Indiana, USA suggests three mechanisms of lowering body weight due to frequent consumption of peanuts (Mattes 2004). Firstly, peanuts have high satiety value which means that eating peanuts satisfies hunger and gives the feeling of fullness. Secondly, peanuts have raising metabolic rates or they burn more energy. And lastly, peanuts have low absorption rates or tend to pass through our digestive system. Peanuts are a rich source of monosaturated fatty acids, magnesium and folate, vitamin E, copper, arginine, and fiber, all of which have cardiovascular disease risk reducing properties.

The nutritive value of peanut protein is a function of its protein content (Peanuts = 15.4-30.2%, peanut flour = 47.0-55.0%, and peanut protein concentrates = 70.2%), amino acid composition (Table 2.2), and protein digestibility. Although, the peanut protein is deficient with respect to certain essential amino acids, for example, lysine, tryptophan, threonine and sulphur containing amino acids, its true digestibility is comparable with that of the animal protein (Singh and Singh 1991). During the past decade, research directions concerning the evaluation of plant proteins as human foods have considerably changed and emphasis is being placed on designing the protein blends of cereals and legumes to correct imbalances between amino acids from the nutritional point of view (Singh and Singh 1991). Keeping this trend and the essential amino acid profile of peanut protein in mind, other oilseed rich in protein- soybean, either in the form of soy flour or soy protein isolate- was thought of as a supplementary source of protein**.**

**Soybean as a source of protein**

Soybeans are 30% carbohydrate, 18% oil, 14% moisture, and 38% protein. Soybean is the only legume that contains nine essential amino acids at the levels that meet the human requirements. Thus, soy proteins are categorized as a source of high-quality protein, making them equivalent to meat, milk, fish, and eggs as a source of important nutrients (Tockman 2002). Soy foods provide excellent nutrition, they are low in saturated fat, are lactose free and the quality of soy protein is equal to the quality of animal protein (Schaafsma 2000; Sarwar and others 1985; Sarwar 1997; and U.S.D.A. 2000; Riaz 1999). Soybean is a very rich source of essential nutrients suitable for all ages from infants to the elderly that provides an alternative source of easily digestible protein for people who are allergic to the protein in cow's milk (Riaz 1999). Although relatively low in methionine, it is a good source of lysine (Riaz 1999). Various soy ingredients have different nutritional and functional properties which enable developers to give consumers healthful, high-protein snacks, while allowing the processor to optimize nutrition, functionality and cost.

Soy is a source of high quality protein that helps one eat less often thereby decreasing hunger and maintaining muscle mass during weight loss (Anonymous 2003). One emerging trend is the design of cereal and other food products formulated with soy protein because of its proven health benefits; women in particular represent new markets for food manufacturers developing products to meet specific nutritional needs (Tockman 2002). Whether due to its health benefits or its ability to solve a variety of formulation challenges, soy protein can be found in foods ranging from ice cream to burgers, from milk to nut products, and tofu to a variety of meat alternatives (Roberts 2003b). Soy protein can be incorporated in food in many different forms (Anonymous 2003) such as soy flour, relecithinated soy flour, textured soy flour/

concentrate, enzyme active soy flour, soy grits, soy protein concentrate, soy isoflavones, and isolated soy protein.

*Soy Flour*: The full-fat flakes or defatted soy flakes are ground into soy flour. The heat treatment, processing method, particle size and amount of fat govern different types of soy flours. Typical soy flours available are 20, 70 and 90 PDI. The PDI is the Protein Dispersibility Index and indicates the amount of heat treatment received by the flour. Soy flour is 40-50% protein. Tables 2.3 and 2.4 show mean proximate analyses and mean concentrations of amino acids in soy flours, respectively.

*Isolated Soy Protein*: Isolated soy protein (ISP) or soy protein isolate (SPI) has the most of the non-protein components removed from defatted flake. ISP is the most concentrated form of soy protein and is about 90% protein. Isolates are highly dispersible, contain all amino acids, and usually sold as fine powder. Isolates can be lecithinated to improve dispersibility or extruded to yield a wet fibrous protein. New processing methods have improved the flavor of the isolates making it easier to choose an isolate with reduced beany flavor. Processing method affects the functionality, so different isolates have various viscosities, emulsification and whipping properties.

The isolated soy proteins (minimum 90% protein content) are virtually pure, bland-flavored and the most functional of the soy proteins having excellent nutritional qualities. Some can emulsify fat and bind water and are designed to function in a given system in exactly the same way as animal proteins. Some isolates can be used to provide an elastic gel texture, imparting characteristic mouthfeel, while others control viscosity in drinks, making them creamier or full bodied. Various commercial products now take advantage of these characteristics offered by soy proteins (Riaz 1999). Some useful characteristics of soy protein

concentrates and isolates as ideal source of highly digestible protein in a beverage– an easy way for consumers to add soy protein into their diets (Riaz 1999) are:

- Soy protein is low in viscosity
- Soy protein helps achieve desired mouthfeel
- Viscosity contributed by isolates makes it ideal for other nutritious liquid products, such as infant formulas, creamers, milk replacers, and spray-dried products
- Soy products add fiber, mineral, and other nutrients to the formulation
- Water-washed special proteins are available which deliver significant amount of isoflavones and fiber
- Soy isolates are blandest traditionally

Soy protein isolates are highly digestible source of amino acids which are regarded as the protein building blocks needed for proper human growth and maintenance (Roberts 2003b).

Some of the reasons for the use of soy proteins include their relatively low cost and easy availability compared to other competing food ingredients and wide range of functional properties that help to stabilize food systems as well as provide sensory properties such as texture that consumer demands (Myers and others 2003a). Studies have undertaken to develop soymilk and soymilk powder from defatted soymeal with protein content as high as factors such as stability, whiteness and consumer acceptability would allow (Ang and others 1985). Studies have been done to observe various functional properties of the soy protein fractions and simplified ways to obtain them (Myers and others 2003a, 2003b). Numerous isolated soy proteins with a variety of viscosity profiles provide varying degrees of consistency in the finished beverages, for example, a soy shake possessing a smoothie-like consistency demands a high viscosity protein however a high-protein shake with a milky consistency requires a low-

viscosity product, and in the juice based beverage applications, special stabilization is needed; pectin often serving as a stabilizer (Roberts 2003a). As an added benefit, soy protein is complementary to many other essential ingredients, such as calcium, fiber, and probiotics. Different soy proteins have different functionalities, for example, isolated soy protein and soy protein systems can be added to provide a silky mouthfeel and can be used in traditional milk and egg formulas to improve the overall costs of producing a finished product (Tockman 2002).

The compositions of essential amino acids for infant/adult requirements, and of whole eggs, cow's milk, human milk and soy products from defatted meal are shown in Table 2.5. From such compositions it will be seen that the quality of soy protein compares favorably with that of the foods listed. A considerable amount of research has been conducted to explore the health benefits of soy protein (Messina 2003; Ohr 2003; Roberts 2003b, and Riaz 1999). Based on past and ongoing global clinical studies (Tockman 2002), soy protein ingredients may reduce cholesterol, reduce blood pressure, improve bone health, protect against heart disease, improve athletic performance, help with weight management, and ease menopausal symptoms.

Consumer enthusiasm regarding soy protein-based foods and beverages is growing, and retailers are responding by offering more products. In October 1999, the United States Food and Drug Administration (FDA) issued a heart health claim stating that consuming a minimum of 25 g of soy protein per day as a part of a low-fat, low-cholesterol diet may reduce the risk of coronary heart disease. In addition to qualifying as low-fat and low-cholesterol, soy-based foods bearing this heart health claim must provide at least 6.25 g of soy protein per serving (Tockman 2002). The health claim is based on clinical trials showing that consumption of soy protein can lower total and low-density-lipoprotein (LDL) cholesterol levels (Ohr 2003). Nearly 1000 new soy products have been introduced since the FDA approved the heart health claim in 1999. The

evidence of mainstream consumer interest in soy foods is obvious in the most recent study published by the United Soybean Board, "Consumer Attitudes About Nutrition: National Report 2001-2002." The percentage of Americans who consume soy foods weekly increased from 24% in 1999 to 27% in 2001 (Tockman 2002). A survey in 2003 reported heat 28% of Americans use soy products at least once a week (Messina 2003). Additional research and clinical trials are pointing to other potential health benefits of soy protein and soy isoflavones: Bone health, prostate cancer, and menopause are three main areas where consumers may soon be learning more about soy's benefits (Ohr 2003).

Since soybeans have such health benefits, it can be considered as a functional food. Functional food is a product that provides health benefits beyond the traditional nutrients it contains or a food containing significant levels of biologically active components that impart health benefits beyond basic nutrition (Riaz 1999). The interest in health has led to the development of so-called New Age beverage market in the United States, which is estimated to be worth about $1 billion a year at wholesale prices (Hilliam 1993). The beverage industry is the biggest market producing functional foods (Ohr 1997).

Soy beverages look like milk, have good mouthfeel, and clean aftertaste; however, heat and processing may change the flavor profile of the beverage (Riaz 1999). As these soy based foods are consumed in the national diets they are suitable vehicles for fortification to meet known deficiencies in the essential nutrients and such fortifications would serve to provide a complete nutritional supplement in the form of a beverage for children of primary school age and for specific groups of people (Ang and others 1985). Besides providing an alternative to the traditional dairy products, soy protein is being incorporated into many of today's nutritious meal-

replacement beverages, offering 'on-the-go' consumers a way to skip a sit-down meal for the convenience that a liquid option offers (Riaz 1999).

**Key ingredients of the nutritional beverage**

Due to various advantages of incorporating soy proteins in the beverage products and considering their functional and nutritional properties, two sources: defatted soy flour and soy protein isolate were thought to be suitable protein supplements for a peanut-based beverage. Soy protein was considered to be the best in accomplishing two major objectives- supplement the amino acid profile of peanut protein in order to get a nutritious drink and formulate a novel functional drink suitable to the demand of current trend of health aware consumers.

However, soy proteins have few drawbacks; they can not be used in a clear liquid-based beverage because of the presence of suspended long-chain protein molecules. Also some soy protein ingredients impart undesirable beany flavor. Flavor is a major factor that limits the use of many vegetable proteins in foods (Rackis and others 1979). Combining roasted, partially defatted peanut flour and soy protein (defatted soy flour/soy protein isolate) was thought to be a potential approach to overcome the flavor problem to some extent. Also, formulation of peanut/ milk protein blends can be a technique for expanding the use of peanut proteins and these blended food ingredients could be of superior nutritional and sensory quality to either protein individually (Schmidt 1978). Beverage and yoghurt systems prepared from peanut and soybean protein/ milk blends are generally more acceptable than those prepared from soybean or peanut milk (Schmidt and Bates 1976; Schmidt and others 1977). Cereal and snack foods fortified with 17% defatted peanut flour appear to have no adverse effect on sensory acceptability; a peanut-fortified corn-base cereal received a hedonic score of 6.1, which compared favorably with that for a commercial breakfast corn product; meat patties containing peanut flour were rated equal to

or better than similar patties containing equivalent amounts of soy flour (Rackis and others 1979). However, the use of peanut-soy combination in the beverage product is a relatively new concept. Recognition of acceptable sensory qualities involves taste, odor, color, texture, and other factors; the successful introduction of a new protein food becomes especially difficult if its sensory qualities are different (Rackis and others 1979). As consumers have become better informed about nutrition, food manufacturers face the challenge of providing nutritious food, while at the same time, ensuring that the product has an appealing taste, texture, and appearance (Riaz 1999). Acceptance may require a long period of consumption so that one can acquire a taste for the new protein food otherwise, agents that impart acceptable sensory properties must be added to mask objectionable flavors of the new protein food (Rackis and others 1979). As stated by Daniele Karleskind there are several issues with proteins and flavors- one is the taste of the protein itself and the other is how the protein interacts with the flavor (Brandt 2002). The effect of flavor binding on perceived flavor intensity depends on the flavor molecule and the type, amount and composition of the protein, as well as the presence of ingredients such as lipids or polysaccharides (Brandt 2002).

Chocolate has proven to be a popular flavor for soy-based beverages, due to its ability to mask protein flavor and provide a smooth flavor profile. Formulators have more difficulty getting subtle flavors such as vanilla and fruit flavors to come through (Brandt 2002). Addition of chocolate, almond, and vanilla flavorings diminished the beany flavor of soymilk, but the chocolate flavoring exhibited the best effect in enhancing overall sensory quality (Wang and others 2001). Hence in case of peanut-soy protein-based beverage the most accepted beverage flavor was thought to be introduced using cocoa powder and artificial chocolate flavor. The idea

was to use roasted peanut, soy and chocolate flavors at the optimum levels resulting in the most acceptable flavor of the new beverage.

The important factor while using cocoa powder as an ingredient in liquid products is the stability. Preventing physical instability of chocolate milk is both a technological and a scientific challenge (Boomgaard and others 1987). In industrial production three types of instability are important:

1) Sedimentation of cocoa particles forming a densely packed layer at the bottom
2) Formation of large flocs and
3) Formation of light and dark chocolate layers or segregation

The inhibition of sedimentation in chocolate milk by adding stabilizers has been partially successful; the floc formation can be prevented by adjusting the concentration of added stabilizers or lowering the sterilization time; and maintaining a continuous network formed by an interaction of protein and protein-covered cocoa particles in the presence of a suitable stabilizer (carrageenan) can help preventing the sedimentation and segregation to some extent (Vliet and Hooydonk 1984; Boomgaard and others 1987).

A family of carrageenan products that bring special functional properties to many applications are offered by CP Kelco, San Diego, CA (Pszczola 2003). Figure 2.1 shows a range of different stabilizers available as per its application. Genuvisco is one of their carrageenan products designed to thicken and stabilize water- and protein-based formulations; Genulacta, another carrageenan, primarily used in milk-based systems and suitable for ice cream, chocolate milk, and dairy dessert applications, providing excellent texture, mouthfeel, and shelf-life stability; and several others, including a new technology for meat applications (Pszczola 2003). High degree of reactivity of carrageenan with proteins results in a three-dimensional network

holding cocoa powder in suspension at levels as low as 0.25%. This makes it especially valuable and for this reason, kappa-carrageenan is found in majority of flavored refrigerated dairy-and soy-based beverages with its effectiveness being enhanced through the addition of other hydrocolloids that impart viscosity such as xanthan gum, guar gum and others (Klahorst 2002). Sodium alginate, xanthan gums, locust bean gums, kappa, lambda, and iota carrageenan gums were tested in the preliminary studies done by Wang and others (2001) and iota-carrangeenan was found to be the most ideal gum that was not only able to help suspend chocolate powder, but also compatible with soymilk without forming curds or bringing the off-flavor. It also had the ability to improve consistency and mask the beany flavor of soymilk.

Hydrocolloids or more commonly gums are long-chain polymers that dissolve or disperse in water to give a thickening or viscosity-building effect. The gums are also used for secondary effects like stabilization or emulsions, suspension of particulates, control of crystallization, inhibition syneresis, encapsulation, and formation of a film (Dziezak 1991). Building texture, stability, and emulsification are just some of the ways in which gums aid product development. The United States Food and Drug Administration regulates gums, classifying these compounds as either food additives or "generally recognized as safe" (GRAS) substances. The most common sources of gums are

- Plant materials

  Seaweed extracts (alginates, agar, and carrageenan)

  Seed gums (locust bean gum and guar gum)

  Tree exudates (gum arabic, gum ghatti, gum karaya, and gum tragacanth)
- Products of microbial biosynthesis (xanthan gum and gellan gum); and

- Products produced by chemical modification of natural polysaccharides (cellulose derivatives and pectins)

Carrageenan falls under the first category, an extract from red seaweeds, the most important source being *Chondrus crispus* or Irish moss growing abundantly along the North Atlantic coasts. They are sulfated polymers which consist of galactose and anhydrogalactose units. The structure and molecular weight of the fractions- iota, kappa, and lambda carrageenan identified based primarily on the content and distribution of sulfate ester groups- determine their functional properties (Dziezak 1991; Penna and others 2003). Carragennan has been used for its gelling, thickening, stabilizing, emulsifying, and suspending properties in milk and other food products (Dziezak 1991; Penna and others 2003). Because of its reactivity with certain proteins, the gum has found use at low concentrations (typically 0.01 to 0.03%) in a number of milk-based products such as chocolate milk, ice cream, puddings, and cheese analogues (Dziezak 1991).

Soy lecithin can be used as an emulsifier. An emulsifier consists of water soluble hydrophilic parts and water-insoluble/ oil soluble lipophilic parts within it. When an emulsifier is added to a mixture of water and oil, the emulsifier is arranged on the interface, anchoring its hydrophilic part into water and its lipophilic part into oil. The interfacial tension is thus reduced by emulsifier which means that the force separating oil and water is weakened, resulting in the easily mixing oil and water (Anonymous 2002). Only food emulsifiers defined as food additives are usable by law, lecithin being one among them. Lecithin is a mixture containing phospholipid as the major component, widely found in animals and plants and has long been used as a natural emulsifier (Anonymous 2002). Lecithin is available in the market as paste lecithin and powdered lecithin of high purity. It is classified into plant lecithin (derived from soybeans, corn, rapseed, and others); fractionated lecithin (isolated from special components of the raw

materials); and yolk lecithin (made by excluding the phospholipids) which occupies about 30% of an egg yolk.

Several nutritional beverages are commercially available for supplemental use with or between meals, or as a sole source of nutrition (Osborn and others 2003). In addition to flavors, beverage formulations often contain sweeteners, acidifiers, emulsifiers, colors, and botanicals in many different bases- from coffee and tea to dairy and soy proteins (Brandt 2002). Sweeteners are necessary to enhance the palatability of soymilks (Wang and others 2001). Deciding on which sweetening system to use often is a factor of cost and labeling requirement (Brandt 2002). Each sweetener and sweetener blend has its own profile in different beverage bases and greatly impacts overall flavor profile (Brandt 2002). Commonly used sweeteners in the beverages include dry sugar, liquid sucrose, and high fructose corn syrup (Brandt 2002). Many beverage manufacturers are using sweetener blends, one of the good approaches, because many sweeteners have synergies and when used together round out each other's sweetness profiles (Brandt 2002).

**Processing parameters for the nutritional beverage**

The beverage creation involves balancing the effects of sweeteners, acidifiers, and other ingredients to maximize the flavor impact (Brandt 2002). The processing variables and many ingredients to consider make a good-tasting beverage formulation an increasingly complex task.

**Soymilk processing**

Soybean "milks" are conventionally made in the Orient by soaking soybeans, grinding in water, filtering to remove sediment and then heating the extract (Piper and Morse 1923). The product thus obtained can be taken as such or flavored with syrup and taken as a drink (Ang and others 1985). Although this process is simple, the resulting beverage has a distinct painty (linseed oil) off-flavor and odor (Nelson and others 1976). Soymilk has not gained popularity in the Western

countries, chiefly because of its 'beany' flavor and availability of cheap cow's milk, and is used only as a milk substitute by a group of people who cannot tolerate cow's milk. So, much effort has been directed to elimination of this 'beany' flavor to produce a bland product that is acceptable (Ang and others 1985). To reduce beany flavor, Wilkens and others (1967) developed the Cornell method and Nelson and others (1976) developed the Illinois method. Numerous modifications of this traditional Oriental process have been reported to partially improve the off-flavor and odor defects. Some of these modifications of the cold water extracts gave some flavor improvement but resulted in the lower protein recovery than the traditional Oriental process (Nelson and others 1976). Further attempts to improve flavor or protein recovery or both included the methods of using dehulled soybeans, often partially desludged, and some with added stabilizers for colloidal stability (Nelson and others 1976). The Illinois method is touted as "the greatest milestone in soymilk history" because it produces a very bland soymilk (Wilson 1989).

Another characteristic problem of soymilk and soymilk-based beverage products making them unacceptable to consumers is chalkiness: a defect in a liquid food which coats mouth and throat with fine, grainy particles (Kuntz and others 1978). Several attempts to improve the mouthfeel characteristic include application of high pressure homogenization (34456-55130 kPa), colloid milling, and centrifugal clarification methods (Kuntz and others 1978). The Illinois process soymilk made from whole soybeans was reported to have good mouthfeel when the soybeans were sufficiently softened by blanching and homogenization was done at 93.3º C and 24119 kPa (Nelson and others 1976). Another approach to reduce the chalky sensation as well as to improve the physical stability and global flavor of soymilk was giving an enzyme treatment (Rosenthal and others 2003).

A different approach than the traditional way of soymilk formulation is using an extract of defatted soy meal (1:10 ratio of meal:water), sucrose (12%), palm oil (2.0%), emulsifier (0.2%), lecithin (0.2%), and total solids (19.2%). This soymilk formulation (Ang and others 1985) was spray dried to give a reconstitutable soymilk powder. The protein beverages based on soybeans have been tested and found acceptable in several countries (Mustakas 1974). A process was also developed for using full-fat soy flours by extrusion to make a low-cost, spray dried infant beverage that can be reconstituted with water (Mustakas and others 1971). Alternate methods have used the water soluble protein isolates (preparing an emulsion by adding water, emulsifier, oil, minerals, and sugars). Although more expensive, the isolate route permits better control of composition of the ultimate beverage (Mustakas 1974). A soy protein based beverage can also be prepared from a lipid protein concentrate-LPC (Figure 2.3).

Colloid milling can serve as a wet-grinding step before homogenizing. High-pressure homogenization (55130 kPa) reduced particle size and gave beverages with better mouthfeel than low-pressure homogenization (24119 kPa). Also without the use of two mills (colloid mill and homogenizer), lesser solids were dispersed in the liquid, mouthfeel was poor, viscosity was higher, and some sedimentation occurred (Mustakas 1974). Two types of beverages having acceptable sensory properties were prepared from the LPC beverage base (200 ml) by adding:

A) Sugar (3.2 g), salt (0.25 g), and synthetic milk flavoring (0.02 g)

B) Dutch chocolate-flavored prepared mix (30 g)

Another trend in manufacturing vegetable milk is to fortify it with a vegetable oil (for adjusting the ratio of protein and fat to either that of cow's milk or mother's milk), and to fortify further with vitamins and minerals (Mustakas 1974). A study on the flavored soymilks (Figure 2.4) reported that chocolate and almond flavorings improved aroma of soymilks ($P<0.05$) and

addition of gum partially masked beany flavor and off-flavor. In order to extend shelf-life and facilitate distribution, soymilks were typically subjected to intense heat treatment for sterilization (Kwok and Niranjan 1995). From the preliminary investigation done by Ang and others (1985), it appeared that soymilk of high protein content (~2%) generally underwent precipitation during sterilization at 120º C for 10 to 15 min and there was a tendency for the soymilk of high protein content to impart a darker color to product which was slightly intensified during sterilization. Also protein denaturation or unfolding of a three-dimensional protein structure resulting from a chemical or heat treatment, altered the binding sites for flavor molecules; proteins could bind more or less of a flavor compound, depending on the amount of heat treatment (Brandt 2002). Since health-conscious consumers generally like fresh food, soymilks with mild thermal treatment (pasteurization) would conceivably better suit consumers than those that receive intense thermal treatment (Wang and others 2001). The pasteurized soymilks were stable for one month with refrigeration storage. The formulations with a combination of chocolate flavoring and iota-carrageenan gum permitted the production of low-heat-treated soymilk acceptable to consumers (Wang and others 2001).

**Peanut milk processing**

Early in 1970's different methods of supplementation of cow's milk and various terminologies associated with it were introduced: Filled milk, imitation milk, and toned milk being used in different parts of the world for different reasons. According to the U.S. Filled Milk Act, the filled milk is a product resembling milk, made by combining skim milk solids with fat other than butter fat (Chandrasekhara and others 1971). The imitation milk is a product resembling milk but of non-dairy origin (Rubini 1969) and United Nations Protein Advisory Group recommended that the imitation milk should contain 3.5% fat, 3.5% protein, and 5.0% carbohydrate (all w/w)

which necessitates the ratio of partially defatted peanuts to water to be greater than 1:6 (Rubico and others 1987). The peanut milk having composition lower than these imitation milk specifications can be reclassified as a peanut beverage (Rubico and others 1987). And according to the Prevention of Food Adulteration rules of 1959, the toned milk contains 3.0% fat and 8.5% nonfat solids, and double toned milk contains 1.5% fat and 9.0 % nonfat solids (Chandrasekhara and others 1971).

Various reasons for emergence of such products include dietary concerns, scarcity of cow's milk, lower cost, and extension of available milk supply. In India, a process for Miltone vegetable toned milk preparation was developed for the purpose of replacement of imported skim milk powder by indigenously available vegetable protein- peanut protein isolate (Chandrasekhara and others 1971; Figure 2.5). A nutritious milk-like beverage-Miltone- consequently had other applications such as for yoghurt and other milk based preparations, in coffee or tea or as a mildly flavored drink.

Peanut milk preparation methods developed needed further modifications to improve flavor, texture, emulsion stability and shelf-life of peanut beverage (Rubico and others 1987). A non-defatted peanut beverage (NDPB) and a partially defatted peanut beverage (PDPB) were processed (Figure 2.6). Chalkiness, a defect related to large size particles which cause creaming and layering due to flocculation and coalescence, was evident in the peanut milk, even after homogenization and addition of stabilizers (Rubico and others 1987). High pressure double homogenization can reduce these defects like in soymilks.

Aside from beany flavor, the most common problem that limited the consumption of peanut beverage was its short shelf-life, particularly if processing was done at a temperature below 85 ºC (Rubico and others 1988). Refrigeration and higher processing temperature for

longer time can extend the shelf-life of peanut beverage. A peanut beverage was prepared using a modified Illinois method and deskinned full-fat peanut kernels by Rubico and others (1988) as shown in Figure 2.6 with slight modification at the high temperature heating stage. It was observed that temperature had a significant effect on cooked flavor, raw and cooked odors, viscosity and color whereas time significantly affected only cooked flavor and color. Processing at 100 ºC gave the beverage with sensory quality better than the product processed at either 121 ºC or 85 ºC. Rubico and others (1989) conducted another study for nutritional, microbiological and sensory qualities of the peanut beverages prepared by the modified Illinois process for non-defatted peanuts as shown in Figure 2.6 with four different high temperature treatments

1) Processed at 85 ºC for 15 min, bottled and stored
2) Processed at 100 ºC for 15 min, bottled and stored
3) Bottled, processed at 121 ºC for 15 min and stored
4) Processed at 121 ºC for 3 sec, bottled, and stored

The amino acid analysis data of these different treatments are shown in Table 2.7. The microbiological results in this study indicated that refrigeration was required to preserve the peanut beverage processed at 85 ºC for 15 min however, no microbial growth was observed in the products processed at 100 ºC and 121 ºC followed by storage at 4 ºC and 30 ºC for 20 days. Beany flavor was least pronounced in the beverages processed at 100 ºC for 15 min and the most intense at 121 ºC for 3 sec. This indicated that pasteurization at a temperature lower than 100 ºC for a short time followed by refrigerated storage will be the best possible heat treatment in order to achieve the balance between microbial and sensory characteristics.

Chompreeda and others (1989) developed a chocolate flavored peanut beverage using defatted peanut flour (Figure 2.7). The quality attributes such as color, aroma, viscosity, and

flavor of this chocolate flavored peanut beverage were similar to that of chocolate milk; also total aerobic population of the beverage before and after refrigeration for 7 days was less than 10 and 200 colonies/g, respectively and no coliform bacteria was detected in this product. Sensory evaluation and response surface methodology indicated that the optimum formulation of the beverage was obtained by using protein isolate 3.5%, butter fat 3.5%, sugar 8%, cocoa powder 0.7%, stabilizer 0.1%, and water with all sensory characteristics acceptable in the range of like to extremely like (on a 5-point hedonic scale).

Response surface methodology was employed by Galvez and others (1990) to optimize the process reported by Rubico and others (1988). They used different homogenization pressures: 13782, 27565 and 41347 kPa. One portion of the homogenized mixture was cooked at 100 ºC for 10, 15, and 20 min, bottled then capped and other portion was bottled, capped and processed at 121 ºC for 5, 10, and 15 min; all the samples were cooled and stored at 4 ºC (Galvez and others 1990). It was observed that sulfur aromatic was sufficient to discriminate between the samples processed at 100 ºC whereas sulfur aromatic, cooked peanut flavor, and bitterness provided the most efficient combination for discriminating between the samples processed at 121 ºC. The optimum conditions for processing were found to be homogenization pressure >21363 kPa and process time >16 min at a processing temperature of 100 ºC.

The homogenized peanut extract was found to be a suitable base for production of a nutritious beverage with addition of other ingredients such as sugar, colors and flavors to improve its overall acceptability (Rustom and others 1995a; Figure 2.8). Various types of emulsifiers, homogenization temperatures and pressures were studied to observe the effect on physical stability of the extract. The clarified extract prepared as shown in Figure 2.8 was further utilized for UHT-sterilized peanut beverages (Rustom and others 1996; Figure 2.9). The

UHT peanut beverage would be a suitable product in developing countries, since its quality remained good for up to 5 months of storage at 37 ºC (Rustom and others 1995b).

Various milk-like beverages have been formulated from the aqueous extracts of unroasted peanuts or soybeans, however, undesirable characteristics such as beany or green flavors, suspension stability and chalky mouthfeel have been associated with these beverages (Hinds and others 1997b). A method developed using partially defatted, roasted peanuts gives an acceptable peanut beverage formulation (Hinds and others 1997a, 1997b, 1997c; Figure 2.10). A low-fat beverage with roasted peanut flavor developed was a potential milk-substitute containing 11.8% total solids, 2.0% fat and 3.7% protein with whitish orange-yellow color. The suspension stability was improved by the addition of a carrageenan [Benlacta CM61-B (CM), 0.02-0.04%] or a hydrogenated mono- and diglyceride [Emuldan HV52K (HV), 0.2-0.4%]. Treatments yielding the best combination, compared to cow's milk values, of high suspension stability (0.5±0.03, 1.0=Maximum stability) and low viscosity (3.7±0.89 cps) were those containing 0.2% HV and homogenized at $13.8x10^6$ Pa (Hinds and others 1997b). The sensory attribute 'smoothness' or 'absence of chalkiness' is influenced by size, mass, density and distribution of particles and by the nature of the medium in which particles are dispersed; as frictional resistance increases, smoothness decreases and chalky or grainy sensation of mouthfeel becomes prominent (Hinds and others 1997c). The imitation milks prepared from oilseeds contain protein, fat and cellulose particles from cell wall fragments which contribute to particulates and thus to the chalkiness. Chalkiness is also related to size distribution of particles which may be influenced by the nature of the complexes formed during heat-processing (Hinds and others 1997b). Suspension stability of proteins in animal milks and oilseed beverages is a complex phenomenon (Hinds and others 1997a) influenced by heating and cooling protocols, protein and lipid

concentration, type of stabilizer or emulsifier, and pH, ionic strength, and dielectric constant of the medium. Various heat processing protocols, homogenizing parameters, filtration, and microparticulation techniques have been used to improve smoothness in milk products and oilseed beverages (Hinds and others 1997b).

In the milk and yoghurt fortification by oilseed proteins study (Schmidt and others 1980), cow's milk systems were fortified to 15.0% and 18.0% total solids with nonfat dry milk (NDM), peanut flour (PF), or soy protein isolate (SPI) and heated at 70, 80, 85, and 90 ºC for 30 min. It was observed that all the milks exhibited pseudoplastic (or shear thinning) flow behavior as would be expected for cow's milk at 4 ºC. Consistency index (K), or apparent viscosity (η) at shear rate of unity, of PF and SPI milks was generally higher than that of similarly processed NDM milk. Also increased total solids and increased heat treatment more dramatically increased the K value for oilseed milks than for NDM milk and storage (10 days at 4 ºC) increased K value of SPI milk heated at 80 ºC or above (Schmidt and others 1980). Ramanna and Ramannathan (1992) observed that degree of heat treatment, total solids content, and storage have a pronounced effect on the apparent viscosity, consistency index, and yield stress of the fortified milk systems. They found that heat treatment and refrigerated storage could be used to modify the rheological properties of peanut protein/ milk beverages. Also, beverage and curd prepared from peanut flour/ milk blend fortified to 7.7% protein content were found to have more favorable rheological properties than those prepared from a peanut protein isolate/ milk blend.

In order to encourage wide production of bambara groundnut (*Vigna subterranea*), an underutilized but important African legume, the possibility of producing a vegetable milk for local use or an extracted protein with functional properties for use in food processing applications was investigated by Brough and others (1993) (Figure 2.11). To evaluate the

bambara groundnut milks, a comparison with other vegetable milks made from cowpea, pigeonpea and soybean under the same conditions (Figure 2.11) was done. It was observed that the simple vegetable milk produced from bambara groundnut was as acceptable as the milks from other common legumes, and the taste of bambara groundnut milk was preferred to the other legume milks tested.

Another innovative beverage product prepared from peanut butter was Peanut Punch, a beverage popular in the West Indies Islands (Abdul 1988; Figure 2.12). The ingredients of peanut punch (by weight) include liquid skim milk (85.41%), fine sugar (6.50%), stabilizer (carrageenan; 0.04%), peanut butter (natural, smooth; 8.0%) and liquid caramel (0.05%).

**Analyzing beverage products: Important physical attributes**

**Viscosity**

A relationship between the stress required to induce a given rate of shear defines rheological behavior of a fluid. For a Newtonian fluid, the shear stress is directly proportional to the rate of shear, the proportionality constant being viscosity. The term viscosity can only be correctly applied to Newtonian fluids. For fluids that deviate from this behavior, the term 'apparent viscosity' is used as an index of fluid consistency. Figure 2.13 shows curves representing different relationships between the shear stress and rate of shear (Toledo 1980).

The primary purpose of viscosity in the keenly competitive beverage market is delivering sensory attributes, including eye appeal and creaminess which is one of the desirable attributes to denote flavor, richness, mouthfeel, and ultimately, satisfaction (Klahorst 2002). Beverage viscosity is an important property because

- It adds body to a beverage product by producing the sensation of substance in the mouth and upon swallowing

- It enhances and releases the flavor, since flavor components shun the hydrophilic phase of the beverage and seek out association with colloidal molecules
- It determines the stability which is important for maintaining appearance, since beverage water phases suspend fiber, flavor, pulp and protein
- Also it is a primary factor in the prevention of settling and the aggregation of solids suspended in drinks
- And the molecules that lend viscosity also have other important properties as foam stabilizers in beverages where foam plays an important role in the overall sensory experience

Various ingredients such as proteins, sugar, stabilizers, emulsifiers and process conditions especially heat processing and homogenization (the process that keeps fat droplets from aggregating and rising to the top) control the flow properties of beverage products. Viscosity was significantly affected by different types of stabilizing agents and homogenization pressure (Hinds and others 1997b). The beverages which contain hydrocolloids exhibit the property of shear thinning, possessing high viscosity until the force is applied and after which the viscosity decreases. Like stabilizers and emulsifiers, source of sweetness also changes the flow properties and hence affect the viscosity of beverages. Sugars, whether sucrose, corn syrup, high-fructose corn syrup, fructose, rice syrup, cane sugar or honey, contribute to viscosity, depending on the level added and other components of the solution (Klahorst 2002). Proteins form gel thus they have been used as a source to influence texture and viscosity of foods. For beverages, the primary role of protein is to provide nutritive value in a pleasant matrix, and that usually results in increased viscosity (Klahorst 2002). Protein viscosity is largely affected by heat, and proteins vary in their ability to withstand high-temperature processing. Sometimes

when heated to a point of denaturation, irreversible gels form from coagulated protein. Heat, homogenization and pH are important factors that influence the functional characteristics of isolated soy proteins (Klahorst 2002). As technology has developed, soy has joined dairy as one of the principle proteins for beverage fortification and viscosity modification. As soy protein use increases, especially in beverages that serve as quick, high-protein meals, the challenge has been to mimic the consistency of a milkshake or fresh-blended smoothie. One solution is to enhance the viscosity contributed by soy protein with combinations of other stabilizers, emulsifiers and proteins to make the soy more palatable (Klahorst 2002).

**Visual Stability Index**

The stability of chocolate flavored milk can be determined from sedimentation of cocoa particles, formation of large flocs and formation of light and dark colored layers also called as segregation (Boomgaard and others 1987). Addition of stabilizers helps in reducing formation of densely packed layer at the bottom. The suspension stability of beverage was improved by the addition of stabilizer CM or emulsifier HV as compared to the control without CM or HV (Hinds and others 1997b). Sedimentation or creaming of suspended particles in liquids can be retarded by diminishing the size of the particles and prevented by changing the rheological properties of a continuous phase by the addition of stabilizers to form a continuous network of sufficient strength (Boomgaard and others 1987). The presence of protein molecules also plays an important role in the stability of beverage products. Amount of heat treatment, homogenization and particle size of ingredient molecules are crucial in order to monitor the stability (Hinds and others 1997b). Heat processing has considerable effect on the stabilizer-protein interactions, protein denaturation, and formation of long chains of these molecules. Denaturation and aggregation of protein molecules are heat dependent processes. The process of aggregation

between oilseed proteins and properties of their aggregates differ from those of other common proteins like milk (Hinds and others 1997a).

When the beverage product is stored overnight under refrigerated conditions, a layer will appear separating the beverage into two parts if it has particulate material in suspension. The stability of beverage products can be determined by visual observation of the beverage for the layer formation. Visual stability index can be calculated by measuring the total height of the beverage and taking the ratio of height of sediment to the total height of beverage.

Visual Stability Index = Height of the sludge/ Total height of the beverage

One way of determination of quality of a beverage product can be a comparison with cow's milk which ideally will show no separation and hence has visual stability index of 1.00. A similar procedure was used to determine the visual stability of peanut beverage formulations prepared from partially defatted, roasted peanuts (Hinds and others 1997b).

**Analyzing beverage products: Important sensory attributes**

Appearance and color are important visual attributes deciding the liking of a new product being considered for consumer acceptability. These properties will mainly depend on composition, added coloring agents, stabilizing agents and processing parameters, especially heat treatment. In case of a chocolate flavored protein-based beverage, amount of cocoa powder and stabilizer will mainly control color and appearance of the product. Starch, sugars, proteins, and fats interact significantly in a product to set its texture and flavor. Manipulation of these interactions to produce desirable product attributes tests the creativity of food scientists in food product development (Toledo and Brody 2000). Texture, flavor and appearance are perhaps the most important characteristics of foods because they are the attributes consumer can readily assess (Lund 1982). The instrumental measurement of texture is usually in terms of viscosity for liquid

products and the same characteristic can be judged by observing the consistency or thickness as a sensory attribute. The compounds present in trace quantities in food contribute to the color and flavor of foods. When volatile, these compounds are responsible for the aroma of food and when nonvolatile they contribute to the taste sensation. Aroma, taste, and mouthfeel together constitute the flavor (Toledo and Brody 2000).

Flavor is very important factor in products formulated using oilseed proteins because raw legumes and oilseeds enriched with respect to lipoxygenases and other metallo-proteins possess lipid-derived, objectionable flavor compounds. N-hexanal, 3-cis-hexanal, n-pentylfuran, 2(1-pentenyl) furan, and ethyl vinyl ketone are major contributors to grassy-beany and green flavor. Geosmin, an oxygenated hydrocarbon may contribute to musty, moldy, earthy flavor in soy protein isolates. Oxidized phosphatidylcholine most likely accounts for a bitter taste of soy products. Grassy-beany, bitter flavor compounds preexist in the maturing soybeans and are also generated during processing (Rackis and others 1979). In some legumes development of off-flavors can be readily controlled by rapid inactivation of lipoxygenase with heat, alcohol, or acid treatment (Rackis and others 1979). Also agents that impart acceptable sensory properties can be added to mask the objectionable flavors in protein foods. The beany flavor in flavored soymilks was defined as the offensive beany or grassy flavor related to lipoxygenases whereas aroma was defined as the desirable flavor with respect to soy, chocolate, almond and vanilla (Wang and others 2001). In addition to the beany flavor, astringent and bitter aftertaste contributes to low acceptability of products like soymilk. Aftertaste was defined as the undesirable feeling such as bitterness and astringent that remained in the mouth after expectorating (Wang and others 2001). Aftertaste can be rated as acceptable if desirable flavor is persistent; there is no coating on throat cavity and no sensation of bitterness once the liquid product is swallowed completely.

An important component which participates in flavor development during heating is the group of compounds called carbonyls (Toledo and Brody 2000). Carbonyl compounds usually participate in reactions which result in roasted flavors. The flavor of coffee and cocoa, roasted nuts, and roast beef may be attributed to the reactions involving these carbonyls. Although these compounds may be naturally present, formulations may be developed where these compounds are added to intensify the desired effect. The roasted flavors develop as a result of reactions between the carbonyl compounds and free amino acids in a reaction called the Maillard reaction (Toledo and Brody 2000). This reaction results in the formation of large complex molecules which have brown color and a characteristic roasted flavor. Flavor development through the Maillard reaction depends on the concentration of carbonyls and amino acids in the food, moisture content, temperature, and time. Also, defatted peanut flours contain significant amounts of residual oil and phospholipids that can give rise to oxidative deterioration and in roasted peanuts, metalloproteins can initiate formation of off-flavors derived from lipid oxidation (Rackis and others 1979).

Mouthfeel is a property of great importance for the sensory impression of many foods and is thus associated with consumer acceptability (Folkenberg and others 1999). Szczesniak, among others (1979), investigated that mouthfeel property of a beverage is affected by 11 different categories of underlying properties as shown in Table 2.8. Mouthfeel can be defined in various different ways but sense of touch is the most important for the perception of mouthfeel. According to the ISO-standard, mouthfeel is a tactile sensation perceived at the lining of the mouth, including, tongue, gums, and teeth (Folkenberg and others 1999). Mouthfeel of instant cocoa drinks was found to be positively correlated with the cocoa properties (cocoa content, cocoa odor, cocoa flavor, color) and the viscosity properties (viscosity, thickness appearance,

thickness in mouth and resistance), and the addition of stabilizer can significantly change the mouthfeel characteristics of the beverage since it increases the viscosity of the product (Folkenberg and others 1999).

Mouthfeel sometimes refers to absence of chalkiness or smoothness of a beverage product and this sensory attribute can be used to describe presence or absence of particulate matter in the product. Chalkiness is a defect used to describe a food which coats the mouth and throat with fine, grainy particles and investigators have claimed that beverages produced by boiling-water extraction and filtration lack the chalky mouthfeel that is characteristic of nonextracted products (Kuntz and others 1978). The degree of chalkiness is controlled by the particle size of suspended molecules in the liquid product and subsequent processing operations such as filtration and heat treatment. Peanut beverages were less chalky compared to commercial chocolate low-fat milk and chocolate drink, and similar in smoothness to commercial low-fat cows milks since all the particles were ≤ 125 μm (Hinds and others 1997c).

Overall acceptability is a sensory attribute which defines combination of all the different attributes together. Higher overall acceptability scores indicate that the product has good chances of being purchased and tried by consumers if launched in the market. Most of the times the consumer response is judged on the overall acceptability in optimization studies along with other characteristics such as flavor.

**Sensory Evaluation**

**Definition and consumer sensory acceptance tests**

Sensory analysis is the definition and scientific measurement of the attributes of a product perceived by the senses: sight, sound, smell, taste and touch (Lyon and others 1992). Sensory Analysis or Sensory Evaluation is a useful tool in the development of new products and new

food resources, in storage and shelf-life studies, and in quality assurance of products, especially with changes of formulation, processing, and packaging (O'Mahony 1988). Sensory acceptance tests indicate the acceptance of a product without the package, label, price, etc. The difference between consumer sensory and market research testing is that the sensory test is generally conducted with coded, unbranded products whereas market research is most frequently done with branded products (Resurreccion 1998). Sensory tests are used for grading and pricing of products and in more basic research to determine exactly what chemical changes in a food affect its flavor. While sensory evaluation is used to determine "What is the flavor of the food?" consumer testing is used to determine "Do the customers like the food? Will they buy it?" (O'Mahony 1988). Consumer testing is one of the most important activities in product development. The primary purpose of consumer affective tests is to assess personal response by current and potential customers of a product, product ideas, or specific product characteristics (Resurreccion 1998).

Various applications of sensory evaluation include new product development, product matching, product improvement, process change, cost reduction and/or selection of a new source of supply, quality control, storage stability, product grading or rating, consumer acceptance and or preference, panelist selection and training and correlation of sensory with chemical and physical measurements (IFT/SED 1981). The uses of sensory evaluation are diverse, encompassing a range of disciplines of which food science forms only one part (O'Mahony 1988). The implicit goal behind any and all sensory evaluation efforts in the food industry is to enhance quality, improve appearance, flavor and texture as perceived by consumers in order to influence their food choices (translated into purchases) at the point of sale (Resurreccion 1998). Now-a-days there is more integration of sensory analysis and consumer research methods into an

array of complementary product tests focusing on sensory questions. This integration has been encouraged by customer choice-driven markets, need to identify customers' ideals and expectations of sensory quality to help set sensory targets in the design and development of products (Lyon and others 1992).

Consumer evaluation concerns itself with testing certain products using untrained people who are or will become the ultimate users of the product (Resurreccion 1998). There are two major classifications of sensory tests- Analytical (includes two major types: discriminative and descriptive tests) and Affective tests (IFT/SED 1981). The sensory affective tests evaluate preference and or acceptance and/or opinions of a product using paired-preference, ranking or rating (Hedonic scale or Food Action scale) in which untrained panelists representative of a target population and consumers of test product are randomly selected; minimum 24 and 50-100 panelists considered adequate (IFT/SED 1981). The recommended sensory test method for new product development, consumer acceptance and/or opinions and consumer preference is the hedonic scale rating in which the suggested number of samples per test is 1-18 (the larger the number only if mild-flavored or rated texture only) and analysis of variance or rank analysis are suggested methods for analyzing the data (IFT/SED 1981).

Acceptance tests are used to evaluate product acceptability or to determine whether one or more products are more acceptable than others but acceptance and preference are two different things because a person may prefer one sample to another sample, still find both of them unacceptable (Lyon and others 1992). Consumer acceptance of a food may be defined as an experience, or feature of experience, characterized by a positive attitude toward the food; and/or actual utilization (such as purchase or eating) of food by consumers (Resurreccion 1998). There are three main types of acceptance test presentations (Lyon and others 1992):

1) Monadic tests: Samples are presented one at a time
2) Paired tests: Samples are presented two at a time
3) Sequential monadic tests: Samples are presented in sequence to be assessed one at a time

The monadic test method is appropriate for determining the acceptability of a new or unusual food product where there are no similar products for comparison (IFT/SED 1981). There are a number of different methods and scales used to determine and/or measure acceptance, including rank tests, paired preference tests, hedonic scaling and magnitude estimation (Lyon and others 1992). The two most frequently used tests to measure consumer preference and acceptance are the paired preference and the hedonic scale, respectively (Lyon and others 1992). In hedonic rating the assessor is asked to indicate the extent of liking for the product from extreme dislike to extreme like; a popular scale being the nine-point hedonic scale (Peryam and Pilgrim 1957). The 9-point hedonic scale (Figure 4.3; Appendix C) is a rating scale that has been used for many years in sensory evaluation in the food industry to determine the acceptance of a food and to provide a benchmark on which to compare results and its use has been validated in the scientific literature (Resurreccion 1998).

The essential features of the hedonic scale are its assumption of a continuum of preference and the direct way it defines the categories of response in terms of *like* and *dislike* (Peryam and Pilgrim 1957). In the analysis, each descriptor is assigned a value and it is usually assumed that it is an equal interval scale. Another approach is to score on a continuous line scale with the extremes at either end, the distance of the mark along the line is then used as a rating (Lyon and others 1992). It can be observed from Figure 4.3 that the instructions given on a sensory ballot with hedonic scale have two functions: first, to describe the mechanism of the test; second, to encourage freedom of response. The intent is to have the subject answer on the

basis of his first impression and to minimize the intellectual approach, that is, one involving conscious reasoning and judgment, though, of course, these cannot be entirely avoided (Peryam and Pilgrim 1957). The hedonic scale ratings are converted to numerical scores, and statistical analysis is applied to determine difference in degree of liking between or among samples (IFT/SED 1981).

It must be considered whether products being tested are safe to consume or inhale by considering the safety factors like microbiological status, chemical or toxic residues and ingredients which cause allergic responses or other health hazards. It is the primary responsibility of those carrying out sensory analysis to ensure that the assessors are not exposed to unacceptable risk as a result of participating in the tests and they should be aware of any statutory regulations which exist with respect to the control of substance which are considered hazardous to health (Lyon and others 1992). Also it is important to get permission from the regulatory authority controlling the use of human subjects as a part of research. The procedure to get permission for using the human subjects at the University of Georgia is given at the website http://www.ovpr.uga.edu/hso.

The preference or acceptance tests are usually conducted 'in-house' -carried out on company premises with company staff- with the suitable assessors being any member of the staff who is likely to eat the product, provided they are not involved in any way with the product under test, or have been previously selected and trained for discrimination and descriptive tests (Lyon and others 1992). The number of assessors required depends on the test procedure, the purpose of the test, the amount of assessor training, the reproducibility of their results and the variability of the product; the panel should be large enough to overcome such variability (Lyon and others 1992). For laboratory consumer tests usually twenty-five to fifty responses are

obtained; at least forty responses per product are recommended by Stone and Sidel (1993). In a test consisting of 24 or lesser number of panelists, it may be difficult to establish a statistically significant difference. However, it is still possible to identify trends and provide direction; whereas with 50-100 responses per product, statistical significance increases to a large extent (Resurreccion 1998). The consumer panelists should be selected from the target population and screening can be done based on various criteria such as demographic characteristics. The overall objective is to select a relatively homogenous group from a fairly broad cross section, all of whom like and use the product or the product category, and exclude those individuals who exhibit extreme or unusual response patterns (Stone and Sidel 1993). Specific training is not required for acceptance test; however, assessors should be made familiar with the test procedure and be clear about the instructions given (Lyon and others 1992). The orientation of consumer panelists should consist only of describing the mechanics of the test that they need to know, for example, orientation regarding the booth area, explanations about the sample pass-through door, signal lights etc (Resurreccion 1998; Appendix B).

**Experimental design for sensory evaluation**

The development of any new food product involving more than one ingredient requires some form of mixture experimentation as opposed to factorial experimentation (Hare 1974). In a mixture experiment, two or more ingredients are mixed or blended together in varying proportions to form some end product, quality characteristics of which are recorded for each blend, to see if the characteristics change from one blend to the next (Cornell and Harrison 1997). In mixture experimentation, it is impossible to vary one ingredient or component while holding all the others constant because as soon as the proportion of one component is altered, so is that of at least one other component since the sum of all components is always 1.0.

$$\sum_{i=1}^{q} X_i = 1.0$$

Where, q is the number of components in the mixture.

Mathematical models are used to analyze data generated in the mixture experimentation, a familiar two variable model applied to a mixture space by subjecting it to the constraint $X_1+X_2=1$, can be expressed as:

$$E(y) = \beta'_1 X_1 + \beta'_2 X_2 + \beta'_{12} X_1 X_2$$

Estimates of the coefficients can be obtained from most multiple regression computer programs by forcing the intercept through 0 (Hare 1974). Similar calculations applied to other factorial models have lead to following model for a mixture data as suggested by Scheffe (1958) and presented by Snee (1971):

Linear:

$$E(y) = \sum_{i=1}^{q} \beta_i X_i$$

Quadratic:

$$E(y) = \sum_{i=1}^{q} \beta_i X_i + \sum_{1 \le i < j}^{q} \beta_{ij} X_i X_j$$

Frequently in the mixture experimentation, it is impossible to vary the proportion of each component from 0 to 1.0. Each component is constrained within certain limits. Mathematically,

$$0 \le a_i \le X_i \le b_i \le 1.0,\ i = 1, 2, \ldots, q$$

Because constraints on the individual factors determine the design, the choice of constraints is crucial (Gorman 1966). Often in exploratory work the constraints can not be set precisely and, in fact, may have to be estimated experimentally (Gorman 1966). Once the constraints are decided, the experimental points can be found by using extreme vertices design method. The extreme

vertices design is developed as a procedure for conducting experiments with mixtures when several factors have constraints placed on them (McLean and Anderson 1966). According to McLean and Anderson (1966) the selection of vertices and various centroids of the resulting hyper-polyhedron as the design is a method of determining unique set of treatment combinations (the design of the experiment) which may be used to estimate the response surface. In case of the quadratic model, a minimum of ½ q (q+1) points are required. The maximum number of design points for a q-component design will be q $2^{q-1}$. In case additional points are desired in any given design they may be obtained, for example, by using mid points of the edges of the hyper-polyhedron or repeating some of the existing points (McLean and Anderson 1966). There are computer software programs available to get extreme vertices design points and dealing with the data analysis of such mixture experiments.

**Conducting a well organized sensory test**

Design, organization, implementation, and management of the consumer sensory test require meticulous planning in order to obtain the desired information (Resurreccion 1998). The planning for testing include definition of objectives, selection of an appropriate test and experimental design, identification of the tests, screening of samples, selection of sample preparation method, data collection, data processing and analysis, interpretation, and reporting of results in timely manner. Lyon and others (1992) gives detailed guidelines on carrying out a well organized sensory test.

The conditions of sample preparation and presentation should be identical to those under which a product is usually consumed, for example, cold beverages such as carbonated beverages, chocolate drinks, and milk, are best served at approximately 5-9 ºC (Resurreccion 1998). The test objectives determine the sample size. It should be adequately large so that consumers will

be able to evaluate product acceptance and any other attributes being evaluated. Preliminary testing will help to determine the amounts of sample to be consumed during evaluation. It is often necessary to provide instructions to panelists to ensure that enough products are evaluated to give a reliable result. The preliminary testing needs to be conducted whenever possible to determine if the selected sample preparation and presentation procedures are appropriate for a specific test, to specify any special requirements related to preparation and serving, and to identify any additional procedures required as they relate to the product or test (Resurreccion 1998). Appropriate modifications should be done as a result of pre-testing. It is recommended that a complete dry-run of all testing procedures be conducted on the test date, from the preparation of samples, and orientation of panelists, to actual test procedures conducted using two or three untrained individuals as panelists one week before the test date (Resurreccion 1998). This will allow sufficient time for changes to be made if necessary.

Once consumer acceptance for the product has been quantified, the sensory practitioner defines the lower boundary for consumer acceptance: some companies that pride themselves on the quality of their products may be unwilling to produce a product that is only "liked slightly" (=6) on a 9-point hedonic scale and may opt to accept a higher limit set at "like moderately or higher" (=7) for the product (Resurreccion 1998). An action standard specifies a performance requirement for a new or improved product. If there is no competitive framework, the optimal formulation must receive a score equal to or greater than a chosen value that has, in the past, been associated with a product success (Fishken 1983).

**Response surface methodology and product optimization**

Optimization can be defined as the determination of values for process and formulation variables (factors) that result in a product or products with physical properties and sensory characteristics

that satisfy some specific predetermined values which make them acceptable to consumers (Galvez and others 1995). Modeling of quality or consumer acceptance using a response surface methodology (RSM) can be done in a simplified manner when the effect of two or more variables on the product acceptance and its interrelationship with sensory attribute intensities is of interest (Resurreccion 1998). When RSM is utilized, product optimization time is greatly reduced from the traditional "cook and look" optimization techniques that depend on subjective formulation and evaluation procedures (Rudolph 2000).

RSM is a designed regression analysis meant to predict the value of a response variable, or dependent variable, based on the controlled values of the experimental factors, or independent variables (Meilgaard and others 1991). The samples are evaluated by the consumer and descriptive panels, and regression analysis results in the predictive equations. From the parameter estimates, it can be determined which variable contributes the most to the prediction model, thereby allowing the product researcher to focus on the variables that are most important to the product acceptance (Schutz 1983). The dependent variable is the acceptance rating and is the only rating that is absolutely necessary to obtain optimal formulations. Contour plots of the prediction models allow the researcher to determine the predicted value of the response at any point inside the experimental region without requiring that a sample be prepared at the point (Meilgaard and others 1991). RSM is applicable in wide variety of areas including food research and has constantly been successfully demonstrated to be used in optimizing ingredients (Henselman and others 1974; Johnson and Zabik 1981; Vaisey-Genser and others 1987; Chow and others 1988; Shelke and others 1990) and process variables (Oh and others 1985; Floros and Chinnan 1988; Mudahar and others 1990; Galvez and others 1990; Vainionpaa 1991) or both (Bastos and others 1991).

Optimization of all the aspects of a product is the goal in product development. Sensory evaluation is often called upon to determine whether or not the optimum product has been developed and response surface methodology seems to be a popular method of discussion for product optimization within the sensory evaluation field (Giovanni 1983). In optimization research, the like/prefer measure is the dependent variable, while the independent variables are the properties of the products (sensory characteristics, ingredients and others) that were the basis for differentiation (Sidel and Stone 1983). To achieve the objective of developing products with optimal sensory acceptance, one must identify those properties and levels that are important to acceptance (Schutz 1983). Methods of optimization range from the individual specialist's orchestrating optimization on the basis of his or her professional skill and experience to structured statistical approaches of response surface methodology (Sidel and Stone 1983). According to Giovanni (1983) the classical sensory evaluation approaches are inefficient because

- A large number of experiments is required, which can be expensive and time consuming
- Optimum product might not be determined because the experimenter must use educated guesses to specify the levels of ingredients to test and it is difficult for the experimenter to consider the interactions among the variables without assistance of the computer and
- Neither of these approaches establishes mathematical relation which describes the relationship between the variables and responses to these variables

Resources in sensory evaluation and availability of statistical models are intended to expand the intellectual limits about products and to provide a perspective that is not readily apparent by other means. Creative efforts are still an integral part of the system and the intention of optimization is to provide a more precise map of the path that has the highest probability for success (Sidel and Stone 1983). RSM is a statistical method that uses quantitative data from

appropriate experimental designs to determine and simultaneously solve multivariate equations graphically represented as response surfaces which can be used in three ways (Giovanni 1983):

1) To describe how the test variables affect the response;
2) To determine the interrelationships among the test variables; and
3) To describe the combined effect of all test variables on the response

Basically RSM is a four step process. First, two or three factors that are the most important to the product or process under study are identified. Second, the ranges of factor levels which will determine the samples to be tested are defined. Third, the specific test samples are determined by the experimental design and then tested. Fourth, the data from these experiments are analyzed by RSM and then interpreted (Giovanni 1983).

Response surface methodology was used to optimize formulations of a chocolate peanut spread by using a three-component constrained simplex lattice design (Chu and Resurreccion 2004). The optimum formulations (consumer acceptance rating of ≥6.0 for all attributes) for the chocolate peanut spread were all combinations of 29-65% peanut, 9-41% chocolate, and 17-36% sugar, adding up to 100%, at a medium roast. In an optimization study done by Galvez and others (1995), consumer acceptance scores for two commercial mungbean noodle samples were used in order to attain the optimum formulation. The commercial samples A and B were preferred by consumers in the earlier study. In order to prepare better quality noodles than these commercial samples, the process was optimized using the acceptance scores for samples A and B as constraints. The contour plots were developed using predictive models. The shaded areas represented the optimum ranges for consumer acceptance of attributes tested which satisfied acceptance ratings described for commercial samples and the optimum region was found by outlining the regions representing the overlap of the shaded areas. Response surface

methodology using the mixture design was used to determine the optimum ratio of pineapple, papaya, and carambola in the formulation of reduced calorie tropical mixed fruit jam (Abdullah and Cheng 2001). In this study, the limit of acceptance for viscosity, aroma, sourness, color and overall acceptability were set 3.4-4.4, 4.1, 3.7-4.3, 3.7 and 4.8 respectively. This was based on the closeness of the attributes to the acceptance of the commercial product. To obtain the optimum region, contour plots with limits of acceptance were superimposed; the shaded region where contours within limits of acceptance overlapped was considered the predicted optimum region. Thus, a commercial sample can be used as a control and the consumer acceptance scores can be set as criteria to attain the optimum. The three dimensional plots as well as the contour maps provide a useful visual aid for examining the behavior of the response surface and location of the optimum (Vatsala and others 2001). For product development, RSM can be used to establish the optimum level of the primary ingredients in a product, once these ingredients have been identified. This information helps the product developer to understand ingredient interactions in the product which guide the final product formulation and future cost and quality changes (Giovanni 1983).

Table 2.1: Some important food uses of peanut (Singh and Singh 1991)

| Food uses | Regions |
|---|---|
| Raw dry nuts | South and Central Asia, Africa |
| Fresh boiled and salted | Southeast Asia, Africa |
| Fried and mixed with sugar syrup | Asia particularly in India, Pakistan & Bangladesh |
| Fried and coated with chickpea flour | Southeast Asia and Mediterranean regions |
| Nuts fermented and fried | Southeast Asia, particularly Indonesia, Philippines & Thailand. |
| Roasted and salted | Asia, Africa, North & Central America, and South America |
| Peanut butter | Europe, North & Central America, and South America |
| Candies and confections | North & Central America, Some European, Asian and African Countries |

Table 2.2: Essential amino acid composition (g/100 g protein) of peanut-based products (Natarajan 1980)

| Amino Acid | Peanut kernel | Peanut flour | Protein concentrate | Protein isolate | FAO pattern |
|---|---|---|---|---|---|
| Lysine | 3.5 | 4.0 | 2.9 | 3.0 | 5.5 |
| Leucine | 6.4 | 6.4 | 6.8 | 6.4 | 7.0 |
| Valine | 4.2 | 5.3 | 4.7 | 4.4 | 5.0 |
| Isoleucine | 3.4 | 3.2 | 3.5 | 3.5 | 4.0 |
| Threonine | 2.6 | 2.6 | 2.5 | 2.4 | 4.0 |
| Phenylalanine | 5.0 | 4.7 | 5.5 | 5.4 | - |
| Tyrosine | 3.9 | 3.7 | 4.1 | 4.4 | - |
| Total sulfur amino acids | 2.5 | 1.9 | 2.5 | 2.7 | 3.5 |
| Cystine | 1.3 | 1.0 | 1.4 | 1.8 | - |
| Methionine | 1.2 | 0.9 | 1.1 | 0.9 | - |
| Tryptophan | 1.0 | 1.0 | 0.9 | 0.8 | 1.0 |

Table 2.3: Mean proximate analyses of soy flours collected at Cedar Rapids, IA (Porter and Jones 2003)

| Analyte | Mean value |
|---|---|
| Protein (% as is) | 52.05 |
| Moisture | 5.20 |
| Ash (% as is) | 6.31 |
| Fat (by extraction) | 0.80 |
| Fat (by acid hydrolysis) | 2.39 |

Table 2.4: Mean concentrations of amino acids in soy flours collected at Cedar Rapids, IA (Porter and Jones 2003)

| Amino acid | Mean concentration | Amino acid | Mean concentration |
|---|---|---|---|
| Glutamic acid | 8.97 | Glycine | 2.17 |
| Aspartic acid | 6.02 | Isoleucine | 2.20 |
| Leucine | 3.94 | Alanine | 2.12 |
| Arginine | 3.71 | Threonine | 2.05 |
| Lysine | 3.30 | Tyrosine | 1.68 |
| Serine | 2.76 | Histidine | 1.37 |
| Proline | 2.90 | Cystine | 0.74 |
| Phenylalanine | 2.51 | Methionine | 0.71 |
| Valine | 2.30 | Tryptophan | 0.62 |

Table 2.5: Essential amino acids pattern of various protein sources (Ang and others 1985)

| Essential amino acid | Requirement | | | A/E ratio [a] of whole egg protein | Whole egg | Cow's milk | Human Milk | Soy products from defatted meal | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Infant mg/kg | Adult mg/day | | (mg/g total essential amino acid) | (mg/g of protein) | (mg/g of protein) | (mg/g of protein) | (mg/g of protein) | | |
| | | M | F | | | | | Soy milk | Soy milk powder | |
| | | | | | | | | | Spray-dried | Reconstituted |
| Histidine | 32 | - | - | - | 24 | 26 | 25 | 36 | 30 | 39 |
| Isoleucine | 90 | 450 | 700 | 129 | 63 | 46 | 40 | 44 | 39 | 50 |
| Leucine | 150 | 620 | 1110 | 172 | 88 | 93 | 86 | 72 | 68 | 83 |
| Lysine | 105 | 500 | 800 | 125 | 70 | 76 | 87 | 76 | 72 | 89 |
| Phynylalanine | 90 | 220 | 300 | 114 | 99 | 53 100 | 34 66 | 48 80 | 42 75 | 56 89 |
| Tyrosine | | 900 | 1100 | 81 | | 47 | 32 | 32 | 33 | 33 |
| Methionine | 85 | 350 | 200 | 61 | 58 | 25 33 | 16 29 | 16 16 | 24 24 | 17 17 |
| Cystine | | 200 | 810 | 46 | | 8 | 13 | trace | trace | trace |
| Threonine | 60 | 305 | 500 | 99 | 51 | 44 | 44 | 36 | 32 | 39 |
| Tryptophan | 22 | 157 | 250 | 31 | 15 | 14 | 16 | ? | ? | ? |
| Valine | 93 | 650 | 800 | 141 | 69 | 57 | 45 | 40 | 37 | 50 |
| Nitrogen (%) | | | | | 1.78 | 0.55 | 0.19 | 0.40 | 2.43 | 0.29 |
| Factor | | | | | 6.25 | 6.38 | 6.38 | 6.25 | 6.25 | 6.25 |
| Protein | | | | | 12.4 | 3.50 | 1.2 | 2.5 | 15.2 | 1.8 |

[a] A/E ratio = [(each essential amino acid content/total essential amino acid content including cystine and tyrosine)×1000]

Table 2.6: Formulation for chocolate-flavored nutritional beverages (Osborn and others 2003)

| COMPONENT | WEIGHT PERCENT |
|---|---|
| Water | 75.92 |
| Corn Syrup | 5.69 |
| Sucrose | 3.80 |
| Maltodextrin | 3.80 |
| Calcium caseinate | 3.41 |
| Canola oil or SL[a] | 3.41 |
| Cocoa powder | 1.90 |
| Whey protein concentrate | 1.52 |
| Salt | 0.19 |
| Vanilla extract | 0.19 |
| Soy lecithin | 0.08 |
| Artificial fudgey chocolate flavor | 0.05 |
| Carrageenan | 0.04 |

[a]SL, canola oil/caprylic acid structured lipid synthesized at a substrate mole ratio of 1:5

Table 2.7: Amino acid profiles of peanut beverage processed at different temperature and time treatments (g/100g protein normalized to 100% recovery) (Rubico and others 1989)

| Amino Acid | Controls | | Treatments (Mean values) | | | |
|---|---|---|---|---|---|---|
| | Raw peanut extract | 71 ºC homogenized peanut milk | 85 ºC 15 min | 100 ºC 15 min | 121 ºC 3 sec | 121 ºC 15 min |
| Threonine | 2.44 | 2.23 | 2.93 | 2.73 | 2.85 | 2.95 |
| Valine | 4.48 | 3.68 | 4.68 | 4.12 | 4.84 | 4.40 |
| Methionine | 1.05 | 1.89 | 1.37 | 1.55 | Not detectable | Not detectable |
| Isoleucine | 4.03 | 4.04 | 4.35 | 4.51 | 4.75 | 4.04 |
| Leucine | 8.02 | 7.52 | 8.07 | 7.82 | 8.50 | 7.74 |
| Tyrosine | 5.29 | 5.00 | 5.25 | 5.19 | 4.84 | 5.08 |
| Phenylalanine | 6.25 | 5.35 | 5.74 | 5.98 | 6.07 | 6.07 |
| Lysine | 4.31 | 5.16 | 3.05 | 3.81 | 2.88 | 3.60 |
| Aspartic acid | 13.79 | 13.43 | 15.34 | 14.32 | 13.56 | 14.32 |
| Serine | 4.5 | 4.83 | 5.15 | 5.10 | 4.71 | 5.67 |
| Glutamic acid | 22.97 | 22.32 | 23.51 | 23.20 | 21.06 | 23.63 |
| Proline | 4.67 | 4.15 | 3.71 | 3.05 | 4.12 | 3.57 |
| Glycine | 7.44 | 7.16 | 5.66 | 5.33 | 5.36 | 5.47 |
| Alanine | 4.22 | 4.95 | 4.14 | 4.23 | 4.78 | 3.94 |
| Histidine | 3.05 | 4.97 | 2.70 | 2.38 | 2.34 | 3.05 |
| Arginine | 15.86 | 15.33 | 17.34 | 17.22 | 16.01 | 17.52 |

Table 2.8: Classification of sensory mouthfeel properties for beverages (Szczesniak 1979)

| CATEGORY | TYPICAL WORDS |
|---|---|
| Viscosity-related terms | Thin, thick, viscous |
| Feel on soft tissue surfaces | Smooth, pulpy, creamy |
| Carbonation-related terms | Bubbly, tingly, foamy |
| Chemical effects | Astringent, burning, sharp |
| Body-related terms | Heavy, watery, light |
| Coating of oral cavity | Mouth-coating, clinging, fatty, oily |
| Resistance to tongue movement | Slimy, syrupy, pasty, sticky |
| Afterfeel-mouth | Clean, drying, lingering, cleansing |
| Afterfeel-physiological | Refreshing, warming, thirst-quenching, filling |
| Temperature-related | Cold, hot |
| Wetness-related | Wet, dry |

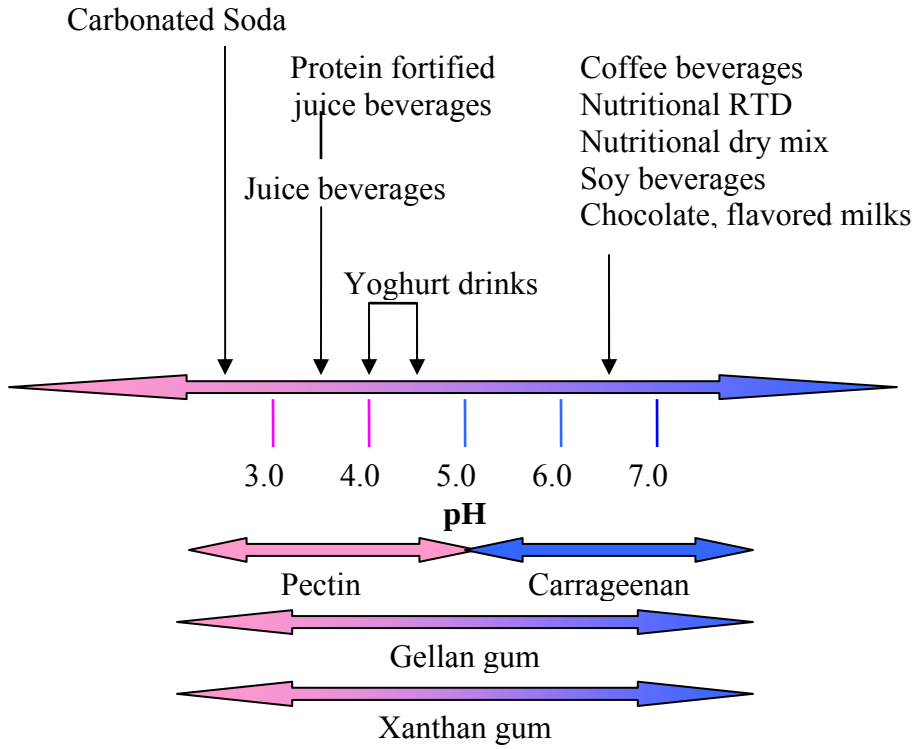


Figure 2.1: Different types of stabilizers available over a broad pH spectrum (Kelco 2003)

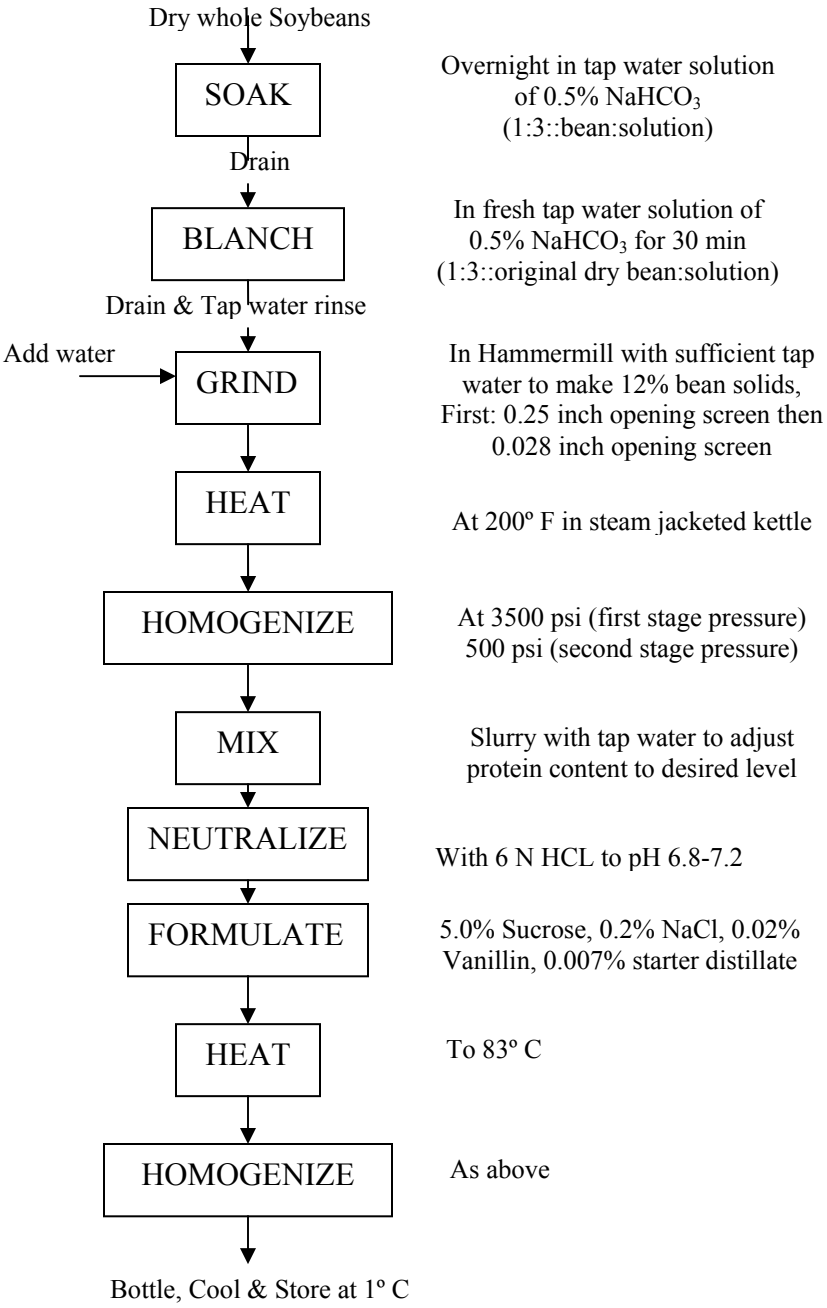


Figure 2.2: Preparation of soybean beverage
(Nelson and others 1975)

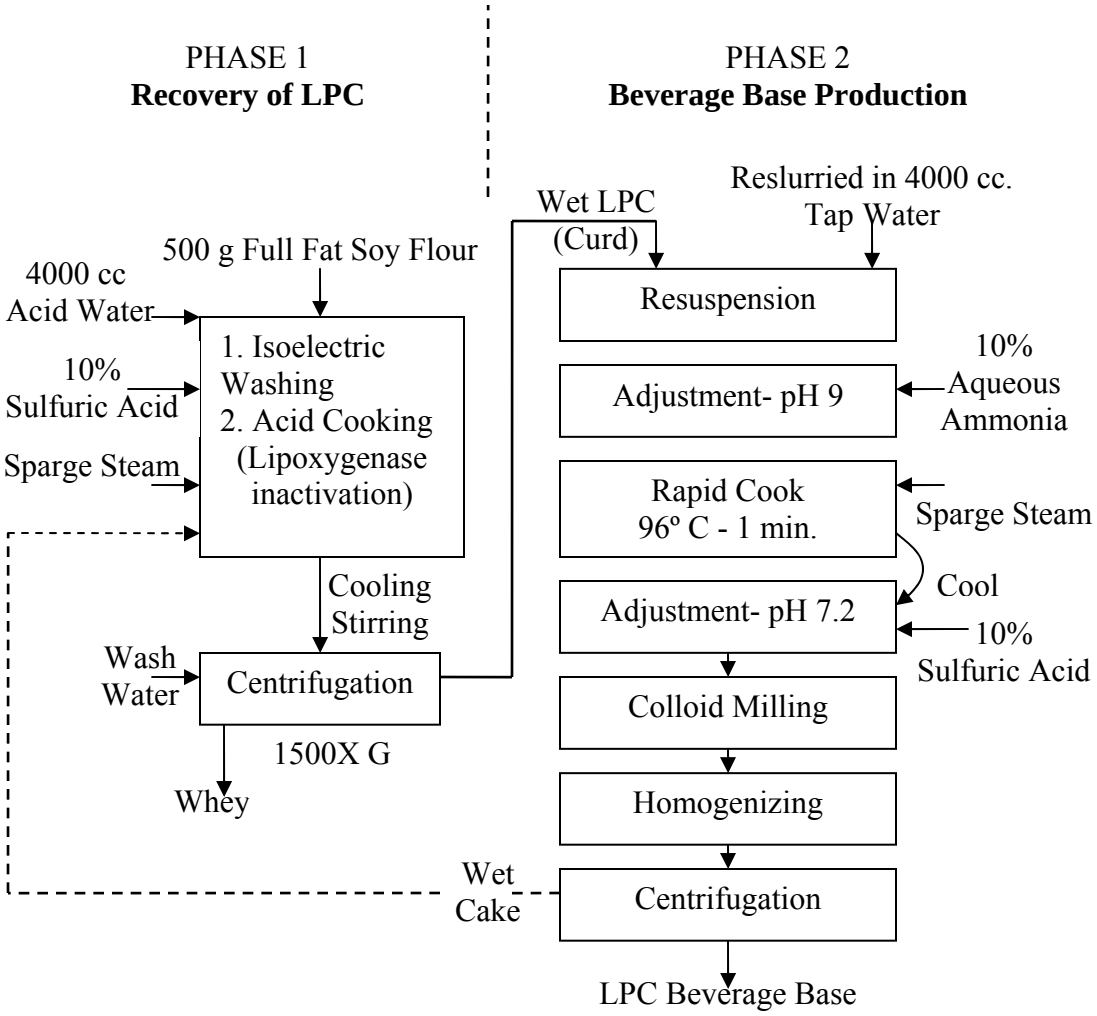


Figure 2.3: Process flow sheet for LPC (lipid protein concentrate) and LPC beverage base preparation
(Mustakas 1974)

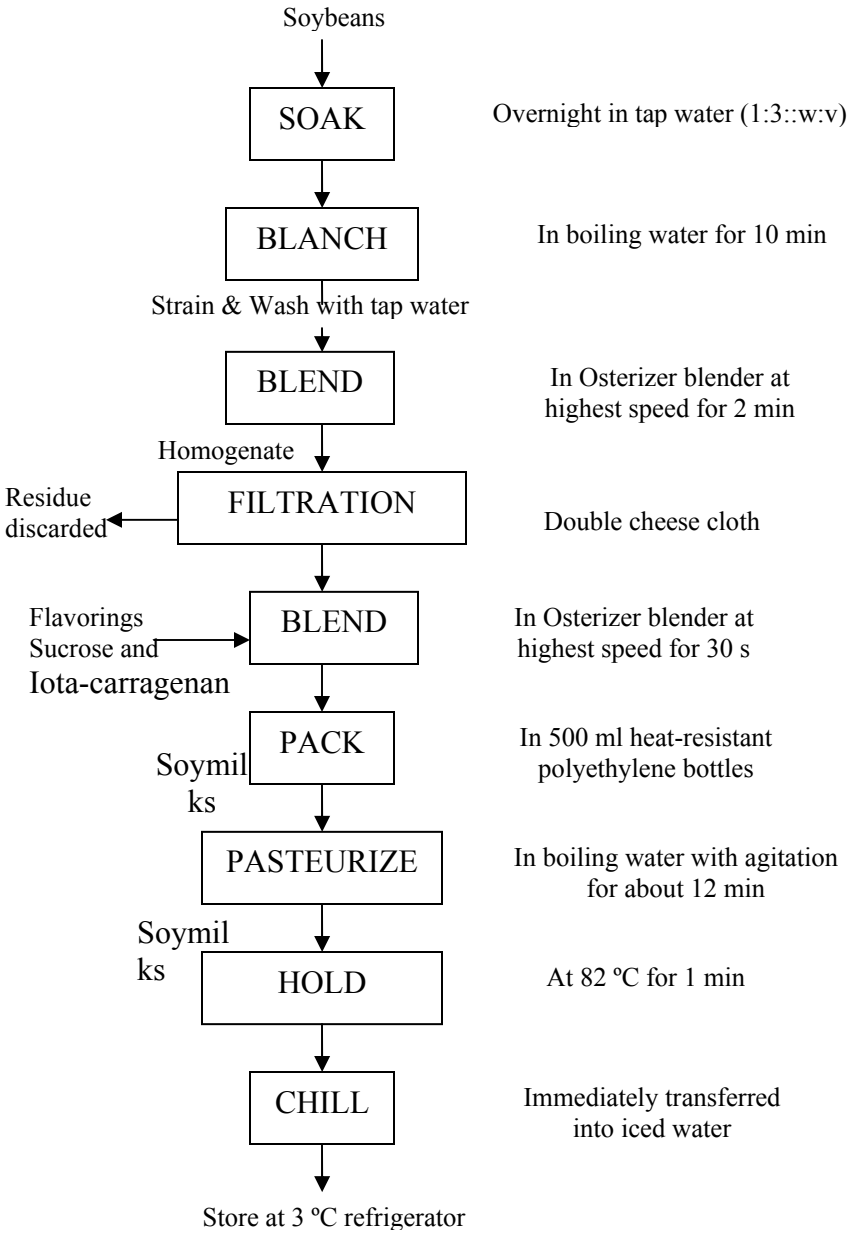


Figure 2.4: Modified Illinois method for preparation of flavored soymilk (Wang and others 2001)

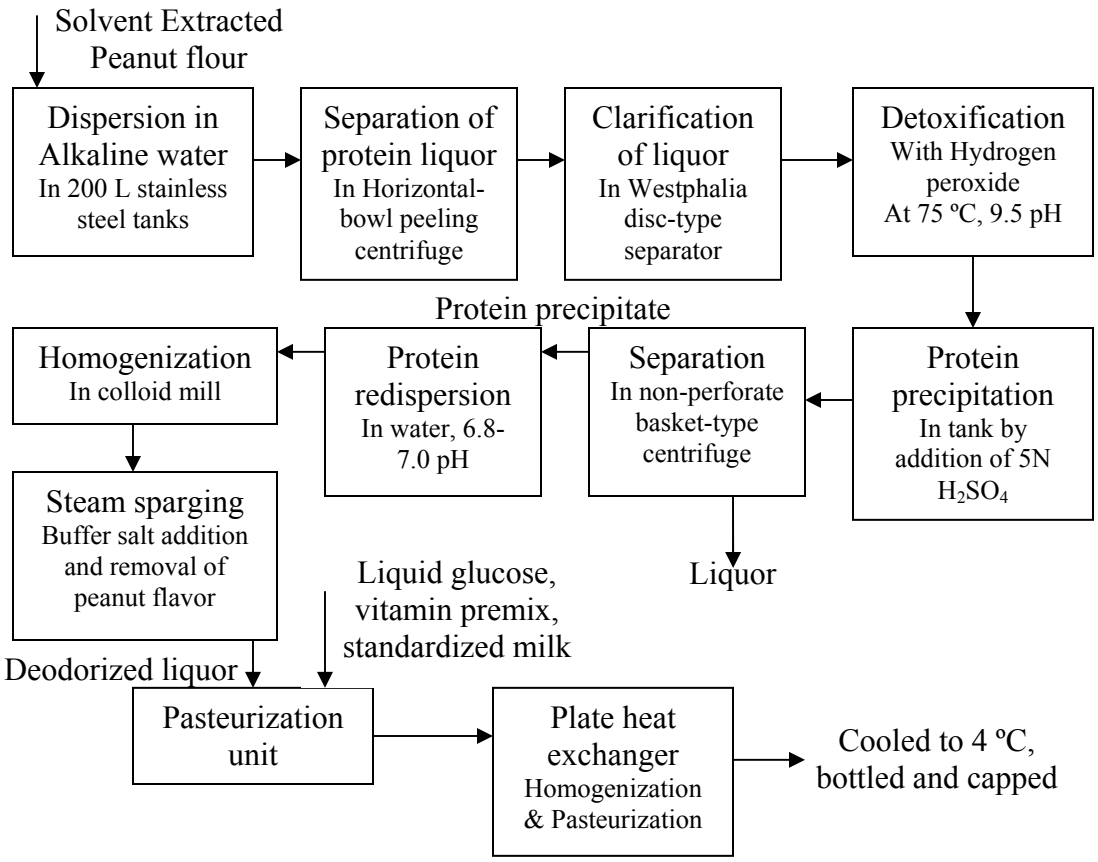


Figure 2.5: Miltone- Vegetable toned milk preparation
(Chandrasekhara and others 1971)

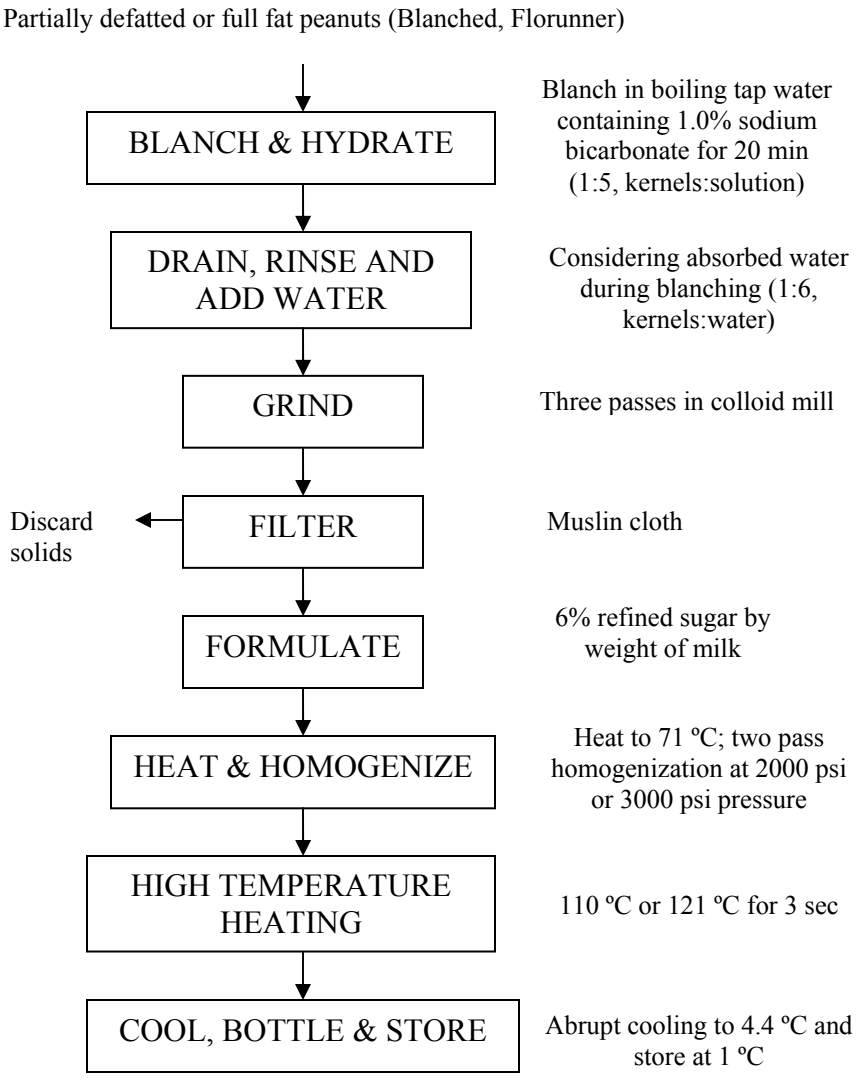


Figure 2.6: Modified Illinois process for peanut beverage preparation (Rubico and others 1987)

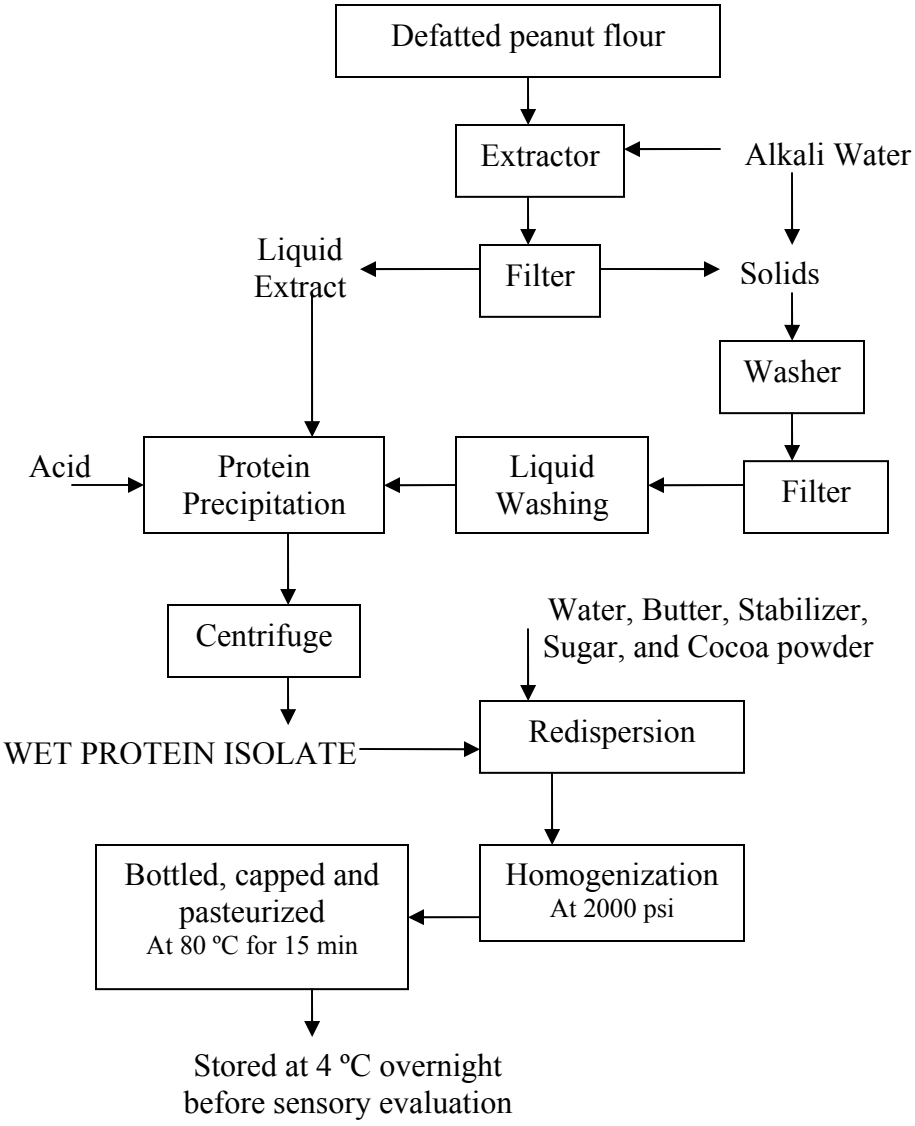


Figure 2.7: Preparation of peanut protein isolate and chocolate flavored peanut beverage (Chompreeda and others 1989)

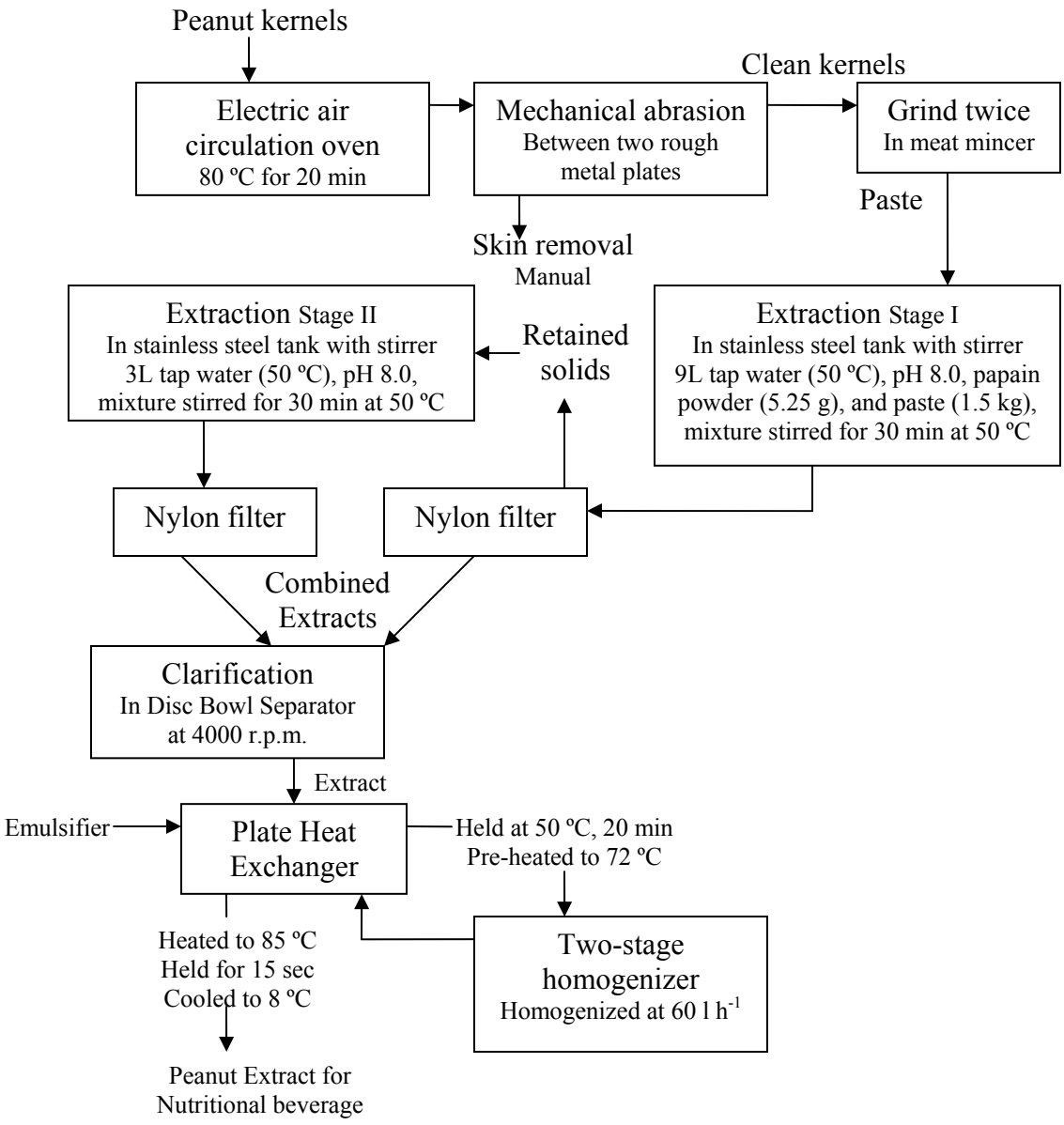


Figure 2.8: Preparation of peanut extract for nutritional beverage formulation (Rustom and others 1995a)

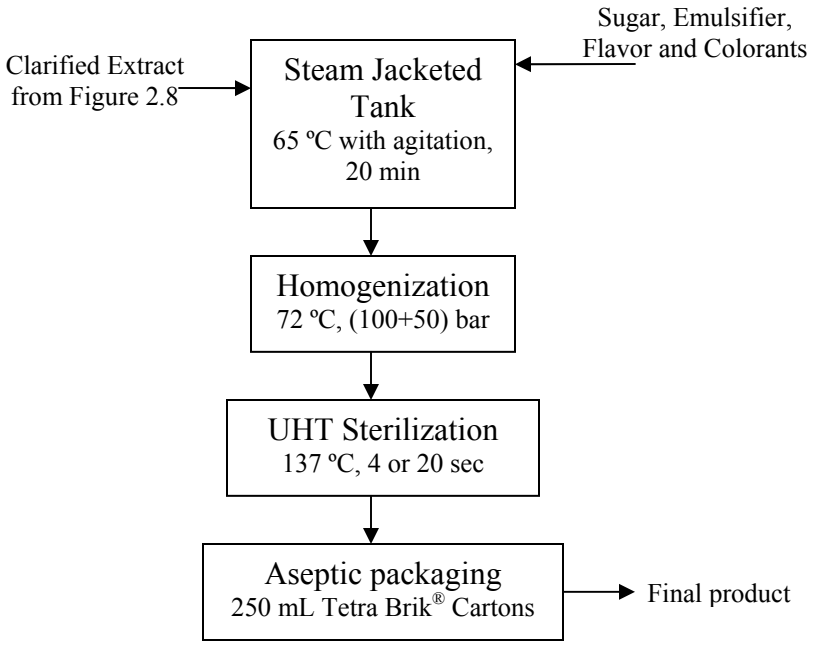


Figure 2.9: UHT-sterilized peanut beverage preparation
(Rustom and others 1996)

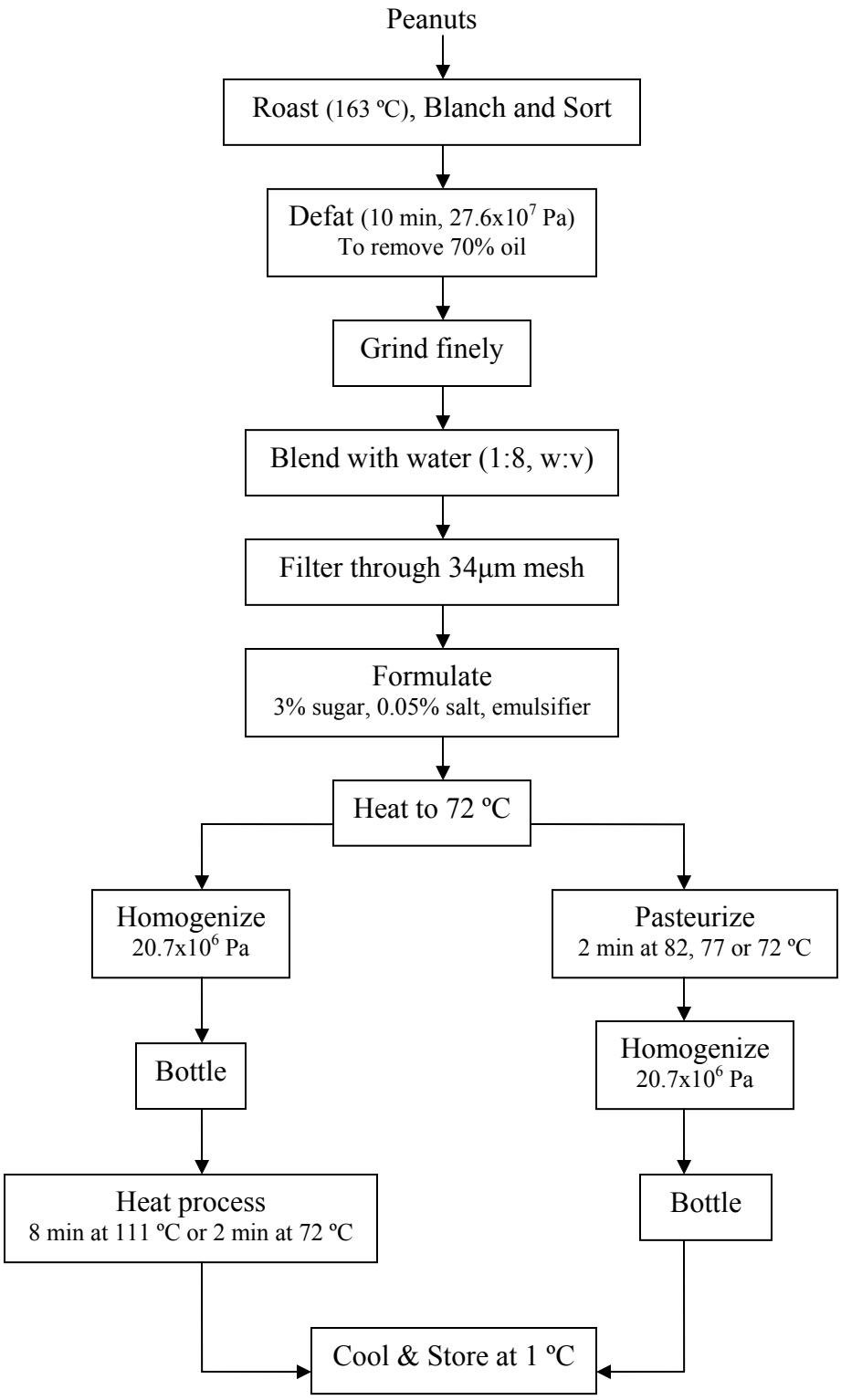


Figure 2.10: Peanut beverage preparation
(Hinds and others 1997a)

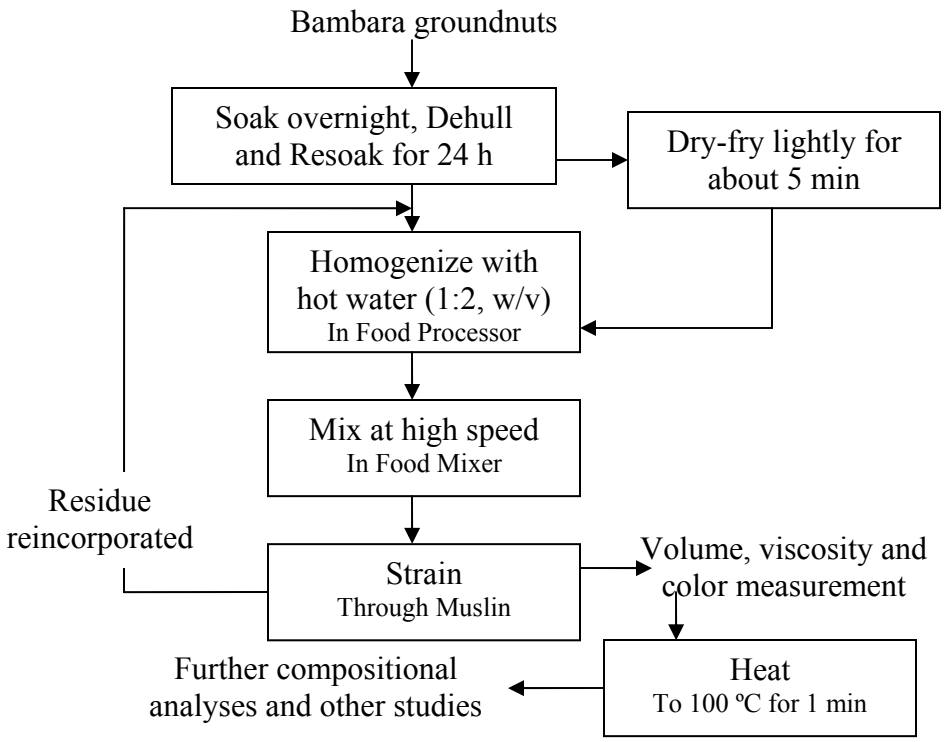


Figure 2.11: A vegetable milk preparation from bambara groundnut (Brough and others 1993)

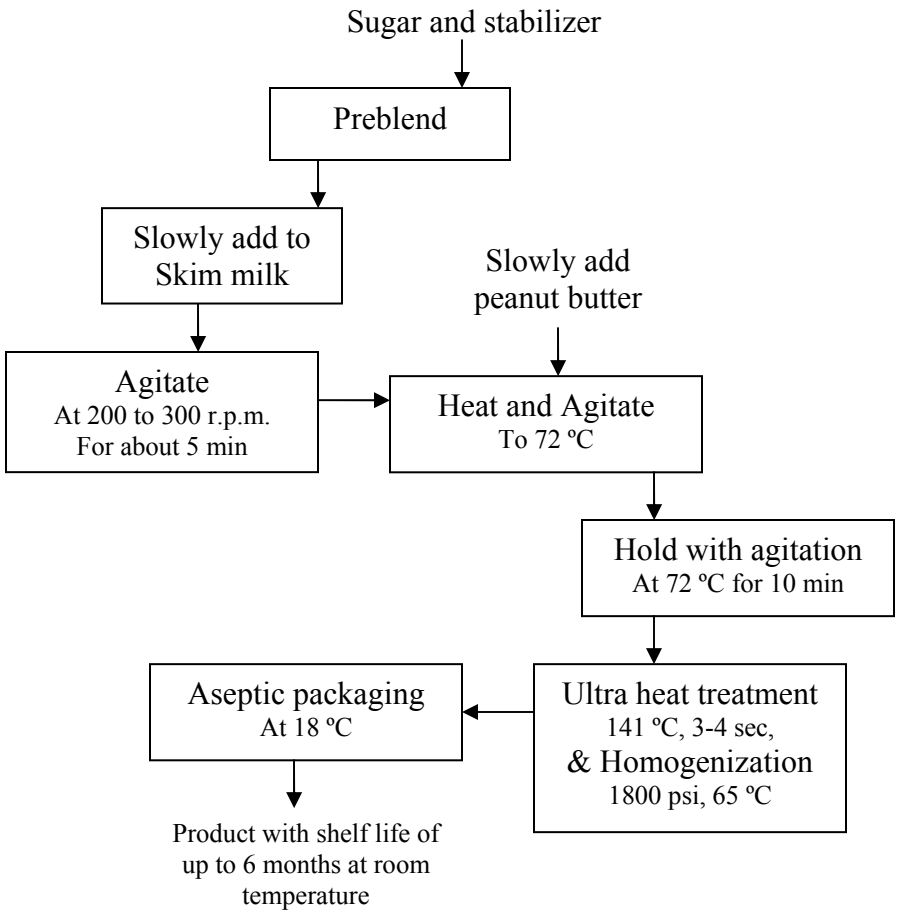


Figure 2.12: Making a natural food peanut punch (Abdul 1988)

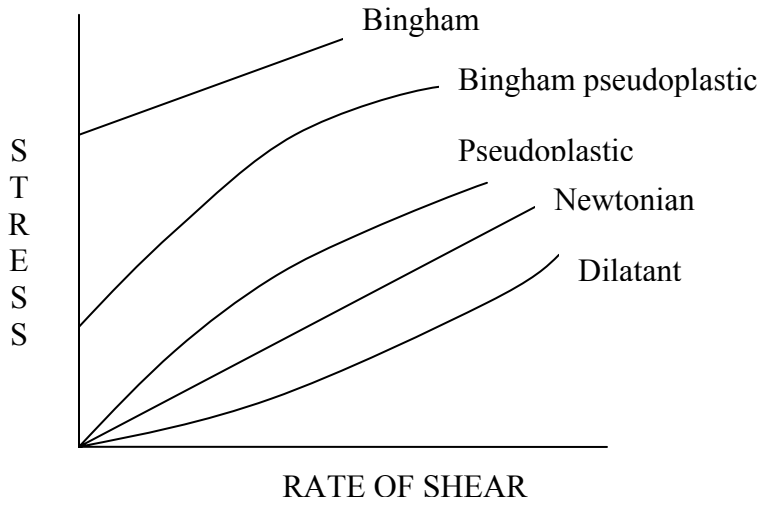


Figure 2.13: Classification of fluids based on the relationship between the shear stress required to induce flow at various rates of shear (Toledo 1980)

# CHAPTER 3

# FORMULATION AND PROCESSING PROTOCOL OF A CHOCOLATE-FLAVORED PEANUT-SOY BEVERAGE[1]

[1] Deshpande, R. P., Chinnan, M. S., and Phillips, R. D. To be submitted to the *Journal of Food Science.*

# ABSTRACT

A new beverage product was developed utilizing two protein rich oilseed sources namely peanut and soy. Medium roasted peanut flour and chocolate flavor were incorporated to offer pleasant flavor profile. The peanut-soy combination would also improve essential amino acid profile especially that of lysine, compared to an all-peanut product. A pilot-plant scale beverage processing protocol involved filtration, homogenization, and pasteurization as the major operating steps. Beverage formulation employed a three-component constrained mixture design. Low and high bound constraints were determined for peanut (30.56%-58.70%), soy (28.26%-43.52%) and chocolate syrup (13.04%-25.93%) based on lysine content, viscosity and visual stability index values of 51 mg/g protein, 36.9 mPa s, and 1.00, respectively.

INDEX WORDS: Chocolate-flavored peanut-soy beverage, Three-component constrained mixture design, Lysine content, Viscosity, Visual stability index

# INTRODUCTION

The United States ranks third in world peanut production with about 39% of the total crop grown in Georgia. Apart from the ever increasing range of traditional peanut-based products, recently, there is growing interest in expanding the utilization of peanuts through protein-based beverage products. Earlier studies on Miltone vegetable toned milk (Chandrasekhara and others 1971), peanut beverage (Rubico and others 1987, 1988, 1989; Galvez and others 1990), chocolate flavored peanut beverage (Chompreeda and others 1989), UHT-Sterilized peanut beverage (Rustom and others 1995, 1996), and partially defatted, roasted peanut beverage (Hinds and others 1997a, 1997b, 1997c) show that peanut has been exploited in several milk-type products with continual advancements with respect to physicochemical, nutritional and sensory characteristics as well as beverage preparation methods.

Several cereal- and legume-based foods using peanuts as protein supplement have been developed to alleviate malnutrition (Singh and Singh 1991). Dietary deficiencies of protein and calories, particularly among preschool children and lactating women in developing countries, is one of the essential factors causing food scientists and nutritionists to undertake development of nutritionally balanced protein-based foods. The nutritive value of peanut protein is function of its protein content (peanut flour = 47.0-55.0%), amino acid composition, and protein digestibility. Peanut protein is deficient with respect to certain essential amino acids, for example, lysine, tryptophan, threonine and sulphur containing amino acids but its true digestibility is comparable with that of animal protein (Singh and Singh 1991). When plant proteins are consumed as human foods considerable emphasis is given on designing protein blends of cereals and legumes to correct imbalances among amino acids from the nutritional point of view (Singh and Singh 1991). As soy-based foods are increasingly included in the

national diets they are suitable vehicles for fortification to meet known deficiencies in essential nutrients. Such fortification would serve as a complete nutritional supplement in the form of a beverage for children and specific groups of people (Ang and others 1985). Besides providing an alternative to traditional dairy products, soy protein is being incorporated into many nutritious meal-replacement beverages, offering convenience to on-the-go consumers (Riaz 1999). Soybean is the only legume that contains all nine essential amino acids at the levels that meet the human requirements (Ang and others 1985). It is categorized as a source of high-quality protein – equivalent to meat, milk, fish, and eggs – that also provides other essential nutrients (Tockman 2002). The soy ingredients have different nutritional and functional properties which enable developers to formulate high-protein foods and allow processors to optimize product nutrition, functionality and cost. Whether due to its health benefits or its ability to solve a variety of formulation challenges, soy protein can be found in foods ranging from ice cream to burgers, from milk to nut products, and tofu to a variety of meat alternatives (Roberts 2003). Therefore, two sources of soy protein: soy flour (40-50% protein) and soy protein isolate (90% protein) were selected to combine with the peanut protein.

Although advances are being made in the utilization of soy proteins in beverages, they can not be used in a clear liquid-based product (because of suspended particles resulting from long-chain protein molecules) and they also impart a beany flavor (Roberts 2003). The undesirable bitter or green, beany notes of soy proteins have led to low consumer acceptability limiting its use in vegetable protein-based foods (Rackis and others 1979). Still, consumer enthusiasm for soy protein-based beverages is growing as attempts to combine flavors and ingredients are on the rise. Flavor masking, using several approaches, is the best tool that has been developed to compensate for bitter, green, beany, and other off-flavors of individual protein

sources or combinations in various applications (Gonzalez and Draganchuk 2003). Chocolate has proven to be a popular flavor for soy-based beverages, due to its ability to provide a smooth and pleasant flavor profile. Formulators have more difficulty getting subtle flavors such as vanilla and fruit flavors to come through since soy proteins have tendency to "soak up" flavors (Brandt 2002). Wang and others (2001) observed that addition of chocolate, almond, and vanilla flavorings diminished beany flavor of soymilk, but the chocolate flavoring exhibited the best effect in enhancing overall sensory quality. When determining a flavor for a new product, consideration should be given at the outset to the types of flavors that are appropriate for the target consumer, as well as flavors that will work well with other ingredients in the formulation (Gonzalez and Draganchuk 2003). Hence, in this study of peanut-soy protein-based beverage the most popular beverage flavor – chocolate – in the form of added ingredients (cocoa powder and artificial chocolate flavor) was used. As consumers have become better informed about nutrition, food manufacturers face the challenge of providing nutritious food that ensures appealing taste, texture, and appearance (Riaz 1999). The beverage business is beginning to focus more on consumer needs as both are seeking new product options. Thus, innovative terminologies such as flavored milks, energy beverages, ready-to-drink meals and functional beverages have been created (Sloan 2003). Keeping the current consumer trend of convenience and nutrition in mind, this study to develop a new chocolate-flavored, peanut-soy-based, nutritious beverage was undertaken.

The objective of this study was to develop a nutritional beverage formulation utilizing three major components: peanut, soy and chocolate. The idea was to achieve nutritional and functional benefits of roasted peanut flour, soy (either flour or protein isolate) and chocolate at optimum levels so as to complement each other giving the most acceptable new beverage

product. The ranges of three components were based on viscosity, visual stability, and essential amino acid content, especially lysine. The use of various other ingredients (stabilizers, emulsifiers, peanut oil, flavoring agents, and sweeteners), variations in beverage formulation, and various beverage processing parameters were identified through extensive literature review and preliminary beverage preparation trials. The new beverage product developed is expected to have improved nutritional and sensory attributes.

## MATERIALS AND METHODS

**Materials** Medium roast, partially defatted (12% fat) peanut flour was obtained from Golden Peanut Company, Alpharetta, Ga., U.S.A. Soy protein isolate (Prolisse™) and defatted soy flour were obtained from two different plants of Cargill Soy Protein Solutions, one of them at Sidney, OH and another at Cedar Rapids, IA, U.S.A., respectively. Maltodextrin (AVEBE MD 20) was donated by AVEBE America Inc., Princeton, N.J., U.S.A. Soy lecithin (LECIGRAN™ 5750) was provided by Riceland Foods, Inc., Lecithin Division, Stuttgart, Ark., U.S.A. Artificial chocolate flavor was supplied by Carmi Flavor & Fragrance Co., Inc., Commerce, Calif., U.S.A. The carrageenan stabilizer (Satiagel X-amp 4000) was provided by Degussa Texturant Systems Sales, LLC, Atlanta, Ga., U.S.A. Commercial chocolate milk (Hershey®'s creamy chocolate milk shake), corn syrup (light), vanilla extract (pure), cocoa powder (Hershey®'s European style, Dutch processed cocoa), sucrose, peanut oil, and salt were purchased locally.

**Formulation and processing parameters** The selection of key ingredients was based on a survey of popular nutritious drinks and flavored soymilks available in the market. Ingredient proportions and necessary processing parameters for the beverage preparation were derived from an extensive literature review on peanut and soy – based milk-type products. Preliminary trials done were also instrumental in deriving a pilot-plant scale beverage preparation protocol.

**Product design** A three-factor constrained mixture design was developed using peanut (30.56%-58.70%), soy (28.26%-43.52% either soy flour, SF or soy protein isolate, SPI) and chocolate syrup (13.04%-25.93%). The lower and upper bound constraints for the primary components were based on lysine content (51.0 mg/ g protein), viscosity (36.9 mPa s), and visual stability index (VSI = 1.00). The physical property, VSI is a measure of stability of beverage sample which is based on the visual separation of solid and liquid phases. The viscosity of samples was measured instrumentally. The lysine content was obtained from the dietary reference intake guidelines on essential amino acid consumption (Table 3.2). The viscosity of commercial chocolate milk (Hershey®'s creamy chocolate milk shake) and visual stability of commercial cow's milk were the basis of determination of physical properties of the beverage developed.

**Beverage preparation** Base stocks of filtered slurries of peanut flour, soy flour (SF) and soy protein isolate (SPI) were prepared separately for use in beverage formulations. The pilot-plant scale processing protocol as shown in Fig. 3.1a, 3.1b and 3.1c was designed.

*Preparation of base stocks* All the three base stocks, peanut, soy flour, and soy protein isolate, were prepared separately as per the following steps. Medium roast partially-defatted peanut flour, defatted soy flour and soy protein isolate were finely ground in a Morehouse mill (Model M-MS-3, Morehouse Industries, Los Angeles, Calif., U. S. A.). The ground flours (250 g) were mixed with tap water (2400 g) and homogenized (34474 kPa, room temperature, five passes) in a laboratory homogenizer (APV Gaulin Homogenizer Model 15 15MR-8TBA, Everett, Mass., U. S. A.). The homogenized slurries were filtered separately, through 273-mesh filter screen fabricated from PVC pipe and polyester mesh material (McMaster Co., Fulton Industrial Blvd.,

Atlanta, Ga., U. S. A.) having quoted mesh size of 53 microns. The residues collected on the filter screen were discarded and filtrates stored for use as the base stocks (Fig. 3.1a).

*Preparation of chocolate syrup* Various ingredients such as water, sucrose, corn syrup, matlodextrin, cocoa powder, peanut oil, vanilla extract, salt, soy lecithin, artificial chocolate flavor, and stabilizer (Fig. 3.1b) were mixed in appropriate quantities. [The exact proportions of these ingredients have not been disclosed since the product being developed is an invention under review by The University of Georgia Research Foundation, Inc., Athens, Ga., U. S. A.]. The mixture was heated to 75 ºC in a steam kettle, mixing continuously with a hand-held blender. It was allowed to cool to room temperature and then ready to be used in the beverage.

*Processing of beverage* The final beverage formulations were prepared using the base stocks of peanut slurry, soy slurry (either SF or SPI) and chocolate syrup (a liquid blend of various other ingredients prepared as described in Fig. 3.1b) mixed in predetermined proportions (Fig. 3.1c). The mixture was heated to 75 ºC in a steam kettle, stirring constantly with a hand-held blender. It was homogenized (34474 kPa, 72 ºC, three passes) in a laboratory homogenizer and pasteurized (93 ºC for 1 s) followed by cooling (to about 35 ºC) in a plate heat exchanger (Armfield FT74, UHT Unit, Airfield Ltd., Ringwood, Hampshire, England, BH24 1 DY). The beverage was then filled into sterililized, labeled 250 ml bottles and stored at 4 ºC.

**Measurement of nutritional and physical properties** Nutritional properties of the beverage were based on lysine content (mg/ g protein) of final beverage formulation. The lysine contents were calculated based on the compositions of peanut flour, soy flour and soy protein isolate (Table 3.3) and the estimated percent protein recovery (Table 3.4) in the base stocks. Different beverage samples prepared using the pilot-plant scale processing protocol were evaluated after storing overnight as well as for a period of one week. Physical properties - viscosity (η) and

visual stability index (VSI) were measured. The viscosity was measured using a Brookfield Viscometer (Model LVDV-II+, Brookfield Engineering Laboratories, Inc., Stoughton, Mass.) equipped with small sample adaptor (SSA 18/13R, 25 ºC, 20 rpm). The visual stability index was measured by taking the ratio of the total height of the beverage to the height of the sediment collected after separation (Hinds and others 1997a, 1997b).

**Percentage protein recovery** Percentage protein recovered in the base stocks (filtered protein slurries) were calculated as shown in Table 3.4. It was based on the amount of flour used as a starting material, amount of water added and average weight of dry residue collected after filtration. Following formula was used for the calculation of recovery:

$$\text{Protein recovery (\%)} = \frac{\text{(g of flour as a starting material)} - \text{(g of dry residue after filtration)}}{\text{(g of total filtrate collected)}} \times 100$$

The respective protein recovery values of peanut (7.69%), soy flour (8.19%), and soy protein isolate (9.43%) were used to calculate protein and lysine content of mixture of peanut and soy slurries for different experimental formulations.

**Low and high level constraints from lysine content** Based on lysine content of 51 mg/g protein (Table 3.2) as a reference value, high and low constraints of lysine content were determined as 58.7 mg/g protein (115% of reference value) and 43.4 mg/g protein (85% of reference value), respectively. In order to get these desired high and low levels of lysine contents in the final beverage, different amounts of peanut and soy slurries containing the known amount of protein and lysine contents were used. Details of calculations are given in the APPENDIX A. From various combinations of peanut and soy slurries it was found that a combination of 1350 g of peanut slurry and 650 g of soy flour slurry resulted in lysine content of 44.1 mg/ g protein (a value close to lower limit of 43.4 mg/g protein) and another combination of

1175 g of peanut slurry and 825 g of soy protein isolate slurry resulted in lysine content of 57.1 mg/g protein (value close to higher limit of 58.7 mg/g protein). Based on these lysine content values the low and high bound constraints were determined as 825 g and 1350 g for peanut; 650 g and 1175 g for soy, respectively (Table 3.5).

**Beverage preparation trials** Using the beverage processing protocol described in Fig. 3.1a, 3.1b, and 3.1c preliminary beverage formulation trials were conducted. In high peanut (1350 g) low soy (650 g) and low peanut (1175 g) high soy (825 g) experimental combinations different levels of chocolate syrup (300 g, 500 g, and 700 g) were added. These preliminary formulations were analyzed for viscosity and VSI values (data not reported). Viscosity of commercial chocolate milk (36.9 m Pa) and VSI of commercial cow's milk (1.00) was the basis to obtain 300 g as the lower limit and 700 g as the higher limit of chocolate syrup in the beverage. These limits gave the low and high level constraints of chocolate in the mixture design (Table3.6).

Using the lower and upper bound constraints for peanut, soy and chocolate as given in Table 3.6 a three component constrained mixture design was obtained. Six mixture proportions (3 with 13.04% and 3 with 25.93% of chocolate syrup) were selected for experimental beverage preparation trials. Using these proportions and the beverage processing protocol 12 experimental beverage formulations were prepared (6 soy flour based and 6 soy protein isolate based). All these formulations were analyzed for viscosity and VSI properties (Table 3.7).

## RESULTS AND DISCUSSION

### Development of new beverage product

*Processing parameters* Earlier work done in our laboratory on peanut beverage preparation (Hinds and others 1997a, 1997b, 1997c) guided the development process of chocolate-flavored, peanut-soy beverage. The selection of type of stabilizer (carrageenan) and choice of milling and

homogenization operations were based on particle recovery and stabilizer evaluation study (Deshpande and others 2003). Initial trials for determination of processing steps were done using mixtures of 50% soy (either flour or isolate) and 50% dark roasted peanut flour. Different treatments such as Morehouse milling and filtration, homogenization and filtration, and Morehouse milling, homogenization and filtration indicated that the combination of all three operations was the best treatment giving smooth mouthfeel to the beverage. These observations were in agreement with the findings of Mustakas (1974) during the study on LPC (lipid protein concentrate)-based beverages which mentioned that high pressure homogenization (55158 kPa) reduced particle size and yielded beverages with better mouthfeel than low-pressure homogenization (24132 kPa). Also, without the use of both colloidal mill and homogenizer, poor mouthfeel, higher viscosity, and some sedimentation occurred. Hence peanut flour, soy flour, and soy protein isolate powder were ground in Morehouse mill, homogenized after mixing with water, and the slurries were filtered to give base stock slurries of peanut, soy flour and soy protein isolate (Figure 3.1a). Thus, milling, homogenization, and filtration were important operating stages of beverage preparation.

Pasteurization in a plate heat exchanger was done in order to increase shelf-life of the beverage and allow instantaneous cooling while the product is in motion thereby, improving stability and consistency of the final product. The pasteurization temperature-time conditions were obtained by referring to Grade "A" Pasteurized Milk Ordinance (PMO). From the various suggested temperature-time combinations, 93 ºC and 1 s combination was selected. Since the product contains added ingredients (sweeteners, cocoa powder, flavors etc.) the operating temperature (93 ºC) was slightly higher (by 4 ºC) than that suggested for milk (89 ºC). Health-conscious consumers generally like fresh food, so soymilks with mild thermal treatment

(pasteurization) would conceivably better suit consumers than those receiving intense thermal treatment (Wang and others 2001). Wang and others (2001) observed that pasteurized soymilks were stable for one month with refrigeration storage, and formulations with a combination of chocolate flavoring and iota-carrageenan gum permitted production of low-heat-treated soymilk acceptable to consumers. Also, Ang and others (1985) observed that soymilk of high protein content (~2%) generally undergoes precipitation during sterilization at 120 ºC for 10 to 15 min. They also observed that there is a tendency to impart a darker color to product which is slightly intensified during sterilization. Considering such observations, mild heat treatment (pasteurization at 93 ºC) for short time (1 second) was finalized for this study.

Peanut milk preparation studies done by Rubico and others (1987, 1988, 1989), a chocolate flavored peanut beverage study by Chompreeda and others (1989), peanut beverage optimization study by Galvez and others (1990), a study on utilization of peanut extract for beverage preparation by Rustom and others (1995, 1996), and a roasted peanut beverage investigations done by Hinds and others (1997) were instrumental in deciding final pilot-plant scale protocol of the chocolate-flavored, peanut-soy beverage being developed in this study.

*Selection of key ingredients* Various milk-like beverages have been prepared from aqueous extracts of oilseeds such as peanuts or soybeans (Lee and Beuchat 1992; Nelson and others 1976). These oilseeds usually contain the base ingredient but sugar, emulsifiers, stabilizers, additives, and flavors are added to improve the stability and acceptance of the product (Lee and Rhee 2003). Apart from off-flavor, astringent or bitter aftertaste is another factor contributing to low acceptability of soymilks, and may explain the reason that many commercial soymilks in the U. S. are formulated with a variety of adjunct ingredients, cocoa powder, vanilla extract, malt extract, seaweed extract, β-carotene, gums, and others to improve the palatability of soymilk

(Wang and others 2001). In general, physicochemical properties of the beverages are influenced by many factors including functionality of ingredients, processing conditions, and interactions between colloidal ingredients (Cano-Ruiz and Richter 1998). Protein-flavor interactions are a hot topic that continues to spur research at both supplier and research institutes (Brandt 2002).

Sensory analysis results by Wang and others (2001) showed that chocolate and almond flavorings improved the aroma of soymilks ($p < 0.05$), and addition of gum partially masked the beany off-flavor. Sensory evaluation and response surface methodology study done by Chompreeda and others (1989) indicated that the optimum formulation of the chocolate flavored peanut beverage was obtained by using protein isolate 3.5%, butter fat 3.5%, sugar 8%, cocoa powder 0.7%, stabilizer 0.1%, and water with all sensory characteristics acceptable in the range of like to extremely like. A low fat beverage with roasted peanut flavor developed by Hinds and others (1997a, 1997b, 1997c) was a potential milk-substitute containing 11.8% total solids, 2.0% fat, and 3.7% protein with whitish orange-yellow color. A formulation developed by Osborn and others (2003) for chocolate-flavored nutritional beverages had various ingredients like corn syrup, sucrose, maltodextrin, soy lecithin, cocoa powder and others in water.

To our knowledge, a combination of peanut and soy in the beverage product has not been previously reported. Also, soy proteins have a major problem of imparting beany flavor (Rackis and others 1979; Roberts 2003). Wang and others (2001) observed that panelists preferred flavored soymilk over plain soymilk, and they liked chocolate flavor better than other flavors. Also, chocolate has ability to mask protein flavor and provide a smooth profile (Brandt 2002). However, the important factor, while using cocoa powder as an ingredient in liquid products, is the stability. Maintaining a continuous network formed by interaction of protein and protein covered cocoa particles in the presence of a suitable stabilizer (carrageenan) helps to prevent

sedimentation and to some extent segregation (Vliet and Hooydonk 1984; Boomgaard and others 1987). Carrageenan's high degree of reactivity with proteins which results in a three-dimensional network holding cocoa powder in suspension at levels as low as 0.25% makes it especially valuable, and for this reason, kappa-carrageenan is found in majority of flavored refrigerated dairy-and soy-based beverages, its effectiveness being enhanced through addition of other hydrocolloids imparting viscosity (Klahorst 2002).

Hence, after finalizing the operating conditions for the beverage processing, various pilot-plant scale beverage preparation trials were performed to derive suitable ingredient proportions [The exact proportions of these ingredients and data related to these trials have not been reported since the product being developed is an invention under review by The University of Georgia Research Foundation, Inc., Athens, Ga., U. S. A.]. The key ingredients (Table 3.1) for the experimental trials were selected from the most common ingredients listed on nutritional beverage products available in the market and their respective functional advantages.

The ingredient proportions except for the protein sources were based on the beverage preparation trials using 50% peanut (dark and medium roasted flours) and 50% soy sources. The preliminary sensory evaluation by the members of our laboratory (data not reported) indicated that medium roasted peanut flour gave better flavor and aftertaste. The sensory attributes along with viscosity and visual stability measurements were helpful in deciding the amounts of stabilizer, emulsifier, oil, sweeteners and flavoring agents. However, it was observed that the proportions of peanut, soy and all other ingredients were interdependent. This observation led to a mixture design approach for the experimental trials. A mixture experiment is an experiment in which the response is a function only of the proportions of the components (constituents) present in the mixture and is not a function of the total amount of the mixture (Cornell 1973). Therefore,

the beverage ingredients were grouped into three major components as peanut, soy and chocolate. The two components peanut and soy represent the base stocks and the third component chocolate represent chocolate syrup obtained by the definite protocols developed as shown in Fig. 3.1a,3.1b,3.1c. The determination of acceptable ranges of these three major components in the mixture design (Table 3.6) based on physical properties of beverage (Table 3.7) was done by further trials on the beverage preparation.

**Mixture design approach and determination of high and low level constraints** It was observed from the beverage preparation trials (Table 3.7) that very low levels of chocolate syrup resulted in poor suspension of particles in the beverage, on the other hand very high amounts resulted in very thick formulations as compared to the control (Hershey®'s creamy chocolate milk shake). Thus, the chocolate syrup (the liquid blend of various other ingredients prepared as described in Fig. 3.1b) had a functional as well as flavor role. However, it is most probably specific components of the syrup such as sucrose, corn syrup, maltodextrin, soy lecithin, and stabilizer that had this effect. Higher proportions of peanut and soy also resulted in thicker formulations due to increased concentrations of proteins which play important role during heat treatment. In a peanut beverage study done by Rubico and others (1988) viscosity (55-1950 mPa s) was dependent on composition, homogenization pressure, and temperature; temperature significantly affecting viscosity probably due to changes in protein and carbohydrate structures. In agreement to findings of Rustom and others (1996) a possible cause of gelation in chocolate-flavored peanut beverages could be interaction of peanut protein with polysaccharide thickening agent (carragennan) leading to aggregation of molecules and subsequently increasing the viscosity. As observed by Hinds and others (1997a, 1997b, 1997c) stability, viscosity, and mouthfeel characteristics of peanut beverage were dependent upon amount and type of stabilizer

used, heating and cooling protocols, protein-lipid concentrations, and particle size. In agreement to the fact stated by Brandt (2002) that protein denaturation or the unfolding of the three-dimensional protein structure resulting from chemical or heat, altered the binding sites for flavor molecules, and protein could bind more or less of a flavor compound depending upon the amount of heat treatment. The protein sources also imparted off-flavor at higher proportions.

Therefore, desirable range of all the key ingredients which can result in an acceptable beverage product was of interest. The choice of constraints is crucial because the constraints on the individual factors influence the design (Gorman 1966). Often in exploratory work the constraints can not be set precisely and, in fact, may have to be estimated experimentally (Gorman 1966). Hence, the lower and upper bound constraints of three major components (peanut, soy, and chocolate syrup) in this study were based on three factors:

1) Lysine content
2) Viscosity and
3) Visual stability index

*Lysine content* Since peanut is mainly deficient in lysine and soy is a good complementary lysine source, an essential amino acid, lysine was chosen as the basis for deriving a range of the best combinations of peanut and soy that can be expected to result in a nutritionally superior beverage product. Lysine content in mg/g protein of experimental beverage formulations was calculated as explained in APPENDIX A.

The comparison of different lysine content values of different combinations of peanut and soy slurries was done. The high (58.7 mg/g protein) and low (43.4 mg/g protein) constraints of lysine content were basis for this comparison. This resulted into maximum and minimum quantities of peanut and soy slurries that can be used in the beverage. The highest amount of

peanut slurry (1350 g) that can be added to the lowest amount of soy slurry (625 g) resulted into 44.1 and 50.2 mg/g protein for SF and SPI formulations, respectively. Similarly the lowest amount of peanut slurry (825 g) added to the highest amount of soy slurry (1125 g) resulted into 50.8 and 57.1 mg/g of protein for SF and SPI, respectively. The calculated values were close to the reference value of 51 mg/g protein.

Essential amino acid composition of peanut protein products showed that lysine contents (g/100 g protein) of peanut kernel, flour, protein concentrate, protein isolate were 3.5, 4.0, 2.9, and 3.0, respectively, as against the FAO pattern of 5.5 (Natarajan 1980). Another study done by Ang and others (1985) showed that lysine content (mg/g of protein) was 70, 76, 87, 76, and 72 in whole egg, cow's milk, human milk, soymilk, and spray dried soymilk powder, respectively, with lysine requirement for an adult being 500 (male)-800 (female) mg/day. From the amino acid profiles of peanut beverages developed by Rubico and others (1989), it was observed that raw peanut extract had lysine content (g/100 g protein) of 4.31, for 71 ºC homogenized peanut milk it was 5.16, and it decreased to the range of 2.88-3.81 for samples processed at higher temperatures and time conditions (85 ºC, 100 ºC, and 121 ºC for 15 min, and 121 ºC for 3 sec). It was suggested that various heat treatments can affect some amino acids; however, actual significance of the apparent changes in the lysine content was not clear in the absence of biological data. These lysine content values in the literature indicated that those estimated in this study (Table 3.5) were in the expected range and close to the reference lysine content value of 51 mg/g protein (Table 3.2).

*Viscosity and visual stability index* It was found that visual stability index (VSI) and viscosity (η) values ranged from 0.87-0.99 and 17.7-131.8 mPa s, respectively (Table3.7). It can be observed that the visual stability was improved by the addition of carrageenan since VSI

values closer to commercial cows milk (VSI = 1.00) as compared to VSI of peanut beverage (0.49-0.71) prepared by Hinds and others (1997a, 1997b, and 1997c). Table 3.7 showed that the new product being developed had stability values similar to that of cow's milk (VSI = 1.00), and viscosity values comparable to that of commercial chocolate milk ($\eta$ = 36.9 mPa s).

The estimated range of lysine contents from 44.1 to 57.1 mg/g protein resulted in the trial formulations having viscosity and visual stability values ranging from 17.7 to131.8 mPa s and 0.63 to 0.99, respectively. In this way, based on the estimated lysine contents and measured values of physical properties of beverage formulations, peanut range of 825 g-1350 g, soy range of 650 g-1175 g, and chocolate range of 300 g-700 g ultimately gave the lower and upper bound constraints of the mixture design as 30.56%-58.70%, 28.26%-43.52%, and 13.04%-25.93%, respectively (Table 3.6).

The purpose of finding low and high level constraints for three major components of the mixture design was obtaining the range of possible number of mixture formulations in order to study the acceptability of the new product being developed in this study. The pilot-plant scale protocol developed in this study will help in obtaining different beverage formulations repetitively without variations in the method of preparation. The chocolate-flavored, peanut-soy beverage thus developed is expected to have improved nutritional and sensory properties because of the combination of low cost, easily available peanut protein, high quality soy protein and popular chocolate flavor. Further study on consumer acceptability and nutritional characteristics of the chocolate-flavored, peanut-soy beverage will help ensuring the success of the new product developed in this study.

**Future work**

The extreme vertices design will be developed as a procedure for conducting experiments with mixtures since several factors have constraints placed on them (McLean and Anderson 1966). The experimental points will be obtained by using such extreme vertices design. All those formulations will then be subjected to consumer affective sensory evaluation in order to study sensory attributes of the new product developed and know its acceptability among the target consumers. Hence, the future work of this study will be conducting sensory evaluation of the new product developed and studying its consumer acceptability.

## CONCLUSIONS

A chocolate-flavored, peanut-soy beverage was developed by utilizing two oilseed protein sources: peanut and soy. The key ingredients were selected; the formulation and processing parameters were obtained to develop a pilot-plant scale beverage processing protocol useful for further study on sensory attributes of the beverage. The lysine contents were estimated to be in the range of 44.1-57.1 (mg/g protein) as compared to the desired value of 51.0 mg/g protein. The viscosity (η) values of the trial formulations were in the range of 17.7-131.8 mPa s as compared to commercial chocolate milk (Hershey®'s creamy chocolate milk shake) viscosity measured as 36.9 mPa s. The visual stability index (VSI) values of trial formulations were also found to be in the range of 0.63-0.99 which was close to that of commercial cow's milk value of 1.00. The ranges of lysine content, η and VSI values resulted into the low and high level constraints for a three factor mixture design. The three major components of this mixture design were peanut, soy and chocolate syrup (a liquid blend of various other ingredients) with low and high level constraints as 30.56%-58.70%, 28.26%-43.52% and 13.04%-25.93%, respectively.

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Table 3.1: Chocolate flavored peanut-soy based beverage ingredients and their functions

| **Ingredient** | **Function** |
|---|---|
| Medium roasted, partially defatted peanut flour | Protein source and roasted flavor enhancement |
| Soy flour/ soy protein isolate | Source of good quality protein |
| Water | Suspension medium |
| Sucrose, corn syrup, and maltodextrin | Flavor, sweetness, consistency and body enhancement |
| Peanut oil | Peanut flavor enhancement and emulsification |
| Cocoa powder and vanilla extract | Flavor enhancement |
| Soy lecithin | Emulsification and consistency enhancement |
| Artificial chocolate flavor | Flavor enhancement |
| Carrageenan | Stabilization, and viscosity, body, consistency, and mouthfeel enhancement |
| Salt | Taste improvement |

Table 3.2: Summary FNB/IOM 2002 amino acid scoring pattern for use in children ≥1 year of age and in all other older age groups (NAP 2002)

| Amino Acid | mg/g protein |
|---|---|
| **Histidine** | **18** |
| **Isoleucine** | **25** |
| **Leucine** | **55** |
| Lysine | 51 |
| **Methionine + cysteine** | **25** |
| **Phenylalanine + tyrosine** | **47** |
| **Threonine** | **27** |
| **Tryptophan** | **7** |
| **Valine** | **32** |

Table 3.3: Amino acid profile (USDA-NAL 2003)

| Nutrient | g/100 g of edible portion | | |
|---|---|---|---|
| | **Defatted peanut flour** | **Defatted soy flour (SF)** | **Soy protein isolate (SPI)** |
| Proteins | 52.2 | 51.5 | 80.7 |
| **Amino Acids** | | | |
| **Tryptophan** | **0.51** | **0.68** | **1.12** |
| **Threonine** | **1.79** | **2.04** | **3.14** |
| **Isoleucine** | **1.84** | **2.28** | **4.25** |
| **Leucine** | **3.38** | **3.83** | **6.78** |
| Lysine | 1.87 | 3.13 | 5.33 |
| **Methionine** | **0.64** | **0.63** | **1.13** |
| **Cystine** | **0.67** | **0.76** | **1.05** |
| **Phenylalanine** | **2.71** | **2.45** | **4.59** |
| **Tyrosine** | **2.12** | **1.78** | **3.22** |
| **Valine** | **2.19** | **2.35** | **4.10** |
| **Arginine** | **6.24** | **3.65** | **6.67** |
| **Histidine** | **1.32** | **1.27** | **2.30** |
| **Alanine** | **2.08** | **2.22** | **3.59** |
| **Aspartic acid** | **6.37** | **5.91** | **10.20** |
| **Glutamic acid** | **10.91** | **9.11** | **17.45** |
| **Glycine** | **3.15** | **2.17** | **3.60** |
| **Proline** | **2.30** | **2.75** | **4.96** |
| **Serine** | **2.57** | **2.73** | **4.59** |
| **Tryptophan** | **0.51** | **0.68** | **1.12** |

Table 3.4: Protein recovery in base stocks of peanut, soy flour, and soy protein isolate

| # | Description | Peanut | Soy flour (SF) | Soy protein isolate (SPI) |
|---|---|---|---|---|
| 1 | Amount of ground flour as starting material (g) | 250 | 250 | 250 |
| 2 | Amount of water (g) | 2400 | 2400 | 2400 |
| 3 | Average weight of dry residue collected after filtration (g) | 50.02 | 35.90 | Negligible |
| 4 | Average weight of protein solids recovered in the filtrate (g) = (#1 - #3) | 200 | 214 | 250 |
| 5 | Average weight of total filtrate collected (g) = (#2 + #4) | 2600 | 2615 | 2650 |
| 6 | Percentage proteins recovered (%) | 7.69 | 8.19 | 9.43 |

Table 3.5: Calculation* of lysine content for determination of low and high level constraints

| Nutrient | Units | Experimental beverage formulations** | | | |
|---|---|---|---|---|---|
| | | High peanut[a] + Low soy[a] | | Low peanut[b] + High soy[b] | |
| | | SF*** | SPI*** | SF | SPI |
| $Protein_1$ | g in total beverage | 81.58 | 103.65 | 82.63 | 122.52 |
| $Protein_2$ | g/100 g edible portion | 4.08 | 5.18 | 4.13 | 6.13 |
| $Lysine_1$ | g in total beverage | 3.61 | 5.21 | 4.20 | 7.09 |
| $Lysine_2$ | g/100 g edible portion | 0.18 | 0.26 | 0.21 | 0.35 |
| Lysine content | mg/g protein | 44.11 | 50.23 | 50.82 | 57.09 |
| | g/100 g protein | 4.41 | 5.02 | 5.08 | 5.71 |
| Reference lysine content | | 51.0 mg/g protein or 5.10 g/100 g protein | | | |

* Refer to APPENDIX A for detailed understanding of calculating values given in this table

** For a total of 2000 g of peanut-soy filtrate –

a The highest amount of peanut filtrate (1350 g) that can be added to the lowest amount of soy filtrate, either flour or isolate (650 g)

b The lowest amount of peanut filtrate (825 g) that can be added to the highest amount of soy filtrate either flour or isolate (1175 g)

– in order to get the range of lysine values (mg/ g protein) close to that of reference value

*** SF= Soy flour formulations and SPI= Soy protein isolate formulations

Table 3.6: Calculation of lower and upper bound constraints for mixture design

| **Base stocks** | **Upper limit g (percentage)** | **Lower limit g (percentage)** |
|---|---|---|
| Peanut | 1350 (58.7%) | 825 (30.6%) |
| Soy | 1175 (43.5%) | 650 (28.3%) |
| Chocolate syrup | 700 (25.9%) | 300 (13.0%) |
| Total | 2700 (100.0%) | 2300 (100.0%) |
| **Total weight of peanut and soy base stocks together = 2000 g** | | |

Table 3.7: Physical characteristics of trial experimental formulations (Average values)

| Mixture proportions | | | | Viscosity | Visual stability index |
|---|---|---|---|---|---|
| **Peanut** | **Soy flour** | **Soy protein isolate** | **Chocolate syrup** | **(mPa s)** | **(Ratio)** |
| 0.5869 | 0.2827 | 0 | 0.1304 | 17.7 | 0.89 |
| 0.5107 | 0.3590 | 0 | 0.1304 | 32.2 | 0.87 |
| 0.4344 | 0.4352 | 0 | 0.1304 | 18.3 | 0.96 |
| 0.4580 | 0.2827 | 0 | 0.2593 | 62.8 | 0.98 |
| 0.3818 | 0.3589 | 0 | 0.2593 | 48.4 | 0.98 |
| 0.3056 | 0.4352 | 0 | 0.2593 | 37.9 | 0.99 |
| 0.5869 | 0 | 0.2827 | 0.1304 | 42.4 | 0.98 |
| 0.5107 | 0 | 0.3590 | 0.1304 | 42.9 | 0.99 |
| 0.4344 | 0 | 0.4352 | 0.1304 | 29.8 | 0.63 |
| 0.4580 | 0 | 0.2827 | 0.2593 | 100.5 | 0.99 |
| 0.3818 | 0 | 0.3589 | 0.2593 | 131.8 | 0.99 |
| 0.3056 | 0 | 0.4352 | 0.2593 | 128.7 | 0.99 |

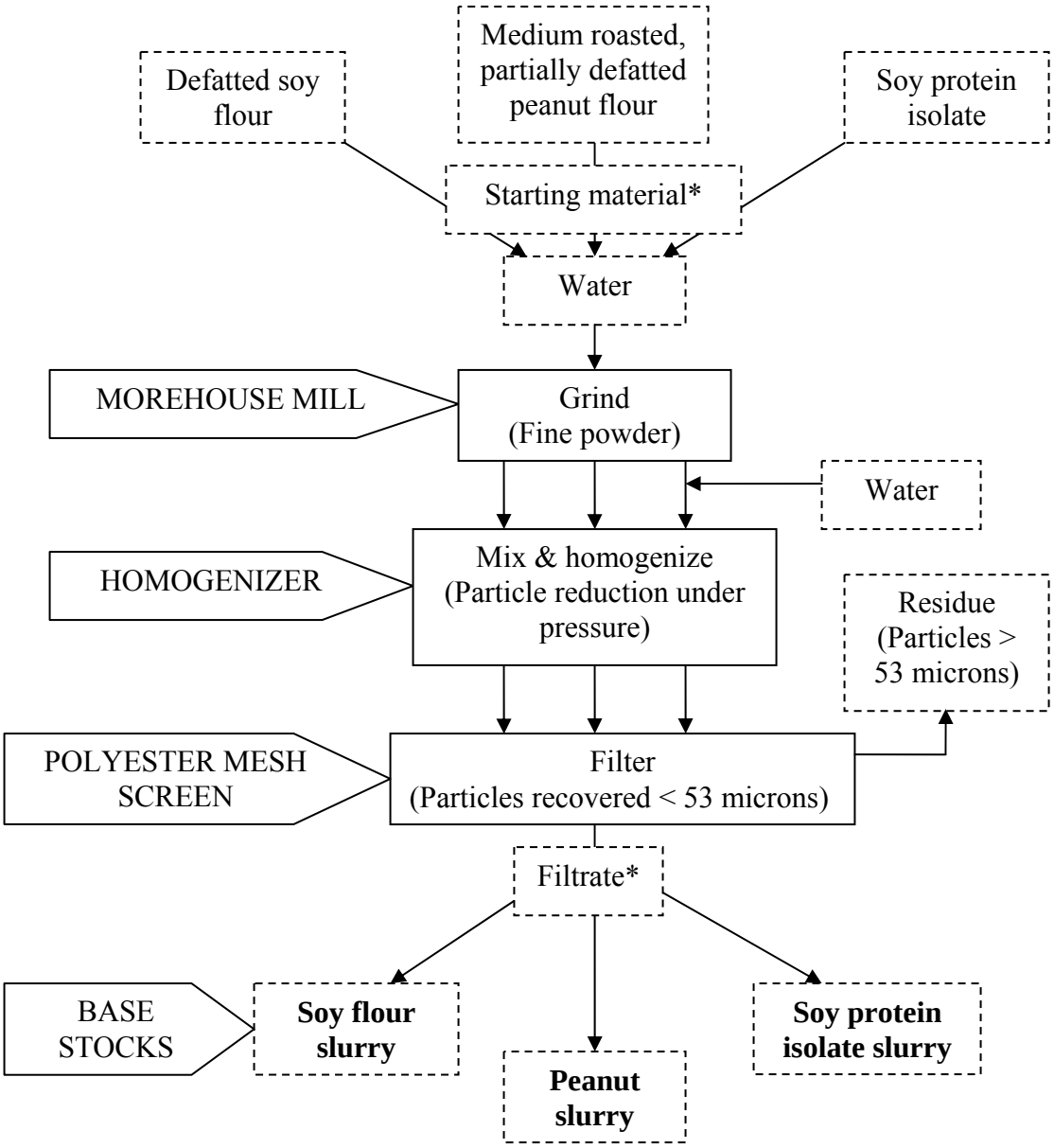


Figure 3.1a: Base stocks of peanut, soy flour (SF) and soy protein isolate (SPI)

*Base stock is obtained form the corresponding starting material. For example, starting with defatted soy flour as a starting material will result into base stock of soy flour slurry.

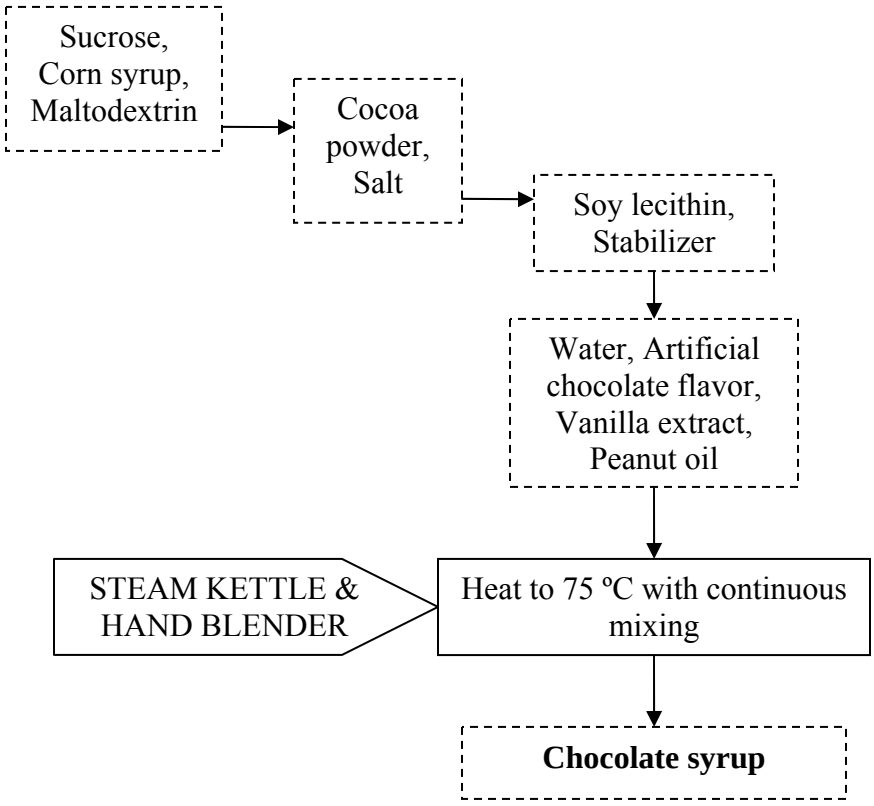


Figure 3.1b: Preparation of chocolate syrup for chocolate-flavored, peanut-soy beverage

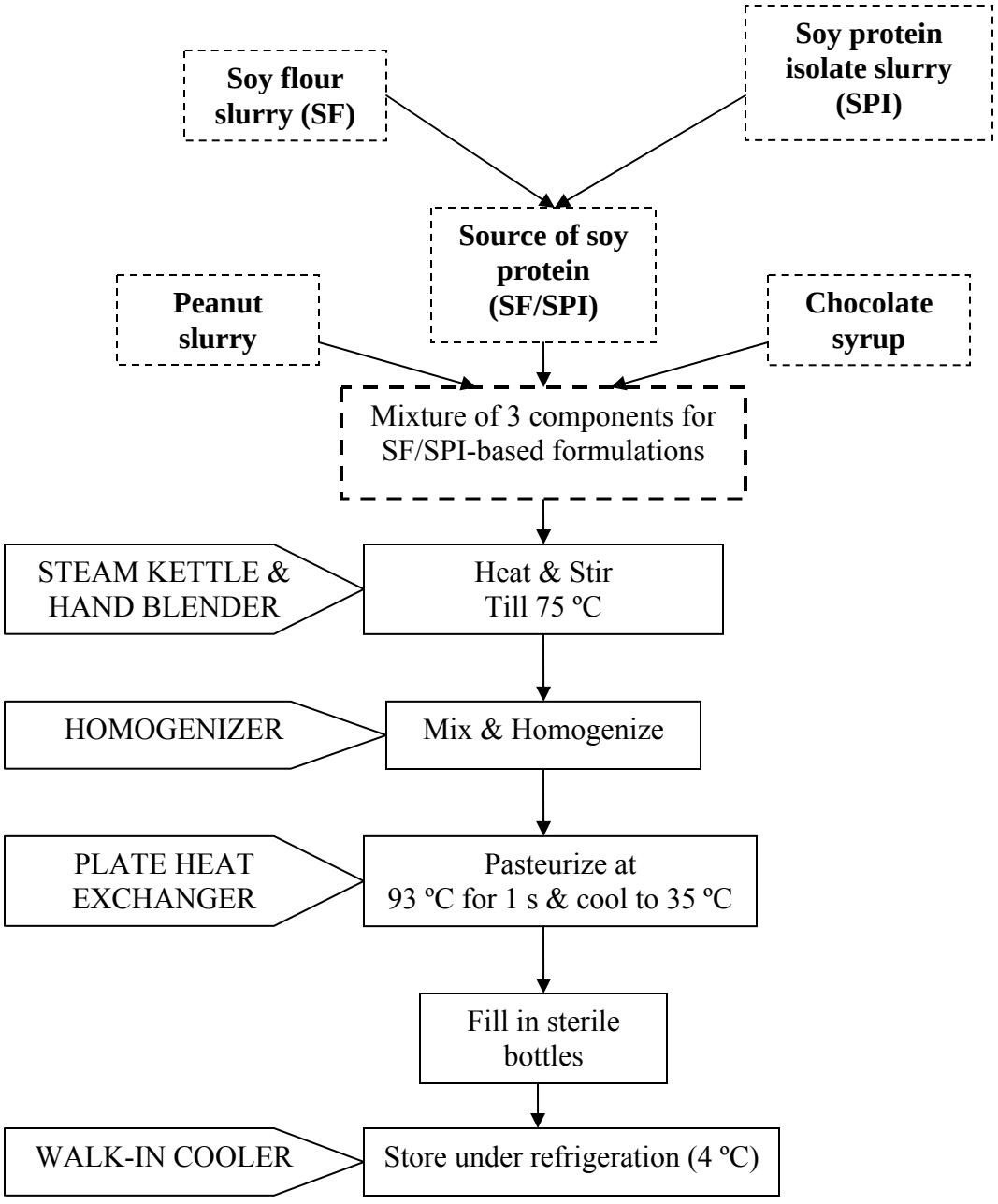


Figure 3.1c: Processing steps for chocolate-flavored, peanut-soy beverage

# CHAPTER 4

# SENSORY ATTRIBUTES AND CONSUMER ACCEPTABILITY OF NEW CHOCOLATE-FLAVORED PEANUT-SOY BEVERAGE[1]

[1] Deshpande, R. P., Chinnan, M. S., and McWatters, K. H. To be submitted to the *Journal of Food Science.*

# ABSTRACT

A chocolate-flavored peanut-soy beverage was investigated for nine sensory attributes employing consumer affective sensory evaluation. Twenty-eight beverage formulations with commercial chocolate milk as a control, were evaluated by untrained panelists (n=41) using a 9-point hedonic scale (1=dislike extremely; 9=like extremely). Mean ratings for the control were between 6 (like slightly) and 7 (like moderately). Formulation #8 having 43.9% peanut, 36.3% soy protein isolate (SPI), and 19.8% chocolate syrup had highest consumer acceptability. As compared to the control, it was rated significantly higher for appearance, color, and sweetness. SPI- based formulations #7, #6, and soy flour-based formulation #14 had the highest mean ratings for color (7.0), aroma (6.2), and flavor (6.0), respectively.

INDEX WORDS: Chocolate-flavored peanut-soy beverage, Sensory evaluation, Chocolate milk, Hedonic scale, Consumer acceptability

# INTRODUCTION

Product development is a part of business strategy as well as a research method in the food industry. It is the starting point for the introduction of a new product and the continuous improvement of a product (Earle 1997). It has always been the heart of the food industry where rapid technological changes, accompanied by a steady increase in the standard of living, resulted in even greater opportunities for product development. Insight into new product development is hinged on insights into human needs (Desrosier and Desrosier 1971). Consumer needs/desires are a major driving force of new food product development, and perceptions about food form the basis of those perceived desires (Bursey 1983). Today's food consumers have adopted different patterns of food consumption suitable to their more mobile and diversified lifestyles; hence the task of the food system is changing from "bringing the consumer to the food" to "bringing the food to the consumer" (Brody and others 2000). The sequence of activities leading to the introduction of a successful new product into today's highly competitive marketplace is founded on extensive and ongoing market research which elicits and defines the changing consumer needs (Bursey 1983).

The beverage business is taking a new shape. The beverage industry is focusing more on consumer desires and looking for alternative product options suitable for a changing marketplace. Recently, beverage products have created new categories and a whole new business through innovative product options. Ready-to-eat, packaged for on-the-go, high protein drinks and meal replacers are some of the top trends consumers are willing to try over the next few years (Sloan 2003a). As a part of convenience and single serving choices, "meals in motion" are becoming more and more popular. Changing life-styles and eating preferences have inspired a new generation of portable products. Health awareness follows the trend of

convenience while choosing suitable food products from a variety of options available. Consumers want access to novel and interesting foods that are fresh, convenient, and tasty. Health is important but not at the expense of flavor. The explosion of scientific information relating diet to health, coupled with the rapid communication of that knowledge to the consumer, has provided a challenge to the American food industry (Bursey 1983). The desire for good health and the concern over carbonated soft drink consumption have driven consumers to juice and juice drinks (Sloan 2003b). Healthier product alternatives, including juice/soy combinations, juice/fruit/dairy smoothies, fortified juices/drinks, and organic drinks, are among those enjoying rapid growth (Sloan 2003b) with 28% of Americans consuming soy foods or soy beverages once a week or more (Ohr 2003). Functional beverages is an altogether new category showing increase in sales indicating that today's health issues drive sales as well. "Better for you" products can help boost beverage sales and have great applications in the beverage industry. Overall, consumers are looking to lead healthier lives, and the beverage industry has tremendous potential for future developments; this is creating a wave of innovative terminologies such as flavored milks, energy beverages, liquid meal-replacements, weight directed products, ready-to-drink meals, and functional beverages.

The nature of change in the beverage industry is remarkable; this changing scenario poses a challenge for the industry as well as opportunity for new product development. However, if a food product cannot be re-engineered or modified to fulfill the consumer desire and ultimately the demand for the product, thus meeting the benchmark goals, it will not succeed (Robinson 2000). Food quality is in the mind of the observer, response to food quality may be a conditioned response which is subject to many variables outside the control of the food processor (Lund 1982). Although commercially processed foods must attract the consumer by the

appearance of the package, repeat purchases will depend on how pleasing a sensory experience is perceived when the product is eaten. And based on the feedback from sensory testing, adjustments then must be made to optimize the sensory properties of the food product, such as, flavor, sweetness, texture, thickness, color, and many such aspects of the product (Pyne 2000). Hence, sensory evaluation can be the best tool to understand consumer preferences in order to direct the process of successful product development.

Sensory evaluation has been defined as "a scientific discipline used to evoke, measure, analyze, and interpret reactions to those characteristics of foods and materials as they are perceived by the senses of sight, smell, taste, touch, and hearing" (IFT 1975). In other words, the use of human senses to measure flavor and other sensory characteristics of foods and other products is called Sensory Analysis or Sensory Evaluation, a useful tool in the development of new products and new food resources (O'Mahony 1988). Sensory acceptance tests indicate the acceptance of a product without the influence of package, label, price and other such factors. Consumer testing is one of the most important activities in product development; the primary purpose of consumer affective tests is to assess the personal response by current and potential customers of a product, product ideas, or specific product characteristics (Resurreccion 1998). The implicit goal behind any and all sensory evaluation efforts in the food industry is to enhance quality, to improve appearance, flavor and texture as perceived by consumers in order to influence their food choices (translated into purchases) at the point of sale (Resurreccion 1998).

Keeping the current consumer trend of convenience and nutrition in mind, this study to develop a new chocolate-flavored, peanut-soy protein-based, nutritious beverage was undertaken. The main objective was to evaluate this new beverage product for its sensory characteristics. It has been developed from two important vegetable protein sources: peanut and

soy, complementing each other to benefit the goal of a nutritionally superior product. However, it is challenging to provide a nutritious food that ensures appealing taste, texture, and appearance. Hence, it was desired to achieve balance between nutrition and acceptability by subjecting a number of possible product formulations to consumer affective sensory testing. The consumer response to experimental product formulations in comparison to a commercial control sample was expected to ultimately lead to the development of a successful product. Sensory evaluation was essentially conducted to obtain first-hand information on consumer acceptability to guide the development process.

## MATERIALS AND METHODS

**Materials** The source of peanut protein was medium roast, partially defatted (12% fat) peanut flour, obtained from Golden Peanut Company, Alpharetta, Ga., U.S.A. Two soy protein sources were soy protein isolate (Prolisse™) and defatted soy flour, obtained from Cargill Soy Protein Solutions, Sidney, OH and Cedar Rapids, IA, U.S.A., respectively. The carrageenan stabilizer (Satiagel X-amp 4000) was provided by Degussa Texturant Systems Sales, LLC, Atlanta, Ga., U.S.A. Soy lecithin (LECIGRAN™ 5750) was offered by Riceland Foods, Inc., Lecithin Division, Stuttgart, Ark., U.S.A. Artificial chocolate flavor was donated by Carmi Flavor & Fragrance Co., Inc., Commerce, Calif., U.S.A. Maltodextrin (AVEBE MD 20) was provided by AVEBE America Inc., Princeton N.J., U.S.A. Corn syrup (light), sucrose, cocoa powder (Hershey®'s European style, Dutch processed cocoa), peanut oil, vanilla extract (pure), salt and commercial chocolate milk (Hershey®'s creamy chocolate milk shake) were purchased locally.

**A new chocolate-flavored peanut-soy beverage** Two important protein sources, peanut and soy, were used to prepare a nutritional beverage. The initial formulation and processing steps for beverage formulations were based on extensive literature review and preliminary beverage

preparation trials (Deshpande and others Unpublished). A pilot-plant scale beverage processing protocol involved a three-component constrained-mixture-design approach. Upon finalizing the operating protocol, the experimental design was created to determine the total number of beverage formulations required to conduct sensory evaluation.

**Mixture design** A three-component constrained mixture design was developed using Peanut ($X_1$: 30.56%-58.70%), Soy ($X_2$: 28.26%-43.52% either soy flour, SF or soy protein isolate, SPI) and Chocolate syrup ($X_3$: 13.04%-25.93%). The third component of the mixture, chocolate syrup, was a liquid blend of various other ingredients such as stabilizers, emulsifiers, sweeteners, and flavor additives (Deshpande and others Unpublished). Preliminary studies on the calculation of lysine content (Reference lysine content = 51.0 mg/g protein), measurement of viscosity ($\eta$ = 36.9 mPa s), and visual stability index (VSI = 1.00) were the basis of determination of lower and upper bound constraints (Deshpande and others Unpublished). The reference lysine content (51 mg/g protein) was obtained from the guidelines on essential amino acid consumption (NAP 2002). The acceptable range of viscosity for design formulations was based on the measurement of viscosity of Hershey®'s creamy chocolate milk shake. Similarly the VSI was based on the stability of commercial cow's milk.

The extreme vertices design for the mixture experiment (McLean and Anderson 1966) was obtained using design of experiment (DOE) software: STATISTICA™, Version 6.0 for windows (StatSoft®, Tulsa, OK). The lower and upper bound constraints determined for each mixture component ($X_1$: 0.3056-0.5870; $X_2$: 0.2826-0.4352; and $X_3$: 0.1304-0.2593) were used to generate this design, which gave 11 experimental design points. As shown in Figure 4.1, the parallelogram bound by dashed (-------) lines represented an experimental region on a ternary plot with the three components, peanut ($X_1$), soy ($X_2$), and chocolate or chocolate syrup ($X_3$),

being respective triangular coordinates. There were three overlapping points shown as a vertex point '$V_2$' in the figure. Since all three points had similar coordinates, only one was selected resulting in a parallelogram as the experimental region. It had four corner points representing extreme vertices outlying the constrained region, four midpoints of each edge, and a point inside the parallelogram representing the centroid of the constrained region. The relative proportions of each component were constrained to add up to the same value of 1 where:

$$\sum_{i=1}^{3} X_i = X_1 + X_2 + X_3 = 1$$

The minimum number of points needed to estimate the parameters for the quadratic model for optimization by response surface methodology were determined using following equation:

$$y = q\,(2^{q-1})$$

Where, the variable q described as the number of mixture variables in the design was three ($X_1$, $X_2$, and $X_3$) in this case resulting in a y value of twelve.

Hence, the experimental region was represented separately in Figure 4.2. Five additional design points ($C_1$, $C_2$, $C_3$, $C_4$, and $C_5$) were selected as the centroids of smaller parallelograms, for example, point $C_1$ was the centroid of smaller parallelogram formed by points $V_1$, $M_3$, $C_5$, and $M_1$. All of these design points represented fourteen mixture proportions of three major components as given in Table 4.1.

Two sets of formulations were prepared using the 14 mixture proportions. One set had soy flour as a source of soy protein (SF-based formulations), and another soy protein isolate (SPI-based formulations); all other ingredients and processing parameters were kept constant. The twenty-eight experimental beverage samples were formulated in small batches containing

2000g fixed weight of peanut($X_1$) – soy($X_2$) base stocks blended in predetermined mixture proportions (Table 4.1). The amount of chocolate syrup required in each batch was based on the total weight of beverage formulation and the proportion of chocolate syrup (or chocolate: $X_3$) from the corresponding mixture proportion. These twenty-eight beverage formulations served along with the control in each sensory sub-session resulted in a total of 32 samples for sensory evaluation.

**Beverage processing protocol** A pilot-plant scale processing protocol was used. This protocol was designed based on the preliminary study of development of a new chocolate-flavored, peanut-soy beverage (Deshpande and others Unpublished).

Base stocks of filtered peanut, soy flour (SF) and soy protein isolate (SPI) slurries were prepared separately for use in beverage formulations as per the three component mixture design. Morehouse mill (Model M-MS-3, Morehouse Industries, Los Angeles, Calif.) grinding of medium roast partially-defatted peanut flour, defatted soy flour and soy protein isolate gave finely ground flours as the starting materials for base stock preparation. The fine flours (250 g each), mixed separately with tap water (2400 g), were homogenized (34474 kPa, room temperature, five passes) in a laboratory homogenizer (APV Gaulin Homogenizer Model 15 15MR-8TBA, Everett, MA). The homogenized slurries were filtered using 273-mesh (53 microns), polyester filter screen (McMaster Co., Fulton Industrial Blvd., Atlanta, Ga.), residues discarded and filtrates stored for use as base stocks.

The key ingredients (sucrose, corn syrup, maltodextrin, cocoa powder, peanut oil, vanilla extract, salt, soy lecithin, artificial chocolate flavor, and carrageenan) were mixed in water for making the chocolate syrup. The mixture was heated to 75 ºC in a steam kettle, mixing

constantly with a hand-held blender while heating. At this stage the syrup was ready to be used in the beverage formulations.

Twenty-eight beverage formulations were prepared using the base stocks of peanut slurry, soy slurry (either SF or SPI) and chocolate syrup mixed in predetermined proportions as explained in the mixture design. The mixture was heated to 75 ºC in a steam kettle, mixing constantly with a hand-held blender while heating and then homogenized (34474 kPa, 72 ºC) in a laboratory homogenizer. Pasteurization at 93 ºC for 1 s followed by cooling to about 35 ºC was done in a plate-heat-exchanger (Armfield FT74, UHT Unit, Armfield Ltd., Ringwood, Hampshire, England, BH24 1 DY). The beverage was then filled into labeled 250 ml bottles and stored at 4 ºC until presented in sensory evaluation sessions.

**Sensory evaluation** Sensory evaluation was conducted by performing a consumer acceptance test in the sensory laboratory of the Department of Food Science and Technology, University of Georgia, Griffin Campus. Untrained panelists were recruited from the staff and students of the Griffin campus. The panelists (n = 41) were screened and enlisted by ensuring that they were not allergic to any of the ingredients used in the beverage formulations, consumed chocolate milk or similar flavored non-carbonated milk-type drink, and liked eating peanuts. Panelists participated voluntarily in the sensory evaluation.

There were five sensory sessions; each session was administered twice a week (two sub-sessions/ week), and each sub-session was monitored at three different time slots (three sensory tests/ sub-session). Thirty-two samples including Hershey®'s creamy chocolate milk shake as the control were presented in four sensory sessions (8 samples served per session). Apart from these four sessions, one sensory session conducted in the beginning of the actual evaluation was considered as a 'dry-run'. All procedures of sample preparation and sensory evaluation were

similar in the case of the dry-run as well as the other four sessions. The panelists were allowed to select any of the available time slots on one of the sub-sessions per week. They were instructed to evaluate the test samples for nine different sensory attributes using a nine-point hedonic scale. A complete randomized block design was used whereby each panelist evaluated all 32 samples over a period of one month. Each panelist evaluated eight samples (7 experimental beverage formulations and one control) served one at a time with a compulsory break of 3-5 min after evaluation of the 4$^{th}$ sample. Panelists were requested to complete brief questionnaires during the break in each sensory test. Some refreshments were provided at the end of each sensory test.

The sensory evaluation was carried out by performing three 30-minute sensory tests at 9:30 AM, 10:30 AM, and 11:15 AM, every Tuesday and Friday, for five consecutive weeks. Every test began by greeting the panelists and giving them a brief overview of how the sensory tests would be conducted. In the first week, they were given instructions regarding the use of the sensory booths, signal light buttons, and ballot forms (sensory data collection sheets, APPENDIX C) with a short summary of sample evaluation procedures. They were then requested to read and sign two copies of a consent form approved earlier by the University of Georgia's Institutional Review Board (IRB). The greeter confirmed that panelists had no allergies towards any of the product ingredients by recording their answer in the space provided on the consent form and then co-signed the form. The panelists were then led to well-equipped, environmentally-controlled partitioned booths in the sensory laboratory for the evaluation.

The bottled beverage formulations stored at 4 ºC in a walk-in cooler were transferred into two ice-filled boxes prior to the sensory sub-session. Care was taken that the samples were maintained at approximately 5-9 ºC. Each sample was partially filled into 90 ml plastic cups

(about 60 ml) and immediately served for sensory evaluation. The sample cups were labeled using three-digit sample identification numbers obtained from a random number table. The samples were presented in a monadic sequential order accompanied by paper ballots to record corresponding sensory responses. The ballot (APPENDIX C) was designed to have nine questions asking the panelists to express their feeling about each sensory attribute using a 9-point hedonic scale, where 1 = dislike extremely, 5 = neither like nor dislike, and 9 = like extremely (Peryam and Pilgrim 1957) as shown in Figure 4.3. They were also asked whether they would like to buy the product if available in the marketplace and were given a further option of writing comments about it. The sensory attributes evaluated included overall liking, appearance, color, aroma, consistency, flavor, sweetness, mouthfeel, and aftertaste. The panelists were instructed to visually observe, smell, and drink at least four sips of each sample before finishing the evaluation. They were provided unsalted crackers and drinking water to cleanse their palate in between samples as well as cups with lids for expectoration. The sensory data thus collected was statistically analyzed, and the mean ratings for beverage formulations were compared to those of the control to determine the best formulation.

**Measurement of nutritional and physical properties** The nutritional characteristics of the beverage were evaluated on the basis of lysine contents (mg/g protein) calculated from the compositions of peanut flour, soy flour and soy protein isolate, and the expected percentage protein recovery in the respective slurries/base stocks (Deshpande and others Unpublished). Physical properties such as viscosity (η) and visual stability index (VSI) were also measured. A Brookfield Viscometer (Model LVDV-II+, Brookfield Engineering Laboratories, Inc., Stoughton, MA) equipped with a small sample adaptor (SSA 18/13R, 25 ºC, 20 rpm) was used

for measurement of η, and a method suggested by Hinds and others (1997b) was used to determine VSI of various samples.

**Statistical analysis** The experimental design was obtained using a statistical software STATISTICA™, Version 6.0 for windows (StatSoft®, Tulsa, OK, USA). The statistical analysis software (SAS® Proprietary Software, 1999-2001), Release 8.2 for Windows (SAS® Institute Inc., Cary, NC, USA) was used for data analysis. The ANOVA and Duncan's Multiple Range Test were used to compare different attributes at the 95% significance level ($\alpha = 0.05$).

## RESULTS AND DISCUSSION

**Physical properties of beverage formulations** The ranges of lysine contents in experimental beverage formulations were 44.1-57.1 mg/g protein (equivalent to lysine content of 4.41-5.71 g/100 g protein) compared to the reference lysine content of 51.0 mg/g protein or 5.10 g/100 g protein (Deshpande and others Unpublished). A study done by Ang and others (1985) showed that whole egg, cow's milk, human milk, soymilk, and spray dried soymilk powder had lysine content (mg/g of protein) of 70, 76, 87, 76, and 72, respectively. According to them the lysine requirement for an adult was 500 (male)-800 (female) mg/day. Also, the amino acid profiles of various peanut beverage treatments studied by Rubico and others (1989) indicated that the lysine content (g/100 g protein) of raw peanut extract was 4.31, that of 71 ºC homogenized peanut milk was 5.16, and it was below 4 (in the range of 2.88-3.81) for samples processed at different temperature and time conditions (85 ºC, 100 ºC, and 121 ºC for 15 min, and 121 ºC for 3 sec). They suggested that it can be expected to see various heat treatments affecting some amino acids; however, actual significance of these apparent changes was not clear in the absence of biological data. In general, the lysine contents of beverage formulations prepared in this study were close to the range observed in the literature.

The values for two physical properties, viscosity (η) and visual stability index (VSI), were in the range of 17.7-131.8 mPa s and 0.63-0.99, respectively, compared to the viscosity of Hershey®'s creamy chocolate milk shake (36.9 mPa s) and the VSI of commercial cow's milk (1.00). The viscosity measurements of various beverage formulations (Figure 4.4) indicated that the formulations containing higher amounts of soy protein isolate (SPI) and chocolate syrup were significantly more viscous (100.5-131.8 mPa s) as compared to the respective soy flour (SF)-based formulations (37.9-62.8 mPa s). The SF-based formulations followed a viscosity pattern more closely to the control (Hershey®'s creamy chocolate milk shake) as compared to the SPI-based formulations. Besides, the viscosity of SPI-based formulations increased sharply with the subsequent increase in the amount of chocolate syrup resulting in the viscosity values from 42.4 mPa s to 131.8 mPa s for formulations 8, 9, 10, 11, 12, 13, and 14, all of the formulations being more viscous than the control.

However, the visual stability index values (Table 4.2) for both SPI- and SF-based formulations containing a low amount of chocolate syrup were low (0.63-0.89). It was speculated that since the chocolate syrup was a liquid blend of various ingredients, there was a simultaneous decrease in the amount of stabilizer and emulsifier with a decrease in the amount of chocolate syrup in the beverage formulations resulting in lower VSI values. At low chocolate syrup concentrations, the amount of stabilizer and emulsifier might not be enough to form a stable suspension, causing separation and settling of most of the particles.

On the other hand, at higher chocolate syrup concentrations, the SPI proteins might also have played an important role of forming larger protein molecules, producing more viscous formulations. Heat, homogenization and pH are important factors that influence the functional characteristics of isolated soy proteins (Klahorst 2002). The process of aggregation between

oilseed proteins and the properties of their aggregates differs from those of other common proteins like milk, and when liquids containing soy protein are heated at ≥ 100 ºC, gel-type aggregates involving covalent disulphide bonds are formed (Hinds and others 1997a). As soy protein use increases, especially in the beverages that serve as quick, high-protein meals, the challenge has been to mimic the consistency of a milkshake or fresh-blended smoothie. One solution is to enhance the viscosity contributed by soy protein with combinations of other stabilizers, emulsifiers and proteins to make the soy more palatable (Klahorst 2002).

The type and amount of stabilizer or emulsifier, heating-cooling protocols, temperature, protein-lipid concentration, and particle size are important factors influencing viscosity, stability, and mouthfeel or chalkiness of peanut beverages (Hinds and others 1997c). Since heat treatment is a major factor altering physical and chemical properties of homogenized liquids containing protein and fat, it can be thought to play a role in governing the physical properties of the beverage. Consistency index (K), or apparent viscosity (η) at shear rate of unity, of PF (peanut flour) and SPI milks was generally higher than that of similarly processed NDM (non-fat dry milk) milk (Schmidt and others 1980). Also increased total solids and increased heat treatment more dramatically increased the K value for oilseed milks than for NDM milk, and storage (10 days at 4 ºC) increased K value of SPI milk heated at 80 ºC or above. Degree of heat treatment, total solids content and storage were shown to have a pronounced effect on the apparent viscosity, consistency index, and yield stress of the fortified milk systems (Ramanna and Ramanathan 1992). Also, sugars whether sucrose, corn syrup, high fructose corn syrup, fructose, rice syrup, cane sugar or honey, contribute to viscosity, depending on the level added and other components of the solution (Klahorst 2002).

Temperature significantly affected viscosity of peanut beverages prepared by Rubico and others (1988) probably due to changes in protein and carbohydrate structures. A possible cause of gelation in the chocolate-flavored peanut beverages could be interaction of peanut protein with polysaccharide thickening agents (carragennan and guar gum) leading to aggregation of molecules (Rustom and others 1996). Hinds and others (1997b) observed that the visual stability and viscosity values of their preliminary experimental beverages ranged from 0.49-0.71 and 3.7-23.6 mPa s depending on the type of stabilizer or emulsifier used. Soybean beverages heated to 82 ºC then homogenized twice at 24.1 x $10^6$ Pa had suspension and visual stability indices of 0.9 and 1.0, respectively, after a 7-day quiescent storage time at 1.1 ºC (Nelson and others 1976; Priepke and others 1980). The physical properties of the peanut-soy beverage product under study were found to be in close correlation to these findings.

Among all the SF- and SPI-based formulations, only #8, #9, and #10 had viscosity as well as VSI close to the desired values. The SPI-based formulation #8 had the best balance of both the physical properties ($\eta$ = 41.5 mPa s; VSI = 0.99). For beverages, the primary role of protein is to provide nutritive value in a form that has desirable physical as well as sensory attributes. Because protein viscosity is largely affected by heat, proteins vary in their ability to withstand high-temperature processing; when heated to a point of denaturation, irreversible gels form from coagulated protein (Klahorst 2002). Evidently, the concentration of SPI and chocolate syrup appeared to govern the viscosity pattern of the various beverage formulations.

**Correlation of physical and sensory properties** Figure 4.5 shows that the SPI-based formulations having higher viscosity values were rated lower than the corresponding SF-based formulations for four sensory attributes: appearance (SPI: 5.6-6.2; SF: 6.3-6.5), color (SPI: 5.7-6.2; SF: 6.2-6.5), consistency (SPI: 5.4 -5.8; SF: 6.2-6.3), and mouthfeel (SPI: 5.6-5.8; SF:

5.7-6.0). It can be observed that higher amounts of chocolate syrup not only increased viscosity of the beverages resulting in lower ratings for consistency, but also resulted in visibly darker formulations having lower ratings for appearance and color. Since chocolate syrup was a blend of various other ingredients, increase in the concentration of chocolate syrup can be correlated to the increased concentration of stabilizer, sweeteners, and cocoa powder resulting in thicker and visually darker formulations. Higher amounts of SPI might also be responsible for lower ratings of consistency and mouthfeel because of a simultaneous increase in protein concentration giving thicker formulations. Higher viscosity resulted in lower sensory acceptability but lower viscosity consequently resulted in lower visual stability, hence the formulation that had balanced physical and sensory characteristics was of interest.

**Comparison of sensory properties of SF and SPI formulations** All of the sensory attributes were significantly different with the exception of appearance ($\alpha = 0.05$). Graphs plotted for mean hedonic ratings against various formulations arranged in increasing amount of chocolate syrup (Figure 4.5) indicated that formulations prepared with SPI were better than those with SF, especially for the sensory attributes aftertaste, aroma, flavor and overall acceptability. Aftertaste, flavor, overall acceptability, and sweetness of SPI formulations showed a gradual increase in the mean ratings as the amount of chocolate syrup increased. For all of the sensory attributes except mouthfeel, formulations #7, #8, and #9 prepared from SPI had higher mean ratings compared to the corresponding formulations prepared using SF. SPI-based formulation #8 was the best formulation receiving the highest ratings for all sensory attributes.

The higher the concentration of SPI, the greater the stability and nutritional benefits due to a higher concentration of high quality soy proteins. However, such formulations containing high amounts of both SPI and chocolate syrup resulted in higher viscosity and sweetness, thicker

consistency, visibly darker color, and less appealing appearance, ultimately lowering the consumer acceptability. This was in agreement with the observation that protein denaturation and aggregation, carragennan interaction with water, hydrated polysaccharide-protein networks, and hydrated lipid-protein complexes play important roles in controlling beverage characteristics. With respect to all sensory attributes, the formulations which had consumer acceptability close to the control (Hershey®'s creamy chocolate milk shake) were those containing a medium range of chocolate syrup. And among those again, the SPI-based formulations were rated higher compared to the SF-based formulations. It was evident that the SPI-based formulations were preferred compared to the SF-based formulations.

Proteins are added as ingredients to foods to achieve functional and nutritional goals (Giese 1994), and the beverage industry is the biggest market producing functional foods (Ohr 1997). Isolated soy proteins (minimum 90% protein content) are virtually pure, bland-flavored, and the most functional of the soy proteins having excellent nutritional qualities. Some can emulsify fat, bind water, and are designated to function in a given system in exactly the same way as animal proteins. Some isolates can be used to provide elastic gel texture, imparting interesting mouthfeel, while others control viscosity in drinks, making them creamier or full bodied. Various commercial products now take advantage of the characteristics offered by soy proteins (Riaz 1999). A sensory evaluation of 18 commercial soy flours, concentrates, and isolates confirmed that these products are not bland; had odor scores ranging from 5.8 to 7.7 and flavor scores, from 4.2 to 7.0 (scores rated on a 10-point scale where 10 was bland and 1 was strong) (Rackis and others 1979). It was observed that flavor scores of the products do not show a great reduction in flavor intensity when flours were processed into concentrates and isolates, in

spite of the removal of non-protein constituents; however, differences exist between flavor descriptors of flours, concentrates, and isolates.

**Comparison of beverage formulations with control (Hershey®'s creamy chocolate milk shake) based on sensory data** Mean ratings for Hershey®'s creamy chocolate milk shake were between 6 (like slightly) and 7(like moderately). SPI-based formulation #8, the best formulation with respect to both physical and sensory attributes, received mean ratings comparable to that of the control. As shown in Figure 4.6 it had significantly higher ratings for appearance (7.0), color (6.8), and sweetness (6.4). All sensory attributes except aftertaste were rated close to 6.0 or higher; aftertaste was the lowest-rated attribute with a mean score of 5.8 while appearance was the highest-rated attribute, having a mean score of 7.0. The sensory attribute sweetness was important because it was the attribute for which the control received the lowest mean rating (5.9).

It can be observed that the SPI-based formulation #8 having 43.9% peanut ($X_1$), 36.3% soy ($X_2$), and 19.8% chocolate syrup ($X_3$) had the highest overall acceptability (Figure 4.5 H). Also SPI-based formulation #7 ($X_1$ = 37.0%, $X_2$ = 43.5%, $X_3$ = 19.5%), #6 ($X_1$ = 52.3%, $X_2$ = 28.3%, $X_3$ = 19.5%), and SF-based formulation #14 ($X_1$ = 30.6%, $X_2$ = 43.5%, $X_3$ = 25.9%) received the highest mean ratings for color (7.0), aroma (6.2), and flavor (6.0), respectively (Refer to Table 4.2; Figure 4.5 C, D, and F). Interestingly, sweetness, color, and appearance were some of the main attributes for which the control was rated lower compared to some experimental formulations.

Wang and others (2001) found that chocolate and almond flavorings improved the aroma of soymilks ($p < 0.05$) and that addition of gum partially masked the beany off-flavor. Thus, the combination of chocolate flavoring and iota-carrageenan gum permitted production of low-heat-

treated soymilk acceptable to consumers. Chocolate-flavored soymilk received the lowest off-flavor score (2.3) while vanilla soymilk without gum was given the highest (3.1) ($p < 0.05$) on a scale represented by 1 as minimum and 7 as maximum intensity ratings. It was observed that all samples received a "weak" score for aftertaste and chalkiness, on the other hand, samples with gum were found to be more ($p < 0.05$) viscous (average viscosity score: 4.9) than those without gum (average viscosity score: 3.7). Chompreeda and others (1989) reported that the color of a chocolate-flavored peanut beverage was affected mainly by cocoa powder whereas sweetness was affected by both sugar and cocoa powder; sugar and cocoa powder had little influence on aroma, however. Also, the overall flavor was more greatly influenced by level of sugar than level of cocoa powder.

In the present study, the mean ratings for the sensory attributes were obtained from 41 consumer responses for each formulation on a 9-point hedonic scale. The responses for the first sensory session (dry-run) were not considered in the final analysis. However, the purpose of the dry-run was to avoid any major inconsistencies due to sample preparation or sensory procedures. Also it was helpful in becoming familiar with the organization and conduct of sensory testing, allowing sufficient time to make the necessary changes before carrying out the actual tests. Since it is recommended to conduct a complete dry run of all the testing procedures, one week before the test date (Resurreccion 1998), the first sensory session out of five total sessions was considered as the dry run.

**Consumer responses in terms of buying tendency and common descriptors used for the new beverage** Based on the consumer comments about different beverage formulations and their responses to the question whether they would like to buy the new beverage product (APPENDIX C), overall consumer opinion about the chocolate-flavored, peanut-soy beverage

was judged qualitatively. Consumers were less willing to buy formulations having lower amounts of chocolate syrup (about 5-15% saying yes) than high chocolate syrup formulations (45-52% saying yes). About 51.2% said that they would buy SF-based formulation #13 followed closely by 48.8% opting for SPI-based formulation #8. Almost all of the participants agreed that nutritional benefit could help boost the acceptability of the product under study; a few suggested that some changes in sweetness level, thickness, and flavor of some of the formulations might change their opinion about buying the product. Various descriptors used by the panelists in the comments option on the sensory ballot were summarized and grouped as shown in Table 4.3.

In general, the best blends or good balance of peanut and chocolate giving the smoothest mouthfeel in the case of SPI-based formulations were those containing medium peanut, medium SPI and high chocolate syrup concentrations. Some grainy or chalky mouthfeel was recognized in formulations having higher concentrations of soy flour along with medium to high levels of peanut. Such formulations were also described as having noticeable bitterness. Higher peanut concentrations were perceived to have an undesirable burnt aftertaste or somewhat odd flavor described as coffee beans/ tea/ mocha flavor. In some SPI-based formulations having lower concentrations of chocolate syrup, a slight beany flavor was perceived. At higher chocolate syrup concentrations, sweetness and chocolate flavor overpowered the peanut flavor which was considered undesirable by some consumers. Sweetness was the major concern for such high chocolate syrup formulations.

**Consumer preferences and awareness of 'good for you' product options available in the market** Based on the survey done with the help of questionnaires (APPENDIX E) provided during a 3-5 min break in the sensory test, consumer preferences and health awareness were judged. About 95% of the consumers knew that peanut is a good source of nutrition with 35%

eating peanuts at least twice a week; 65% believed that including peanuts in a healthy diet can help people lose weight. About 80% knew that diets high in monounsaturated fat from foods like peanuts, peanut butter, peanut oil and olive oil are superior to low-fat diets for heart health.

Among the sensory test consumers, 89% consumers drank milk at least once a week whereas the remainder did not prefer milk at all. All of the panelists liked chocolate milk; however, only 48% consumers preferred consuming chocolate milk or a milk-type, non-carbonated beverage once a week; the remainder bought such products very rarely. Almost 75% said that they never drank soymilk, even though 92% thought that it could be a protein-rich, low-fat, nutritious component of the diet; 75% knew that soymilk is an excellent lactose-free alternative to milk. Those who consumed soymilk preferred it at least once a week or more.

The most important attribute associated with consumption of health-directed products was taste. Other attributes such as color, consistency, sweetness, flavor and, nutrition were given more or less equal preference. The preferred serving style for nutritional beverages similar to the one under study was 'ready off-the-shelf but requiring refrigeration' followed by 'refrigerated packs sold in the grocery stores', 'instant powdered mix to be added in milk/water', and 'cold packs from vending machine'. If available, most of the panelists (47.6%) preferred consuming the chocolate-flavored, peanut-soy beverage at home. About 24% said that they could consume it anywhere, some preferring it on a bike ride or on a long drive, while a few selected the vending machine option. Chocolate was the most popular beverage flavor with 56% people choosing it as their favorite followed by vanilla and strawberry. Consumers showed more or less equal interest in 'low-fat' and 'good to eat' kinds of products with the remainder showing greater interest in 'low-calorie' than 'high-protein' foods. Although 85% believed that 'Nutritional Facts' information would definitely or might influence their purchase decision of the peanut-

based beverage under study, 51% said they would consider buying and 31% said they might buy it if a 'health claim' accompanied the product. For a single serving (240 ml) bottle of chocolate-flavored, peanut-soy beverage, 95% of the consumers were willing to pay about $1.69 or less. Many of the panelists expressed that the more often they consumed the product under study, the more they got used to the taste. Even though they did not like the taste at first, their liking gradually seemed to increase as they became more familiar with the product. Since the product was relatively a new concept, it took time for the panelists to become accustomed to the taste. Overall, the new concept was very favorably received. The panelists showed enthusiasm and interest in evaluating and assisting with the development of the new chocolate-flavored, peanut-soy beverage.

**Future work** Optimization of chocolate-flavored, peanut-soy beverage using response surface methodology (RSM) can be the next objective of this work. The optimization procedure for product development has been found to save time, costs less than repeated consumer testing of one or two products at a time, and provides a level of certainty about the performance of the product formulation (Fishken 1983). The sensory response in terms of various attributes can be modeled to give the optimum range of the three mixture components, leading to the most acceptable product formulation.

## CONCLUSIONS

Peanut flour was successfully combined with soy (either soy flour-SF or soy protein isolate-SPI) and chocolate syrup to obtain a new beverage product with acceptability ratings comparable to that of Hershey®'s creamy chocolate milk shake. Based on consumers' willingness to buy the new product, suggestions provided, and responses to the questionnaires, preferences of target consumers towards the new product were studied. Overall, the concept was well received and

consumers showed enthusiasm in evaluating the new beverage. The nutritional, physical, and sensory properties of twenty-eight chocolate-flavored, peanut-soy beverage formulations, and a control (Hershey®'s creamy chocolate milk shake) were studied effectively. The sensory properties revealed that soy protein isolate (SPI)-based formulation #8, having 43.9% peanut, 36.3% SPI, and 19.8% chocolate syrup, was the best overall. It received significantly higher ratings than the control for appearance, color, and sweetness. SPI- based formulations #7, #6, and SF-based formulation #14 had highest mean ratings for color (7.0), aroma (6.2), and flavor (6.0), respectively. Involving the target consumer early in the development process is expected to enhance the probability of the product's success in the marketplace.

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Table 4.1: Fourteen mixture proportions obtained from three component mixture design

| Design points[a] | Formulation# | Three Mixture Components | | |
|---|---|---|---|---|
| | | Peanut ($X_1$) | Soy ($X_2$) | Chocolate syrup ($X_3$) |
| **$V_1$** | **1** | 0.5870 | 0.2826 | 0.1304 |
| **$V_2$**[b] | **14** | 0.3056 | 0.4352 | 0.2592 |
| **$V_3$** | **3** | 0.4344 | 0.4352 | 0.1304 |
| **$V_4$** | **12** | 0.4581 | 0.2826 | 0.2593 |
| **$M_1$** | **6** | 0.5225 | 0.2826 | 0.1949 |
| **$M_2$** | **7** | 0.3700 | 0.4352 | 0.1948 |
| **$M_3$** | **2** | 0.5107 | 0.3589 | 0.1304 |
| **$M_4$** | **13** | 0.3818 | 0.3589 | 0.2593 |
| **$C_1$**[c] | **4** | 0.5095 | 0.3246 | 0.1658 |
| **$C_2$**[d] | **11** | 0.3689 | 0.4009 | 0.2303 |
| **$C_3$**[e] | **5** | 0.4333 | 0.4009 | 0.1658 |
| **$C_4$**[f] | **10** | 0.4451 | 0.3246 | 0.2303 |
| **$C_5$**[g] | **8** | 0.4392 | 0.3628 | 0.1981 |
| **$C_6$** | **9** | 0.4181 | 0.3742 | 0.2077 |

a Refer to Figures 4.1 and 4.2

b Two vertex and a centroid design point overlap in the ternary plot and show similar mixture proportions. Hence, only one was selected for sensory formulation, disregarding the other two.

c Design point $C_1$ is a centroid of a parallelogram formed by points $V_1$, $M_3$, $C_5$ and $M_1$

d Design point $C_2$ is a centroid of a parallelogram formed by points $V_2$, $M_2$, $C_5$ and $M_4$

e Design point $C_3$ is a centroid of a parallelogram formed by points $V_3$, $M_3$, $C_5$ and $M_2$

f Design point $C_4$ is a centroid of a parallelogram formed by points $V_4$, $M_4$, $C_5$ and $M_1$

g Design point $C_5$ is a centroid of a parallelogram formed by points $V_1$, $V_2$, $V_3$ and $V_4$

Table 4.2: Visual stability index (VSI) of various beverage formulations

| 28 BEVERAGE FORMULATIONS | | | | | | | |
|---|---|---|---|---|---|---|---|
| Formulation[a]# | Mixture proportions | | | SOY FLOUR (SF) | | SOY PROTEIN ISOLATE (SPI) | |
| | Peanut ($X_1$) | Soy ($X_2$) | Chocolate syrup ($X_3$) | Sample # | VSI | Sample # | VSI |
| 1 | 0.5869 | 0.2827 | 0.1304 | 446 | 0.89 | 728 | 0.98 |
| 2 | 0.5107 | 0.3590 | 0.1304 | 218 | 0.87 | 287 | 0.99 |
| 3 | 0.4344 | 0.4352 | 0.1304 | 824 | 0.96 | 191 | 0.63 |
| 4 | 0.5095 | 0.3246 | 0.1658 | 259 | 0.99 | 792 | 0.99 |
| 5 | 0.4333 | 0.4009 | 0.1658 | 475 | 0.98 | 624 | 0.98 |
| 6 | 0.5225 | 0.2827 | 0.1949 | 116 | 0.99 | 643 | 0.99 |
| 7 | 0.3700 | 0.4352 | 0.1948 | 172 | 0.96 | 572 | 0.72 |
| 8 | 0.4392 | 0.3628 | 0.1981 | 538 | 0.95 | 653 | 0.99 |
| 9 | 0.4181 | 0.3742 | 0.2077 | 847 | 0.97 | 741 | 0.98 |
| 10 | 0.4451 | 0.3246 | 0.2303 | 721 | 0.98 | 354 | 0.99 |
| 11 | 0.3689 | 0.4009 | 0.2303 | 688 | 0.99 | 749 | 0.98 |
| 12 | 0.4580 | 0.2827 | 0.2593 | 967 | 0.98 | 894 | 0.99 |
| 13 | 0.3818 | 0.3589 | 0.2593 | 646 | 0.98 | 132 | 0.99 |
| 14 | 0.3056 | 0.4352 | 0.2592 | 381 | 0.99 | 532 | 0.99 |

a Formulations were arranged such that the amount of chocolate syrup added to each formulation increased with the increasing formulation number

Table 4.3: Comments by sensory panelists describing beverages containing soy protein isolate (SPI) or soy flour (SF)

| Soy Type | Type of a formulation | Most common comments/descriptors |
|---|---|---|
| SPI | Low chocolate | Lacks sugar, weak flavor, bland, mouth drying, washed-out color |
| | Medium chocolate, medium peanut, high SPI | Good taste but too thick |
| | High chocolate, high SPI | Too thick, creamy or lumpy mouthfeel, too dark, sugar overdose, mouth watering |
| | Medium peanut, medium SPI, low chocolate | Unacceptable aroma, mocha flavor, slight beany flavor |
| | Medium peanut, high SPI, medium chocolate | Beany flavor/aftertaste, too thick, makes mouth watery |
| | Medium peanut, medium SPI, high chocolate | Best blend, good balance of peanut and chocolate, smoothest, no chalkiness |
| | High peanut, medium SPI, low chocolate | Burnt flavor/aftertaste, coffee taste, some chalkiness, too peanutty, bitter, beany, strong peanut and soy taste, coating after swallowing, medicinal aftertaste |
| SF | Low chocolate, high peanut, low-medium SF | Too watery/ thin body, lacks sugar, weak chocolate flavor, bland, coffee bean taste, bitter, burnt aftertaste, chalky, astringent, mouth drying |
| | Medium chocolate, medium peanut, high SF | Granular mouthfeel, no noticeable chocolate flavor/ aroma, mouth drying, consistency, color, and appearance good but bitter taste |
| | High chocolate, medium peanut, medium SF | Good, flavorful but too sweet and chocolaty, strong chocolate flavor |
| | High chocolate, medium peanut, high SF | Tastes burnt, rich, sweet and chocolaty, too thick, mouth coating |
| | High chocolate, high peanut, medium SF | Overpowering sweetness, too dark and thick, strong aftertaste, persistent bitterness |

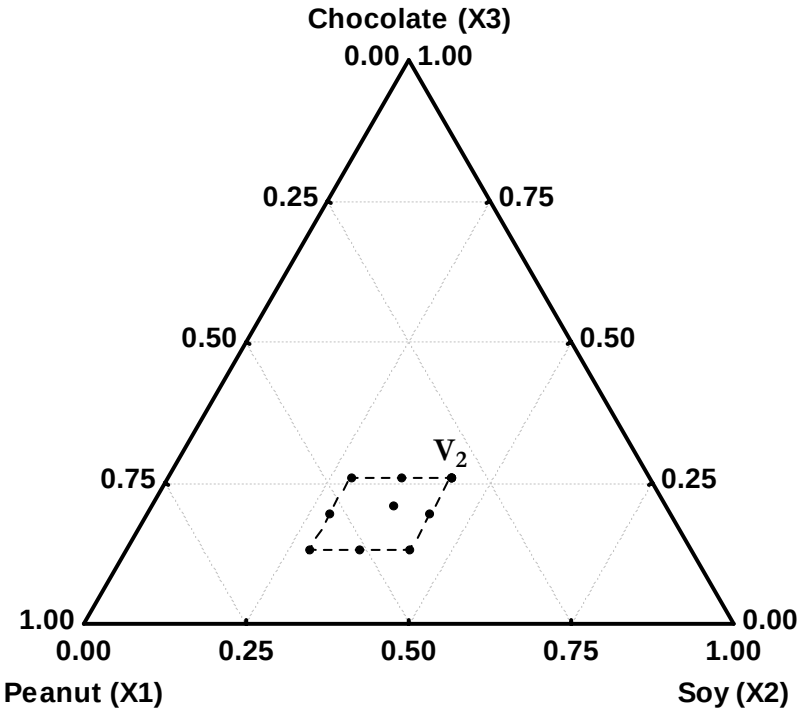


Figure 4.1: Experimental region on a ternary plot showing vertex and centroid points of a three-component constrained mixture design

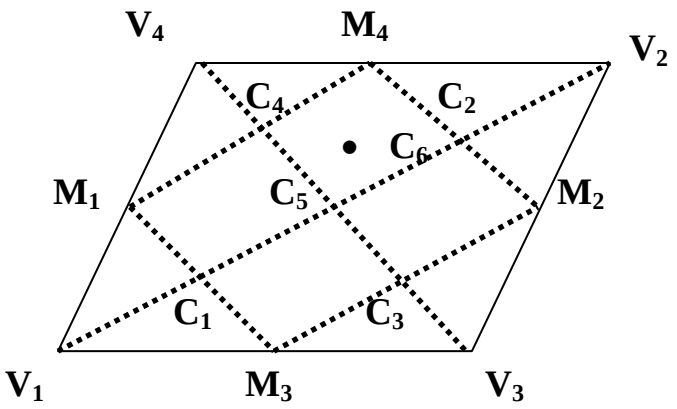


Figure 4.2: Selection of different design points in the experimental region for various formulations in order to conduct sensory evaluation

**Panelist Code: _____ Sample Code: _____ Date: _____**

**Instructions:**

- Visually observe, smell and drink **AT LEAST** four sips of each sample to complete this ballot.
- Please **CHECK THE SPACE** that best reflects your feeling about the product.
- Answer **ALL TEN** questions.
- Use the space provided at the end for additional comments.

1. **OVERALL**, how do you **"LIKE"** this product?

| Dislike | Dislike | Dislike | Dislike | Neither Like | Like | Like | Like | Like |
|---|---|---|---|---|---|---|---|---|
| Extremely | Very Much | Moderately | Slightly | Nor Dislike | Slightly | Moderately | Very Much | Extremely |
| [ ] | [ ] | [ ] | [ ] | [ ] | [ ] | [ ] | [ ] | [ ] |
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |

Figure 4.3: An example of a hedonic scale for overall acceptance

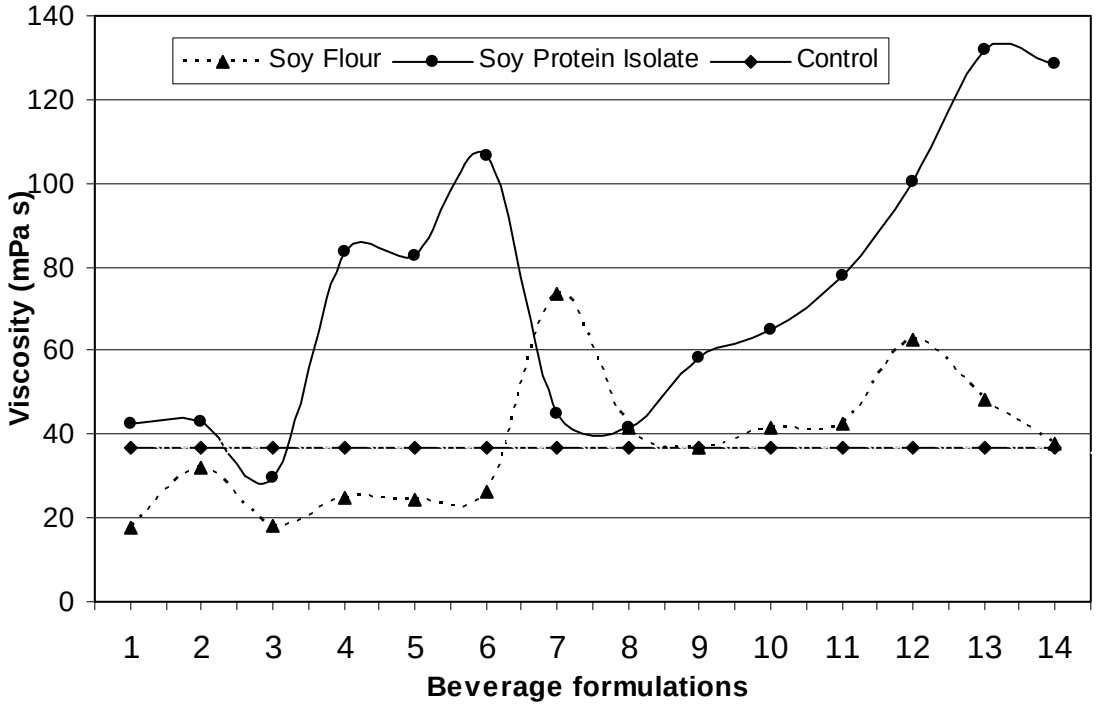


Figure 4.4: Viscosity values of various soy flour and soy protein isolate – based formulations in comparison to the control (Hershey®'s creamy chocolate milk shake; η=36.9 mPa s)

[Amount of chocolate syrup added to each formulation increased as we move from left to right on X-axis]

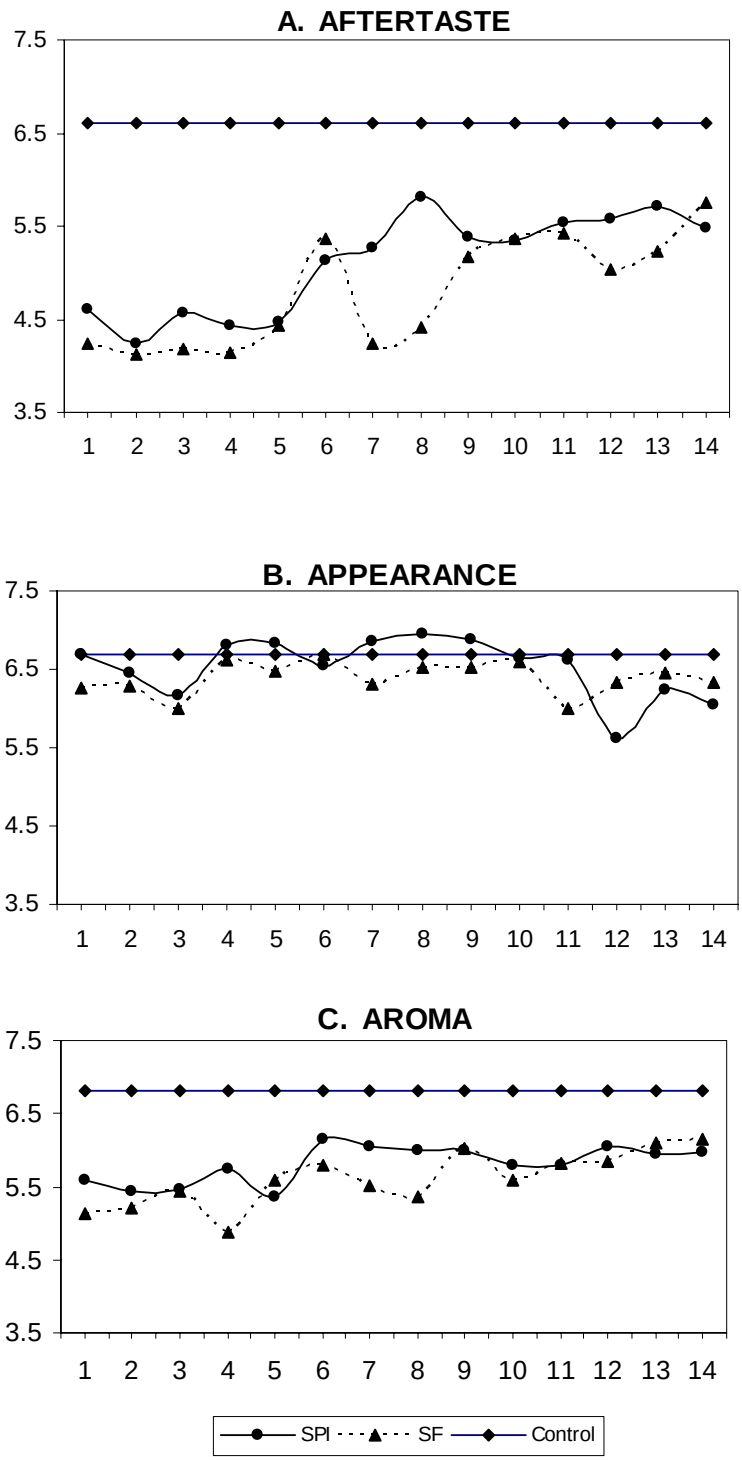


Figure 4.5: Trends of sensory attributes (aftertaste, appearance, and aroma) for soy flour (SF) and soy protein isolate (SPI) formulations compared to control (Hershey®'s creamy chocolate milk shake)

[Amount of chocolate syrup in the beverage formulations increased with the increasing formulation number on the X-axis. The Y-axis shows the range of mean hedonic ratings]

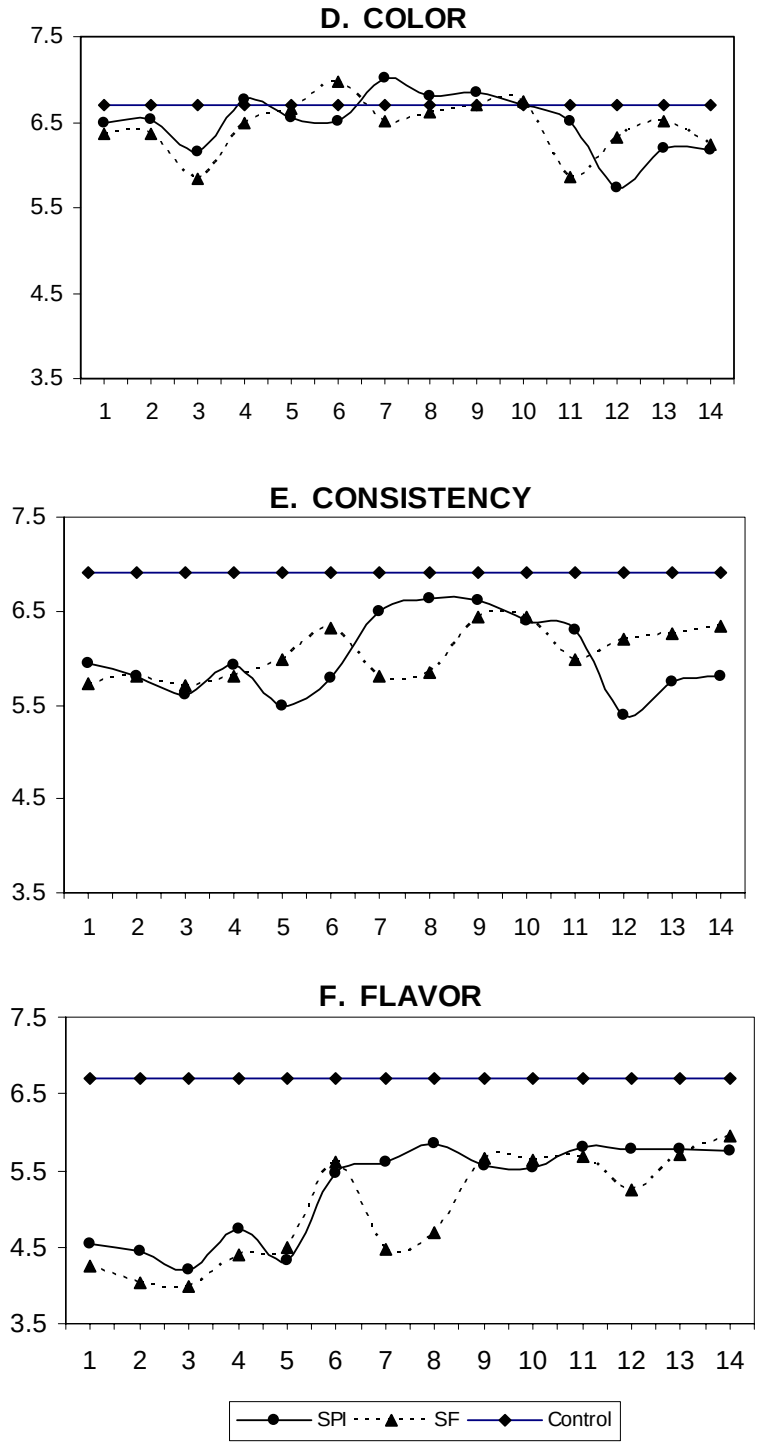


Figure 4.5: Trends of sensory attributes (color, consistency, and flavor) for soy flour (SF) and soy protein isolate (SPI) formulations in comparison to control (Hershey®'s creamy chocolate milk shake)

[Amount of chocolate syrup in the beverage formulations increased with the increasing formulation number on the X-axis. The Y-axis shows the range of mean hedonic ratings]

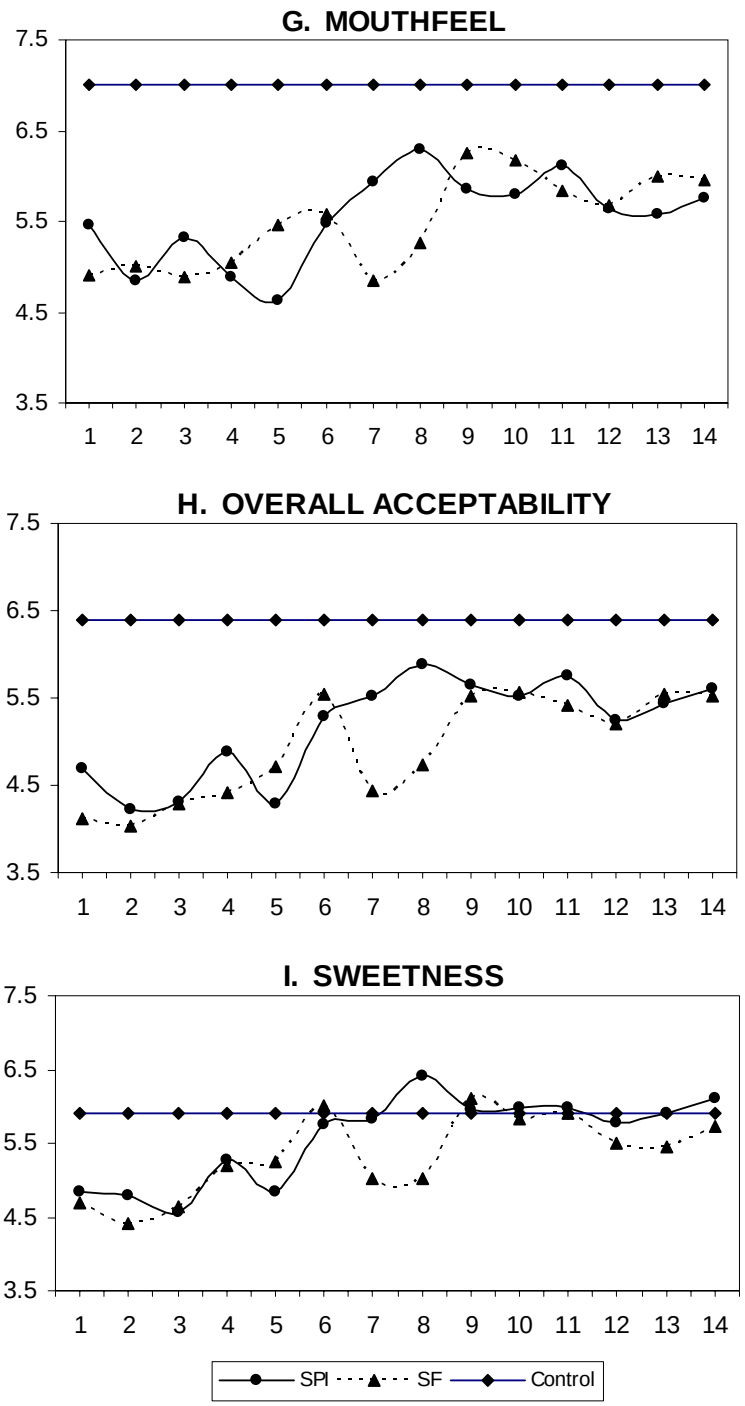


Figure 4.5: Trends of sensory attributes (mouthfeel, overall acceptability, and sweetness) for soy flour (SF) and soy protein isolate (SPI) formulations compared to control (Hershey®'s creamy chocolate milk shake)

[Amount of chocolate syrup in the beverage formulations increased with the increasing formulation number on the X-axis. The Y-axis shows the range of mean hedonic ratings]

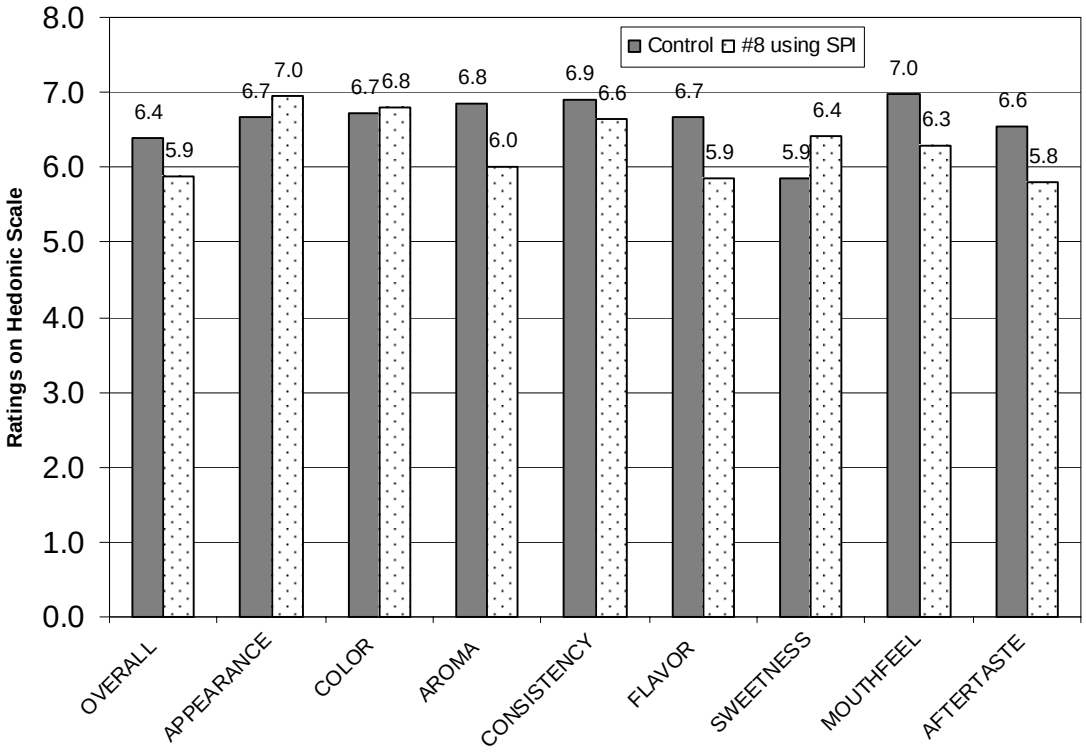


Figure 4.6: Comparison of the best formulation (#8 using SPI) with control (Hershey®'s creamy chocolate milk shake) for mean (n=41) hedonic ratings of various sensory attributes

# CHAPTER 5

# OPTIMIZATION OF A CHOCOLATE-FLAVORED, PEANUT-SOY BEVERAGE USING RESPONSE SURFACE METHODOLOGY (RSM) AS APPLIED TO CONSUMER ACCEPTABILITY DATA[1]

[1] Deshpande, R. P., Chinnan, M. S., and McWatters, K. H. To be submitted to the *Journal of Food Science.*

## ABSTRACT

Optimization of a chocolate-flavored, peanut-soy beverage was done using response surface methodology (RSM). Twenty-eight beverage formulations were processed by mixing peanut ($X_1$ = 30.6%-58.7%), soy ($X_2$ = 28.3%-43.5%), and chocolate syrup ($X_3$ = 13.0%-25.9%) in the proportions obtained using a three component, constrained mixture design where, source of soy was either flour (SF) or protein isolate (SPI). Consumer acceptability was measured in terms of nine response variables by 41 consumers using a 9-point hedonic scale. Parameter estimates were determined by performing regression analysis with no intercept option. L-pseudocomponents were introduced to get equivalent second degree models used to generate contour plots. Superimposition of contour plots corresponding to each response variable resulted in optimum regions having consumer acceptability ratings $\geq$ 5.0; the control (commercial chocolate milk) ratings were 6.0-7.0. Optimum formulations were all the combinations of 34.1%-45.5% $X_1$, 31.2%-42.9% $X_2$, and 22.4%-24.1% $X_3$ in SF-based formulations and 35.8%-47.6% $X_1$, 31.2%-43.5% $X_2$, and 18.3%-23.6% $X_3$ in SPI-based formulations.



INDEX WORDS: Optimization, Chocolate-flavored, peanut-soy beverage, Response surface methodology (RSM), L-pseudocomponents, Contour plots

# INTRODUCTION

Optimization of all aspects of a product is an effective strategy of accomplishing successful development of the product. The process of food product development requires intimate blending of research findings, science, technology, imagination, experience, and skills. It is the process of initiation and advance, error, iteration, adaptation, and reiteration directed towards an exclusive goal of a nearly perfect manifestation of the product concept (Segall 2000). The optimization procedure for product development saves time, ultimately costs less than repeated consumer testing of one or two products at a time, and provides a level of certainty about the performance of the product formulation (Fishken 1983). Sensory evaluation is often called upon to determine whether or not the optimum product has been developed, and response surface methodology (RSM) is employed for product optimization within the sensory evaluation field (Giovanni 1983).

Among various categories of new products such as classical innovations, line extensions, clones and several others (Segall 2000) the chocolate-flavored, peanut-soy beverage developed in this study falls under the category of 'Invention'. It has not been previously marketed or produced but by definition is a novel, unique, and distinctly untried, unfamiliar, or even previously nonexistent product (Segall 2000). The main intention of development of this product was utilization of two important vegetable protein sources, peanut and soy, which are readily available in abundance and at a reasonable price. Proteins are added as ingredients to foods to achieve functional and nutritional goals (Giese 1994). Peanut protein is deficient with respect to certain essential amino acids, for example, lysine, tryptophan, threonine and sulfur-containing amino acids but its true digestibility is comparable with that of animal protein (Singh and Singh 1991). Soybean is very rich in essential nutrients. It is an excellent source of good quality

protein equivalent to other animal proteins, such as milk, eggs, meat and fish, and is suitable for all ages from infants to the elderly (Schaafsma 2000; Sarwar and others 1985; Sarwar 1997; U.S.D.A. 2000; Riaz 1999). Chocolate has proven to be a popular flavor for soy-based beverages, due to its ability to mask protein flavor and provide a smooth flavor profile (Wang and others 2001). Hence, the final product developed as a combination of two proteins with a chocolate flavor can be expected to have improved essential amino acid profile and pleasant flavor. However, it is challenging to provide nutritious food that ensures appealing taste, texture, and appearance. If a food product cannot be re-engineered or modified to fulfill consumer need and ultimately the demand for the product, thus meeting the benchmark goals, it will not succeed (Robinson 2000). Thus, involving target consumers in the development process will enhance the likelihood of obtaining a successful product for the current market place. To obtain first-hand information on consumer acceptability, the new product developed in this study was evaluated by consumer sensory analysis.

Data from consumer affective tests are often difficult to understand, hence, graphical representations are useful in increasing comprehension, for example, scatter plots, bar graphs, and histograms are especially useful in this task (Resurreccion 1998). Once consumer acceptance for the product has been quantified, the lower boundary for consumer acceptance such as "liked slightly" ( = 6) on a 9-point hedonic scale or sometimes an action standard that specifies a performance requirement for a new or improved product can be set for further analysis (Resurreccion 1998). The optimal formulation is the one that receives a score equal to or greater than a chosen value that has been associated with a product's success (Fishken 1983). Optimization can be defined as the determination of values for process and formulation variables that result in a product or products with physical properties and sensory characteristics satisfying

such specific predetermined values making them acceptable to consumers (Galvez and others 1995). Modeling of quality or consumer acceptance using response surface methodology (RSM) can be done in a simplified manner when the effect of two or more variables on product acceptance and its interrelationship with sensory attribute intensities is of interest (Resurreccion 1998). When RSM is utilized, product optimization time is greatly reduced from traditional "cook and look" optimization techniques that depend on subjective formulation and evaluation procedures, and often stop short of fully realized product improvements (Rudolph 2000).

RSM is a designed regression analysis meant to predict the value of a response or dependent variable based on the controlled values of the experimental factors or independent variables (Meilgaard and others 1991). RSM is applicable in a wide variety of areas including food research and has constantly been successfully demonstrated to be used in optimizing: a) Ingredients (Henselman and others 1974; Johnson and Zabik 1981; Vaisey-Genser and others 1987; Chow and others 1988; Shelke and others 1990); b) Process variables (Oh and others 1985; Floros and Chinnan 1988; Mudahar and others 1990; Galvez and others 1990; Vainionpaa 1991); c) Both ingredient and process variables (Bastos and others 1991).

The objective of this study was to find an optimum chocolate-flavored, peanut-soy beverage formulation that would have consumer acceptance close to that of commercial chocolate milk. Since three-dimensional plots as well as contour maps provide useful visual aids for examining behavior of the response surface and location of the optimum (Vatsala and others 2001), response surface methodology employing mixture design was used to determine the optimum ratio of peanut, soy (either soy flour, SF, or soy protein isolate, SPI), and chocolate syrup in the respective SF and SPI formulations.

# MATERIALS AND METHODS

**Materials** Soy protein isolate (Prolisse™) and defatted soy flour were obtained from two plants of Cargill Soy Protein Solutions, one at Sidney, OH, and another at Cedar Rapids, IA, respectively. Medium roast, partially defatted (12% fat) peanut flour was obtained from Golden Peanut Co., Alpharetta, Ga. Maltodextrin (AVEBE MD 20) was donated by AVEBE America Inc., Princeton N.J. Soy lecithin (LECIGRAN™ 5750) was provided by Riceland Foods, Inc., Lecithin Division, Stuttgart, Ark. Artificial chocolate flavor was supplied by Carmi Flavor & Fragrance Co., Inc., Commerce, Calif. The carrageenan stabilizer (Satiagel X-amp 4000) was provided by Degussa Texturant Systems Sales, LLC, Atlanta, Ga. Sucrose, corn syrup (light), cocoa powder (Hershey®'s European style, Dutch processed cocoa), vanilla extract (pure), peanut oil, and salt were purchased locally. The control formulation, commercial chocolate milk (Hershey®'s creamy chocolate milk shake), was also purchased locally.

**New beverage product** A new nutritious peanut- and soy-based beverage was developed with the help of a pilot-plant scale beverage processing protocol involving mixture design (Deshpande and others Unpublished). A three-component constrained mixture design was developed using Peanut ($X_1$), Soy ($X_2$; either soy flour, SF or soy protein isolate, SPI) and Chocolate syrup ($X_3$). The lower and upper bound constraints were determined (Deshpande and others Unpublished) based on lysine content (Reference lysine content = 51.0 mg/g protein), viscosity ($\eta$ = 36.9 mPa s), and visual stability index (VSI = 1.00). The essential amino acid consumption guidelines (NAP 2002) on lysine gave reference lysine content of 51 mg/g protein. The acceptable range of viscosity and VSI for the design formulations was based on the viscosity of Hershey®'s creamy chocolate milk shake and the stability of commercial cow's milk, respectively.

Using design of experiments software: STATISTICA™, Version 6.0 for windows (StatSoft®, Tulsa, OK, USA), the extreme vertices design for the mixture experiment (McLean and Anderson 1966) was obtained. The lower and upper bound constraints for each mixture component ($X_1$: 0.3056-0.5870; $X_2$: 0.2826-0.4352; and $X_3$: 0.1304-0.2593) were used to generate the design. Joining the vertex points ($V_1$, $V_2$, $V_3$, and $V_4$) resulted in a parallelogram as an experimental region on a ternary plot with the three components, peanut ($X_1$), soy ($X_2$), and chocolate or chocolate syrup ($X_3$), representing respective triangular coordinates as shown in Figure 5.1. The experimental design gave 9 design points (4 vertices, 4 midpoints of the edges of the parallelogram, and a centroid point). But the minimum number of points (y) needed to estimate the parameters for the quadratic model for optimization using response surface resulted in a value of twelve where,

$$y = q\,(2^{q-1})$$

For q = 3; described as the number of mixture variables in the design ($X_1$, $X_2$, and $X_3$).

Hence the experimental region was represented separately and five additional design points ($C_1$, $C_2$, $C_3$, $C_4$, and $C_5$) were selected as the centroids of smaller parallelograms. For example, point $C_1$ was the centroid of smaller parallelogram formed by points $V_1$, $M_3$, $C_5$, and $M_1$. All of these design points together represented fourteen mixture proportions of the three components. Two sets of formulations were prepared using such 14 mixture proportions. One set had soy flour as a source of soy, and another soy protein isolate; all other ingredients and processing parameters were kept constant. The twenty-eight experimental beverage samples were formulated in small batches containing 2000 g fixed weight of peanut-soy base stocks blended in predetermined mixture proportions. These beverage formulations served along with the control in each sensory sub-session resulted in a total of 32 samples for sensory evaluation.

**Beverage preparation** A pilot-plant scale processing protocol based on the preliminary study of development of a new chocolate-flavored, peanut-soy beverage (Deshpande and others Unpublished) was designed for formulating various beverage mixtures. Base stocks of filtered peanut, soy flour (SF) and soy protein isolate (SPI) slurries were prepared separately for use in the formulations. Medium roast partially-defatted peanut flour, defatted soy flour, and soy protein isolate were finely ground. A Morehouse mill (Model M-MS-3, Morehouse Industries, Los Angeles, Calif.) was used for the dry grinding stage. The ground flours (250 g) and tap water (2400 g) were mixed and homogenized at room temperature in a laboratory homogenizer (APV Gaulin Homogenizer Model 15 15MR-8TBA, Everett, Mass.) set at 34474 kPa pressure by passing five times. The homogenized slurries were filtered through a polyester filter screen with quoted mesh size of 53 microns (273-mesh). The residues collected on the screen were discarded and filtrates stored for use as the base stocks.

The chocolate syrup was prepared by mixing the following ingredients in water: sucrose, corn syrup, maltodextrin, cocoa powder, peanut oil, vanilla extract, salt, soy lecithin, artificial chocolate flavor, and carrageenan. The dry ingredients were mixed first followed by the addition of liquid ingredients. The mixture was heated to 75 ºC in a steam kettle with constant stirring by a hand-held blender. Once the temperature was reached and the syrup was homogenous, it was ready to be used in the beverage formulations.

The base stocks of peanut slurry, soy slurry (either SF or SPI) and chocolate syrup were mixed in predetermined proportions suggested by the mixture design. It resulted in twenty-eight beverage formulations, which were processed further. Each formulation was heated to 75 ºC in a steam kettle, stirred using a hand-held blender while heating, and then homogenized at 34474 kPa pressure and 72 ºC in a laboratory homogenizer. Pasteurization at 93 ºC for 1 s was done

followed by cooling to about 35 ºC in a plate-heat-exchanger (Armfield FT74, UHT Unit, Armfeild Ltd., Ringwood, Hampshire, England, BH24 1 DY). The beverage thus prepared was then filled into labeled 250 ml bottles and stored at 4 ºC until presented in sensory evaluation sessions.

**Sensory analysis** The consumer acceptance test was conducted in the sensory laboratory of the Department of Food Science and Technology, University of Georgia, Griffin Campus. Untrained panelists (n = 41) were recruited from the staff and students of the Griffin campus and participated voluntarily in the sensory evaluation. Criteria for participation were that panelists were not allergic to any of the beverage ingredients, consumed chocolate milk or similar flavored non-carbonated milk-type drink, and liked eating peanuts.

There were five sensory sessions monitored twice a week (two sensory sub-sessions/ week) over a period of one month. The sensory session conducted in the beginning was considered as a 'dry-run' for which all of the sample preparation and sensory evaluation procedures were similar. Thirty-two samples (8 samples served per sub-session: 7 experimental beverage formulations and 1 control) were presented in four sessions. Hershey®'s creamy chocolate milk shake served as the control. A complete randomized block design was used whereby each panelist evaluated all 32 samples over a period of one month.

Three sensory tests at 9:30 AM, 10:30 AM, and 11:15 AM were performed each Tuesday and Friday for five consecutive weeks. At the beginning of each test, the panelists were greeted and briefed about the overall sensory test procedures and use of the sensory booth signal lights. In the first week, they were requested to read and sign two copies of a consent form. After confirming that the participants had no allergies towards any of the product ingredients, their answer was recorded in the space provided on the consent form which was then co-signed by the

greeter. Panelists were then led to well-equipped, environmentally-controlled partitioned booths for the sensory test.

The bottled beverage formulations stored at 4 ºC in a walk-in cooler were transferred into two ice-filled Styrofoam boxes prior to the sensory sub-session. Care was taken that the samples were maintained at approximately 5-9 ºC. Each sample was partially filled (about 60 ml) into 90 ml plastic cups and immediately served. The sample cups were labeled using three-digit sample identification numbers obtained from a random number table. The samples were presented in a monadic sequential order accompanied by paper ballots (APPENDIX C) to record corresponding sensory responses. The panelists were instructed to visually observe, smell, and drink at least four sips of each sample to evaluate overall acceptability, appearance, color, aroma, consistency, flavor, sweetness, mouthfeel, and aftertaste. A nine-point hedonic scale was used where, 1 = dislike extremely, 5 = neither like nor dislike, and 9 = like extremely (Peryam and Pilgrim 1957). Statistical analysis of sensory data and comparison of mean ratings of beverage formulations with those of the control determined the best formulation.

**Statistical methods** Statistical software STATISTICA™, Version 6.0 for windows (StatSoft®, Tulsa, OK, USA) was used for the experimental design and SAS® Proprietary Software,1999-2001, Release 8.2 for Windows (SAS® Institute Inc., Cary, NC, USA) for data analyses. The raw data was analyzed by performing regression analysis procedure (PROC REG) with no intercept option (NOINT) because the model components were required to sum to 100% (or the three component mixture design in this study had the limitation of $X_1 + X_2 + X_3 = 1.0$). Two separate sets of parameter estimates, one for soy flour (SF) and another for soy protein isolate (SPI) formulations, were obtained by modeling each response variable. Aftertaste, appearance, aroma, color, consistency, flavor, mouthfeel, overall acceptability, and sweetness were

considered as response or dependent variables whereas, peanut, soy (either SF or SPI), chocolate (or chocolate syrup), peanut*soy, peanut*chocolate, and soy*chocolate as independent variables.

An alternative system of coordinates involving lower bound pseudocomponents or L-pseudocomponents was utilized to set up a design for fitting a model over a sub-region of the original constrained surface (Cornell and Harrison 1997). A second-degree or quadratic Scheffe-type mixture model in the L-pseudocomponents of the form

$$Y = \beta_1 * X_1' + \beta_2 * X_2' + \beta_3 * X_3' + \beta_{12} * X_1' * X_2' + \beta_{13} * X_1' * X_3' + \beta_{23} * X_2' * X_3' + \varepsilon$$

was fitted to raw data on nine sensory attributes. The two data sets, one for SF and another for SPI, were analyzed separately. The fitted models for all the attributes were used to generate three-dimensional response surfaces as well as contour plots using STATISTICA™. Superimposition of contour plot regions of interest (within which each attribute received hedonic ratings $\geq 5.0$) resulted in optimum regions for SF and SPI formulations.

## RESULTS AND DISCUSSION

**Analysis of sensory data** Based on the mean hedonic ratings of overall acceptability, sweetness, color, and appearance, the soy protein isolate (SPI) formulations were more acceptable to the consumers compared to the corresponding soy flour (SF) formulations. From Table 5.1 it can be observed that the mean ratings for the control (Hershey®'s creamy chocolate milk shake) were between 6 (like slightly) and 7 (like moderately). These values were obtained by averaging the mean ratings (n = 41) of 4 control samples served in four sensory sessions. SPI-based formulation #9 received mean ratings comparable to that of the control and significantly higher ratings for appearance, color, and sweetness. Also, this SPI-based formulation #9 (Peanut = 43.9%, Soy = 36.3%, Chocolate syrup = 19.8%) had the highest overall acceptability compared to all other beverage formulations. Two other SPI-based formulations, #6 (Peanut = 37.0%, Soy

= 43.5%, Chocolate syrup = 19.5%), and #8 (Peanut = 52.3%, Soy = 28.3%, Chocolate syrup = 19.5%) received highest mean ratings for color (7.0) and aroma (6.1), respectively. SF-based formulation #19 (Peanut = 30.6%, SF= 43.5%, Chocolate syrup = 25.9%) had the highest flavor rating of 6.0. Interestingly, sweetness, color, and appearance were some of the main attributes for which several experimental formulations were rated higher compared to the control.

It can also be observed from Table 5.1 that all of the formulations having low levels of chocolate syrup (13%) were rated low (< 5.0) for the attributes of flavor, sweetness, and aftertaste, lowering the overall scores. High levels of soy flour (43.5%) in formulation #20 and peanut (50.9%) in formulation #27 were also rated low (< 5.0) overall. This indicated that more than 13% of chocolate syrup was required (in a mixture of peanut, soy and chocolate syrup) to mask the undesirable flavor, with sweetness also playing a role in overall taste of the product. Upper extremes of soy as well as peanut were not preferred. Thus, flavor, sweetness, and aftertaste were some of the important attributes helpful in assessing consumer acceptability of the test products.

It was observed by Folkenberg and others (1999) that sweet, milk flavor, and milk odor were highly positively correlated, and "cocoa properties", the "viscosity properties", and mouthfeel highly negatively correlated with overall liking, suggesting that consumers preferred cocoa drinks with a sweet and milky flavor; however, beverages with a high content of cocoa flavor and thick (stabilized) consistency were not preferred. According to Osborn and others (2003) significant differences ($p < 0.05$) were found in experimental as well as commercially available chocolate-flavored nutritional beverages for foam/bubbly appearance and sweet flavor attributes. In case of a chocolate-flavored peanut beverage (CFPB) studied by Chompreeda and others (1989) it was observed that color was affected mainly by cocoa powder; sweetness was

influenced by both sugar and cocoa powder; and overall flavor was more greatly influenced by level of sugar than level of cocoa powder. The optimum formula of this CFPB acceptable by 96% consumers had color, aroma, flavor, and overall quality scores (on a 5-point hedonic scale) of 4.7, 4.8, 4.9, and 4.7, respectively.

Rustom and others (1996) found that the use of cacao powder and cocoa flavor seemed to mask the "peanut" and "beany" off-flavors in chocolate-flavored beverages, with high ratings of aroma significantly contributing to high ratings for taste and overall acceptability. On a 7-point intensity scale, chocolate-flavored soymilks prepared by Wang and others (2001) had the highest aroma ratings of 5.3 and sweetness ratings being between 4.2-4.6. Also, they suggested that in their preliminary study, most panelists considered unsweetened soymilk as having low acceptability, indicating that sweetness was an important factor determining the acceptability of soymilk. A preliminary sensory evaluation of the peanut beverages by Hinds and others (1997) indicated that bottle-processed (111 ºC for 8 min) beverages were the most viscous, very chalky and imparted slightly bitter and beany aftertaste, however, those containing emulsifier and kettle-pasteurized at 72 ºC for 2 min had the strongest roasted flavor, smooth mouthfeel and viscosity similar to cow's milk.

**Parameter estimates for SF and SPI formulations** In mixture experiments, the focus of attention is on the blending properties of the components in the mixture (Cornell and Harrison 1997). These blending properties are called linear or nonlinear blending depending on whether the response changes linearly or nonlinearly upon changing the composition of the ingredients in the blend. The blending properties are determined by fitting a special type of mixture models to data collected from the various mixtures used. The quadratic model (Cornell and Harrison 1997) fitted to mixture data in this study was

$$\text{Response} = \beta_1*X_1 + \beta_2*X_2 + \beta_3*X_3 + \beta_{12}*X_1*X_2 + \beta_{13}*X_1*X_3 + \beta_{23}*X_2*X_3 + \varepsilon$$

Where, the linear quadratic variables $X_1$, $X_2$, $X_3$, $X_1*X_2$, $X_1*X_3$, and $X_2*X_3$ represent three components and their cross product terms. The quantities $\beta_1$, $\beta_2$, $\beta_3$, $\beta_{12}$, $\beta_{13}$, and $\beta_{23}$ are unknown parameters or coefficients whose values were estimated from the sensory data as shown in Tables 5.2 and 5.3. The term $\varepsilon$ represents a random error.

With respect to the blending properties of peanut ($X_1$), soy ($X_2$), and chocolate or chocolate syrup ($X_3$), the coefficient estimates of the first three terms, called linear blending terms in the model equation, represented the average acceptance ratings for the beverage blends located at three vertices of the triangle. These blends were 100% peanut ($X_1 = 1$), 100% soy ($X_2 = 1$), and 100% chocolate syrup ($X_3 = 1$). Comparisons of corresponding values for all sensory responses in the case of SF-based formulations (Table 5.2) revealed that the sensory attributes of color and sweetness were significantly affected at the highest chocolate syrup concentrations; this in turn affected two nonlinear blending terms (the coefficients of cross product terms) $X_1*X_3$ and $X_2*X_3$. This suggested that higher concentrations of chocolate syrup in SF-based beverage formulations might significantly reduce the acceptability of the product with respect to color and sweetness. However, similar observations in the case of SPI-based formulations (Table 5.3) indicated that there were five sensory attributes affecting consumer acceptability of the final beverage product. These were appearance, color, consistency, overall acceptability, and sweetness. Also in SPI-based formulations, aroma was significantly affected at 100% peanut ($X_1 = 1$) which indicated that higher concentrations of peanut might also reduce acceptability in terms of aroma of the final product. This suggested that consumers might not prefer blends with higher chocolate syrup and peanut concentrations. These predictions were in accordance with the results of sensory analysis as discussed in a previous section.

**Introduction of L-pseudocomponents** Since the experimental region or the region of interest for optimization purpose was relatively small with lower and upper bound constraints (Figure 5.1), introduction of lower bound pseudocomponents (L-pseudocomponents) was thought of as an alternative system for model fitting. The L-pseudocomponents ($X_i'$ values) were calculated as per the definition (Cornell and Harrison 1997):

$$\mathbf{X_i' = \frac{X_i - L_i}{1 - L}}$$

where, i = 1, 2, 3,……., q

$$L = \sum_{i=1}^{q} L_i = L_1 + L_2 + \ldots + L_q$$

$$X_1' + X_2' + X_3' + \ldots + X_q' = 1$$

Using this definition, the L-pseudocomponents for three original mixture components were calculated (Lpeanut = $X_1'$, Lsoy = $X_2'$, and Lchocolate = $X_3'$). In the definition, the L-values represented lower bound constraints of three components ($L_1$ = 0.3056, $L_2$ = 0.2826, and $L_3$ = 0.1304), and the range of X-values represented the original lower and upper bound constraints ($X_1$: 0.3056-0.5870; $X_2$: 0.2826-0.4352; and $X_3$: 0.1304-0.2593). Substituting these values in the definition resulted in redefined constraints for the mixture design as Lpeanut or $X_1'$ = 0 - 1.0, Lsoy or $X_2'$ = 0 - 0.5423, and Lchocolate or $X_3'$ = 0 - 0.4581. The new experimental design points and region bound by these redefined constraints are shown in Figure 5.2 and Table 5.4) (Refer to Figure 5.1 and Table 5.1 for the original constraints).

**Modeling and model fitting** Using the new design points with lower bounds, the raw data obtained from the consumer affective sensory evaluation for soy flour (SF) and soy protein isolate (SPI) formulations was separately analyzed using STATISTICA™ software. The

equivalent second degree or Scheffe-type mixture models in the L-pseudocomponents thus obtained are shown in Tables 5.5 and 5.6.

The quadratic model in the three component proportions was assumed adequate for describing the shape of the response surface above the constrained region and thus was fitted to nine sensory attributes or response variables. This resulted in two sets of model equations, one each for SF and SPI formulations. Since soy flour and soy protein isolate are two separate ingredients representing source of soy protein (soy: $X_2'$), the consumer acceptability data was analyzed separately for both sets.

**Three dimensional response surfaces for overall acceptability** The equivalent second degree models were further used for generation of contour plots for optimization purpose. Also, the quadratic models for overall acceptability of SF and SPI formulations were utilized to generate the three dimensional response surface plots shown in Figure 5.3. The average acceptance scores were the heights of the estimated general acceptance surface above each of the three vertices of the triangle in this figure. Comparison of these heights for SF and SPI formulations revealed that the overall acceptability of SPI formulations was significantly more affected at higher concentrations of chocolate syrup, whereas for the other two components there was no significant difference.

Also the comparison of amount or magnitude of the curvature in the shape of the surface above the $X_1' - X_3'$ edge of the triangle revealed that change in the proportion of peanut and chocolate syrup was significant to reduce the overall acceptability scores in the case of SPI formulations. Similar observations above the $X_2' - X_3'$ edge showed that changes in chocolate syrup proportion ultimately affected changes in the proportion of soy in order to achieve

maximum overall acceptability of the product. However, this change again was significant only in the SPI formulations.

Soy beverages look like milk, have good mouthfeel, and clean aftertaste, however, heat and processing may change the flavor profile of the beverage (Riaz 1999). Temperature significantly affected viscosity of peanut beverages probably due to changes in protein and carbohydrate structures (Rubico and others 1988). Protein viscosity is largely affected by heat, and proteins vary in their ability to withstand high-temperature processing; when heated to a point of denaturation, irreversible gels form from coagulated protein (Klahorst 2002). Protein denaturation and aggregation, carragennan interaction with water, hydrated polysaccharide-protein networks, and hydrated lipid-protein complexes play important roles in controlling beverage characteristics (Hinds and others 1997). Also, type and amount of stabilizer can change the physical properties of a peanut-based beverage. It was observed by Schmidt and others (1980) that increased total solids and increased heat treatment more dramatically increased the K value (consistency index) for oilseed milks than for NDM (non-fat dry milk) milk, and storage (10 days at 4ºC) increased K value of SPI milk heated at 80ºC or above. Degree of heat treatment, total solids content and storage were shown to have a pronounced effect on the apparent viscosity, consistency index, and yield stress of fortified milk systems (Ramanna and Ramanathan 1992). Sugars whether sucrose, corn syrup, high fructose corn syrup, fructose, rice syrup, cane sugar or honey, contribute to viscosity, depending on the level added and other components of the solution (Klahorst 2002). In this study, since chocolate syrup was prepared by blending various other ingredients, the increase in chocolate syrup level indicated the proportional increase in other ingredients. From all of these observations it can be anticipated that at higher chocolate syrup level together with higher concentrations of other ingredients such

as sugar, stabilizer, and cocoa powder, physical and sensory properties of beverage formulations and overall acceptability would be adversely affected. Similarly, at higher levels of SPI which is 90% protein, the total solids, as well as type of protein interactions, might have been playing roles in governing the properties of the beverage formulations affecting the overall acceptability.

**Generation of contour plots and limit of acceptance** The optimization was done by generating contour plots for each attribute and comparing the SF and SPI plots for respective attributes (Figures 5.4, 5.5, and 5.6). Since optimization is essentially the determination of values of formulation variables that result in a product having physical and sensory characteristics satisfying specific predetermined values making them acceptable to consumers (Galvez and others 1995), the lower limit of consumer acceptability in this study was determined from the acceptability scores of the control (Hershey®'s creamy chocolate milk shake). It was observed that the average consumer acceptability ratings for the control when all nine attributes were considered ranged from 6.0 (like slightly) to 7.0 (like moderately) on a 9-point hedonic scale (Table 5.1). Hence, the lower limit of acceptability for both SF and SPI formulations was decided to be consumer ratings $\geq$ 5.0 for all of the attributes.

When many factors and interactions affect a desired response, response surface methodology (RSM) is an effective tool for optimizing the process, and contour plots can be usefully employed to study the response surface and locate the optimum (Rustom and others 1991). To achieve the objective of developing products with optimal sensory acceptance, one must identify those properties and levels that are important for acceptance (Schutz 1983). RSM was used to optimize formulations of chocolate peanut spread by using a three-component constrained simplex lattice design where the optimum formulations were those having consumer acceptance rating of $\geq$ 6.0 for all attributes (Chu and Resurreccion 2004). In an optimization

study done by Galvez and others (1995), consumer acceptance ratings for two commercial mungbean noodle samples were used in order to attain the optimum formulation. Contour plots were developed, having shaded areas representing the optimum ranges for consumer acceptance of attributes tested which satisfied acceptance ratings described for commercial samples; the optimum region was found by outlining the regions representing the overlap of the shaded areas. RSM using mixture design was also used to determine the optimum ratio of pineapple, papaya, and carambola in the formulation of reduced calorie tropical mixed fruit jam (Abdullah and Cheng 2001). In this study, the sensory rating limit of acceptance for viscosity (3.4-4.4), aroma (4.1), sourness (3.7-4.3), color (3.7), and overall acceptability (4.8) was based on the closeness of the attributes to the acceptance of the commercial product. To obtain the optimum region, contour plots with limits of acceptance were superimposed, and the shaded region where contours within limits of acceptance overlapped was considered the predicted optimum region.

**Comparison of SF and SPI formulations from contour plots** Once the lower limit of acceptance for experimental formulations was decided, the regions of acceptability (the region with ratings $\geq 5.0$) for each attribute were determined. These regions were marked as separate areas represented as circular boundaries as shown in the contour plots. The dashed lines in these diagrams represent the constrained region of experimental formulations. The numbers represent hedonic mean ratings corresponding to each contour line in ternary graphs plotted with L-pseudocomponents. The comparison of such areas in each case for SF and SPI formulations revealed that SPI formulations had a larger range of acceptability, especially for the attributes of consistency, flavor, overall acceptability, and sweetness (Figure 5.5 and 5.6). For the attributes of appearance, aroma, color, consistency, and sweetness, SPI formulations received ratings $\geq 6.0$ indicating that they were rated similar to the control; for SF formulations, appearance, color and

mouthfeel were the attributes with ratings ≥ 6.0. The attributes which can be considered responsible for low acceptability of SF formulations might be aftertaste, consistency, flavor, and overall acceptability, since the areas of acceptability were smaller compared to other attributes. Similarly, in the case of SPI formulations, attributes that limited acceptance were aftertaste, aroma, flavor, and mouthfeel. On the whole, SPI formulations seemed to have a better chance of success compared to the SF formulations when judged for all of the sensory attributes considered together.

**Optimization with the help of contour plots** In order to determine the optimum formulation, the regions of acceptability in the contour plot for each attribute were superimposed. The area of overlap thus obtained is represented as the shaded region in both SF and SPI formulations (Figure 5.7). The shaded regions indicate that any point within this area represents a combination of peanut, soy (either SF or SPI), and chocolate syrup (a liquid blend of various other ingredients) that would result in consumer acceptance ratings ≥ 5.0 for all of the sensory attributes tested, and would be comparable to the commercial chocolate milk (Hershey®'s creamy chocolate milk shake). An optimal formulation maximizes consumer acceptance which means that it is the best formulation possible with a fixed set of ingredients, and any other formulation will not perform as well as the optimal (Fishken 1983).

Formulations incorporating SPI resulted in a larger area of overlap. Aroma and aftertaste were the most important attributes limiting the level of maximum consumer acceptability. It can be observed that higher levels of peanut or lower levels of chocolate syrup reduced acceptability ratings for both aftertaste and aroma. Concentration of soy protein isolate was acceptable over a larger range than soy flour, however, peanut and chocolate syrup levels both at high and low ends were the limiting factors deciding consumer preferences. The optimum formulations for

SPI-based beverage formulations were determined as all combinations of 35.8%-47.6% peanut, 31.2%-43.5% soy protein isolate, and 18.3%-23.6% chocolate syrup, adding up to 100%.

Formulations incorporating SF resulted in a smaller area of overlap than SPI. In this case also, aroma and aftertaste were the most important attributes limiting the level of maximum consumer acceptability. Observations similar to SPI formulations indicated that these attributes were governed by peanut and chocolate syrup levels limiting the consumer acceptability. The optimum formulations for SF-based beverage formulations were determined as all combinations of 34.1%-45.5% peanut, 31.2%-42.9% soy flour, and 22.4%-24.1% chocolate syrup, adding up to 100%.

The levels of third mixture component chocolate or Lchocolate ($X_3$ or $X_3'$) represented the levels of chocolate syrup added in the mixture to obtain the final beverage formulation. The chocolate syrup was prepared by mixing various other ingredients such as stabilizers, emulsifiers, sweeteners, and flavor additives in the predetermined proportions. [The exact proportions of these ingredients have not been disclosed since the product being developed is an invention under review by The University of Georgia Research Foundation, Inc., Athens, Ga., U. S. A.]. Any change in the level of chocolate or Lchocolate thus indicated proportional change in all those ingredients that form the chocolate syrup. From the two optimum regions, it can be seen that the acceptable range of chocolate syrup was significantly lower in beverages prepared from soy flour as compared to those from soy protein isolate. Even though the range was low, the level of chocolate syrup acceptable in SF formulations was high. Comparatively higher levels of peanut and soy were acceptable in SPI formulations, whereas lower levels of chocolate syrup were preferred. In general, a larger number of acceptable formulations can be prepared using soy protein isolate as a source of soy protein than soy flour in combination with peanut and

chocolate syrup (all three adding up to 100%) when a chocolate-flavored, peanut-soy beverage is prepared.

**Utilization of soy protein isolate as a soy protein source** The advantages of using of soy protein isolate (SPI) as against soy flour in the beverage product can be recognized due to higher processing efficiency, better nutritional properties, and higher consumer acceptability of the final product prepared from SPI. From the preliminary studies (data not shown), it was observed that SPI was completely soluble in water. The milling treatment given at the dry powder stage followed by homogenization with water reduced the size of SPI particles such that a negligible amount of residue was discarded after filtration. Since filtration was the most time-consuming operation, use of SPI not only saved processing time but also gave maximum protein recovery. Since the main objective of utilizing soy was to supplement essential amino acids, the final beverage can be expected to have an improved nutritional profile because of minimum processing losses. The price (per kg) of SPI, however, was higher compared to that of soy flour. But considering the other advantages, it can be concluded that SPI would be a better option than soy flour as a source of soy protein in the chocolate-flavored, peanut-soy beverage.

Besides providing an alternative to traditional dairy products, soy protein is being incorporated into many of today's nutritious meal-replacement beverages, and various commercial products now take advantage of these characteristics offered by soy proteins (Riaz 1999). Isolated soy proteins (minimum 90% protein content) are virtually pure, bland-flavored, and the most functional of the soy proteins having excellent nutritional qualities. Some isolates can be used to provide an elastic gel texture, imparting an interesting mouthfeel, while others control viscosity in drinks, making them creamier or full bodied. Some can emulsify fat and bind water and are designed to function in a given system in exactly the same way as animal

proteins (Riaz 1999). Soy protein isolates are a highly digestible source of amino acids which are regarded as the protein building blocks needed for proper human growth and maintenance (Roberts 2003).

**Future studies** Optimization using response surface methodology resulted in a combination of ingredients which can be expected to give a beverage product with maximum consumer acceptability and a product similar to commercial chocolate milk. However, validation of the optimum region can be done by choosing some combinations in the optimum region and some outside, for both soy flour- and soy protein isolate-based formulations. Also, a shelf-life study of the new protein-based nutritious beverage would be a good approach to determine keeping quality. This would be essential for introduction of the new product into the competitive market. Another important aspect is studying nutritional profile of the new product, which will also help in marketing the product based on its compositional and functional benefits.

## CONCLUSIONS

The optimization of chocolate-flavored, peanut-soy beverage was accomplished by the use of a consumer affective sensory evaluation study. Response surface methodology was applied to obtain quadratic or second degree response surface model equations. The contour plots were generated, and superimposition of the regions of acceptance (consumer ratings $\geq 5.0$ for all of the sensory attributes) gave optimum regions for soy flour (SF) and soy protein isolate (SPI) formulations. They were combinations of 34.1%-45.5% peanut, 31.2%-42.9% soy, and 22.4%-24.1% chocolate syrup in the case of SF formulations, and 35.8%-47.6% peanut, 31.2%-43.5% soy, and 18.3%-23.6% chocolate syrup, in the case of SPI formulations. The chocolate syrup used in this study was a liquid blend of various other ingredients and hence change in the levels of chocolate syrup indicated proportional change in all those other ingredients. The optimum

ranges as well as comparison of contour plots indicated that SPI formulations were more acceptable than those with SF and that a greater number of acceptable formulations can be prepared using SPI in combination with peanut and chocolate syrup such that all the three components add up to 100%. The SPI was a better choice than SF as a source of soy protein since it increased the processing efficiency; SPI can also be expected to give a nutritionally superior beverage product with high consumer acceptability and sensory characteristics comparable to commercial chocolate milk. The goal of utilizing of peanut, soy, and chocolate together in a new beverage product acceptable to target consumers was accomplished.

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Table 5.1: Twenty-eight formulations obtained from mixture design and corresponding mean hedonic ratings for various sensory attributes

| Formulation* # | Mixture Design Proportions** | | | Mean values over 41 panelists (n = 41) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Peanut($X_1$) | Soy($X_2$) | Chocolate syrup($X_3$) | Overall | Appearance | Color | Aroma | Consistency | Flavor | Sweetness | Mouthfeel | Aftertaste |
| **Control (Hershey®'s creamy chocolate milk)*** ** | | | | **6.4** | **6.7** | **6.7** | **6.8** | **6.9** | **6.7** | **5.9** | **7.0** | **6.6** |
| 1 | 0.3818 | 0.3589 | 0.2593 | 5.4 | 6.2 | 6.2 | 6.0 | 5.8 | 5.8 | 5.9 | 5.6 | 5.7 |
| 2 | 0.4344 | 0.4352 | 0.1304 | 4.3 | 6.2 | 6.1 | 5.5 | 5.6 | 4.2 | 4.6 | 5.3 | 4.6 |
| 3 | 0.5107 | 0.3590 | 0.1304 | 4.2 | 6.5 | 6.5 | 5.4 | 5.8 | 4.4 | 4.8 | 4.9 | 4.2 |
| 4 | 0.4451 | 0.3246 | 0.2303 | 5.5 | 6.6 | 6.7 | 5.8 | 6.4 | 5.5 | 6.0 | 5.8 | 5.3 |
| 5 | 0.3056 | 0.4352 | 0.2592 | 5.6 | 6.0 | 6.2 | 6.0 | 5.8 | 5.8 | 6.1 | 5.8 | 5.5 |
| 6 | 0.3700 | 0.4352 | 0.1948 | 5.5 | 6.9 | 7.0 | 6.0 | 6.5 | 5.6 | 5.8 | 5.9 | 5.3 |
| 7 | 0.4333 | 0.4009 | 0.1658 | 4.3 | 6.8 | 6.6 | 5.4 | 5.5 | 4.3 | 4.9 | 4.6 | 4.5 |
| 8 | 0.5225 | 0.2827 | 0.1949 | 5.3 | 6.5 | 6.5 | 6.1 | 5.8 | 5.5 | 5.8 | 5.5 | 5.1 |
| **9** | **0.4392** | **0.3628** | **0.1981** | **5.9** | **7.0** | **6.8** | **6.0** | **6.6** | **5.9** | **6.4** | **6.3** | **5.8** |
| 10 | 0.5869 | 0.2827 | 0.1304 | 4.7 | 6.7 | 6.5 | 5.6 | 6.0 | 4.5 | 4.9 | 5.5 | 4.6 |
| 11 | 0.4181 | 0.3742 | 0.2077 | 5.7 | 6.9 | 6.9 | 6.0 | 6.6 | 5.6 | 6.0 | 5.9 | 5.4 |
| 12 | 0.3689 | 0.4009 | 0.2303 | 5.8 | 6.6 | 6.5 | 5.8 | 6.3 | 5.8 | 6.0 | 6.1 | 5.5 |
| 13 | 0.5095 | 0.3246 | 0.1658 | 4.9 | 6.8 | 6.8 | 5.7 | 5.9 | 4.7 | 5.3 | 4.9 | 4.4 |
| 14 | 0.4580 | 0.2827 | 0.2593 | 5.2 | 5.6 | 5.7 | 6.0 | 5.4 | 5.8 | 5.8 | 5.6 | 5.6 |
| 15 | 0.3818 | 0.3589 | 0.2593 | 5.5 | 6.5 | 6.5 | 6.1 | 6.3 | 5.7 | 5.4 | 6.0 | 5.2 |
| 16 | 0.4344 | 0.4352 | 0.1304 | 4.3 | 6.0 | 5.8 | 5.4 | 5.7 | 4.0 | 4.6 | 4.9 | 4.2 |
| 17 | 0.5107 | 0.3590 | 0.1304 | 4.0 | 6.3 | 6.4 | 5.2 | 5.8 | 4.0 | 4.4 | 5.0 | 4.1 |
| 18 | 0.4451 | 0.3246 | 0.2303 | 5.6 | 6.6 | 6.7 | 5.6 | 6.4 | 5.6 | 5.8 | 6.2 | 5.4 |
| 19 | 0.3056 | 0.4352 | 0.2592 | 5.5 | 6.3 | 6.2 | 6.1 | 6.3 | 6.0 | 5.7 | 6.0 | 5.8 |
| 20 | 0.3700 | 0.4352 | 0.1948 | 4.4 | 6.3 | 6.5 | 5.5 | 5.8 | 4.5 | 5.0 | 4.9 | 4.2 |
| 21 | 0.4333 | 0.4009 | 0.1658 | 4.7 | 6.5 | 6.7 | 5.6 | 6.0 | 4.5 | 5.2 | 5.5 | 4.4 |
| 22 | 0.5225 | 0.2827 | 0.1949 | 5.5 | 6.7 | 7.0 | 5.8 | 6.3 | 5.6 | 6.0 | 5.6 | 5.4 |
| 23 | 0.4392 | 0.3628 | 0.1981 | 4.7 | 6.5 | 6.6 | 5.4 | 5.9 | 4.7 | 5.0 | 5.3 | 4.4 |
| 24 | 0.5869 | 0.2827 | 0.1304 | 4.1 | 6.3 | 6.4 | 5.1 | 5.7 | 4.2 | 4.7 | 4.9 | 4.2 |
| 25 | 0.4181 | 0.3742 | 0.2077 | 5.5 | 6.5 | 6.7 | 6.0 | 6.4 | 5.7 | 6.1 | 6.2 | 5.2 |
| 26 | 0.3689 | 0.4009 | 0.2303 | 5.4 | 6.0 | 5.9 | 5.8 | 6.0 | 5.7 | 5.9 | 5.8 | 5.4 |
| 27 | 0.5095 | 0.3246 | 0.1658 | 4.4 | 6.6 | 6.5 | 4.9 | 5.8 | 4.4 | 5.2 | 5.0 | 4.1 |
| 28 | 0.4580 | 0.2827 | 0.2593 | 5.2 | 6.3 | 6.3 | 5.9 | 6.2 | 5.2 | 5.5 | 5.7 | 5.0 |

* Formulation #1 to #14 correspond to soy protein isolate (SPI) formulations and #15 to #28 correspond to soy flour (SF) formulations

** These mixture proportions were obtained using original lower and upper bound constraints

*** Ratings for control represent average values of mean ratings (n=41) of 4 control samples presented in four sensory sessions

Table 5.2: Parameter estimates for mixture variables for soy flour (SF) formulations before introducing L-pseudocomponents

| RESPONSE | Parameter estimates: **SOY FLOUR FORMULATIONS** | | | | | |
|---|---|---|---|---|---|---|
| | Peanut ($X_1$) | Soy ($X_2$) | Chocolate ($X_3$) | Peanut*Soy ($X_1$*$X_2$) | Peanut* Chocolate ($X_1$*$X_3$) | Soy* Chocolate ($X_2$*$X_3$) |
| Aftertaste | 7.4141 | 4.1866 | 0.0399 | -16.3359 | 6.6173 | 28.0306 |
| Pr > \| t \| | 0.1024 | 0.7558 | 0.9989 | 0.6148 | 0.8843 | 0.6211 |
| Appearance | 3.8038 | -3.1234 | -27.9441 | 13.8289 | 49.7114 | 69.5674 |
| Pr > \| t \| | 0.3173 | 0.7820 | 0.2643 | 0.6114 | 0.1925 | 0.1438 |
| Aroma | 5.7251 | 12.2939 | 22.1492 | -14.2893 | -13.5830 | -32.8552 |
| Pr > \| t \| | 0.1001 | 0.2339 | 0.3333 | 0.5660 | 0.6968 | 0.4500 |
| **Color** | 3.2673 | -4.1935 | -54.4412 | 11.0079 | 90.4374 | 110.0391 |
| Pr > \| t \| | 0.4056 | 0.7191 | **0.0355** | 0.6954 | **0.0219** | **0.0254** |
| Consistency | 4.3258 | 1.4107 | 0.0303 | 6.3668 | 13.4187 | 20.8475 |
| Pr > \| t \| | 0.2828 | 0.9060 | 0.9991 | 0.8251 | 0.7395 | 0.6788 |
| Flavor | 4.2837 | -6.9588 | -25.6363 | 2.7311 | 42.2748 | 93.2527 |
| Pr > \| t \| | 0.3470 | 0.6067 | 0.3924 | 0.9332 | 0.3546 | 0.1019 |
| Mouthfeel | -2.1481 | -18.7805 | 0.9619 | 52.1318 | 4.3505 | 63.2210 |
| Pr > \| t \| | 0.6090 | 0.1323 | 0.9722 | 0.0833 | 0.9177 | 0.2289 |
| Overall | 0.8073 | -2.0815 | -23.4537 | 4.7454 | 55.2348 | 63.3752 |
| Pr > \| t \| | 0.8574 | 0.8759 | 0.4276 | 0.8827 | 0.2202 | 0.2594 |
| **Sweetness** | 2.7315 | 2.4609 | -68.4158 | -14.2796 | 120.6920 | 122.3125 |
| Pr > \| t \| | 0.5480 | 0.8553 | **0.0225** | 0.6608 | **0.0083** | **0.0318** |

Table 5.3: Parameter estimates for mixture variables for soy protein isolate (SPI) formulations before introducing L-pseudocomponents

| RESPONSE | Parameter estimates: **SOY PROTEIN ISOLATE FORMULATIONS** | | | | | |
|---|---|---|---|---|---|---|
| | Peanut ($X_1$) | Soy ($X_2$) | Chocolate ($X_3$) | Peanut*Soy ($X_1$*$X_2$) | Peanut* Chocolate ($X_1$*$X_3$) | Soy* Chocolate ($X_2$*$X_3$) |
| Aftertaste | 2.4333 | 4.2274 | -16.2124 | -6.6926 | 45.8080 | 40.2094 |
| Pr > \| t \| | 0.5739 | 0.7435 | 0.5723 | 0.8315 | 0.2992 | 0.4528 |
| **Appearance** | 1.0967 | -16.7019 | -103.3798 | 33.0277 | 139.0361 | 217.0805 |
| Pr > \| t \| | 0.7743 | 0.1441 | **<.0001** | 0.2351 | **0.0004** | **<.0001** |
| **Aroma** | 8.2780 | 15.8626 | -27.4338 | -35.7842 | 60.7838 | 31.9783 |
| Pr > \| t \| | **0.0116** | 0.1047 | 0.2065 | 0.1326 | 0.0687 | 0.4297 |
| **Color** | 2.0761 | -10.2700 | -93.8389 | 20.2796 | 131.4110 | 188.8005 |
| Pr > \| t \| | 0.5872 | 0.3686 | **0.0002** | 0.4656 | **0.0008** | **<.0001** |
| **Consistency** | 0.3141 | -15.2463 | -87.5273 | 29.9373 | 120.6330 | 192.0115 |
| Pr > \| t \| | 0.9425 | 0.2416 | **0.0026** | 0.3447 | **0.0068** | **0.0004** |
| Flavor | 2.9282 | 0.1733 | -46.7621 | -9.2967 | 86.3584 | 101.4451 |
| Pr > \| t \| | 0.5176 | 0.9898 | 0.1198 | 0.7774 | 0.0616 | 0.0705 |
| Mouthfeel | 6.0078 | 9.0832 | -37.4408 | -22.5970 | 67.7516 | 67.0262 |
| Pr > \| t \| | 0.1541 | 0.4703 | 0.1808 | 0.4605 | 0.1150 | 0.1989 |
| **Overall** | 3.6792 | -1.8558 | -80.1911 | -12.5764 | 125.5364 | 158.9900 |
| Pr > \| t \| | 0.4188 | 0.8914 | **0.0081** | 0.7037 | **0.0070** | **0.0049** |
| **Sweetness** | 0.4669 | -9.6931 | -72.0511 | 11.6467 | 115.0188 | 161.1067 |
| Pr > \| t \| | 0.9170 | 0.4688 | **0.0156** | 0.7206 | **0.0120** | **0.0038** |

Table 5.4: Experimental beverage formulations after introduction of L-pseudocomponents

| **Formulation** | **Redefined mixture proportions*** | | |
|---|---|---|---|
| # | Lpeanut ($X_1'$) | Lsoy($X_2'$) | Lchocolate($X_3'$) |
| 1 | 0.2708 | 0.2711 | 0.4581 |
| 2 | 0.4577 | 0.5423 | 0.0000 |
| 3 | 0.7289 | 0.2715 | 0.0000 |
| 4 | 0.4957 | 0.1493 | 0.3550 |
| 5 | 0.0000 | 0.5423 | 0.4577 |
| 6 | 0.2289 | 0.5423 | 0.2289 |
| 7 | 0.4538 | 0.4204 | 0.1258 |
| 8 | 0.7708 | 0.0004 | 0.2292 |
| 9 | 0.4748 | 0.2850 | 0.2406 |
| 10 | 0.9996 | 0.0004 | 0.0000 |
| 11 | 0.3998 | 0.3255 | 0.2747 |
| 12 | 0.2249 | 0.4204 | 0.3550 |
| 13 | 0.7246 | 0.1493 | 0.1258 |
| 14 | 0.5416 | 0.0004 | 0.4581 |
| 15 | 0.2708 | 0.2711 | 0.4581 |
| 16 | 0.4577 | 0.5423 | 0.0000 |
| 17 | 0.7289 | 0.2715 | 0.0000 |
| 18 | 0.4957 | 0.1493 | 0.3550 |
| 19 | 0.0000 | 0.5423 | 0.4577 |
| 20 | 0.2289 | 0.5423 | 0.2289 |
| 21 | 0.4538 | 0.4204 | 0.1258 |
| 22 | 0.7708 | 0.0004 | 0.2292 |
| 23 | 0.4748 | 0.2850 | 0.2406 |
| 24 | 0.9996 | 0.0004 | 0.0000 |
| 25 | 0.3998 | 0.3255 | 0.2747 |
| 26 | 0.2249 | 0.4204 | 0.3550 |
| 27 | 0.7246 | 0.1493 | 0.1258 |
| 28 | 0.5416 | 0.0004 | 0.4581 |

* Refer to Table 5.1 for original mixture proportions for peanut ($X_1$), soy ($X_2$), and chocolate ($X_3$)

Table 5.5: Second degree models in terms of nine sensory attributes for soy flour (SF) formulations after introduction of L-pseudocomponents

| **Response (Y) For SF** | **Peanut ($X_1'$)** | **Soy ($X_2'$)** | **Chocolate ($X_3'$)** | **Peanut*Soy ($X_1' * X_2'$)** | **Peanut* Chocolate ($X_1' * X_3'$)** | **Soy* Chocolate ($X_2' * X_3'$)** |
|---|---|---|---|---|---|---|
| $Y = \beta_1 * X_1' + \beta_2 * X_2' + \beta_3 * X_3' + \beta_{12} * X_1' * X_2' + \beta_{13} * X_1' * X_3' + \beta_{23} * X_2' * X_3' + \varepsilon$ | | | | | | |
| Aftertaste = $4.3706 * X_1' + 4.1486 * X_2' + 6.1579 * X_3' - 1.3052 * X_1' * X_2' + 0.5113 * X_1' * X_3' + 2.1922 * X_2' * X_3'$ | | | | | | |
| Appearance = $6.369 * X_1' + 5.2322 * X_2' + 4.3096 * X_3' + 1.105 * X_1' * X_2' + 3.9509 * X_1' * X_3' + 5.5371 * X_2' * X_3'$ | | | | | | |
| Aroma = $5.1027 * X_1' + 6.1557 * X_2' + 7.585 * X_3' - 1.1394 * X_1' * X_2' - 1.0863 * X_1' * X_3' - 2.6227 * X_2' * X_3'$ | | | | | | |
| Color = $6.4374 * X_1' + 5.1237 * X_2' + 2.5234 * X_3' + 0.8796 * X_1' * X_2' + 7.1762 * X_1' * X_3' + 8.7403 * X_2' * X_3'$ | | | | | | |
| Consistency = $5.7935 * X_1' + 5.2853 * X_2' + 6.3948 * X_3' + 0.5071 * X_1' * X_2' + 1.0677 * X_1' * X_3' + 1.6604 * X_2' * X_3'$ | | | | | | |
| Flavor = $4.3309 * X_1' + 3.0585 * X_2' + 5.1979 * X_3' + 0.2107 * X_1' * X_2' + 3.342 * X_1' * X_3' + 7.3719 * X_2' * X_3'$ | | | | | | |
| Mouthfeel = $4.8679 * X_1' + 2.6828 * X_2' + 6.8375 * X_3' + 4.1334 * X_1' * X_2' + 0.3474 * X_1' * X_3' + 5.0155 * X_2' * X_3'$ | | | | | | |
| Overall = $4.1782 * X_1' + 3.6965 * X_2' + 4.7391 * X_3' + 0.3727 * X_1' * X_2' + 4.3706 * X_1' * X_3' + 5.011 * X_2' * X_3'$ | | | | | | |
| Sweetness = $4.7545 * X_1' + 4.6438 * X_2' + 1.5405 * X_3' - 1.1284 * X_1' * X_2' + 9.5677 * X_1' * X_3' + 9.6997 * X_2' * X_3'$ | | | | | | |

Table 5.6: Second degree models in terms of nine sensory attributes for soy protein isolate (SPI) formulations after introduction of L-pseudocomponents

| **Response (Y) For SPI** | **Peanut ($X_1'$)** | **Soy ($X_2'$)** | **Chocolate ($X_3'$)** | **Peanut*Soy ($X_1' * X_2'$)** | **Peanut* Chocolate ($X_1' * X_3'$)** | **Soy* Chocolate ($X_2' * X_3'$)** |
|---|---|---|---|---|---|---|
| $Y = \beta_1 * X_1' + \beta_2 * X_2' + \beta_3 * X_3' + \beta_{12} * X_1' * X_2' + \beta_{13} * X_1' * X_3' + \beta_{23} * X_2' * X_3' + \varepsilon$ | | | | | | |
| Aftertaste = $4.3875 * X_1' + 4.6504 * X_2' + 5.1377 * X_3' - 0.5423 * X_1' * X_2' + 3.614 * X_1' * X_3' + 3.1539 * X_2' * X_3'$ | | | | | | |
| Appearance = $6.564 * X_1' + 4.6263 * X_2' - 1.3519 * X_3' + 2.6295 * X_1' * X_2' + 11.0224 * X_1' * X_3' + 17.2197 * X_2' * X_3'$ | | | | | | |
| Aroma = $5.6599 * X_1' + 6.5073 * X_2' + 3.996 * X_3' – 2.8377 * X_1' * X_2' + 4.8141 * X_1' * X_3' + 2.5286 * X_2' * X_3'$ | | | | | | |
| Color = $6.4601 * X_1' + 5.2169 * X_2' - 0.659 * X_3' + 1.6182 * X_1' * X_2' + 10.4209 * X_1' * X_3' + 14.9812 * X_2' * X_3'$ | | | | | | |
| Consistency = $5.7383 * X_1' + 4.1709 * X_2' - 0.147 * X_3' + 2.3748 * X_1' * X_2' + 9.558 * X_1' * X_3' + 15.215 * X_2' * X_3'$ | | | | | | |
| Flavor = $4.4772 * X_1' + 4.2025 * X_2' + 3.5653 * X_3' – 0.7496 * X_1' * X_2' + 6.829 * X_1' * X_3' + 8.0065 * X_2' * X_3'$ | | | | | | |
| Mouthfeel = $5.1196 * X_1' + 5.8221 * X_2' + 3.3723 * X_3' – 1.8078 * X_1' * X_2' + 5.3474 * X_1' * X_3' + 5.2653 * X_2' * X_3'$ | | | | | | |
| Overall = $4.5607 * X_1' + 4.1524 * X_2' + 0.7942 * X_3' – 1.0015 * X_1' * X_2' + 9.9391 * X_1' * X_3' + 12.5808 * X_2' * X_3'$ | | | | | | |
| Sweetness = $4.813 * X_1' + 3.7237 * X_2' + 1.9648 * X_3' + 0.9166 * X_1' * X_2' + 9.1056 * X_1' * X_3' + 12.7481 * X_2' * X_3'$ | | | | | | |

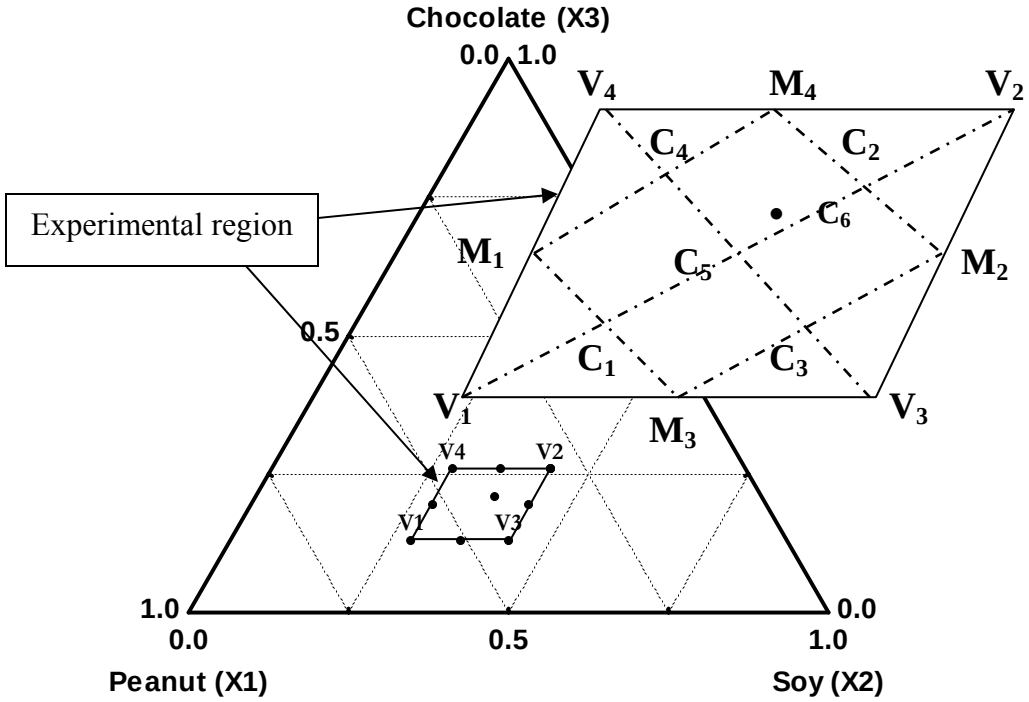


Figure 5.1: Experimental region showing design points those can be used as sample formulations

The magnified version of the experimental region shows additional 5 points ($C_1$, $C_2$, $C_3$, $C_4$, and $C_5$) to give a total of 14 mixture formulations possible for sensory evaluation

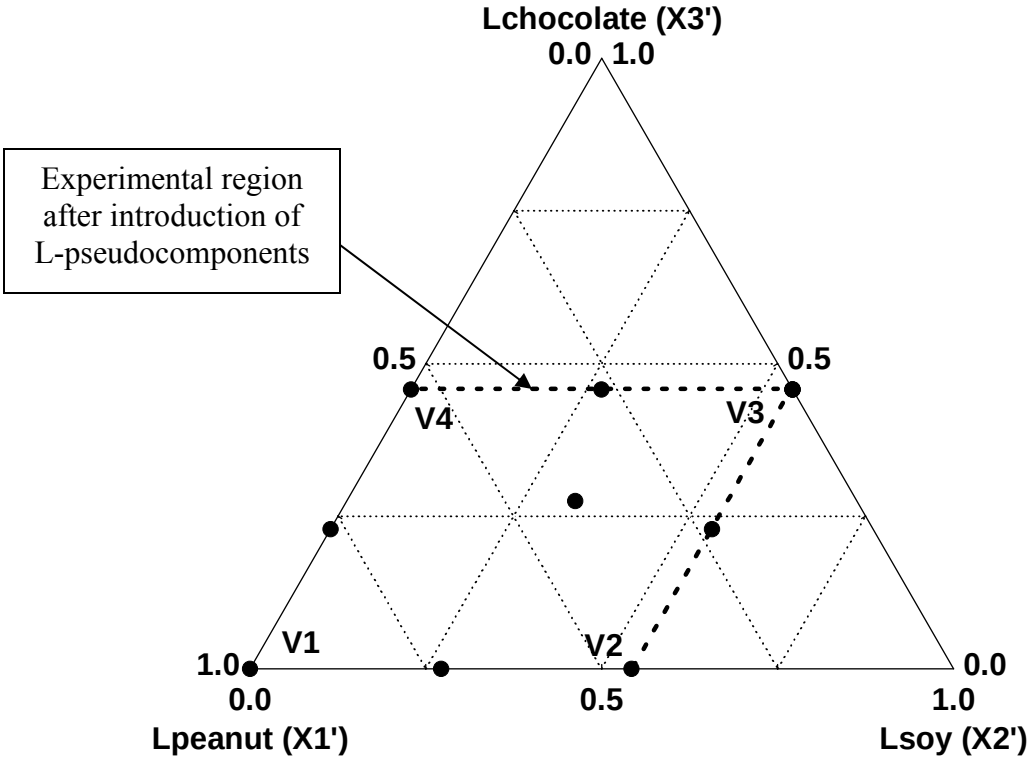


Figure 5.2: Experimental design points and the beverage formulation region after introducing lower bound pseudocomponents (L-pseudocomponents)

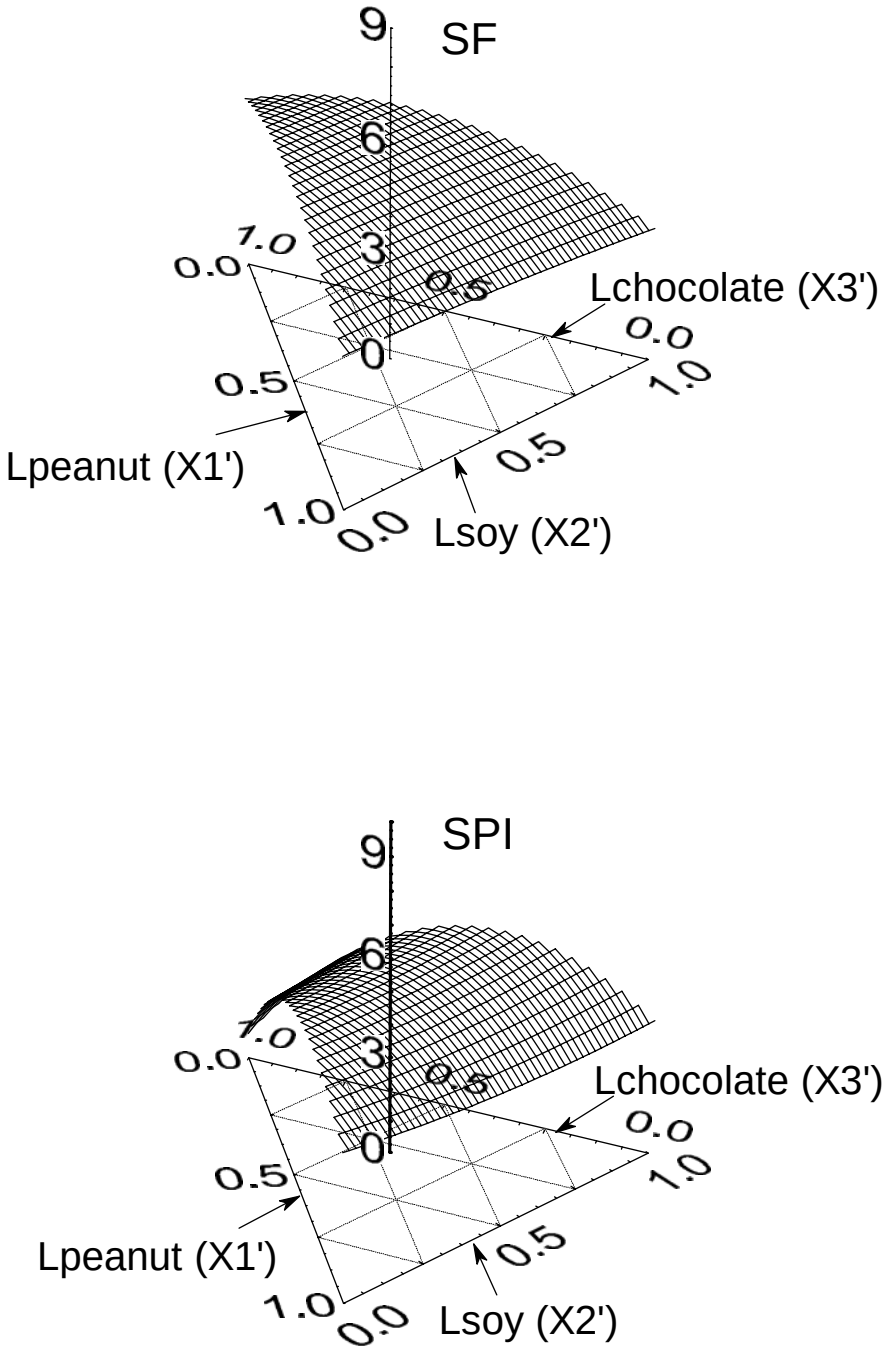


Figure 5.3: Response surfaces (three-dimensional plots) for overall acceptability obtained using L-pseudocomponents for soy flour (SF) and soy protein isolate (SPI) formulations

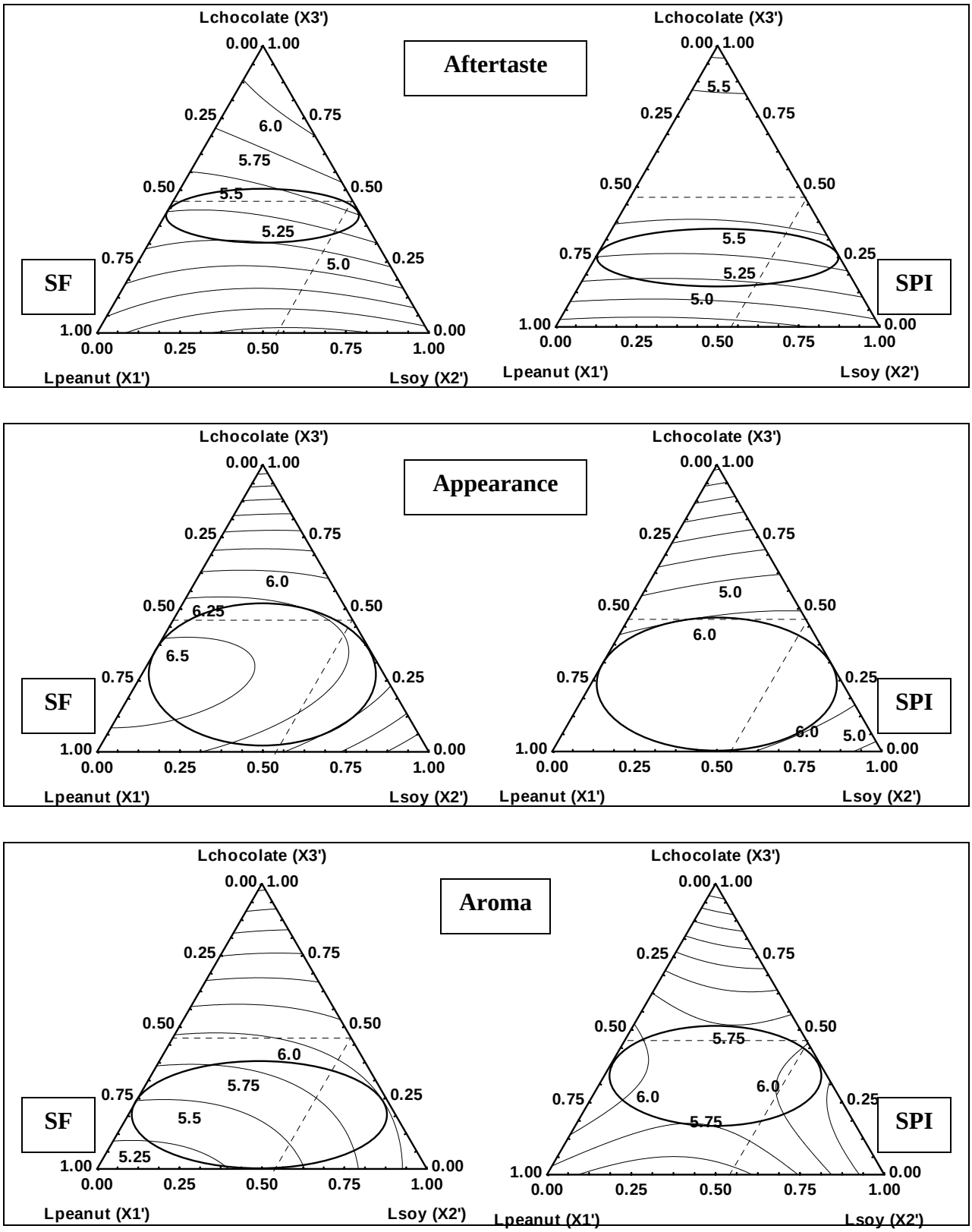


Figure 5.4: Contour plots for aftertaste, appearance, and aroma for soy flour (SF) and soy protein isolate (SPI) formulations

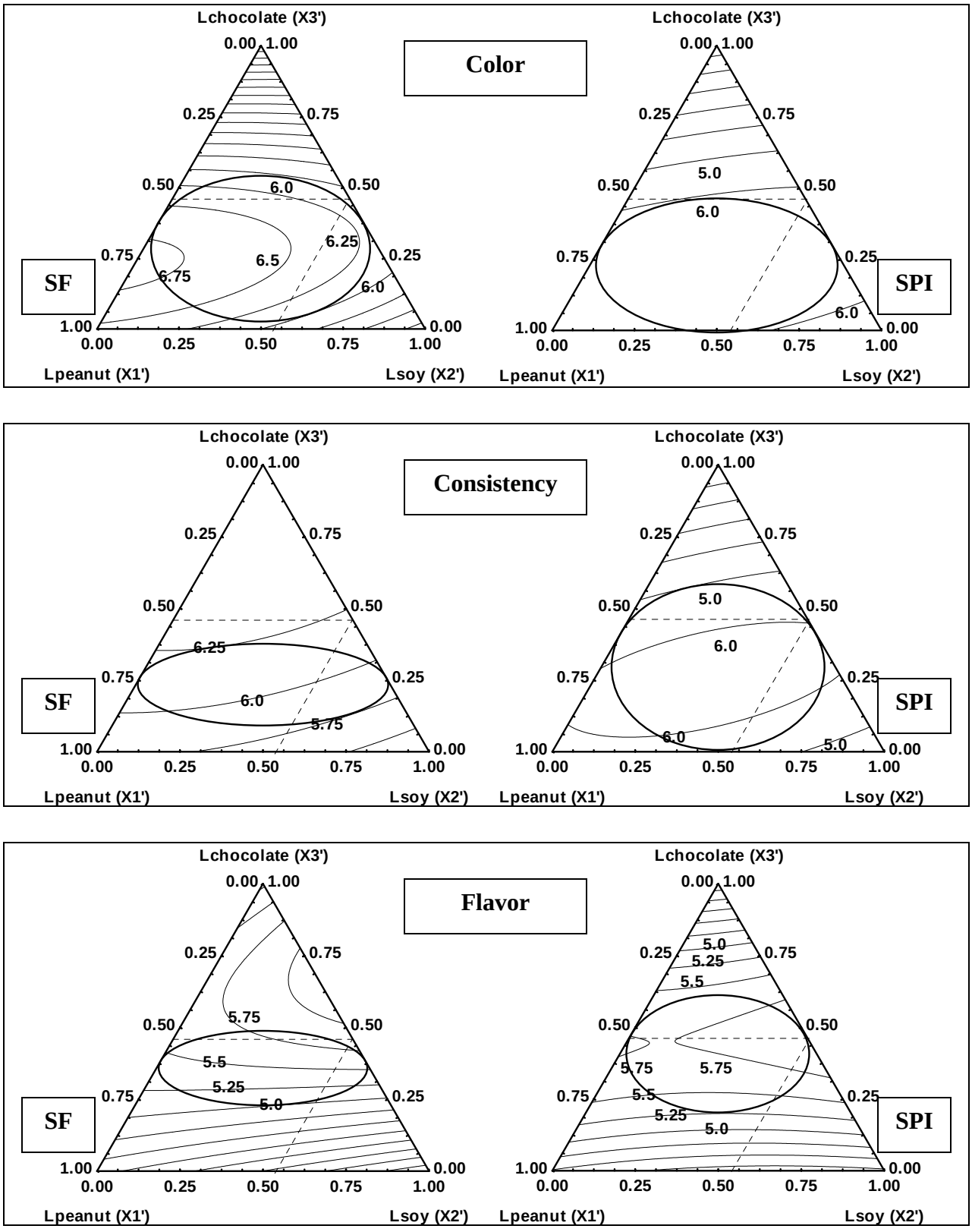


Figure 5.5: Contour plots for color, consistency, and flavor for soy flour (SF) and soy protein isolate (SPI) formulations

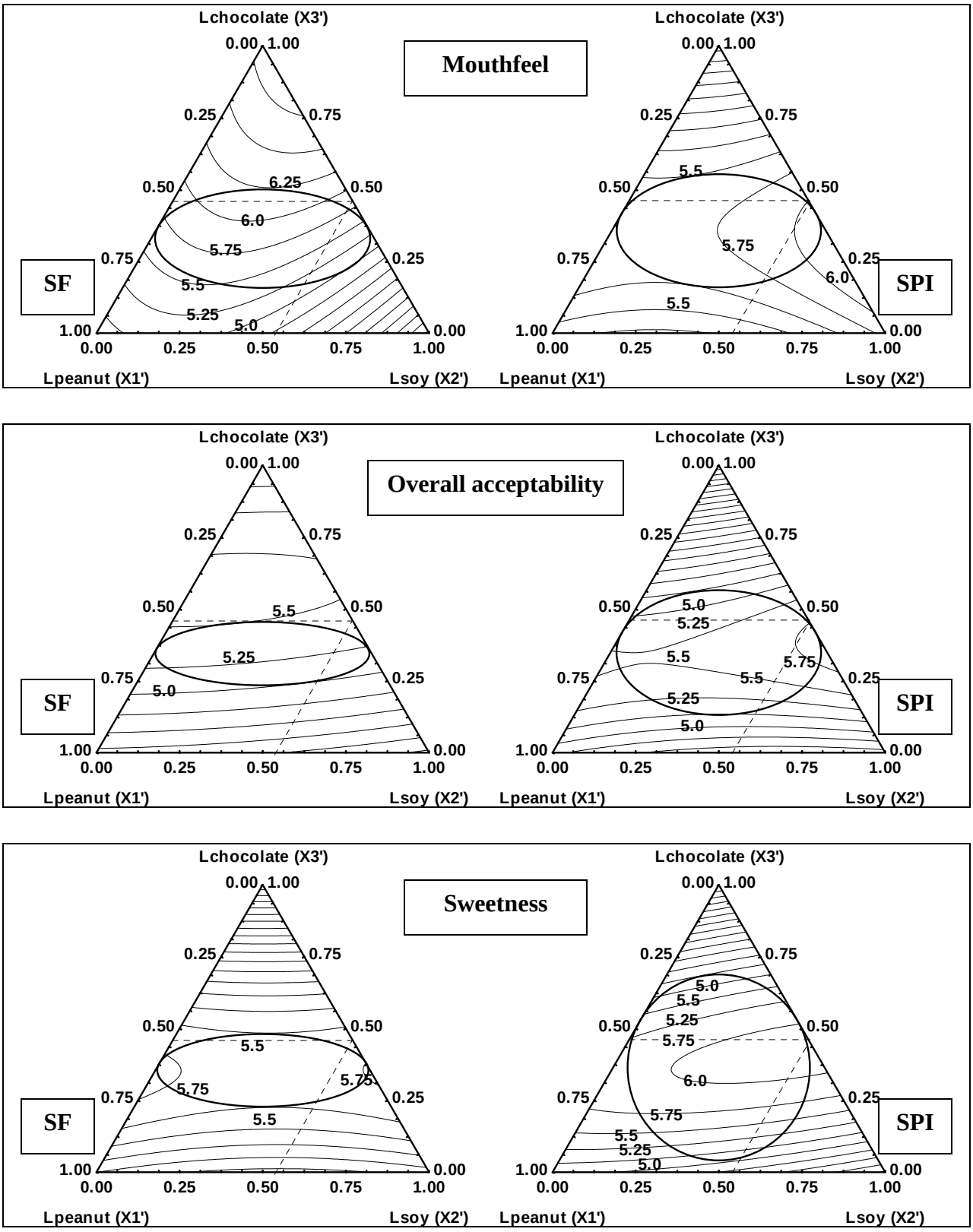


Figure 5.6: Contour plots for mouthfeel, overall acceptability, and sweetness for soy flour (SF) and soy protein isolate (SPI) formulations

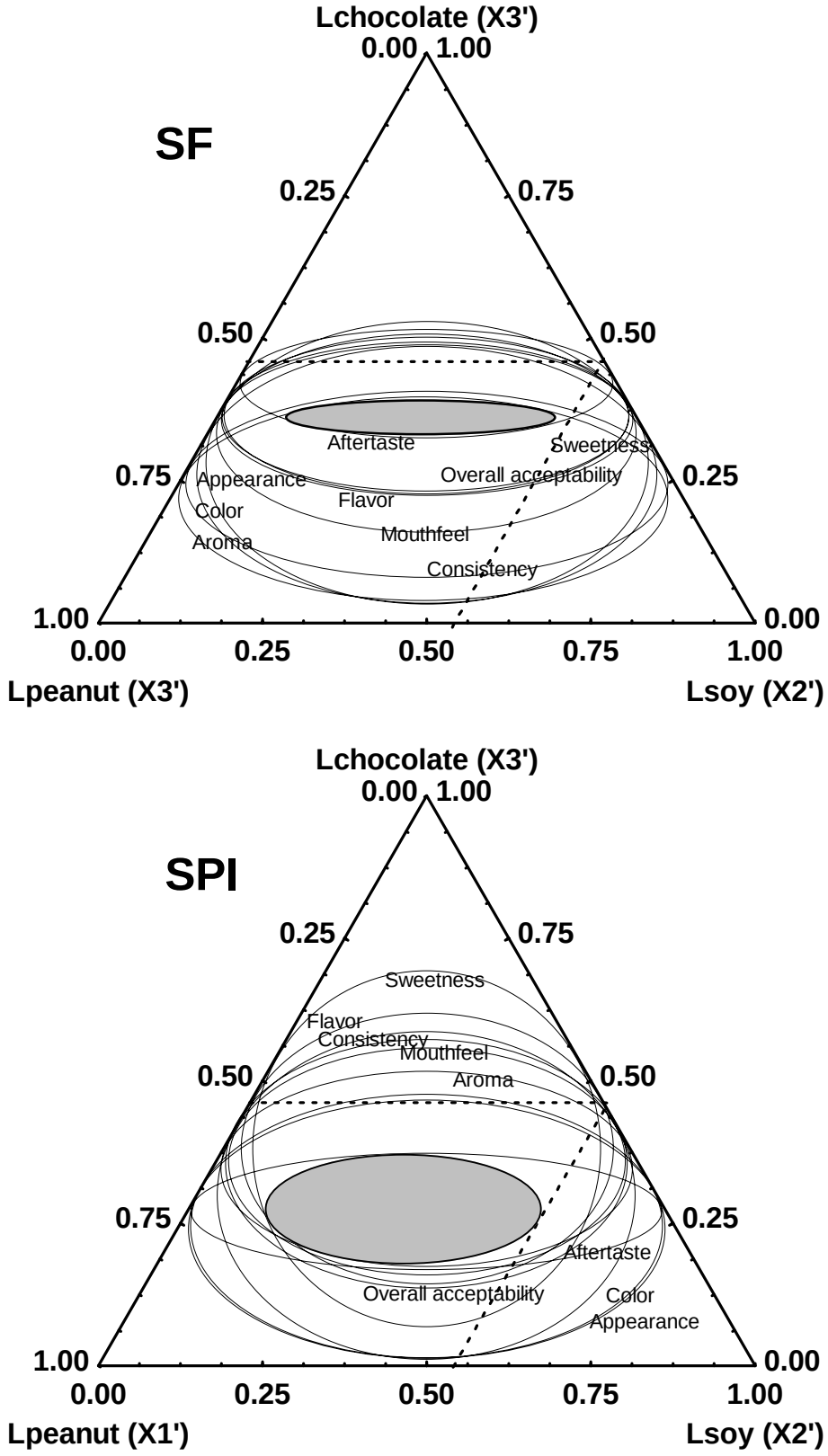


Figure 5.7: Optimum regions (shaded areas) obtained by superimposing contour plots for all nine attributes for soy flour (SF) and soy protein isolate (SPI) formulations

# CHAPTER 6

# SUMMARY AND CONCLUSIONS

A chocolate-flavored, peanut-soy beverage was developed by utilizing two important oilseed proteins: peanut and soy. The main objectives of enhancing sensory acceptability and nutritional properties of new beverage were accomplished by incorporating roasted peanut flour, chocolate flavor, and soy protein. Thorough review of earlier studies, market survey of available ingredients and milk-type products, and preliminary beverage preparation trials were crucial for selection of key ingredients, beverage formulation, and development of a pilot-plant scale beverage processing protocol.

Medium roasted peanut flour, chocolate syrup, and soy protein either in the form of soy flour (SF) or soy protein isolate (SPI) were processed as per the developed protocol. Physical characteristics of the beverage such as viscosity ($\eta$) and visual stability index (VSI) were measured. Nutritional properties were estimated based on lysine content (mg/g protein). The range of lysine contents (mg/g protein): 44.1-57.1 (reference lysine content = 51.0), $\eta$ (mPa s): 17.7-131.8 (commercial chocolate milk = 36.9), and VSI: 0.63-0.99 (commercial cow's milk = 1.00) resulted into low and high level constraints for a three-component, constrained mixture design. Peanut ($X_1$), soy ($X_2$), and chocolate syrup ($X_3$) were major components with constraints as $X_1$ = 30.56%-58.70%, $X_2$ = 28.26%-43.52%, and $X_3$ = 13.04%-25.93%. An extreme vertices design for the three-component, constrained mixture resulted into 14 possible beverage formulations. Two separate sets, one using SF and another using SPI resulted into twenty-eight chocolate-flavored, peanut-soy beverage formulations for sensory evaluation.

Twenty-eight beverage formulations and control (Hershey®'s creamy chocolate milk shake) were effectively studied for sensory properties by 41 untrained panelists. Nine sensory attributes including overall acceptability were evaluated using a 9-point hedonic scale (1 = dislike extremely, 5 = neither like nor dislike, and 9 = like extremely). Consumer response indicated by 'likings' on the hedonic scale was analyzed by comparing mean ratings of various sensory attributes. The formulation having 43.9% peanut, 36.3% SPI, and 19.8% chocolate syrup was the best overall. The mean ratings of this SPI-based formulation for various sensory attributes (as compared to the control) were: overall acceptability 5.9 (6.4), appearance 7.0 (6.7), color 6.8 (6.7), aroma 6.0 (6.8), consistency 6.6 (6.9), flavor 5.9 (6.7), sweetness 6.4 (5.9), mouthfeel 6.3 (7.0), and aftertaste 5.8 (6.6). The ratings for appearance, color, and sweetness were observed to be higher than the control. Two SPI-based formulations, one prepared with 37.0% $X_1$, 43.5% $X_2$, and 19.5% $X_3$; and another with 52.3% $X_1$, 28.3% $X_2$, and 19.5% $X_3$ were rated highest for color (7.0) and aroma (6.2), respectively. Another SF-based formulation (30.6% $X_1$, 43.5% $X_2$, and 25.9% $X_3$) had the highest mean ratings for flavor (6.0). Thus, the beverage had acceptability ratings comparable to that of commercial chocolate milk.

Optimization using response surface methodology (RSM) was done next. The consumer response in terms of various sensory attributes was modeled to give the optimum range of three mixture components leading to the most acceptable product formulation. Contour plots were generated and superimposition of regions of maximum acceptance (consumer ratings $\geq$ 5.0 for all the sensory attributes) gave optimum regions for SF and SPI formulations. It was observed that 34.1%-45.5% $X_1$, 31.2%-42.9% $X_2$, and 22.4%-24.1% $X_3$ in case of SF; and 35.8%-47.6% $X_1$, 31.2%-43.5% $X_2$, and 18.3%-23.6% $X_3$, in case of SPI were the ranges that can result into optimum formulations. In general, the SPI-based formulations were more acceptable than SF-

based formulations, and greater number of acceptable formulations can be prepared from SPI in combination with peanut and chocolate syrup such that all the three components add up to 100%. Also, SPI was a better choice as a source of soy protein, since it can be expected to give nutritionally superior beverage product with higher consumer acceptability and characteristics comparable to that of commercial chocolate milk.

The levels of third mixture component chocolate ($X_3$) represented the levels of chocolate syrup added in the mixture to obtain the final beverage formulations. The chocolate syrup was prepared by mixing various other ingredients such as stabilizers, emulsifiers, sweeteners, and flavor additives in the predetermined proportions. [The exact proportions of these ingredients have not been disclosed since the product being developed is an invention under review by The University of Georgia Research Foundation, Inc., Athens, Ga., U. S. A.]. Any change in the level of chocolate syrup thus indicated proportional change in all those ingredients that form the chocolate syrup.

Thus, the optimization using response surface methodology was an efficient statistical technique of handling several factors influencing the acceptability of the chocolate-flavored, peanut-soy beverage. Involving target consumers in the development process resulted into a novel beverage suitable to current consumer trend of convenience and nutrition. Using commercial chocolate milk as the control helped in achieving desired product characteristics close to that of similar product available in the market. In future, validation, shelf-life, and nutritional profile studies will complete the process of development of acceptable beverage product.

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APPENDICES

## APPENDIX A

### Calculation of lysine content of experimental beverage formulations

Lysine content in mg/g protein for SF (soy flour based) or SPI (soy protein isolate based) formulation containing '*a*' g of peanut slurry and '*b*' g of soy slurry was calculated as follows:

$$\text{Lysine content (mg/g protein)} = (\text{Lysine}_2 \times 1000)/\text{Protein}_2 \quad \text{.....(1)}$$

Where,

$$\text{Protein}_2 \text{ (g/100 g edible portion)} = (100 \times \text{Protein}_1)/2000 \quad \text{.....(2)}$$

$$\text{Lysine}_2 \text{ (g/100 g edible portion)} = (100 \times \text{Lysine}_1)/2000 \quad \text{.....(3)}$$

Note: In Eq.2 and Eq.3, basis is 2000 g of total beverage

And

$$\text{Protein}_1 \text{ (g in total beverage)} = (A_P + A_S)/10000 \quad \text{.....(4)}$$

$$\text{Lysine}_1 \text{ (g in total beverage)} = (B_P + B_S)/10000 \quad \text{.....(5)}$$

Here, $A_P$ and $A_S$ represent protein content and $B_P$ and $B_S$ represent lysine content of peanut and soy (either soy flour or soy protein isolate) slurries/base stocks. These terms in Eq.4 and Eq.5 were calculated as follows:

$$A_P = a \times R_P \times P_P \quad \text{.....(6)}$$

$$A_S = b \times R_S \times P_S \quad \text{.....(7)}$$

$$B_P = a \times R_P \times L_P \quad \text{.....(8)}$$

$$B_S = b \times R_S \times L_S \quad \text{.....(9)}$$

Where,

*a* = Amount of peanut slurry in g

*b* = Amount of soy slurry in g (either soy flour or soy protein isolate)

$R_P$ = Percentage protein recovered in peanut slurry from Table 3.4

$R_S$ = Percentage protein recovered in soy (either SF or SPI) slurry from Table 3.4

$P_P$ = Protein content in g/100 g edible portion of peanut flour from Table 3.3

$P_S$ = Protein content in g/100 g edible portion of soy (either SF or SPI) from Table 3.3

$L_P$ = Lysine content in g/100 g edible portion of peanut flour from Table 3.3

$L_S$ = Lysine content in g/100 g edible portion of soy (either SF or SPI) from Table 3.3

**Example** (Refer to Table 3.5):

Lysine content (mg/g protein) of a SF (soy flour based) experimental formulation is illustrated below using equations 1 through 9. Let us consider a combination of peanut-soy slurries that contains 1350 g of peanut slurry (*a*) and 650 g of soy slurry (*b*) (refer to high peanut + low soy, SF experimental beverage formulation given in Table 3.5). The lysine content (44.1 mg/ g protein) of this particular peanut-soy combination is calculated as follows:

Since,

*a* = Amount of peanut slurry in g = 1350 g

*b* = Amount of soy flour slurry in g = 650 g

$R_P$ = Percentage protein recovered in peanut slurry from Table 3.4 = 7.69%

$R_S$ = Percentage protein recovered in soy flour slurry from Table 3.4 = 8.19%

$P_P$ = Protein content in g/100 g edible portion of peanut flour from Table 3.3 = 52.2

$P_S$ = Protein content in g/100 g edible portion of soy flour from Table 3.3 = 51.5

$L_P$ = Lysine content in g/100 g edible portion of peanut flour from Table 3.3 = 1.87

$L_S$ = Lysine content in g/100 g edible portion of soy flour from Table 3.3 = 3.13

Hence,

$A_P$ = 1350 x 7.69 x 52.2 = 541914.3.....(from Eq.6)

$A_S$ = 650 x 8.19 x 51.5= 274160.3.....(from Eq.7)

$B_P$ = 1350 x 7.69 x 1.87= 19413.4.....(from Eq.8)

$B_S$ = 650 x 8.19 x 3.13= 16662.6.....(from Eq.9)

Substituting these values in equations 4 and 5 we get

$Protein_1 = (A_P + A_S)/10000$ = 81.6 g in total beverage.....(from Eq.4)

$Lysine_1 = (B_P + B_S)/10000$ = 3.6 g in total beverage.....(from Eq.5)

Substituting the values for $Protein_1$ and $Lysine_1$ in Eq.2 and Eq.3 we get

$Protein_2$ = (100 x 81.6)/2000 = 4.08 g/100 g edible portion…….....(from Eq.2)

$Lysine_2$ = (100 x 3.6)/2000 = 0.18 g/100 g edible portion…….....(from Eq.3)

Note: In Eq.2 and Eq.3, basis is 2000g of total beverage

Finally, using Eq.1, we get the lysine content (mg/g protein) as

Lysine content = (0.18 x 1000)/4.08 = 44.1 mg/ g protein…….....(from Eq.1)

In this way, lysine content of different combinations of peanut and soy slurries in various experimental beverage formulations can be calculated.

## APPENDIX B Sensory Analysis Instructions Sheets

B.1 Instructions given to the panelists during the sensory test sessions

**~~~~ATTENTION~~~~**

1. WHEN YOU SIT DOWN PLEASE PRESS **GREEN BUTTON.**
2. PLEASE **DO NOT** CONVERSE IN THIS ROOM.
3. FILL A GLASS OF WATER USING THE PLASTIC WATER CUP.
4. VISUALLY OBSERVE, SMELL AND DRINK A SMALL PORTION OF THE SAMPLE IN ORDER TO EVALUATE VARIOUS ATTRIBUTES.
5. DRINK **AT LEAST** FOUR SIPS OF THE SAMPLE TO COMPLETE THE **"BALLOT FORM"**.
6. YOU MAY USE THE STYROFOAM SPIT CUP IF YOU DO NOT WISH TO SWALLOW THE SAMPLES. IF YOU USE IT FOR ONE SAMPLE YOU WILL NEED TO USE IT FOR ALL YOUR SAMPLES. PLEASE COVER WITH LID AND THROW AFTER YOU FINISH THE SESSION.
7. PLEASE **USE SEPARATE "BALLOT FORMS"** FOR DIFFERENT SAMPLES.
8. PLEASE WRITE **"PANELIST CODE"** AND **"SAMPLE CODE"** ON THE **"BALLOT FORM"**
9. PLEASE **RATE VARIOUS ATTRIBUTES** AND **CHECK THE SPACE** THAT BEST REFLECTS YOUR LIKING.
10. DRINK ENOUGH **WATER** AND **CRACKERS** BETWEEN SAMPLES TO RINSE YOUR MOUTH.
11. PLACE REMAINING SAMPLE AND **"BALLOT FORM"** IN THE TRAY AND RETURN IT THROUGH **"PASS THROUGH"** DOOR.

12. GET A **NEW SAMPLE** AND **"BALLOT FORM"** FOR EVALUATION.
13. REPEAT **STEPS 4 THROUGH 12** UNTILL YOU FINISH THE SESSION.
14. PLEASE PLACE ALL THE USED CUPS AND NAPKINS INSIDE THE **"PASS THROUGH"** DOOR **AT THE END OF THE SESSION**.
15. PLEASE PRESS **GREEN BUTTON** AND **WHITE BUTTON** WHEN YOU LEAVE.
16. PLEASE LEAVE THE ROOM QUETLY AND RETURN TO THE CONFERENCE ROOM.
17. PLEASE MAKE SURE THAT YOU HAVE SIGNED THE **SIGN UP SHEET**, **CONSENT FORM** AND COMPLETED THE **QUESTIONNARE** BEFORE YOU FINALLY LEAVE.
18. PLEASE **COME AGAIN** IF THIS IS NOT YOUR **FIFTH SENSORY SESSION**.

**~~~~~~~~~~~THANK YOU FOR YOUR PARTICIPATION~~~~~~~~~~~**

B.2 Instructions for the person-in-charge to greet and welcome the panelists:

1. Please make sure that you have folders ready for the panelists coming for a particular sensory sub-session.
2. Please have panelists sign in.
3. Please check the panelist code and give it to each panelist attending a particular sub-session.
4. Tell them to sign the consent form keeping a copy for their record.
5. Please check that they have signed the "sign up" sheet and consent form.
6. Please go over the instruction sheet once in brief and tell panelists to follow it during the sample evaluation.
7. Tell them that instruction sheet is posted on the booth wall. Sensory score ballots and pencil will be provided along with the sample being served once they occupy the booth area.
8. Tell them that once they occupy the booth they will pass the panelist code to the server.
9. Tell them that they will receive one sample at a time along with a sensory score ballot which they have to return once all the questions are marked and evaluation of that particular sample has been finished.
10. Tell them that they have to fill up a questionnaire during a break of 3 min between two groups of sample servings (4 samples + 3 min break + 4 samples) per session.
11. Show the signboard and explain the functioning of buttons, using dummy tray explain water cup, spit cup, ballot and the sequence of events.
12. Explain the test protocol: The test will be divided into two parts.

a. Panelist will receive a tray with water cup, spit cup, napkins, ballot, pencil and first cup of sample after they pass the panelist code to the server.
b. After finishing evaluation of first sample they will pass the remaining sample and "ballot form" through "PASS THROUGH" door.
c. They will receive next sample and "ballot form" for evaluation.
d. Total of 4 such samples will be evaluated one by one.
e. Their will be a break of 3 min after finishing group of 4 sample evaluation.
f. They will fill out the questionnaire provided during the break.
g. After the break they will return the questionnaire and repeat the procedure for evaluation of remaining four samples one by one as described b through d.

13. Please be sure to explain them the purpose of spit cup, water cup and remind to drink water and eat crackers between each sample during sensory evaluation to rinse the mouth.
14. Please make sure that panelists are comfortable in the booth area and know they have understood the procedure.
15. Please give following instructions to the panelists for taste test:

a) Panelist will use separate "ballot forms" for each sample and they will return it with the remaining sample.
b) Panelist will write his/her panelist code and sample code as shown on the sample cup at the top space provided on the "ballot form".
c) They will receive one sample at a time along with the "ballot form".
d) Please evaluate all the samples by marking on the "ballot form" at the space that best reflects their liking.

e) Please return the remaining sample and the completed "ballot form" in "PASS THROUGH" door before pressing white button for next set of sample and "ballot form".

f) Please drink a small portion of sample and start your evaluation.

g) Visually observe, smell and take some more sips to answer all different questions.

h) You may use spit cup to avoid swallowing it completely. You will have to use spit cup for all the samples if used for one. Caution: Drinking 8 different samples of the beverage may make them feel full.

i) Eat ½ cracker and drink several sips of water between the samples.

j) After evaluating 4 samples you will have a short break of 3 min. During this break you have to fill out a short questionnaire. When finished please place it in the doorway and press white button.

k) You will be served next four samples one at a time. Please evaluate them as described earlier.

l) When finished you have to place tray, all used cups, napkins if any and pencils in the "PASS THROUGH" door. Press white button, and then press green button to turn light off.

m) Please quietly return to the greeting area for some refreshments.

n) Please make sure that you have completed all the paperwork before you finally leave.

o) Please remind the next scheduled session date and time unless this is their fifth and last session.

## APPENDIX C Sensory data collection sheet (Ballot Form)

**Panelist Code: _____ Sample Code: _____ Date: _____**

**Instructions:**

- Visually observe, smell and drink **AT LEAST** four sips of each sample to complete this ballot.
- Please **CHECK THE SPACE** that best reflects your feeling about the product.
- Answer **ALL TEN** questions.
- Use the space provided at the end for additional comments.

1. **OVERALL**, how do you **"LIKE"** 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 **"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 |

3. 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 |

4. How would you rate the **"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. How would you rate the **"CONSISTENCY"** 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 |

**……..Please Turn Page Over to CONTINUE……..**

6. How would you rate the **"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. How would you rate the **"MOUTHFEEL (Absence of chalkiness)"** 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. How would you rate the **"AFTER-TASTE"** 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. Would you be willing to buy this product if available in the marketplace?

| Yes | [ ] | No | [ ] |
|---|---|---|---|

Comments (optional) _____

_____

~~~~~.....~~~~
~~~~~

## APPENDIX D The consent form for sensory test

## CONSENT FORM

I, _____, agree to participate in a research study entitled "HATCH PROJECT: Approaches to improve processing and packaging techniques for snack and other processed foods-Development of flavored peanut beverages and study of physical and sensory properties" conducted by Rashmi P. Deshpande from the Department of Food Science and Technology at the University of Georgia, Griffin, GA (770-412-4747 ext. 125) under the direction of Dr. Manjeet S. Chinnan, Department of Food Science and Technology, University of Georgia, Griffin, GA (770-412-4741).

I understand that my participation is voluntary. I can stop taking part at any time without giving any reason, and without penalty. I can ask to have all of the information about me returned to me, removed from the research records, or destroyed.

The reason for this study is to test a peanut-based-beverage for overall acceptability and preference among different protein sources used in combination with peanut flour and added flavor. I will not benefit directly from this research. However, my participation in this research may lead to information that could help to identify the characteristics the consumer prefers in the case of milk-type, protein-based beverage products.

The following points have been explained to me:

1) The procedures are as follows: The coded samples will be placed in front of me and I will evaluate them by normal standard methods (visual observation, smelling, tasting, and swallowing) and indicate my evaluation on score ballot. All procedures are standard methods as published by the American Society for Testing and Materials.

2) Participation entails the following risks: The flavor additives, various protein sources, emulsifier, stabilizer and other ingredients are approved by FDA and are known as GRAS (generally recognized as safe). The researchers are not aware of any adverse effects on pregnancy. Other risk which may be envisioned is that of an allergic reaction to peanut seeds and other additives used. However, because I will know the nature of the products beforehand, the situation can normally be avoided. In the event of an allergic reaction, emergency services may be obtained by dialing 911. My insurance company or I will be responsible for the costs incurred. The medical treatment will be available from the Family Medical Center or the Spalding Regional Hospital, Griffin, GA.

3) It is my responsibility to make known to the investigators any allergies I may have toward the food products being tested when they occur. The foods to be tested are protein-based beverages having defatted soy flour, soy protein isolate, and defatted peanut flour as basic ingredients along with water, sucrose, corn syrup, maltodextrin, cocoa powder, peanut oil, pure vanilla extract, salt, soy lecithin, artificial chocolate flavor, and carrageenan.

   (Allergies: _____ )

4) The results of the participation will be kept confidential and will not be released in any individually identifiable form without my prior consent unless required by law.

5) I will be assigned an identifying number and this number will be used on all of the questionnaires I fill out.

6) The investigator will answer any further questions about the research, either now or during the course of the project (770-412-4747 ext. 125).

I understand that I am agreeing by my signature on this form to take part in this research project and understand that I will receive a signed copy of this consent form for my records.

| _____ | _____ | _____ |
|---|---|---|
| Name of Researcher | Signature | Date |
| Telephone: _____ | Email: _____ | |

| _____ | _____ | _____ |
|---|---|---|
| Name of Participant | Signature | Date |

Please sign both copies, keep one and return one to the researcher.

Additional questions or problems regarding your rights as a research participant should be addressed to Chris A. Joseph, Ph.D. Human Subjects Office, University of Georgia, 606A Boyd Graduate Studies Research Center, Athens, Georgia 30602-7411; Telephone (706) 542-3199; E-Mail Address IRB@uga.edu.

## APPENDIX E Questionnaires (I through IV) for sensory test

### QUESTIONNAIRE I

### CONSUMPTION OF READY-TO-SERVE (RTS) BEVERAGE PRODUCTS

**Please answer ALL TEN questions by circling the option that best describes your preference.**

1. How often do you drink milk?
    a. Twice a day
    b. Once a day
    c. Once a week
    d. Not at all

2. How often do you consume ready-to-drink, non-carbonated beverages? For e.g. Bottled refrigerated coffee drink, milk shake or chocolate milk etc.
    a. Everyday
    b. Once a week
    c. Rarely

3. What is the most important attribute you look for in the 'ready-to-drink' beverages?
    a. Color
    b. Taste
    c. Consistency
    d. Sweetness
    e. Flavor
    f. Nutrition
    g. All of the above

4. How do you prefer the serving style of a beverage?
    a. Ready off the shelf but requiring home-refrigeration
    b. Cold pack from vending machine
    c. Refrigerated packs sold in grocery stores
    d. Instant mix with water/milk

5. How do you prefer the serving package of a beverage?
    a. On the go plastic bottle
    b. Glass bottle
    c. Can
    d. Carton pack
    e. Stand alone pouch

6. What is your favorite flavor for beverages?
    a. Vanilla
    b. Strawberry
    c. Chocolate
    d. Other (Specify) _____

**……Please Turn Over to CONTINUE……**

7. Have you tasted various soymilks available in the market? For e.g. Original, Vanilla, Strawberry, and Chocolate flavored soymilk.
    a. Yes
    b. No

8. If Yes,
   (i)How do you like the taste and flavor of soymilk?
    a. Good
    b. Very good
    c. Bad
    d. Very bad
   (ii)How often do you drink soymilk?
    e. Twice a day
    f. Once a day
    g. Once a week
    h. Never

9. Did you know that soymilk is an excellent lactose-free alternative to milk?
    a. Yes
    b. No

10. Did you know that soymilk can be a protein rich, low fat nutritious component of your diet?
    a. Yes
    b. No

**……THANK YOU……**

**QUESTIONNAIRE II**

**Peanuts: History, nutrition and consumption information**

Sensory Session III November 4 and November 7, 2003

**Please answer <u>ALL TWELVE</u> questions by circling the option that best describes your preference.**

1. I eat peanuts
    a. Everyday
    b. At least twice or more a week
    c. Rarely
    d. Never (because……)
2. Peanuts are
    a. Nuts
    b. Legumes (e.g. Soybeans, Peas etc.)
    c. Fruits
    d. Vegetables
    e. None of the above
3. Peanuts are also called:
    a. Earth nuts
    b. Monkey nuts
    c. Grass nuts
    d. Ground nuts
    e. All of the above
    f. None of the above
4. What is the most popular form of peanut consumption in the US?
    a. Peanut butter
    b. Snack peanuts
    c. Candies
    d. Peanut oil
    e. Peanut flour in confectioneries
5. Do you know of any peanut based BEVERAGE type product other than the one being tested in this study?
    a. Yes
    b. No

    If yes, Please specify _____

    _____
6. Are peanuts a good source of nutrition?
    a. Yes
    b. No

**……Please Turn Page Over to CONTINUE……..**

7. Peanut varieties produced in the U.S. are
    a. Runner
    b. Virginia
    c. Spanish
    d. Valencia
    e. All of the above
    f. No idea
8. World's largest peanut producing country is
    a. United States
    b. China
    c. India
    d. Mexico
    e. Bulgaria
9. Which of the following states in the US contribute to peanut production?
    a. Florida
    b. Oklahoma
    c. Alabama
    d. Texas
    e. Virginia
    f. North Carolina
    g. Georgia
    h. All of the above
10. Do you know that Georgia is the largest producer of peanuts in the U.S.? Georgia produces almost 1/2 the total U.S. peanut crop of which >50% is used to make peanut butter (2002).
    a. Yes
    b. No
11. Did you know that diets high in monounsaturated fat (like those constituting peanuts, peanut butter, peanut oil and/or olive oil) are superior to low-fat diets for a healthy heart?
    a. Yes
    b. No
12. Do you think peanuts can be a part of healthy diet that will help people lose weight?
    a. Yes
    b. No

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**QUESTIONNAIRE III**

**Sensory Evaluation of New Products: Art and Science**

Sensory Session IV November 11 and November 14, 2003

**Please answer <u>ALL TEN</u> questions by circling the option that best describes your preference.**

1. Have you participated in sensory evaluation study before?
   a. Yes
   b. No

   If yes,

   i) When did you last participate?

   a. Once in the past six months
   b. Once in the past year
   c. Other: Specify_____

   ii) What was the product you evaluated?

_____

_____

2. What in your opinion is the most important factor in sensory analysis?
   a. Surrounding environment
   b. Product serving conditions (e.g. Temperature, sample size and cup etc.)
   c. Serving order
   d. Sensory ballot sheet sequence and presentation
   e. Characteristics of product being studied
   f. Other (Please specify_____)
3. The rating scale being used in this study which has choices from "Like extremely" to "Dislike extremely" is known as
   a. Five-point Hedonic Scale
   b. Nine-point Hedonic Scale
   c. Food Action Rating Scale
   d. Pictorial Rating Scale
   e. All of the above
   f. None of the above
   g. No idea
4. The 'in-house' sensory analysis tests are those carried out in the:
   a. Homes of the consumers
   b. Company/department premises with their staff
   c. Restaurant or department store
   d. All of the above
   e. None of the above
   f. No idea

**……Please Turn Page Over to CONTINUE……..**

5. The method of sensory data collection using computers compared to paper ballots is perhaps
    a. More efficient and appropriate
    b. Same/ equivalent
    c. Less efficient
    d. Not comparable
    e. No preference
    f. Other (Please Specify_____)
6. Sensory analysis is the definition and scientific measurement of a product perceived by _____
    a. Sight
    b. Sound
    c. Smell
    d. Taste
    e. Touch
    f. All of the above
    g. None of the above
7. Sensory evaluation is done during:
    a. Shelf-life study
    b. Product reformulation
    c. New product development
    d. Product maintenance
    e. All of the above
    f. None of the above
8. _____is one of the most important activities in product development.
    a. New concept generation
    b. Selecting recipe or formulation
    c. Consumer acceptance
    d. Advertising and Marketing
    e. All of the above
    f. None of the above
9. Sensory acceptance tests are conducted:
    a. To determine market demand of product being tested
    b. To collect demographic information
    c. To screen products and identify those that are significantly disliked or liked
    d. All of the above
    e. None of the above
10. In food industry, the ultimate goal of sensory evaluation is to:
    a. Enhance quality and increase sales
    b. Know consumer opinion
    c. Launch new product
    d. Compare different products
    e. None of the above

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**QUESTIONNAIRE IV**

**Sensory study of a 'Peanut Beverage'**

Sensory Session V November 18 and November 21, 2003

**Please answer <u>ALL TEN</u> questions by circling the option that best describes your preference.**

1. What do you like to have in the breakfast?
   a. Milk and Cereal
   b. Cereal bar
   c. Sandwich
   d. Tea/Coffee/Juice
   e. Other (Please specify_____)
2. If available, you would like to consume the 'Peanut Beverage' under study
   a. In the restaurant/ cafeteria
   b. At home
   c. From the vending machine
   d. Anywhere
   e. Other (Please specify_____)
3. What type of product do you preferably purchase?
   a. Low fat
   b. Low calorie
   c. High protein
   d. Good to eat (Do not care as long as you like it)
   e. Other (Please specify_____)
4. Would the 'Nutritional Facts' information (such as Fat, Protein or Calorie content) influence your purchase in case of 'Peanut Beverage'?
   a. Yes
   b. No
   c. May be
5. If 'Peanut Beverage' was available in the market in refrigerated section (like chocolate milk) with 'Health Claim' such as

   "While many factors affect heart disease, diets low in saturated fat and cholesterol may reduce the risk of this disease".

   OR

   "25 grams of soy protein a day, as part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease. A serving of [*name of food*] supplies __ grams of soy protein".

   Would you consider buying it?
   a. Yes
   b. No
   c. May be

**……Please Turn Page Over to CONTINUE……..**

6. In future, the beverage product under study if available in the retail grocery stores' refrigerated section as a single serving bottle (240ml size) costing $1.69, what would you be willing to pay for a bottled peanut drink?
   a. More
   b. Less
   c. About the same
   d. Other (Please specify_____)
7. If rated on a scale of 10, how would you overall rate the 'Chocolate flavored peanut-based beverage'?

_____

Suggestions if any_____

_____

_____

8. How was your overall sensory experience during this study?

a. Good
b. Bad
c. No comments
d. Other (Please specify_____)

Suggestions if any_____

_____

_____

9. If rated on a scale of 10, how would you rate the performance on our side?

_____

10. We can improve on_____

a. The sensory facility
b. Product presentation
c. Greetings and guidelines
d. Refreshments
e. Scheduling appointments and giving reminders
f. Other (Please specify_____)

Comments (optional)

_____

_____

**~~~~~…Thank you...~~~~**