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Quality characterization of cholesterol-free mayonnaise-type spreads containing rice bran oil

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QUALITY CHARACTERIZATION OF CHOLESTEROL-FREE MAYONNAISE-TYPE SPREADS CONTAINING RICE BRAN OIL

A Thesis

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DEDICATION

I dedicate this thesis to my parents. Words can not express the love, admiration, and appreciation I have for you. You have provided me with everything I have, made me the person I am, and helped me achieve all I have today. You have always believed in me and supported my decisions. I am truly blessed to have such supportive and loving parents. THANK YOU VERY MUCH FOR EVERYTHING!

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ABSTRACT

Traditional mayonnaise is manufactured with soybean oil (SBO) and egg-yolk containing ingredients. About 1/4 of American consumers have some forms of cardiovascular disease, accounting for >40% of all deaths in USA. Rice bran oil (RBO), a healthy lipid source, has cholesterol-lowering effects, and could be used to replace SBO in mayonnaise preparation. To take advantage of the health benefits associated with RBO, food products containing RBO need to be developed and characterized.

Cholesterol-free mayonnaise-type spreads containing RBO were developed using a constrained mixture design. Two studies were performed to determine sensory attributes driving acceptance and purchase intent and to optimize the formulation. In the first study, following a Balanced Incomplete Block design, consumers evaluated the products. The attributes that differentiated the formulations were color, odor, spreadability and mouthfeel. Taste, mouthfeel, and overall liking were identified as the attributes influencing purchase intent. Purchase intent increased after consumers were informed of RBO health benefits. The overall liking odds ratio decreased, meaning that consumers were willing to sacrifice product liking in favor of RBO health benefits. Combinations of 37-42% RBO, 53-57% water, and 1-6% SPC, were determined as yielding optimum formulations. For advanced product refinement taste and mouthfeel must be focused.

In study two, three flavored products were developed based on Formulation E: Sour Cream & Onion, Cheddar & Sour Cream, and Monterrey Jack. Consumers evaluated all flavored samples and a control based on preference ranking. There were significant differences among flavored spreads and control. Consumers were able to correctly differentiate between the flavored samples and the control. These differences were present among all flavors except

among Sour Cream & Onion and Monterrey Jack. All flavored products were found acceptable and there was an increase in purchase intent after consumers were aware of the potential health benefits associated with product consumption.

The quality of the spreads was characterized through the development of sensory descriptors and determination of several physicochemical properties. Colorimetry, pH, and viscosity measurements showed no differences among the formulations over time. Oryzanol concentration increased with increased RBO content of the formulations.

CHAPTER 1. INTRODUCTION

About one fourth of the American population has some form of cardiovascular disease, that can result in heart disease and stroke. These two are the first and third causes of death in the United States for both males and females, accounting for more than 40% of all deaths (Centers for Disease Control and Prevention 2005), with high blood cholesterol being one of the risk factors for heart disease. The American Heart Association (2006) reported that more than 2,600 Americans die of cardiovascular disease each day, an average of one death every 33 seconds. A healthy diet low in saturated fat, among others, is necessary for reducing the risk of heart disease.

Mayonnaise, one of the oldest and most used sauces worldwide and normally used as a sandwich spread in North America, is a mixture of oil, egg, vinegar and spices. United States law requires that mayonnaise contains at least 65% oil. Commercial mayonnaise contains between 70-80% oil. The market for this product is mounting as different and interesting flavors and ingredients are launched, and healthy versions are developed (McClements 2005). Development of these healthier versions has aroused due to increased consumer alertness of the over-consumption of cholesterol and saturated fats and under-consumption of healthier food components.

Observational studies on diverse populations show overwhelming evidence of a "linear relationship between plasma lipid levels and cardiovascular disease-induced death rate" (Cicero and Gaddi 2001). It is recognized that the cardiovascular disease death rate is higher in Northern Europe and North America than in Mediterranean countries. Trichopoulou and others (1999) reported that the Mediterranean diet is low in cholesterol, saturated and oxidized fatty acids. Tikkanen and Adlercreutz (2000) reported that the Far Eastern Asian diet, also low in cholesterol and fatty acids and rich in rice and soy derived proteins, is related with a low level of

cardiovascular-related mortality. There are numerous reports on the antihypercholesterolemic effects of vegetable oils rich in polyunsaturated fatty acids (Cicero and Gaddi 2001).

There are no available data about the production of a cholesterol-free mayonnaise-type spread containing soy protein concentrate and an LDL cholesterol lowering oil. Therefore, the objective s of this thesis research were to develop a cholesterol-free, low fat, rice bran oil based mayonnaise-type spread and to determine the consumer sensory characteristics that determine product acceptance and drive purchase intent. A cholesterol free product was achieved by replacing eggs, a cholesterol containing ingredient, with soy protein concentrate. This product also contained less fat, which was achieved by lowering the amount of oil. The rice bran oil used in this formulation has serum cholesterol lowering properties. This product was developed with the challenge of incorporating these new ingredients without detrimentally influencing the physical, chemical and sensory qualities.

This thesis is divided into six chapters. Chapter one provides a summarized introduction and discusses this research's justification. Chapter two presents a literature review with concepts associated with this thesis work. Chapter three presents the product development process, the consumer study and the product optimization of the cholesterol-free mayonnaise-type spreads containing rice bran oil. The third chapter also presents the product's optimization process. Chapter four discusses discrimination testing of flavored products based on a ranking test and the acceptability and consumers' purchase intent of the product. In chapter five, sensory descriptors for the product are presented and the product physicochemical properties are characterized. Chapter six consists of a brief summary of all the findings of this research and possible future work. All cited references and appendices containing the survey questionnaires for all consumer

studies, research consent forms, SAS codes and other figures are included. To conclude, the VITA of the author of this work is provided.

CHAPTER 2. LITERATURE REVIEW

2.1 Introduction

Heart disease and stroke are the nation's leading causes of death. Heart disease and stroke are both the principal components of cardiovascular disease; they are the first and third leading causes of death in the United States. One American dies each second of cardiovascular disease, amounting to 927,000 deaths a year of approximately 70 million Americans (roughly one fourth of the population) live with a cardiovascular disease (CDC 2006). Apart from the death rate caused by this disease, the economic impact is experienced. According to the Center for Disease Control and Prevention, approximately $394 billion was projected to be the health expenditures for cardiovascular disease in 2005. The two major risk factors for cardiovascular disease are high blood pressure and high blood cholesterol. Mayonnaise, a staple American food high in fat, is defined by the FDA as the emulsified semisolid food prepared from vegetable oil(s), acidifying ingredients, egg-yolk containing ingredients, and spices. Traditional mayonnaise contains at least 65% fat. Soy protein concentrate provides an alternative to cholesterol containing eggs due to its emulsifying properties. Rice bran oil (RBO), a healthy lipid source, has cholesterol-lowering effects among other health benefits attributed to the unsaponifiable components.

2.2 Rice Bran Oil

Rice, *Oryza sativa*, is the second largest cereal grain produced worldwide after wheat and is the primary source of food for nearly half of the world's population. The origin of rice is attributed to Southeast Asia, i.e. eastern India, Indo-China and southern China or Africa (Salunkhe and others 2000). For the most part, rice production is concentrated in developing

Asiatic countries, mainly in China, India and Indonesia (FAO 1985). Figure 1 summarizes rice area, yield production and value for the United States from 1995-2004.

Year	Area planted	Area harvested	Yield per acre	Production	Marketing year average price per cwt. received by farmers	Value of production
	1,000 acres	*1,000 acres*	*Pounds*	*1,000 cwt.*	*Dollars*	*1,000 dollars*
1995	3,121.0	3,093.0	5,621	173,871	9.15	1,587,236
1996	2,824.0	2,804.0	6,120	171,599	9.96	1,690,270
1997	3,125.0	3,103.0	5,897	182,992	9.70	1,756,136
1998	3,285.0	3,257.0	5,663	184,443	8.89	1,654,157
1999	3,531.0	3,512.0	5,866	206,027	5.93	1,231,207
2000	3,060.0	3,039.0	6,281	190,872	5.61	1,049,961
2001	3,334.0	3,314.0	6,496	215,270	4.25	925,055
2002	3,240.0	3,207.0	6,578	210,960	4.49	979,628
2003	3,022.0	2,997.0	6,670	199,897	8.08	1,628,948
2004	3,347.0	3,325.0	6,942	230,818	7.40	1,676,020

Figure 1: Rice, rough: Area, Yield Production, and Value, United States. 1995-2004 **(USDA-NASS** *Agricultural Statistics 2005)*

An interest in the production of rice bran oil (RBO) aroused in Asian countries due to the shortage of edible oils. Rice bran production is estimated to be 47 million tons. The bran is separated during milling and is a potential source of edible oil for rice-producing countries, estimated to produce over 3.5 million tons of bran oil (Salunke and others 2000). Currently, about 450,000 metric tons of rice bran oil is produced worldwide, where Japan produces almost 25% of this amount. According to Gopala Krishna (2002), India ranks first in the production of edible RBO. RBO is now extensively used in Asian countries (Kahlon and others 1992); but in the United States the interest in RBO was recently renewed, since production investigations first started in the 1950s. This interest was generated due to nutritional value of the oil and export opportunities, where the potential production is 41,000 metric tons (Orthoefer 1996).

Most of the oil in rough rice is concentrated in the germ and bran layers, which together are referred to as “bran” (Figure 2) and make up only 10% of the rough rice weight. These rice bran layers have an oil content of about 20% (Cicero and Gaddi 2001, Orthoefer 1996). Figure 2 illustrates the location of the bran layer in the *Oryza sativa* seeds.

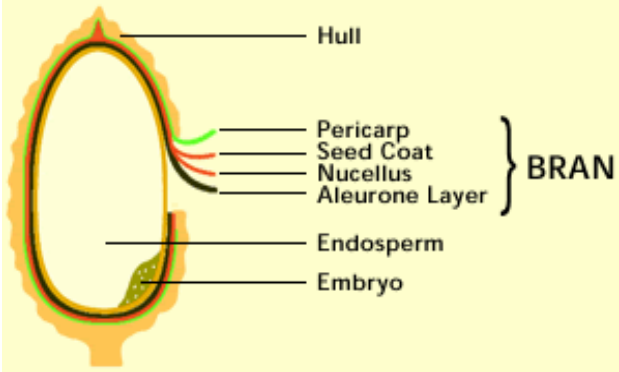

Figure 2- Bran Layer of *Oryza Sativa* Seeds (Orthoefer, 1996)

The oil is extracted from raw or stabilized bran by a solvent extraction method, where hexane and petroleum are the most preferred solvents (Salunkhe and others 1992, Orthoefer 1996). The extraction process consists of soaking the bran in the solvent, removing the oil by percolation and filtration. The solvent is removed from the miscella (oil plus solvent) by stripping, and then it is condensed and recovered. The crude RBO has higher free fatty acid (FFA) content than many other vegetable oils (3-20%) and the biologically active components are concentrated in the unsaponifiable fraction (5-8%) of the oil. The refining method used to remove the FFA depends on the quality of the crude oil (Orthoefer 1996). The refining steps consist of degumming, dewaxing, removal of FFA, bleaching and deodorization (Salunkhe and others 1992). Upon refining, oil loss is 18% to 20% and there is about a 50% loss of active components, the unsaponifiable content in the oil (Dunford and King 2000). The final concentration of gamma-oryzanol in RBO depends on the processing conditions (Saska and Rossiter 1998). Gopala Krishna (2003) reported that the oxidative stability of physically refined RBO is higher than that of chemically refined RBO. The crude RBO is not apt for human consumption, but the refined oil is principally used as shortening, cooking oil, and salad oil. The refined and winterized oil is excellent for mayonnaise, salad dressings and other emulsified products (Salunkhe and others 1992).

Refined RBO is light in color (usually pale yellow), odorless, and limpid at 20°C. It has a density of 0.920-0.930 at 20°C and has a pleasant, light sweet flavor (Sugano and Tsuji 1997). Rice bran oil is a stable oil that exhibits similar properties to soy or soy plus cottonseed oils, it does not solidify as easily when compared to cottonseed and peanut oil, and it can be hydrogenated to a semi-solid fat. Rice bran oil, typically an oleic-linoleic-type fatty acid, contains mainly oleic, linoleic, and linolenic acids as unsaturated fatty acids, and mostly palmitic and stearic acids as saturated fatty acids. Table 1 summarizes the fatty acid composition of RBO. Neutral lipids in the oil comprise 88-89%, glycolipids 6-7%, and phospholipids 4.5-5% (Hemavathy and Prabhakar 1987). It contains over eight different sterols, amongst which β-sitosterol (50-60%), campesterol (15-25%) and sigmasterol (10-13%) are the major compounds (Gaydou and Raonizafinimanana 1980).

RBO is an excellent source of nutritionally beneficial compounds. These compounds include oryzanol, lecithin and the unsaponifiable matter (Sugano and Tsuji 1997; Dunford and King 2000). Rice bran oil is characterized by its comparatively high content of unsaponifiable material when compared to other edible oils (Sugano and Tsuji 1997). The biologically active compounds are concentrated in the unsaponifiable fraction of the oil (Dunford and King 2000).

Table 1: Free Fatty Acid Profile of Rice Bran Oil (Orthoefer, 1996)

Free Fatty Acid	**Content (%)**
C16:0 – Palmitic	15.0
C18:0 – Stearic	1.9
C18:1 – Oleic	42.5
C18:2 – Linoleic	39.1
C18:3 – Linolenic	1.1
C20:0 – Arachidic	0.5
C22:0 – Behenic	0.2

2.2.1 Properties of Gamma-Oryzanol

The most characteristic component of RBO is gamma-oryzanol, the mixture of ferulic acid esters of triterpene alcohols and sterols (Itoh and others 1973; Xu and Godber 1999). Figure 3 shows the chemical structure of cycloartenyl ferulate, one of several plant sterols esterified to ferulic acids that form part of Oryzanol. The three major components of gamma-oryzanol, which account for approximately 80% of the oryzanol in rice bran are cycloartenyl ferulate, 2,4-methylenecycloartanyl and campesteryl ferulate. The fundamental molecular structure is the ferulic aromatic phenolic nucleus sterified to cyclopentanperihydrophenanthrene (Seetharamiah and Prabhakar 1986).

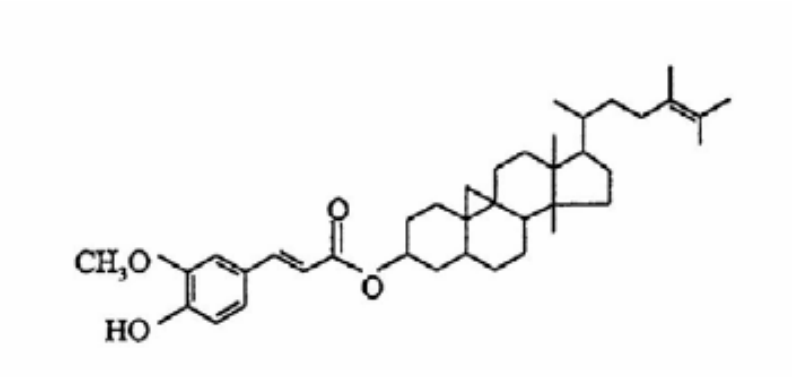

Figure 3: Cycloartenyl ferulate, one of the several plant sterols esterified to ferulic acids that form part of Oryzanol.

The antioxidant property of gamma-oryzanol, possibly due to its ferulic acid structure, accounts for the nutritional function of this compound. Xu and Godber (2001) investigated the antioxidant activity of the three major components of gamma-oryzanol and reported that their antioxidant activity was lower than that of α-tocopherol in protecting against linoleic acid oxidation.

The content of gamma-oryzanol (115-780 ppm) differs with the source of RBO, depending on the degree and possibly the method of processing (Rogers and others 1993).

Oryzanol is known to be a powerful inhibitor of the formation of the iron-driven hydroxyl radicals and also a natural antioxidant that possess potential antioxidant activity both *in vivo* and *in vitro* (Kim and others 2001).

2.2.2 Rice Bran Oil Health Benefits

The beneficial effects of RBO are well known. One of the most investigated properties of RBO is its antihypercholesterolemic property. The cholesterol-lowering effects of RBO are either attributed to the unsaponifiable fraction or the free fatty acid composition, with more findings reporting that the hypolipidemic effect of RBO is not entirely explained by its fatty acid composition.

According to Most and others (2005), the cholesterol-lowering effects of RBO are credited to the unsaponifiable components and not entirely to the free fatty acid composition. Most and others (2005) reported results in which LDL cholesterol decreased by 7% in healthy, moderately hypercholesterolemic subjects who consumed RBO over a 10 week period even though high-density lipoprotein (HDL) cholesterol remained unchanged. According to Watkins and others (1999), the cholesterol level decreased by 14.1% and low-density lipoprotein (LDL) cholesterol declined by 20.6% in hypercholesterolemic subjects consuming rice bran oil non-saponifiables for a one year period. Watkins and others (1999) also reported an increase in HDL cholesterol levels and a decrease in triglyceride levels on the same subjects. Wilson and others (2000) reported a significant contribution of the unsaponifiable fraction (non-fatty acid components) of RBO to its cholesterol-lowering properties. Qureshi and others (1991) reported that tocotrienols present in RBO inhibit cholesterol synthesis. Similar results were reported by Sugano and Tsuji (1997), where it was stated that the occurrence of gamma-oryzanol and

tocotrienols (components of the unsaponifiable fraction) could be responsible for the hypocholesterolemic effect of RBO.

Vissers and others (2000) reported that RBO sterols' effects in lowering serum total cholesterol are probably due to 4-desmethylsterols. Similar findings were reported by Hendricks and others (1998) and Sierksma (1999). Cholesterol-lowering properties of RBO have been reported in rats, non-human primates, and humans (Wilson and others 2000; Sugano and Tsuji 1997; Cicero and Gaddi 2001; Vissers and others 2000). Aside from LDL cholesterol-lowering properties, other potential health benefits of RBO include modulation of pituitary secretion, inhibition of gastric acid secretion, antioxidant action and inhibition of platelet aggregation (Cicero and Gaddi 2001).

2.3 Soy

Soy food utilization around the world varies widely. Soybeans, *Glycina maxima*, are native to eastern Asia and grown in several countries of the world (O'Brien 2004). Soybeans have been an important part of the East Asian diet for centuries, due to its well-balanced amino acid composition. In the Asian diet soybeans are traditionally used in foods such as tofu, soymilk, and fermented products. In Western nations soybeans are consumed in the form of refined soy protein ingredients used in food processing (Riaz 2006). Soybeans in food applications became very popular after a soy protein health claim was approved in 1999 by the United States Food and Drug Administration. The use of soy is important to the food industry due to its many applications in food. Soy ingredients are being regarded as versatile ingredients due to their applications in a food system. Aside from the health benefits one can attain from soybean ingredient consumption, these ingredients also play a role in food functionality.

2.3.1 Soy Bean Morphology and Composition

Dry soybeans are close to spherical in shape with wide variability in size. The size varies with growing conditions and variety. The morphology of the bean can be described with respect to the seed coat, cotyledon, and the germ. The seed coat (or testa) is the outermost layer of the bean and makes up to 9% of the soybean by dry weight. The seed coat color can widely vary. According to the United States classification the seed coat can be yellow, green, brown, and black beans. During early maturity all beans are green due to chlorophyll; but as the bean matures and the chlorophyll disappears, the residual flavonoid pigments predominate. It is important to note that certain varieties do not loose chlorophyll and consequently they have green seed coats (Snyder and Kwon 1987). Soybeans contain two cotyledons, which become the first pair of leaves for the young seedling and contain the nutrients required before the seedling can carry on photosynthesis. The predominant features of the cotyledon cells are the protein bodies, lipid bodies, starch grains, and cell walls. The third part is the germ, which is about 2.5% of the bean by weight. The germ, upon germination becomes the new soybean plant (Snyder and Kwon 1987). Soybean proximate chemical composition varies depending on variety and growing conditions. Soybeans contain approximately 40% protein, 35% carbohydrates, 20% fat, and 5% ash on a dry weight basis (Riaz 2006; Snyder and Kwon 1987).

2.3.2 Soy Protein

Protein is the second major chemical component of the soybean that has commercial value; where the first major component is the oil. Soybean protein is valuable due to its amino acid composition that complements that of cereals. The protein content of soybeans, usually varying between 38-40%, is larger than that of other legumes (20-30%), and much larger than that of cereals (8-15%) (Snyder and Kwon 1987).

Soy protein products, which are derived from defatted flakes, are divided into three groups based on their protein content (Figure 3). Soybean flours comprise the soybeans from which the hull and oil has been removed; soy protein concentrates are defatted flour from which sugars and oligosaccharides have been removed; and soy protein isolated are defatted flour from which fiber, acid-soluble proteins, sugars, and oligosaccharides have been removed (Riaz 2006). As a results of the abovementioned fractionations, these three soy protein products have a minimum of 50, 70, and 90% protein (on a dry basis), respectively. Soybean protein contains proteinaceous substances known as trypsin inhibitors. These substances inhibit the digestion of protein and the nutritionally important hemagglutinins.

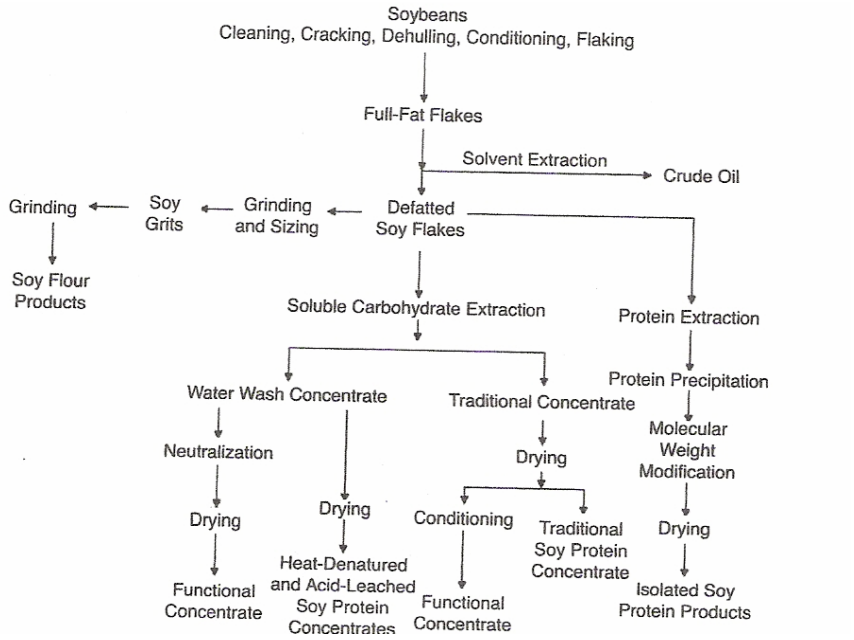

Figure 4: Soybean Processing Pathways

Soy protein can be produced by several methods of which alcohol extraction is the most frequently used, even though water extraction results in the better retention of isoflavones.

Diverse varieties of concentrated or isolated protein goods are prepared by milling, toasting, extraction of fat and saccharides, and isolation of protein fractions (Hui 1992).

2.3.3 Soy Protein Functionality

Functional properties of soy proteins are of great importance. Proteins can interact with other food ingredients to form desirable food properties; interaction which is called functionality. The following are examples of such interactions in food applications: added protein can prevent fat or water separation during heating of a meat product, can prevent staling by controlling moisture redistribution in baked goods, can form stable emulsions and foams, etc.

The interactions of soy proteins with water and lipids are not mutually exclusive (Snyder and Kwon 1987). In food products containing both water and lipids, protein interaction determination is difficult. These are two manners in which protein and lipid may interact: lipid absorption and emulsions. In lipid absorption the functionality involved is that of preventing lipid separation during heating of lipid-containing products. The emulsion type that makes use of soy protein functionality is the oil-in-water emulsion. Soy protein increases the emulsion capacity, i.e., the amount of oil in an emulsion, and it can also stabilize the emulsion from creaming or from separating into two phases. Creaming refers to the phenomenon when the oil droplets aggregate and rise to the top of the emulsion. Phase separation occurs when the oils droplets coalesce and the two original phases of oil and water form (Snyder and Kwon 1987).

Proteins successfully promote emulsion formation due to the presence of both hydrophobic and hydrophilic regions. During normal conditions, the hydrophilic region is exposed to the aqueous phase and the hydrophobic part is exposed to the interior of the globular protein in the solution. When in an oil-in-water emulsion, protein molecules located at the oil-water interface unfold, allowing the hydrophobic region to associate with the oil phase while the

hydrophilic region remains associated with the aqueous phase. It is important to note that a protein that is soluble in the aqueous phase during emulsion formation has greater possibility of appropriately orienting at the interface to stabilize the emulsion.

2.3.4 Soy Protein Concentrate (SPC)

Soy protein concentration can be increased by removing soluble carbohydrates, resulting in soy protein concentrate, which should contain at least 70% protein on a dry weight basis (Snyder and Kwon 1987). SPCs are made by four different processing pathways. These processes are: acid leaching (at pH 4.5, because soy protein has minimum solubility at this pH and as a result mainly soluble carbohydrates are extracted), aqueous alcohol extraction (60 to 90%), protein denaturizing with moist heat before extraction with water, and size exclusion separation by membranes (Riaz 2006; Snyder and Kwon 1987). Soluble sugars, certain bean flavors, anti-nutritional factors, and enzymes than can cause off-flavors are removed during processing. However, the resultant proteins do not have good solubility properties due to protein aggregation during isolation. Poor solubility is observed in all resultant proteins, except those which have been membrane processed. Compared to SPCs produced by traditional processing methods, membrane-processed proteins offer improved solubility, emulsification, flavor, and naturally occurring isoflavones.

SPC is made from dehulled, defatted soybeans from which a portion of the carbohydrates has been removed. SPC retains most of the fiber originally present in the soybean. It is commercially available as granules, flour or spray dried. SPC is widely used both as a functional and nutritional ingredient in a variety of food products and used for some non-food applications. Such food products include, but are not limited to, baked goods, breakfast cereals and certain

meat and poultry products. SPC is very digestible making it suitable for children, pregnant and lactating women, and the elderly.

2.4 Mayonnaise

Mayonnaise is probably one of the most widely used sauces worldwide and commonly used as a sandwich spread in North America. It was first produced commercially in eastern United States in the early 1900's where it was introduced as Hellman's Mayonnaise. The market for this product is mounting as different and interesting flavors and ingredients are launched and healthy versions are developed (McClements 2005).

Mayonnaise is an oil-in-water emulsion despite containing between 70-80% fat. Oil in water emulsions consist of finely dispersed droplets of oil in a continuous phase of water or a dilute aqueous solution. Droplet size range is from less than 1μm to 20μm or more (Snyder and Kwon 1987). This emulsion is formed by mixing the eggs, vinegar and spices, and then slowly feeding the oil, resulting in a closed-packed foam of oil droplets or coarse emulsion. Dissimilarly, if the aqueous and oil phases are mixed at once the result is a water-in-oil emulsion, whose viscosity is similar to the oil from which it was made (Depree and Savage 2001).

2.4.1 Regulations

Mayonnaise is defined under the United States Food and Drug Administration (FDA) Standards as an emulsified semisolid food prepared from edible vegetable oil(s) (not less than 65% by weight), acidifying ingredient(s) including vinegar and/or lemon/lime juice (not less than 2.5%), and egg yolk-containing ingredients. Optional ingredients include salt, nutritive carbohydrate sweeteners, spices, monosodium glutamate, sequestrants, and crystallization inhibitors (21CFR169.140). These ingredients have limitations imposed on them. For example,

added seasonings cannot simulate the color of added egg yolk and EDTA salts are permitted as metal chelators at levels up to 75ppm to protect the oil form oxidizing or reverting in flavor and to protect the mayonnaise from color loss. Mayonnaise may be blended and packed in an atmosphere in which air is replaced in whole or in part by carbon dioxide or nitrogen.

2.4.2 Mayonnaise Production

For mayonnaise production, a combination of a high speed blender and a homogenizer is usually used (Hui 1992). After production the product may then be heat treated to inactivate microbes prior to packaging and storage. With the purpose of avoiding product breakdown, freezing, heating and excessive mechanical agitation must be avoided during storage and transport (Dickinson and Stainsby 1982).

Traditionally, a wide variety of edible oils has been used for mayonnaise preparation. These oils include soybean, cottonseed, corn, canola, olive, sesame, safflower and sunflower (Hui 1992). Recent trends show the demand of reduced fat, low fat, or fat free versions of traditional food products. Ford and others (2004) reported that the total fat content of emulsified products can be reduced by replacing the fat droplets with nonfat ingredients. These ingredients are usually biopolymers, such as gums, starch and proteins (Clegg 1996). When the fat content is reduced the flavor profile of the product is affected, which is one of the toughest quality attributes to mimic. For that reason, the supplementation of the biopolymer fat replacers with surfactants or flavorings is necessary. Another trend is to replace the oils traditionally used with "health-promoting oils", particularly polyunsaturated lipids (Watkins and German 2002).

A wide variety of thickening agents are used in oil-in-water emulsions, which can either be natural or chemically modified polysaccharides. The majority of these thickening agents include xanthan, starch, modified starch, cellulose gum, cellulose gel, carrageenans, alginates,

locust bean gum, gum arabic, pectin and guar gum (McClements 2005). These thickening agents can be used alone or in combinations with others in order to achieve the textural, mouthfeel and stability characteristics that are wanted. The quantity required of the thickening agent(s) solely depends on the preferred texture of the product. According to Frank (2000) when trying to achieve a highly viscous product, such as mayonnaise, the lower the fat content the larger the quantities of thickening agent(s) required to produce the same texture. The desired taste and aroma of the final product is achieved by the contribution of sugars, salts, acids, and flavorings.

Mayonnaise is relatively resistant to microbial spoilage due to the inability of pathogens to grow under acidic conditions, pH < 4.4 (Smittle 2000). In order to aid in microbial growth prevention, the pH of the aqueous phase is controlled between 2.4 - 4.5 by means of acids such as acetic, citric, lactic or phosphoric (McClements 2005). The growth of bacteria is slow and/or inhibited by ingredients such as vinegar, lemon juice, and salt. Preservatives and antimicrobials can also be added to the product to slow the bacterial growth.

The properties of the interfacial membrane that surrounds the oil droplets depend on the surface active compounds present. The preparation kinetically stable of emulsions that are of practical use for the food industry requires the incorporation of substances such as emulsifiers and/or thickening agents (McClements and Demetriades 1998). Different emulsifiers and/or thickening agents can act at the droplet interface, where the main function is to prevent droplet coalescence. These emulsifiers or surface-active substances can be added as specific emulsifying ingredients or they may be present in more complex ingredients, such as egg yolk. Emulsifiers that are commonly used to stabilize mayonnaise include phospholipids, proteins, and particulate matter, all of which are surface-active components found in eggs (Le Denmat and others 2000).

2.4.3 Physicochemical Properties of Mayonnaise

Physicochemical properties of mayonnaise include stability, rheology, appearance and flavor. Commonly, the term emulsion stability refers to the capacity of an emulsion to resist changes in its physicochemical properties with time. According to Harrison and Cunningham (1985), the factors that affect the oil-in-water emulsion stability of mayonnaise include the amount and stability of thee oil, amount of egg yolk used, relative volume of the oil phase to the aqueous phase, types and amounts of emulsifiers, methods of mixing, water quality, temperature, and viscosity. The droplet size distribution and the nature of the stabilizing interfacial film influence the rate destabilization of a food emulsion (Tung and Jones 1981). Mayonnaise is a thermodynamically unstable system due to the energetically unstable contact between oil and water molecules, and due to the difference in densities of oil and water. (McClements and Demetriades 1998). In order to preserve the stability (appearance, texture, taste) of the emulsion, the prevention of droplet coalescence, flocculation, and/or creaming is necessary (Rao 1999). Coalescence is the process by which two or more droplets merge together to form a single larger droplet. Creaming becomes a problem in low fat products, i.e., those containing less than 50-60% fat. Creaming is defined as the process by which droplets move upward due to gravity because they have a lower density compared to the surrounding liquid (McClements and Demetriades 1998).This phenomenon can be prevented through the addition of thickening or gelling agents, such as gums or starches, to the aqueous phase of the emulsion. Flocculation is the process by which two or more droplets stick together to form an aggregate in which the droplets retain their individual integrity. In mayonnaise, the driving force for droplet flocculation is attributed to the screening of electrostatic repulsion between droplets.

Lopez (1981) reported that oxidation or hydrolysis reactions may also lead to quality deterioration of the product. All fat-containing foods, such as mayonnaise, are susceptible to spoilage through auto-oxidation of the unsaturated and polyunsaturated fats in the oil, resulting in a rancid flavor. In mayonnaise, oxidation appears to initiate at the droplet interface. Emulsified lipids are often oxidized quicker than bulk oil because of the large exposure area to air (Coupland and McClements 1996). Depree and Savage (2001) stated that light is a cause of oxidation of fats by acting on photosensitizing agents. The stability of mayonnaise to oxidation also depends on the type of oil utilized. Oils high in linoleic acid and linolenic acid (such as corn oil and soybean oil) oxidize less rapidly when compared to oils containing higher polyunsaturated fatty acids. Hseih and Regestein (1992) reported that mayonnaise prepared with corn oil was less susceptible to oxidation and the mayonnaise made with soybean oil was the least susceptible.

The perceived quality of mayonnaise is greatly determined by product rheology. The rheology of a product is determined by the way that it flows or deforms in response to the application of a force (McClements and Demetriades 1998). Rheological properties such as texture, consistency, firmness, and smoothness are difficult to evaluate reliably. It has been shown that, like polymeric systems, food emulsions such as mayonnaise exhibit non-Newtonian viscoelastic properties (Holdsworth 1971, Atkin and Sherman 1980). Giasson and others (1997) showed that full fat, light and fat free mayonnaise can be differentiated through thin-film, morphology, tribiology and wetting studies; studies which provided important data which may be relevant to mouthfeel. Rheology has great impact on product quality, functional and sensory characteristics such as creaminess, smoothness, pourability, spreadability, thickness, and shelf life due to gravitational separation (Wendin and Hall 2001, Juszczak and others 2003).

Mayonnaise tends to be optically opaque due to light scattering caused by high droplet concentration. The flavor of a food is one of the most important quality attributes, it determines whether the product will be found desirable and therefore purchase it another time. Flavor is due to the combination of volatile odor molecules, nonvolatile taste molecules and mouthfeel (Depree and Savage 2001). The water-soluble components, such as acidulants, sweeteners, and seasonings, determine the taste of mayonnaise. The aroma is determined by the major ingredients (oil, lemon juice) or added flavorings. The flavor profile may be altered due to chemical degradation reactions, such as lipid oxidation, during storage (Jacobsen and others 1999). The emulsion droplets and thickening agents are the ones that contribute to the desirable mouthfeel (Wendin and Hall 2001). Upon the development of a reduced fat product, the creamy or fatty mouthfeel is lost due to the removal of fat droplets (Mela and others 1994), which, in turn, changes the flavor profile of the product.

2.5 Functional Foods

The author of the first book pertaining to functional foods stated that "It is becoming increasingly clear that there is a strong relationship between the food we eat and our health" (Goldberg 1994). The unfolding of functional food science as a new nutritional agenda over the recent years, represents one of the most controversial areas of food and health. This controversy has awakened because it suggests using food and the components of food in relation to treatment or prevention of disease which has been characteristically the territory of drug development rather than food consumption. Functional food science aims to maintain health, improve well-being and create the conditions for reducing the risk of disease (Heasman and Melletin 2001). The target is the alleged diseases of affluence – particularly cardiovascular diseases and certain cancers.

Consistent definitions for functional foods and nutraceuticals have challenged academics, scientists, business analysts and policy experts (Heasman and Melletin 2001). Although there is no consensus on the exact definition of the term, according to the US Institute of Medicine, functional foods are defined as: "any modified food or food ingredient that may provide a health benefit beyond the traditional nutrients it contains" (American Dietetic Association 1995). Goldberg (1994) defined functional foods as: "any food that has a positive impact on an individual's health, physical performance or state of mind in addition to its nutritive values". Even though a vast number of definitions have been proposed, there is currently no legal definition for functional food, beverage or nutraceutical in the US (Heasman and Mellentin 2001). A vast subject matter regarding the challenges surrounding functional foods lies behind the straightforward definitions; it encompasses food industry challenges (development and marketing), consumer challenges (acceptance), regulatory and policy challenges, and scientific/technology and nutritional challenges. Food companies worldwide are reforming their operations and are "spending hundreds of millions of dollars to develop and market functional food and beverage products" (Heasman and Mellentin 2001). According to Heasman and Mellentin (2001) there are three major factors driving the functional foods revolution: (1) an ambitious fundamental change in diet for the developed and developing world, (2) the potential of a new type of health-prioritizing consumers brought into the market by food companies and (3) crucial investors drive corporate purpose in functional foods.

Goldberg (1994) stated that there has been a rapid accumulation of scientific knowledge concerning the beneficial function(s) of a variety of food ingredients for the prevention and treatment of particular diseases. The National Academy of Sciences, Food and Nutrition Board's (1989) report on diet and health concluded that the amounts and the types of fats and other lipids

consumed in a diet influence the risk of atherosclerotic heart disease. It was reported that any reduction in saturated fatty acid consumption is likely to reduce coronary heart disease.

CHAPTER 3. SENSORY OPTIMIZATION OF CHOLESTEROL-FREE MAYONNAISE TYPE SPREADS CONTAINING RICE BRAN OIL

3.1 Introduction

About one fourth of the American population has some form of cardiovascular disease, accounting for more than 40% of all deaths in the United States; with high blood cholesterol being one of the risk factors for these heart-related diseases. Traditional mayonnaise, currently being manufactured using soy bean oil (SBO), contains at least 65% fat. Therefore, one of the main objectives of this study is the development of a cholesterol free product containing rice bran oil (RBO) as a functional ingredient potentially used for its reported cholesterol lowering properties. Soy protein concentrate (SPC) was used to replace egg yolk. A RBO-based mayonnaise-type spread is a spreadable oil-in-water emulsion that does not comply with the standard of identity for mayonnaise by containing less than 65% oil and not contain egg-yolk (21CFR169.140).

3.1.1 Consumer Acceptance Testing

Acceptance testing is an important component in sensory evaluation in which liking or preference for a product is measured. According to Stone and Sidel (1993), acceptance testing is a valuable and necessary component of every sensory evaluation program; where in product evaluation it is typically followed by discrimination and descriptive testing. The evaluation task is referred to as acceptance, preference or consumer testing (Stone and Sidel 1993). The main principle of affective tests is the assessment of personal preference and/or acceptance of a product, a product idea or specific product characteristics either by current or potential consumers (Meilgaard and others 1999). Being used mainly by producers of consumer goods, consumer tests are used more and more each year due to their effectiveness as a tool in designing

products or services that will retail in larger quantities and/or at higher prices (Meilgaard and others 1999). According to Meilgaard and others (1999), the reasons for conducting a consumer test usually fall within one of the following categories: product maintenance, product improvement/optimization, development of new products, assessment of market potential, product category review or support of advertising claims.

According to Stone and Sidel (1993) there are two methods which are commonly used for measuring product liking or preference: paired-comparison and the nine-point hedonic scale, where the latter is the most useful for measuring product liking and preference. As an instrument for the assessment of food likes and dislikes by consumers, the nine-point hedonic scale has proven to be durable and useful (Lawless and Klein 1991). Inexperienced consumers/judges are able to understand the scale with minimal instruction (Stone and Sidel 1993; Lawless and Klein 1991). This scale is stable and product differences are reproducible among diverse sets of panelists. This scale possesses several relevant properties: it is balanced, contains a neutral point, and has approximately equal psychological spacing between scale points, giving it more or less interval scale properties (Lawless and Klein 1991).

For purposes of conducting a sensory test, a group of subjects is selected as a sample of the larger population for whom the product is intended (Meilgaard and others 1999). According to Stone and Sidel (1993), the subjects involved in the acceptance test should be qualified based on demographic information and usage criteria or preference from collected survey information. Among the demographic information to be considered for panelist selection are: user group, age, sex, income, geographic location, nationality, region, race, religion, education, and employment (Meilgaard and others 1999). Currently, the vast majority of acceptance tests involve employees and residents local to the company offices, technical center, or plants (Meilgaard and others

1999; Stone and Sidel 1993). According to Meilgaard and others (1999), employees and local residents are acceptable subjects when the objective is product maintenance. However, if the objective is new product development, product optimization, or product improvement, employees or local residents are not representative of the consumer and should not be used as such.

The testing site or location affects the results of the sensory test due to: (1) the length of time the products are used/tested, (2) controlled preparation vs. normal-use preparation of the product, (3) perception of the product alone in a central location vs. in conjunction with other foods or personal care items in the home, (4) influence of family members on each other in the home, and (5) length and complexity of the questionnaire (Meilgaard and others 1999). Acceptance testing can be conducted in one of three primary settings: laboratory, central location, home use (Meilgaard and others 1999, Stone and Sidel 1993).

The laboratory environment is most frequently used location for sensory acceptance tests (Stone and Sidel 1993). Meilgaard and others (1999) stated that the advantages associated with laboratory tests are the following: (1) control of product preparation and presentation, (2) employees can be contacted on short notice, and (3) color and other visual aspects can be masked so that subjects can concentrate on flavor or texture differences. In addition to these advantages Stone and Sidel (1993) include (4) rapid data feedback and (5) low cost.

The central location test (CLT) is one of the most frequently used consumer tests, especially for market research. CLT is usually conducted in a place highly accessible to a large number of potential purchasers, which were pre-recruited or intercepted (Stone and Sidel 1993). When intercepted, respondents are screened in the open and those that qualify are led to a closed-off area (Meilgaard and others 1999). The quantities of responses that are typically collected per

location are 50-300 (Meilgaard and others 1999) and 100 responses per product are usual as stated by Stone and Sidel (1993). For CLT the samples are prepared out of consumers' sight and served on uniform plates, cups or glasses labeled with three digit codes. Stone and Sidel (1993) state that the number of samples presented to the consumer should be limited to 5 or 6, taking into consideration that fewer samples will minimize test time. Scoresheet instructions and questions accompanying the samples should be clear and concise due to high potential distraction (Meilgaard and others 1999). The advantages of using CLT are: (1) product evaluation is conducted under controlled conditions, (2) the results are validated because the product is tested by the end-users themselves, (3) favorable conditions for a high response return from a large sample population, and (4) one consumer can evaluate several products during one test session (Meilgaard and others 1999; Stone and Sidel 1993). Among the disadvantages associated with CLT are: (1) testing of product under semi-artificial conditions in regards to normal use in terms of preparation, amount used, etc. and (2) limited amount of information is obtained by the data due to limited amount of questions that can be asked during the test session (Meilgaard and others 1999).

The home use test (HUT) represents the ultimate consumer test (Meilgaard and others 1999). For HUT, the environment in which the product is tested and other test factors are not controlled, meaning that the panel size should be doubled in size (50-100 families) compared to the laboratory test (Stone and Sidel 1993). According to Meilgaard and others (1999), characteristic panel sizes range from 75-300 per city in 3 or 4 cities. Usually two products are compared. The first product is tested for 4-7 days, after which time the second product is supplied once the scoresheet has been filled by the consumer (Meilgaard and others 1999; Stone and Sidel 1993). Even though HUT has the disadvantages of being expensive, time consuming

and lacking environmental control; the product is tested under actual usage conditions and all family member's opinions are obtained as well as marketing information (Stone and Sidel 1993).

Bias responses resulting from taste fatigue result when panelists are required to judge several food samples at a time. For such situations an incomplete block design is used, where panelists are considered as blocks and the samples to be tested as the treatments. Balanced incomplete block (BIB) design achieves homogeneity within the block and estimates the treatment differences with superior precision (Gacula and Singh, 1984). The BIB design is specified by its parameters: t = number of treatments, k = number of experimental units per block, r = number of replications of each treatment, b = number of blocks, and λ = number of blocks in which each pair of treatments occurs together (Gacula and Singh, 1984). The drawback associated with BIB is that number of replications per treatment is restricted; meaning that for a given number of treatments and number of experimental units per block the required number of replications per treatment and the number of blocks are fixed by the design and are not specified by the researcher.

3.1.2 Product Optimization

A class of statistical procedures which maximize a product's overall acceptability is referred to as product optimization (Moskowitz 1983). According to Moskowitz (1983) there are two methods of achieving product optimization: (1) finding the mixture of ingredients that generates the highest attainable acceptance score, at physically manageable ingredient levels and (2) finding the ingredient combination that generates a sensory perception similar to a pre-designated sensory profile. Stone and Sidel (1993) defined optimization as a method for developing the best achievable product in its class. In product optimization ingredients are interconnected through a quantitative, mathematical model developed by the sensory analyst.

The model shows the anticipated changes in perception and acceptance which are the outcome of explicit changes in ingredient formulations. The model recaps the interrelations and permits the marketer to diminish the effort when developing the new product (Moskowitz 1983). Being able to determine the particular combination of physical variables which correspond to the highest rating on an item is a major benefit of modeling (Hui 1992).

Curve fitting methods are used to develop equations which interrelate two or more variables. Least squares regression is the statistical analysis used to estimate the parameters of the equation(s). With the values of the parameters a curve can be produced which illustrates the experimental data obtained by the consumers. This approach to curve fitting techniques is referred to as response surface methodology (RSM). RSM is highly effective in permitting the reduction of the number of trials that must be carried out (Hui 1992). A simple equation, that best fits the data, is developed by the sensory analyst. This equation becomes in reality a model of the interrelations linking the ingredients and consumer perceptions. The response surface refers to the equation or the geometrical area the equation illustrates. The response surface is a smooth representation of the data whereas the empirical data are represented as uneven points on the surface.

3.2 Materials and Methods

3.2.1 Mayonnaise-Type Spread Preparation

Ten different spread formulations were prepared following the three-component coordinates mixture design. The products were prepared based on six total ingredients consisting of three dry ingredients (SPC, stabilizer, and salt) and three liquid ingredients (RBO, water, and lemon juice).

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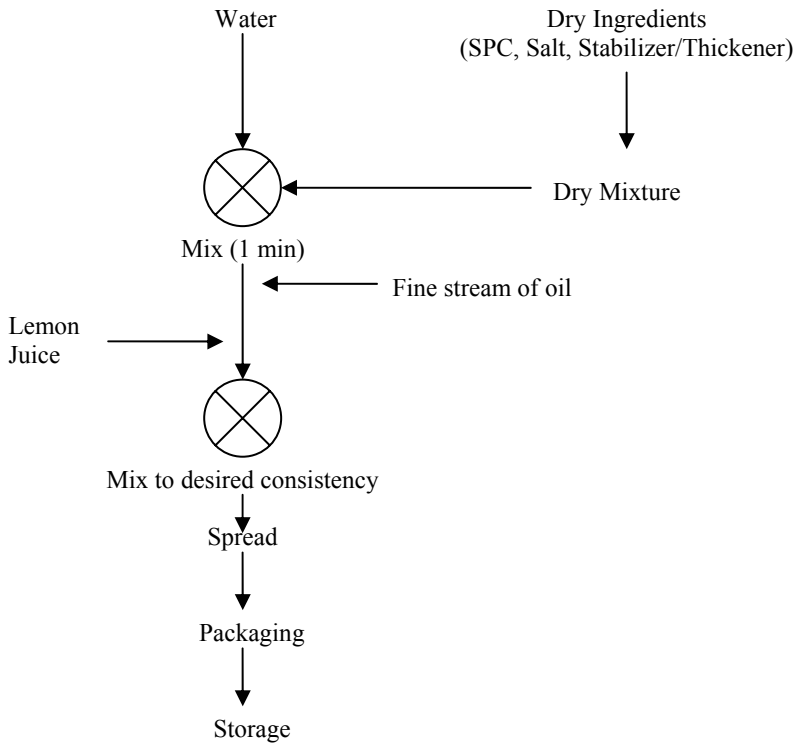

Figure 5: Flow Diagram for Spread Preparation

Oryzan^{TM} RBO, which is a high oryzanol refined bleached dewaxed deodorized RBO, was obtained from Rito Partnership (Stuttgart, Arkansas) [color, 28Y 2.3R; free fatty acid (% as oleic, AB), 0.034; peroxide value (PV), 0.39 mequiv/kg; moisture, 0.0050 %; flavor, 7.0; iodine value, 104.3; and oryzanol (spectrophotometric), 1.1]. The soy SPC was provided by Archer Daniels Midland Company (Decatur, Illinois); it is available in the market under ACRON® S [moisture, 6%; protein, 72 %; fat, 4%, ash, 5%; total dietary fiber, 20%; and calories, 290/100g]. The thickening or gelling agent used, which is a blend of xanthan gum, guar gum and sodium alginate, was obtained from Tic Gums, Inc. (Belcamp, Maryland); it is available in the market under the name TIC PRETESTED® Pre-Hydrated® SALADIZER® 250 Powder [percent calories from fat, 1%; calories from fat, 3.6 Kcal; total fat 0.4 g; sodium, 1888 mg; total

carbohydrate, 84 g; soluble dietary fiber 84 g; and protein, 1g (all quantities per 100 grams)]. The lemon juice (ReaLemon®) and salt (Morton®) were purchased from a local grocery store.

As the first step in formulating the spread all ingredients were measured out. The dry ingredients were mixed and then placed in a food processor. To this mixture, water was gradually added and blended together until a uniform clump-free paste was obtained. Then, the oil and lemon juice were added, alternating among the two and ensuring that the oil was added in the form of a fine thread. The product was then transferred to a sterilized container and stored under refrigeration at 4^{o}C. Figure 5 illustrates the spread preparation process

3.2.2 Mixture Experimental Design

Experimental design has been used by sensory analysts and product developers with great success to comprehend consumer reactions to test prototypes involving known ingredients and processes (Hui 1992). The three component constrained simplex lattice mixture design (Cornell 1983) was used for the experimental design, of which ten different formulations resulted (Figure 6 and Table 2). Here, three (3) of the formula ingredients were varied in a way that allows the researcher to assess the effects of each ingredient and the interactions on attribute perceptions and acceptance. RBO (X_1), SPC (X_2), water (X_3) were the variables comprised in the mixture design (Figure 6 and Table 2). These three constituents, being the only variables, made up 90.4% of the total formulation and the remaining ingredients (9.6%) were constant throughout the 10 formulations as follows: salt (0.7%), lemon juice (8.9%), and stabilizer (0.75% of total weight). Table 3 summarizes the actual 10 formulations used in this study. In the mixture design the component partitions (X1, X2, and X3) presented the following upper and lower boundaries: RBO (37% - 57%), water (37% - 57%) and SPC (1% -11%).

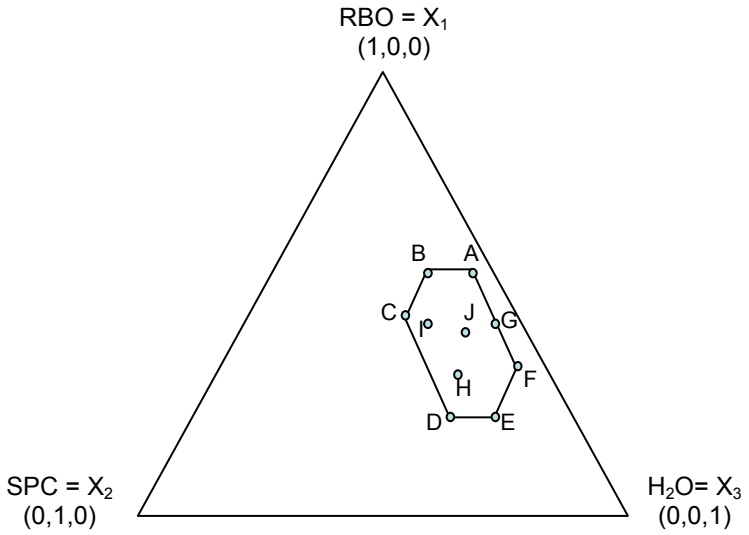

Figure 6: The constrained region in the simplex coordinate system. X_1 = rice bran oil, X_2 = soy protein concentrate, and X_3 = water. Letters within the hexagon represent the 10 formulations, corresponding to lettering A-J.

Table 2: Mixture Design Representing Varying Ingredients for the Ten Formulations for Mayonnaise-Type Spread*

Formulation	% RBO	% Water	% SPC
A	57	42	1
B	57	37	6
C	52	37	11
D	37	52	11
E	37	57	6
F	42	57	1
G	47	52	1
H	42	50	8
I	50	42	8
J	48	48	4

* These three ingredients sum up to 100%, as per the mixture design. Lemon juice, salt, and stabilizer are the complements in the actual formulations.

Table 3 summarizes the ten formulations with the actual percentages used for all ingredients for the product preparation. In this case the three varying ingredients (RBO, SPC, water) constitute 90.4% of the formulation total. The complementary ingredients consist of lemon juice, salt, and the stabilizing agent.

Table 3: Ten Formulations for Mayonnaise-Type Spreads*

Formulation	% RBO	% Water	% SPC	%LJ	%Salt
A	51.5	38.0	0.9	8.9	0.7
B	51.5	33.4	5.4	8.9	0.7
C	47.0	33.4	9.9	8.9	0.7
D	33.4	47.0	9.9	8.9	0.7
E	33.4	51.5	5.4	8.9	0.7
F	38.0	51.5	0.9	8.9	0.7
G	42.5	47.0	0.9	8.9	0.7
H	38.0	45.2	7.2	8.9	0.7
I	45.2	38.0	7.2	8.9	0.7
J	43.4	43.4	3.6	8.9	0.7

*The three varying ingredients (RBO, water, SPC) represent 90.4% of the total formulation. Complementary ingredients are: lemon juice (LJ) (8.9%), salt (0.7%) and stabilizer (0.75% of total weight).

3.2.3 Consumer Acceptance Test

For purposes of evaluating the sensory quality of the product, a consumer acceptance test was conducted. Three hundred and sixty (360) untrained consumers, randomly chosen from Louisiana State University, Baton Rouge Campus, participated in the consumer acceptance test. The following criteria were essential for recruitment of all participants: 18 years of age or older, not allergic to rice and/or soy products, and willingness for participation for approximately 15 minutes to complete the survey. Consumers rated appearance, color, odor/aroma, smoothness, spreadability, taste, mouthfeel, and overall liking of the product based on the 9-point hedonic scale (1= dislike extremely, 5 = neither like nor dislike, 9= like extremely) (Figure 7). Graininess, aftertaste, acceptability, purchase intent, and purchase intent after consumers were provided with more information about the product were evaluated using a binomial (yes/no) scale.

Figure 7: Panelists evaluating the spread samples during the consumer acceptance study

Based on the Balanced Incomplete Block Design (BIB) (Cochran 1957), the consumers were simultaneously presented with 3 out of the 10 sample formulations. These formulations were randomly coded with the letter A to J for a total of 108 observations per formulation. The consumers were given samples of 30 g placed in lidded transparent containers which were accompanied with white bread (onto which the product was spread by the panelists) and room temperature water for palate cleansing purposes between sample tasting. Consumers were presented with a questionnaire and instructed on proper filling. Consumers were required to complete and sign a consent form approved by the Louisiana State University Institutional Review Board prior to participation on the testing.

3.2.4 Statistical Data Analysis

All data were analyzed with a predetermined confidence level of 95% ($\alpha = 0.05$) using the Statistical Analysis Software System, Version 9.1 (SAS® Institute, Cary, NC).

3.2.4.1 ANOVA

The analysis of variance (ANOVA) is a statistical technique applied to determine which of the various effects operating concurrently on a process or development are important and the influence that these effects have on the results (Piggot 1996). Generally, the analysis of variance is suitable for the study of the effects of qualitative factors on a quantitative measurement. The measure of the total variation of a data set expressed as a sum of terms is the basic idea behind ANOVA (Freund and Perles 1999). The assumptions behind this statistical technique are normal distribution of the studied variables, variance equality and independence of the errors.

Analysis of variance was used in this study to determine consumers' views and acceptability of all sensory attributes and the overall liking of the products. From consumers' responses it can be determined if any significant differences existed among the ten spread samples. In the presence of significant differences, it needs to be determined where these differences lie; and for such purposes Tukey's studentized range test was performed. This test is defined as "a method of multiple-comparison for pairwise comparisons of k means and for the simultaneous estimation of differences between the means by confidence intervals" (Gacula and Singh 1987).

3.2.4.2 MANOVA and DDA

The partitioning of the total variation into pieces of variation attributable to the treatments sources and error is known as the multivariate analysis of variance (MANOVA)

(Johnson and Wichern 2002). MANOVA, akin to ANOVA, is a procedure used for analyzing multicomponent data. MANOVA is used to determine if there is a significant difference of the measurement values between the classes, i.e., to determine if treatments applied to a product, such as different ingredient quantities used, cause significant differences. Hui (1992) states that this determination is accomplished by the yield a global estimate as to whether there are any significant differences among the different variables or their correlations. This means that if the multivariate F-value is not significant, there is no significant difference among the variables. Conversely, if the F-value is significant, there is statistical significance somewhere. Therefore; for this purpose, the analyst must apply other tests to determine where the significance exists.

MANOVA is occasionally used in combination with discriminant analysis for data analysis. When used in conjunction, MANOVA is first used to determine treatment effects, i.e., if differences are present, and the discriminant analysis is then used to determine whether the variables, all combined, are correlated within the classes. Descriptive discriminant analysis (DDA), usually performed after MANOVA, identifies explanatory variables that are the cause of significant differences among samples or units understudy (Huberty 1994).

MANOVA was used in this study to establish if significant differences exist when all sensory attributes are compared simultaneously. DDA was used to determine, when all attributes compared, which of the attributes were accountable for the principal differences among the ten spread formulations in terms of consumers' perceptions.

3.2.4.3 Logistic Regression

Logistic regression is a predictive analysis which uses binomial probability theory. This analysis involves the prediction of the likelihood of the outcome, a dichotomous dependent variable (yes/no), based on the predictor variables which are quantitative or categorical. Logistic

regression is not limited to a single predictor, making it suitable for use in this study, in which the acceptability and purchase intent (independent variables) are predicted by the eight sensory attributes. Logistic regression calculates the probability of success (event) over the probability of failure (non event), therefore the results of this analysis are in the form of a likelihood, i.e., the odds ratio, which must be equal to zero or greater. An odds ratio of 1 indicates that event and non event are both equally likely to occur. An odds ratio greater than 1 indicates that the event is more likely to occur and an odds ratio less than 1 indicates that the condition of non event is more likely to occur. Logistic regression analysis was employed to predict both product acceptability and purchase intent based on the odds ratio point estimate.

3.2.4.4 McNemar Test

The McNemar test is a simple way to test marginal homogeneity for matched binary responses in 2 x 2 tables. Marginal homogeneity implies that row totals are equal to the corresponding column totals. It represents a comparison of dependent proportions for dual response variables. It studies the change in consumer response measured twice as a dichotomous variable, i.e., it compares the same individuals before and after a treatment. The McNemar test is a variation of the Chi-square test for binomial (yes/no) data; therefore it is a Chi-square distribution, two-tailed test, with one (1) degree of freedom. Significant results are obtained when the marginal frequencies or proportions are not homogeneous.

In addition to the chi-squared value, a 95% confidence interval (CI) was calculated using marginal sample proportions (p_{+1} - p_{1+}), which can be used to estimate the actual differences in the means. The following equation was used to calculate the marginal sample proportions:

$$p_{ij} = n_{ij}/N$$

where n_{ij} is the number of consumers making decision i before and decision j after the additional information was provided about the product and N is the total number of consumer responses. The 95% CI was calculated using the following formula:

$$(p_{+1} - p_{1+}) \pm Z_{\alpha/2}(\text{ASE})$$

where $(p_{+1} - p_{1+})$ represents the difference in proportions between the consumers who would purchase the product after additional information was provided (p_{+1}) and those who would also purchase the product before the information was provided (p_{1+}). The term $Z_{\alpha/2}$ is the standard normal percentile having a right-tail probability equal to $\alpha/2$, which for a 95% CI $Z_{\alpha/2} = 1.96$. ASE is the estimated standard error for the proportion difference, and was calculated using the following formula:

$$\text{ASE} = \{[p_{1+}(1-p_{1+}) + p_{+1}(1-p_{+1}) - 2(p_{11}p_{22}-p_{12}p_{21})]/N\}^{1/2}$$

where p_{11} is proportion of consumers who would purchase the product before and after information was provided, p_{12} is the proportion of those who would purchase before but not after, p_{21} is the proportion of those who would not purchase the product before but would be willing to purchase afterwards, and p_{22} indicates the number of subjects who answered negatively prior to and after tasting the product.

The McNemar test was used in this study to determine if a significant change existed in purchase intent before and after additional information about health benefits was given to the consumer.

3.2.4.5 Principal Component Analysis

Principal components analysis (PCA) is a method of internal analysis, used to study the relation of variables within the same data set (Hui 1992). PCA, as a statistical technique, is used to simplify datasets; it decreases the dimensionality of the dataset at the same time as retaining

those characteristics that contribute most to its variance. PCA can be applied to any multivariate data set as a descriptive modus operandi, making PCA a helpful technique (Piggott and Sharman 1986). PCA examines the variance of the data to determine which variables go together and which others belong to a different group (Hui 1992). PCA has two main functions: it indicates any correlation among the variables in the data set and it shows relationships among the objects and then it attempts to group those things that are correlated with each other (Hui 1992).The data matrix can be envisioned as illustrating a multi-dimensional space or a two-dimensional plot for more simple cases. When there are many variables the visualization of the sample space becomes intricate, in this situation is when PCA can aid the interpretation of multivariate data. The sensory descriptors that are correlated and contribute to the greatest variance in ratio to the total are grouped into the first PC. The second PC, derived in the same manner, is a measure of the variance remaining after the first PC has been extracted and accounts for the next greatest amount of variance. The process carries on based on the same approach until all the variances have been accounted for (Hui1992). Since the first few components account greatly for the total amount of the variance the rest of the components can be ignored. These last principal components do not supply enough additional information to justify their use. Therefore, the investigator is rarely interested in all the components.

Principal component analysis was used to illustrate any existing relationship among the sensory attributes and the relationship between these attributes and the different formulations as illustrated in a product-attribute bi-plot. The first principal component (PC) covers as much of the variation in the data as possible and the second PC is orthogonal to the first and covers as much of the remaining variation as possible.

3.2.5 Product Optimization

For this study, Response Surface Methodology (RSM) was used in conjunction with least squares regression analysis to determine the effects of the response variables on the consumer acceptance of cholesterol-free mayonnaise-type spreads containing RBO. Prediction models obtained were used to construct contour maps representing the combination of the independent factors that were found to have a significant effect. These contour maps were used to characterize the optimal formulation. Logistic regression was used to show which sensory attributes are critical to overall product acceptance and purchase intent, which are the limiting factors in obtaining the optimal formulation. The scores selected within the plots were those equal to or greater than 5.0 (neither like nor dislike). The optimal formulation was determined through the superimposition of mixture response surface (MRS) plots.

3.3 Results and Discussion

3.3.1 Consumer Acceptability

ANOVA results for consumer acceptance rating for appearance, color, odor/aroma, and smoothness for the ten different formulations are presented in Table 4. Scores for spreadability, taste, mouthfeel, and overall liking are presented in Table 5. From these tables it can be observed that all the sensory attributes received a mean score of no less than 4.0. Regarding appearance, the ten formulations were not perceived as significantly different from each other by the consumers. Formulation E (33.4 % RBO, 51.5 % water, 5.4 % SPC) received the highest appearance mean score (5.94) whereas formulation C (47.0% RBO, 33.4 % water, 9.9% SPC) received the lowest score (5.48). Mean scores concerning color are significantly different with respect to certain formulations. Formulations A, B, G, H, I, and J had scores that were not significantly different. Formulation F (38.0% RBO, 51.5% water, 0.9% SPC) received the

highest mean score for color (6.50) and formulation D received the lowest score (5.61). Based on the results from Tukey's Studentized range test, these two formulations (F and D) were found to have significantly different mean scores. For odor, formulations E and F received the highest mean scores (5.44) with sample C receiving a mean score of 4.69. A similar trend was observed within the smoothness results, formulation E received the highest score (6.34) and formulation C received the lowest score (5.65). All smoothness mean scores are not significantly different from each other in accordance with Tukey's test.

Table 4: Mean Acceptability Scores for Appearance, Color, Odor and Smoothness

Formulation[a]	Mean Scores of Sensory Attributes*			
	Appearance	**Color**	**Odor**	**Smoothness**
A	5.58 ± 1.83^{A}	6.22 ± 1.51^{ABC}	5.49 ± 1.40^{A}	6.29 ± 1.78^{A}
B	5.85 ± 1.66^{A}	6.05 ± 1.57^{ABC}	4.94 ± 1.29^{AB}	6.04 ± 1.69^{A}
C	5.48 ± 1.66^{A}	5.70 ± 1.58^{BC}	4.69 ± 1.57^{B}	5.65 ± 1.79^{A}
D	5.55 ± 1.68^{A}	5.61 ± 1.62^{C}	4.73 ± 1.64^{B}	6.04 ± 1.66^{A}
E	5.94 ± 1.75^{A}	6.34 ± 1.45^{AB}	5.44 ± 1.66^{A}	6.34 ± 1.71^{A}
F	5.63 ± 2.00^{A}	6.50 ± 1.59^{A}	5.44 ± 1.38^{A}	6.23 ± 1.95^{A}
G	5.88 ± 1.71^{A}	6.23 ± 1.49^{ABC}	5.21 ± 1.32^{AB}	6.33 ± 1.64^{A}
H	5.58 ± 1.69^{A}	5.89 ± 1.41^{ABC}	4.67 ± 1.47^{B}	5.65 ± 1.67^{A}
I	5.67 ± 1.80^{A}	5.93 ± 1.56^{ABC}	4.85 ± 1.62^{AB}	5.78 ± 1.65^{A}
J	5.81 ± 1.87^{A}	5.99 ± 1.67^{ABC}	4.97 ± 1.80^{AB}	5.89 ± 1.72^{A}

[a]Sample formulations are specified in Table 3. Data is represented as mean ± standard deviation and all values are based on a nine-point hedonic scale where 1= dislike extremely, 5 = neither like nor dislike, 9 = like extremely.

* mean values in the same column not followed by the same letter are significantly different at $p \leq 0.05$

Spreadability was rated the highest of all sensory attributes for all formulations, except A, F, and G. All these three samples (A, F, G) consist of 0.9% SPC; the formulations possibly did not present the standard consistency for a mayonnaise-type product. Formulation E received the highest score (7.06) for spreadability. Spreadability might have been scored as such due to consumers' familiarity with the product, being mayonnaise a common household item. Taste was the lowest rated sensory attribute, where formulation H (38.0 % RBO, 45.2 % water, 7.2 % SPC) received the highest mean score (4.77) and formulation C received the lowest score (4.04). According to Tukey's test, the mean scores for taste are not significantly different form each other. This means that all formulations were perceived as equal in regards to the taste of the product. For formulation H, only 35.85% of the consumers perceived an aftertaste; comparably for formulation C, 35.29% of the subjects perceived an aftertaste. For formulations A, G and F, 48.57%, 47.62%, and 46.15% of the consumers, respectively, detected an aftertaste. All these formulations contained 0.9% SPC.

For mouthfeel, Formulation E received the highest mean score (5.93) and formulation C the lowest score (4.76). Graininess was perceived mostly for formulation C (49.53%) and slightly for formulation F (2.80%). Formulation C contained 9.9% SPC and formulation F contained 0.9% SPC. Formulation E, which received the highest mean score for mouthfeel, was identified to be grainy by 38.32% of the consumers. Samples B (51.5 % RBO, 33.4 % water, 5.4 % SPC) and E received the highest mean overall liking score of 4.97. These two samples were followed by formulation H with an overall liking mean score of 4.94. Formulation C received the lowest mean overall liking score (4.35). All other formulations received comparable mean overall liking scores ranging from 4.35 – 4.88. Formulation E received the highest ratings for all

sensory attributes, except taste and color, when compared simultaneously to all other nine formulations.

Table 5: Mean Acceptability Scores for Spreadability, Taste, Mouthfeel, and Overall Liking

Formulation[a]	Mean Scores of Sensory Attributes*			
	Spreadability	**Taste**	**Mouthfeel**	**Overall Liking**
A	5.93 ± 2.04^{B}	4.10 ± 2.01^{A}	5.64 ± 1.93^{ABC}	4.51 ± 1.81^{A}
B	6.20 ± 1.98^{B}	4.69 ± 1.90^{A}	5.54 ± 1.85^{ABCD}	4.97 ± 1.67^{A}
C	5.98 ± 1.91^{B}	4.04 ± 2.07^{A}	4.76 ± 2.16^{D}	4.35 ± 2.02^{A}
D	6.45 ± 1.76^{AB}	4.33 ± 1.98^{A}	5.00 ± 1.89^{CD}	4.57 ± 1.77^{A}
E	7.06 ± 1.62^{A}	4.74 ± 2.08^{A}	5.93 ± 1.79^{A}	4.97 ± 1.86^{A}
F	5.90 ± 2.20^{B}	4.42 ± 2.39^{A}	5.65 ± 1.94^{ABC}	4.78 ± 2.14^{A}
G	5.97 ± 1.89^{B}	4.40 ± 2.09^{A}	5.85 ± 1.77^{AB}	4.68 ± 1.93^{A}
H	6.56 ± 1.51^{AB}	4.77 ± 1.98^{A}	5.22 ± 1.90^{ABCD}	4.94 ± 1.83^{A}
I	6.30 ± 1.72^{AB}	4.36 ± 1.95^{A}	5.08 ± 2.02^{BCD}	4.49 ± 1.93^{A}
J	6.15 ± 1.77^{B}	4.69 ± 2.06^{A}	5.66 ± 1.97^{ABC}	4.88 ± 1.98^{A}

[a] Sample formulations are specified in Table 3. Data is represented as mean ± standard deviation and all values are based on a nine-point hedonic scale where 1= dislike extremely, 5 = neither like nor dislike, 9 = like extremely.
* mean values in the same column not followed by the same letter are significantly different at $p \leq 0.05$

In conclusion, appearance, smoothness, taste, and overall liking were not found to be significantly different among all the formulations. However color, odor, spreadability, and mouthfeel were found to be different among the majority of the formulations.

3.3.2 Overall Product Differences

Multivariate analysis of variance was employed in order to determine if the ten formulations differed considering all the sensory attributes simultaneously. A Wilk's Lambda P-

Value of < 0.0001 (Table 6) indicates that a difference exists among all ten formulations when all eight sensory attributes are concurrently compared. With the aim of determining which sensory attributes are accountable for the underlying differences among the formulations, descriptive discriminative analysis (DDA) was used.

Table 6: Multivariate Analysis of Variance

MANOVA	Test Criteria and F Approximations for the Hypothesis of No Overall Form Effect				
H = Type III SSCP Matrix for Forms E = Error SSCP Matrix S = 8 M = 0 N = 518.5					
Statistic	**Value**	**F Value**	**Numerator DF**	**Denominator DF**	**Pr>F***
Wilk's Lambda	0.80707	3.15	72	6327.6	<.0001
Pillai's Trace	0.20415	3.04	72	8368	<.0001
Hotelling - Lawley Trace	0.22547	3.25	72	4246.3	<.0001
Roy's Greatest Root	0.15051	17.49	9	1046	<.0001

*P-Value < 0.0001 indicates that a difference exists among all ten formulations

According to pooled within canonical structure in the first dimension (Can 1), odor/aroma (0.487), color (0.382) and mouthfeel (0.365) are the sensory attributes that significantly contribute to the differences among the ten formulations. In accordance to the second dimension (Can 2), spreadability (0.872) also makes a significant contribution to the overall differences between the formulations (Table 7). Altogether, these four sensory attributes which best differentiate the products, explain 95% of the cumulative variance. These results agree with those obtained by performing Tukey's Studentized Range test, in which these four sensory attributes (odor, color, mouthfeel, and spreadability) were found to be significantly different among the majority of the formulations.

Table 7: Canonical Structure r's Describing Group Differences among the Ten Formulations [a]

Sensory Attribute	Can 1**	Can 2**
Appearance	0.056	0.317
Color	**0.382***	0.371
Odor/Aroma	**0.487***	0.419
Smooth	0.327	0.301
Spreadability	-0.224	**0.872***
Taste	-0.062	0.437
Mouthfeel	**0.365***	0.577
Overall Liking	0.003	0.395
Cum. Variance Explained	66.75%	81.78%

[a] Based on Pooled Within-Group Variances
*Sensory attributes accountable for the difference among the samples
** Can = Canonical Structure, Pooled within canonical structure in the first and second dimension

3.3.3 Product Acceptability and Purchase Intent

Product acceptability, purchase intent, purchase intent of a cholesterol free product, and purchase intent with knowledge of the health benefits provided by RBO were evaluated based on a binomial (yes/no) scale. Results for affirmative responses for the abovementioned questions are presented in Table 8. Sample E, with the highest number of positive responses was the most acceptable formulation (72.12%). This formulation (E) consisted in 33.4 % RBO, 51.5% water and 5.4 % of SPC. This formulation also rated highest for all mean responses for all attributes, except taste (see Tables 4 and 5). Regarding product acceptability, sample E is followed by sample B (51.5 % RBO, 33.4 % water, 5.4 % SPC) with 68.87% positive responses. Formulation H follows the two aforementioned samples with 66.99% acceptability. These acceptability results agree with those for overall liking for samples E, B and H. Sample C received the lowest acceptability score, which coincides with the lowest overall liking score expressed by the mean responses (Tables 4 and 5).

Purchase intent results do not precisely coincide with those for product acceptability. Purchase intent was highest for formulations H, J and F, with 30.48%, 29.91% and 29.25%, respectively. Formulation J consisted in 43.4 % RBO, 43.4 % water, and 3.6% SPC, formulation H in 38.0 % RBO, 45.2 % water, and 7.2 % of SPC; and formulation F in 38.0% RBO, 51.15% water, and 0.9% SPC.

Table 8: Affirmative Responses (in percentages) for Product Acceptability and Purchase Intent of Mayonnaise-Type Spread Formulations

Formulation[a]	Acceptability	Purchase Intent	Purchase Intent Product With No Cholesterol**	Purchase Intent w/ Knowledge of Health Benefits**
A	59.81	20.75	34.58	51.40
B	68.87	25.00	37.04	48.15
C	52.38	20.56	32.41	50.00
D	56.31	23.81	35.24	46.23
E	72.12	25.23	36.79	52.34
F	62.38	29.25	36.79	47.17
G	65.38	23.58	29.25	41.51
H	66.99	30.48	38.46	46.15
I	55.24	24.30	32.71	42.06
J	58.25	29.91	39.25	47.66

[a] Sample formulations can be found in Table 3.
**Consumers were asked about their purchase decision if the product was cholesterol free and with the knowledge of the potential health benefits from the consumption of a product containing RBO.

When consumers were asked of their intent of purchasing a product that was cholesterol free, responses given changed from the initial intent. Purchase intent increased, meaning that consumers were willing to sacrifice overall liking of the product for its cholesterol-free characteristic. Formulations J, H, and B received the highest purchase intent percentages, being 39.25%, 38.46%, and 37.04%, respectively. Similarly, the responses regarding the ten formulations also changed when the consumers were questioned about their intent to purchase a product once they were informed of the potential health benefits associated with the consumption

of a RBO containing product. Purchase intent was highest for formulation E, followed by A and C, with 52.34%, 51.40%, and 50.00%, respectively. Once again, consumers are willing to sacrifice overall product acceptability for a product they could benefit from.

3.3.4 Logistic Regression Analysis for Product Acceptability and Purchase Intent

In order to correlate acceptability and purchase intent with the 9-point hedonic scale scores, logistic regression analysis was employed. Table 9 presents the predictive models that were used to predict consumer acceptability and purchase intent. Purchase intent was evaluated before additional information was given to the consumers and after additional information was given regarding the product. All four prediction models were obtained from the intercept and point estimates for each sensory attribute through logistic regression analysis.

Table 9: Full Logistic Regression Models for Predicting Acceptability and Purchase Decisions

Attributes	Predictive Model*
Acceptability	y = -5.0538 + 0.0587(Appearance) – 0.0194(Color) + 0.0146 (Odor) +0.0155(Smooth) – 0.0686 (Spread) +0.0187 (Taste) + 0.1394 (Mouthfeel) + 1.0980 (Overall Liking)
Purchase Intent	y = -7.9013 – 0.1278 (Appearance) + 0.0371(Color) – 0.0515 (Odor) - 0.0770 (Smooth) + 0.1115 (Spread) +0.4908 (Taste) – 0.1783 (Mouthfeel) + 1.0513 (Overall Liking)
Purchase Intent / Cholesterol-Free	y = -5.4905 – 0.0229 (Appearance) + 0.0268 (Color) – 0.00622 (Odor) - 0.0469 (Smooth) + 0.0762 (Spread) +0.3524 (Taste) – 0.1576 (Mouthfeel) + 0.7524 (Overall Liking)
Purchase Intent / RBO Health Benefits	y = -4.2540 – 0.0220 (Appearance) + 0.0979 (Color) – 0.0235 (Odor) - 0.1145 (Smooth) – 0.0322 (Spread) +0.2089 (Taste) – 0.1222 (Mouthfeel) + 0.5793 (Overall Liking)

*Predictive models based on estimates for each of the sensory attributes that resulted from logistic regression analysis

Based on the regression analysis, the most influential sensory attributes regarding product acceptability and purchase intent are determined based on a $Pr > \chi 2$ value less than $\alpha = 0.05$. Overall liking is the most influential sensory attribute for product acceptability determination and prediction, with an odds ratio point estimate of 2.998 (Table 10). Subsequently, mouthfeel is the second most important attribute in determining and predicting consumers' product acceptability, with an odds ratio of 1.150. This means that for every one-point increase in the 9-point hedonic scale for overall liking and mouthfeel, overall product acceptability will increase by 199.8% and 15.0%, respectively. This means that overall liking and mouthfeel (in that order) would affect the probability of the consumers' decision on product acceptability more than the other six sensory attributes. Overall product acceptability can be predicted with 80.33% accuracy based on percent hit rate (Table 11).

Likewise, overall liking was the determining sensory attribute for purchase intent, purchase intent of a cholesterol free product, and purchase intent with knowledge of the health benefits that can be provided by RBO; with the highest odds ratios of 2.861, 2.122 and 1.785, respectively. For these three purchase intent scenarios, overall liking, as the most influential sensory attribute, is followed by taste and mouthfeel. In the scenario order abovementioned, the odds ratios for taste are 1.634, 1.422, and 1.232. In the same manner, mouthfeel odds ratios are 0.837, 0.854, and 0.885. Based on percent hit rate, purchase intent can be predicted with 86.79% accuracy. Similarly purchase intent of a cholesterol free product can be predicted with 79.50% accuracy and purchase intent with knowledge of the potential benefits of RBO can be predicted with 75.24% accuracy.

Table 10: Probability $>\chi^2$ and Odds Ratio Point Estimates for Acceptance and Purchase Intent

Consumer Acceptance			
Parameter	**Estimate**	**Pr > χ2***	**Odds Ratio****
Appearance	0.0538	0.4396	1.061
Color	-0.0194	0.8057	0.981
Odor/Aroma	0.0146	0.8327	1.015
Smooth	0.0155	0.8252	1.016
Spreadability	-0.0686	0.1963	0.934
Taste	0.0187	0.8382	1.019
Mouthfeel	0.1394	0.0253	1.150
Overall Liking	1.0980	<.0001	2.998
Consumer Purchase Intent			
Parameter	**Estimate**	**Pr > χ2***	**Odds Ratio****
Appearance	-0.1278	0.1808	0.880
Color	0.0371	0.6918	1.038
Odor/Aroma	-0.0515	0.4900	0.950
Smooth	-0.0770	0.3781	0.926
Spreadability	0.1115	0.0939	1.118
Taste	0.4908	<.0001	1.634
Mouthfeel	-0.1783	0.0374	0.837
Overall Liking	1.0513	<.0001	2.861
Consumer Purchase Intent of a Cholesterol-Free Product			
Parameter	**Estimate**	**Pr > χ2***	**Odds Ratio****
Appearance	-0.0229	0.7679	0.977
Color	0.0268	0.7319	1.027
Odor/Aroma	-0.00622	0.9228	0.994
Smooth	-0.0469	0.5127	0.954
Spreadability	0.0762	0.1624	1.079
Taste	0.3542	<.0001	1.422
Mouthfeel	-0.1576	0.0203	0.854
Overall Liking	0.7524	<.0001	2.122
Consumer Purchase Intent with Knowledge of RBO Health Benefits			
Parameter	**Estimate**	**Pr > χ2***	**Odds Ratio****
Appearance	0.0220	0.7362	1.022
Color	0.0979	0.1500	1.103
Odor/Aroma	-0.0235	0.6843	0.977
Smooth	0.1145	0.0615	1.121
Spreadability	-0.0322	0.4911	0.968
Taste	0.2089	0.0056	1.232
Mouthfeel	-0.1222	0.0309	0.885
Overall Liking	0.5793	<.0001	1.785

*Probability values < 0.05 determine which attributes are significant.

**Odds Ratios predict the increase in acceptability and purchase intent due to a point increase in the 9-point hedonic scale

Table 11: Percent Hit Rate for Product Acceptability and Purchase Decisions

Attribute	% Hit Rate*
Acceptability	80.33
Purchase Intent	86.79
Purchase Intent / Cholesterol-Free	79.50
Purchase Intent / RBO Health Benefits	75.24

*Percent hit rate refers to the to thee accuracy with which each of the attributes can be predicted for the product

3.3.5 Change in Probability of Purchase Intent

Evaluation of change in purchase intent probability was evaluated by means of the McNemar test. The probabilities of purchase intent by consumers were evaluated previous and subsequent to being informed that the product was cholesterol free (Table 12) and of the possible health benefits that could be associated with product consumption (Table 13).

The null hypothesis being tested states that the purchase intent probability is the same before (π_{1+}) and after (π_{+1}) additional information concerning the product was provided, i.e. H_o: $\pi_{1+} = \pi_{+1}$. In other words, on the average there is no change in purchase intent after extra information is given about the product. From the results of the McNemar test, the probability of purchase intent of the product after the consumer was informed that the product was cholesterol-free is significant (p-value $< \alpha = 0.05$) for all 10 formulations, with the exception of formulation G (p-value = 0.083). The results for the probability change of purchase intent after knowing the potential health benefits of a RBO-based product indicate that the intent before and after being informed are significantly different for all 10 formulation (p-value <0.0001). Therefore, for both scenarios the consumer's purchase decision was influenced by additional information provided after the product had been tasted.

It can be predicted with 95% confidence that purchase intent will increase at least by that value stated by the lower confidence limit and at the most by that value stated by the upper

confidence interval (Table 12). For instance, for formulation A there will be a purchase intent increase of at least by 6.3% and at the most by 20.2% after the consumer is aware that the spread is a cholesterol-free product. The sample for whose purchase intent prediction establishes that the most increase would happen is formulation A (at the most 20.2% increase) and that for which the least increase will happen would be formulation F (at the most 12.6% increase). The formulations that have a broader predicted purchase intent range once the consumer is aware that the product is cholesterol free are samples B, D, and E. Formulations B and D with a 14.3 point range and sample E with a 14.2 point range. Conversely, formulation F has the narrowest purchase intent range (10.1) of all ten formulations. Over all, purchase intent will increase at the most between 12.6-20.2% comparing all formulations.

Table 12: Changes in Purchase Intent Probability after Knowledge that the Product Contained No Cholesterol [a]

Formulation [b]	$\chi 2$	p-value	95% CI-L*	95% CI-U**
A	12.250	0.001	0.063	0.202
B	9.941	0.016	0.049	0.192
C	12.000	0.001	0.052	0.172
D	9.000	0.003	0.043	0.186
E	9.000	0.003	0.042	0.184
F	8.000	0.005	0.025	0.126
G	3.000	0.083	-0.007	0.120
H	5.333	0.021	0.013	0.141
I	7.364	0.007	0.025	0.143
J	8.333	0.004	0.033	0.154

[a] All probabilities calculated by means of the McNemar Test
[b] See Table 3 for formulations
* 95% Confidence Interval – Lower Bound
** 95% Confidence Interval – Upper Bound

When the consumer was aware of the RBO-related health benefits (Table 13), purchase intent increased overall, at the most between 22.3-40.3%. This means that the consumer is more

willing to sacrifice overall liking of the product for the health benefits that RBO can potentially provide than for a cholesterol-free product. In relation to RBO health benefits, the percent purchase intent increase was the greatest for formulation A (21.9) in terms of the lower confidence level. Formulation A also presented the greatest purchase intent increase in terms of the upper confidence level (40.3). The outcome of formulation A presenting a greater purchase intent increase is also observed in the results for a cholesterol-free product.

Table 13: Changes in Purchase Intent Probability after Knowledge of the Potential Health Benefits Associated with Product Consumption [a]

Formulation [b]	$\chi 2$	p-value	95% CI-L*	95% CI-U**
A	31.114	<.0001	0.219	0.403
B	20.161	<.0001	0.140	0.323
C	31.000	<.0001	0.204	0.376
D	19.593	<.0001	0.132	0.307
E	25.485	<.0001	0.179	0.363
F	19.000	<.0001	0.106	0.252
G	19.000	<.0001	0.106	0.252
H	16.000	<.0001	0.085	0.223
I	19.000	<.0001	0.105	0.250
J	15.696	<.0001	0.096	0.259

[a] All probabilities calculated by means of the McNemar Test
[b] See Table 3 for formulations
* 95% Confidence Interval – Lower Bound
** 95% Confidence Interval – Upper Bound

3.3.6 Principal Component Analysis

The product-attribute bi-plot, constructed using PC 1 and PC 2, is shown in Figure 8. As illustrated by Figure 8, overall liking, mouthfeel, and taste are the discriminating attributes for the RBO-based mayonnaise-type spreads. This result agrees with those attributes which were found to be accountable for determining purchase intent (in all three scenarios) using logistic regression analysis. Therefore, it can be stated that consumers probably rated the products'

attributes based on purchase intent. Formulations B and J fall within the same area as taste, overall liking, and mouthfeel. Both of these formulations contain comparable amounts of SPC (5.4% and 3.6%, respectively) and received the following mean scores: taste (4.69 B, 4.69 J), mouthfeel (5.54 B, 5.66 J) and overall liking (4.97 B, 4.88 J).

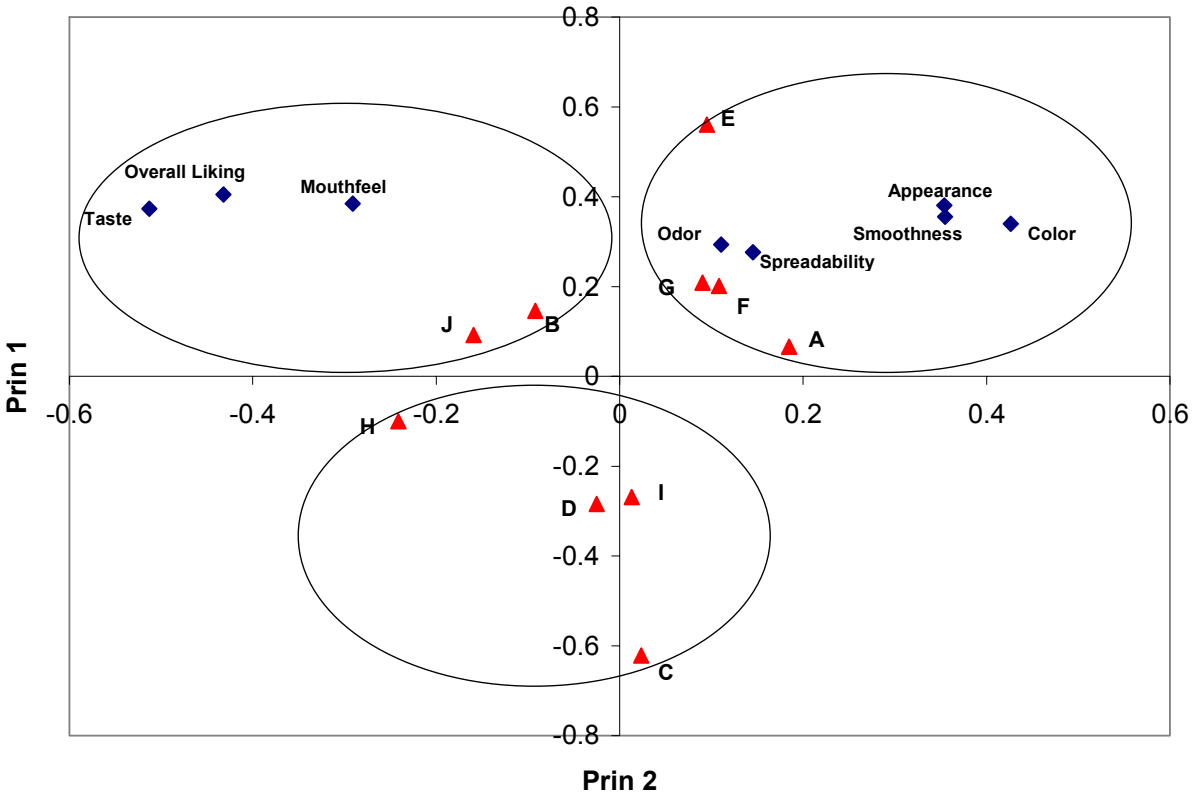

Figure 8: Principal Components Analysis*
*Refer to Table 3 for complete product formulations and Tables 4 and 5 for mean scores for sensory attributes for all 10 formulations. See Appendix A for Prin1*Prin3 and Prin2*Prin3 bi-plots.

Appearance, color, odor/aroma, smoothness, and spreadability are all clustered within the top right hand quadrant. The formulations also found within this quadrant are A, E, F, and G. Formulations A, F, and G, found to be clustered closer together, all contain 0.9% SPC. These three formulations also received comparable acceptability scores (65.38 G, 62.38 F, 59.81 A).

Formulations E, containing 5.4% SPC, ranked highest on acceptability (72.12) among all ten formulations. Sample E also received the highest mean ratings for all attributes, except spreadability. Formulation A contains the highest percentage of RBO (51.5%) and a small amount of water (38.0%) and formulation E contains the lowest percentage of RBO (33.4%) and the highest percentage of water (50.8%).

Formulations C, D, H, and I are represented in the two lower quadrants. Also, in these two quadrants no sensory attributes are present. All these formulations contain the highest percentage of SPC among all 10 formulations. Formulations H and I contain 7.2% SPC and formulations C and D contain 9.9% SPC. Analyzing the quadrants separately, the formulations on the left hand side D and H contain 47.0% water and those samples found on the right hand side (C and I) contain 47 % and 45.2 % RBO, respectively. Product C is illustrated as the least accepted, in accordance with mean scores for all samples regarding specific sensory attributes formulation C received the lowest score for overall liking (4.35). The acceptability scores for formulations D, I and C, were also comparable: 56.31 D, 55.24 I, and 52.38 C.

3.3.7 Product Optimization

Product optimization was performed using the three-component mixture design experiment in combination with logistic regression analysis. The predictive models obtained using restricted regression analysis, without intercept, are presented in Table 14. These predictive models were used to plot the mixture response surface (MRS) for each of the sensory attributes under discussion (Figure 9). The optimal formulation was determined by the superimposition of the all the sensory attributes critical to consumer acceptance and purchase intent, as determined by logistic regression analysis. Superimposition was determined by mean acceptance scores of 5.0 and above.

Table 14: Parameter Estimates for Variables Used in Final Prediction Models for Consumer Acceptance

Variables	Prediction Model*	R-Square
Appearance	-10.11*x1 - 380.16*x2 - 9.84*x3 + 739.47*(x1*x2) + 63.35*(x1*x3) + 741.47*(x2*x3) -1345.71*(x1*x2*x3)	0.9124
Color	17.09*x1 + 62.73*x2 + 19.43*x3 - 158.65*(x1*x2) - 48.54*(x1*x3)-182.88*(x2*x3) + 404.76*(x1*x2*x3)	0.9389
Odor/Aroma	17.77*x1 + 63.79*x2 + 18.32*x3 - 140.80*(x1*x2) - 51.15*(x1*x3) - 135.24*(x2*x3) + 234.43*(x1*x2*x3)	0.9172
Smoothness	3.71*x1 -170.17*x2 +3.11*x3+ 378.62*(x1*x2) +12.11*(x1*x3) + 406.80*(x2*x3) - 910.27*(x1*x2*x3)	0.9246
Spreadability	6.39*x1 - 233.17*x2 +6.84*x3 + 339.88*(x1*x2) - 3.72*(x1*x3) + 376.78*(x2*x3) -395.97*(x1*x2*x3)	0.9200
Taste	-3.55*x1 - 269.32*x2 - 2.42*x3 + 423.19*(x1*x2) + 28.96*(x1*x3) + 428.43*(x2*x3) - 510.35*(x1*x2*x3)	0.8251
Mouthfeel	-7.14*x1 - 404.12*x2 - 7.04*x3 + 750.26*(x1*x2)+ 52.04*(x1*x3) + 770.94*(x2*x3) -1363.18*(x1*x2*x3)	0.8892
Overall liking	1.39*x1 -206.58*x2 + 2.31*x3 + 324.06*(x1*x2) + 10.90*(x1*x3) + 327.24*(x2*x3) - 399.38*(x1*x2*x3)	0.8619

*Calculation of parameter estimates based on raw data with no intercept option.
**Calculation of R-square values is based on reduced regression models for each attribute.

Critical sensory attributes were determined based on the probability greater then chi-square ($Pr > \chi^2$). If the $Pr > \chi^2$ was less than 0.05, then the attribute was considered significant in terms of consumer acceptance, purchase intent, or both. The $Pr > \chi^2$ for each sensory attribute is presented in Table 9. For consumer acceptance, mouthfeel, and overall liking are significant. In terms of purchase intent, before and after additional information was provided to the consumers about the product, taste, mouthfeel, and overall liking are significant. As a result, the MRS of taste, mouthfeel, and overall liking were used to determine the optimal formulations. The superimposition of these critical attributes is shown in Figure 10, indicating that any formulation containing 37-42% RBO, 1-6% SPC, and 50-57% water (in respect to the three component mixture design), will yield an acceptable product that could be potentially purchased.

3.4 Conclusions

ANOVA and MANOVA results showed no significant difference among appearance, smoothness, taste and overall liking; however color, odor, spreadability and mouthfeel were found to be different among the majority of the formulations. DDA indicated that when all attributes compared; the attributes accountable for 95% of the difference are color, odor, spreadability, and mouthfeel. LRA results showed that mouthfeel and overall liking were the most discriminating sensory attributes for overall acceptance. Purchase intent responses, in the absence and presence of a health claim, were also affected by these two attributes in addition to taste. The odds ratio point estimate decreased in the presence of the health claim; therefore this claim affected the likelihood of buying. This is in accordance with an overall purchase intent increase of 10% once the consumer was aware that the product was cholesterol free, whereas there was a 22% increase once the consumer was informed of RBO health benefits. Based on percent hit rate it can be predicted that a new formulation will be 80.33% acceptable, with

86.79% purchase intent, 79.5% intent knowing the product is cholesterol free, and 75.24% intent knowing the health benefits provided by the rice bran oil. PCA indicated that sample C was significantly different from all other samples, with the lowest acceptance and purchase intent, and having mouthfeel as the most discriminating attribute. Samples D, H and I can also be clustered with sample C, all having high SPC content as the parallel. Regression analysis was performed and using RSM, contour maps were constructed to characterize the optimal formulation, determined as 37-42% RBO, 1.0-6.0% SPC, and 50-57% water. This study indicated that color, odor, spreadability and mouthfeel are the most discriminating sensory attributes, and overall liking is the best acceptance and purchase intent predictor. Consumers purchase intent increases with the presence of a health claim, therefore there is a willingness to sacrifice product liking in favor of health benefits.

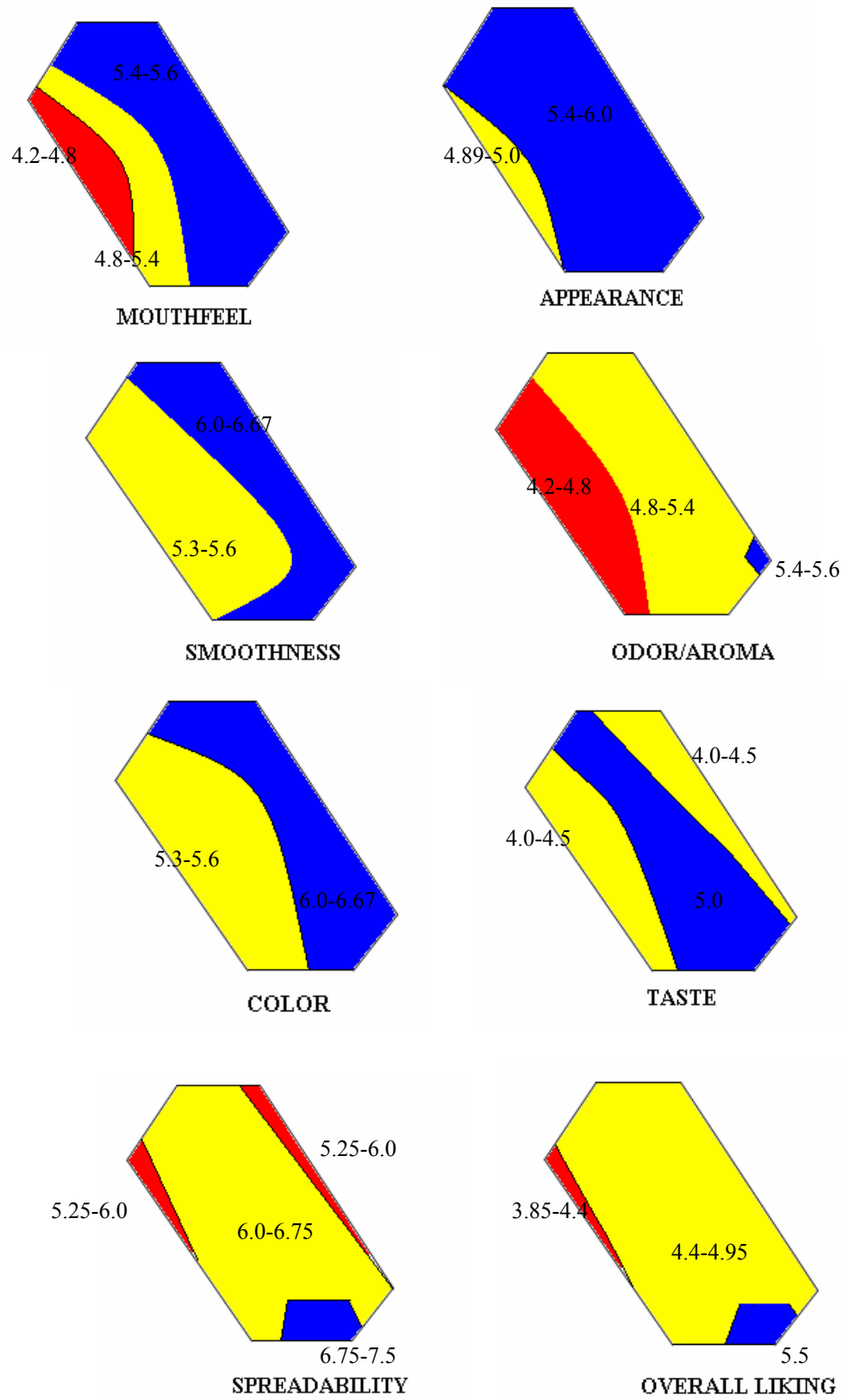

Figure 9: Response Surface Methodology (RSM) for Contributing Sensory Attributes Representing Mean Sensory Attributes as Evaluated by Consumers

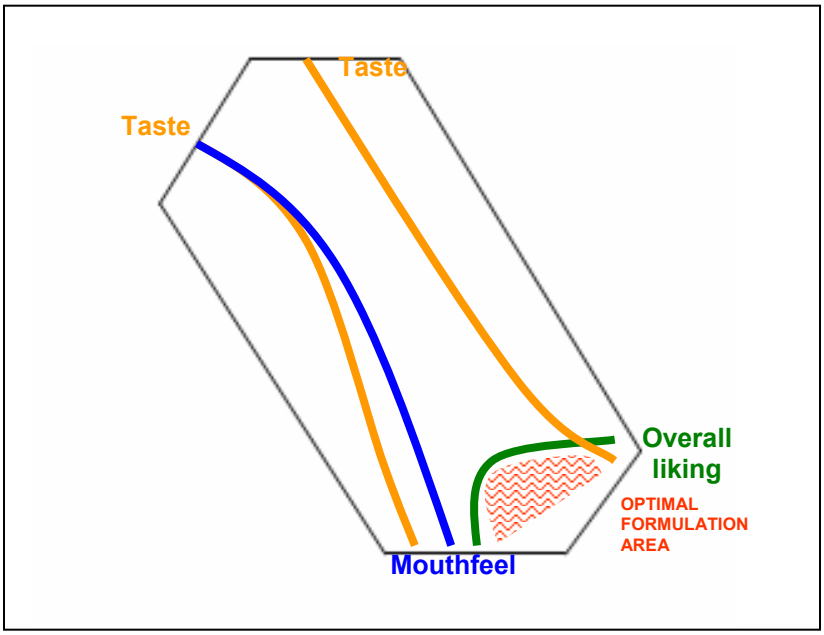

Figure 10: Superimposition of Critical Product Attributes to for Optimal Formulation Determination

CHAPTER 4. PREFERENCE RANKING ANALYSIS OF NOVEL CHOLESTEROL-FREE MAYONNAISE-TYPE SPREADS CONTAINING RICE BRAN OIL

4.1 Introduction

Once the optimal formulation range has been established, it is important to determine if a flavored enhanced product is preferred over the plain formulation; i.e., if consumers can distinguish or discriminate among two samples from the same formulation for which the only difference is added-flavor. This is of special interest based on the results from the previous study, i.e., consumer-oriented optimization of the product, where it was concluded that consumers were willing to sacrifice taste and overall liking for the health benefits provided by the product. If consumers can differentiate among the samples, with preference towards the flavored product, then there would be no taste sacrifice for a healthier product.

The objectives of this study were to determine (1) if consumers prefer a flavored product over a plain one from the same formulation and (2) to compare results with the previous study to determine if there was a significant difference in product acceptability and purchase intent of the chosen formulation.

4.1.1 Discriminative Sensory Tests

When performing discriminative sensory tests, the major question is whether or not differences exist amid the samples, where the similarity or difference testing approach can be used. In some instances the researcher may want to demonstrate that two samples are perceptibly different form one another. In other cases the researcher may be interested in determining if the two samples are amply comparable to be used interchangeably (Meilgaard and others 1999). An assortment of tests exist that can be performed to determine if panelists can detect overall difference and/or differences regarding a specific attribute among two or more samples of a food

product (Prinyawiwatkul 2004). Difference tests are classified into overall difference tests and attribute difference tests. Overall difference tests (which answer if a sensory difference exists between samples) include tests such as the Triangle, Duo-trio, A-not A, Difference-from-Control, etc (Meilgaard and others 1999). Attribute difference tests (which answer how a specific attribute differ between samples) include tests such as paired comparison, n-AFC, and various types of multiple comparison tests (Meilgaard and others 1999).

Prinyawiwatkul (2004) reported that discriminative sensory tests have several applications, among which are: (1) to establish if products differ as a result of changes in ingredients, processing, packaging, storage, etc. (2) to determine if an overall product differentiation can be detected, but that can not be accredited to any specific attribute, (3) to establish if a differentiation exists due to a specific attribute, (4) to monitor the panelists ability to discriminate between tests samples, and (5) to select and screen panelists for descriptive analyses.

4.1.2 Signal Detection Theory

Signal detection theory (SDT) is a measurement theory that allows for the separation of an evaluator's true sensitivity from response bias (Prinyawiwatkul 2004). Using the SDT, the subject's decision process becomes unambiguous and can also be represented statistically, which is a major advantage of this procedure (Meilgaard and others 1999). Signal detection, in a simple experiment, involves two levels of stimulus. The background stimulus is referred to as the noise (N) and the weaker but higher level of stimulus near the threshold is referred to as the signal (S). When performing food sensory tests, the signal can be new, reformulated, or improved products and the noise can be the control, existing or the current product being produced (Prinyawiwatkul 20004). In a signal detection experiment, an asserted decision (referred to as "hit") is made when

a signal is presented and perceived as so. However, an incorrect decision (referred to as "miss) is made when the signal is presented and perceived as noise. When the noise is presented and perceived as a signal a "false alarm" results and when it is correctly perceived as the noise a "correct rejection" results (Lawless and Heymann 1998). Figure 11 illustrates these responses.

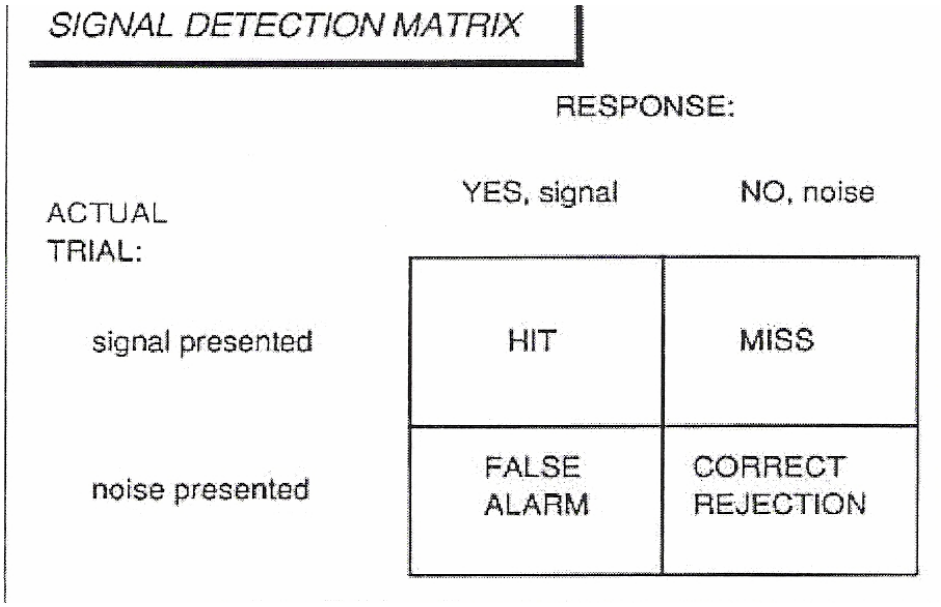

Figure 11: Signal Detection Matrix (Lawless and Heymann 1998)

Meilgaard and others (1999) defined SDT as "a system of methods based on the idea that the point of interest is not the threshold as such, but rather 'the size and the psychological difference between the two stimuli', which has the name of *d'* ". This sensory difference between signal and noise stimuli, *d'*, represents the separation of the means of the two distributions in standard deviation units. The d' value is calculated as the difference between the Z-scores from the proportion of "hits" and the Z-scores from the proportion of "false alarms" (Lawless and Heymann 1999). The higher the *d'* value the better the discrimination.

The limitation for *d'* is that normal distribution is required; therefore SDT assumes normal distribution of the signal and the noise, with equal variances. This theory also assumes

the existence of variability in the signal and the noise due to variation in the background levels in sensory nerves and other factors.

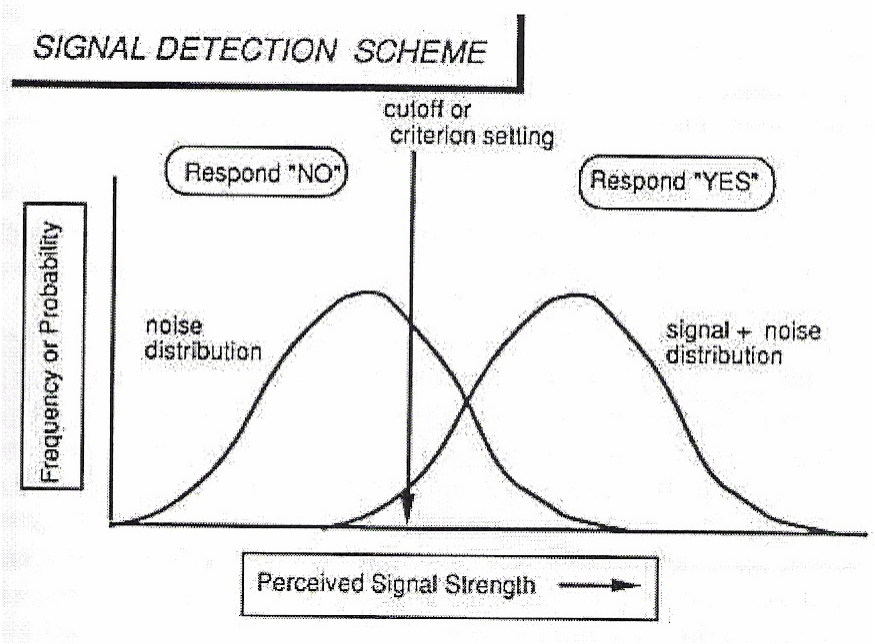

Figure 12: Signal Detection Scheme (Lawless and Heymann 1998)

4.1.3 ROC Curve-Differing Sensitivities

One measure of discrimination which does not depend on the exact forms of the signal and noise distributions is the area under the Receiver Operating Characteristic (ROC) Curve (Figure 13). According to Lawless and Heymann (1998), the ROC curve is useful in that it allows for the definition of a judge's ability to detect stimuli across the different levels of criterion. The level of discrimination, a measure related to *d'*, is proportional to the area under the ROC curve. There is no discrimination when the hit rates and false alarm rates are equal and *d'* is equal to zero. Higher levels of discrimination between stimuli (higher *d'* values) are illustrated by curves that arch more towards the upper left hand side corner of the graph.

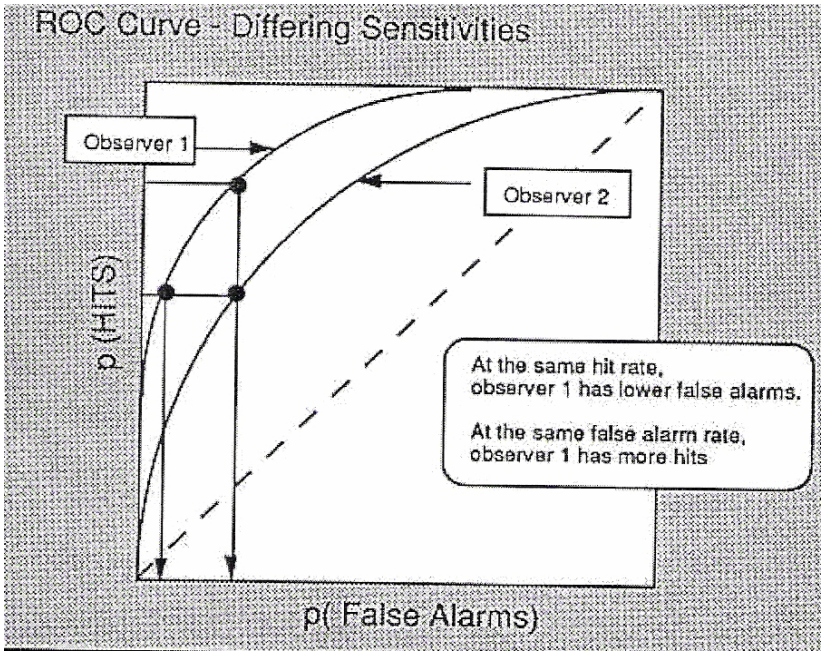

Figure 13: ROC Curve-Differing Sensitivities

4.1.4 R-Index Approach

R-Index is a means of applying signal detection of stimuli to foods; it is an alternative measure used to provide an index of discrimination ability but without assuming equally and distributed variances from signal and noise distributions (Prinyawiwatkul 2004). R-Index is a measure of the degree of difference between the control and treatment samples and states the probability value of a particular judge appropriately distinguishing between the two samples. The R-Index and the differentiating probability are directly proportional, i.e. as the degree of difference (R-Index) increases so does the probability of distinguishing between the two samples. The R-Index is extremely useful when testing food products because it is intricate to perform numerous trials that are necessary in order to obtain an accurate estimate of d' using the SDT (Lawless and Heymann 1998).

When using a rating scale, R-Index converts the rating scale to an index related to the percent of the area under the ROC (Receiver Operating Characteristic) curve, which is a measure

of discrimination. An R-Index value of 100% indicates perfect discrimination by the judge. An R-Index value of 50%, a chance value, indicates that the samples cannot be discriminated. Intermediary values, between 100 % and 50%, specify a probability of discrimination between chance and correct choice (Cliff and others 2000).

Using the R-Index procedure presents several advantages: (1) it is a powerful parametric statistical analysis, particularly when more than two samples are compared, (2) if a judge is considered a measuring instrument, a large number of judges is not required, and (3) only a few sensitive/accurate judges are needed with multiple replications. Hence the mentioned advantages; this procedure is time consuming, it requires more samples than simpler paired comparison, and does not provide a direction of the difference in regards to the sensory attribute in question. In addition, the traditional R-Index only gives the probability of the judge being able to differentiate between the samples; nonetheless it does not provide the direction or magnitude of the difference.

4.2 Materials and Methods

4.2.1 Spread Preparation

Based on the consumer-oriented product optimization of the ten spread formulations described in Chapter 3, formulation E was chosen to carry on the discriminative analysis. This formulation was chosen based on the high ratings received for all sensory descriptors and the ratings for acceptability and purchase intent. This formulation also meets the optimal formulation range of 37-42% RBO, 1-6% SPC and 50-57% water. Formulation E contains 37 % RBO, 6 % SPC, and 57% water. A lower quantity of required RBO decreases the cost of the product and the amount of SPC makes the product of a desirable consistency as discussed in the previous chapter.

The spread was prepared based on seven (7) total ingredients consisting of four (4) dry ingredients (SPC, stabilizer, salt, and flavor) and three (3) liquid ingredients (RBO, water, and lemon juice). As the first step in formulating the spread all ingredients were measured out. The dry ingredients were mixed and then paced in a food processor. To this mixture, water was gradually added and blended together until a uniform clump-free paste was obtained. Then, the oil and lemon juice were added, alternating among the two and ensuring that the oil was added in the form of a fine thread. Forty (40) grams of flavor were added to the plain formulation and then blended for 2 minutes. The final product was then transferred to a sterilized container and stored under refrigeration at 4^{o}C.

Oryzan^{TM} RBO , which is a high oryzanol refined bleached dewaxed deodorized rice bran oil, was the oil used and obtained from Rito Partnership (Stuttgart, Arkansas) [color, 28Y 2.3R; free fatty acid (% as oleic, AB), 0.034; peroxide value (PV), 0.39 mequiv/kg; moisture, 0.0050 %; flavor, 7.0; iodine value, 104.3; and oryzanol (spectrophotometric), 1.1]. The soy protein concentrate used in this study was provided from Archer Daniels Midland Company (Decatur, Illinois); it is available in the market under ACRON® S [moisture, 6%; protein, 72 %; fat, 4%, ash, 5%; total dietary fiber, 20%; and calories, 290/100g]. The thickening or gelling agent used, which is a blend of xanthan gum, guar gum and sodium alginate, was obtained from Tic Gums, Inc. (Belcamp, Maryland); it is available in the market under the name TIC PRETESTED® Pre-Hydrated® SALADIZER® 250 Powder [percent calories from fat, 1%; calories from fat, 3.6 Kcal; total fat 0.4 g; sodium, 1888 mg; total carbohydrate, 84 g; soluble dietary fiber 84 g; and protein, 1g (all quantities per 100 grams)]. The lemon juice (ReaLemon$^{®}$) and salt (Morton$^{®}$) were purchased from a local grocery store. The flavors used were obtained from Land O'Lakes, Inc. (St Paul, Minnesota); they are available in the market under the

following names: Cheddar and Sour Cream, Sour Cream and Onion, and Monterrey Jack Dried Cheese. Cheddar and Sour Cream [moisture, 0-5%; pH, 5.2-5.6; salt, 7.1 – 10.1%; coliform, <10CFU/g; mold, <300CFU/g; yeast <300CFU/g; *E Coli*, <10 CFU/g; standard plate count (SPC), <100000 CFU/g; *Salmonella*, 1500g] contains whey, cheese[Cheddar and Blue(cultured milk, salt, enzymes)], partially hydrogenated soybean oil, buttermilk, saslt, monosodium glutamate, maltodextrin, onion powder, sour cream (cultured cream, nonfat milk), nonfat dry milk, natural and artificial flavors, disodium phosphate, citric acid, garlic powder, color (including yellow 6), disodium inosinate and disodium guanylate, lactic acid, silicon dioxide (added at not more than 2% as an anti caking agent). Sour Cream and Onion [fat, 3-7%; moisture, 0-5%; pH , 4.5-4.9; salt, 8– 11%; coliform, <100CFU/g; mold, <300CFU/g; yeast <300CFU/g; *Staphylococcus Aureus*, Coagulase (+), <10CFU/g; *E Coli*, <3 MPN/g; standard plate count (SPC), <100000 CFU/g; *Salmonella*, not present] contains whey, dextrose, nonfat dry milk, sour cream solids (cultured cream, non dry milk), salt, onion powder, monosodium glutamate, cultured nonfat milk solids, food starch-modified, dehydrated parsley, artificial flavor, citric acid, lactic acid, tocopherols and ascorbyl palmitate (added to improve stability). Monterrey Jack Dried Cheese [fat, 38-42%; moisture, 0-4%; pH , 5.5-5.9; salt, 1-2%; coliform, <10CFU/g; mold, <50CFU/g; yeast <50CFU/g; *Staphylococcus Aureus*, Coagulase (+), <10CFU/g; *E Coli*, <3 MPN/g; standard plate count (SPC), <50000 CFU/g; *Salmonella*, not present] contains cheese [Monterrey Jack and Swiss(milk, salt, cheese cultures, enzymes)], buttermilk, partially hydrogenated soybean oil, maltodextrin, natural flavor, disodium phosphate, artificial flavor, lactic acid.

Table 15: Flavored Spread Formulations

Formulation	RBO (%)	SPC (%)	Water (%)	Flavor*
A	33.4	5.4	51.5	Sour Cream & Onion
B	33.4	5.4	51.5	Plain
C	33.4	5.4	51.5	Cheddar & Sour Cream
D	33.4	5.4	51.5	Monterrey Jack

*All formulations (based on original formulation E) were prepared plain with 40 grams of flavor added

4.2.2 Consumer Test

For purposes of ranking the product based on preference, a ranking test was conducted. One hundred (100) untrained consumers, randomly chosen from the Louisiana State University, Baton Rouge Campus, participated in the test. The following criteria were essential for recruitment of all participants: 18 years of age or older, not allergic to rice and/or soy products and willingness for participation for approximately 15 minutes to complete the survey. The consumers were simultaneously presented with 4 samples which were randomly coded from A through D for a total of 100 observations per formulation. Three (3) of these formulations were flavored and one was the plain formulation used as control (Table 15). The consumers were given samples of 30 g placed in lidded transparent containers which were accompanied with unsalted crackers and room temperature water for palate cleansing purposes between sample tasting. Consumers were presented with a questionnaire and instructed on proper filling. Consumers were required to complete and sign a consent form approved by the Louisiana State University Institutional Review Board prior to participating on the testing. After the evaluation, the panelists were instructed to rank the four samples based on their preference from 1-4, where 1 was the least preferred and 4 was the most preferred. The panelists were forced to make a choice, i.e, no ties were given. The consumers also rate the acceptability and purchase intent for all four (4) samples based on a binomial (yes/no) scale.

4.2.3 Statistical and Data Analysis Methods

The validity of statistical procedures lies on underlying statistical assumptions. Statistical methods used in experimental design analysis require that the observations be normally and independently distributed (Gacula and Singh 1984). Methods that rest on specific distributional assumptions are parametric methods. There are methods that do not depend on specific distributional assumptions, such methods are nonparametric methods. Gacula and Singh (1984) stated that most nonparametric methods use ranks assigned to experimental observations in decision-making rules.

4.2.3.1 Friedman's Test and the Analog to Fisher's LSD

When using a randomized block design and the data are in the form of ranks a nonparametric analysis is performed using a Friedman-type statistic (Meilgaard and others 1999). This procedure assumes that numerous observations were gathered; it is reasonably correct for studies concerning 12 judges or more. Friedman's Test is the non-parametric equivalent to the two-way analysis of variance without interaction, which is based on a chi-square distribution (Lawless and Heymann 1998). The Friedman statistic for rank data is:

$$T = \{[12/bt(t + 1)]\Sigma x_{.j}^2\} - 3b(t + 1)$$

where b = the number of panelists, t = the number of samples, and $x_{.j}$ = the rank sums .

The test procedure is to reject the null hypothesis (H_o) of no sample differences at the preset α-level if the value of T exceeds $\chi^2_{\alpha,t-1}$, and to accept H_o otherwise (Meilgaard and others 1999). This means that the solution to the χ^2 based equation (χ^2 statistic) is compared to the critical χ^2 value. If the value of T is greater than the χ^2 critical value, the samples in question are considered as different.

A significant χ^2 statistic implies different samples. Then a multiple comparison procedure is performed with the purpose of determining which if the samples differ significantly. This procedure is the nonparametric analog to Fisher's LSD (Least Significant Difference) for rank sums from a complete randomized block design. It is defined as:

$$LSD_{rank} = z_{\alpha/2}\sqrt{[bt(t + 1)/6]} = t_{\alpha/2,\infty}\sqrt{[bt(t + 1)/6]}$$

If two sample's rank sum difference is greater than the value of LSD_{rank} it is concluded that the two samples are significantly different at the α-level.

4.2.3.2 Wilcoxon Rank Sum Test/Kruskal-Wallis H Test

The Wilcoxon rank sum test is appropriate when two random samples from population X and from population Y are taken independently of each other (Gacula and Singh 1984). The null hypothesis is that both populations (X and Y) are alike. The Wilcoxon rank sum method requires the ranking of each of the observations in both samples in order of magnitude. When there are more than two populations the Kruskal-Wallis *H* test can be used (Gacula and Singh 1984), which is a generalization of the Wilcoxon rank sum test. The null hypothesis states that all the populations (*k*) means are equal. The alternative hypothesis states that at least one member is different from each other. It has been shown that when there are large samples the *H* statistic approximately follows the Chi-square distribution with *k*-1 degrees of freedom. Critical values are then obtained from χ^2 distribution tables. The null hypothesis is rejected if the calculated χ^2 is greater that the critical χ^2 value, for the α level of significance.

4.2.3.3 R-Index

In a traditional R-Index procedure the judges are familiarized with the signal (S) and the noise (N) samples. When served an equal number of S and N samples, the judges are

required to determine if the randomly presented samples are definitely signal (S), perhaps signal but not sure (S?), perhaps noise (N?), or definitely noise (N). From these responses, the R-Index calculation is as follows:

Table 16: R-Index Response Format for Calculation Procedure

	Judge's Response				**Total**
Sample	**S**	**S?**	**N?**	**N**	
S	*a*	*b*	*c*	*d*	n_S=a+b+c+d
N	*e*	*f*	*g*	*h*	n_N=e+f+g+h

$$\text{R-Index} = \frac{[a(f+g+h) + b(g+h) + ch] + [1/2\,(ae+bf+cg+dh)]}{(n_S)(n_N)}$$

When the R-Index procedure is carried using ranking, a preset attribute is compared among N and S1, S2, S3, etc. and the degree of differentiation is presented as a percentage among N and each S independently. Once the R-Index is determined, its significance needs to be tested. It needs to be determined if the R-Index (expressed as a percent) is greater than by chance (50%) at a given sample size (SS) and level of significance (α). The null hypothesis (H_o) states that the R-Index (%) is equal to chance (50%). H_o is rejected if the obtained deviation from 50% is equal or greater than the value in the table.

4.3 Results and Discussion

4.3.1 Friedman's Test and the Analog to Fisher's LSD

In terms of preference the null hypothesis (H_o) was stated as no sample differences at $\alpha = 0.05$. The T-value (based on b = 100, t = 4, and the rank sums in Table 17) was equal to 152.6 and the critical value at $\alpha = 0.05$ was 7.82. Since the T-value exceeds the critical value the null hypotheses is rejected and there are sample differences at $\alpha = 0.05$.

Table 17: Rank Response Frequency and Rank Sums

Sample*	Response Frequency for Ranks**				Rank Sum[a]
	4	3	2	1	
A	40	34	19	7	307
B (control)	0	2	18	80	122
C	45	32	20	3	319
D	16	32	42	10	254

* A= Sour Cream & Onion, B = plain, C = Cheddar Cheese & Sour Cream, D = Monterrey Jack
**Ranks: 4 = like the most and 1 = like the least
[a] Rank Sum = Σ(rank*response frequency)

Being that there are samples differences (the $\chi 2$ statistic is significant), it was determined among which samples the differences were present. The nonparametric analog to Fisher's LSD procedure was performed and the LSD_{rank} was found to have a value of 35. 78 (based on b = 100, t = 4, $\alpha = 0.05$, $t_{\alpha/2,\infty} = 1.96$). Two samples are declared significantly different if their rank sums differ by more than the value of LSD_{rank}. The rank sum differences are presented in Table 18.

Table 18: Rank Sum Differences*

Sample (Rank Sum)**	C (319)	A (307)	D(254)	B(122)
C (319)				
A (307)	12 (NS)[a]			
D (254)	65 (S)[b]	53(S)		
B (122)	197 (S)	185(S)	132 (S)	

*Values less than 35.78 signify that the two samples in question are significantly different from each other, values greater then 35.78 indicate the opposite.
**Values in parenthesis are the rank sums for each sample.
[a]NS = Not Significantly Different
[b]S =Significantly Different

Based on the aforementioned specifics for establishing the existence of a difference between two samples, all three flavored samples (A, C, D) were found to be significantly different from the control (B). Among the flavored samples, formulation D was found to be significantly different from formulations A and C. The only pair that was not declared as significantly different from one another was A-C. In conclusion, sample differences were present among the all flavored samples and the control/plain sample. Within the flavored samples,

differences were present among the Cheddar & Sour Cream Flavor and both the Sour Cream & Onion and the Monterrey Jack. No differences were present, as perceived by the judges, among the Sour Cream & Onion and Monterrey Jack flavors.

4.3.2 Wilcoxon Rank Sum Test/Kruskal-Wallis *H* Test

For the Kruskal-Wallis test the null hypothesis (H_o) stated that all the samples were perceived as equal by the consumers and the alternative hypothesis stated that all the samples were perceived as different. The critical value for this test is 7.82 at $\alpha = 0.05$ and DF = 3. The chi-square value (χ^2 statistic) of 193.7011 is greater than the critical value; therefore, the null hypothesis was rejected and it was concluded that the samples were not perceived as equal by the panelists. This conclusion can also be verified by means of Pr > Chi-Square (<0.0001) which is less than $\alpha = 0.05$, meaning that H_o is rejected and the samples were not perceived as the same.

Table 19: Kruskal-Wallis Test

Chi-Square[a]	DF	Pr> Chi-Square[b]
193.7011	3	<0.0001

[a] Chi-Square >7.82 implies H_o is rejected
[b] Probability < 0.05 implies H_o is rejected

4.3.3 R-Index

Using the traditional R-Index approach it was determined that consumers can correctly distinguish between the flavored samples (A, C, D) and the control (B) in terms of preference (Table 20). From Table 20 it is observed that the panelists were better at distinguishing among samples C and B (R-Index = 95.23%), i.e. among the control and the Cheddar & Sour Cream flavor. Sample B was followed by samples A and D; with R-Indices of 92.97% and 88.90%, respectively. Distinguish among the flavored samples was not as successful. Judges were able to distinguish mainly among samples C-D (69.51%), followed by A-D (66.16%), and lastly by A-C

(46.84%). This means that the panelists were better at distinguishing the Sour Cream & Onion and Cheddar & Sour Cream flavors from the Monterrey Jack flavor. In the case of A-C where the R-Index is below 50%, i.e., the judge not being able to distinguish the S and N; in this case among the Sour Cream & Onion and Cheddar & Sour Cream flavors.

When testing the significance of these R-Indices (for a 2-tailed test with N=100 and α = 0.05) the critical values are as shown in Table 20. Any value between 40.34 and 59.66 is not significant. H_o (R-Index equal to chance) is rejected if the R-Index value is above 59.66 or below 40.34. Only pair A-C has an R-Index value that is not significant, meaning that the consumers were not able to differentiate among the samples. For all other sample pairs the null hypotheses were rejected. This means that the R-Index is not equal to chance. We can conclude that consumers were able to correctly differentiate between the flavored samples and the plain (control) and also among samples A-D and C-D but not between A-C.

Table 20: R-Indices for Combinations Presented

Compared Samples*	R-Index (%)[a]	R-Critical[b]
A-**B**	92.97	59.66
C-**B**	95.23	59.66
D-**B**	88.90	59.66
A-C	46.84	40.34
A-D	66.16	59.66
C-D	69.51	59.66

*A= Sour Cream &Onion, B = plain, C = Cheddar Cheese & Sour Cream, D = Monterrey Jack
[a]Sample pairs with R-Index value greater than 59.66 or below 40.34 are different from each other.
[b]R-Critical determined for N=100 and a 2-tailed test with α = 0.05.

4.3.4 Acceptability and Purchase Intent

Product acceptability, purchase intent, and purchase intent of the product with knowledge of the health benefits provided by RBO were evaluated based on a binomial (yes/no) scale. Results for affirmative responses for the abovementioned questions are presented in Table 21.

Sample C, with the highest percentage of positive responses was the most acceptable formulation (97%). This formulation (C) consisted in the Cheddar & Sour Cream flavor. Regarding product acceptability, sample C was closely followed by sample A (Sour Cream & Onion flavor) with 96% acceptability. Formulation D (Monterrey Jack flavor) follows the two aforementioned samples with 84% acceptability. Sample C (control/plain) received the lowest acceptability score (49%). Purchase intent results closely coincided with those for product acceptability. Purchase intent was highest for formulations A (65%), closely followed by sample C (63%). Sample D received a purchase intent score of 49% and sample C received the lowest score (9%). All flavored samples were found to be acceptable by the consumers. Samples A and C received the highest acceptability, and likewise, purchase intent for these two formulations received the highest scores. Sample C was found to be the least acceptable with a minute possibility of purchase intent by the consumer.

Table 21: Affirmative Responses for Acceptability and Purchase Intent

Sample	Acceptability (%)	Purchase Intent (PI) (%)	PI -Health Benefits (%)*
A	96	65	77
B	49	9	22
C	97	63	77
D	84	49	60

* Purchase Intent after consumers were informed of the potential health benefits attributed to the consumption of a RBO-containing product

Purchase intent increased for all four formulations when consumers were informed of the potential health benefits they could receive from through the consumption of a RBO containing product. Purchase intent was the same for samples A and C (77%), followed by sample D (60%) and sample B (22%). Purchase intent increased the most for formulation C (14%), followed by formulations B (13%), A (12%), and D (11%). Overall, difference in purchase intent increase among the samples was not different. In the case of sample C, this means that consumers are

willing to sacrifice preference/overall liking for health benefits, due to comparable purchase intent increase among all four formulations.

4.3.5 Change in Probability of Purchase Intent

Evaluation of change in purchase intent probability was evaluated by means of the McNemar test. The probabilities of purchase intent by consumers were evaluated previous and subsequent to being informed of the possible health benefits that could be associated with product consumption (Table 22).

The null hypothesis being tested states that the purchase intent probability is the same before (π_{1+}) and after (π_{+1}) additional information concerning the product was provided, i.e., H_o: $\pi_{1+} = \pi_{+1}$. In other words, on the average there is no change in purchase intent after extra information is given about the product. From the results of the McNemar test, the probability of purchase intent of the product after the consumer was informed of the possible health benefits that could be associated with product consumption is significant (p-value < $\alpha = 0.05$) for all four formulations. Therefore, consumer's purchase decision was influenced by additional information provided after the product had been tasted.

Table 22: Changes in Purchase Intent Probability after Knowledge of the Potential Health Benefits Associated with Product Consumption [a]

Formulation [b]	χ^2	p-value	95% CI-L*	95% CI-U**
A	8.000	0.0047	0.040	0.200
B	13.000	0.0003	0.064	0.196
C	14.000	0.0002	0.072	0.208
D	9.308	0.0023	0.043	0.179

[a] All probabilities calculated by means of the McNemar Test
[b] See Table 3 for formulations
* 95% Confidence Interval – Lower Bound
** 95% Confidence Interval – Upper Bound

It can be predicted with 95% confidence that purchase intent will increase at least by that value stated by the lower confidence limit and at the most by that value stated by the upper confidence interval. For instance, for formulation A there will be a purchase intent increase of at least by 4.0% and at the most by 20.0% after the consumer is aware of the potential health benefits. The sample for whose purchase intent prediction establishes that the most increase would happen is formulation C (at the most 20.8% increase) and that for which the least increase will happen would be formulation D (at the most 17.9% increase). The formulation that has a broader predicted purchase intent range, once the consumer is aware of the potential health benefits, is sample A (16% difference). Conversely, formulation B has the narrowest purchase intent range (13.2%) of all four formulations. Overall, purchase intent will increase at the most between 17.9-20.8% comparing all formulations.

4.4 Conclusion

From this study it was concluded that consumers were able to correctly differentiate among the control sample and the flavored samples. According to Friedman's Test and the Analog to Fisher's LSD, the Wilcoxon Rank Sum Test/Kruskal-Wallis *H* Test, and R-Index values sample differences were present among the flavored spreads and the control (B). According to the Analog to Fisher's, differences were present among the Cheddar & Sour Cream Flavor (C) and both the Sour Cream & Onion (A) and the Monterrey Jack (D). There was no differentiation among the Sour Cream & Onion and Monterrey Jack flavors. According to R-Index values, consumers were able to correctly differentiate between the flavored samples and the control and also among samples A-D and C-D but not between A-C. Consumers found all flavored products acceptable (>84%) and also presented a purchase intent increase of 12.5% on the average for all four samples. Preference for sample A and C was expressed by the consumers.

There was an increase in purchase intent probability after the consumers were aware of the potential health benefits associated with product consumption.

CHAPTER 5. QUALITY CHARACTERIZATION OF NOVEL CHOLESTEROL-FREE MAYONNAISE-TYPE SPREADS CONTAINING RICE BRAN OIL

5.1 Introduction

5.1.1 Descriptive Analysis

Descriptive analysis, when compared with acceptance and discrimination methods, is the most refined of the procedures available to the sensory analyst (Stone and Sidel 1993). Descriptive analysis results give complete sensory descriptions of an assortment of products and provide a starting point for establishing which sensory attributes are important to acceptance. Descriptive information is essential in product development. Information provided by application of this methodology is essential in focusing efforts on those product variables that are recognized as different, and from which one can establish cause and effect relationships (Stone and Sidel 1993).

Descriptive analysis methods include qualitative and quantitative methods. The Flavor Profile is a qualitative technique and quantitative methods include the Texture Profile, Qualitative Descriptive Analysis (QDA), Spectrum Descriptive Analysis and Free Choice Profiling. Among the abovementioned methods, the Texture Profile, the Flavor Profile, QDA, and the Spectrum Descriptive Analysis require a consensus among

Unlike discrimination and acceptance tests, descriptive tests require the subject to provide numerous judgments for each product. Descriptive tests involve relatively few subjects, as few as 10 and as many as 20. One of the steps involved in a descriptive analysis is the development of a descriptive language for the array of products being described.

The aim of this study was to develop a list of terms, along with their definitions and references that would serve as the descriptors in any future descriptive analysis (Spectrum Descriptive Analysis) of the cholesterol-free mayonnaise-type spread containing rice bran oil.

5.1.2 Color

It is difficult for human beings to describe objectively the colors of materials using everyday language (Hutchings 1994). For this reason, standardized methods have been developed for consistent color measurement and specification. These methods are based on a trichromatic principle, which means that it is possible to describe any color in terms of three mathematical variables, i.e. hue, value and chroma (Francis and Clydesdale 1975). For an emulsion, the color is determined by the absorption and scattering of light waves from the continuous and dispersed phase. Through spectrophotometry one can measure the transmission and reflection of light from objects as a function of wavelength in the visible region.

In the late 1920's the Commission Internationale de l'Eclairage (CIE) system of color measurement was adopted (Piggott 1984). This technique of tristimulus colorimetry is now increasingly being used in the food industry as a quality control tool. The color spectrum is a combination of different parameters. The L*, a* and b* parameters express the color based on a descriptor of color, known as luminance, which is not visible to the human eye. L* refers to the lightness, a* to redness and b* to yellowness. L* may have values between 0 and 100. a* and b* are the chroma coordinates, as seen in a 2 dimension form, Figure 14. a* and b* have values between -80 and +80, but more common values encompass -60 to +60. The negative values of a* and b* refer the greenness and blueness of the sample. In addition to these parameters c* is the derived quantities' saturation and is defined as a right triangle $(a^{*2} + b^{*2})^{1/2}$. The hue angle (H^{o}) is defined as $\tan^{-1}(b^*/a^*)$.

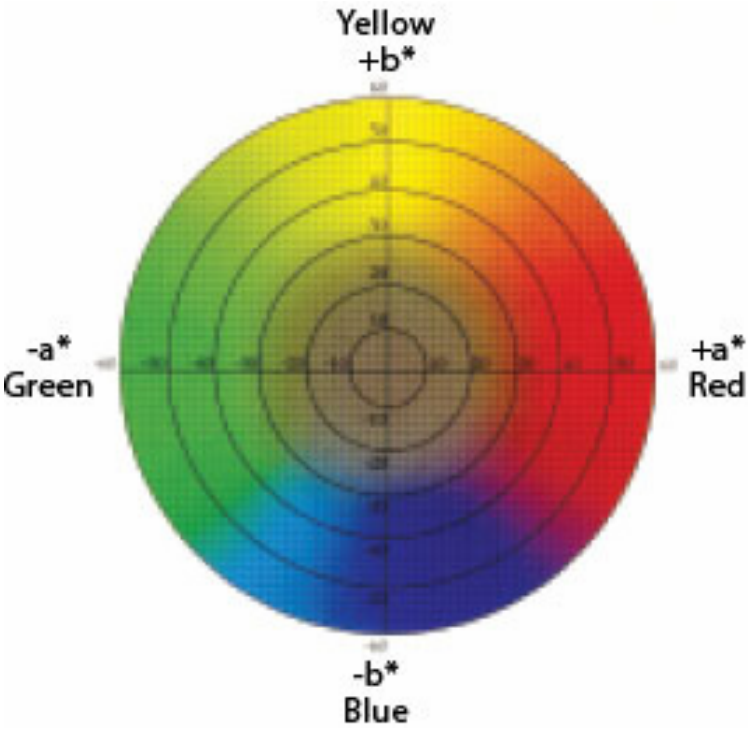

Figure 14: 2-Dimension Color Spectrum

5.1.3 pH

The measure of the activity of hydrogen ions in a solution is defined as pH. pH determines a solution's acidity or alkalinity. A neutral pH (7.0) signifies equal hydrogen ion and hydroxide ion activity. Aqueous solutions with a pH lower than 7.0 are considered acidic, whereas a pH higher than 7.0 implies an alkaline solution. Even though pH values have no unit, it is not an arbitrary scale. The number arises from a definition based on the activity of hydrogen ions in the solution. Being an experimental value, pH has an associated error with the precise formula being:

$$pH = -\log_{10}(a_{H+})$$

with a_{H+} (unitless) denoting the concentration of hydrogen ions.

5.1.4 Viscosity

Viscosity is defined as the resistance of a fluid to flow. Normally measured in response to a shear stress, viscosity relates the stress to the strain rate, i.e., the ratio of the shear force applied to the amount of resulting deformation (Rosenthal 1999; Rao 1999). In the case of emulsions such as mayonnaise, the shearing stress is not directly proportional to the rate of shear and is therefore categorized as a Non-Newtonian fluid. Non-Newtonian fluids change viscosity when they are stirred, shaken, or otherwise agitated (Rosenthal 1999). Mayonnaise presents thixotropic characteristics, meaning that it becomes less viscous when agitated. Viscosity can be measured in different unit systems. The International System of Units' (SI) unit known as the poiseuille (PI) is N s/m^2. It is commonly expressed, in American Society of Testing Materials (ASTM) standards as the poise (dyne s/cm^2).

5.2 Materials and Methods

5.2.1 Lexicon Development

For the development of a preliminary list of lexicon two (2) commercial samples of mayonnaise and two (2) experimental samples were used, for a total of five samples. For the actual product evaluation 3 experimental samples were used. The experimental samples used consisted in the formulations presented in Table 20.

Table 23: Formulations used as Experimental Samples for Lexicon Development*

Formulation	% RBO	% Water	% SPC
A	51.5	38.0	0.9
C	47.0	33.4	9.9
J	43.4	43.4	3.6

*The three varying ingredients (RBO, water, SPC) represent 90.4% of the total formulation. Complementary ingredients are: lemon juice (LJ) (8.9%), salt (0.7%) and stabilizer (0.75% of total weight).

For lexicon development a ten member panel of participants from Louisiana State

University was assembled. The panelists consisted of 7 females and 3 males. With all judges present, a total of 9 sessions (15 hours) were required for training and development of the list of terms, definitions, and references. During the orientation session an introduction and explanation concerning descriptive analysis and specifically lexicon development was given to the panelists. The subjects developed the preliminary list of descriptors by evaluating the five (5) samples aforesaid. The samples (30 g) were presented simultaneously at room temperature in plastic lidded containers coded as mentioned in Table 23. The panelists were then instructed to evaluate each sample individually concerning aroma, taste, mouthfeel, and aftertaste. After this was performed, a discussion followed in order to reach a consensus. From the individual sessions a collective list of terms with definitions was prepared. The panelists were then presented with the list of terms with the definitions to establish an agreement between the descriptors and established definitions for such terms. Once the panelists agreed on the appropriate terms and their definitions, they were presented with the proper references. In this case they evaluated the three experimental samples during several sessions and asked to indicate which descriptors were perceived in the cholesterol-free mayonnaise-type spreads containing RBO. The panelists were instructed to smell the samples in short deep sniffs. Afterwards, they were asked to taste the sample and record any aftertaste perceptions after 60 seconds. After evaluating each of the samples the panelists were requested to rinse their palates with spring water and unsalted crackers. Once all samples were evaluated by the subjects, a general consensus was reached.

5.2.2 Physicochemical Properties

5.2.2.1 Color

The color of the spread samples was measured with a spectrophotometer, Minolta model CM-508d Series (Osaka, Japan) with a 10^{o} standard observer and D_{65} illuminant. The

spectrophotometer was calibrated to white with the standard supplied by the manufacturer. The following parameters were recorded from the apparatus: L*, a*, b*, and H^o. The spread samples were placed in 2 oz cups, in which once completely filled the spread was smoothed out and the color measurements then taken using the spectrophotometer. Three different batches were prepared with duplicate measurements taken for each of the ten formulations. Measurements were taken every 7 days for a 28 day period.

5.2.2.2 pH

The pH of the mayonnaise-type spread samples was measured with an IQ Scientific Instruments pH meter. The spread samples were placed in 2 oz cups and the pH measurements taken and recorded. Three different batches were prepared with duplicate measurements taken for each of the ten formulations. Measurements were taken every 7 days for a 28 day period.

5.2.2.3 Viscosity

The viscosity of the mayonnaise-type spread was measured with a mechanical viscometer (Brookfield Model DV-II +). The spread samples were placed in 2 oz cups and the viscosity measurements taken at 10 RPM using a T-C spindle from the Helipath Spindle Set (Brookfield Engineering Labs, Inc.). Data was gathered in Wingather V2.1 Software (Brookfield Engineering labs, Inc.) Three different batches were prepared with duplicate measurements taken for each of the ten formulations, with all values recorded in centipoises (cP).Measurements were taken every 7 days for a 28 day period.

5.2.2.4 Oryzanol Content

The lipid fraction of the spread formulation was first extracted for the analysis because gamma-oryzanol is a fat soluble compound based on the procedures described by Xu and Godber

(1999). Fifteen mL of hexane (solvent) was added to a flask containing approximately 0.5g of spread and then mixed using a Sonic Dismembrato (Model 60, Fisher Scientific) at 10 watts for several seconds (until complete destruction of the spread sample). The mixture was then transferred to a glass test tube and then centrifuged at 3500 RPM in a Hermle Labnet Centrifuge (Model Z 383K) and 20^{o}C for 10 minutes. Five mL of distilled water were then added to the blend. The organic layer was then transferred to a clean glass test tube and placed in a rotary evaporator (Labconco CentriVap Console) under vacuum at 55^{o}C to obtain the crude RBO. Five mL of hexane (solvent) was then added to the extracted oil and mixed in a vortex for several seconds. The solution was then transferred to an HPLC vial.

The dissolved samples were injected into the HPLC system consisting of a Waters^{TM} 486 tunable absorbance detector, a Waters^{TM} 717 plus autosampler, Waters^{TM} 474 scanning fluorescence detector, and a Waters^{TM} 510 HPLC pump for separation and analysis of gamma-oryzanol in the lipid extraction.

The obtained chromatograms were utilized to determine the concentration of gamma-oryzanol present in the spread samples. The software calculated the area under the oryzanol peaks and its actual concentration in the samples was calculated using the following calibration curve equation: peak area = 138652 x oryzanol content µg; where the calibration curve is between the area under the peak and the oryzanol content. With the calibration curve, dilution factor of 40 and the spread sample size utilized, the following equations were used to determine the concentration of oryzanol as ppm in the samples:

$$\text{Oryzanol concentration (µg)} = \frac{\text{peak area}}{138652}$$

$$\text{Oryzanol (ppm)} = \frac{\text{Oryzanol concentration (µg) x 40}}{\text{Sample size weight (g)}}$$

5.3 Results and Discussion

5.3.1 Lexicon Development

The descriptors detected and agreed upon by the 10 judge panel during the lexicon development process are the following:

ODOR/AROMA [a]

1.- Sour
Definition: A sharp aromatic associated with products that have a sour taste or are fermented
Reference: ReaLemon® lemon juice

2.- Beany
Definition: Aromatic characteristic of soybeans and other legumes
Aromatic characteristic of soybean oil in the early stages of oxidation
Reference: Camellia® Large limas (large butter beans)

3.- Nutty
Definition: Aromatic associated with nuts or nut meats
Reference: Diamond of California hazelnuts

4.- Oily
Definition: An overall term for the aroma and flavor notes reminiscent of vegetable oil or mineral oil products
Reference: Light tasting olive oil

5.- Rancid
Definition: Aromatic associated with oxidized fats and oils
Reference: Rancid RBO

6.- Sweet Aromatic
Definition: Aromatic associated with materials that also have a sweet taste, such as molasses, caramelized sugar, cotton candy, maple syrup, maltol
Reference: Shure Fine ® sweet pickles

FLAVOR [a]

1.- Beany
Definition: Flavor characteristic of Soybeans and other legumes
Reference: Great Value™ organic original soymilk

2.- Metallic
Definition: A flat chemical feeling factor stimulated on the tongue by metal coins
Reference: Campbell's ® tomato juice (canned)

3.- Oily
Definition: An overall term for the aroma and flavor notes reminiscent of vegetable oil or mineral oil products
Reference: Light tasting olive oil

4.- Rancid
Definition: Flavor associated with oxidized fats and oils
Reference: Rancid RBO

5.- Salty
Definition: Taste on tongue stimulated by sodium salt, especially sodium chloride
Reference: Solutions of sodium chloride

6.- Sour
Definition: Basic taste on the tongue associated with acids
Reference: Campbell's ® tomato juice (canned)

7.- Sweet
Definition: Taste on the tongue stimulated by sugars and high potency sweeteners
Reference: Sucrose solutions

MOUTHFEEL (Mouth Texture)

1.- Creamy [b]
Definition: Smooth mouthfeel of stirred yogurt
Reference: Plain Yogurt

2.- Grainy [a]
Definition: A grainy character in the soybean
Example: Great Value™ - vanilla frosting

3.- Melting Rate [b]
Definition: Rate at which the product turns from solid to liquid
Reference: Breyers® - light vanilla bean ice cream

4.- Oily[b]
Definition: Overall feeling factor associated with vegetable oil or mineral oil products
Reference: All Seasons - fresh buttermilk ranch dressing

AFTERTASTE

1.- Astringent[a]
Definition: The chemical feeling factor on the tongue or other skin surfaces of the oral cavity described as puckering/dry and associated with tannins and alum
Reference: Yellow Tail ® Cabernet Sauvignon

2.- Mouth coating [c]
Definition: The mouthfeel associated with the covering of the inside of the mouth
Reference: Kraft -creamy Italian dressing

3.- Powdery
Definition: Feeling factor associated with fine particles
Reference: Great Value [TM] - light strawberry nonfat yogurt

Defenitions: [a] Civille and Lyon 1996, [b] Leveaux and Resureccion1996, [c] Santa Cruz and others 2002.

5.3.2 Physicochemical Properties

5.3.2.1 Color

The values for whiteness (L*), redness (a*), yellowness (b*), and hue angle (H^o) for all 10 formulations (A-J) are illustrated in Figures 15-18. The lightness (L* values) slightly decreased when compared over time for all formulations with values ranging between 83 and 89, except for sample C. A decrease in L* implies that there was a faint change in the whiteness of the samples during storage time. Formulations C (containing 9.9% SPC) retained its L* value over time. From Figure 14 it can be observed that no differences exist among the treatments over time.

A similar trend was observed for a* values, redness was essentially the same over the 28 day period for all samples. A slight increase was observed for sample C, over time. Again, a* values were not observed to be different among treatments and through storage time. b* values basically remained the same over time, presenting only a slight increase. However, samples B and C essentially remained the same over time, no increase was observed by the end of the 28 day-period. An increase in b* values signifies that the yellowness of the samples slightly increased over time. b* values, ranging overall between -1.6 and 0.2, were observed not to be different form each other. Hue angle (H^o) values do not show any changes with time for all ten formulations. From the obtained results for the color parameters (L*, a*, b*, and H^o), it can be

concluded that no color differences were observed among the ten formulations and no changes occurred over the 28-day period for each individual sample.

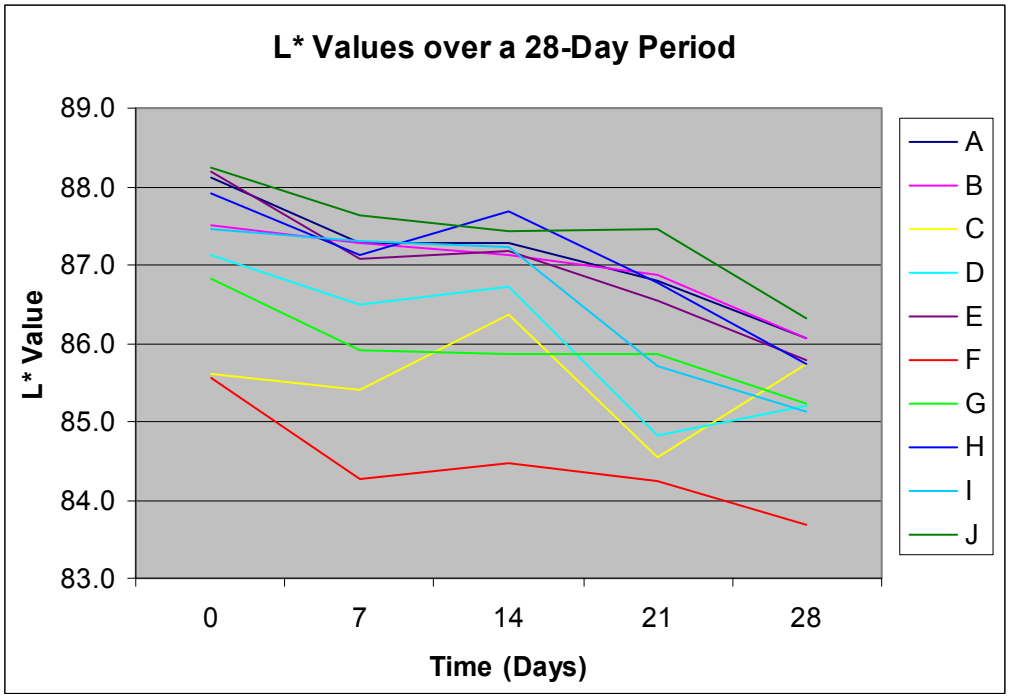

Figure 15: Lightness (L*) Values for all 10 Mayonnaise-Type Spread Formulations

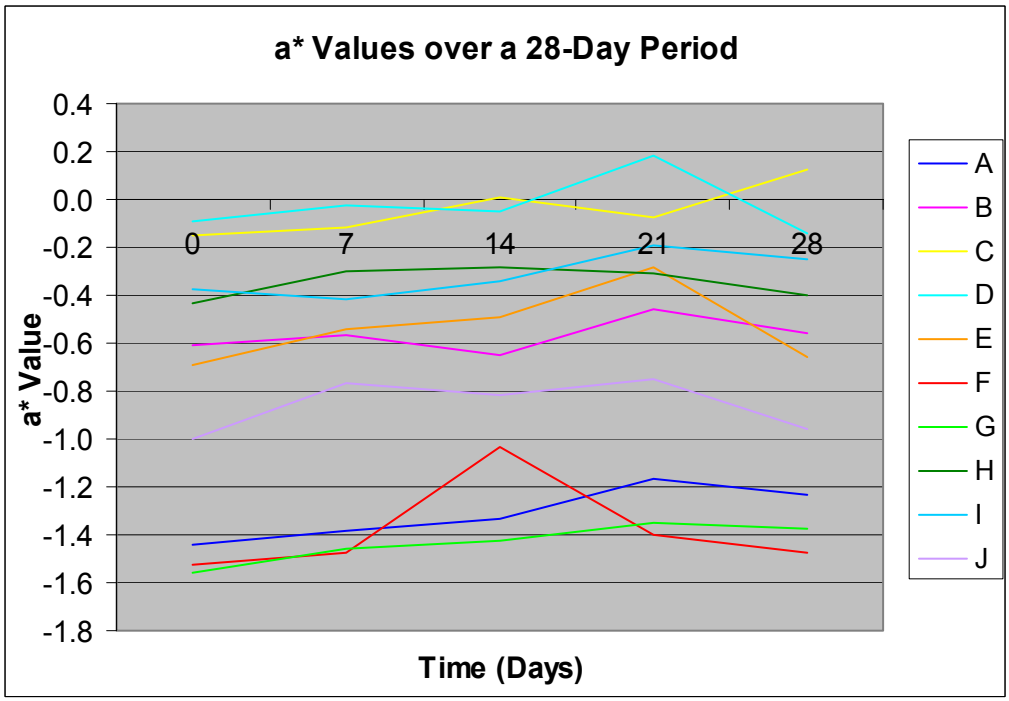

Figure 16: Redness (a*) Values for all 10 Mayonnaise-Type Spread Formulations

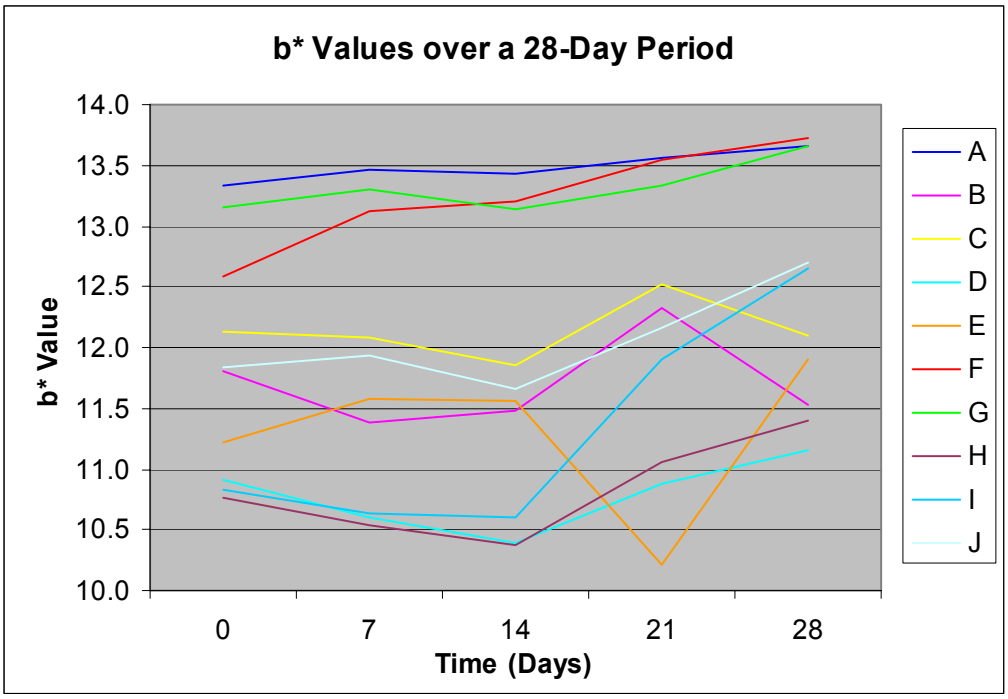

Figure 17: Yellowness (b*) Values for all 10 Mayonnaise-Type Spread Formulations

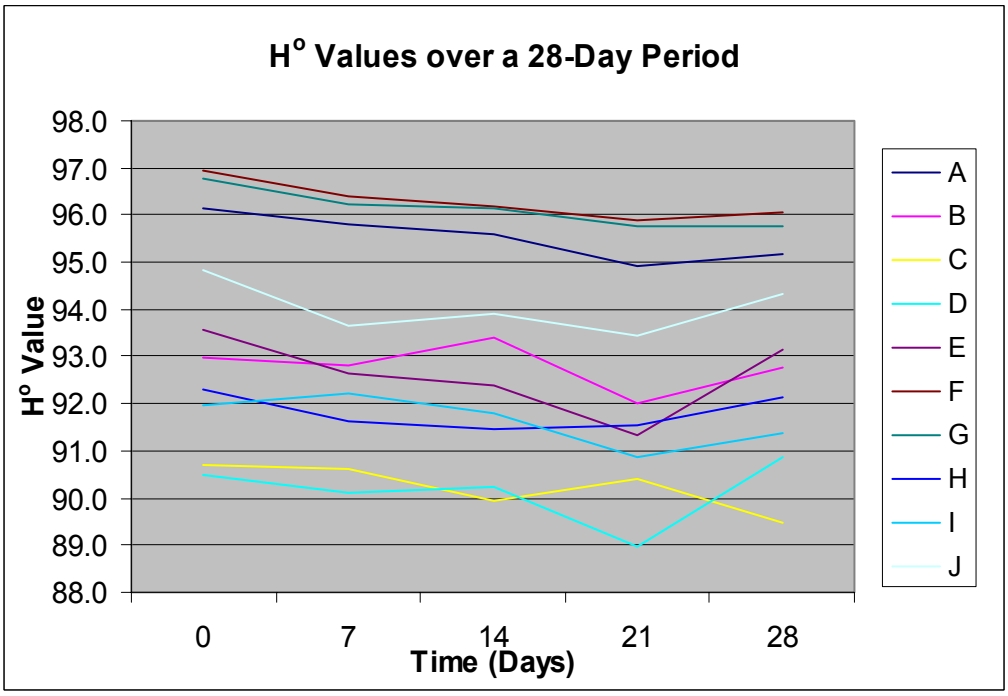

Figure 18: Hue angle (H^o) Values for all 10 Mayonnaise-Type Spread Formulations

5.3.2.2 pH

The results for pH experimental values obtained are presented in Figure 19 (see Appendix B for mean numerical values). Results are shown for all samples and within the same sample over a 28-day period. The pH range for all formulations is approximately 3.0-5.0. There was no significant change in pH for any of the ten formulations. Formulations A, F, and G presented the most acidic pH values; ranging between 3.1-3.2. These three formulations contained 0.9% SPC; this means that the lower the SPC content of the formulation, the lower the pH of the product. Formulation J (3.6% SPC) follows the three aforesaid samples, with a pH range of 3.9-4.1. The samples with the highest SPC contents also presented the highest pH values of all the ten formulations. In conclusion, from the observed pH values in Figure 19, pH increased with increasing SPC content and the pH value did not fluctuate greatly for each individual sample over time.

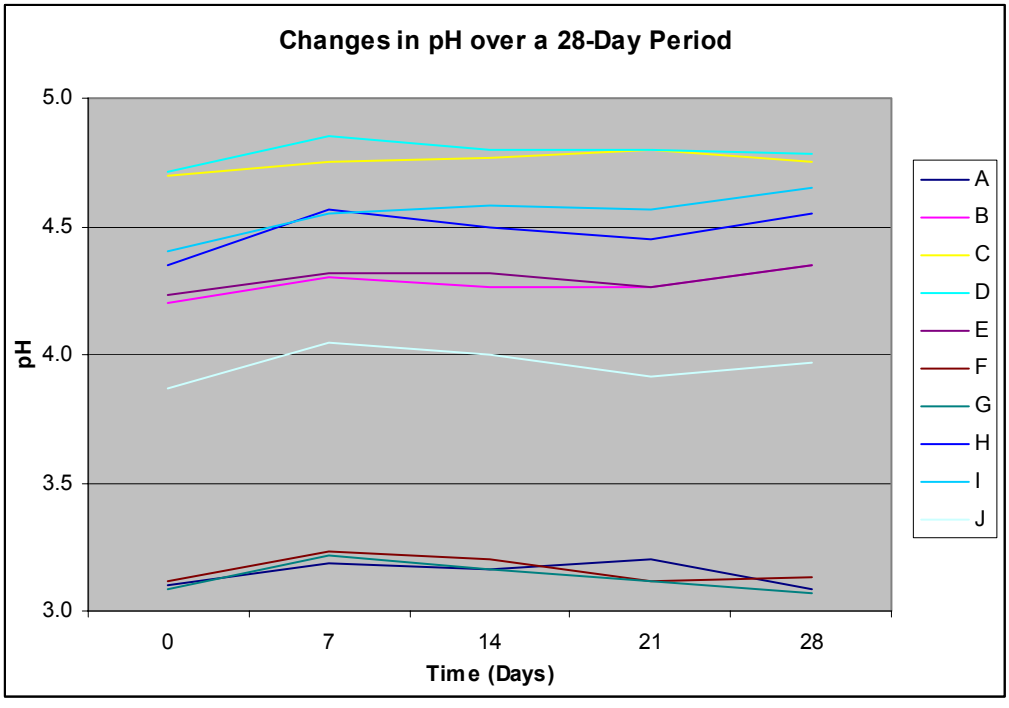

Figure 19: pH values for all 10 mayonnaise-type spread formulations over a 28-Day Period

5.3.2.3 Viscosity

Viscosity measurements for the spread formulations are presented in Figure 20 (see Appendix B for mean numerical values); except for formulations B and I which presented a consistent high viscosity above the instrumental measurement range over the 28-day period. On the day of emulsion preparation (Day 0) the highest viscosities observed where for formulations B and I. Exclusive of these two formulations (B and I), the highest viscosity was observed for formulation B (90694.7 cP) and the lowest viscosity for formulation F (13525.0 cP). When comparing formulations with equal SPC content and varying RBO it was observed that as the RBO content increased so did the viscosity of the formulation. For example, for those formulations containing 1% SPC (F, G, A) as the RBO content increased (42%, 47%, 57%, respectively) so did the viscosity (13525.0 cP, 19041.0 cP, 33161.5 cP). The same trend is observed for the lingering formulations. When comparing the viscosity values across the 28-day period, rather consistent values were observed.

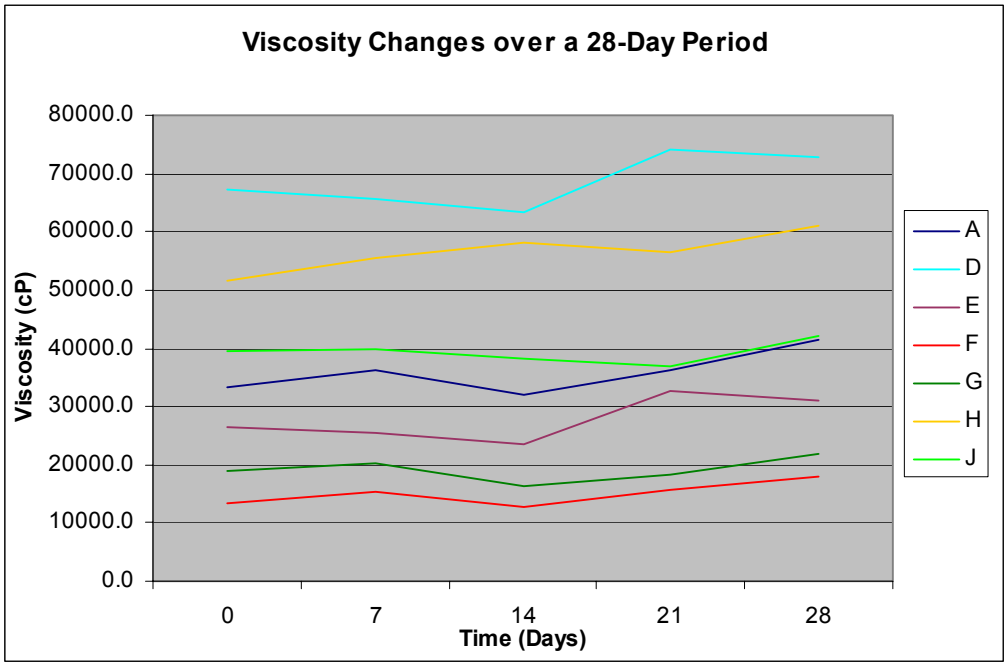

Figure 20: Viscosity values (cP) for Spread Formulations over a 28-Day Period

5.3.2.4 Oryzanol Content

The oryzanol content in the spread sample's chromatograms was presented as two adjoining peaks. The result of oryzanol concentration in all ten formulations is presented in Figure 21 (See Appendix B for the mean numerical values obtained). Oryzanol content was lower for samples D, E, G, and H. Samples D and E had an oryzanol content of 1104.46µg/g and 1083.73µg/g, respectively. These two formulations presented the lowest RBO content of all ten (33.4% RBO) and approximately the same water content, 47.0% and 51.5%, respectively. Sample H (38.0% RBO) contained 1254.24µg/g oryzanol and sample G (42.5% RBO) contained 1139.39µg/g oryzanol.

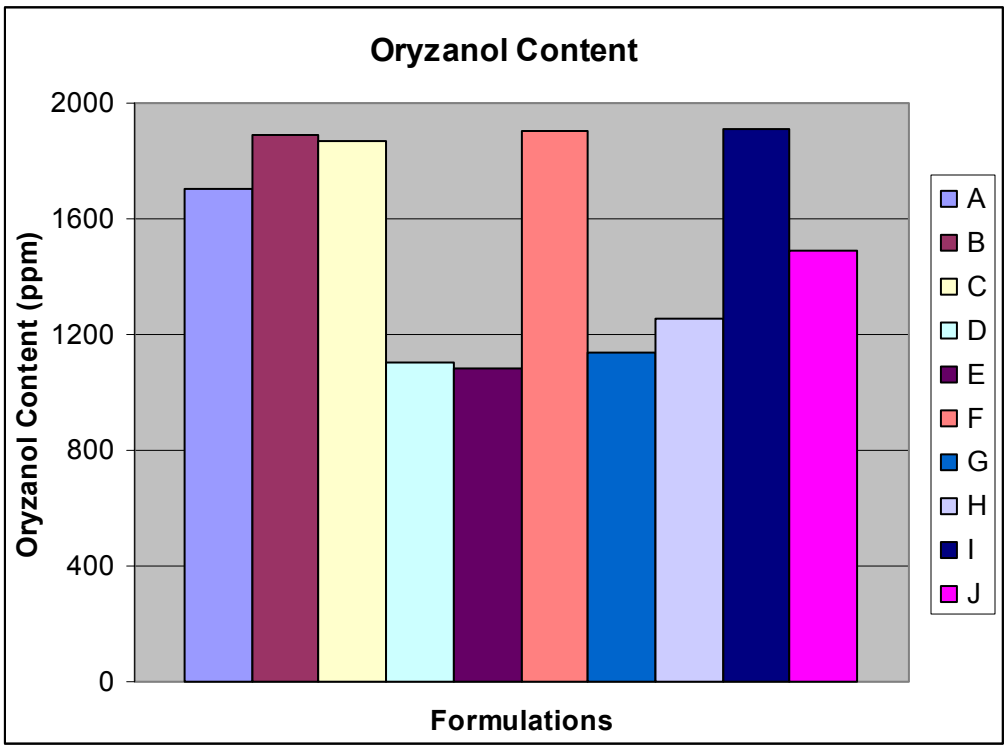

Figure 21: Oryzanol Content (ppm) for all ten Spread Formulations

Formulations A, B, and C had the highest RBO content (ranging between 47.0 and 51.5%) presented the some of the highest oryzanol contents; 1704.52µg/g, 1887.79µg/g and

1866.46µg/g, respectively. Formulation I (45.2% RBO) presented the highest oryzanol content (1911.10µg/g). Formulations J followed with 43.4 %RBO, this formulation presented 1488.15µg/g oryzanol. It can be observed as a general trend, that as the RBO content increased so did the oryzanol content of the formulations. The only inconsistency with the observed trend is formulation F, which regardless of its content of 38.0% RBO presented a much higher oryzanol concentration (1904.64µg/g) than formulation H (which had the same RBO content). It can be concluded that as RBO content increased in the formulations so did the oryzanol concentration in the product.

5.4 Conclusion

Quality characterization of the cholesterol-free mayonnaise type spreads was successful. A sensory descriptive language was developed that covers a lexicon that can potentially be used for a detailed descriptive analysis. Color, pH, viscosity, and oryzanol content specifications were effectively determined for all ten formulations. Color parameters were observed not to be different between formulations and neither changed with time. pH was found to be directly proportional to SPC content in the formulations and did not change over the 28-day period. Viscosity was found to correlate with RBO content (at constant SPC content) and did not show any changes over time. Likewise, oryzanol concentration increased with increased RBO present in the formulations.

CHAPTER 6. SUMMARY AND CONCLUSIONS

A consumer acceptance study was performed to determine consumer acceptability and to determine the optimal formulation of novel cholesterol-free mayonnaise-type spreads containing rice bran oil. For this consumer test ten different spread formulations were prepared. Each consumer (n = 365) evaluated three of the ten spread formulations (based on a Balanced Incomplete Block design) for appearance, color, odor/aroma, smoothness, spreadability, taste, mouthfeel, and overall liking of the product based on the 9-point hedonic scale. Graininess, aftertaste, acceptability, purchase intent, and purchase intent after being provided with more information about the product were evaluated using a binomial (yes/no) scale. Consumers preferred formulation E (33.4% RBO, 51.5% water, 5.4% SPC) with an overall liking score of 4.97. This formulation also received the highest acceptability score (72.12%) and the third highest purchase intent score (25.23 %). With a Wilk's Lambda p-value of <0.0001, it was concluded that a difference existed among all ten formulations when all eight sensory attributes were simultaneously compared. The attributes responsible for this difference are odor/aroma, color, mouthfeel, and spreadability. For product acceptability, mouthfeel and overall liking were the most influential attributes; whereas taste, mouthfeel, and overall liking were the most influential attributes for purchase intent. There were significant changes in purchase intent when consumers were informed that the product was cholesterol free and also when they were informed of the potential health benefits associated with the rice bran oil in the product. Product optimization (based on taste, mouthfeel and overall liking) indicated that any formulation containing 37-42% RBO, 1-6% SPC, and 50-57% water, will yield an acceptable product that could be potentially purchased by consumers.

The formulation with the highest acceptability and within the ingredient content boundaries established through product optimization (E) was chosen to be further analyzed. For this purposes a second consumer study was conducted. Based on formulation E, three different flavored formulations were prepared: Sour Cream & Onion, Cheddar & Sour Cream, and Monterrey Jack. Based on a Randomized Complete Block design, each consumer (n = 100) evaluated all of the flavored samples and a control based on preference ranking. Acceptability and purchase intent were also evaluated for all four samples based on a binomial (yes/no) scale. Also, a series of binomial type questions were compared regarding purchase intent when providing additional information to the consumer regarding rice bran oil health benefits. According to Friedman's Test and the Analog to Fisher's LSD, the Wilcoxon Rank Sum Test/Kruskal-Wallis *H* Test, and R-Index values sample differences were present among the flavored spreads and the control (B). According to the Analog to Fisher's LSD, differences were present among the Cheddar & Sour Cream Flavor (C) and both the Sour Cream & Onion (A) and the Monterrey Jack (D). There was no differentiation among the Sour Cream & Onion and Monterrey Jack flavors. According to R-Index values, consumers were able to correctly differentiate between the flavored samples and the control and also among samples A-D and C-D but not between A-C. Consumers found all flavored products acceptable and also presented a purchase intent increase. Preference for sample A and C was expressed by the consumers. There was an increase in purchase intent probability after consumers were aware of the potential health benefits associated with product consumption.

Finally, the quality of the cholesterol-free mayonnaise-type spreads was characterized through the development of sensory descriptors and determination of several physicochemical properties. A sensory descriptive language was developed that covers a lexicon that can

potentially be used for a detailed descriptive analysis. Color, pH, viscosity, and oryzanol content were the physicochemical properties evaluated. Their specifications were effectively determined for all formulations. Color parameters were not different between formulations and neither changed with time. pH was found to be directly proportional to SPC content in the formulations and did not change over time. Likewise, oryzanol concentration increased with increased RBO present in the formulations.

Cholesterol-free mayonnaise-type spreads containing rice bran oil and soy protein concentrate were successfully developed. Product refinement of the optimal formulation would guarantee acceptability and purchase intent of this novel product.

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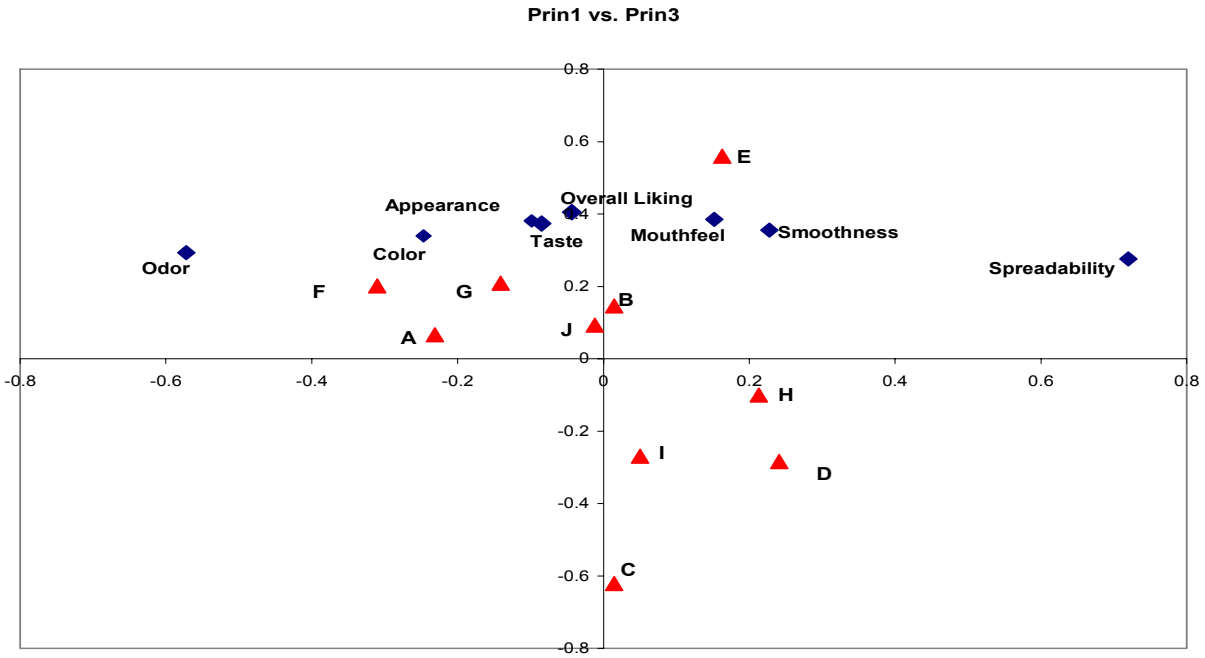
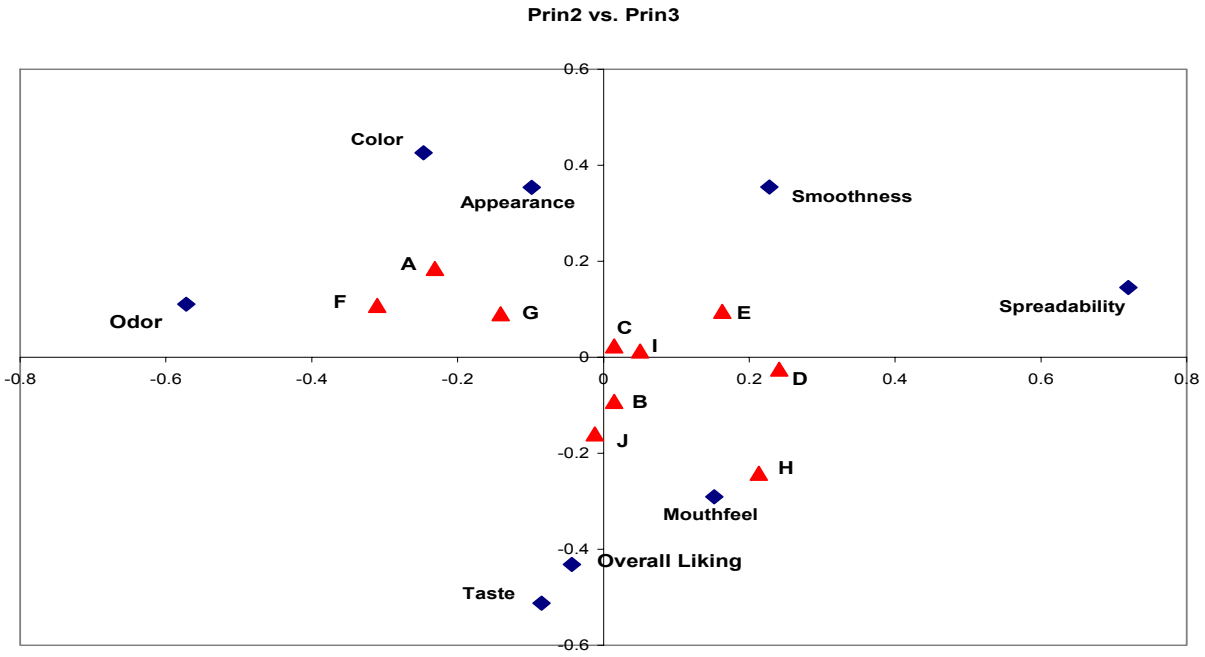
APPENDIX A. PRINCIPAL COMPONENT ANALYSIS BI-PLOTS

A.1 PCA bi-plot involving PC1 and PC3



A.2 PCA bi-plot involving PC2 and PC3



APPENDIX B. PHYSICOCHEMICAL PROPERTIES ANALYSES RESULTS

B.1 Color Parameters' Mean Numerical Values

FORMULATION A					
Day	**L***	**a***	**b***	**c***	**H^o**
0	88.10	-1.44	13.34	13.49	96.15
7	87.27	-1.38	13.47	13.54	95.79
14	87.29	-1.33	13.44	13.51	95.59
21	86.79	-1.17	13.56	13.62	94.91
28	86.06	-1.23	13.67	13.72	95.17
FORMULATION B					
Day	**L***	**a***	**b***	**c***	**H^o**
0	87.51	-0.61	11.81	11.82	92.96
7	87.28	-0.57	11.38	11.40	92.83
14	87.14	-0.65	11.49	11.52	93.38
21	86.88	-0.46	12.33	12.34	92.02
28	86.07	-0.56	11.53	11.55	92.75
FORMULATION C					
Day	**L***	**a***	**b***	**c***	**H^o**
0	85.61	-0.15	12.13	12.13	90.72
7	85.41	-0.11	12.09	12.09	90.62
14	86.36	0.01	11.85	11.86	89.95
21	84.54	-0.08	12.52	12.53	90.39
28	85.73	0.13	12.09	12.10	89.48
FORMULATION D					
Day	**L***	**a***	**b***	**c***	**H^o**
0	87.13	-0.09	10.90	10.90	90.50
7	86.51	-0.02	10.61	10.61	90.13
14	86.73	-0.05	10.39	10.40	90.23
21	84.82	0.19	10.88	10.89	88.96
28	85.20	-0.14	11.16	11.17	90.86
FORMULATION E					
Day	**L***	**a***	**b***	**c***	**H^o**
0	88.19	-0.70	11.21	11.24	93.56
7	87.08	-0.54	11.57	11.59	92.64
14	87.18	-0.49	11.56	11.57	92.40
21	86.55	-0.28	10.21	11.89	91.33
28	85.79	-0.66	11.90	11.92	93.15
FORMULATION F					
Day	**L***	**a***	**b***	**c***	**H^o**
0	85.55	-1.53	12.59	12.68	96.94
7	84.27	-1.48	13.13	13.21	96.40
14	84.48	-1.03	13.21	13.27	96.20
21	84.24	-1.40	13.55	13.62	95.89
28	83.69	-1.47	13.73	13.82	96.05

FORMULATION G					
Day	**L***	**a***	**b***	**c***	**H^o**
0	86.81	-1.56	13.15	13.24	96.79
7	85.90	-1.46	13.30	13.38	96.22
14	85.87	-1.43	13.14	13.24	96.13
21	85.86	-1.35	13.34	13.39	95.76
28	85.22	-1.38	13.66	13.73	95.78
FORMULATION H					
Day	**L***	**a***	**b***	**c***	**H^o**
0	87.92	-0.43	10.77	10.84	92.30
7	87.13	-0.30	10.54	10.55	91.61
14	87.68	-0.29	10.37	10.38	91.47
21	86.78	-0.31	11.07	11.07	91.54
28	85.74	-0.40	11.39	11.40	92.14
FORMULATION I					
Day	**L***	**a***	**b***	**c***	**H^o**
0	87.46	-0.38	10.83	10.84	91.98
7	87.31	-0.42	10.63	10.64	92.22
14	87.23	-0.34	10.60	10.61	91.80
21	85.71	-0.19	11.90	11.90	90.89
28	85.14	-0.25	12.66	12.66	91.38
FORMULATION J					
Day	**L***	**a***	**b***	**c***	**H^o**
0	88.25	-1.00	11.85	11.89	94.83
7	87.64	-0.76	11.94	11.96	93.64
14	87.43	-0.82	11.67	11.70	93.91
21	87.47	-0.75	12.16	12.18	93.46
28	86.31	-0.96	12.70	12.74	94.34

B.2 pH Mean Numerical Values

	DAY				
Formulation	**0**	**7**	**14**	**21**	**28**
A	3.1	3.2	3.2	3.2	3.1
B	4.2	4.3	4.3	4.3	4.4
C	4.7	4.8	4.8	4.8	4.8
D	4.7	4.9	4.8	4.8	4.8
E	4.2	4.3	4.3	4.3	4.4
F	3.1	3.2	3.2	3.1	3.1
G	3.1	3.2	3.2	3.1	3.1
H	4.4	4.6	4.5	4.5	4.6
I	4.4	4.6	4.6	4.6	4.7
J	3.9	4.1	4.0	3.9	4.0

B.3 Viscosity Mean Numerical Values

	Mean Viscosity Measurements in centipoises (cP)				
Formulation	**Day 0**	**Day 7**	**Day 14**	**Day 21**	**Day 28**
A	33161.5	36114.5	32155.0	36214.0	41355.5
B	90694.7	96168.7	95013.7	BIM*	BIM
C	BIM	BIM	BIM	BIM	BIM
D	67167.0	65773.5	63410.0	73985.5	72682.0
E	26287.0	25562.3	23510.0	32551.3	30915.5
F	13525.0	15305.5	12670.0	15699.5	18101.5
G	19041.0	20113.1	16375.0	18163.5	22008.0
H	51583.5	55672.9	58173.0	56533.5	61047.5
I	BIM	BIM	BIM	BIM	BIM
J	39443.5	39802.5	38076.0	36817.0	42179.0

*BIM = Values beyond instrumental measurement

B.4 Oryzanol Content Numerical Values

Formulation	%RBO	µg/g
A	51.5	1704.52
B	51.5	1887.79
C	47.0	1866.46
D	33.4	1104.46
E	33.4	1083.73
F	38.0	1904.64
G	42.5	1139.39
H	38.0	1254.24
I	45.2	1911.10
J	43.4	1488.15

APPENDIX C. CONSUMER STUDY CONSENT FORMS

C.1 Acceptance Test

Research Consent Form

I, _____, agree to participate in the research entitled "Consumer Acceptance of Rice Bran Oil Based Mayonnaise-Type Spread," which is being conducted by Witoon Prinyawiwatkul of the Department of Food Science at Louisiana State University, phone number (225)578-5188.
I understand that participation is entirely voluntary and whether or not I participate will not affect how I am treated on my job. I can withdraw my consent at any time without penalty or loss of benefits to which I am otherwise entitled and have the results of the participation returned to me, removed from the experimental records, or destroyed. 360 consumers will participate in this research. For this particular research, about 10-15 minute participation will be required for each consumer.

The following points have been explained to me:
1. In any case, it is my responsibility to report prior participation to the investigator any allergies I may have.

2. The reason for the research is to gather information on consumer sensory acceptability of a rice bran oil based mayonnaise-type spread. The benefit that I may expect from it is a satisfaction that I have contributed to solution and evaluation of problems relating to such examinations.

3. The procedures are as follows: Three coded samples will be placed in front of me, and I will evaluate them by normal standard methods and indicate my evaluation on score sheets. All procedures are standard methods as published by the American Society for Testing and Materials and the Sensory Evaluation Division of the Institute of Food Technologists.

4. Participation entails minimal risk: The only risk which can be envisioned is that of an allergic reaction to rice and soy products, lemon juice, xanthan gum, guar gum, and sodium alginate. However, because it is known to me beforehand that the food to be tested contains common food ingredients, the situation can normally be avoided.

5. The results of this study will not be released in any individual identifiable form without my prior consent unless required by law.

6. The investigator will answer any further questions about the research, either now or during the course of the project.

The study has been discussed with me, and all of my questions have been answered. I understand that additional questions regarding the study should be directed to the investigator listed above. In addition, I understand the research at Louisiana State University AgCenter that involves human participation is carried out under the oversight of the Institutional Review Board. Questions or problems regarding these activities should be addressed to Dr. David Morrison,
Associate Vice Chancellor of LSU AgCenter at 578-8236. I agree with the terms above.

_____ _____
Signature of Investigator Signature of Participant

Witness: _____ Date: _____

C.2 Ranking Test

Research Consent Form

I, _____, agree to participate in the research entitled "Consumer Acceptance of Rice Bran Oil Based Mayonnaise-Type Spread," which is being conducted by Witoon Prinyawiwatkul of the Department of Food Science at Louisiana State University, phone number (225)578-5188.

I understand that participation is entirely voluntary and whether or not I participate will not affect how I am treated on my job. I can withdraw my consent at any time without penalty or loss of benefits to which I am otherwise entitled and have the results of the participation returned to me, removed from the experimental records, or destroyed. 360 consumers will participate in this research. For this particular research, about 10-15 minute participation will be required for each consumer.

The following points have been explained to me:
1. In any case, it is my responsibility to report prior participation to the investigator any allergies I may have.

2. The reason for the research is to gather information on consumer sensory acceptability of a rice bran oil based mayonnaise-type spread. The benefit that I may expect from it is a satisfaction that I have contributed to solution and evaluation of problems relating to such examinations.

3. The procedures are as follows: Four coded samples will be placed in front of me, and I will evaluate them by normal standard methods and indicate my evaluation on score sheets. All procedures are standard methods as published by the American Society for Testing and Materials and the Sensory Evaluation Division of the Institute of Food Technologists.

4. Participation entails minimal risk: The only risk which can be envisioned is that of an allergic reaction to rice and soy products, milk and milk products, lemon juice, xanthan gum, guar gum, and sodium alginate. However, because it is known to me beforehand that the food to be tested contains common food ingredients, the situation can normally be avoided.

5. The results of this study will not be released in any individual identifiable form without my prior consent unless required by law.

6. The investigator will answer any further questions about the research, either now or during the course of the project.

The study has been discussed with me, and all of my questions have been answered. I understand that additional questions regarding the study should be directed to the investigator listed above. In addition, I understand the research at Louisiana State University AgCenter that involves human participation is carried out under the oversight of the Institutional Review Board. Questions or problems regarding these activities should be addressed to Dr. David Morrison,
Associate Vice Chancellor of LSU AgCenter at 578-8236. I agree with the terms above.

_____ _____
Signature of Investigator Signature of Participant

Witness: _____ Date: _____

APPENDIX D. CONSUMER STUDY QUESTIONNAIRES

D.1 Acceptance Test

SAMPLE SURVEY FORM **SAMPLE X**

1. Gender: Male_____ Female_____

2. How would you rate the **OVERALL APPEARANCE** of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐
1	2	3	4	5	6	7	8	9

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 **ODOR or 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 **SMOOTHENESS (visual observation)** 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

6. How would you rate the **SPREADABILITY** of this product? ***Please spread product onto the white bread.***

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 **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

8. How would you rate the **MOUTHFEEL/SMOOTHNESS** 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. Is the texture of this product "**GRAINY"**? ☐ **YES** → **IF YES:** ☐ **ACCEPTABLE** ☐ **NOT ACCEPTABLE**
☐ **NO**

10. Did you detect undesirable off-flavor or aftertaste? ☐ **YES** ☐ **NO**

11. Please rate your **OVERALL LIKING** of this product?

Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely
☐	☐	☐	☐	☐	☐	☐	☐	☐
1	2	3	4	5	6	7	8	9

12. Is this product **ACCEPTABLE**? **YES [ ] NO [ ]**

13. Would you **BUY** this product if it were commercially available? **YES [ ] NO [ ]**

14. Would you **BUY** this product knowing it is cholesterol free? **YES [ ] NO [ ]**

15. Would you **BUY** this product knowing it contained health beneficial compounds from rice bran oil, which could reduce your risk for heart disease by lowering LDL/ bad cholesterol? **YES [ ] NO [ ]**

D.2 Ranking Test

Male _____ Female _____

Please circle YES or NO for each question and sample below

	Sample A	Sample B	Sample C	Sample D
Is this product acceptable?	YES NO	YES NO	YES NO	YES NO
Would you purchase this product?	YES NO	YES NO	YES NO	YES NO
Would you purchase this product knowing it could help lower your bad cholesterol?	YES NO	YES NO	YES NO	YES NO

Please rank the 4 samples (A, B, C, D) according to your preference from 1- 4.
1 = like the LEAST
4 = like the MOST
NO TIES!

SAMPLE	A	B	C	D
Rank				

APPENDIX E. SAS CODES

E.1 Product Optimization

E.1.1 ANOVA, MANOVA, PCA, DDA, LRA

```
data one;
input Panelist Gender sample $  Rbo Spc Water Apperance Color Odor Smooth
      Spread Taste Mthfeel Grainy GrainAccep Aftrtaste Oliking Accept Buy
      Bnocholes Buyhealth;
datalines;
proc freq;
tables Buy*Bnocholes  Buy*Buyhealth;
proc sort;
by sample;
proc freq;
by sample;
tables Gender Grainy GrainAccep Aftrtaste Accept Buy Bnocholes
       Buyhealth;
tables Buy*Bnocholes  Buy*Buyhealth;
proc means mean std n maxdec=2;
by sample;
var Apperance Color Odor Smooth  Spread Taste Mthfeel  Oliking;
proc anova;
class sample;
model Apperance Color Odor Smooth  Spread Taste Mthfeel Oliking = sample;
means sample/tukey lines;
Proc candisc out=outcan mah;
class sample;
var Apperance Color Odor Smooth  Spread   Taste  Mthfeel  Oliking;
Proc logistic data = one;
model Accept = Apperance Color Odor Smooth  Spread Taste Mthfeel Oliking/
ctable;
Proc logistic data = one;
model Buy = Apperance Color Odor Smooth  Spread Taste Mthfeel
Oliking/ctable;
Proc logistic data = one;
model Bnocholes = Apperance Color Odor Smooth  Spread Taste Mthfeel
Oliking/ctable;
Proc logistic data = one;
model Buyhealth = Apperance Color Odor Smooth  Spread Taste Mthfeel
Oliking/ctable;
proc princomp out = prin;
var Apperance Color Odor Smooth  Spread Taste Mthfeel Oliking;
proc plot;
plot prin2*prin1 = sample;
plot prin2*prin3 = sample;
plot prin3*prin1 = sample;
proc sort; by sample;
proc print; by sample;
var prin1 prin2 prin3;
proc means; by sample;
var prin1 prin2 prin3;
run;
```

E.1.2 McNemar

```
Data one;
Input Sample $ Buy BuyHealth Count;
datalines;
run;
proc freq; weight Count;
tables Buy*BuyHealth/agree;
by sample;
run;
```

```
Data one;
Input Sample $ Buy BuyNoCholesterol Count;
datalines;
run;
proc freq; weight Count;
tables Buy*BuyNoCholesterol/agree;
by sample;
run;
```

E.1.3 Regression Analysis

```
data one;
input   Panelist Gender  sample $  x1 x2  x3 Apperance  Color  Odor Smooth Spread Taste
        Mthfeel  Grainy  GrainAccep  Aftrtaste  Oliking  Accept  Buy  Bnocholes  Buyhealth;
        *//x1 = rbo, x2 = spc, x3 = water//*;
x4 = x1*x2;
x5 = x1*x3;
x6 = x2*x3;
x7 = x1*x2*x3;
datalines;
proc reg;
model Apperance Color Odor Smooth Spread Taste Mthfeel
        Oliking = x1 x2 x3 x4 x5 x6 x7/noint ;
run;
```

E.1.4 RSM (sample)

```
Data;
DO V1 = 0.02 to 0.3 by 0.005;
      DO V2 = 0.15 to 0.4 by 0.005;
            X1 = (SQRT (6)*V1+1)/3;
            X2 = (1-X1-SQRT(2)*V2)/2;
            X3 = 1-X1-X2;
            color   = 0;
            IF (0.37 LE X1 LE 0.57) and (0.01 LE X2 Le 0.11) and
                  (0.37 LE X3 LE 0.57) then DO;
            color   = -7.14639*X1-404.12599*X2-7.04982*X3+750.26957*(X1*X2)
                                    +52.04929*(X1*X3)+770.94182*(x2*x3)-
1363.18374*(x1*x2*x3);
            END;
            OUTPUT;
            END;
            END;
            Run;
Proc Plot;
Plot V1*V2 = color / VPOS = 40 HPOS = 60 Contour = 10;
Run;
```

E.2 Preference Ranking

E.2.1 Frequency Procedure

```
data one;
input Consumer Gender A B C D;
datalines;
proc freq;
tables A B C D;
proc sort;
by gender;
proc freq;
by gender;
tables A B C D;
run;
```

E.2.2 Wilcoxon

```
data one;
do consumer = 1 to 100;
do sample = 'A', 'B', 'C', 'D';
input rank@@;
output;
end;
end;
datalines;
proc npar1way wilcoxon;
class sample;
var rank;
run;
```

E.2.3 Acceptability and Purchase Intent

```
data one;
input Consumer Gender Sample $ Accept Buy BuyHealth;
datalines;
proc sort; by  Sample;
proc freq; by  Sample;
tables Accept Buy BuyHealth;
proc sort; by  Sample Gender;
proc freq; by  Sample Gender;
tables Accept Buy BuyHealth;
run;
```

E.2.4 McNemar

```
data one;
input Panelist sample $ Buy BuyHealth;
datalines;
proc sort; by sample;
proc freq; by sample;
tables Buy*BuyHealth;
run;

Data one;
Input Sample $ Buy BuyHealth Count;
datalines;
run;
proc freq; weight Count;
tables Buy*BuyHealth/agree;
by sample;
run;
```

VITA

Karen Garcia was born July 10th,1981 in Tegucigalpa, Honduras. At the age of four she moved to La Ceiba, Honduras, where she graduated high school. In May 2004 she graduated from Louisiana State University in Baton Rouge, Louisiana, where she received the degree of Bachelor of Science in Chemical Engineering. She is a candidate for a Master of Science from the Department of Food Science at Louisiana State University and Agricultural and Mechanical College, which will be awarded in August 2006.