DEVELOPING GLUTEN-FREE CAKES USING RESPONSE SURFACE METHODOLOGY

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INTRODUCTION

Introduction

Celiac disease or "gluten-sensitive enteropathy," a common gastrointestinal disease, is known to be activated when the sensitive individual ingests wheat gluten (Compbell, 1992),. An abnormal immune system response to gluten causes damage to the small bowel, which results in malabsorption. The symptoms includes diarrhea, abdominal cramps, malaise, lassitude, weakness, and marked weight loss. In children, failure to thrive can occur. Approximately 140,000 cases of celiac disease have been reported in the United States (Lawrie, 1992) with an estimated prevalence of approximately 1 in 3,000. However, among people of European descent it is more prevalent. For instance, in northern Europe, the incidence is 1 in 1000; and in western Ireland, it is 1 in 300.

The treatment for celiac disease is lifelong adherence to a gluten-free diet (Campbell, 1987). However, the gluten-free diet can be difficult to follow for many reasons. Gluten is ubiquitous. Common sources of gluten include breakfast cereal, breads, pasta, snack foods, desserts, and beer. Gluten is found, often unlabeled, in most processed foods where it is a part of fillers and stabilizers (Saunderlin, 1994). Also, it is found in the binder component of many medications. Adolescents also find adherence to a gluten-free diet difficult for social reasons (Mayer, Greco, Troncone, Auricchio, &Marsh,

1991). Life without hamburgers, doughnuts, and cakes is particularly difficult for this age group. The goals for therapy for celiac patients are to maintain a gluten-free diet, to regain and maintain normal weight, to be free of the symptoms, and to avoid future problem such as intestinal cancer. To achieve these goals requires the cooperation of the physician, dietitian, and, most importantly, the patient. Also, more gluten-free products should be available for patients in order for them to have a varied diet, lead a more normal life, and maintain good health.

Unfortunately, it is a challenge to develop baked goods without gluten because wheat is the major cereal grain in the American diet, and gluten is the major protein in wheat. Gluten is the structure-forming protein (Kulp, Hepburn, and Lehmann, 1974) giving breads and other baked goods their characteristic texture and flavor. The research of Jongh (1961) illustrates that doughs containing only starch and water form a suspension in which repulsive forces exist between the starch granules, causing the suspension to demonstrate the rheological property of dilatancy. Such a system lacks the structural coherency necessary to adequately retain the air that may be incorporated during mixing or the gas generated by baking powder or by yeast fermentation. According to Kulp, *et al.* (1974), native starch granules lack the ability to bind sufficient water at the dough stage to achieve the proper degree of gelatinization during baking. Therefore, gluten substitutes are needed to mimic its function. Research has been done to find suitable gluten substitutes such as xanthan gum, GMC (glycerylmonostearate) and pregelatinized starch. Research at Oklahoma State University has focused on the use a mixture of various waxy and non-waxy starches in combination with gums and pregelatinized corn starch as cake flour. These cakes should be further tested for acceptability.

Purpose and Objectives

The purpose of this research was to develop a statistical model utilizing a response surface design that would identify a formula for acceptable gluten-free cakes. Therefore, this research has the following objectives:

1. To develop cakes and collect data on four different starches and two different stabilizers, and to use those data to generate a response surface model.
2. To test a cake formula suggested by the statistical model for acceptability by a non-celiac panel.

Assumptions

The author assumes the following:

1. That the panelists will use their sensory evaluation skills developed during training to assess the sensory attributes of the products, and the data generated will accurately reflect the perceptions of the panelists.
2. Sensory evaluation generates data that helps determine the attributes and acceptability of developed food products.

Limitations

Only four starches (potato, rice, corn, tapioca), and two stabilizers (xanthan gum and instant starch) were tested in this research.

The test sample was limited to four panelists in the preliminary test and the main test, and 12 panelists in the test of the final product.

Only one celiac patient was available in sensory evaluation for gluten-free cakes.

Hypotheses:

The following hypotheses were used to evaluate the quality of the cake.

1. The cake suggested by response surface model is not rated as acceptable cake by non-celiac panelists.

2. There is no difference in acceptability rating between gluten-free cakes and standard cakes.

CHAPTER II

REVIEW OF LITERATURE

The purpose of this research was to develop a statistical model utilizing a response surface design that would identify a formula for acceptable gluten-free cakes. Therefore, this literature review contains information on celiac disease, treatment and diet for celiac patients, functions of gluten, and development of gluten-free baked products. Since the sensory data are collected in this study, sensory evaluation as a research tool is discussed. In addition, response surface methodology as very special statistical tool to help product development is reviewed.

Celiac Disease

Celiac disease is also known as celiac sprue, nontropical sprue, gluten sensitive enteropathy,and gluten intolerance. All refer to the same problem--the inability to tolerate the gluten found in wheat, barley, rye, oats, and possibly millet and buckwheat grains. It is a condition that is estimated to affect 1 in 2,500 of the population in North America (Canadian Celiac Association, 1987). Lawrie (1992) estimated that 1 in 3,000 in the United States. It is one of the commonest chronic intestinal disorders of Caucasian people. Statistics show that, from many parts of Europe, one person out of every 1,000 to 2,000 individuals suffers from the condition. In the west of Ireland, it is as common as 1 in every 300 persons (Davidson, 1987).

Historical Background of Celiac Sprue

Celiac disease was first recognized as a chronic condition by a physician, Aretaeus of Cappadocia, in the Second Century A. D., but it was not until the 20th Century that causative factors have been known (Canadian Celiac Association, 1987). Thaysen in 1932 provided a clinical description of the disease in adults, although he was likely unaware of the pathology of the intestinal lesion. In 1950, Dicke suggested that certain dietary cereal grains were harmful to children with celiac sprue. He noted that the incidence of celiac sprue in children in Holland during World War II was markedly reduced due to the shortage of cereal grain in World War II. When cereal grains again became plentiful after the war, the incidence of celiac sprue matched the prewar levels. Later, researchers including van de Kamer, Weifers, and Dicke, showed that the water-soluble protein in the gluten of wheat was the substance which damaged the small intestine of patients with celiac sprue (CSA/USA: Celiac Sprue Association/United States of America, 1990).

In 1954, Paulley, studying surgical biopsy material, provided the first accurate description of the characteristic intestinal lesion in patients with celiac sprue. In the 1950's, Rubin and coworkers demonstrated convincingly that celiac disease in children and idiopathic or nontropical sprue in adults were an identical disease with the same clinical and pathological features (CSA/USA, 1990).

Later, a research group led by A. C. Frazer separated gliadin (subfraction of gluten) into several subfractions and found alpha-gliadin to be toxic in a celiac disease patient who had remained free of symptoms on a gluten-free diet (Paveley, 1988; Sturgess, Ellis, & Ciclitira, 1991).

Symptoms of Celiac Sprue

The most common clinical symptoms for adults usually include some of the following: weight loss; chronic diarrhea; abdominal cramping and bloating; intestinal gas; abdominal distention; and muscle wasting. Appetite is often increased to the point of craving for food. Weakness, lack of energy, and fatigue are also common. For children (6 months to 3 years) usually show growth failure and may or may not have diarrhea, projecting vomiting, and bloated abdomen. Other symptoms of nutrient deficiency may occur when the small intestine is damaged and is not able to absorb nutrients normally. These symptoms include changes in the oral mucosa and other tissues due to vitamin deficiencies, anemia due to iron deficiency, or osteoporosis due to calcium deficiency.

A less common problem is a gluten-related skin disorder, dermatitis herpetifomis, which may be present for some patients. The appearance of small, itchy blisters on the skin surface is the clinical sign of dermatitis herpetiformis. A typical case of celiac sprue does not exist. Each individual exhibits any combination and any number of these symptoms (CSA/USA, 1990).

The Causes of Celiac Disease

Since World War II, there has been much interest in and considerable research about celiac disease, but the mechanism by which gluten damages the lining of the small intestine is still not known. In recent years, several theories have been developed. First, an enzyme deficiency may cause incomplete digestion of gluten, causing an accumulation of toxic peptides that damage susceptible mucosa; second, genetic factors may cause redisposition to an immune response that damages the small intestinal mucosa; third, in addition to exposure to cereal glutens, other environmental factors, such as infection by an intestinal adenovirus, may trigger the celiac immune response (Bailey, Freedman, Price, Chescoe, & Ciclitira, 1989; Kagnoff, Paterson, Kumar, Kasarda, Carbone, Unsworth, & Austin, 1987; Trier, 1991).

Diagnose of Celiac Disease

Although there may be many clinical signs and laboratory tests indicating a malabsorption problem, the only means of diagnosing celiac disease is by small intestine biopsy (jejunal biopsy) and response to the gluten-free diet (CSA/USA, 1990).

The Treatment for Celiac Disease

Presently, the only known treatment is complete removal of gluten from the diet. When gluten is removed, the small intestine is able to repair most of the damage. Within three to six days after all gluten is removed from the diet, the cells in the intestinal lining are already reverting toward their normal status. All products containing wheat, barley, rye, oats, millet, and buckwheat are avoided. These substances are common in the normal diet, so the celiac patient will require extensive and repeated dietary instruction. It is necessary (and realistically, imperative) for the family members and the celiac to understand that the diet must be strictly adhered to if the person involved is to remain healthy and well (CSA/USA, 1990).

However, a gluten-free diet is very difficult to follow. According to a recent Canadian national survey, 88% of respondents were unable to adhere to it consistently (Compbell, 1987). Adolescents (Mayer, *et al.*, 1991) also find adherence to a gluten-free diet difficult for social reasons. Therefore, parents of the celiac children should encourage the gluten-free diet and are best advised to obtain vitamins and minerals and other medications by prescription through the celiac's physician to avoid the inappropriate fillers, dyes, emulsifiers and thickeners in medications and foods.

Diet for Celiac Patient

"Once a sprue always a sprue" (CSA/USA, 1990). For the celiac patient, it is a lifelong battle with gluten. Since the gluten-free diet is not easy to follow, patient and family education with carefully planning and implementation require the team work of the physician, dietitian, and , most importantly, the patient. Generally, a diet prepared from fresh meat, vegetables, and fruit is gluten-free and well tolerated. Fresh dairy products are also well tolerated when the small bowel begins to heal if the patient does not have coexisting lactase deficiency. Sources of gluten-free flour and other gluten-free products are available via mail order and are generally less expensive than similar products purchased in health food stores (Saunderlin, 1994).

Two national celiac disease support groups provide detailed information in coping with celiac disease. These are:

Celiac Sprue Association/United States of American, Inc.
P.O. Box 31700
Omaha, Nebraska 68131-0700
(402) 558-0600

The Gluten Intolerance Group of North America
P.O. Box 23053
Seattle, Washington 98102-0353
(206) 325-6980

Gluten

Gluten is a protein found in wheat, rye, oat, and barley (American Dietetic Association, 1985), it comprises two fractions: gliadin and glutenin in approximately equal amounts. Gliadin is the fraction soluble in 70% alcohol and is characterized as being rather sticky and fluid. Glutenin is the fraction consisting of the alcohol-insoluble proteins in gluten and is characterized as very elastic (McWilliams, 1993).

Gliadins of wheat gluten and similar proteins in the other grains are associated with development of intestinal damages. Gliadins are divided into four major electrophoretic fractions--alpha, beta, gamma, and omega--with gliadins of the alpha, beta, and gamma fractions sharing a similar amino acid composition and terminal amine sequence. The alpha-gliadin fraction is known to activate celiac disease, and data suggest that the other wheat gliadin fractions are also capable of disease initiation (Levenson, 1985; Marsh, 1992; Sturgess, 1991).

Structure of Gluten

Gliadins have molecular weights ranging from 30,000 to 75,000 daltons. The proteins in the gliadin complex are probably elliptical, single-polypeptide chains, resulting in quite compact molecules that are held in this shape by internal disulfide bonds. Glutenin, however, has disulfide bonding between polypeptide subunits, resulting in much greater molecular weight ranging from about 100,000 to as high as 15 million (Huebner, 1977). Glutenin has a fibrous nature, providing a sharp contrast to the elliptical character of gliadin.

Function of Gluten in Baked Products

The baking process relies on the leavening of an elastic, extensible, gas-retaining dough. In order to obtain this material, gliadin appears to be plasticiser, promoting viscous flow and extensibility, while glutenin provides the large elastic networks. This process happens when gliadin and glutenin and water are manipulated together, the individual proteins are hydrated by the addition of water or an aqueous liquid. Mixing the hydrated proteins disrupts the dry stage associations of protein with starch, and results in the breaking of many intermolecular secondary bonds and the formation of new bonds. This results in the development of a cohesive gluten matrix that forms the foundation of the structure of baked products. The textural function of gluten allows expansion of cells and provides rigidity of structure after baking (McWilliams, 1993).

Baked Products Without Gluten

Since gluten is a major structure-forming protein in baking, it is a big challenge to develop gluten-free baked products for celiac patients and their families with starches instead of wheat flour. Typically, the gluten-free products have poor palatability, low volume, coarse grain, and texture.

Gluten Substitutes

Research has been done to find suitable gluten substitutes for acceptable gluten-free baked products. In 1954, Rotsch baked breads from wheat starch and carob bean gum which replaced gluten as a binding agent. The research of Jongh (1961) illustrated the principles of formation of bread structure from starch in the absence of gluten. According to Jongh, doughs containing only starch and water form a stable suspension in which repulsive forces exist between the starch granules, causing the suspension to demonstrate the rheological property of dilatancy. Such a system lacks the structural coherency necessary to adequately retain the air that may be incorporated or the gas that is generated by yeast fermentation. A suitable binding agent is necessary to form a coherent system able to retain the leavening gas (Jongh, 1961). While Rotsch used gums for this purpose, Jongh achieved this goal by means of a surfactant GMS (glycerylmonostearate). Jongh's concept was utilized in the development of a composite flour, permitting the use of non-wheat raw materials for breadmaking. In addition to the factors listed by Jongh, Kulp, *et al.* (1974) believed that the binding agent (gluten substitute) should also increase the water-binding capacity of the system. Native starch granules lack the ability to bind sufficient water at the dough stage to achieve the proper degree of gelatinization during baking.

The research of Kulp, *et al.* in 1974 showed that pregelatinized starch and two gums, carboxymethyl cellulose (CMC) and xanthan were satisfactory in replacing gluten and produced acceptable bread structures. These gluten replacers varied, however, in their degree of effectiveness: approximately 14 percent pregelatinized starch (as a percent of total starch) was necessary to perform the role of gluten, while only about 5 percent CMC and 2 percent xanthan gum were required.

Other research done by Haque, *et al.*(1994) showed that bread with satisfactory volume compared to conventional wheat bread can be made with rice flour by incorporation of hydroxypropylmethylcellulose (HPMC) and ispaghula husk (isabgol) from *plantago ovato* Forsk.

Other Methods Used to Improve Gluten-Free Products

In addition to using the gluten substitutes, a number of methods have been used to improve the quality of gluten-free baked products. Treatment with water just before use enhanced the functionality of rice flour in baked products. Hydration with intense mixing and /or holding time improved eating quality, volume, and appearance of layer cakes made from 100% rice flour (Bean, Elliston-Hoops, Nishita, 1983). Also, using extra egg, cottage or ricotta cheese, extra leavening, and, the combination of gluten-free flours instead of single flour yields the better gluten-free baked products (Hagman, 1990). Even though all above methods are beneficial to the gluten-free baked products, they can not solve the major lack-of-structure problem of the gluten-free baked products. More research is needed to be done to provide more acceptable products for celiac patients.

Sensory Evaluation

Sensory evaluation has been performed throughout human history, but the science of sensory evaluation is relatively new. During World War II, the U.S. Army

Quartermaster Food and Container Institute's research projects stimulated interest in sensory evaluation while investigating food acceptance within the armed forces. More recently, scientists have developed sensory testing as a formalized, structured, and codified methodology; and they continue to develop new methods and refine existing ones (Meilgaard, 1987).

Sensory evaluation is defined by the Institute of Food Technologists (IFT) as "a scientific discipline used to evoke, measure, analyze, and interpret reactions to those characteristics of foods and materials as they are perceived by the senses of sight, smell, taste, touch, and hearing." The principal uses of sensory evaluation techniques are in quality control, product development, and research. However, the applications of sensory evaluation extend to other fields such as environmental odors, personal hygiene products, diagnosis of illnesses, testing of pure chemicals, etc. (IFT, 1981; Meilgaard, 1987).

Sensory Evaluation Tests

There are two types of sensory tests: analytical tests and affective tests. Analytical tests are used to identify and describe differences among sensory attributes and to study detectable levels of variance among samples. Affective tests are preference tests or acceptance tests based on a measure of preference or a measure from which relative preference can be determined (IFT, 1981; Meilgaard, 1987).

Discrimination-difference tests can be either analytical or affective, they determine whether a difference exists between samples and include paired comparisons, triangle, duo-trio and ranking, as well as rating difference/scalar difference from a control.

Sensitivity tests and threshold tests are also included in this category (IFT, 1981; Meilgaard, 1987).

Analytical-descriptive tests include attribute rating and descriptive analysis. Ratio scaling is used to estimate the relationship between the quantity of a substance(s) generating a physical characteristic and the sensory perception of the stimulus(i), while descriptive analysis is used to analysis the profile of favor, texture, etc. A flavor profile provides information about a product's aromas, flavors, after-taste and mouth feel. A texture profile describes the sensory components related to texture, such as mechanics, geometry, fat, and moisture (IFT, 1981; Meilgaard, 1987).

Preference and acceptance tests are affective tests and include: paired preference, ranking, hedonic rating scale, and food action rating scale. The purpose of these tests is to select the most acceptable food product based on the sensory attribute. A group's pleasure from and preference for a food product is measured by a hedonic rating scale, while a group's attitudes and anticipated actions toward a food product are scaled by a food action rating scale (IFT, 1981; Meilgaard, 1987).

Choosing and Training Panelists

The panel is the analytical "tool" in sensory evaluation. Before a panel can be used with confidence, the ability of the panelists to reproduce judgments must be determined. Interest, motivation, general attitude, and emotional state of the panelists should be considered. Panelists should be in good health and should absent themselves when suffering from conditions that might interfere with normal functions of taste and smell. To select a reliable panel for research, researchers start with as large a group as possible and rank them according to their ability to detect the differences among samples, so as to choose more reliable panelists from the group (Larmond, 1977).

To make objective decisions in analytical testing, panelists must be trained to disregard their personal preferences. The panelists must become familiar with the testing methods that will be used. All panel members must know and agree upon the exact connotation of each descriptive term used. The use of physical standards during training sessions will help the panelists become more stable in their judgments. During the training period the method of handling and testing of the samples should be discussed and a common procedure agreed upon (Larmond, 1977).

Response Surface Methodology (RSM)

Response Surface Methodology (RSM) is a collection of statistical and mathematical techniques useful for developing, improving, and optimizing processes. It also has important applications in the design, development, and formulation of new products, as well as in the improvement of existing product designs (Myers and Montgomery, 1995).

The most extensive application of RSM is in the industrial world, particularly in situations where several input variables potentially influence some performance measure or quality characteristic of the product or process. This performance measure or quality characteristic is called the response. It is typically measured on a continuous scale, although attribute responses, ranks, and sensory responses are not unusual. The input variables are called independent variables. and they are subject to the control of the engineer or scientist, at least for purpose of a test or an experiment (Myers and Montgomery, 1995).

RSM is very helpful tool in developing food products in the food industry where the response variables of interest in the food product are a function of the proportion of the ingredients used in its formulation. This is a special type of response surface problem called mixture problem. In 1971, Hare suggested that food researchers should apply mixture design to formulate food products instead of using a conventional experimental design such as a factorial design. Recently, RSM has been used in the development and optimization of cereal products by different workers (Vaisey-Genser, 1987; Shelke, 1990; Malcolmson, 1993). Furthermore, RSM has been used in attempts to develop special diet products such as rice flour yeast bread and gluten-free pocket-type flat bread for celiac patients (Ylimaki, 1991; Toufeili, 1994).

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CHAPTER III

METHODS AND PROCEDURES

The purpose of this study was to develop a statistical model utilizing a response surface design that would identify a formula for acceptable gluten-free cakes. This chapter outlines materials, instrumentation, sample preparation, experimental design, data collection, sensory evaluation, and statistical analysis.

Materials

The materials used included potato starch, rice starch, tapioca starch, corn starch, instant starch, xanthan gum, egg, shortening, salt, vanilla, sugar, baking power and milk. All of these ingredients are FDA approved and were available through retail or wholesale food suppliers. The conventional cakes were made from a Betty Crocker cake mix manufactured by General Mills, Inc.

Instrumentation

Gas ovens, electric mixers, electric balance, spatula, sifter, mixing bowls, cake tester, and line spread test equipment were used.

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Sample Preparation

Gluten-Free Cakes

Gluten-free cakes were made of 100g of gluten-free flour mixtures (different combinations of starches, instant starch and xanthan gum according to the statistical design) with other ingredients shown in Table I and Figure 1. Gluten-free cakes were prepared by an adapted conventional method using a Rival Model 455 electric mixer at lowest setting. The steps followed were: mix the shortening well, put in sugar and egg, mix well, add 1/3 flour mixture and 1/3 milk and vanilla at a time, mix the batter until well mixed (about 30 seconds). The batter was transferred to a greased pan and baked at 350°F for 25 minutes.

TABLE I

FORMULA OF GLUTEN-FREE CAKES

Ingredients	Amount
G-F Flour Mixture(includes stabilizers)	100.0g
Sugar	80.0g
Shortening	32.0g
Egg	32.0g
Salt	1.0g
Baking Powder	3.1g
Milk	80.0ml
Vanilla	1.0ml

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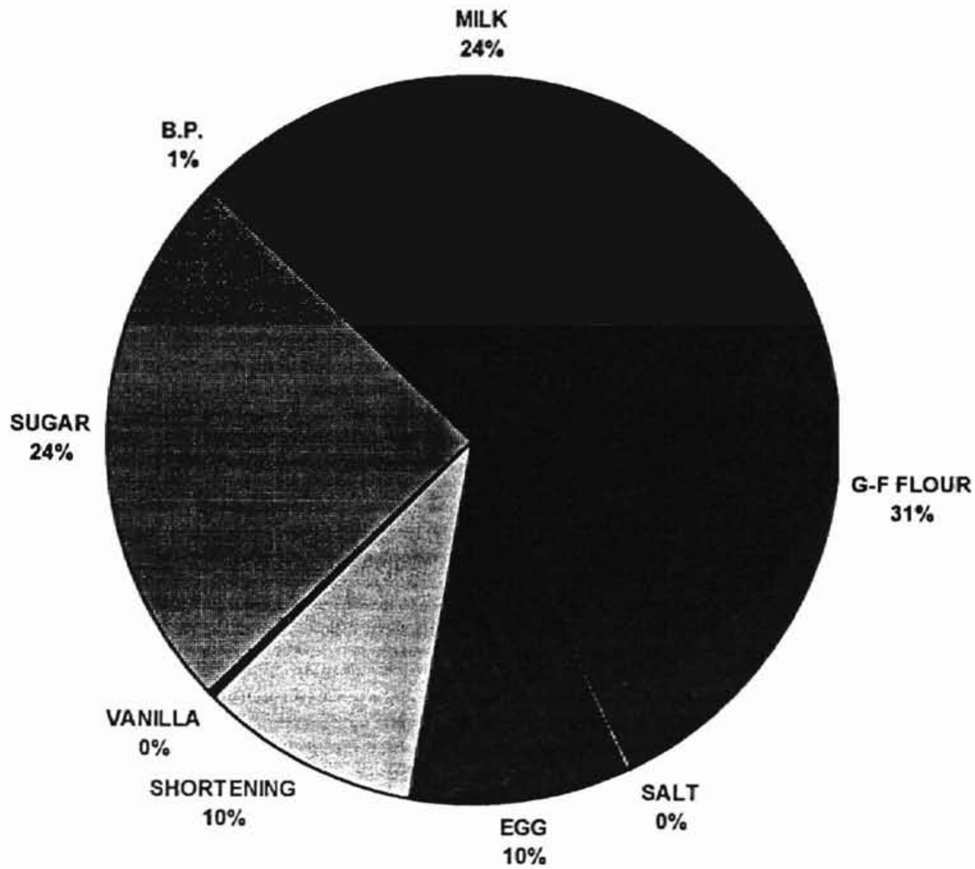

Figure 1. Composition of the Gluten-Free Cake

Standard Cake

The standard cake was a Betty Crocker cake mix manufactured by General Mills, Inc. The major ingredients in the cake mix were bleached enriched flour, egg, sugar, oil, leavening, salt, wheat starch, cellulose gum, xanthan gum and nonfat dried milk. The standard cake was prepared as instructed on box.

1. Heat oven to 350°F.

2. Beat cake mix, 1-1/4 cups water, 1/3 cups vegetable oil and 2 eggs on low speed 30 seconds.

   Beat on Medium speed 2 minutes. Pour into greased pan.

3. Bake 40 minutes at 350°F until done.

Experimental Design

Mixture design methodologies were applied to obtain a final formula yielding optimal or near optimal responses for three dependent variables (powdery, sticky/gummy, and overall acceptability). In a preliminary test, three levels of six independent variables (tapioca starch, rice starch, potato starch, corn starch, instant starch and gum) were tested (see Table II). In this table, alphabets (A-L) represent the 12 different starch ratios. For example, A represents the 2 parts of tapioca starch and 1 part of rice starch used in the formula. The numbers represent instant starch (IS) and gum combinations. For example, 1 represents 6g IS and 0.5g gum. The alphabet and the number together represents the formula. The total weight of all six variables was constant and added up to 100g in each formula. For example:

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TABLE II

MIXTURES EXPERIMENT GLUTEN-FREE CAKE "FLOUR" FORMULAS
SHOWING STARCH RATIOS AND STABILIZER AMOUNTS

Variables	Starch Ratios(g)											
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**
Tapioca	2x	0	0	x	0	x	2x	0	x	0	x	x
Potato	0	2x	x	0	x	x	0	2x	0	x	0	x
Rice	x	x	2x	0	x	0	0	0	2x	0	x	x
Corn	0	0	0	2x	x	x	x	x	0	2x	x	0
Formulas	**1A**	**2B**	**3C**	**4D**	**5E**	**6F**	**7G**	**8H**	**9I**	**10J**	**11K**	**12L**
IS	6	4	5	6	4	5	4	6	5	4	6	5
Gum	.5	.5	.5	.7	.7	.7	1	1	1	.7	.7	.7
Formulas	**13A**	**14B**	**15C**	**16D**	**17E**	**18F**	**19G**	**20H**	**21I**	**22J**	**23K**	**24L**
IS	5	6	4	4	5	6	5	4	6	6	5	4
Gum	.7	.7	.7	.5	.5	.5	.5	.5	.5	1	1	1
Formulas	**25A**	**26B**	**27C**	**28D**	**29E**	**30F**	**31G**	**32H**	**33I**	**34J**	**35K**	**36L**
IS	4	5	6	5	6	4	6	5	4	5	4	6
Gum	1	1	1	1	1	1	.7	.7	.7	.5	.5	.5

0, x, and 2x represent starch ratios and all formulas add up to 100g. x=1/3(100-gum-IS)g.

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Cake 1A:	x=31.2
tapioca =	62.4
potato =	0
rice =	31.2
corn =	0
IS =	6.0
gum =	0.5
	100.0g

All 36 recipes were prepared and the sensory results were analyzed using the multiple regression method.

The three formulas, 10J, 22J, and 34J which all used two parts of corn starch and one part of potato starch were selected because of the consistency to yield an acceptable cake. In these three cakes, gum level ranged from 0.5 to 1g, and instant starch ranged from 4 to 6g. Furthermore, all three formulas called for fewer starches and were simpler to prepare than some of the others. Therefore, the 2:1 ratio of corn:potato was chosen as the starch/flour mixture for the main test.

In the main test, the stabilizer amounts were set using a central composite design. Amounts of xanthan gum were 0.1, 0.35, 0.7g, and amounts of instant starch were 0, 2, 4g. The balance of the 100g mixture was made up of a mixture of two parts of corn starch to one part of potato starch (see Table III). A total of nine formulas with the central point replicated (total 10 runs) were prepared. The entire design was replicated twice.

For final product testing, four formulas identified by the main test model were prepared. These formulas had the following gum/instant starch gram amounts: 0.5/0.8; 0.43/0.2; 0.64/0.2; 0.52/0.5. One of these formulas (0.52/0.5) was chosen to test its overall acceptability as compared to a standard cake. This evaluation used 12 non-celiac panel and one celiac patient.

TABLE III

EXPERIMENTAL DESIGN FOR MAIN TEST

Cake Formulas	Gum (g)	IS (g)	Starch (g)
1	0.10	4	95.90
2	0.35	4	95.65
3	0.70	4	95.30
4	0.10	2	97.90
5	0.35	2	97.65
6	0.70	2	97.30
7	0.10	0	99.90
8	0.35	0	99.65
9	0.70	0	99.30

Formula 5 was replicated four times, all others twice.
*IS=instant starch.
**Starch=one part potato starch:two parts corn starch.

Data Collection:

For physical data, batter viscosity and volume index of baked products were collected. Further sensory data on gummy/sticky, powdery mouth feel, and overall acceptability of the cakes were collected.

Sensory Evaluation

Sensory evaluation played a very important role in this research. It provided data to build the response surface model for acceptable cakes. In the preliminary and main tests, the panelists were four healthy food professionals who were familiar with the characteristics of the gluten-free cakes. In the final product testing, the panelists were 12 healthy non-celiac volunteers and one celiac patient from Oklahoma State University. Before the evaluation, all the panelists attended sensory evaluation training sessions. During these sessions, the panelists received training on basic taste, odor, texture, viscosity, basic taste identification and intensity rankings

Four food professionals (research team) evaluated the 36 cakes in the preliminary test, and the 10 cakes and their replicates in the main test. The panelists received a score sheet with three bipolar-anchored scales. Figure 2 is a copy of the data score sheet. The main test suggested four formulas which were prepared. The research team chose one of those to test using 12 panelists for overall acceptability as compared to a standard cake. Only one celiac patient was available to evaluate the gluten-free cake. The score sheet is shown as Figure 3.

SENSORY EVALUATION SHEET FOR CAKE

Your evaluation data is very important for this research! Please mark the line where you think it best describes your evaluation of this food product.

Powdery

5 ____ 1

Powdery residue in mouth — no powdery residue

Sticky/Gummy

5 ____ 1

Very sticky in your mouth — not sticky

Overall Acceptability

5 ____ 1

Very acceptable — not acceptable

Figure 2. Gluten-Free Cake Score Sheet Used in the Preliminary and the Main Test.

SENSORY EVALUATION SHEET FOR CAKE

Please taste these samples and rate the acceptability of the cakes, thank you!

785 (Gluten-Free Cake)
Overall Acceptability

5 ____ 1

Very acceptable — not acceptable

572 (Standard cake)
Overall Acceptability

5 ____ 1

Very acceptable — not acceptable

Figure 3. Cake Score Sheet to Test Final Product.

Statistical Analysis

In the main test, a quadratic response surface model was fit to the data for four different response variables. Among these response variables, one was objective variables (volume index). The other three were sensory variables (powdery, sticky/gummy and overall acceptability). Optimizing responses for all variables yielded a short range of values for stabilizers. From this range, four formulas were prepared, and one formula was chosen for final acceptability testing as compared to standard cake. SAS was used to analyze all data.

OKLAHOMA STATE

CHAPTER IV

RESULTS AND DISCUSSIONS

The purpose of this study was to develop a statistical model utilizing a response surface design that would identify a formula for acceptable gluten-free cakes. This chapter includes objective and sensory data of gluten-free cakes collected from the nine formulations in the main test, response surface models, and the using of the model.

Objective Measurement of Gluten-Free Cakes

The means of the objective data--volume index and line spread on baked cake and cake batters are shown in Table IV, Figure 4, and Figure 5. The volume index was determined by measuring the height of middle and two cms from sides of the half cake and averaging the sum of the three measures. This measurement is an estimate of the surface area of a center slice of the cake and as such is an index to volume (McWilliams, 1993); and , thus, is indicative of greater volume for comparison purpose. In nine formulations, No. 9 which contained the greatest amount of gum, 0.7g, had the largest volume index, 4.1 cm. The line spread measured the batter viscosity, the smaller the number, the more viscous the batter, the larger the number, the less viscous the batter. The amount of gum and instant starch used in the cake determined the viscosity of the batter, in that the more

TABLE IV

OBJECTIVE DATA OF GLUTEN-FREE CAKES

Cake No.	Cake Formulas (g gum, g IS)	Volume Index (cm)	Line Spread (cm)
1	(0.10, 4)	3.34	5.13
2	(0.35, 4)	3.55	2.63
3	(0.70, 4)	3.38	0.88
4	(0.10, 2)	2.97	8.13
5	(0.35, 2)	3.39	5.35
6	(0.70, 2)	3.47	1.63
7	(0.10, 0)	2.94	7.44
8	(0.35, 0)	3.54	7.66
9	(0.70, 0)	4.10	4.13

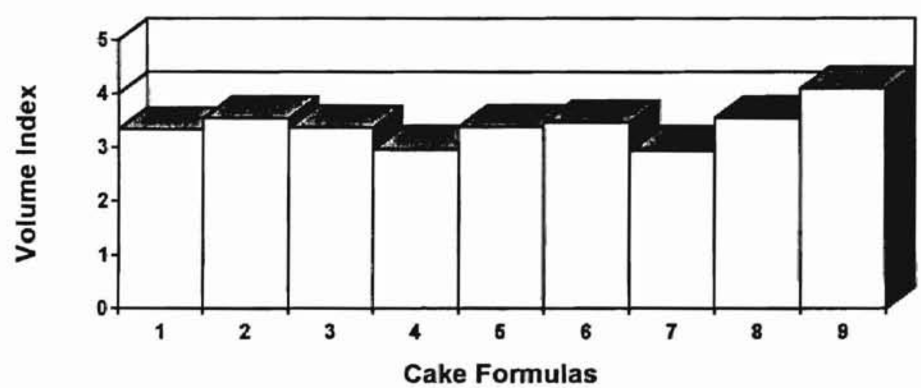

Figure 4. Volume Indexes of the Gluten-Free Cakes with Nine Formulations.

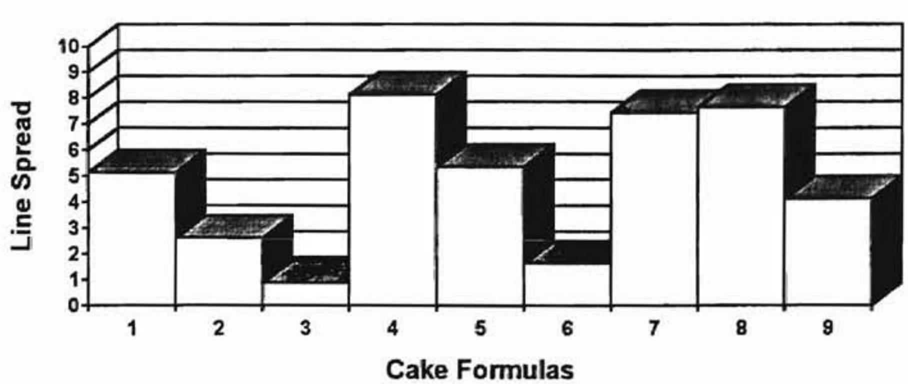

Figure 5. Line Spread of the Gluten-Free Cakes with Nine Formulations.

gum and instant starch was used, the more viscous the batter (see table IV). No. 4 formula, which contained the greatest amount of gum (0.7g) and instant starch (4g) had line spread measurement of 0.88 and produced the most viscous batter. However, a batter that was too thick was hard to handle, would not pour, and tended to incorporate large air spaces in the panned batter. In this research, line spread values that ranged from 4-8 produced easily handled batter.

Sensory Evaluation of Gluten-Free Cakes

The means of sensory evaluation data of the gluten-free cake in powdery, sticky/gummy, and, overall acceptability are shown in Table V and Figures 6, 7, and 8. If the measurement of overall acceptability of a cake was more than 3 out of 5, the cake was considered acceptable. If the measurement of powdery was less than 2.5 out of 5 and sticky/gummy of a cake was less than 2 out of 5, it was considered as acceptable mouth feel in a cake. The sensory attributes of cakes were determined by the amount of gum and instant starch used in the recipes. The No. 9 formula which contained 0.7g gum and no instant starch yielded the most acceptable cake out of the nine formulas prepared in the main test, with overall acceptability 4.41 out of 5. However, the No. 3 formula which contained 0.7g gum but also had 4g instant starch yielded the least powdery cake (powdery 1.53). The No. 8 formula which contained 0.35g gum and no instant starch produced the least sticky/gummy cake (sticky/gummy=1.55). These results showed that using only gum could produce acceptable cakes, but incorporating instant starch seemed to reduce the powdery mouth feel.

TABLE V

SENSORY EVALUATION OF GLUTEN-FREE CAKES

Cake No.	Cake Formulas (g gum, g IS)	Attributes (means) Powdery	Sticky/Gummy	Overall acceptability
1	(0.10, 4)	2.59	2.30	3.54
2	(0.35, 4)	2.22	2.76	3.79
3	(0.70, 4)	1.53	3.88	3.00
4	(0.10, 2)	2.95	2.93	3.00
5	(0.35, 2)	2.11	2.44	3.74
6	(0.70, 2)	2.00	3.13	3.38
7	(0.10, 0)	3.50	2.67	2.80
8	(0.35, 0)	2.18	1.55	3.76
9	(0.70, 0)	2.11	1.89	4.41

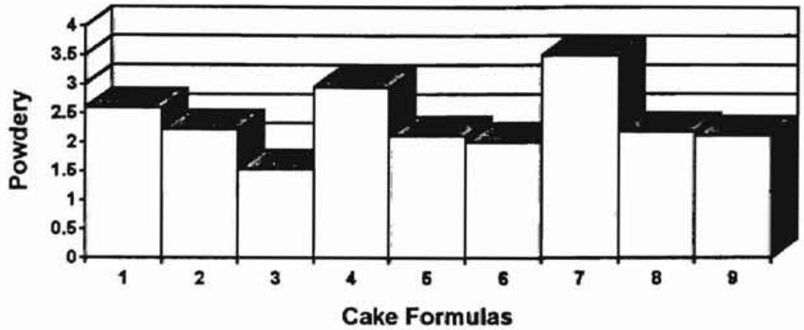

Figure 6. Powdery Mouth Feel of Gluten-Free Cakes.

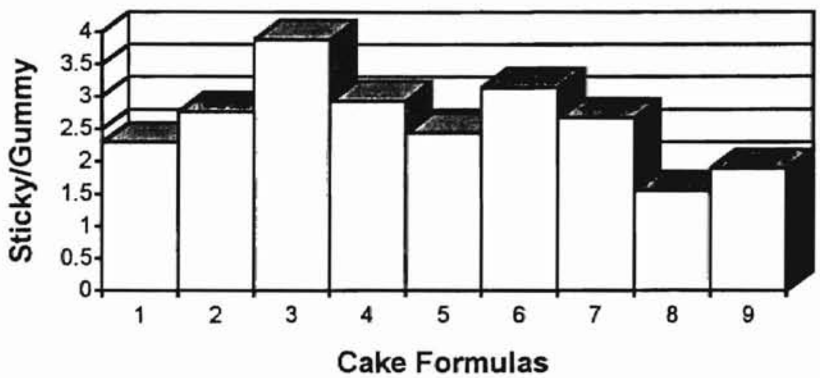

Figure 7. Sticky/Gummy Mouth Feel of Gluten-Free Cakes

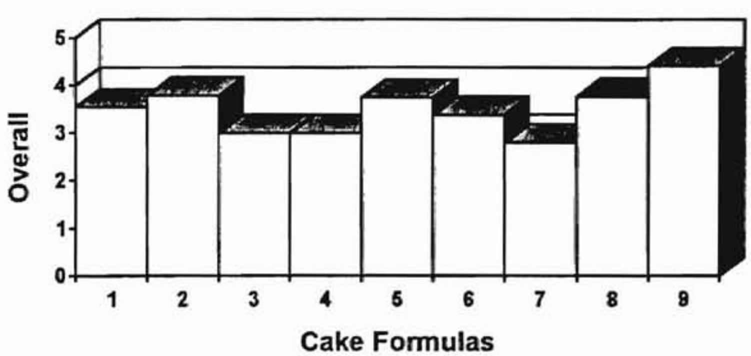

Figure 8. Overall Acceptability of Gluten-Free Cakes.

Response Surface Models

Response surface models of volume index, powdery mouth feel, sticky mouth feel, and overall acceptability of the gluten-free cake were built. For volume index of the gluten-free cake, a quadratic model best fit the data ($R^2 = 0.6623$, MSE = 0.07615). The equation is:

$$Volume\ Index = -1.44 - 5.395gum^2 - 0.906\ \mathrm{IS}*\mathrm{gum} + 0.033IS^2 + 0.2IS + 7gum$$

Differentiation yielded no maximum or minimum critical point in the interval region of IS ($0 \leq IS \leq 4$) and gum ($0 \leq gum \leq 0.7$) used in the experiment. A maximum volume index occurs on the "edge" of the region where IS=0g and gum=0.7g. The three dimensional response surface model that demonstrates this is shown in Figure 9.

The contour plot is shown in Figure 10. Each line or "contour" identifies the IS and gum combinations that yield volume indexes of 3, 3.5, 4. For example, a volume index of 4, which is a desirable volume of gluten-free cake, can be achieved by IS and gum combinations indicated by the area in lower right corner of the contour plot (shown as shadow area). Combinations such as IS=0g, gum=0.4g; IS=0.5g, gum=0.5g; IS=0.8g, gum=0.6g would yield a volume index of at least 4.

For powdery mouth feel of the gluten-free cake, a quadratic model best fit the data ($R^2 = 0.3728$, MSE = 0.5269). The equation is:

$$Powdery = 3.81 + 4.466gum^2 - 5.6gum + 0.0575gum*IS - 0.15IS + 0.0015IS^2$$

which is graphed in Figure 11. Differentiation yielded a minimum critical point. It was IS=43.33g, gum=0.35g. This critical point is far out of the 0-4g range for IS. The important feature of this equation is the interaction of IS and gum on the powdery mouth

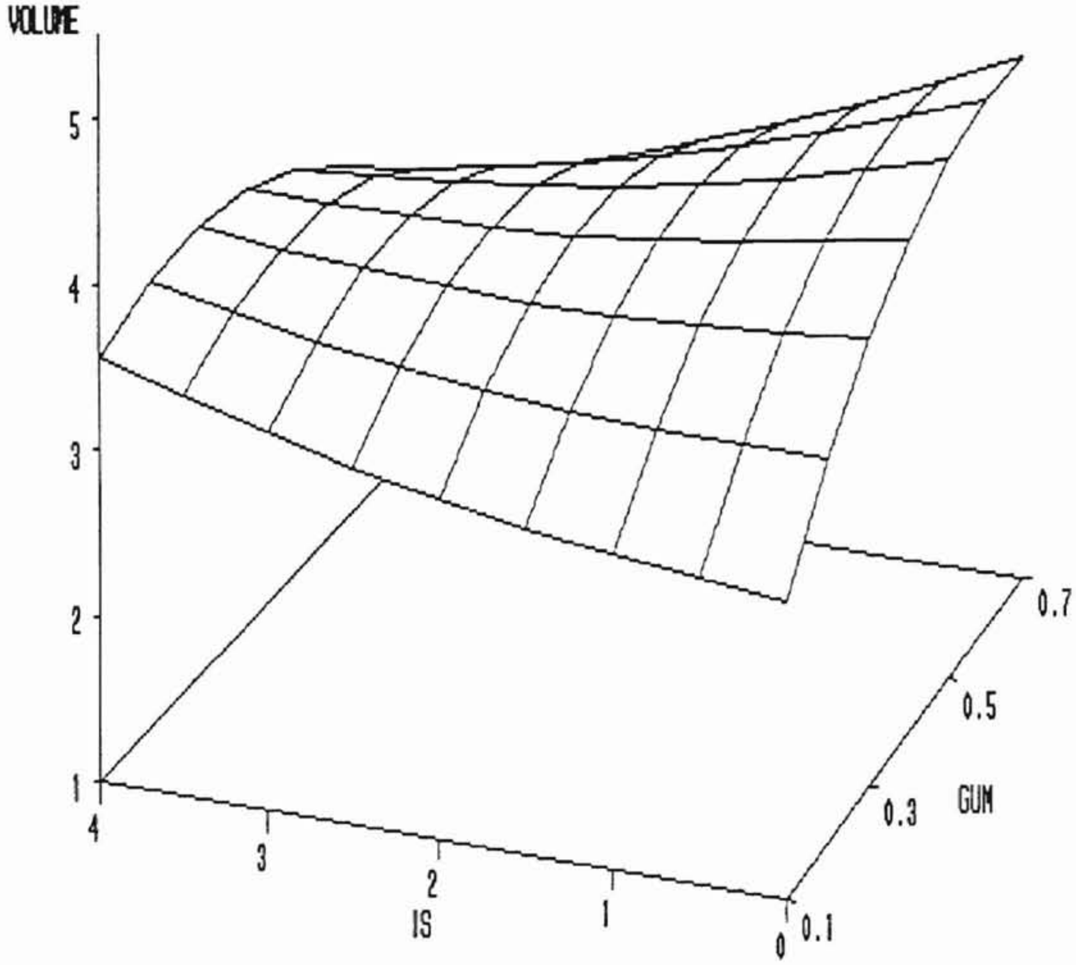

Figure 9. Response Surface Model of Volume.

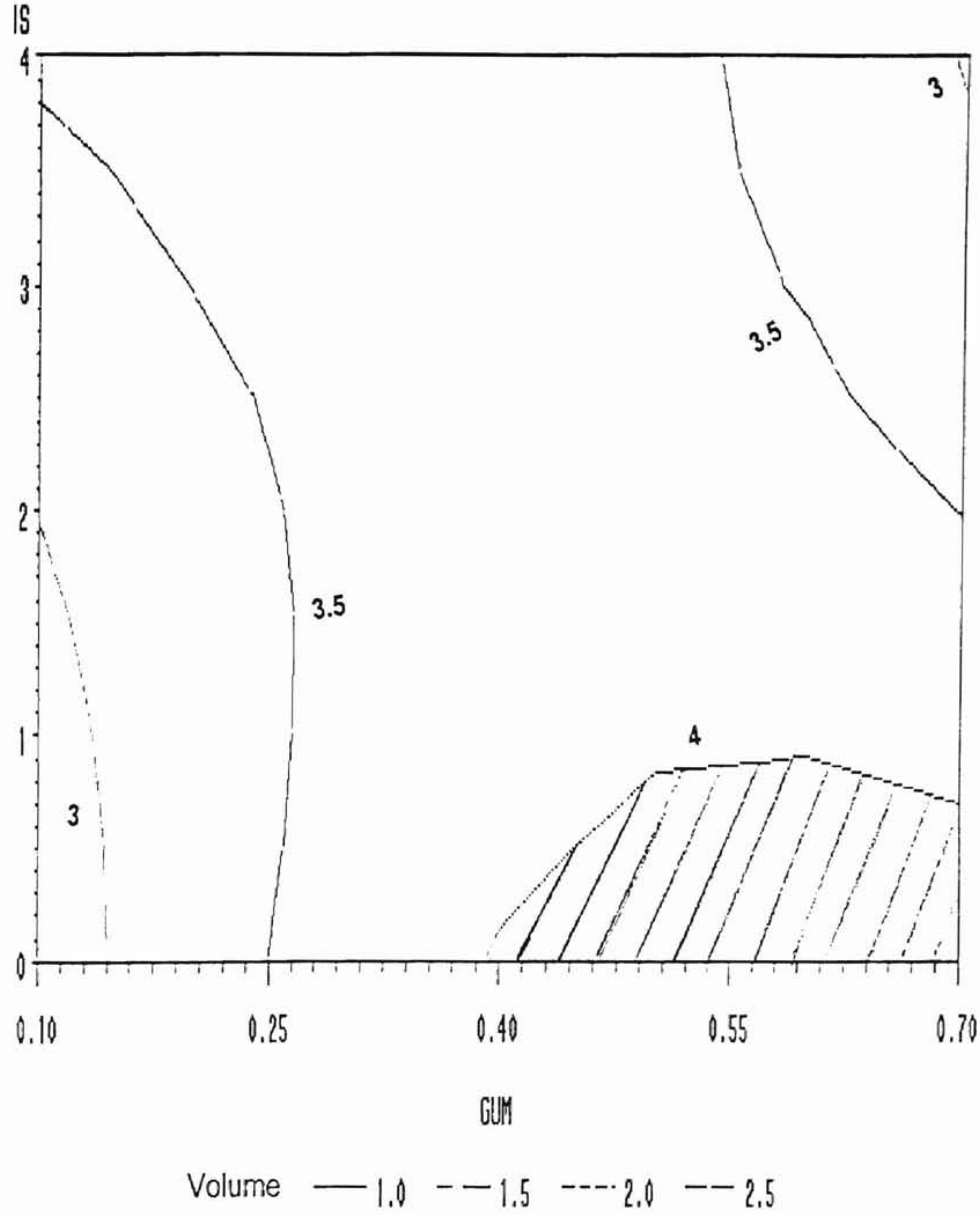

Figure 10. Contour Plot of Volume.

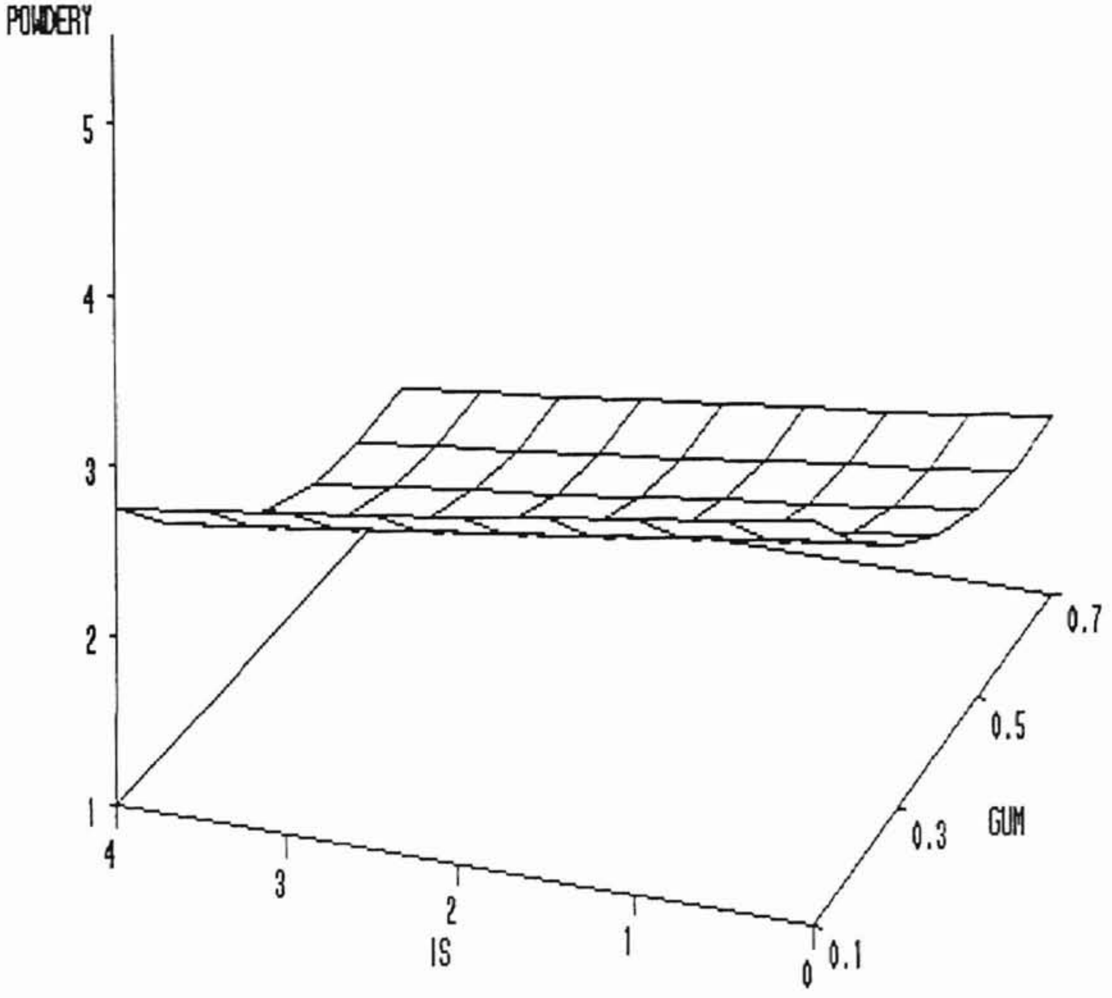

Figure 11. Response Surface Model of Powdery Mouth Feel.

feel.

Acceptable scores for powdery mouth feel are less than 2.5. The contour plot in Figure 12 identifies values of IS and gum that yield acceptable powdery mouth fee. These combinations occur in the right corner (shown as shadow area). For example, combinations such as IS=1g, gum=0.4g; IS=0.5g, gum=0.5g; IS=0.2g, gum=0.7g would yield acceptable powdery mouth feel (powdery<2.5).

For sticky mouth feel of the gluten-free cake, a quadratic model best fit the data ($R^2 = 0.5045$, MSE = 0.40593). The equation is:

$$Sticky = 2.95 + 5.402gum^2 + 0.712\text{gum}*\text{IS} - 5.2gum$$

Differentiation yielded a minimum critical point. When IS=0.73g, gum=0g, the sticky mouth feel of the gluten-free cake was at a minimum. Also, this equation shows that instant starch and gum have an interactive effect on the sticky mouth feel of the gluten-free cake. As both are increased, the sticky mouth feel of the gluten-free cake increased. As more gum was used (from 0 to 0.7g), the sticky mouth feel of the gluten-free cake increased. This three dimensional response surface model is shown in Figure 13.

Acceptable scores for sticky mouth feel are less than 2. The contour plot in Figure 14 identifies values of IS and gum that yield acceptable sticky mouth feel. These combinations occur in lower right corner (shown as shadow area) of the contour plot. For example, combinations such as IS=0.3g, gum=0.4g; IS=0.5g, gum=0.5g; IS=0g, gum=0.7g would yield acceptable sticky mouth feel (sticky<2).

For overall acceptability of the gluten-free cake, a quadratic model best fit the data ($R^2 = 0.5459$, MSE = 0.24419). The equation is:

$$Overall = 3.89 + 6.881gum - 5.382gum^2 + 0.101IS - 0.904\text{IS}*\text{gum} + 0.039IS^2$$

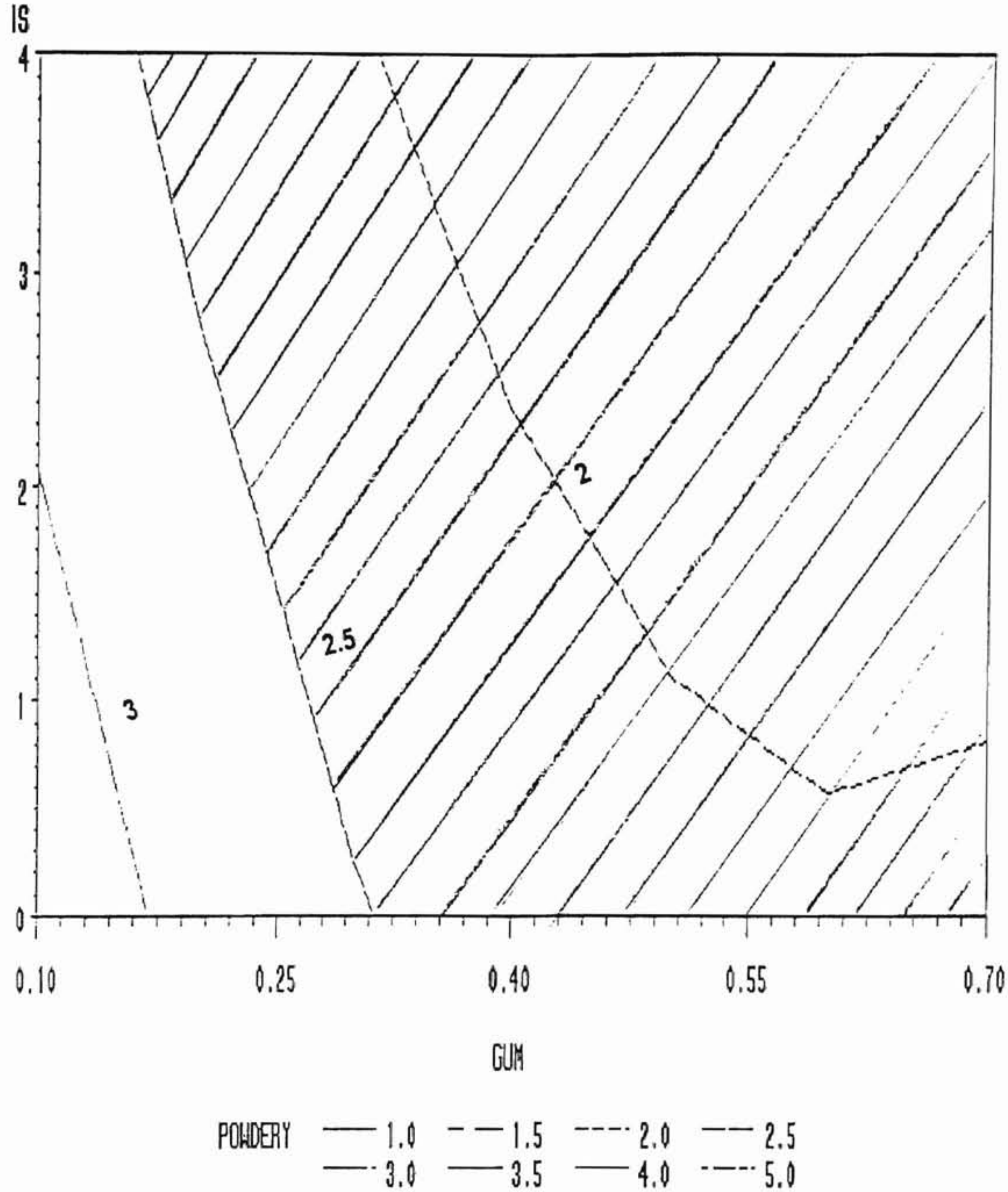

Figure 12. Contour Plot of Powdery Mouth Feel.

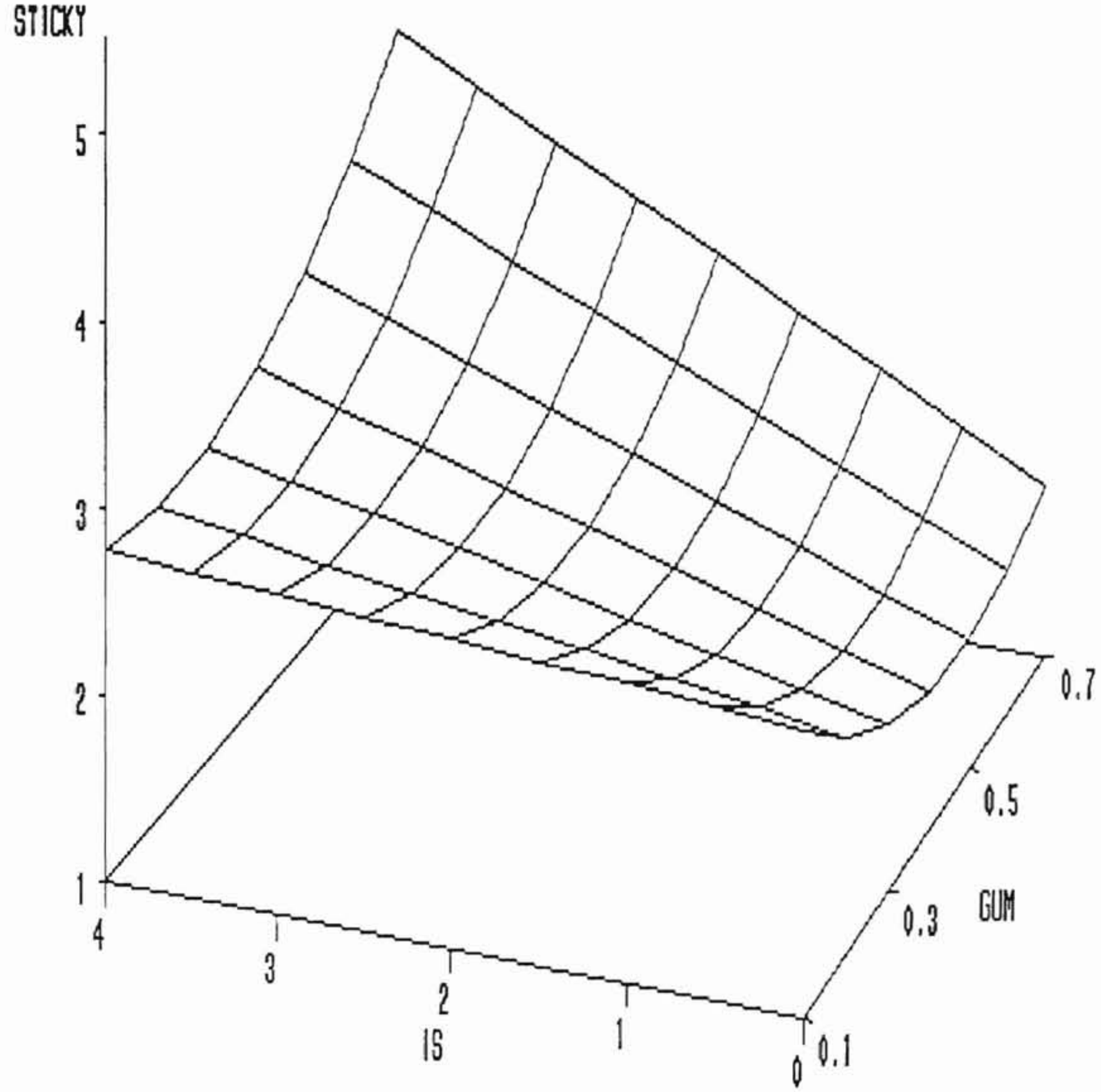

Figure13. Response Surface Model of Sticky Mouth Feel.

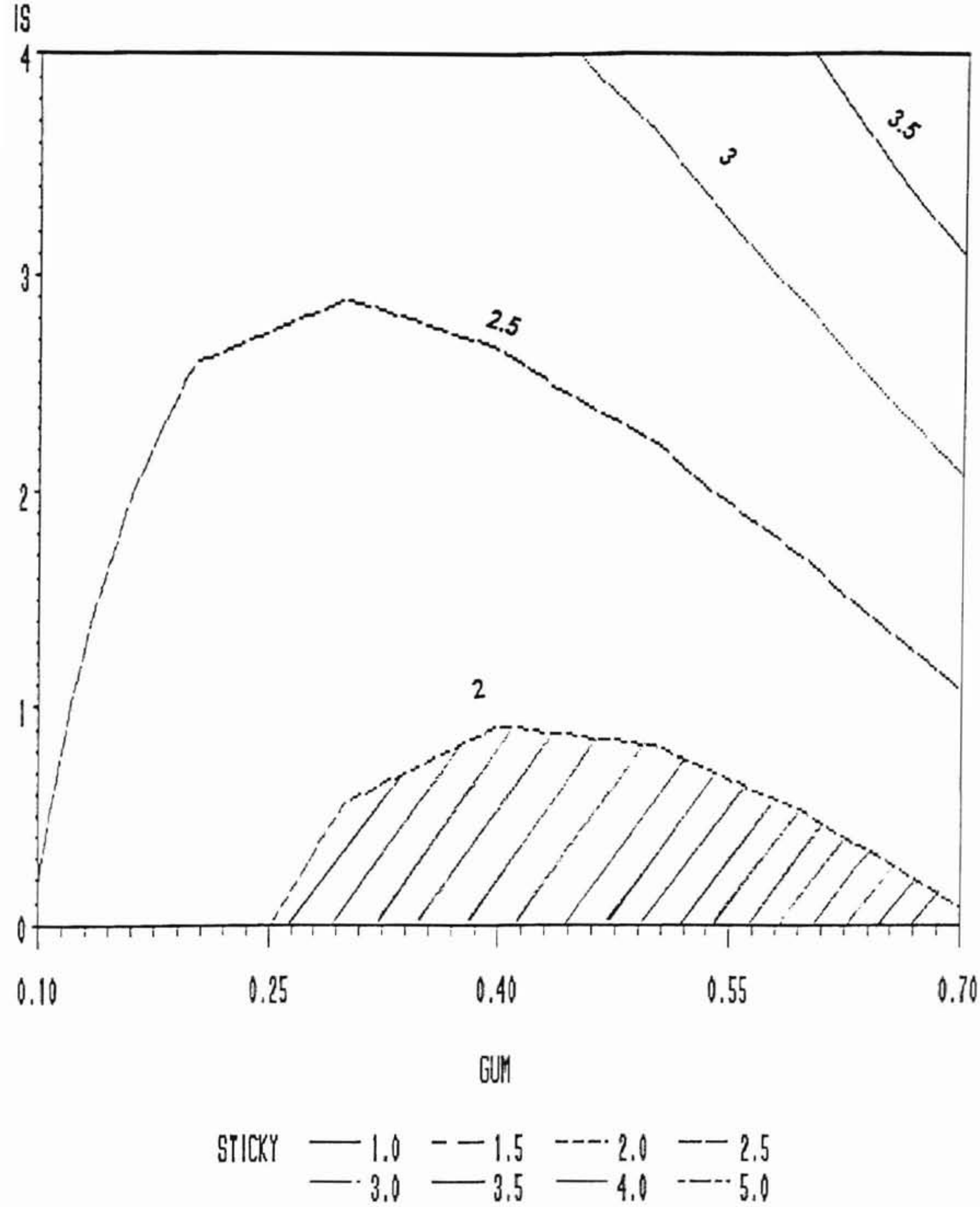

Figure14. Contour Plot of Sticky Mouth Feel.

Differentiation yielded no maximum or minimum critical point in the interval region of IS ($0 \leq IS \leq 4$) and gum ($0 \leq gum \leq 0.7$) used in the experiment. A maximum overall acceptability occurs on the "edge" of the region where IS=0g and gum=0.7g. The three dimensional response surface model that demonstrates this is shown in Figure 15.

The contour plot is shown in Figure 16. Overall scores of 3 or more are acceptable and can be achieved by IS and gum combinations indicated by almost the whole area of the contour plot (shown as shadow area). Combinations such as IS=0g, gum=0.7g; IS=2g, gum=0.5g; IS=4g, gum=0.6g would yield acceptable overall acceptability (overall>3).

Whether a gluten-free cake was acceptable or not was determined mostly by its sensory attributes. Therefore, only the responses of sensory attributes: powdery, sticky/gummy and overall acceptability were used to obtain formulations for acceptable cakes. For each attribute, areas on the contour plots that represented acceptable gluten-free formulations were identified in Figures 12, 14, and 16. For powdery, responses less than 2.5 out of 5 were acceptable; for sticky/gummy, responses less than 2 out of 5 were acceptable; for overall acceptability, responses more than 3 out of 5 were acceptable. Finally, for the collection of the three attributes, the area of acceptable gluten-free cake formulations was produced by overlapping the acceptable areas of the three individual contour plots. The area identifying formulations of acceptable gluten-free cake is shown in Figure 17 in the lower right corner. Within this formulation area, four recipes were selected for further evaluation. These formulas had the following gum/instant starch gram amounts: 0.5/0.8; 0.43/0.2; 0.64/0.2; 0.52/0.5. IS=0 was not chosen in order to reduce the powdery mouth feel.

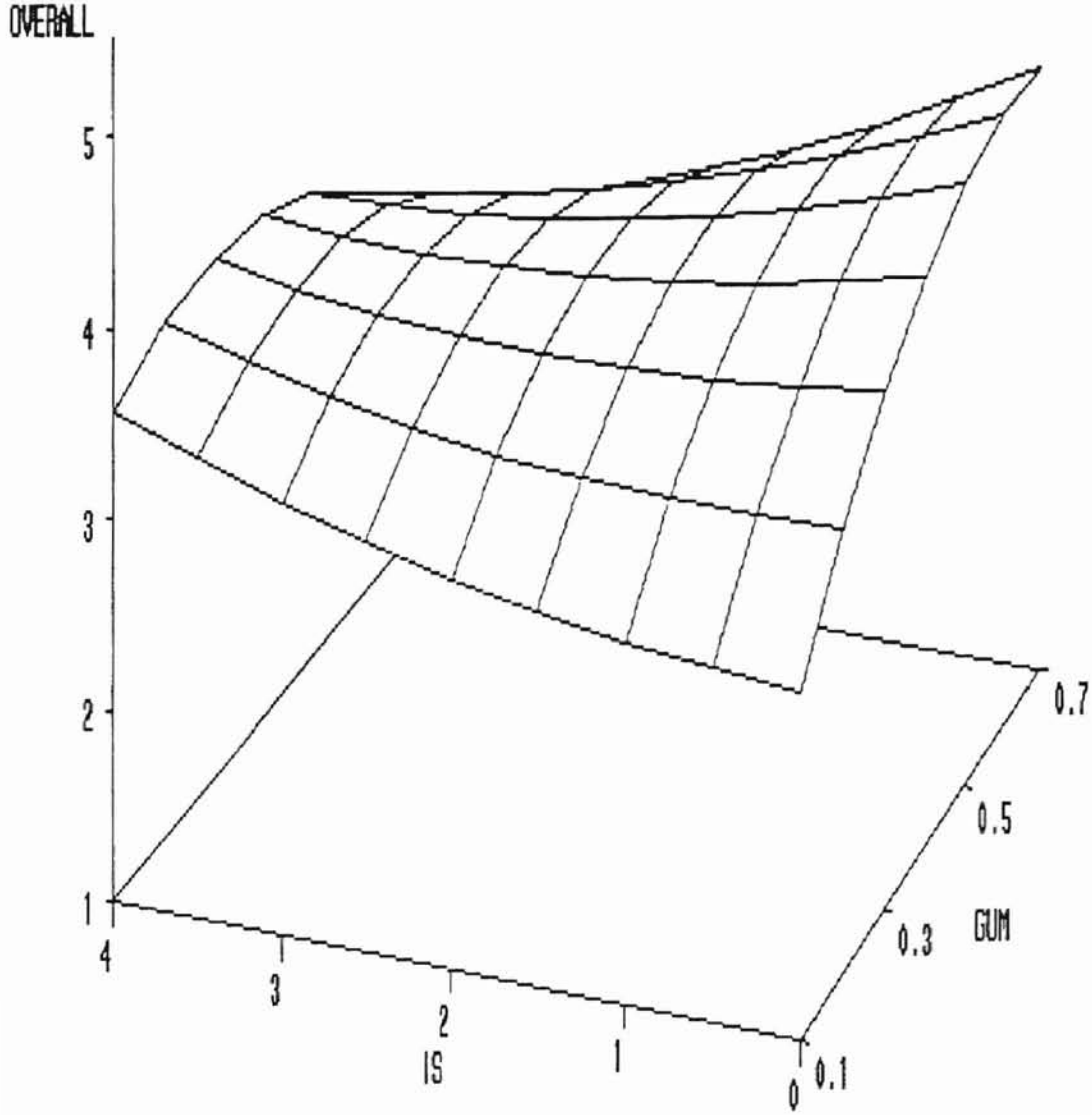

Figure 15. Response Surface Model of Overall Acceptability.

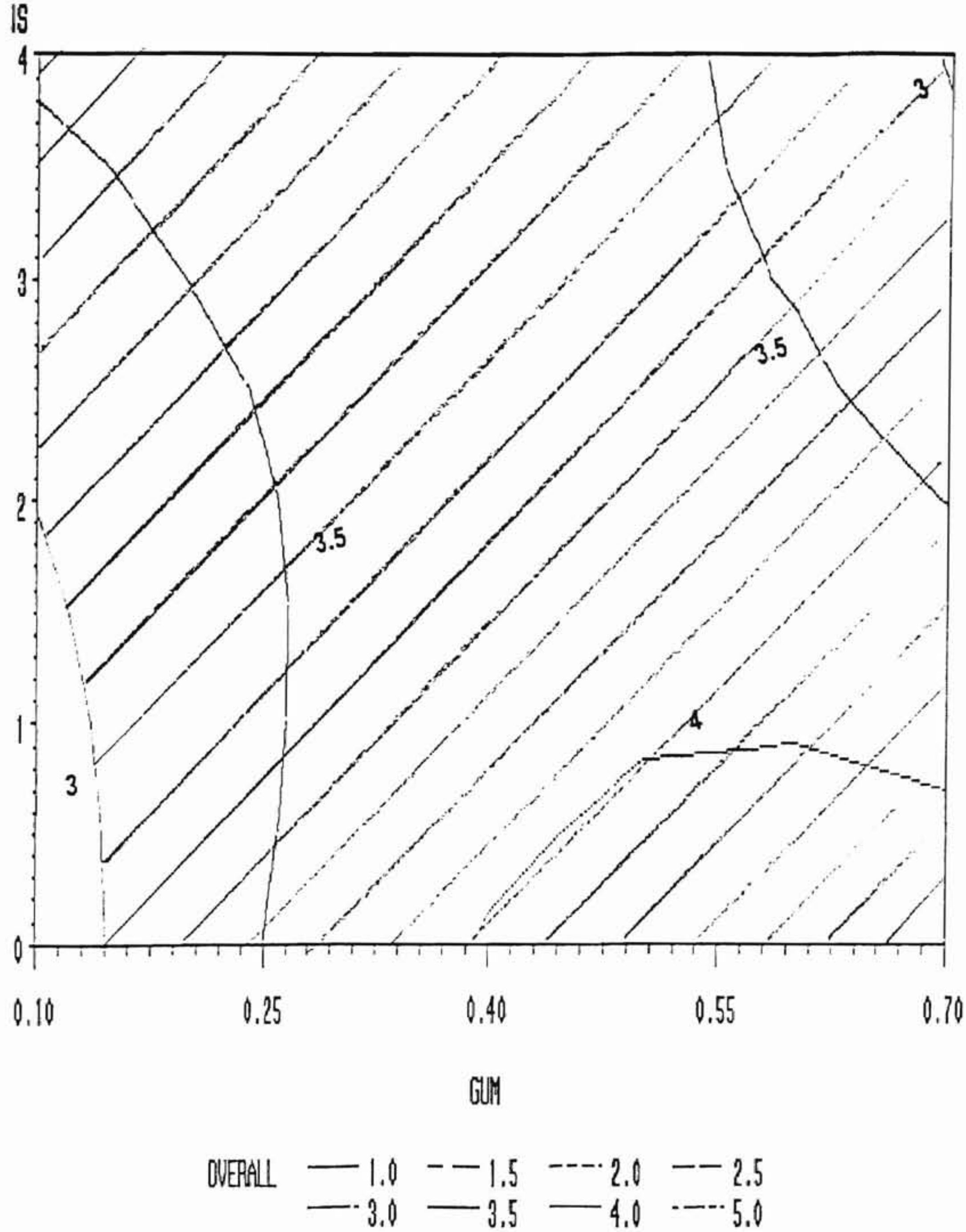

Figure 16. Contour Plot of Overall Acceptability.

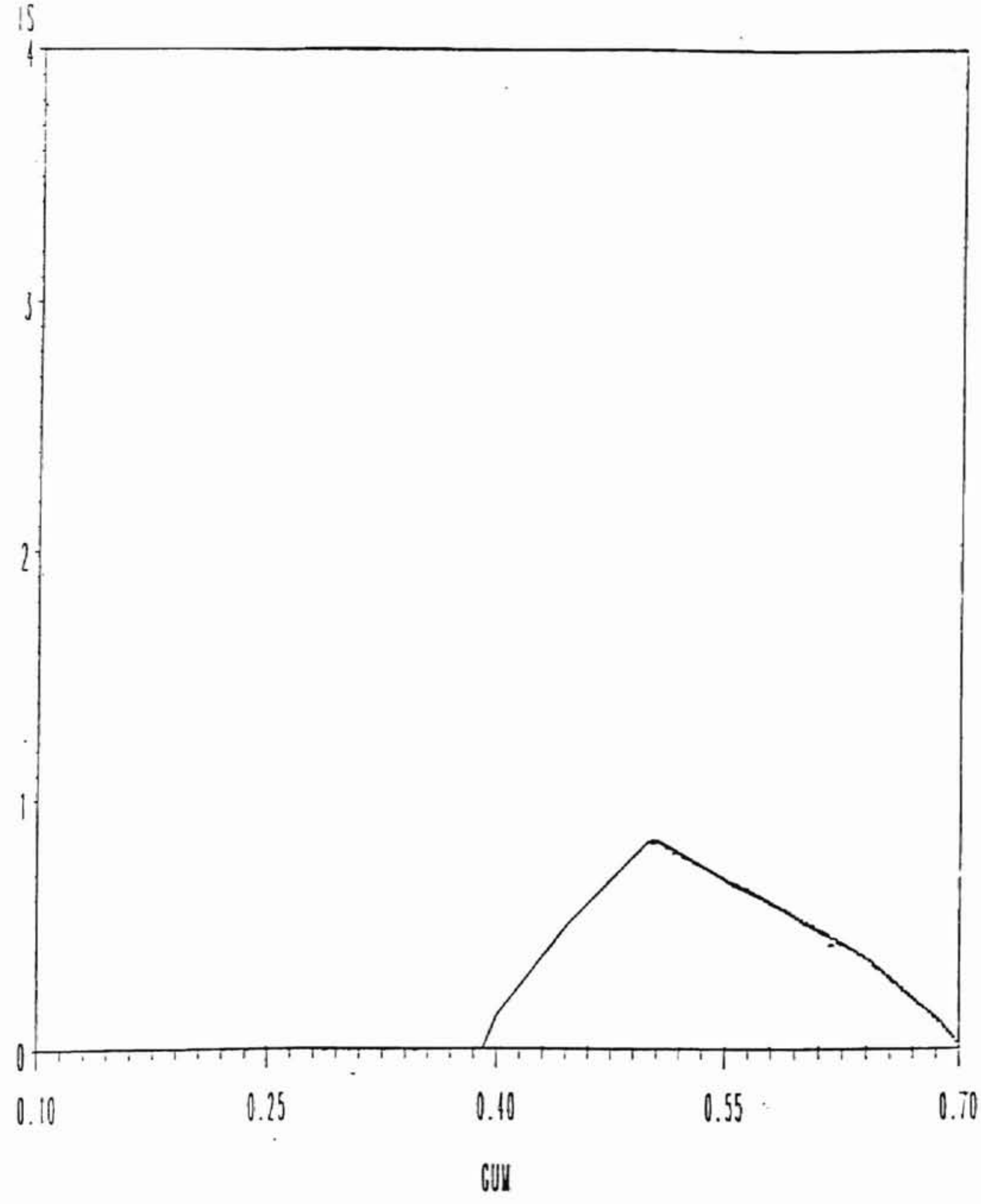

Figure 17. Formulation Area of Suggested Acceptable Gluten-Free Cakes.

Using the Response Surface Model

All four of the formulas suggested by the main test were prepared, and one of those was selected for acceptability testing by the research team. That formula (0.52g gum and 0.5g instant starch) was prepared, and its acceptability compared to that of a standard cake using 12 non-celiac panelists. Also, only one celiac patient was available to evaluate the acceptability of gluten-free cake. For the non-celiacs, the mean of acceptability of the identified gluten-free cake was 3.24 out of 5 while the mean of acceptability of standard cake was 4.33 out of 5. Based on a t-test, on average, the gluten-free cake was judged acceptable (mean acceptability=3.24>3) by the non-celiac panelists ($P \leq 0.05$). The paired t-test was performed to determine whether there was difference between the acceptability of the gluten-free cake and standard cake. The result showed that gluten-free cake was judged less acceptable than the standard cake ($P=0.0049$). For the celiac patient who, of course, was unable to rate the standard cake, the acceptability of the gluten-free cake was 3.8 out of 5.

CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

The purpose of this study was to develop a statistical model utilizing a response surface design that would identify a formula for acceptable gluten-free cakes. A response surface model was built to suggest acceptable gluten-free cake formulations. On average, the formulations suggested by this model produced an acceptable cake. However, the cake was judged less acceptable than the standard cake by the non-celiac panelists.

Recommendations

The following recommendations are for additional research of gluten-free cake.

1. Evaluate the acceptability and related sensory attributes of the gluten-free cake by more celiac patients.
2. Investigate other stabilizers and modified starches to measure their contribution to the quality of gluten-free cake.
3. Test more of the formulas that these response surface methodology tests identified as having a possibility of success.

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APPENDIXES

APPENDIX A

IRB FORM APPROVAL

OKLAHOMA STATE UNIVERSITY
INSTITUTIONAL REVIEW BOARD
HUMAN SUBJECTS REVIEW

Date: 09-16-96 **IRB#:** HE-97-012

Proposal Title: DEVELOPING GLUTEN-FREE CAKES

Principal Investigator(s): Sue Knight, Jingwen chen

Reviewed and Processed as: Exempt

Approval Status Recommended by Reviewer(s): Approved

ALL APPROVALS MAY BE SUBJECT TO REVIEW BY FULL INSTITUTIONAL REVIEW BOARD AT NEXT MEETING, AS WELL AS ARE SUBJECT TO MONITORING AT ANY TIME DURING THE APPROVAL PERIOD.
APPROVAL STATUS PERIOD VALID FOR ONE CALENDAR YEAR AFTER WHICH A CONTINUATION OR RENEWAL REQUEST IS REQUIRED TO BE SUBMITTED FOR BOARD APPROVAL.
ANY MODIFICATIONS TO APPROVED PROJECT MUST ALSO BE SUBMITTED FOR APPROVAL.

Comments, Modifications/Conditions for Approval or Reasons for Deferral or Disapproval are as follows:

Signature: John H. Wheloff

Chair of Institutional Review Board

Date: September 18, 1996

APPENDIX B

STATISTICAL ANALYSIS PROCEDURE

Model: MODEL1
Dependent Variable: VOLUME

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	4	2.24025	0.56006	7.355	0.0017
Error	15	1.14225	0.07615		
C Total	19	3.38249			

Root MSE	0.27595	R-square	0.6623
Dep Mean	3.35450	Adj R-sq	0.5723
C.V.	8.22634		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	-6478.493602	1590.9739335	-4.072	0.0010
IS	1	65.006169	15.97055487	4.070	0.0010
GUM2	1	-1.776776	1.45647744	-1.220	0.2413
GUMSTRCH	1	0.688303	0.16185905	4.252	0.0007
STARCH	1	64.809403	15.90863971	4.074	0.0010

Model: MODEL2
Dependent Variable: POWDERY

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	3	5.01051	1.67017	3.170	0.0531
Error	16	8.43041	0.52690		
C Total	19	13.44092			

Root MSE	0.72588	R-square	0.3728
Dep Mean	2.32800	Adj R-sq	0.2552
C.V.	31.18039		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	3.805055	0.58866360	6.464	0.0001
GUM2	1	4.407933	3.76410588	1.171	0.2587
GUMSTRCH	1	-0.055789	0.03250675	-1.716	0.1054
ISSTRCH	1	-0.001492	0.00110316	-1.353	0.1950

Model: MODEL3
Dependent Variable: STICKY

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	3	6.60514	2.20171	5.431	01.009
Error	16	6.48632	0.40539		
C Total	19	13.09146			

Root MSE	0.63671	R-square	0.5045
Dep Mean	2.59650	Adj R-sq	0.4116
C.V.	24.52171		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	2.952556	0.46320513	6.374	0.0001
GUM2	1	5.349661	3.31236683	1.615	0.1258
GUMIS	1	0.661528	0.19969021	3.313	0.0044
GUMSTRCH	1	-0.051926	0.02831916	-1.834	0.0854

Model: MODEL4
Dependent Variable: OVERALL

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	5	4.11021	0.82204	3.366	0.0332
Error	14	3.41867	0.24419		
C Total	19	7.52888			

Root MSE	0.49416	R-square	0.5459
Dep Mean	3.51600	Adj R-sq	0.3838
C.V.	14.05450		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for H0: Parameter=0	Prob > |T|
INTERCEP	1	-4506.012783	1438.5379686	-3.132	0.0073
GUM	1	6.779886	2.30013094	2.948	0.0106
GUM2	1	-4.929000	2.63235980	-1.872	0.0822
STARCH	1	90.299246	28.87445303	3.127	0.0074
STARCH2	1	-0.452179	0.14492283	-3.120	0.0075
IS2	1	0.491375	0.15579806	3.154	0.0070

VITA

Jingwen Chen

Candidate for the Degree of

Master of Science

Thesis: DEVELOPING GLUTEN-FREE CAKES USING RESPONSE SURFACE METHODOLOGY

Major Field: Nutritional Sciences

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Education: Graduated from Simen High School, Guangdong, China, in July 1985; received Bachelor of Science degree in Pharmacy from Guangdong Medical and Pharmaceutical College, Guangdong, China in July 1989. Completed requirements for Master of Science degree from Oklahoma State University in May 1997.

Experience: Pharmacist, Shenzhen Eye Hospital, Shenzhen, China, 1989-1991. Graduate Research Assistant in Department of Nutritional Science, Oklahoma State University, 1993 to Present.