RESPONSE SURFACE METHODOLOGY WITH MIXTURE EXPERIMENT FOR NON-GLUTEN PASTA DEVELOPMENT

By

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CHAPTER I

INTRODUCTION

Celiac disease, also called gluten-sensitive enteropathy, nontropical sprue, or celiac sprue, is a malabsorption disorder (Saunderlin, 1994). The lining in the small intestines of some people recognizes gluten, a protein complex, as a foreign substance. This causes an immune response and leads to swelling and soreness. Over time, the lining of the intestine loses the villi that absorb the nutrients from the diet. The celiac patient ingesting gluten will experience weakness, diarrhea, abdominal pain, and weight loss; and the person may develop anemia, anorexia, amenorrhea, and low blood calcium levels (Rottmann, 1987). 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.

The only treatment for celiac disease patients is lifelong adherence to a gluten-free diet (Campbell, 1992). Celiac patients should not ingest gluten-containing foods such as wheat, barley, oats, and rye at any level. However, the gluten-containing foods are very difficult to eliminate from the daily diet because many products contain gluten. For example most breads, breakfast cereals, desserts, gravies, and sauces contain gluten. Other processed food which also may contain 'hidden' gluten are some types of instant

coffee, ice cream, catsup, salad dressing, and even some vinegars. Although, by using rice, potato, or other non-wheat starches to make products one can eliminate the gluten from the diet, researchers have reported that non-gluten flour products are less desirable in taste, texture, color, and product variety. These starches can not replace what gluten contributes to products such as breads and pastas: elasticity, structure, textural properties, and sensory characteristics. Therefore, there is a need for gluten replacements. For example, non-starch polysaccharides such as xanthan gum and locust bean gum. These imitate the functional properties of gluten for non-gluten products. Xanthan gum and locust bean gum can contribute elasticity, texture, and sensory characteristics in non-gluten products because of their high viscolasticity properties (Toufeili et al., 1994). Xanthan gum and locust bean together have a synergistic interaction to increase gel strength (Whistler and BeMiller, 1997). This synergism could help non-gluten pasta mimic the functional properties of wheat flour.

Without using wheat flour, other non-gluten flours or starches combine with xanthan gum and locust bean gum to replace gluten in wheat flour. But each one of these has its unique properties that contribute to non-gluten product systems. Therefore, this research used response surface methodology with mixture experiment to investigate the functional properties of xanthan and locust bean gums with non-gluten flours and starches to assess the effect of varying levels of added starch, flour, and gum to non-gluten pasta.

Objectives

This research is designed:

1: To investigate the functional properties of non-gluten pasta.

2: To analyze the sensory characteristics of non-gluten pasta.

3: To visualize the microstructure of non-gluten pasta by using scanning electron microscope.

4: To develop non-gluten pasta using five different starches and flours, and two gums.

5: To develop statistical models by using response surface methodology with mixture design for the optimum formula of non-gluten pasta.

Assumptions

The author assumes the followings:

1: Five different starches and flours with two gums can yield an acceptable non-gluten pasta.

2: The mixture formula suggested by statistical models yields a highly acceptable non-gluten pasta.

3: Locust bean gum and xanthan gum not only interact with each other but also with the five flours and starches.

4: Non-gluten starches, flours, and gums improve functional properties and sensory characteristics of non-gluten pasta.

Limitations

1. There are only five independent variables tested: modified starch, potato starch, tapioca starch, xanthan gum, and locust bean gum.
2. The semi-training panel for sensory evaluation contains twenty-one persons.
3. The consumer panel testing was based on a forced choice with no option to decline making a preferential choice.
4. Appropriate standards are used for the panelists' reference judgement.
5. The experimental is limited to fifteen treatments; an incomplete block design is used. There are two replicates of the fifteen treatments with Treatments 8 and 11 duplicated in each replicate.

Format of dissertation

This dissertation follows the format of the Thesis Writing Manual of the Oklahoma State University Graduate College except that Chapters IV and V are written following the guidelines for authors for the Journal of Food Science. Chapter IV covers objective data collection and microscopy. Chapter V covers sensory data collection and microscopy.

CHAPTER II

LITERATURE REVIEW

Food sensitivity

Food sensitivity is an adverse food reaction defined as any untoward reaction following the ingestion of food (Lifshitz, 1988). There are many types of adverse reactions to foods. A broad meaning of "food sensitivities" is: a reproducible, unpleasant reaction to a specific food or food ingredient (Table 1). Those foods or food ingredients, also called food allergens, can initiate and provoke the immunological reaction of an allergy (Taylor, 1992). Food sensitivities can be divided into two main items: primary food sensitivities and secondary food sensitivities (Figure 1). Further, primary food sensitivities can be classified into immunological reactions (involving the body's immune systems) and non-immunological reaction diseases (Hefle, 1996). In celiac disease, allergic reactions of patients who are sensitive to gluten of wheat, rye, barley, and oats are classed as primary food sensitivities (non-IgE-mediated reaction).

Celiac disease and gluten sensitivity

Celiac disease, also called gluten sensitive enteropathy, is a sensitivity to gluten and is a relatively rare malabsorption disorder (Saunderlin, 1994). Prevalence rates range from a high of 1 in 300 in western Ireland to between 1 in 1000 and 1 in 2000 in

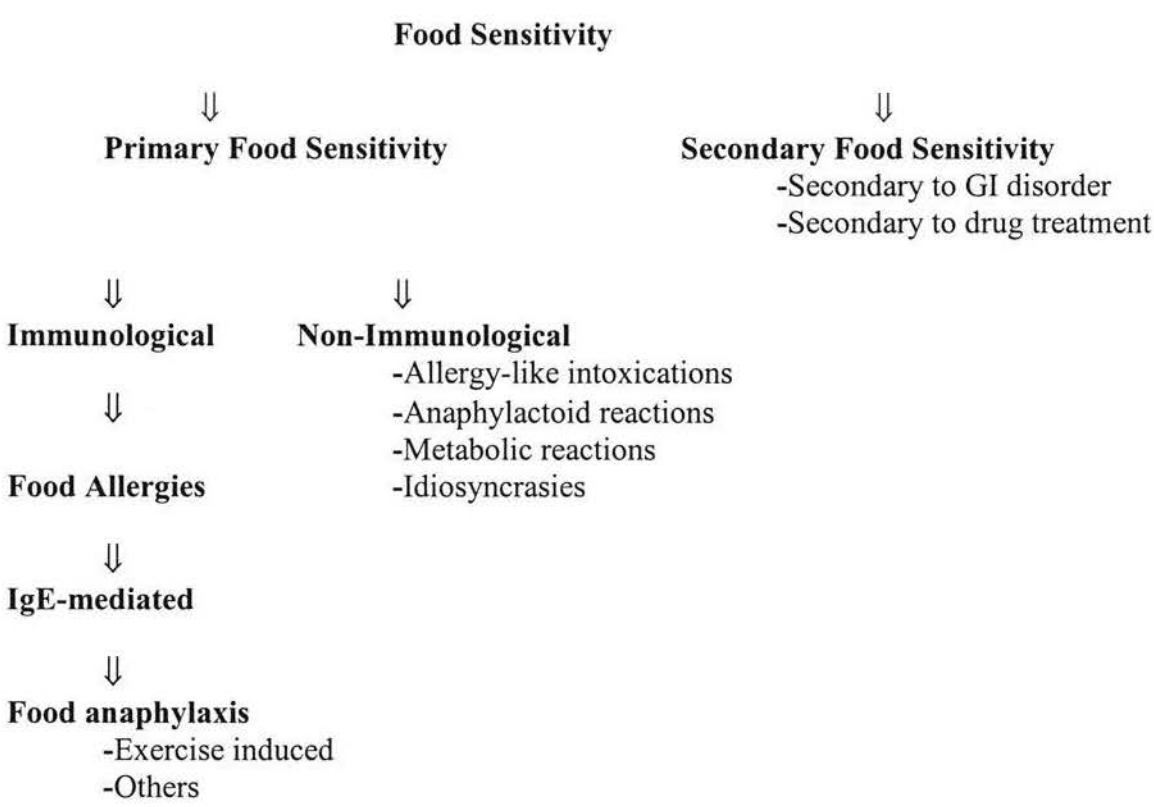
other regions of Great Britain and Northern Europe (Trier, 1991). The individual suffers diarrhea, abdominal pain, and weakness when ingesting gluten. The immunopathology

Table 1. Known food allergens

Brazil nuts
2S High-methionine protein
Cow's milk
β-Lactoglobulin
α-Lactalbumin
Caseins
Egg white
Ovomucoid
Ovalbumin
Ovotransferrin
Egg Yolk
Apovitellin I
Apovitellin VI
Lipoprotein
Livetin
Mustard
Peanut
Concanavalin A-reactive glycoprotein (CARG)
Ara h1
Ara h2
Soybean
β-Conglycinin
Glycinin
Kunitz soybean trypsin inhibitor
Gly m 1
Shrimp
Pen a 1 (tropomyosin)
Codfish
Gad c1 (Parvalbumin)
Rice
Albumin fraction (14-16 kD)
Glutelin fraction
Wheat (gluten)
Albumins
Globulin

The chemistry and biology of food allergens, p. 90. Food Technology-March 1996

Figure 1. Food sensitivity



The chemistry and biology of food allergens, p. 86. Food Technology-March 1996

of celiac disease is very complex and not completely understood. The immune response to nonreplicating lumnial antigens in celiac patients may be abnormal. This causes increasing epithelial permeability and swelling of the intestinal lining. Those symptoms cause a morphological change of the villi (Brandtzaeg et al., 1989). Over time, the lining of the small intestine loses the mucosal layer and the villi that absorb nutrients. However, the submucosa, muscular layer, and serosa are spared. Finally, celiac patients lose weight and reflect the consequences of malabsorption that could include anemia, anorexia, amenorrhea, and low blood calcium levels. Currently, the only treatment is life long abstinence.

Although people who are sensitive to gluten are not considered as celiac disease patients, they experience anaphylaxis that is a reaction of the body's immune system attacking itself. This can cause any or all these symptoms: skin reactions, vomiting, rapid closing of the throat, and sudden fall in blood pressure. Death can result if immediate action is not taken. As in celiac disease, the only treatment for gluten sensitivity patients is also a life-long abstinence from foods containing gluten.

Polysaccharides

Polysaccharides (Table 2), polymers of monosaccharides, have been used as main ingredients of human food such as breakfast cereal, bread, cake, noodle or pasta for many centuries. Polysaccharides in foods include: (a) starches [raw, pregelatinized, and modified]; (b) cellulose and cellulose derivatives; (c) seaweed extracts [alginates, carrageenans, and agar]; (d) plant exudates or gums [arabic, karaya, and tragacanth]; (e) seed gum [locust bean and guar]; (f) plant extracts [pectins]; and (g) microbial gums [xanthan gum and gellan gum] (Glicksman,1979). Polysaccharides along with fat, protein, water, minerals, and vitamins are basic components in food. In addition, water-soluble-polysaccharides such as starches, flours, and non-starch gums that have been used in the food industry, are known as hydrocolloids for thickening, stabilization, emulsification, fat replacement, and gel formation in food systems (Table 3). Because polysaccharides have various functional properties, it is very important for food scientists to understand their properties applied to food products (Sanderson, 1981).

Polysaccharide polymers can be divided into three types: single branched, substituted linear, and branch-on-branch (Figure 2). Each polysaccharide has its unique

functional properties in food systems, depending on molecular weight, linkage type, and polymer type. Because of that, it is important to understand each polysaccharide's specific properties and the concentration needed when each polysaccharide is applied to food. For example, carrageenan is very useful for gelling products, and xanthan gum is used for thickening in salad dressing or stabilizing in cake mixes (Dziezak, 1991). Also, different starch polysaccharides, such as corn flour, rice flour, etc, may contain a mixture of two or even three of the polymer forms.

Table 2. Classification of selected polysaccharides in foods by sources

	Examples
Algal	Agar, algins, carrageenans, furcellaran
Higher plants	
Insoluble	Cellulose
Extract	Pectins
Seeds	Corn starches, rice starches, locust bean
Tubers and roots	Potato starches, tapioca
Exudates	Gum arabics, gum karaya
Microorganisms (fermentation gums)	Xanthans
Derived	
From cellulose	Carboxymethylcelluloses, Hydroxypropylcelluloses
From starch	Starches acetate, starch phosphates
Synthetic	Polydextrose

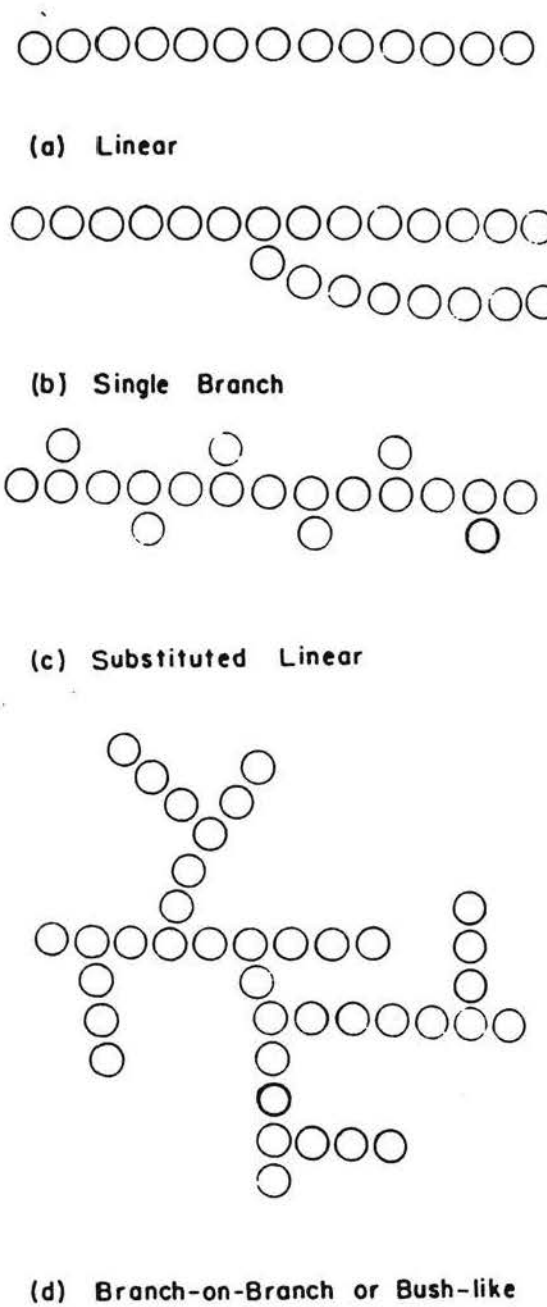
Carbohydrate Chemistry for Food Scientists, p. 64. Whistler and BeMiller, 1997.

Table 3. Functional properties of water-soluble polysaccharides

Function	Example
Adhesive	Glazes, icing, frostings
Binding agent	Pet foods
Bodying agent	Dietetic beverages
Crystallization inhibitor	Ice cream, sugar syrups, frozen foods
Clarifying agent (fining)	Beer, wine
Cloud agent	Fruit drinks, beverages
Coating agent	Confectionery, fabricated onion rings
Dietary fiber	Cereals, bread
Emulsifier	Salad dressing
Encapsulating agent	Powdered flavors
Film former	Sausage casing, protective coatings
Flocculating agent	Wine
Foam stabilizer	Whipped topping, beer
Gelling agent	Pudding, deserts, confectionery
Molding	Gum drops, jelly candies
Stabilizer	Salad dressing, ice cream
Suspending agent	Chocolate milk
Swelling agent	Processed meat products
Syneresis inhibitor	Cheese, frozen foods
Thickening agent	Jams, pie filling, sauces
Whipping agent	Toppings, marshmallows

Food Hydrocolloids, p. 7. Martin Glicksman, 1982.

Figure 2. Types of polysaccharides



Food Hydrocolloids, p. 9. Martin Glicksman, 1982

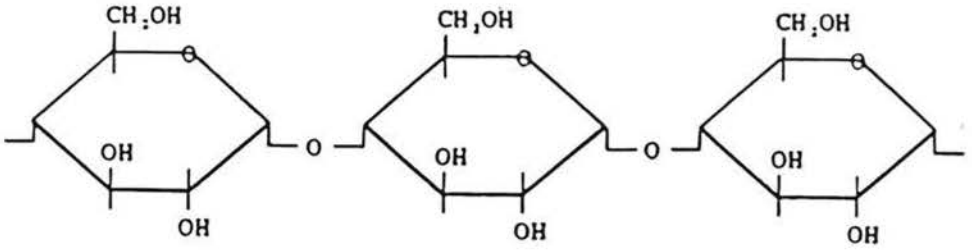
Functional properties of starch polysaccharides

Starch

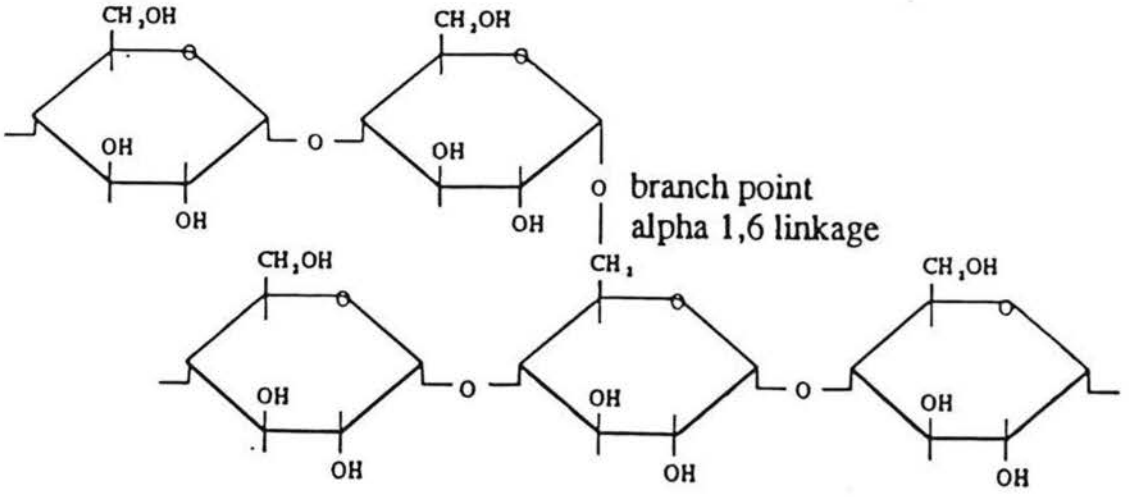
Starch, a polysaccharide, is obtained primarily from cereals, certain roots, or tubers. Starch is composed of amylose and amylopectin (Figure 3). Both amylose and amylopectin are polymers of D-glucose. The unit in amylose is α-d-glucopyranose with an α-1,4-glycosidic linkage. Most amylose molecules are linear, but a very small degree of branching exists (Hoseney, 1986); the side chains on those molecules are very few and act similarly to unbranched amylose molecules. The degree of amylose polymerization (number of glucose units per molecule) ranges from 500 to 2000 (Pomeranz, 1987). Strong internal forces permit different molecular shapes to form a helix structure because many hydroxyl groups contribute a high degree of hydrogen-bonding capability (Whistler and Daniel, 1984). Starches containing amylose are referred to as nonwaxy starches. Amylose constitutes 20-30% of total starch in cereal starches. Starches containing amylopectin are referred to as waxy starches. Amylopectin constitutes 70-80% of total starch in cereal starches. The chains of amylopectin are formed from glucose units in α-1,4-glycosidic linkages; the branches are connected by α-1,6-linkages. The degree of polymerization ranges from 10^4 to 10^5. This makes amylopectin one of the largest naturally occurring macromolecules (Manners, 1985). There are many branches in amylopectin polymerization; the branches average 20-25 glucose units (Manners, 1985). The high degree of polymerization and highly branched structure are responsible for the high viscosity of amylopectin dispersions. The natural physical structural unit of starch is the granule that is constituted of both amylose and amylopectin (Figure 4). Starch

granules are birefringent, indicating a high degree of internal order (Penfield and Campbell, 1990).

Figure 3. The structure of amylose and amylopectin



AMYLOSE



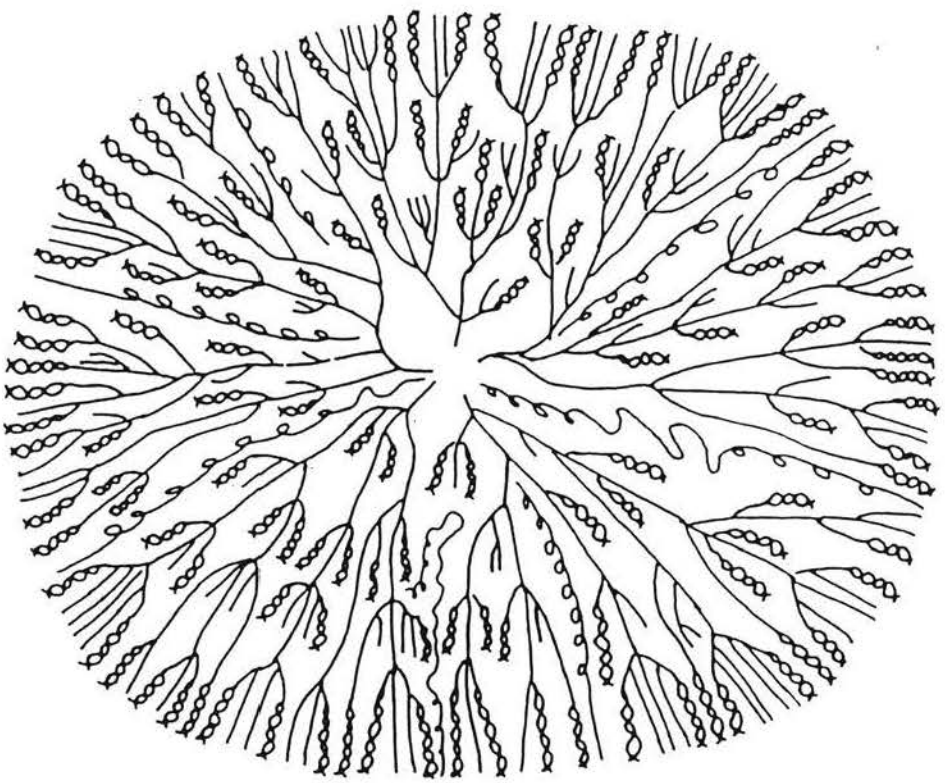
AMYLOPEMN

Carbohydrate Chemistry for Food Scientists, p. 117. Whistler and BeMiller, 1997

Table 4. Properties of amylose and amylopectin

Properties	Amylose	Amylopectin
Molecular weight	50,000-200,00	1- to-several millions
Glycosidic linkage	Mainly α-D-(1,4)	α-D-(1,4), α-D-(1,6)
Retrogradation	High	Low
Molecular shape	Linear	Branched

Figure 4. Schematic of the organization (structure) of a starch granule



Bakers Digest, p. 18. Lineback, 1984

A micellar network formed by association of segments of individual molecules in various patterns imparts durability to the granular structure and controls the swelling behavior of a starch during heating (Christianson et al., 1981). A starch dispersion heated in water can be divided into two steps: the first stage is starch gelatinization and the second stage is pasting. Starch gelatinization is the collapse or disruption of molecular order within the starch granule manifested in irreversible changes in properties such as granular swelling, native crystal melting, loss of birefringence, and starch solubilization. In the pasting stage, granular swelling continues with exudating of molecular components from the granule; and eventually, total disruption of the granules (Atwell et al., 1988). As granules swell, the size of granule increases and thus contributes viscosity. At the same time, some straight chain amylose is released from the granules and dissolves in a colloidal dispersion; the dispersion thus is a sol where the intact granules are in suspension (Miller et al., 1973). If heating is continued to a thickened starch suspension, maximum viscosity is achieved. With continued heating, the viscosity will decrease as the granules disrupt into fragments. After a thickened starch cools, the energy is reduced from the suspension; the viscosity increases. The bindings between the molecules draw them back together again. In some high amylose starches (20-30%), a gel structure forms which is probably due to the free amylose forming hydrogen bonds not only with other amyloses but also with amylopectin branches in the swollen granules, so that a continuous network forms (Penfield and Campbell, 1990). Because of the different molecular weights, and structure between amylose and amylopectin, they contribute different properties (Table 4). Amylose exhibits gelling properties and amylopectin shows a thickening power. Therefore the proportions of amylose and amylopectin are very important to the properties of a specific starch.

In food systems, starches usually interact with protein to form starch-protein complexes. The ratio of starch/protein is important in different food systems. For example, cake needs a lower starch/protein ratio than bread. A higher ratio of protein produces a firm food structure that is good for bread but undesirable for cake texture.

Corn flour. Corn, next to wheat, is the grain most used in the United States. Corn flour is used commercially in dry mixes for various flour products in breading, ready-to-eat breakfast cereals, and extruded snacks (Penfield and Campbell, 1990). A mix of corn-soy milk blend is used in the United States for food aid programs (Rooney and Serna-Saldivar, 1987). Corn starch is the refined starch from corn flour. Generally, corn starch can be divided into two categories: waxy corn starch and nonwaxy corn starch depending on proportion of amylose and amylopectin. Amylopectin in waxy corn starch can be close to 100% (Matz, 1991). In nonwaxy corn starch, amylopectin is about 70% and amylose about 30%. Researchers (Anonymous, 1985) report using corn starch for non-gluten cake, bread, snacks, and pasta; but the quality of these non-gluten products is less desirable because of lacking gluten. Alloncle et al. (1989) suggested that gums such as xanthan and locust bean gum, used as thickeners instead of starches or flours, can be very successfully applied into food systems to modify the taste, texture, and flavor. Xanthan gum and locust bean gum also can interact with starch polysaccharides to increase the thickening properties, gelling ability, and reduce retrogradation in food systems.

Rice flour. Rice flours are a major food in Asian counties, but not in the United States. Rice contains about 6.5-7% protein; the other major components are polysaccharides that are amylose and amylopectin. Rice flour protein does not have as high viscoelastic properties as gluten. Therefore, rice flour can only be a partial

substitution for wheat flour, or may be added with other flours such as potato starch, corn flour, or tapioca starch. Rice flour can be substituted for 5% of the wheat flour in yeast-raised bread with little effect on acceptance; but acceptance of bread decreases as rice is increased up to 30% (Luh and Liu, 1980). Because rice flour does not have gluten which must be avoided by celiac patients, rice flour has been used in non-gluten products for these people. Researchers have examined the quality of bread and cake made with rice flours and other non-wheat flours for non-gluten products. However, these products were not very highly rated by celiac patients (Nishita et al., 1976; Nishita and Bean, 1979; and Bean et al., 1983). Ylimaki et al. (1988) have developed non-gluten bread mixed with rice and potato starch and an added non-starch gum, carboxymethylcellulose (CMC), to provide some of the structure of gluten. The results show that added gum increased the bread volume and tenderness.

Potato starch. Potato starch for food use accounts for 30% of total starch usage in the United States (Whistler and Daniel, 1985). Potato starch is preferred over corn starch and other starches in certain applications, high consistency on pasting and heating, flexible film formation, binding power, and low gelatinization temperature. Unmodified potato starch has an exceptionally high cooked viscosity per dry weight of starch, partially attributed to its high content of starch phosphate esters (Wiesenborn et al., 1994). However, gelatinized potato starch granules are readily disrupted by shear during conveyering and mixing operations, resulting in greatly reduced viscosity. Modification of starch is often needed to tailor functional characteristics to desired applications. In a non-gluten bread, potato starch combined with rice flour has been used to replace wheat flour (Ylimaki et al., 1988). Therefore, potato starch is a potential starch for a non-gluten product to replace part of wheat starch.

Tapioca starch. Tapioca is an underutilized carbohydrate source in the United States and has not been a big agricultural crop, but tapioca does have potential as a carbohydrate producing crop in this country (De Vries et al., 1967). Tapioca comes from the cassava plant. Tapioca starch has higher viscosity and stronger binding ability after cooking than corn starch, rice flour, or potato starch (Manilal et al., 1983). Tapioca starch has been successfully added with other flours for bread (Ciacco and D'Appolonia, 1977). Tapioca starch also is used as a thickening agent in salad dressing and as a water-binding agent in pie filling because of its lower retrogradation and higher water binding ability (Fanta and Christianson, 1996; and Jacobson and BeMiller, 1998). Tapioca starch can be use in non-gluten products to interact with other starches during gelatinization.

Modified starch. In the food industry, food processors generally prefer starches with better behavioral characteristics than those provided by native starches. For example, potato, corn, or waxy corn starch pastes produce weaker-gels and less thickening than needed under food processing and manufacturing conditions (Whistler and BeMiller, 1997). Modification can increase the ability of starch pastes to withstand heat, shear, or acid associated with processing; and modification introduces specific functional properties such as resistance to retrogradation. Now, modified food starches are abundant functional food ingredients and additives in food products. Instant starch or pregelatinlization starch is a precooked starch that has been dried and ground. The starch swells and thickens in cold water without further heating. (Colonnal et al., 1987). This is a very convenient ingredient in products such in instant puddings, cake mixes, and non-gluten products. However, instant starch has less viscosity and adhesiveness, compared to freshly cooked paste. In 1988, some researchers introduced several cold water starches that combined convenient use with greater stability and better texture in food systems

(Anonymous, 1988). Because modified starch has these properties, it can be applied to non-gluten pasta to interact with other ingredients and form a network in the mixing process (Molina et al., 1975).

Functional properties of non-starch polysaccharides

Non-starch polysaccharides

Non-starch polysaccharides can be used in many foods because of their various functional properties. These are long-chain polymers that dissolve or disperse in water to give viscosity or gel building effects (Glicksman, 1982). More than thickening, non-starch based polysaccharides also have a secondary effect on stabilization of emulsions, suspension of particulates, control of crystallization, inhibition of syneresis, encapsulation, and formation of a film. The United State Food and Drug Administration regulates gums as food additives "Generally Recognized as Safe" (GRAS) substances. The regulations covering each specific gum are outlined in Title 21 of the Code of Federal Regulations in Parts 172.580-172.874 and Parts 182.1480-184.1724, respectively. Although generally used at levels less than 2% in foods, the non-starch based polysaccharides (gums) have become more popular in food industry applications because of their various properties.

Locust bean gum. Locust bean gum is a very important thickening polysaccharide for both food and non-food uses (Whistler and BeMiller, 1997). Locust bean gum comes from the ground endosperm of seeds from the locust tree seed pods. Formerly locust bean gum was used mainly in dairy and frozen dessert products. Locust

bean gum is now also used in low fat products as a fat replacer by mimicking the texture contributed by fat. The typical applications in food products are in Table 5.

Table 5. Typical products containing locust bean gum

Bakery products	Dips
Low-calorie salad dressings	Cream cheese
Dairy products	Frozen novelties
Cheese spreads	Ice creams
Cottage cheese	Whipped toppings

Carbohydrate Chemistry for Food Scientists, p. 176. Whistler and BeMiller, 1997

A galactomannan is the main component of locust bean gum. Galactomannan contains a main chain of (1-4)-linked β-d-mannopyranosyl units branched with single α-d-galactopyranosyl units attached to O-6. The branching occurs on about 56% of the main chain units with about 44% of the main chain "naked-no branches" part (Whistler and BeMiller, 1997). A segment of a galactomannan molecule is in Figure 5. The "naked" sections can interact with other non-starch polysaccharides such as xanthan gum to produce a synergism in viscosity and gel strength (Figure 6). Locust bean gum also interacts with starch-based polysaccharides to increase viscosity by forming gels and reduce retrogradation by binding water.

Figure 5. Repeating segment of a galactomannan (locust bean gum) molecule

Carbohydrate Chemistry for Food Scientists, p. 172. Whistler and BeMiller, 1997

Figure 6. Schematic representation of galactomannan (locust bean gum)

Each line represents a sugar unit: the backbone is composed of O-D-mannopyranose units and the side chains are composed of et-D-galactopyranose units.

Schematic representation of galactomannan conformation shows 'smooth' or 'unbranched' regions and 'hairy' regions. (Food Hydrocolloids, p. 142. Martin Glicksman, 1982)

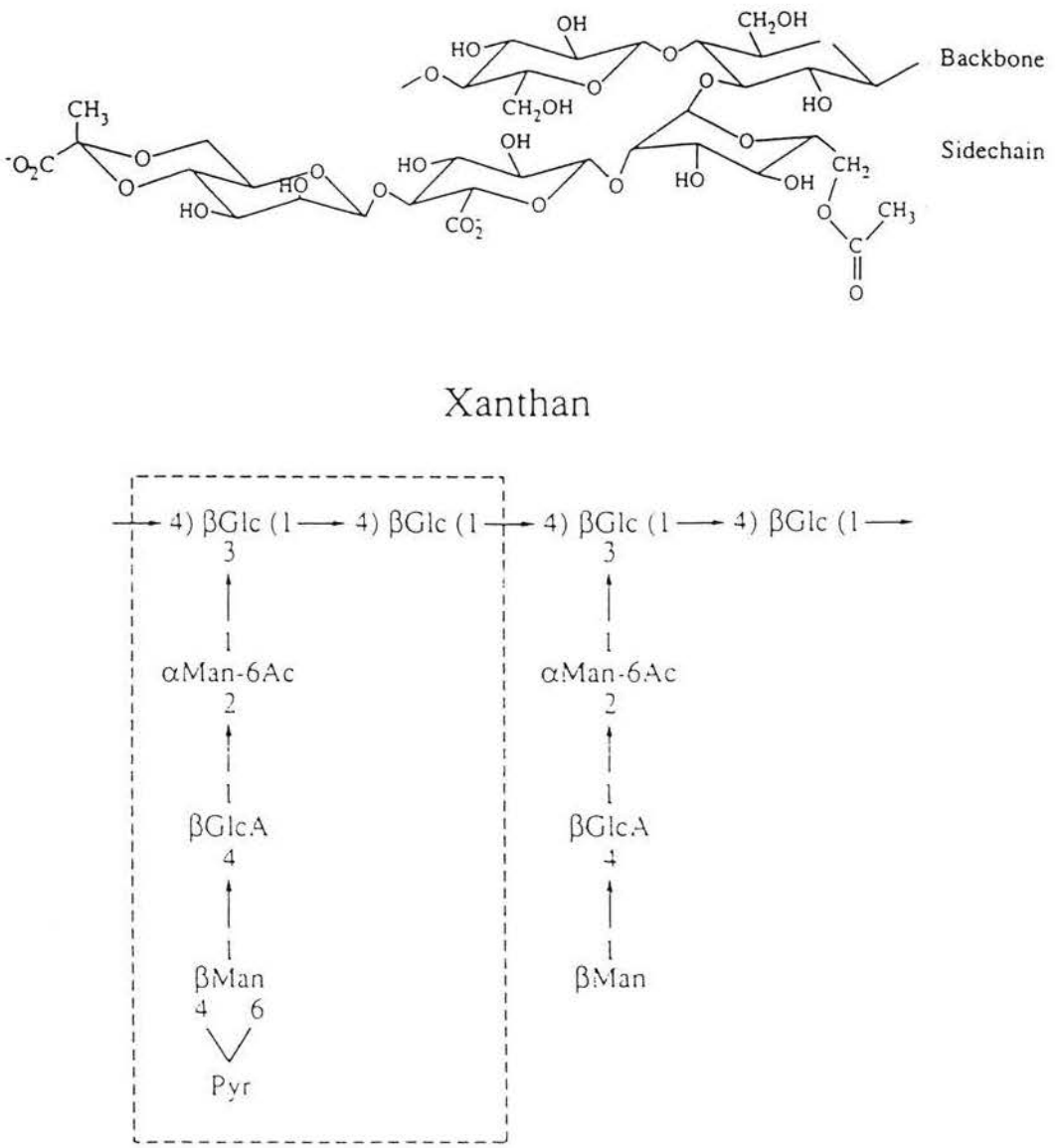
Xanthan gum. This is a polysaccharide produced by the microorganism, *Xanthomonas campestris*. The biosynthesis and isolation of xanthan gum was first reported by Lilly et al. (1958). After that, scientists began to experiment by applying xanthan gum into food systems because of the abundant nature of the microbial polysaccharide and the diverse properties of xanthan gum (Table 6). The primary structure of xanthan gum is shown in Figure 7. The polymer backbone consists of 1,4-linked β-D-glucose identical to that of cellulose. At the 3-position of alternate glucose monomer units, there branches a trisaccharide side chain containing one glucuronic acid and two mannose residues (Figure 8). The estimated molecular weight is about 15 million (Holzwarth, 1978).

Table 6. Typical products containing xanthan gum

Bakery products	Mixes
Cake mixes	Fruit drink mixes
Danish fillings	Gravy mixes
Pie crust	Pudding mixes
Cereal bars	Dressing
Condiments	Low-calorie salad dressing
Salsa	Reduced-calorie mayonnaise
Dairy products	Sauces
Cheese cakes	Barbecue sauce
Cheese spreads	Oriental sauce
Cream cheese	Pizza sauce
Whipped topping	Spread
Egg substitute	Margarine spreads
Frozen foods	Sandwich spreads
Frozen lasagna	Syrup
Ice cream	Chocolate syrup
Frozen pizza	Pancake syrup

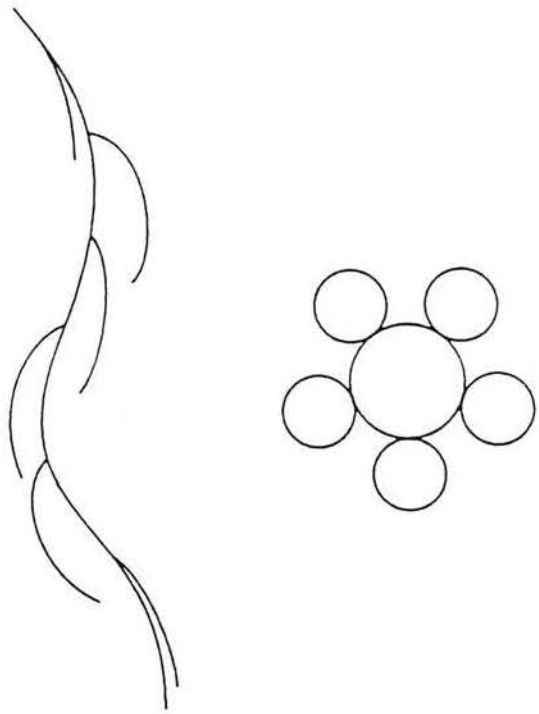
Carbohydrate Chemistry for Food Scientists, p. 184. Whistler and BeMiller, 1997

Figure 7. Structure of xanthan gum units



Structure of the pentasaccharide-repeating unit of xanthan gum. Inside the box is a pyruvylated pentasaccharides building block unit. (Carbohydrate Chemistry for Food Scientists, p. 180. Whistler and BeMiller, 1997)

Figure 8. Relationship of the trisaccharide side chains of xanthan gum molecules



Two representations of the relationship of the trisaccharide side chains to the backbone helix of xanthan molecules. (Carbohydrate Chemistry for Food Scientists, p. 181. Whistler and BeMiller, 1997)

Xanthan gum possesses an extraordinary combination of properties which result in wide applications in food systems as a thickener, emulsion stabilizer, suspending additive, protective colloid, and processing aid (Blanshard and Mitchell, 1978). Much of the activity can be explained on a molecular basis by the evidence provided by X-ray (Morrhouse et al., 1977), nuclear magnetic resonance (NMR), rheometry (Dea et al., 1977 and Morris et al., 1977), and electron microscopy (Holzwarth and Prestridge, 1977). The xanthan gum polymer is composed of a cellulose backbone with trisaccharide branches. In both dissolved and solid states the branches appear to be aligned with the main chain to form a stiff polymer. In native form, xanthan gum exists either single stranded or multistranded (Morrhouse et al., 1977). This conformation is stabilized by intermolecular noncovalent interactions such as hydrogen bonding. Because these bonds are weak, they are very easily disrupted under the effect of applied shear (Figure 9). This may explain the pseudoplasticity of a xanthan gum solution which loosens under shear and retightens when stationary (thixotropicity, Figure 10). In addition, the highly ordered network of entangled stiff molecules accounts for the high suspending property and high yield value in emulsion systems (Pettitt, 1978). This stable helical conformation resists the temperature influence. The enormous branches on the backbone protect the vulnerable glycosidic linkages on the cellulose backbone from hydrolytic cleavage (Blanshard and Mitchell, 1978). The shielding effect provides stability to xanthan gum under strongly acidic or alkaline conditions and also reduces enzymatic degradation.

Xanthan gum, reacting with other non-starch polysaccharides such as locust bean and guar gums, results in a synergism of viscosity and gel strength (Figure 11). Xanthan gum also reacts synergistically with starch-based polysaccharides (Kovacs, 1973). Xanthan gum mixed with starch in solution has an interaction that significantly increases

Xanthan gum mixed with starch in solution has an interaction that significantly increases viscosity of the starch paste during the second stage of swell. Christianson et al. (1981) found that the addition of gum to a starch solution enhanced viscosity by a starch-gum association to contribute a network formation; gum also decreased the degree of retrogradation by interacting with soluble amylose which leached out of the starch granules during the second stage of swelling. Sajjan and Rao (1987) showed that xanthan gum can interact with starch to increase viscosity in a suspension.

Figure 9. Pseudoplastic flow

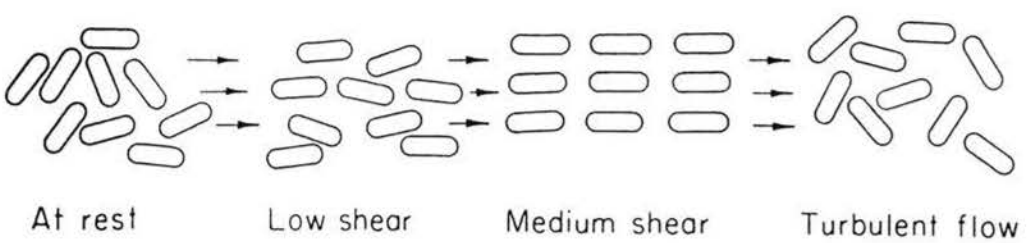
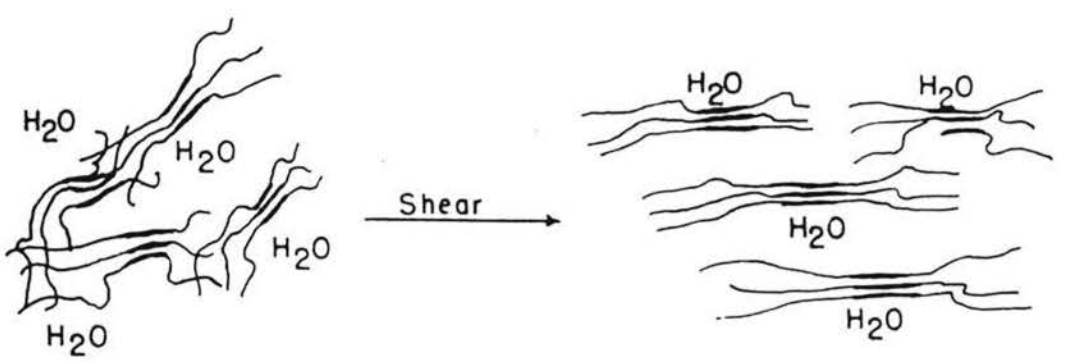
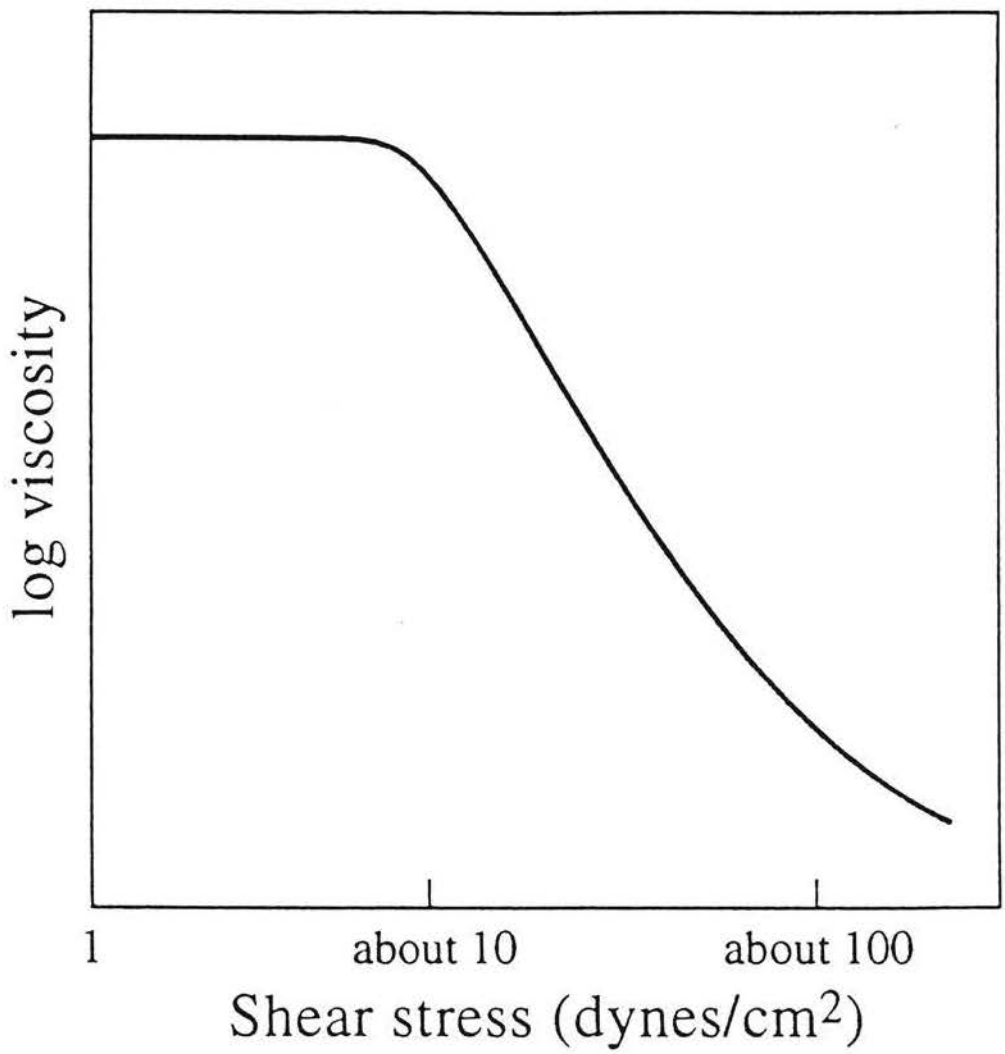


FIGURE 4. Structural changes of pseudoplastic materials.



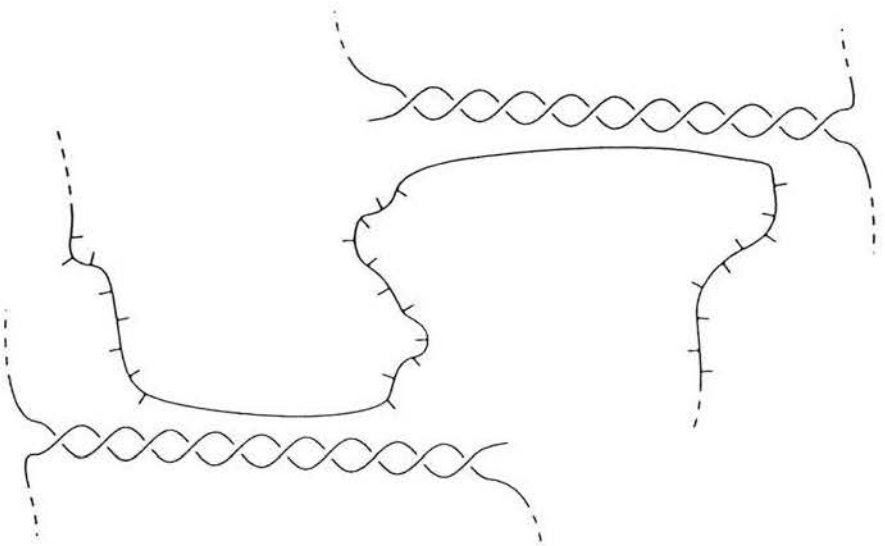
At rest (left), the molecules lie in random arrangement, intertwined and bound to the solvent molecules. Under shear (right), the molecules align and squeeze out the bound water molecules. (Food Hydrocolloids, Martin Glicksman, 1982)

Figure 10. Xanthan gum solution to shear rates and the pseudoplastic region.



The response of a xanthan gum solution to shear rates. (Carbohydrate Chemistry for Food Scientists, p. 182. Whistler and BeMiller, 1997)

Figure 11. Molecular interaction between xanthan gum and locust bean gum



Representation of the hypothesized interaction of a locust bean gum molecule with helical portions of carrageenans and/or xanthan gum to form a three-dimensional structure and a gel. (Carbohydrate Chemistry for Food Scientists, p. 183. Whistler and BeMiller, 1997)

Sensory evaluation for food product development

Sensory evaluation is a tool used by the food industry as a reference to evaluate products, to select or change ingredients for new product development and quality control (Herbert et al., 1991). Compared to instrument measurements, data from sensory evaluation more closely predicts the response of consumers in market acceptance (Goldman, 1994). For example, cake can have a very tender texture measured by texture analyzer but have a very low acceptability scale measured by consumers. Over dependence on objective measures might partially explain the fact that the failure rate of new products has exceeded 80% in the past decade (Hollingsworth, 1994).

Sensory evaluation data are affected by culture, ethnicity, age, and personal habits (Galvin and Waldrop, 1990). Therefore, correctly targeted sensory evaluation is critical for food product development industries. There are three categories of sensory evaluation: discrimination tests (Figure12), analytical/descriptive tests (Figure 13), and affective tests (Figure 14).

Discrimination tests

The discriminative tests are used to determine a difference between samples. Commonly used tests are: paired comparison, triangle, duo-trio, and taste threshold. These tests usually only show the presence of a direction of difference but not the magnitude of difference (Muller, 1977). The panelists evaluating samples usually receive little or no training. Discriminative tests are often used along with affective tests in consumer testing.

Figure 12. Example of triangle test in discrimination test

Triangle Test

Panel code_____ Date_____

Type of sample_____

Instructions:
Taste samples from left to right. Two are identical; determine which is the odd sample. (If no difference is apparent, you must guess.)

Sets of three samples	which is the odd sample	Comment
____ ____ ____	_____	_____
____ ____ ____	_____	_____
____ ____ ____	_____	_____

Figure 13. Example of attributes scaling in analytical/descriptive test

Texture

Hardness

0 (Very soft) (Very hard) 10

Flavor

Meat Aroma

0 (Very light) (Very strong) 10

Figure 14. Example of verbal hedonic scales in affective test

Acceptability

❑	❑	❑	❑	❑
Dislike extremely	Dislike moderately	Neither like or dislike	Like moderately	Like extremely

Analytical/descriptive tests

The analytical/descriptive tests involve both the detection (discrimination) and the description of qualitative and quantitative sensory attributes of a product (Meilgaard et al., 1987). The panels in the descriptive tests vary from semi-trained to very highly-trained. Panelists are expected to detect, indicate, and differentiate the specific attributes in sensory characteristics of a product. Panelists participating in attribute profile, qualitative descriptive analysis, and quantitative descriptive analysis receive many hours of intensive training before formal testing (Meilgaard et al., 1987).

Analytical/descriptive tests that require a high level of training include descriptive analysis and attribute rating. A flavor profile provides information about a product's aroma, flavors, after-taste, and mouth feel; a texture profile describes the sensory components related to texture, such as mechanics, geometry, fat, and moisture (Meilgaard et al., 1987). Ratio scaling is used to estimate the relationship between the quantity of a substance(s) generating physical characteristics and the sensory perception of the stimulus, while descriptive analysis is used to analyze the profile of flavor, texture, etc.

In analytical/descriptive tests, each panelist must be able to detect and describe the perceived sensory attributes of a sample. These qualitative characteristics of a product include all of the appearance, aroma, flavor, texture, or other properties that make it different from others. The panelist has to learn to not only differentiate and rate the quantitative or intensity aspects of a sample but also define degrees of characteristics or qualitative notes present in that sample. Therefore, it is necessary to give panelists hours of training to ensure that they accurately communicate the attributes and sensations they experience (O'Mahony, 1995). This is generally achieved by repeated tasting of the food with discussion to develop descriptions of its sensory attributes. Accuracy is

achieved by developing flavor and texture standards for the panel to measure against; and accuracy is defined by the various attribute labels and testing procedures, so that each panelist will know to which sensation or sensations a given label and method refer (O'Mahony, 1991).

Affective tests

Affective tests measure how well a stimulus of sample is liked (Jellineks, 1964). These tests determine consumer acceptance of a product and consumer preference for a product (Campbell et al., 1979). The affective tests are also called consumer tests; they are often used for market testing of new products as pilot tests before large scale production. The use of an untrained panel to evaluate samples avoids the technical terminology of product attributes used by trained panels and predicts the new product's acceptability in the potential market. The panels for affective tests are usually untrained; however, Cardello et al. (1982) reported that panelists with some training had better discriminative and acceptability judgments of products.

Affective tests assess the personal response (preference or acceptance) and predict potential customers of a product (Meilgaard et al., 1987). These tests are the first steps to prototype production and putting the new products into the market. Affective tests can be divided into preference tests and acceptance tests. Affective tests confirm that a new product's characteristics do confer the expected advantages over the competition in the potential market, review new components to ensure the desired characteristics succeed to large-scale production, and determine the degree of success by competition as it tries to catch up (Moskowitz, 1988).

Acceptance tests are used to determine the "affective status" of a product such as how well it is liked by consumers. A tool often used to measure the degree of

unacceptance or acceptance of a product is the hedonic scale (Figure 14). In hedonic measures, there are three types of scales in common use: 5-point scales, 7-point scales, and 9-point scales.

Scaling and ranking

Scaling and ranking tests use analytical/descriptive procedures to measure degree or compare the sensory attributes and attitudes. These tests are widely used in the food industry for quality control, new product development, and research (Muller, 1977). A scale used in rating and scoring is a continuum divided into spaced successive values. The scales may be unipolar (zero at one end) or bipolar (opposite attributes at each end). Scaling techniques involve the use of numbers or words to express the intensity of a perceived attribute (i.e., sweetness, hardness, or smoothness) in sensory evaluation (Meilgaard et al., 1987). The scales have different levels of measurement that can be classified into four divisions: nominal, ordinal, interval, and ratio. Three types of scales are in common use: category scaling-ordinal data, linear scaling-interval data, and magnitude estimation scaling-ratio data.

Ranking is defined as a method when samples are presented at the same time and must be arranged in order of intensity or degree of some designated attributes. Ranking is only an ordinal process but gives no information on the magnitude of difference. Baker (1962) suggested that ranking is best used when differences between samples are large; and a speedy assessment of many samples can be tested. The advantage of ranking is to offer a rapid and easy method for untrained panels, and it is often used as a part of the preliminary training and selection of panels (Anonymous, 1981). Both scaling and ranking tests are sometime combined with affective testing when panelists are asked to

include a measurement of liking or intent to purchase while doing scaling tests (Meilgaard et al., 1987), or when they are asked to rank samples in order preference.

Electronic microscopy

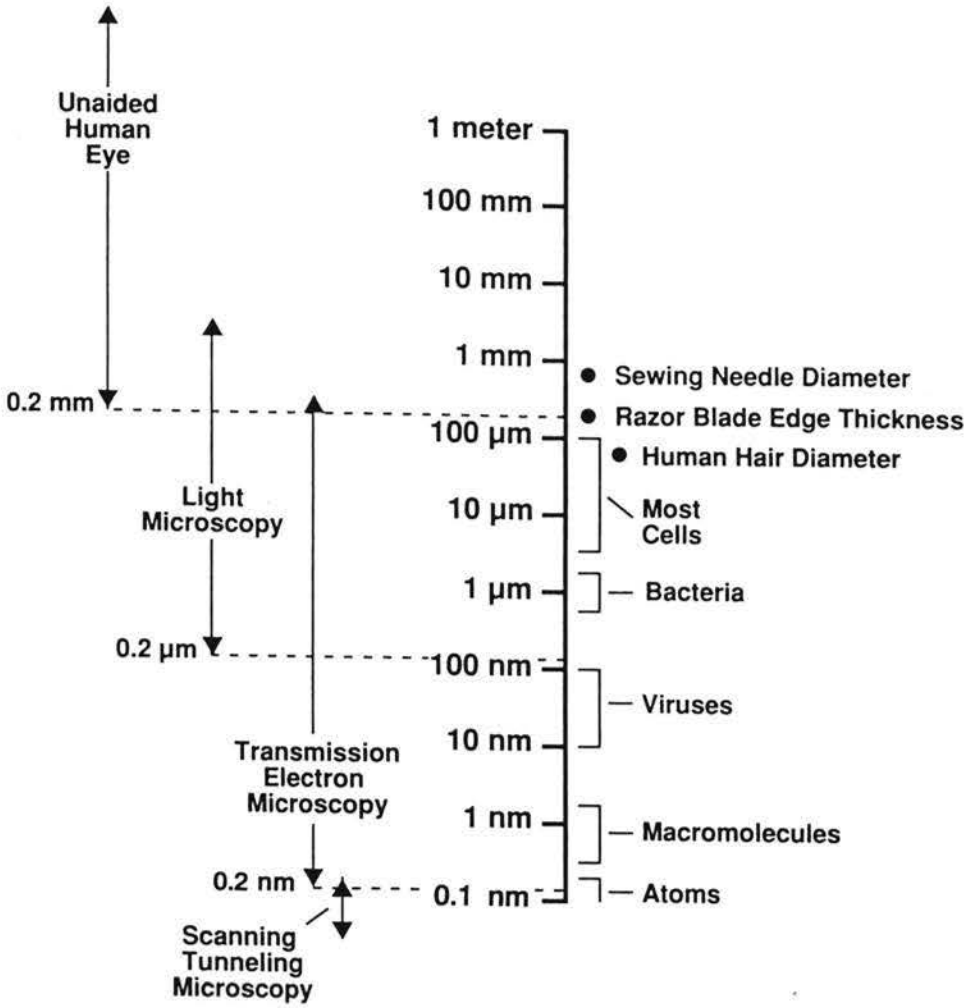
Before 1930, scientists used light microscopy to see the microstructure of a specimen, but some substructures could not be seen clearly (Bozzola and Russell, 1992). Further, they found that as magnification increased, resolution decreased. In 1932, Knoll and Ruska, in Germany invented the first electron microscopy. Since then, scientists have used the electron microscopy intensively to investigate the microstructure of objects. The electron microscope has tremendously influenced our understanding of tissue microstructure. This has given us the ability to visualize molecules and even atoms (Figure 15). There are two basic types of instruments used for electron microscopy (Figure 16): the transmission electron microscope (TEM) and the scanning electron microscope (SEM).

Electron microscopy for food microstructure

Electronic microscopes have been used to investigate the structure of food systems for years, especially in product development. The TEM allows researchers to see through a sample slice to image food microstructure; with SEM they see the three-dimensional view. In 1979, Resmini investigated pasta structure by using electron microscopy. His research provided the basis for illustrating the microstructure of pasta. Tokuya et al. (1991) used the electron microscope to study ultrustructure of polysaccharides in gels used in foods. This research showed that different polysaccharides formed different gel structures. Therefore, using the electron microscope can help us to further realize the functional

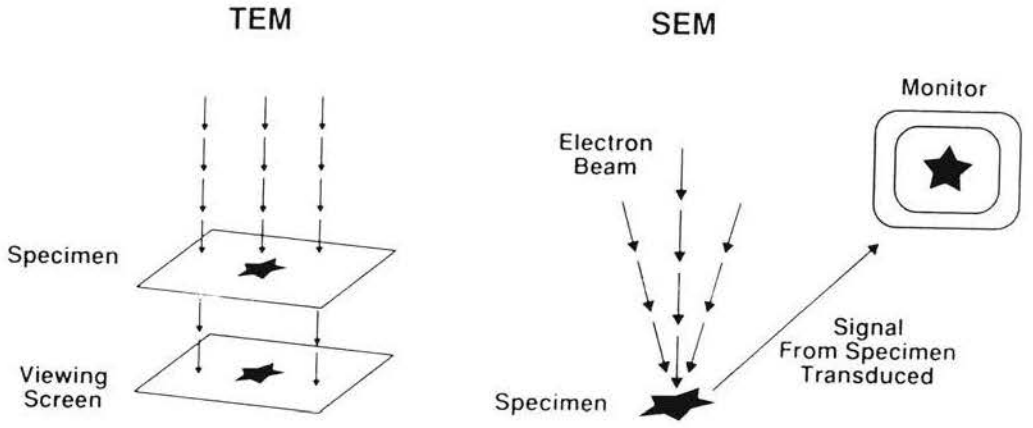
used the electron microscope to study ultrustructure of polysaccharides in gels used in foods. This research showed that different polysaccharides formed different gel structures. Therefore, using the electron microscope can help us to further realize the functional

Figure 15. The range of resolving power



The range of resolving power of various magnifying tools (left), and the structures they are capable of resolving (right). (Electron Microscopy, p. 6. Bozzola and Russell, 1992)

Figure 16. Two basic types of electronic microscopy



The basic difference between (TEM) transmission and (SEM) scanning electron microscopes. (Electron Microscopy, p. 8. Bozzola and Russell, 1992)

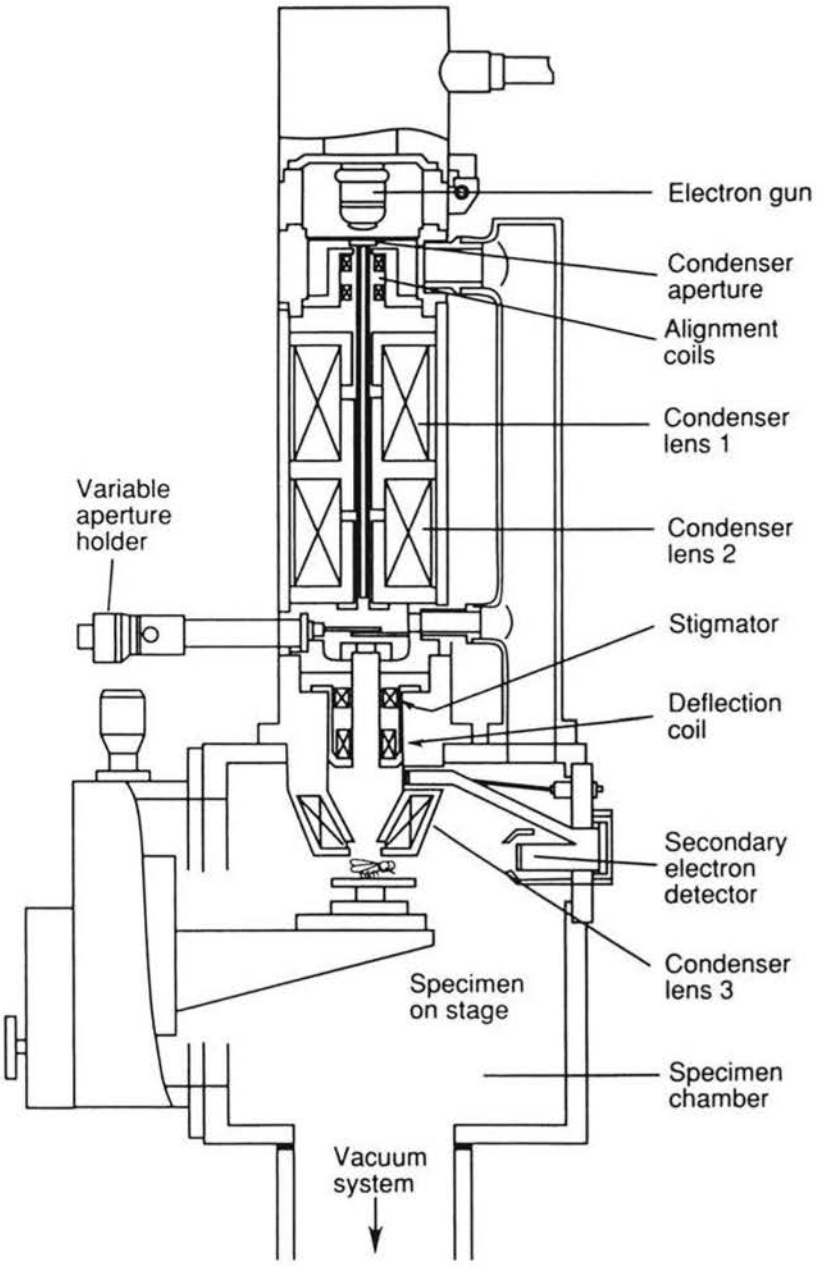
properties of non-gluten products by viewing microstructure images and to explain the behaviors of food systems (Bottcher and Foegeding, 1994).

Scanning electronic microscopy (SEM) for food microstructure

SEM is a useful instrument for studying the three dimensional structure of a variety of food materials. Since commercial SEM became available in 1965, the three dimensional microstructure has been used to explain and predict the physical and functional properties of food materials. SEM also shows changes in microstructure due to different ingredients and processing methods. The SEM produces an image that gives a three dimensional view of a object (Bozzola and Russell, 1992). This type of electron microscopy uses a 2 to 3 nm "spot" of electrons to scan or hit the surface of an object which causes secondary electrons to be ejected from the object's surface. A sensor detects the secondary electrons, and an image is produced over time as the entire object is scanned (Figure 17). The stereostructure and geometry of materials shown by SEM has been used as the basis for the selection and identification of potential food use resources and for process optimization and quality evaluation of manufactured food (Lee and Rha, 1979)

Textural properties of many foods are important in consumer acceptance. Moss et al. (1987) used a scanning electron microscope to investigate the effect of different ingredients on the textural properties on instant noodles. The microstructure image showed the three dimensional gel structures formed by interaction of protein and starch. The SEM also was used to understand the influence of the extrusion process on pasta structure (Pagani et al., 1989 and Evans et al., 1981).

Figure 17. Detailed diagram of column of standard SEM



Electron Microscopy, p. 190. Bozzola and Russell, 1992

Scanning electronic microscopy for non-gluten pasta

Evans et al. (1981) used SEM to reveal the microstructure of the gluten-starch complex in gluten containing products. The image showed how gluten interacts with starch in a matrix. The scanning electron microscope could be used to investigate microstructure and visualize starch, non-starch polysaccharides, and non-gluten protein interaction in non-gluten pasta. However, no study was found that used SEM to study the microstructure of non-gluten pasta. For non-gluten products, it is very important to know how gums interact with starches when mixing them together and what a gel structure contributes to texture of food after cooking. The microstructure image could help food scientists explain the physical behaviors, textural characteristics, and structure of non-gluten pasta related to sensory attributes. Those interactions might help explain the chemical, physical, and sensory characteristics of non-gluten pasta.

Using response surface methodology in mixture experiments for food product development

Response surface methodology

Response surface methodology (RSM) is a collection of statistical and mathematical techniques useful for product development and improvement and for process optimization (Lee and Hoseney, 1982; and Owusu et al., 1982). RSM has important uses in the design, development, and formulation of new products, as well as in the improvement of current products (Myers and Montgomery, 1995). Components of food products or process development systems include minimizing cost, maximizing profits, reducing the use of expensive ingredients or the use of additives and preservatives

without compromising consumer safety, and increasing desirable characteristics (Henika, 1972; and Stanyon and Costello, 1990). The formulation of a new product or the improvement of an old one, and the development of a new process or the optimization of an existing one; can be better understood using response surface methodology (Floros and Chinnan, 1988).

For food scientists, response surface methodology provides many benefits to different fields in food science (Henika, 1982). Ylimaki et al. (1991) used response surface methodology to develop new gluten-free breads and optimized the formula for gluten-free breads based on sensory qualities. The response surface methodology has been used to help researchers to optimize the desired crispness texture of banana chips to meet consumers' acceptance by investigating different blanch temperatures and times (Jackson et al., 1995).

Approximating response functions

In food science research, food scientists are concerned with a product response (Myers and Montgomery, 1995), y [dependent variable such as tenderness], that depends on the factors, $\varepsilon_1, \varepsilon_2, \ldots\ldots\ldots \varepsilon_k$ [independent variables, temperature, ingredient, etc.]. According to Myers and Montogomery (1995, p.3), the relationship is:

$$`y = f(\varepsilon_1, \varepsilon_2, \ldots \varepsilon_k) + e$$

where the true response function "f" is unknown and "e" is an error term that represents other sources of variability not accounted for in "f". We assume "e" has a normal distribution with mean zero and variance σ^2*, then*

$$E(y) = \delta = E[f(\varepsilon_1, \varepsilon_2, \ldots \varepsilon_k)] + E[e]$$

$$\delta = [f(\varepsilon_1, \varepsilon_2, \ldots \varepsilon_k)]$$

where E [e] = 0.

The variables $\varepsilon_1, \varepsilon_2, \ldots \varepsilon_k$ *are usually called the natural variables. While applying response surface methodology, they are transformed into coded variables* X_1 X_2....X_k. *The true function becomes*

$$\delta = [f(X_1, X_2, \ldots X_k)]'$$

Because the true response function "δ" is unknown, it is necessary to develop a suitable approximation for the function "δ". Myers and Montgomery (1995) suggest some models: first-order model (Figure 18), first-order model with interaction (Figure 19), and second-order model (Figure 20).

Mixture experiments

A mixture experiment is a special type of response surface experiment where the factors are the ingredients (by weight or volume) of a mixture, and the response is a function of the proportions of each factor (Myers and Montgomery, 1995). These proportionate amounts of each factor are typically measured by weight or volume, and their sum is equal to 1.

The development of new products involving more than one ingredient (factors) requires the design of a mixture experiment, as opposed to a factorial experiment (Hare, 1974). Because the total amount of a food product is fixed, each factor is not independent; if one of the components changes, the others will change (Won et al., 1997). In a factorial experiment, each factor is independent. As one of the factors changes, the

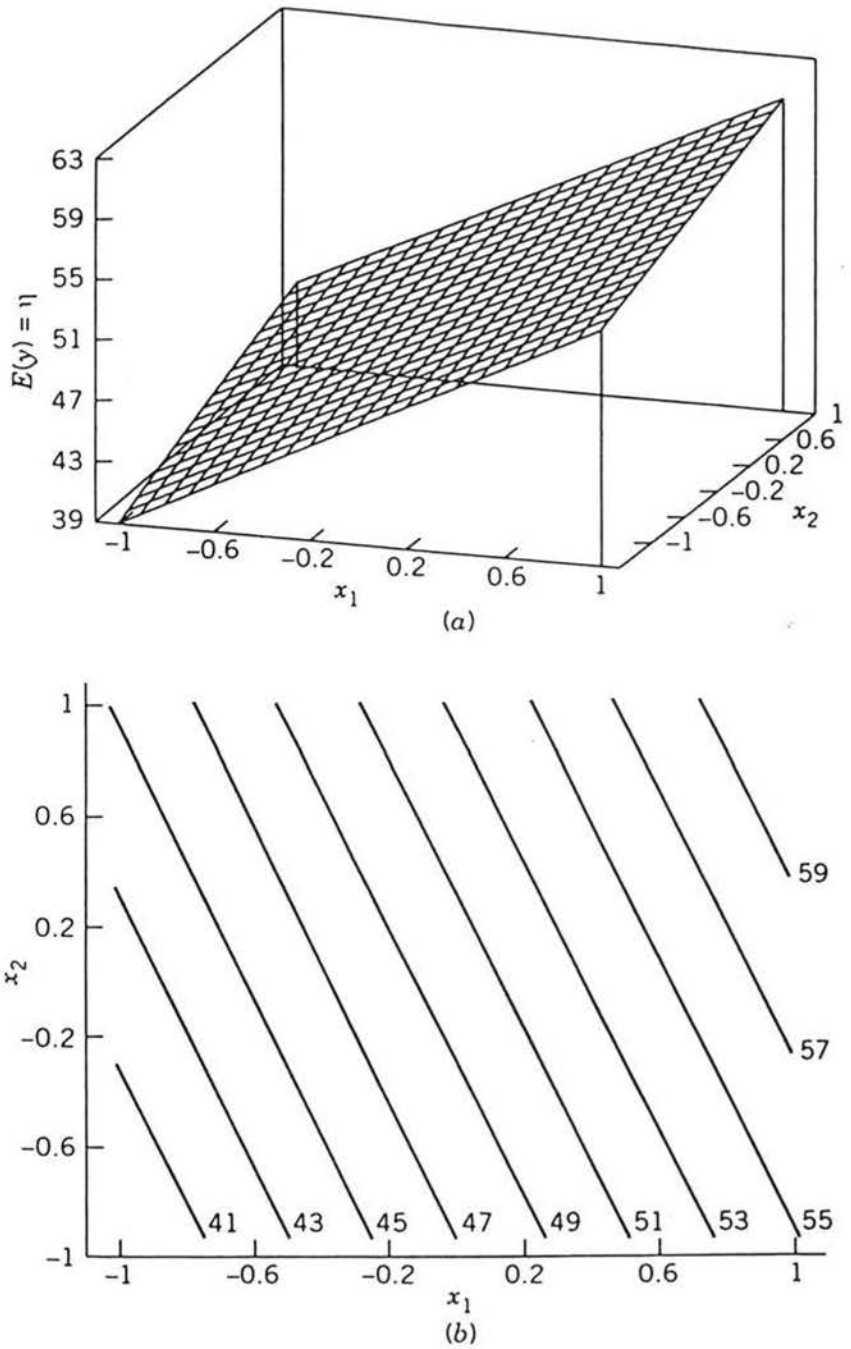
others will not be affected. According to Cornell (1990, p.4), the definition of a mixture experiment is:

> *'The change in the response is assumed to depend only on the relative proportion of the ingredients present in the mixture and not on the amount of the mixture.'*

According to Myers and Montgomery (1995), in a factorial experiment, the response change is measured when one or more of the factor levels are changed while the levels of the other factors are fixed. The change in the response is affected not only by the levels of factors but also by the total amount. Since the total amount in the mixture experiment does not change (always = 1), there are no total amount changes to affect responses (Cornell, 1990).

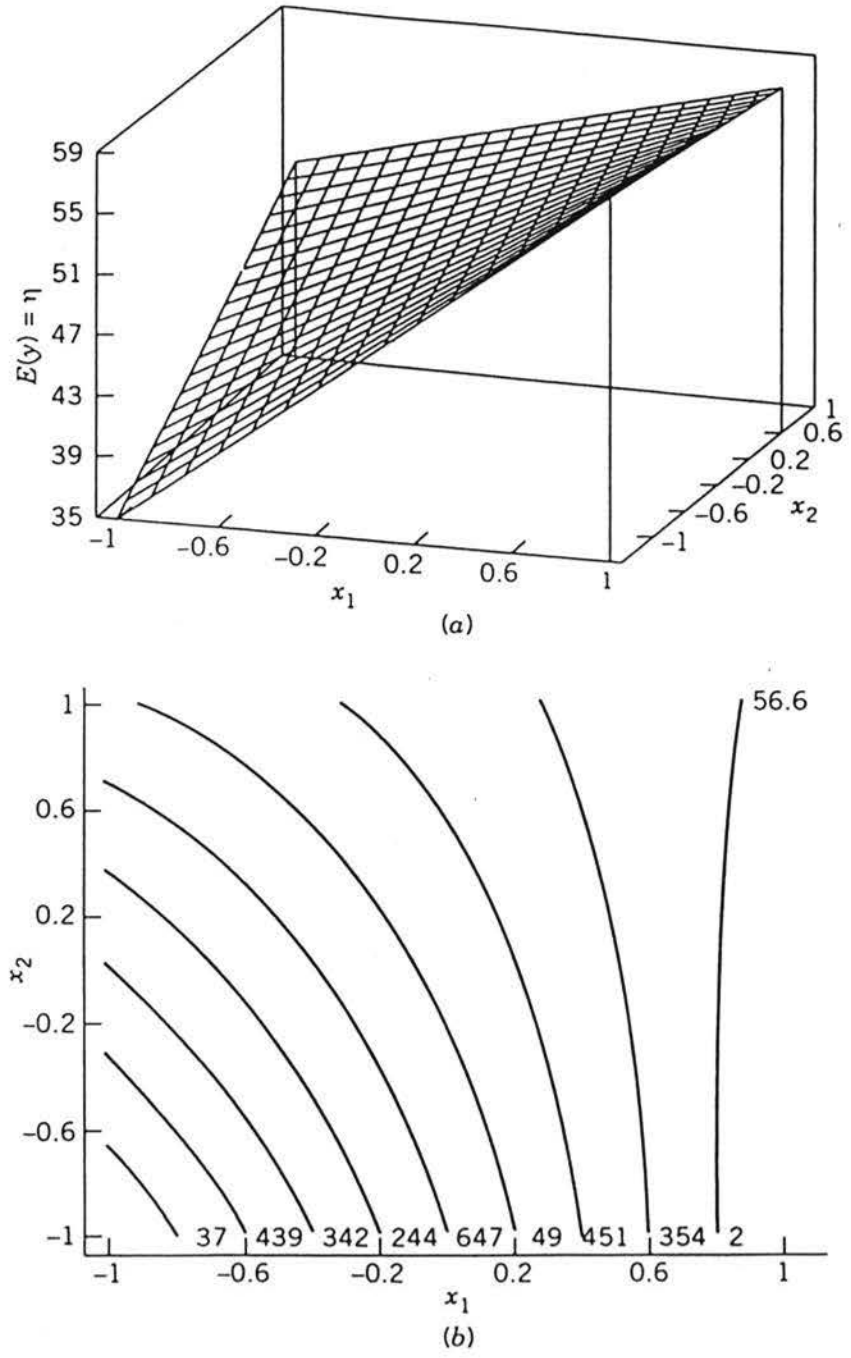
In food science research, most often the amount of a mixture is fixed (Prinyawiwatkul et al., 1997). Therefore, a mixture experiment provides more precise detail about how each ingredient affects responses than the factorial experiment. Arteaga et al. (1993) used a mixture experiment to investigate the interaction of protein functionality among ingredients. Prinyawiwatkul et al. (1997) applied response surface methodology in a mixture experiment to investigate physicochemical properties such as fat content, moisture loss, color changes, and sensory properties of flavor and texture for chicken nuggets extended with fermented cowpea and peanut flours.

Figure 18. Response surface for the first-order model (a) and contour plot (b)



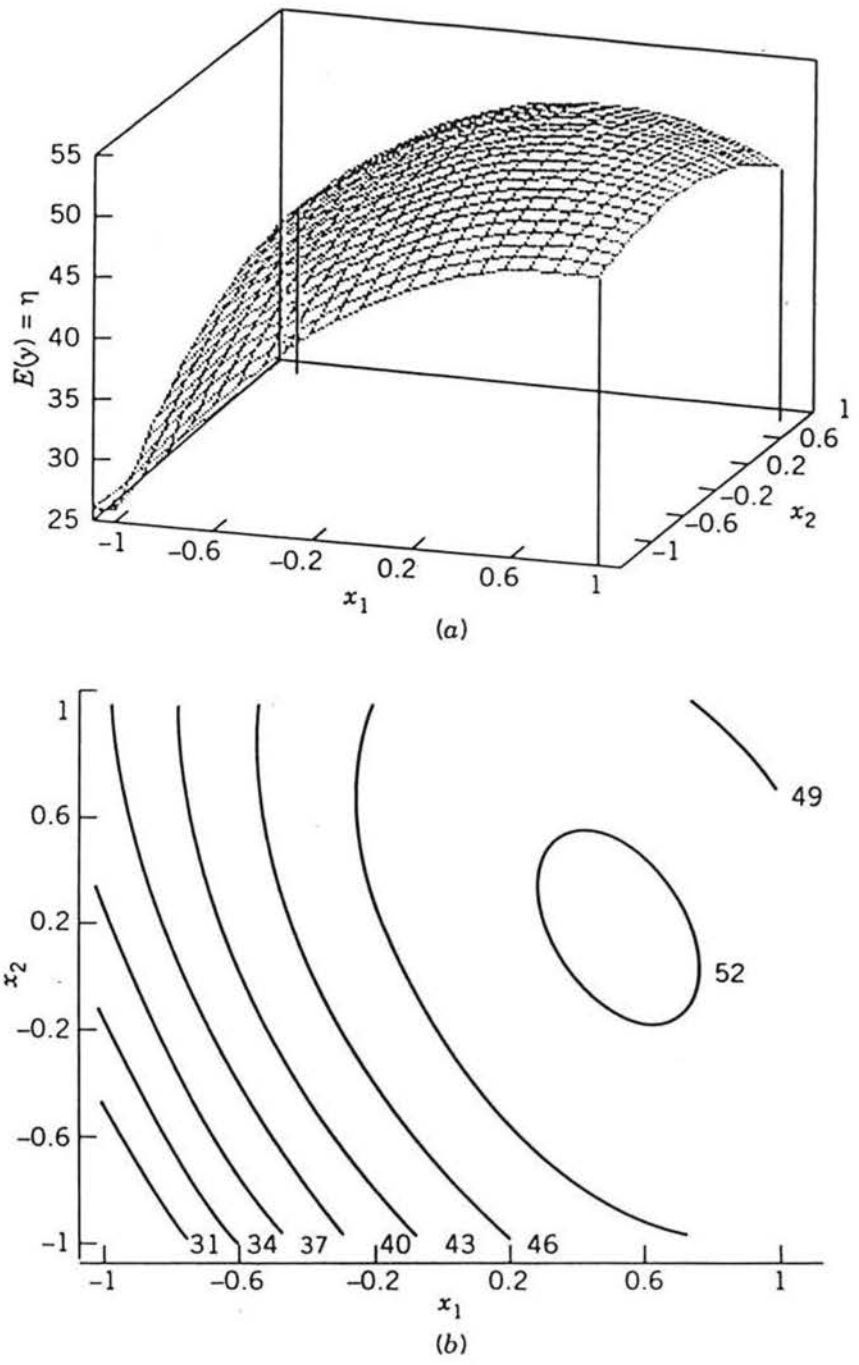
Model $\eta=50+8X_1+3X_2$. (Response Surface Methodology, p. 5. Myers and Montgomery, (1995).

Figure 19. Response surface for the first-order model with interaction (a) and contour plot (b)



Model $\eta=50+8X_1+X_2-4X_1X_2$. (Response Surface Methodology, p. 6. Myers and Montgomery, 1995).

Figure 20. Response surface for the second-order model (a) and contour plot (b)



Model $\eta=50+8X_1+3X_2-7X_1^2-3X_2^2-4X_1X_2$. . (Response Surface Methodology, p. 8. Myers and Montgomery, 1995).

For a mixture experiment, components (factors) always add up to 1: $X_i \geq 0$, $X_1 + X_2 + X_3 + \text{--------} + X_q = 1.0$. That is:

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

where q is the number of components in the mixture. However, in many mixture experiments there are constraints on the component proportions (Myers and Montgomery, 1995). These are often upper- and / or lower-bound constraints of the form $L_i \leq X_i \leq U_i$, i = 1, 2, ……,q, where L_i is the lower bound, $L_i > 0$ and U_i is the upper bound, $U_i < 1$ for the i th component. The general form of the constrained mixture is:

$$X_1 + X_2 + X_3 + \text{--------} + X_q = 1.0$$

$$L_i \leq X_i \leq U_i, \quad i = 1, 2, \ldots\ldots, q$$

Where $L_i \geq 0$ and $U_i \leq 1$ for i = 1, 2, ……, q.

Just like a factorial experiment, mathematical models are used to analyze the data generated in a mixture experiment (Cornell, 1990). For example, a first order regression model for an experiment is:

$$E(y) = \beta_0 + \sum_{i=1}^{q} \beta_i X_i$$

where E(y) is the expected response value, the β_i's are the estimated coefficients, and the X_i's are the independent variables (factors). As this regression model is applied to a mixture experiment (Cornell, 1990), the model becomes:

$$E(y) = \beta_0(X_1 + X_2 + X_3 + \text{---------} + X_q) + \sum_{i=1}^{q} \beta_i X_i$$

$$= \sum_{i=1}^{q} \beta_i^* X_i$$

where $\beta_i^* = \beta_0 + \beta_i$

Scheffe' (1958) suggested and Snee (1971) presented the following set of models for mixture experiment data:

Linear:

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

Quadratic:

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

Special cubic:

$$E(y) = \sum_{i=1}^{q} \beta_i X_i + \sum\sum_{i \leq j}^{q} \beta_{ij} X_i X_j + \sum\sum\sum_{i \leq j \leq k}^{q} \beta_{ijk} X_i X_j X_k$$

Full cubic:

$$E(y) = \sum_{i=1}^{q} \beta_i X_i + \sum\sum_{i \leq j}^{q} \beta_{ij} X_i X_j + \sum\sum_{i \leq j}^{q} \beta_{ij} X_i X_j (X_i - X_j) + \sum\sum\sum_{i \leq j \leq k}^{q} \beta_{ijk} X_i X_j X_k$$

where "q" is the total number of components in the mixture.

In the mixture experiment, each ingredient's proportionate value (X) can be chosen from 0 to unity (1), and all blends among ingredients are possible. The simplex-lattice design was introduced by Scheffe' (1965) to determine points in the blend, with the simplex as a uniformly spaced set of points on a lattice. The notation "{q, m}" was used and implied a simplex-lattice design in "q" components that supported a mixture polynomial of degree "m" (Cornell, 1990).

The number of points in a {q, m} simplex lattice design is:

The number of points in a {q, m} simplex lattice design is:

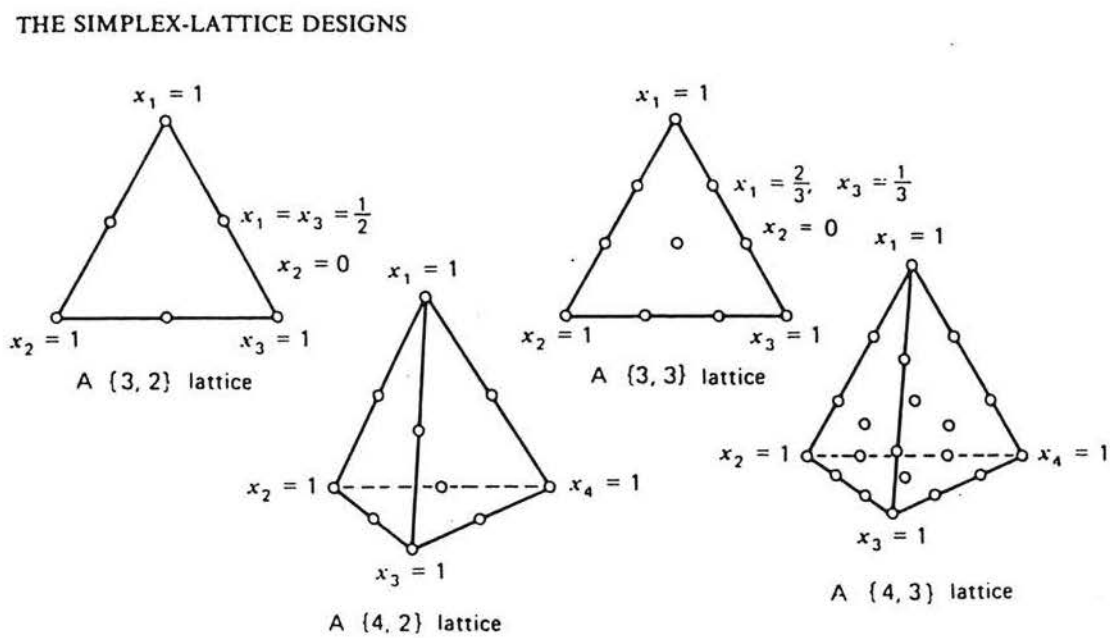
$$N = (q + m - 1) / m!(q - 1)!$$

The proportion taken on by each component is the m+1 equally spaced values from 0 to 1:

$$X_i = 0, 1/m, 2/m, \ldots\ldots, 1, \qquad i = 1, 2, \ldots\ldots..q,$$

and all possible combinations (mixtures) from this equation are used. For example (Figure 21), let q=3 and m=2, {3,2}; and q=3 and m=3, {3,3}.

Figure 21. The simplex-lattice designs



Examples of {3,m} and {4,m} simplex-lattice arrangements, m=2 and m=3. (Response Surface Methodology, p. 540. Myers and Montgomery, 1995).

Non-gluten products

A variety of starch and non-starch polysaccharides have been used in many non-gluten products to replace wheat flour. However, researchers reported that non-gluten flour products were less desirable in taste, texture, color, and product variety because of lacking gluten (Ylimaki, 1989). For example, non-gluten bread without wheat flour had less volume and was tougher in texture, and cakes had a heavy layer on the bottom of cake with less volume and less tenderness (Ylimaki et al., 1988). These reactions were explained by Abecassis et al. (1989) who stated that gluten in wheat flour plays a major role in sensory characteristics in wheat flour products. Gluten, a protein complex, contributes viscoelasticity that can entrap the CO_2 in baked products and bind the starch structure making it more cohesive. Without gluten, pasta has a weak structure and disintegrates during boiling.

For years, researchers applied different starches, flours, and non-starch polysaccharides to non-gluten products and showed increased desirability in the functional properties and sensory characteristics. Toufeili et al. (1994) used methylcellusloe, gum Arabic, and egg albumen to improve gluten-free pocket-type flat breads. Methylcelluloses and egg albumen significantly improved sensory acceptability.

Pasta

Pasta is an ancient food, made from wheat flour or durum wheat flour (Donnelly, 1991). The durum wheat flour gives pasta a light yellow color and contains a very high proportion of gluten, compared to most other wheats. The gluten formation contributes two main functions: (a) dough development during mixing and extrusion; (b) preventing disintegration of pasta during drying and boiling (Feillet, 1984). Dick (1985) reported

that a high gluten content provided a higher quality of pasta with a rubbery or slightly elastic structure and less cooking loss.

Non-gluten pasta

Gluten in pasta is what contributes the desired textural characteristics. Nevertheless, celiac patients can not ingest gluten-containing products. Therefore, wheat or durum wheat can not be used as a ingredient for pasta for these people. But non-gluten pasta has less rubber texture in sensory characteristics. Over the years researchers reported that non-starch polysaccharide polymers were successfully added to non-gluten products such as bread or cake to replace gluten (Ylimaki et al., 1991). Edwards et al. (1995) reported that xanthan gum can be used in whole wheat pasta to enhance the pasta texture. Gums mixed with other non-gluten starches and flours affected gelatinization in cereal-based products (Ferrero et al., 1993). Non-starch polysaccharides such as xanthan gum and locust bean gum have very significant viscoelastic properties and perhaps could be used to mimic properties of gluten to form a rubbery texture of pasta. There were few if any reports of the use of xanthan and locust bean in non-gluten pasta. Therefore, more research is needed in this area.

CHAPTER III

METHODOLOGY

This research was composed of four parts. Part I included preparation of non-gluten pasta and measurement of the physical and chemical properties. Part II included a semi-trained panel that conducted an analytical/descriptive sensory evaluation of pasta. Part III was a study of using a scanning electron microscope to investigate pasta microstructure. Part IV included a consumer panel conducting an acceptance test of pasta for optimum formula.

Part I

Materials

The non-gluten pasta formulas contained seven different polysaccharides (Table 1); five were the independent variables in the research: locust bean gum (TIC GUMS, Inc.), xanthan gum (Kelco, Inc.), modified potato starch (Staley, Inc.), tapioca starch (Staley, Inc.), and potato starch (Staley, Inc). The other two ingredients, yellow corn flour (Shawnee Milling, Co.) and rice flour (Erawan, Co.), were the fixed variables.

Experimental design

This research employed a mixture experiment with five-component constraints, so that the mixture components were locust bean gum (X_1), xanthan gum (X_2), modified starch (X_3), tapioca starch (X_4), and potato starch (X_5) with the corn and rice flours. Based on preliminary tests, the following amounts were chosen for each independent variable:

Independent variables:

locust bean gum (X_1): 10, 25, and 40g

xanthan gum (X_2): 25 and 40g

modified starch (X_3): 30, 35, and 40g

tapioca starch (X_4): 63.35, 66.65, 70.00, 73.35, 76.50, 80.00, 83.40, 86.50, and 90.00g

potato starch (X_5): 31.67, 33.35, 35.00, 36.67, 38.35, 40.00, 41.67, 43.35, and 45.00g

Fixed variables:

corn flour: 250g

rice flour: 50g

The ingredient amounts were selected according to the constraint, $X_1+X_2+X_3+X_4+X_5$ + corn flour + rice flour = 500g (100%). The ratio of tapioca starch to potato starch was held at 2 to 1. Multiple regression analysis was used to fit the model:

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

where y is a measured response.

The treatment structure consisted of 15 combinations. The treatment combinations are shown in Table 1. The design structure was an incomplete block design. The treatment combinations were arranged for each block as shown in Table 2. Each treatment was replicated twice except for Treatments 8 and 11 which were replicated four times (Table 2).

Pasta preparation

Each pasta formula was blended with 330-350g distilled water in a single screw pasta mixer/extruder for 15 min. Pasta was extruded through a 1.5-mm noodle shape die (ABC, Inc., Model D-45 S.H.).

Drying

Fresh pasta was dried at a controlled temperature of 90°C for 5 hours in a food dehydrator (Alternative Pioneering Systems, Inc., Model FD-300T).

pH measurement

To determine the pH of pasta, a 10g sample of fresh pasta was blended with 100 ml distilled water in a blender for 2 min. A Fisher Accument pH meter (Model 610A) was used to measure pH at 30°C.

Heat treatment

The 15g sample of dried pasta was boiled in 1000g tap water for 13 min. Salt (0.5g) was added to increase boiling temperature.

Color measurement

The Minolta chromameter (Minolta, Co., Model-CR-200) was used to measure L*, a*, and b* values. Illuminant C light was used. Color of fresh, dried, and cooked

pasta was measured. Lightness was expressed as L*, the red index as a*, and the yellowness as b*.

Table 1. Experimental treatment structure of the 15 formulas (grams)

Trt	LBG	XG	MS	TS	PS	CF	RF
1	10	25	30	90.00	45.00	250	50
2	10	25	35	86.50	43.35	250	50
3	10	25	40	83.40	41.67	250	50
4	10	40	30	80.00	40.00	250	50
5	10	40	35	76.50	38.35	250	50
6	10	40	40	73.35	36.67	250	50
7	25	25	30	80.00	40.00	250	50
8	25	25	35	76.50	38.35	250	50
9	25	25	40	73.35	36.67	250	50
10	25	40	30	70.00	35.00	250	50
11	25	40	35	66.65	33.35	250	50
12	25	40	40	63.35	31.67	250	50
13	40	25	30	70.00	35.00	250	50
14	40	25	35	66.65	33.35	250	50
15	40	25	40	63.35	31.67	250	50

Trt-treatment, LBG-locust bean gum, XG-xanthan gum, MS-modified starch, TS-tapioca starch, PS-potato starch, CF-corn flour, RF-rice flour.

Table 2. Experimental design structure of 15 treatments in 6 incomplete blocks (grams)

Variable		X1 LBG	X2 XG	X3 MS	X4 TS	X5 PS	--- CF	--- RF
Block 1	Trt 1	10	25	30	90.00	250	50	45.00
	Trt 6	10	40	40	73.35	250	50	36.67
	Trt 8	25	25	35	76.50	250	50	38.35
	Trt 9	25	25	40	73.35	250	50	36.67
	Trt 11	25	40	35	66.65	250	50	33.35
	Trt14	40	25	35	66.65	250	50	33.35
Block 2	Trt 2	10	25	35	86.50	250	50	43.35
	Trt 4	10	40	30	80.00	250	50	40.00
	Trt 7	25	25	30	80.00	250	50	40.00
	Trt 12	25	40	40	63.35	250	50	31.67
	Trt 15	40	25	40	63.35	250	50	31.67
Block 3	Trt 3	10	25	40	83.40	250	50	41.67
	Trt 5	10	25	40	83.40	250	50	41.67
	Trt 8	25	25	35	76.50	250	50	38.35
	Trt 10	25	40	30	70.00	250	50	35.00
	Trt 11	25	40	35	66.65	250	50	33.35
	Trt13	40	25	30	70.00	250	50	35.00
Block 4	Trt 1	10	25	30	90.00	250	50	45.00
	Trt 6	10	40	40	73.35	250	50	36.67
	Trt 8	25	25	35	76.50	250	50	38.35
	Trt 9	25	25	40	73.35	250	50	36.67
	Trt 11	25	40	35	66.65	250	50	33.35
	Trt14	40	25	35	66.65	250	50	33.35
Block 5	Trt 2	10	25	35	86.50	250	50	43.35
	Trt 4	10	40	30	80.00	250	50	40.00
	Trt 7	25	25	30	80.00	250	50	40.00
	Trt 12	25	40	40	63.35	250	50	31.67
	Trt 15	40	25	40	63.35	250	50	31.67
Block 6	Trt 3	10	25	40	83.40	250	50	41.67
	Trt 5	10	25	40	83.40	250	50	41.67
	Trt 8	25	25	35	76.50	250	50	38.35
	Trt 10	25	40	30	70.00	250	50	35.00
	Trt 11	25	40	35	66.65	250	50	33.35
	Trt13	40	25	30	70.00	250	50	35.00

LBG-locust bean gum, XG-xanthan gum, MS-modified starch, TS-tapioca starch PS-potato starch, CF-corn flour, RF-rice flour.

Shear and compression force

A TG4C texture gauge (Food Technology, Co., Model FTA-1000) measured shear/compression force of pasta using the shear/compression cell. Thirty grams of cooked pasta were placed into the cell and pressed at speed 5 with the blade penetrating the cell. The peak force (lbs.) was recorded.

Cooking gain (water absorption)

The 15g of dried pasta were cooked for 13 min and drained for 1 min. The percent water gain was calculated by the following formula:

$$\% \text{ Cooking gain} = \frac{[\text{cooked pasta weight} - \text{dried pasta weight}]}{\text{dried pasta weight}} \times 100$$

Part II

Panel selection and training

The sensory panel was selected from Oklahoma State University students, staff, faculty, and other Stillwater residents. Before they became actual panelists, each person was tested for the ability to identify the four basic tastes, sweet, sour, salt, and bitter (Appendix C). Twenty panelists, after screening, were trained for three hours to identify these sensory attributes: smoothness of surface, hardness of first bite, adhesiveness of chew down, cohesiveness of chew down, and off-flavor (Appendix D and E). First the panelists assigned intensity values to the reference standards and control pasta (regular gluten containing pasta) through discussion and consensus. The

intensity values were assigned to reference standards and the control pasta by making a horizontal line on a numerical scale (0-10). Second, the panelists practiced evaluating sample intensities against reference standards and the control pasta. A control pasta was used as the comparison for each attribute. After training, the panel evaluated the samples.

The definition of each attribute and the evaluation procedure is provided in Appendix D. Some reference standards were obtained from Spectrum Intensity Scales (Meilgaard et al., 1991). Jello (Kraft Foods, Inc.) and cereal-Fiber One (General Mills Sales, Inc.) were used as reference standards for smoothness of surface. Cream cheese (Kraft, Inc.) and carrot (Fresh 1 Marketing, Inc.) were used for hardness of first bite. Tomato (Del Cabo, Inc.) and Rice Krispies (Kellogg's, Co.) were used for adhesiveness of chew down. Muffin and chewing gum (Warner-Lambert, Co.) were used for cohesiveness of chew down. The control pasta (Barilla, Co.) and non-gluten slurry were used for off-flavor.

Sensory evaluation form

The panel used a 10-cm scale (0-10) line scale to evaluate non-gluten pasta smoothness of surface, hardness of first bite, adhesiveness of chew down, cohesiveness of chew down, and off-flavor against reference standards and the control pasta (Figure 1).

Sample preparation and testing

Dried pasta was boiled in 1000g tap water for 13 min. Panelists judged the pasta samples made from the formulas given in Table 8. Testing sessions took place over six days. Sessions were held in a room with ambient temperature (25^{o}C), lighting, and minimized environmental sounds and odors. Panelists were apprised of terminology

definitions and procedure at each session (Appendix D). Fresh reference standards and a control pasta were prepared for each session. In the first and fourth blocks, each panelist received six samples (Treatments 1, 6, 8, 9, 11, and 14). In the second and fifth blocks, each panelist received 5 samples (Treatments 2, 4, 7, 12, and 15). In the third and sixth blocks, each panelist received 6 samples (Treatments 3, 5, 8, 10, 11, and 13). Panelists rated each of the tested samples against reference standards and a control pasta. While testing, they were requested to refrain from discussion and to remain within their individual booths. The panelists had an unlimited supply of distilled water and unsalted crackers to rinse their palates between the samples.

Part III

Specimen preparation for scanning electron microscope

Fixation. Cooked pasta was cut into 1 cm^2 for a surface view and 1 x 0.5 cm^2 for a cross-sectional view. Samples were fixed with 1.6% glutaraldehyde in 0.1M cacodylate buffer at 25°C for 2 hrs and rinsed three times in phosphate buffers (20 min/rinse). After the third rinse the buffer was removed and replaced with 1% osmium in 0.1M cacodylate buffers at 25°C for 2 hrs to fix the samples. The samples were then rinsed three more times in phosphate buffer (20 min/rinse). The samples were allowed to stand in the phosphate buffer after the last rinse and then stored at 4°C overnight.

Dehydration. Before dehydration, the phosphate buffer was removed. The samples were dehydrated in ethanol at concentration: 50%, 70%, 90%, 95%, and 100%. The ethanol (100%) dehydration was repeated three times.

Critical point drying. After dehydration, the samples were critical-point-dried (DENTON DCP-1). This technique allowed sample drying without the surface damage that accompanies air-drying. The critical point of a substance is the specific temperature and pressure where the densities of its liquid and vapor phase are equal, resulting in zero surface tension. The gaseous substance can be released from the sample without surface damage. Liquid carbon dioxide is commonly used because its critical point (36.5°C and 1080 p.s.i.) can be conveniently reached with a single apparatus to dry samples. Dried samples were mounted on stubs and put into the desiccator before gold coating.

Gold coating. Samples were placed into a Hummer II (Technics, Inc., Alexandria, VA) machine for gold coating. Each sample was coated for three minutes and thirty seconds. After coating, the gold-coated specimens were placed in a desiccator to prevent moisture absorption.

Sample scanning

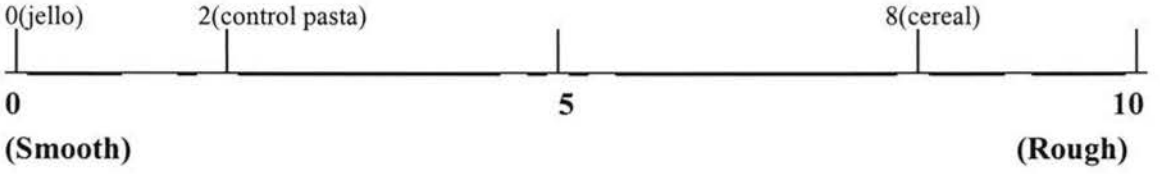
Samples were observed in a JEOL (JSM-35, JEOL LTD., Japan) scanning electron microscope at an acceleration voltage of 25 KeV. Micrographs of the surface and the cross-sections of each sample were taken at magnification of 50X and 1000X for microstructure view.

Figure 1. Sensory evaluation form

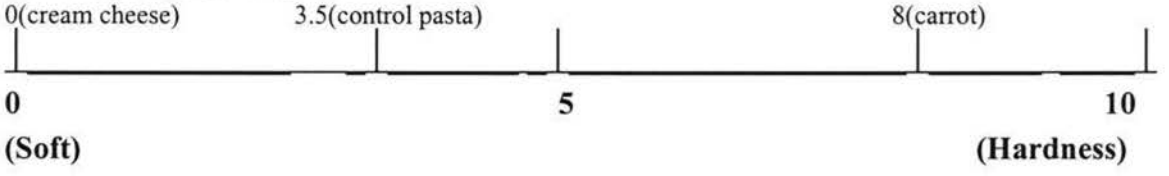
Panelist code: _____

Product: Non-Gluten Pasta

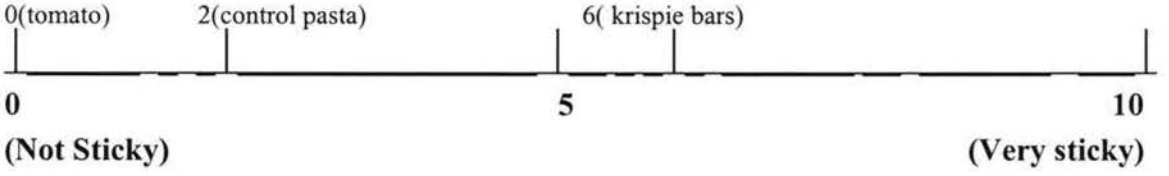
- **Surface**



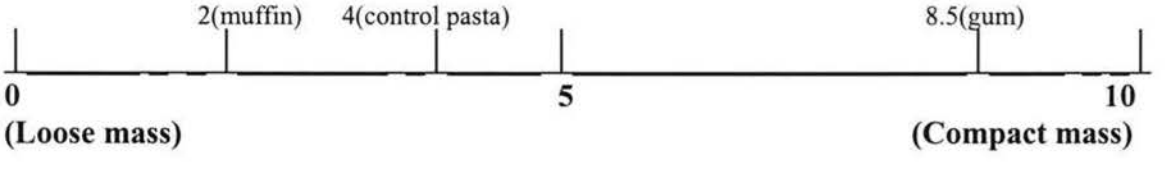
- **Hardness of first bite**



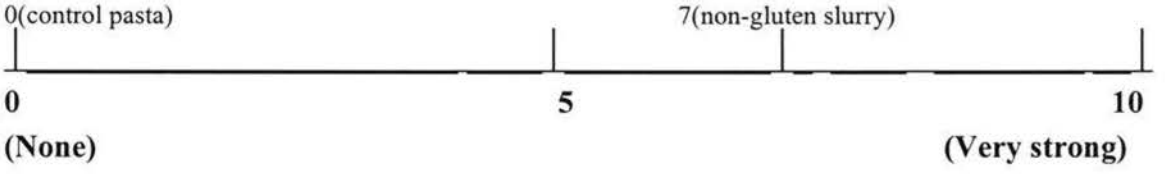
- **Adhesiveness of chew down**



- **Cohesiveness of chew down**



- **Off-flavor**



Part IV

This part of the research tested the validity of the mixture experiment by determining the acceptability of a formula predicted by the fitted model against a control pasta (regular gluten containing pasta) and a pasta made from a previous non-gluten pasta formula.

Optimum regions

The optimum regions were determined by overlapping the contour plots of the physical and sensory properties for non-gluten pasta and comparing with the control pasta.

Formula selection

Three possible non-gluten pasta formulas were selected from the optimum regions by calculating the functions and response of each property. These non-gluten pasta formulas were selected by sorting the most important to the least important properties compared to the control pasta. Each formula was replicated three times and tested for validation (consistency).

Final formula selection

After validation testing, a final formula that possessed the most desirable properties was chosen. Acceptance testing was conducted with this final formula of non-gluten pasta, a previous non-gluten pasta formula, and a control pasta. Appearance, texture, and overall acceptance were tested.

CHAPTER IV

FUNCTIONAL PROPERTIES AND ELECTRON MICROSCOPY OF NON-GLUTEN PASTA USING RESPONSE SURFACE METHODOLOGY WITH MIXTURE EXPERIMENT

ABSTRACT

Non-gluten products are essential for celiac patients and others who are gluten sensitive to replace their gluten-containing foods. Non-starch and starch polysaccharides were investigated using Response Surface Methodology with mixture experiment. Peak force for non-gluten pasta had the highest score at the level of 40g of locust bean gum, 40g of modified starch, and 40g of xanthan gum. For 25g and 40g of xanthan gum peak forces were greater than that of the control (gluten) pasta (76.5) but only if locust bean gum levels were 35g and modified starch was 36g. The peak force was the lowest when locust bean gum was 10g, modified starch was 30g, and xanthan gum was 40g. Cooking gain was enhanced by xanthan gum when the locust bean gum was at 40g and the modified starch at 30g. The mean cooking gain for the non-gluten pasta was more than 200% higher than the control pasta. The cooking gain was greater than 250% when the level of locust bean gum was above 35g, modified starch above 31g, and xanthan gum at 40g. Micrographs showed the matrix structures of the non-gluten pastas were different from the control pasta. Within the non-gluten pastas, the matrices of those with higher

levels of xanthan and locust bean gums appeared denser and also had higher peak force readings and these were more similar to the matrix appearance of the control pasta. The peak force measures of the higher gum pastas were also more similar to the control pasta.

INTRODUCTION

Food sensitivity is an adverse food reaction defined as any untoward reaction following the ingestion of food (Lifshitz, 1988). Certain foods or food ingredients, also called food allergens, can initiate and provoke the immunological reaction of an allergy (Taylor, 1992). Celiac disease, also called gluten-sensitive enteropathy, nontropical sprue, or celiac sprue, is a malabsorption disorder (Saunderlin, 1994). In celiac disease, allergic reactions of patients who are sensitive to gluten of wheat, rye, barley, and oats are classed as food sensitivities (Hefle, 1996). The only treatment for celiac disease patients is lifelong adherence to a gluten-free diet (Campbell, 1992). Although, by using rice, potato, or other non-wheat starches to make products, one can eliminate the gluten from the diet, non-gluten flour products are often less desirable in taste, texture, color, and product variety. These starches can not replace what gluten contributes to products such as pasta: elasticity, structure, textural properties, and sensory characteristics. Therefore, non-starch polysaccharides combined with non-wheat starch have been used in many non-gluten products to replace wheat flour. Researchers reported that non-gluten flour products were improved in taste, texture, and color by adding non-starch polysaccharide (Ylimaki, 1989).

Pasta is an ancient food, made from wheat flour or durum wheat flour. The durum wheat flour gives pasta a light yellow color and contains a very high proportion of gluten, compared to most other wheats. The gluten formation contributes two main functions: (a) dough development during mixing and extrusion; and (b) prevention of disintegration of pasta during drying and boiling (Feillet, 1984). Dick (1985) reported that high gluten content provided a higher quality of pasta with a rubbery or slightly

a rubbery or slightly elastic structure and less cooking loss. Nevertheless, celiac patients can not ingest gluten-containing products. Therefore, wheat or durum wheat can not be used as an ingredient for pasta for these people. But non-gluten pasta has less elasticity texture in sensory characteristics. Over the years researchers reported that different polysaccharide polymers other than starches were successfully added to non-gluten products such as bread or cake to replace gluten (Ylimaki et al., 1991). Edwards et al. (1995) reported that xanthan gum could be used in whole wheat pasta to enhance the pasta texture. Gums mixed with other non-gluten starches and flours affected gelatinization in cereal-based products (Ferrero et al., 1993). Non-starch polysaccharides such as xanthan gum and locust bean gum have very significant viscoelastic properties and perhaps could be used to mimic properties of gluten to form a rubbery texture of pasta.

Response surface methodology (RSM) is a collection of statistical and mathematical techniques useful for developing, improving, and optimizing processes. RSM has important uses in the design, development, and formulation of new products, as well as in the improvement of current products (Myers and Montgomery, 1995). The formulation of a new product or the improvement of an old one, and the development of a new process or the optimization of an existing one, can be better understood using response surface methodology (Floros and Chinnan, 1988). Response surface methodology provides many benefits to different fields in food scientists (Jackson et al., 1995).

Mixture experiment is a special type of response surface experiment in which the factors are the ingredients or components of a mixture, and the response is a function of the proportions of each factor (Myers and Montgomery, 1995). The development of new products involving more than one ingredient (factors) requires the design of a mixture experiment, as opposed to a factorial experiment (Hare, 1974). Because the total amount of a food product is fixed, each factor is not independent; if one of the components changes, the others will change. In a factorial experiment, each factor is independent. As one of factors changes, the others will not be affected. In the mixture experiment, if the total amount of product is held constant (Cornell, 1990), the value of the response changes are made in the relative proportions of those ingredients in the mixture. However, in a factorial experiment, the change in the response is measured when the level of one or more of the factors are changed while holding the levels of the other factors fixed. Not only do the levels of factors affect the change in the response but the total amount is also changed. Therefore, researchers can readily see a response change because of factors, not by varying the total amount of mixture in mixture experiment. Arteaga et al. (1993) used mixture experiment to investigate the interaction of protein functionality among ingredients.

The objective of this study was to use response surface methodology with mixture experiment to develop a non-gluten pasta with physical properties and microstructure similar to a control pasta.

MATERIALS AND METHODS

Non-gluten flours, starches, and gums

The non-gluten pasta formulas contained seven different polysaccharides (Table 1); five were the independent variables in the research: locust bean gum (TIC GUMS, Inc.), xanthan gum (Kelco, Inc.), modified starch (Staley, Inc.), tapioca starch (Staley, Inc.), and potato starch (Staley, Inc.). The other two ingredients, yellow corn flour (Shawnee Milling, Co.) and rice flour (Erawan, Co.), were the fixed variables.

Experimental design

This research employed a mixture experiment with five-component constraints, so that the mixture components were locust bean gum (X_1), xanthan gum (X_2), modified starch (X_3), tapioca starch (X_4), and potato starch (X_5) with corn and rice flours. According to preliminary tests, each independent variable had these constraints: locust bean gum (X_1): 10, 25, and 40g; xanthan gum (X_2): 25 and 40g; modified starch (X_3): 30, 35, and 40g; tapioca starch (X_4): 63.35, 66.65, 70.00, 73.35, 76.50, 80.00, 83.40, 86.50, and 90.00g; potato starch (X_5): 31.67, 33.35, 35.00, 36.67, 38.35, 40.00, 41.67, 43.35, and 45.00g. Fixed variables are: corn flour: 250g and rice flour: 50g where $X_1+X_2+X_3+X_4+X_5$ + corn flour + rice flour = 500g (100%). The ratio of tapioca starch to potato starch was held at 2 to 1. Multiple regression analysis was used to fit the model:

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

where y is a measured response.

The treatment structure consisted of 15 ingredient combinations. The treatment combinations are shown in Table 1. The design structure was an incomplete block

design. The treatment combinations were arranged for each block as shown in Table 2. Each treatment was replicated twice except that Treatments 8 and 11 were replicated four times (Table 2). Each experimental unit had two subsamples

Pasta preparation

Each pasta formula was blended with 330-350g distilled water in a single screw pasta mixer/extruder machine for 15 min. Pasta was extruded through a 1.5-mm noodle shape die (ABC, Inc., Model D-45 S.H.).

Drying

Fresh pasta was dried at a controlled temperature of 90°C for 5 hours in a food dehydrator (Alternative Pioneering Systems, Inc., Model FD-300T).

pH measurement

For the pH of pasta determination, a 10g sample of fresh pasta was blended with 100 ml distilled water in a blender for 2 min. A Fisher Accument pH meter (Model 610A) was used to measure pH at 30°C.

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The 15g sample of dried pasta was boiled in 1000g tap water for 13 min. Salt (0.5g) was added to increase boiling temperature.

Color measurement

The Minolta chromameter (Minolta, Co., Model-CR-200) was used to measure L*, a*, and b* values. Illuminant C light was used. Color of fresh, dried, and cooked pasta was measured. Lightness was expressed as L*, the red index as a*, and the yellowness as b*.

A TG4C texture gauge (Food Technology, Co., Model FTA-1000) measured shear/compression force of pasta using the shear/compression cell. Thirty grams of cooked pasta were placed into the cell and pressed at speed 5 with the blade penetrating the cell. The peak force (lbs.) was recorded.

Cooking gain (water absorption)

The 15g of dried pasta was boiled for 13 min and drained for 1min. The percent water gain was calculated by the following formula:

$$\% \text{ Cooking gain} = \frac{[\text{cooked pasta weight} - \text{dried pasta weight}]}{\text{dried pasta weight}} \times 100$$

Specimen preparation for scanning electron microscope

Fixation. Cooked pasta was cut into 1 cm^2 for a surface view and 1x 0.5 cm^2 for a cross-section view. Samples were fixed with 1.6% glutaraldehyde in 0.1M cacodylate buffer at 25°C for 2 hrs and rinsed three times in phosphate buffers (20 min/rinse). After the third rinse the buffer was removed and replaced with 1% osmium in 0.1M cacodylate buffers at 25°C for 2 hrs to fix the samples. The samples were then rinsed three more times in phosphate buffer (20min/rinse). The samples were allowed to stand in the phosphate buffer after the last rinse and then stored at 4°C overnight.

Dehydration. Before dehydration, the phosphate buffer was removed. The samples were dehydrated in ethanol at concentration: 50%, 70%, 90%, 95%, and 100%. The ethanol (100%) dehydration was repeated three times.

Critical point drying. After dehydration, the samples were critical-point-dried (DENTON DCP-1). This technique allowed sample drying without the surface damage

that accompanies air-drying. The critical point of a substance is the specific temperature and pressure where the densities of its liquid and vapor phase are equal, resulting in zero surface tension. The gaseous substance can be released from the sample without surface damage. Liquid carbon dioxide is commonly used because its critical point (36.5°C and 1080 p.s.i.) can be conveniently reached with a single apparatus to dry samples. Dried samples were mounted on stubs and put into the desiccator before gold coating.

Gold coating. Samples were placed into a Hummer II (Technics, Inc., Alexandria, VA) machine for gold coating. Each sample was coated for three minutes and thirty seconds. After coating, the gold-coated specimens were placed in a desiccator to prevent moisture absorption.

Sample scanning

Samples were observed in a JEOL (JSM-35, JEOL LTD., Japan) scanning electron microscope at an acceleration voltage of 25 KeV. Micrographs of the surface and the cross-sections of each sample were taken at magnification of 50X and 1000X.

RESULTS AND DISCUSSION

Physical and chemical properties

The ranges of physical and chemical property responses for non-gluten pasta and the control pasta are in Table 3. In fifteen treatments, means of a* for dried pasta and a* for cooked pasta were not significantly different ($P > 0.05$). Dried non-gluten pastas had a higher mean L* value but a lower b* value than the dried control pasta. However, the cooked non-gluten pasta mean had a b* value similar to the cooked control pasta. Cooking gain and texture gauge (peak force) readings had high variation among the non-gluten pastas. However, the mean cooking gain of 15 treatments was higher than for the control pasta (Table 4). Xanthan and locus bean gums absorbed large amounts of water while cooking compared to the other ingredients. A mean texture gauge reading of 15 treatments for non-gluten pasta was lower than that of the control pasta although some higher gum-level formulas had peak force means higher than the control pasta.

Choice of model selection

Peak force and cooking gain were selected as model selection responses. Using the Model Selection Procedure (SAS), the model selected is shown in Table 5. The independent variables X_1, X_2, and X_3 were selected with interaction terms X_1X_2, X_1X_3, and X_2X_3 in the model. Coefficients of determination (R^2) indicate that the regression equation explains 52~68% of total variation (Table 5). Five independent variables were introduced so adjusted R-square (R_a^2) was used rather than R^2 (X_i, i = 1, 2, 3,.....).

Surface and contour plots

Texture gauge (peak force). The texture gauge (peak force) readings of the non-gluten pastas were the highest at the levels of 40g for locust bean gum and 40g for

modified starch (Figures1 and 2). With xanthan gum levels of 25g or 40g, the peak force score was 80 lbs (36.4kg) that was greater than the control pasta, 76.5 lbs (34.8kg) but only for locust bean gum levels greater than 35g and modified starch greater than 36g (Figures 1 and 2). Non-starch polysaccharides can interact with starch polysaccharides to increase matrix gel that contributed to the firmness of pasta related to the peak force of pasta (Christianson et al., 1981). However, the peak force of the non-gluten pasta was the lowest for 10g of locust bean gum, 30g of modified starch, and 40g of xanthan gum (Figure 2). The extra amount of xanthan gum from 25g to 40g did not provide firmer structure; instead it weakened the matrix structure. Therefore, the peak force of the non-gluten pastas decreased when xanthan gum increased at the lower levels of locust bean gum and modified starch. With 25g of xanthan gum, peak force fell as low as 65 lbs. at any level of modified starch and locust bean gum less than 15g (Figure 3). When xanthan was increased to 40g, the peak force fell to 55 lbs. when locust bean gum and modified starch were both at the lower levels (Figure 4).

Cooking gain. Cooking gain of the control pasta was 162.7%. Cooking gain was highest at the 40g level for xanthan gum (Figures 5 and 6). Between xanthan gum levels of 25g and 40g, cooking gain increased dramatically (Figures 5 and 6). With 40g the level for both xanthan gum and locust bean gum, there was a high synergism in increased absorption. Cooking gain was lowest at the level of 25g for xanthan gum, 10g for locust bean gum, and 30g for modified starch; these were the lowest levels for each of these ingredients (Figure 5). Cooking gain of greater than 250 % with locust bean gum greater than 35g and modified starch less than 31g, when xanthan gum was at its highest level of 40g (Figures 7 and 8). Compared to the control pasta, the non-gluten pasta absorbed

more water (Table 3). Locust bean and xanthan gums showed high water absorption ability whether alone or in combination; this agrees with the finding of Alloncle et al. (1989) that non-starch polysaccharides can bind a large amount of water to increase water-holding capacity.

Optimum regions. Optimum regions of xanthan gum, modified starch, and locust bean gum were selected by examining the contour plots obtained when modeling the physical properties of non-gluten pasta. At a xanthan gum level of 25g, the optimum region was: texture gauge > 80 lbs (Figure 3) and cooking gain > 220% (Figure 7). At xanthan gum level 40g, the optimum region was: texture gauge > 80 lbs (Figure 4) and cooking gain > 250% (Figure 8). Possible formulas were selected from optimum regions by calculating the functions and responses of each property. Three predicted formulas were selected from the possible formulas by choosing ranges: texture gauge 72 ~ 76 lbs and cooking gain > 220%. Three formulas were replicated three times and tested for validation (consistency) by a small research panel that compared with physical properties. Each formula was consistent among the three replications. This final formula of non-gluten pasta that possessed the most desirable properties among the three formulas was xanthan gum at 40g, modified starch at 35g, locust bean gum at 40g, tapioca starch at 113g, potato starch 57g, corn flour at 250g, and rice flour at 50g.

Scanning electronic microscopy

Scanning electronic micrographs showed the three-dimensional structure of the control and non-gluten pastas. The control pasta had a firm, compact gluten-protein matrix entrapping starch granules (Figures 9 and 10). In Treatments 1 and 3, the surface structure and cross-section of the non-gluten pastas were more compact as modified

starch was increased and locust bean gum and xanthan gum were at their lowest levels (Figures 11, 12, 13, and 14). When modified starch and locust bean gum were at low levels, the surface structure and cross-section of non-gluten pasta were also more compact as xanthan gum increased and (Figures 15, 16, 17, and 18). In general, at higher combined levels of xanthan gum, modified starch, and locust bean gum, the matrix structure was more compact than at the lower levels. The more compact appearance also corresponded to a greater peak force measure. Chinnaswamy and Hanna (1991) also reported that the matrix structure of non-gluten pasta was firmed by non-starch polysaccharides that interact with starch polysaccharides. In general, pasta which produced higher cooking gain and peak force also had a more compact structure. However, some combinations at highest levels of xanthan gum, modified starch, and locust bean caused more stickiness and off-flavor in non-gluten pasta. The micrographs showed difference in appearance between the non-gluten pastas and between the control pasta. In general, the higher gum levels produced a matrix structure more compact in appearance similar to the control pasta. This similarity was also seen in the peak force readings of the pastas.

CONCLUSIONS

The study showed those non-starch polysaccharides, locust bean and xanthan gums, could interact with starch polysaccharides to strengthen the matrix structure of non-gluten pasta. The micrographs of non-gluten pasta showed that higher levels of locust bean gum, modified starch, and xanthan gum had a more compact matrix structure that was more similar to the control pasta matrix. Locust bean and xanthan gum also showed high water absorption ability. Generally, cooking gain and peak force were higher at higher levels of locust bean gum, modified starch, and xanthan gum. However, the highest gum levels produced sticky pastas. Response surface methodology can help researchers optimize the formula of non-gluten pasta. This method provided a clear view of effects of different ingredients in various levels on quality of non-gluten pasta. The mixture experiment investigated the effects of factors on response more precisely and excluded the amount different among treatments.

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Table 1. Experimental treatment structure of the 15 formulas (grams)

Trt	LBG	XG	MS	TS	PS	CF	RF
1	10	25	30	90.00	45.00	250	50
2	10	25	35	86.50	43.35	250	50
3	10	25	40	83.40	41.67	250	50
4	10	40	30	80.00	40.00	250	50
5	10	40	35	76.50	38.35	250	50
6	10	40	40	73.35	36.67	250	50
7	25	25	30	80.00	40.00	250	50
8	25	25	35	76.50	38.35	250	50
9	25	25	40	73.35	36.67	250	50
10	25	40	30	70.00	35.00	250	50
11	25	40	35	66.65	33.35	250	50
12	25	40	40	63.35	31.67	250	50
13	40	25	30	70.00	35.00	250	50
14	40	25	35	66.65	33.35	250	50
15	40	25	40	63.35	31.67	250	50

Trt-treatment, LBG-locust bean gum, XG-xanthan gum, MS-modified starch, TS-tapioca starch, PS-potato starch, CF-corn flour, RF-rice flour

Table 2. Experimental design structure of 15 treatments in 6 incomplete blocks

Blocks	Treatments
Block 1	Trt 1
	Trt 6
	Trt 8
	Trt 9
	Trt 11
	Trt14
Block 2	Trt 2
	Trt 4
	Trt 7
	Trt 12
	Trt 15
Block 3	Trt 3
	Trt 5
	Trt 8
	Trt 10
	Trt 11
	Trt13
Block 4	Trt 1
	Trt 6
	Trt 8
	Trt 9
	Trt 11
	Trt14
Block 5	Trt 2
	Trt 4
	Trt 7
	Trt 12
	Trt 15
Block 6	Trt 3
	Trt 5
	Trt 8
	Trt 10
	Trt 11
	Trt13

Table 3. Range of pH values, chromameter, texture gauge readings, and % cooking gain of 15 non-gluten pasta treatments and the control pasta

Response	Range of non-gluten pasta scores	Control pasta score
pH values	5.10 ~ 5.80	------
Color range readings		------
L*-fresh	69.45 ~ 78.91	------
a*-fresh	-4.36 ~ -2.98	------
b*-fresh	24.84 ~ 34.75	------
L*-dry	70.62 ~ 83.45	53.45
a*-dry	-3.85 ~ -1.09	-1.27
b*-dry	18.77 ~ 30.63	34.72
L*-cooked	60.81 ~ 78.83	63.69
a*-cooked	-5.99 ~ -4.05	-3.03
b*-cooked	20.34 ~ 28.67	24.76
Cooking gain (%)	196 ~ 231	162.7
Texture gauge (lbs.)	58 ~ 81	76.5

L*-lightness, a*-red, b*-yellowness.

Table 4. Means of pH values, chromameter, texture gauge readings, and % cooking gain of 15 non-gluten pasta treatments and the control pasta

Response	Mean of non-gluten pasta	Control pasta
pH values	5.33[a] (F=5.78, P<0.0001)	------
Color readings		
L*-fresh	74.44[a] (F=16.49, P<0.0001)	------
a*-fresh	-3.64[a] (F=13.54, P<0.0001)	------
b*-fresh	30.25[a] (F=11.93, P,0.0001)	------
L*-dry	77.94[a] (F=8.07, P<0.0001)	53.45
a*-dry	-2.62 (F=1.73, P=0.0842)	-1.27
b*-dry	22.52[a] (F=2.74, P=0.0055)	34.72
L*-cooked	70.25[a] (F=12.93, P<0.0001)	63.69
a*-cooked	-4.92 (F=0.98, P=0.4828)	-3.03
b*-cooked	23.61[a] (F=3.06, P=0.0023)	24.76
Cooking gain (%)	210.82[a] (F=34.38, P<0.0001)	162.7
Texture gauge (lbs.)	66.53[a] (F=5.08, P<0.0001)	76.5

[a] indicates significant difference among the 15 treatment means ($P < 0.05$).
L*-lightness, a*-red, b*-yellowness.

Table 5. Regression analysis of texture gauge and cooking gain of non-gluten pasta

	Texture gauge (lbs.)	Cooking gain (%)
Coefficient		
b_0	113.7000	-51.7102
b_1	-0.9217	2.4479
b_2	-1.8950	6.9196
b_3	-1.3833	7.9972
b_{11}	0.0067	-0.0309
b_{12}	0.0363	-0.0943
b_{15}	0.0440	0.2152
Coefficient of Determination		
R^2	0.6881	0.5122
R^2_a	0.6575	0.4642
F-test	$P < 0.0001$	$P < 0.0001$

$E(y)= b_0+b_1X_1+b_2X_2+b_3X_3+b_{11}X_1X_2+b_{12}X_1X_3+b_{15}X_2X_3$, X_1=locust bean gum, X_2=xanthan gum, X_3=modified starch.

Figure 1. Surface plot for peak force at xanthan gum = 25g

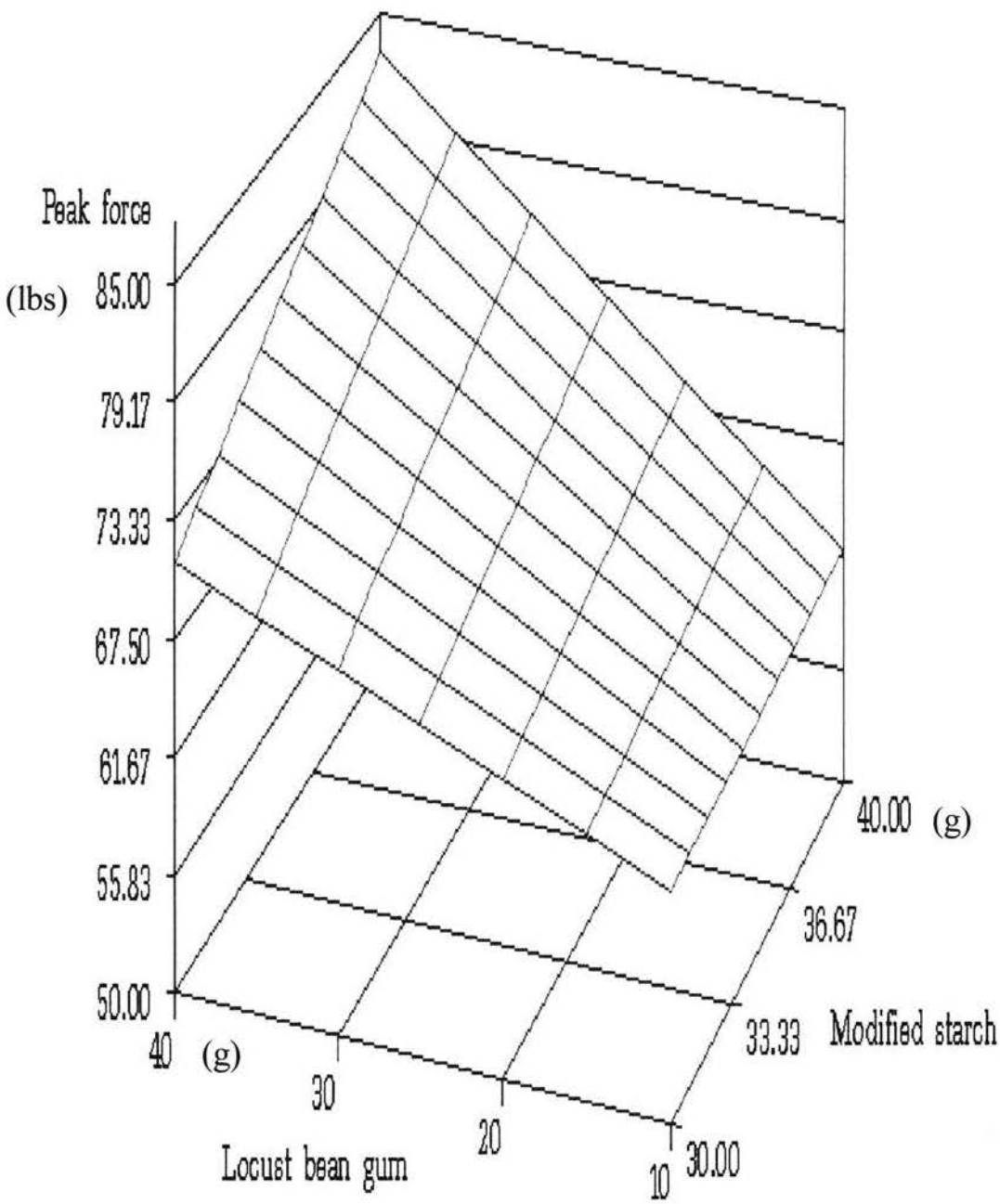


Figure 2. Surface plot for peak force at xanthan gum = 40g

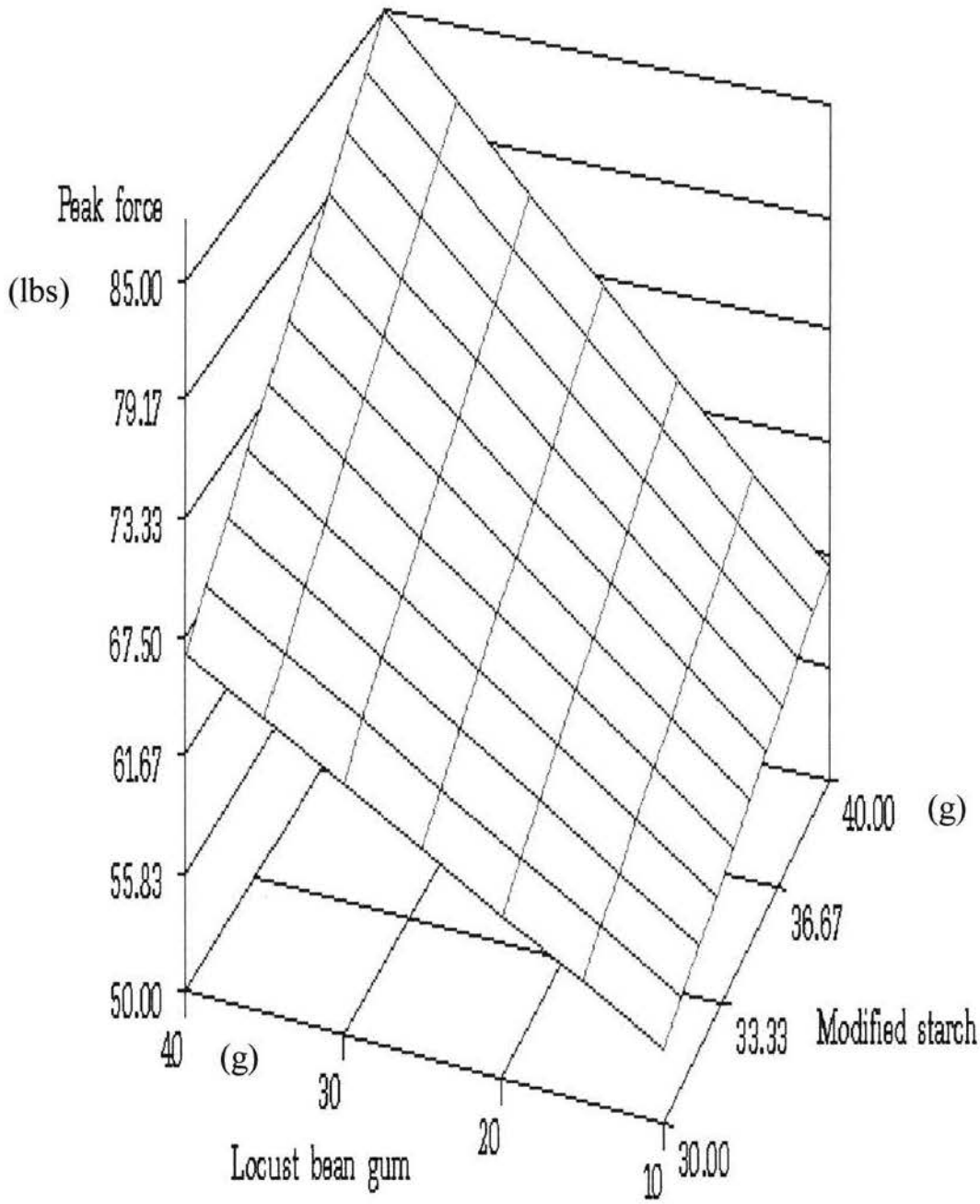


Figure 3. Contour plot for peak force at xanthan gum = 25g

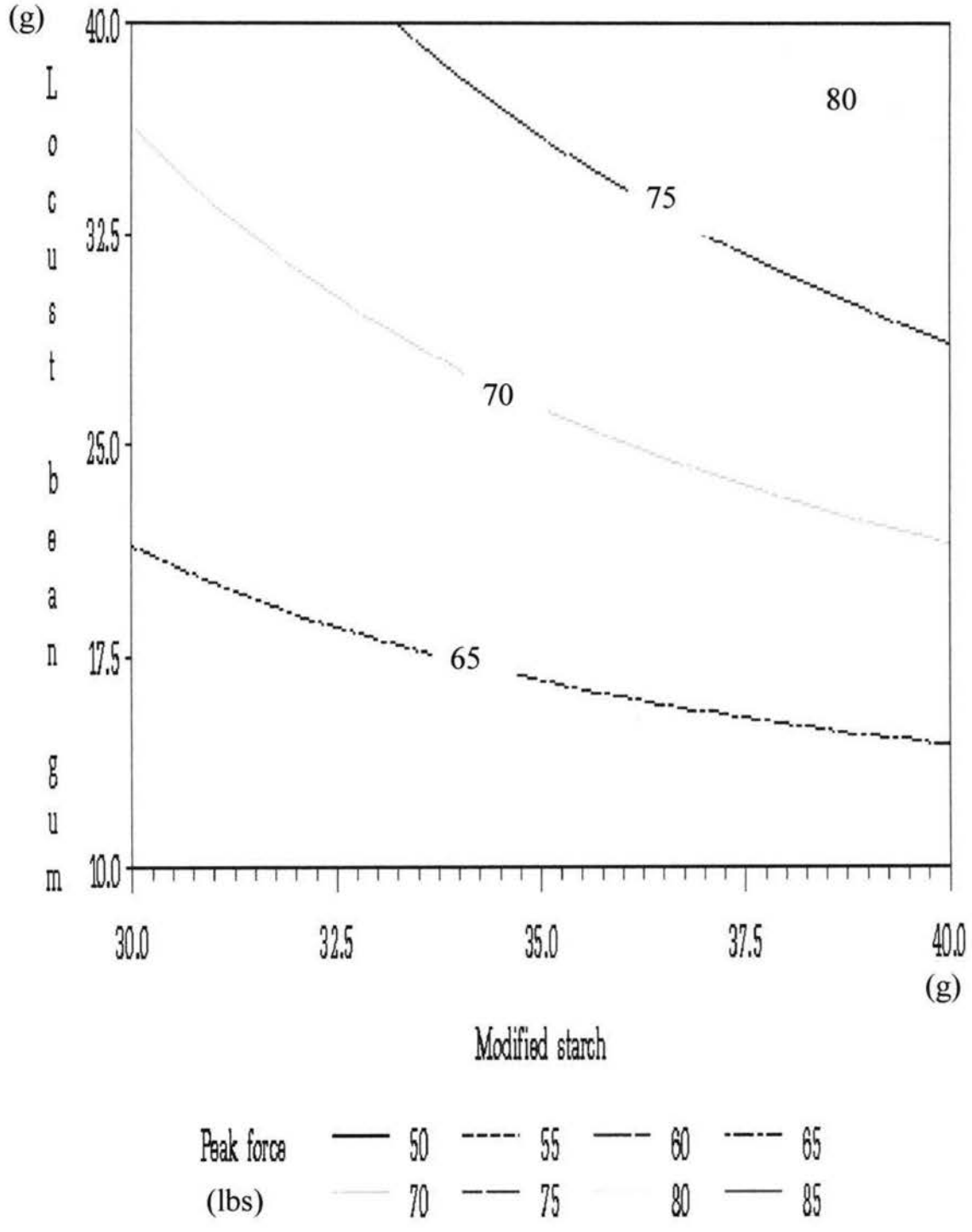


Figure 4. Contour plot for peak force at xanthan gum = 40g

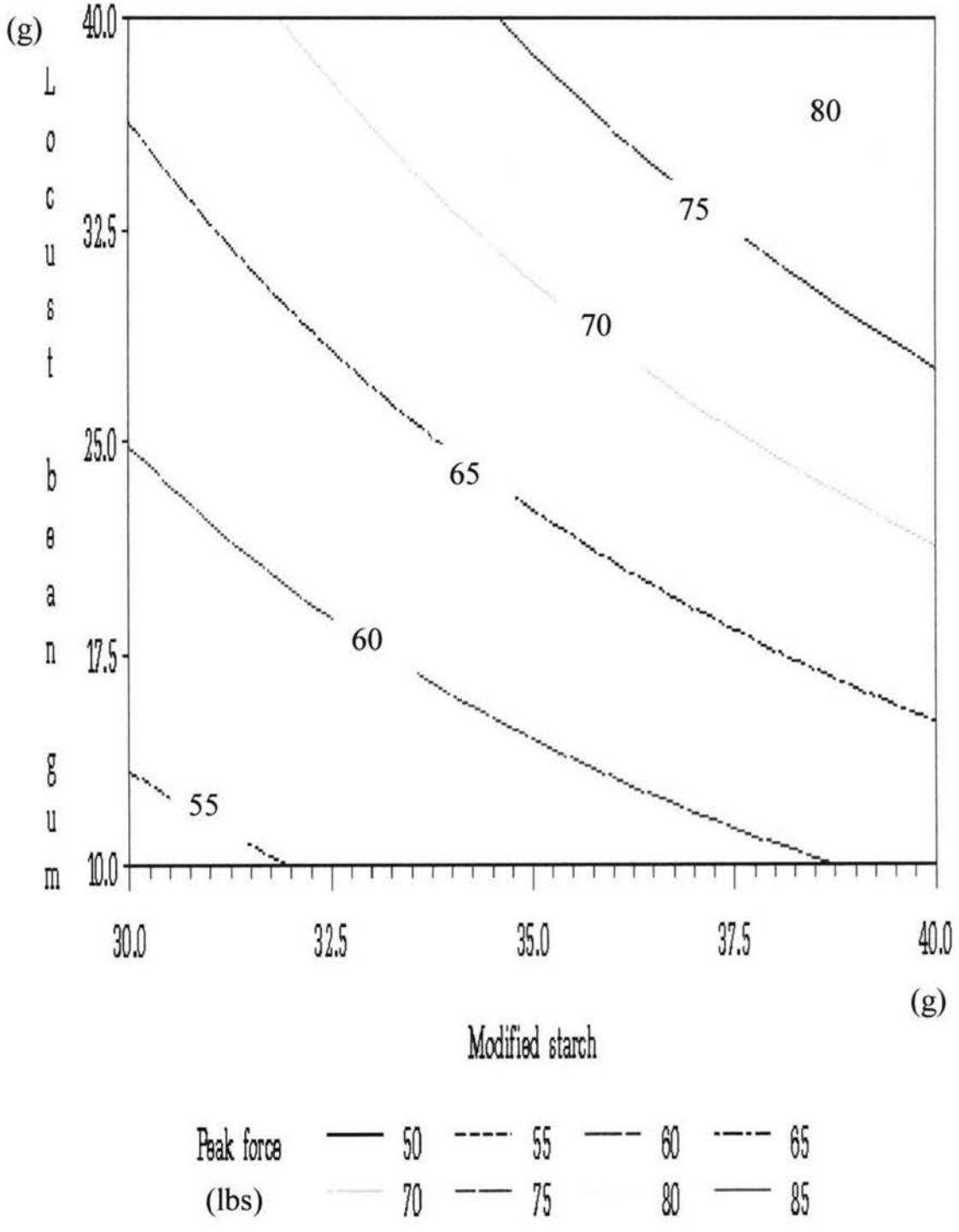


Figure 5. Surface plot for cooking gain at xanthan gum = 25g

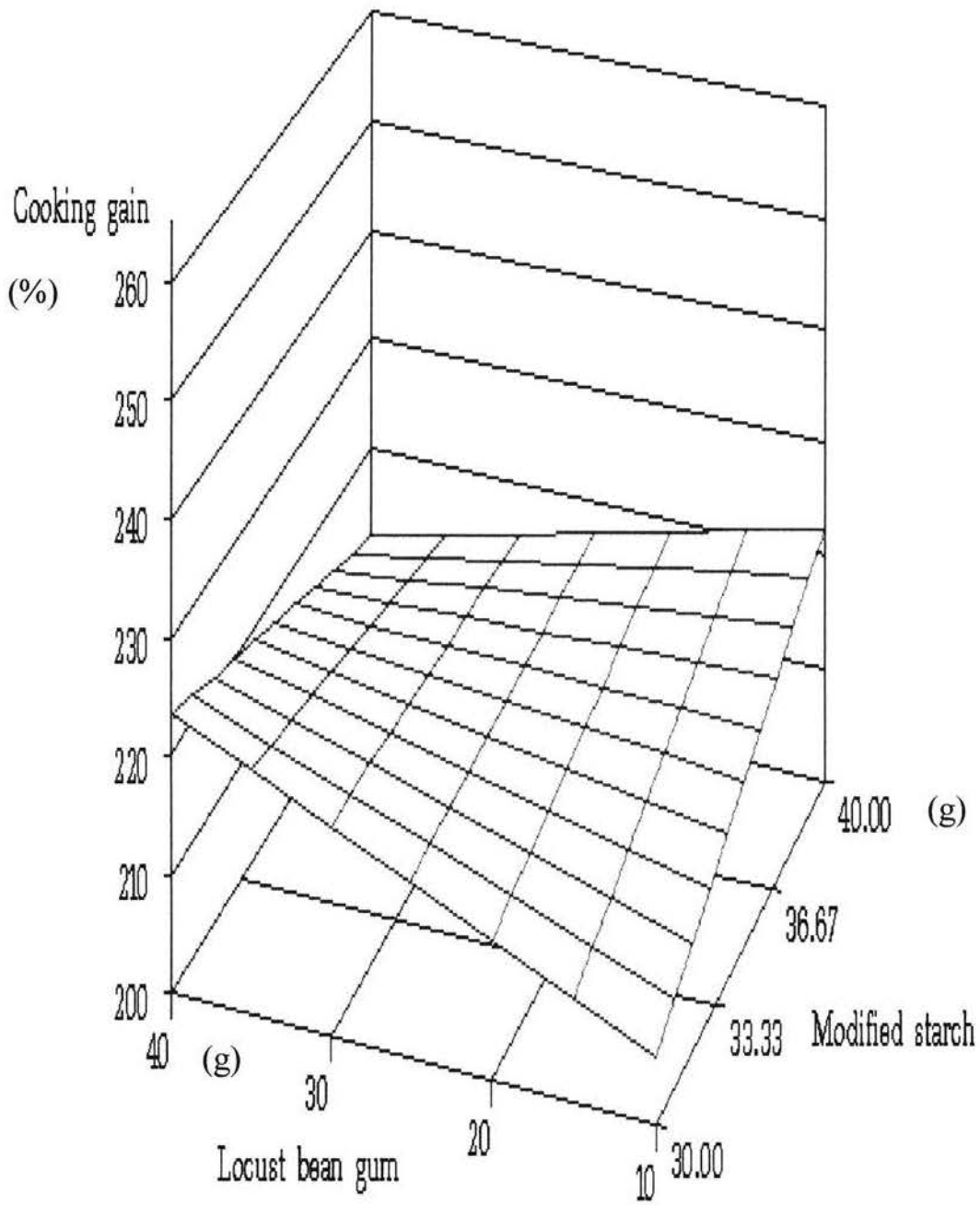


Figure 6. Surface plot for cooking gain at xanthan gum = 40g

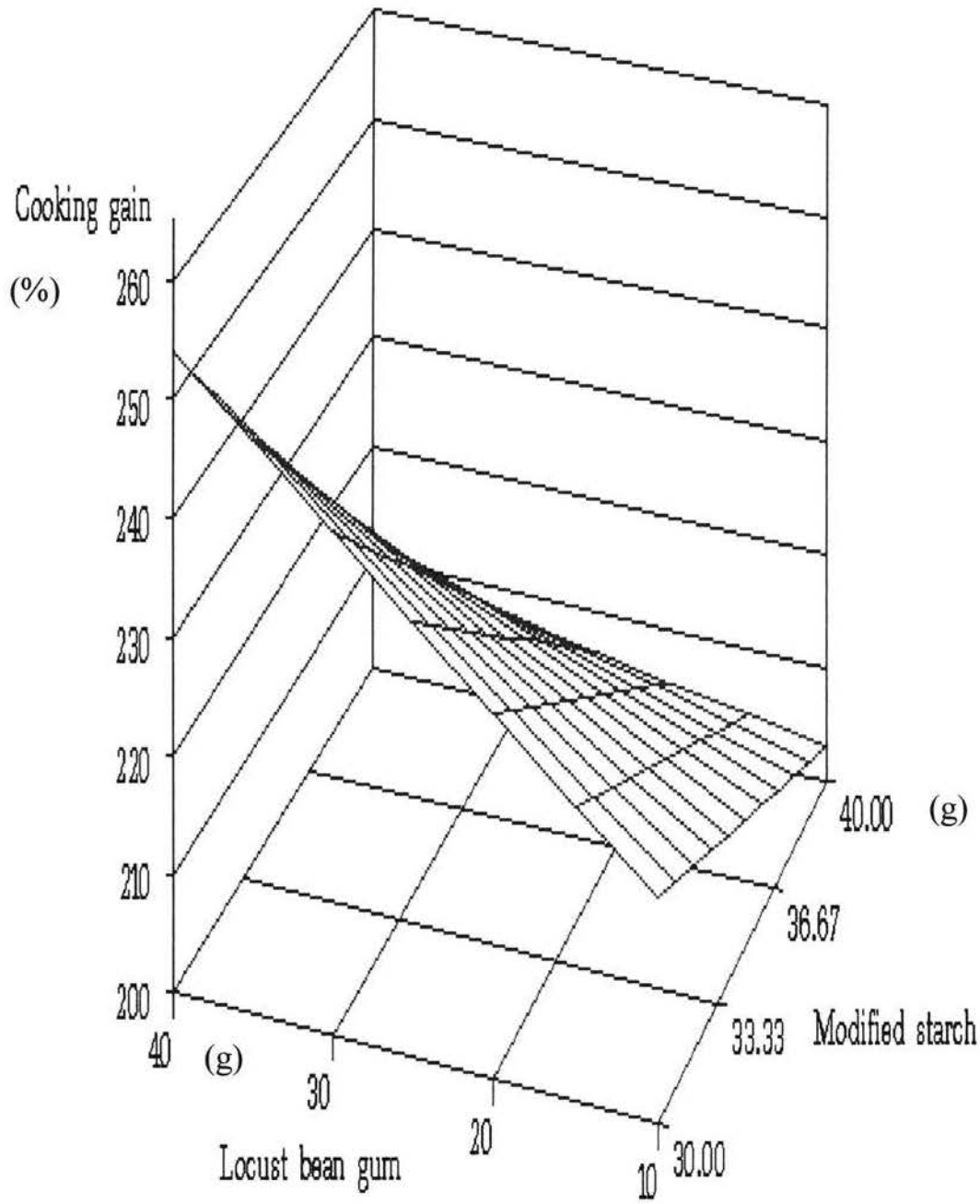


Figure 7. Contour plot for cooking gain at xanthan gum = 25g

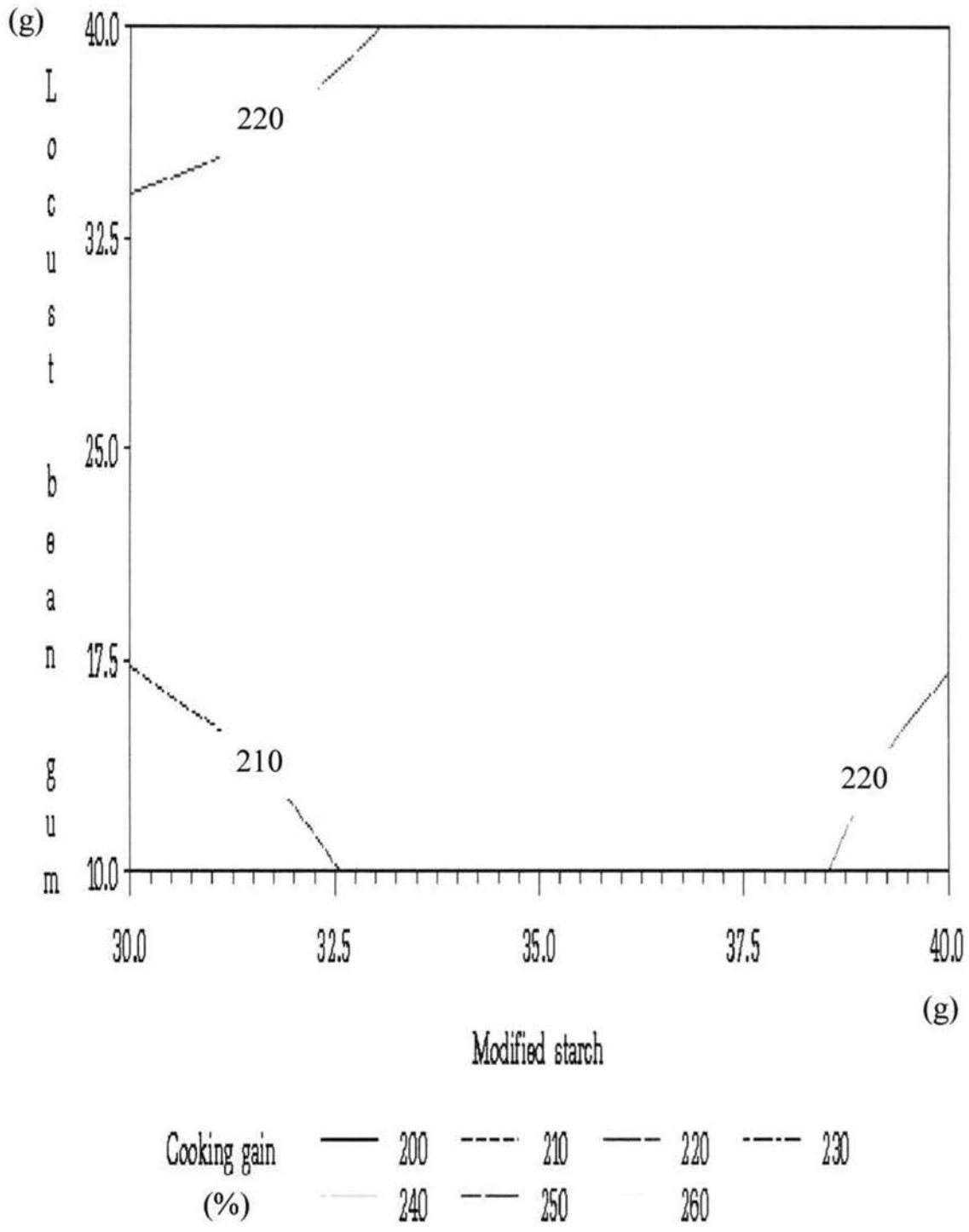


Figure 8. Contour plot for cooking gain at xanthan gum = 40g

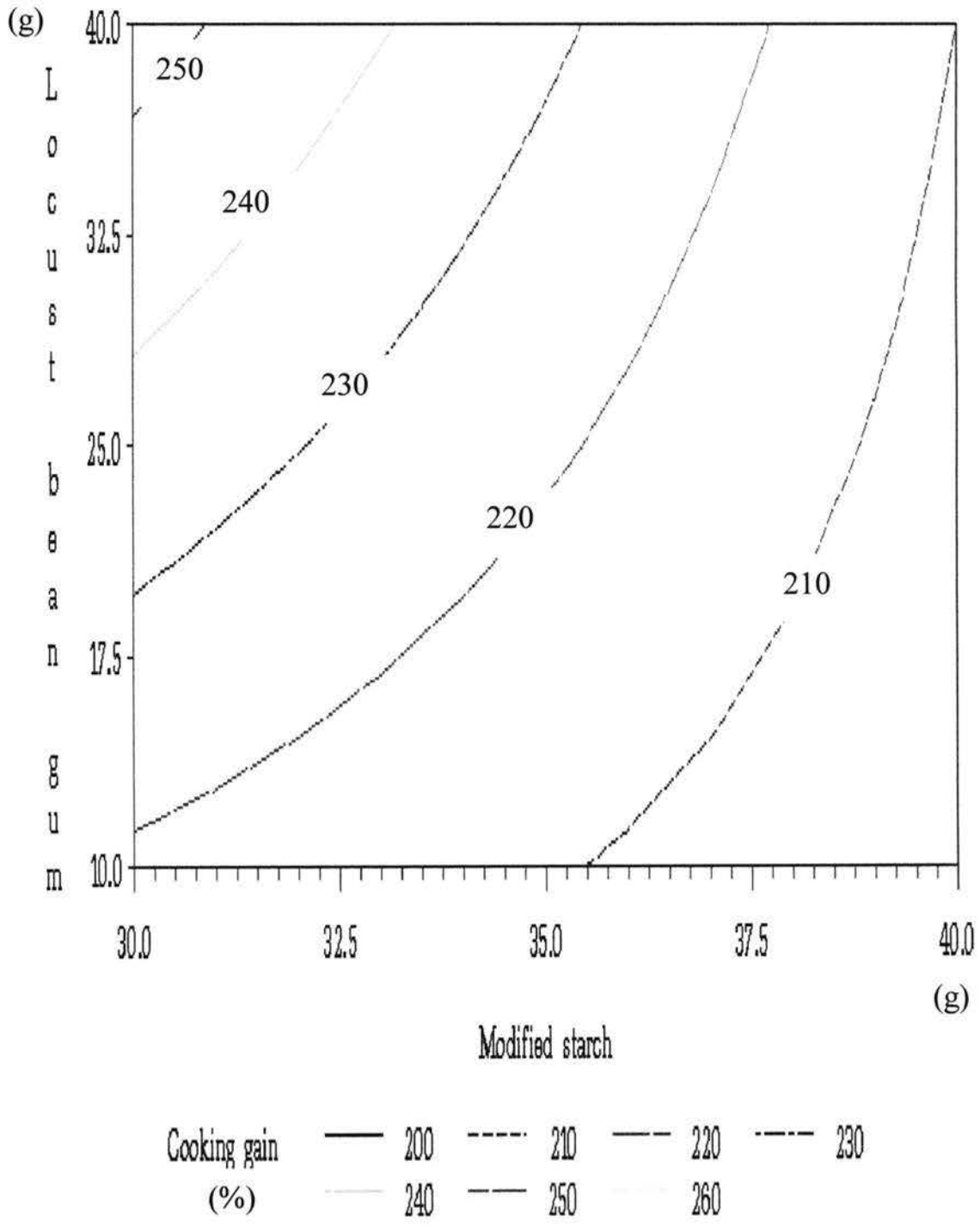
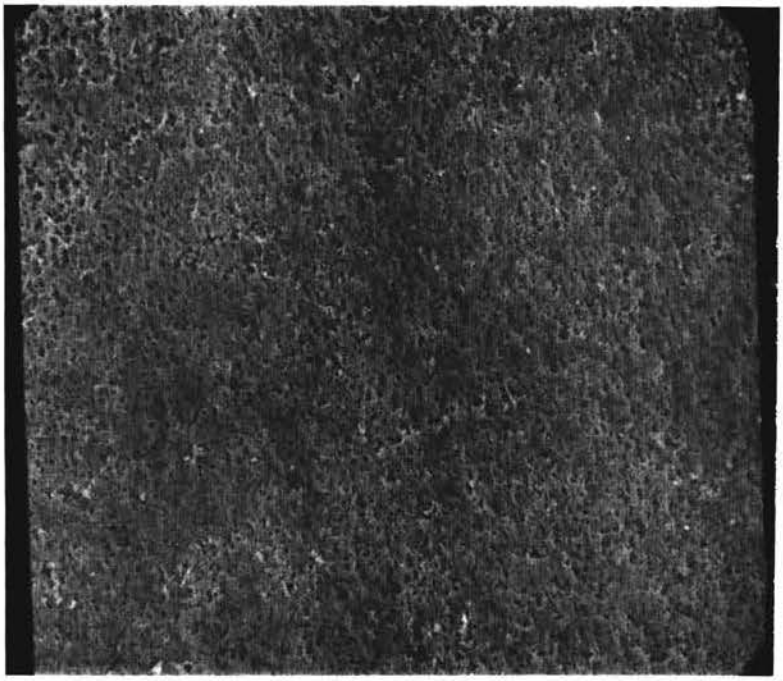
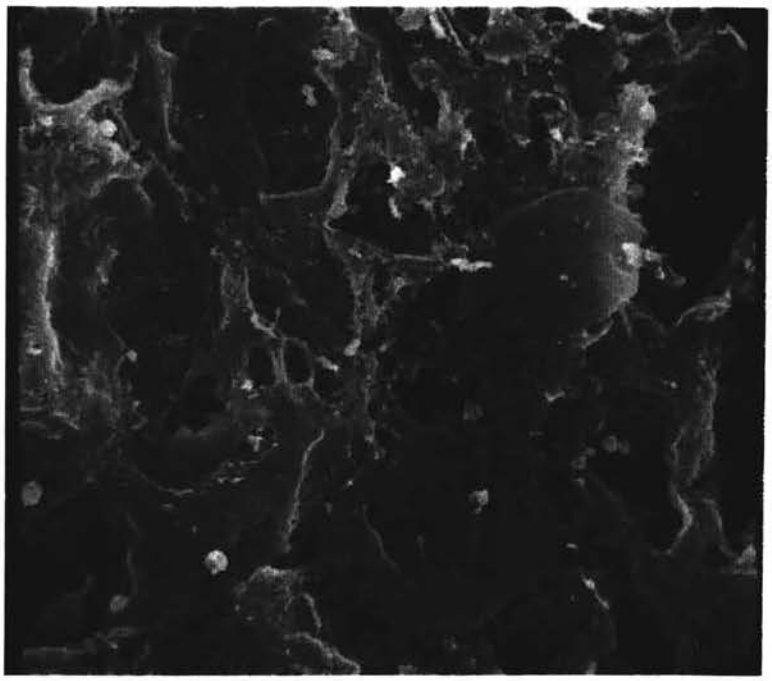


Figure 9. Control pasta (surface)

a



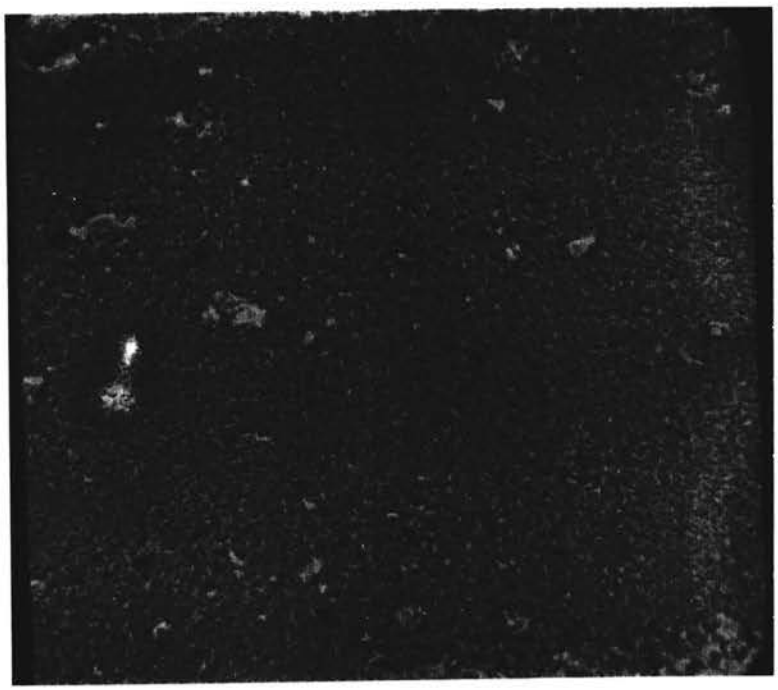
b



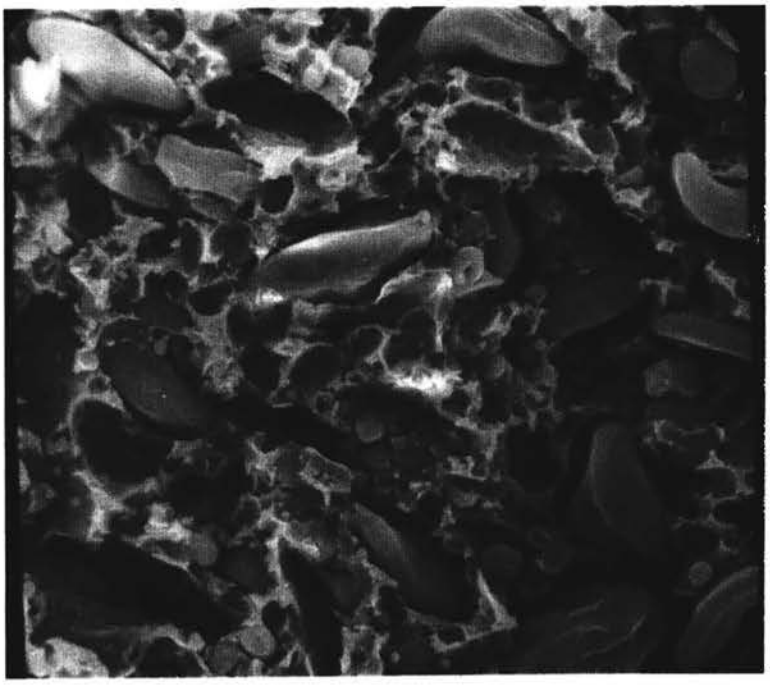
Surface of control pasta. a) 50X; b) 1000X

Figure 10. Control pasta (cross-section)

a



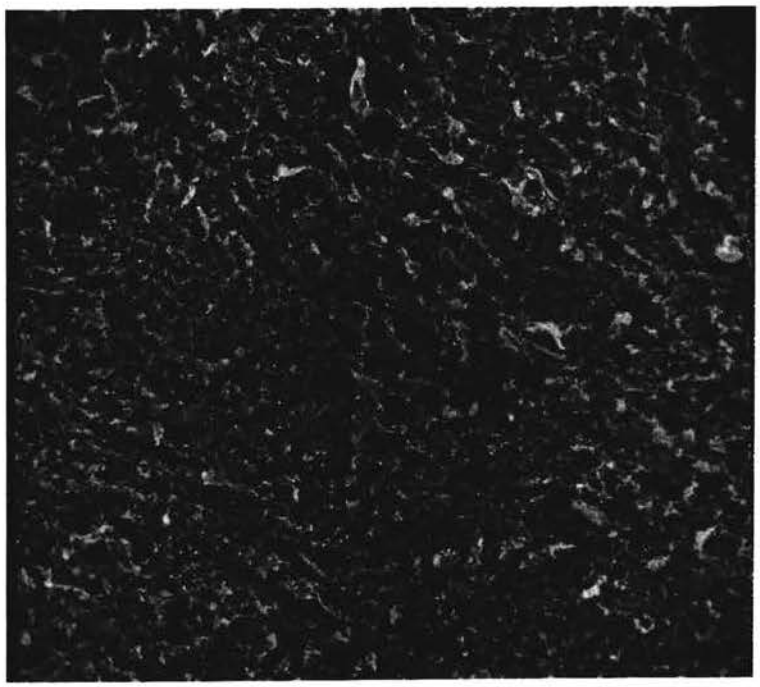
b



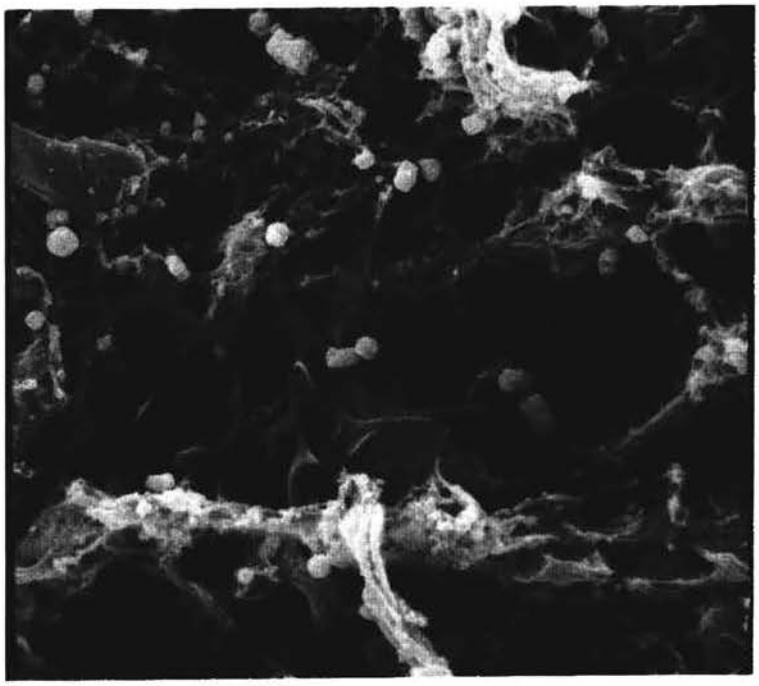
Cross-section of control pasta. a) 50X; b) 1000X

Figure 11. Treatment 1 at modified starch = 30g (surface)

a



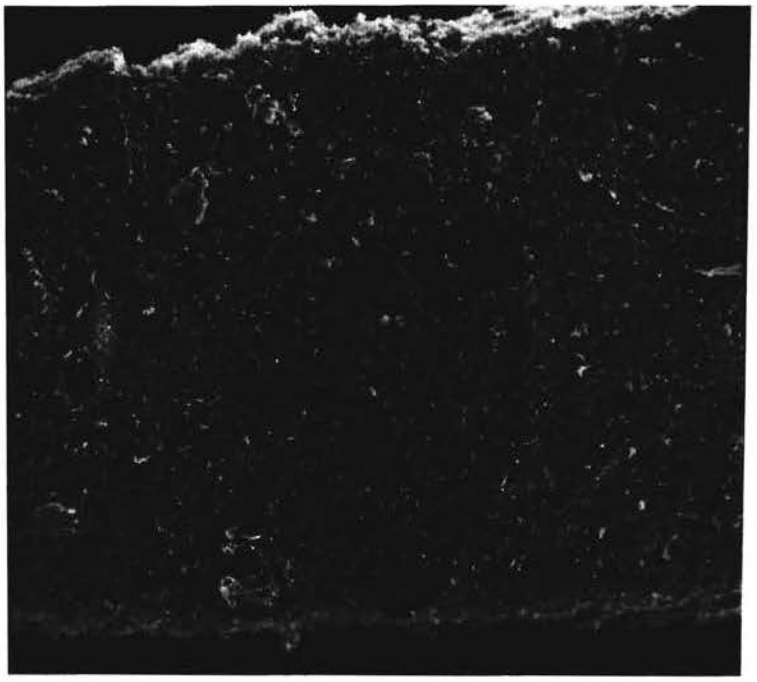
b



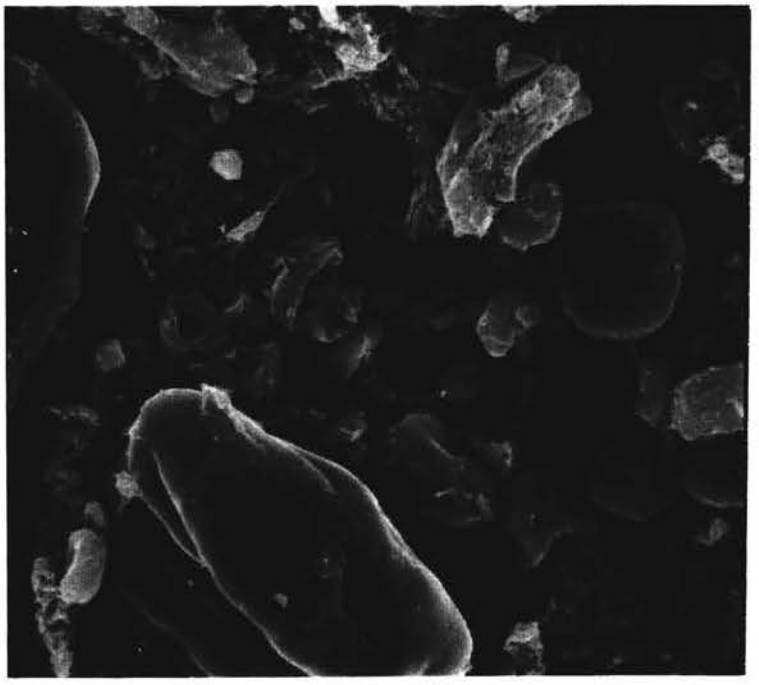
Surface of non-gluten pasta. a) 50X; b) 1000X

Figure 12. Treatment 1 at modified starch = 30g (cross-section)

a



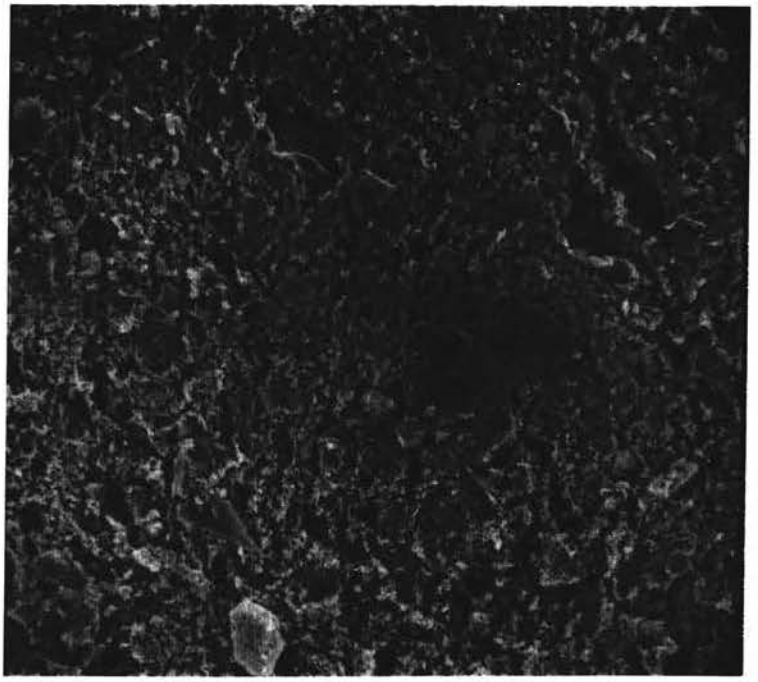
b



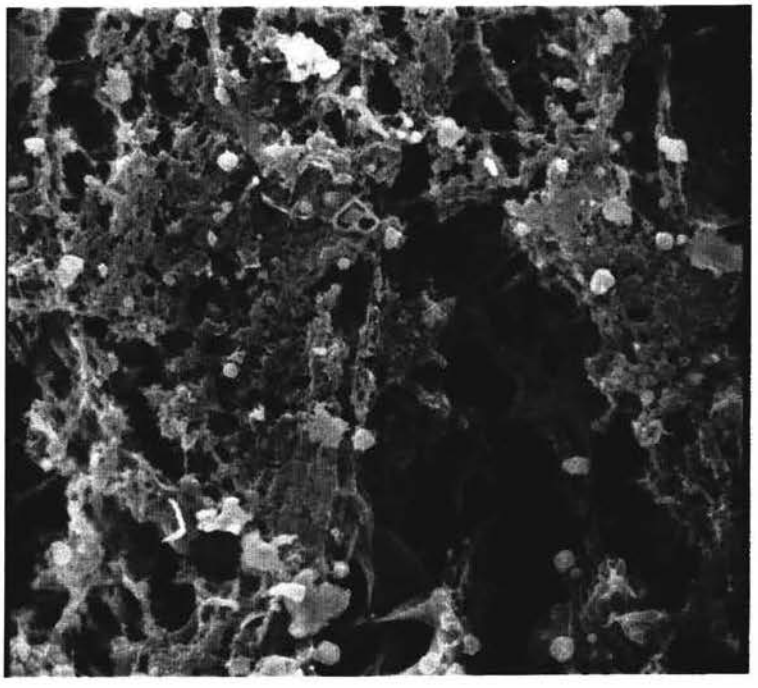
Cross-section of non-gluten pasta. a) 50X; b) 1000X

Figure 13. Treatment 3 at modified starch =40g (surface)

a



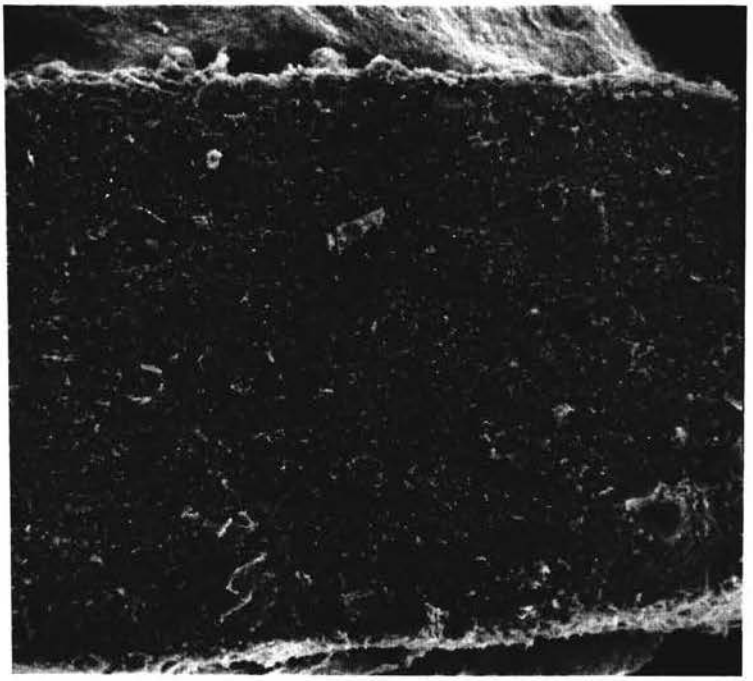
b



Surface of non-gluten pasta. a) 50X; b) 1000X

Figure 14. Treatment 3 at modified starch = 40g (cross-section)

a

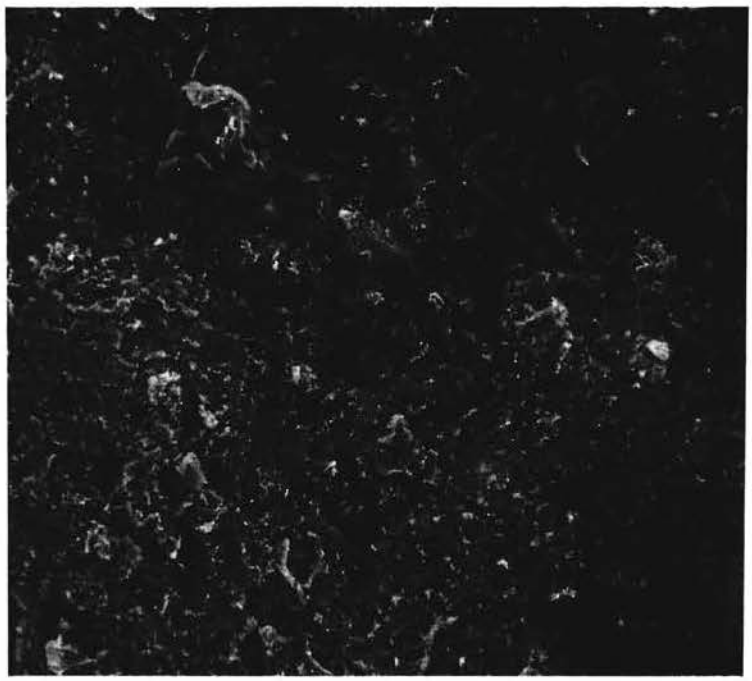


b

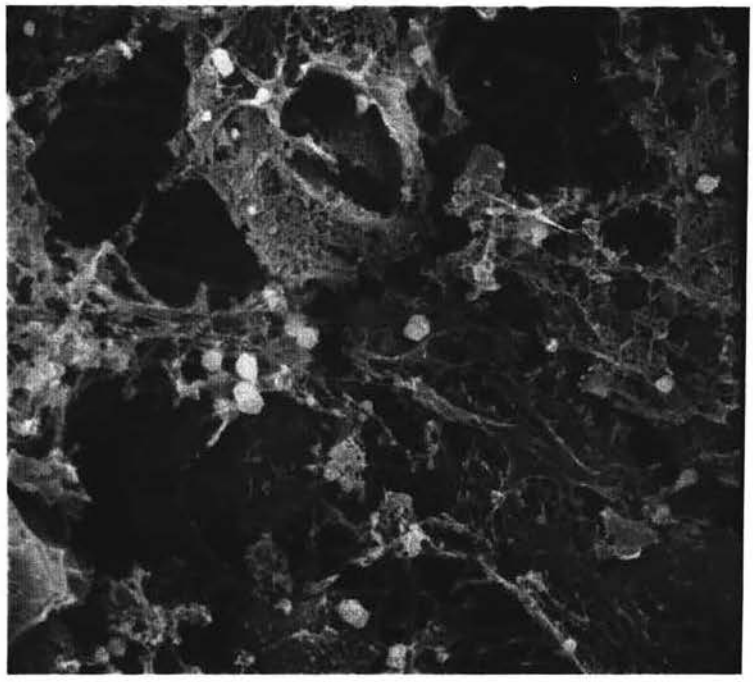
Cross-section of non-gluten pasta. a) 50X; b) 1000X

Figure 15. Treatment 8 at xanthan gum = 25g (surface)

a



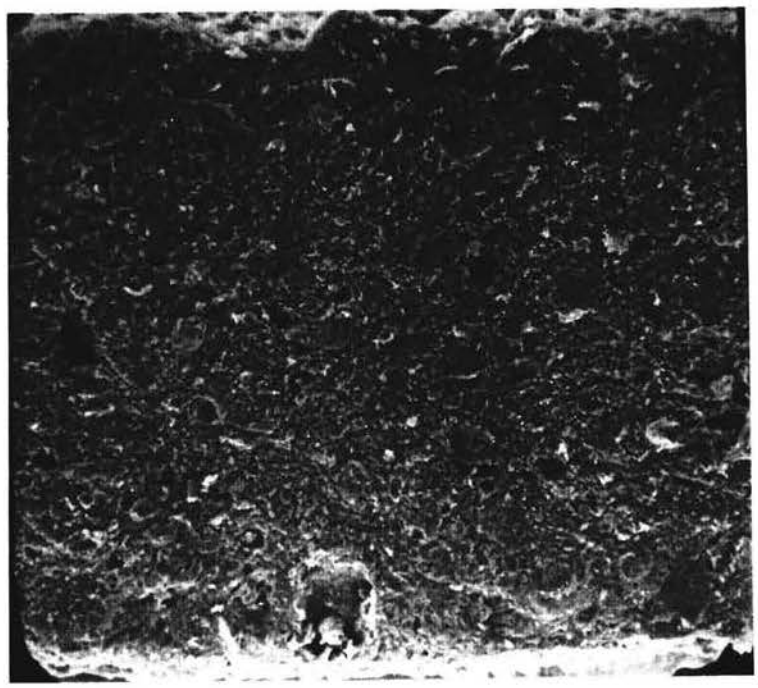
b



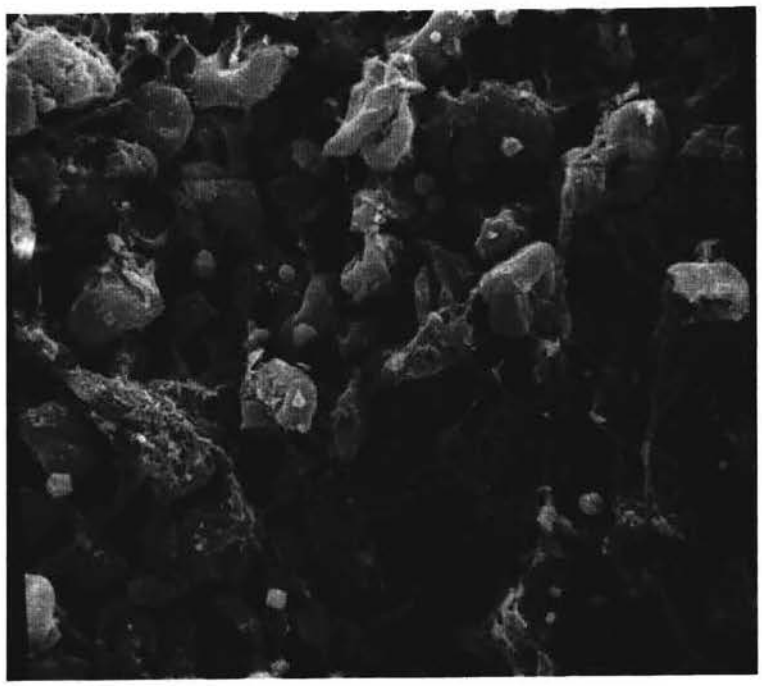
Surface of non-gluten pasta. a) 50X; b) 1000X

Figure 16. Treatment 8 at xanthan gum = 25g (cross-section)

a



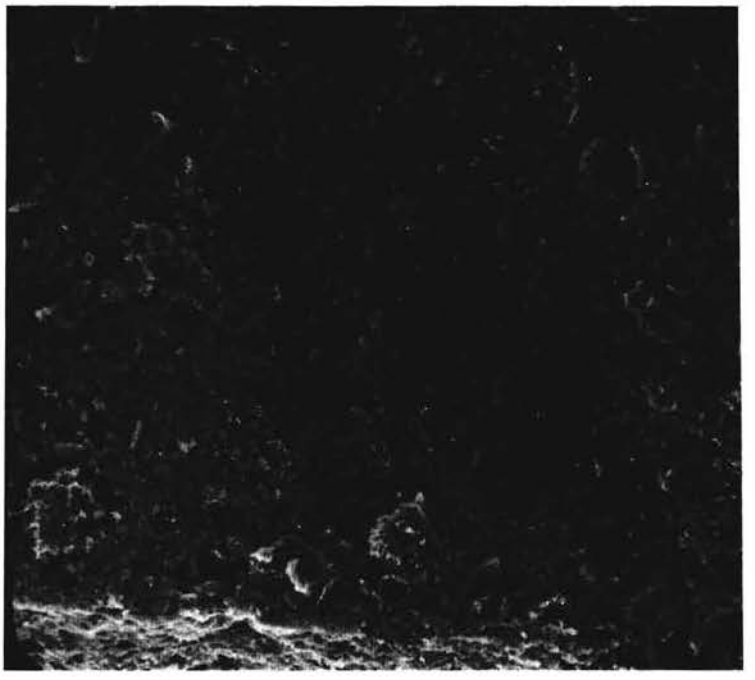
b



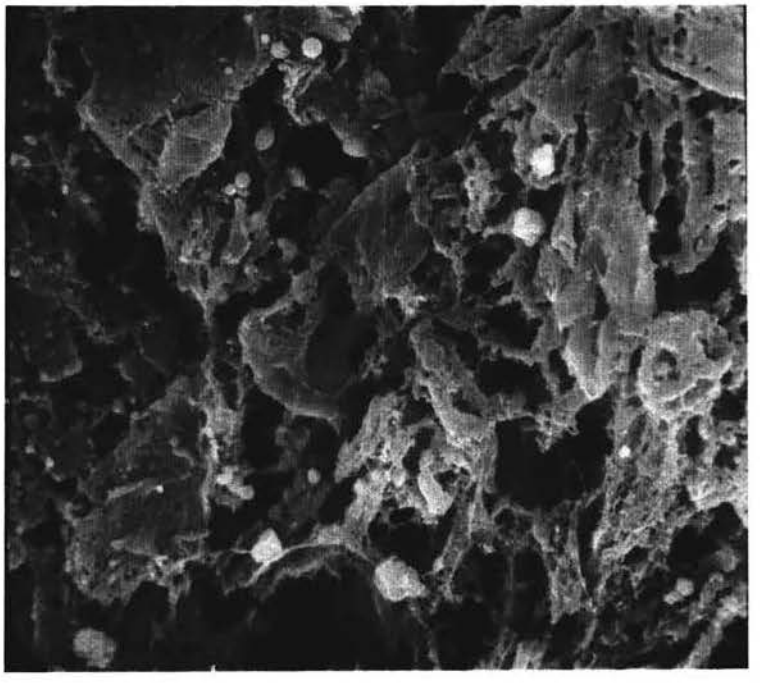
Cross-section of non-gluten pasta. a) 50X; b) 1000X

Figure 17. Treatment 11 at xanthan gum = 40g (surface)

a



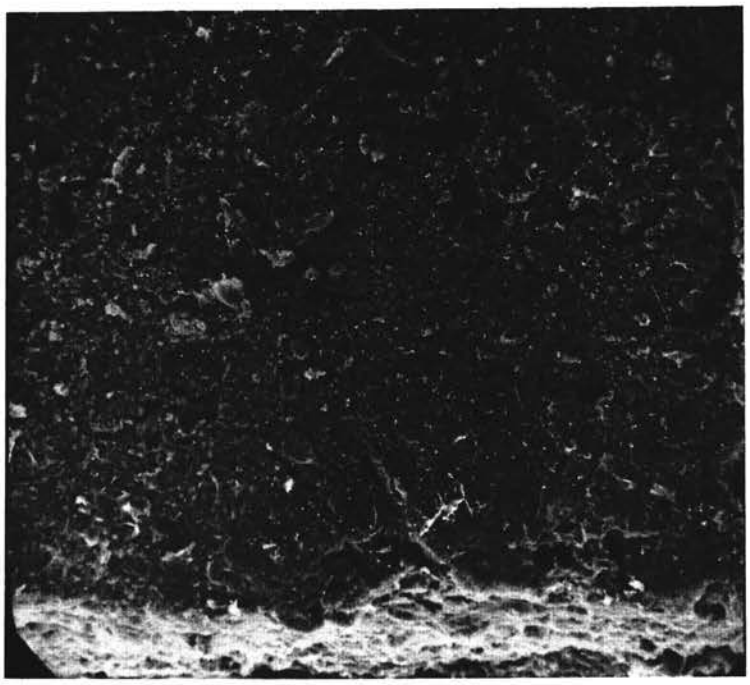
b



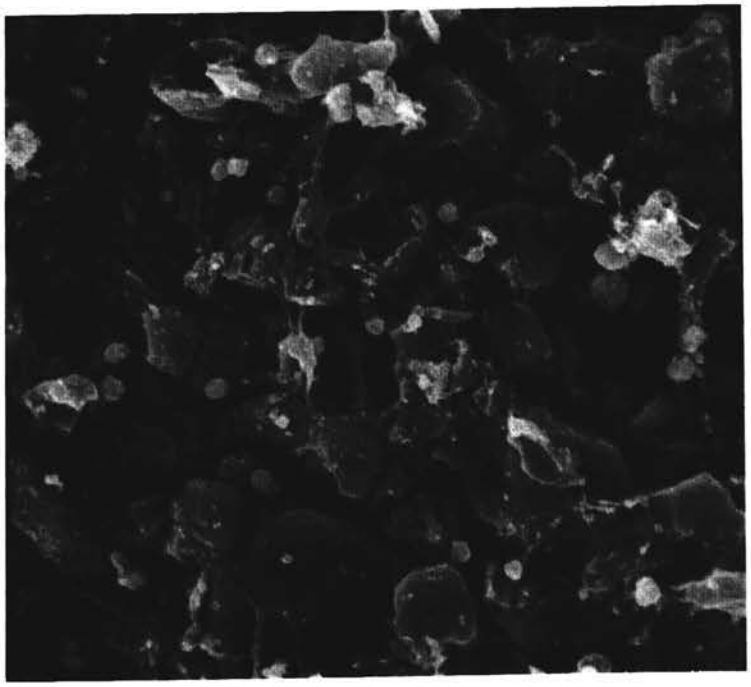
Surface of non-gluten pasta. a) 50X; b) 1000X

Figure 18. Treatment 11 at xanthan gum = 40g (cross-section)

a



b



Cross-section of non-gluten pasta. a) 50X; b) 1000X

CHAPTER V

SENSORY EVALUATION AND ELECTRON MICROSCOPE OF NON-GLUTEN PASTA BY USING RESPONSE SURFACE METHODOLOGY WITH MIXTURE EXPERIMENT

ABSTRACT

Response surface methodology was used to predict sensory attributes of a non-gluten pasta and develop response surface plots to help visualize the optimum region among ingredient ranges. A semi-trained panel was use to establish sensory attributes most similar to a durum semolina pasta which served as the control pasta. Analysis of the selected model statement showed that the smoothness of surface did not increase when xanthan gum and locust bean gum increased. With higher levels of xanthan gum, modified starch, and locust bean gum, hardness of first bite and cohesiveness of chew down had better sensory characteristics. Hardness of first bite and cohesiveness of chew down both had the highest score when locust bean gum, modified starch, and xanthan gum were at the highest levels of 40g. Adhesiveness of chew down (stickiness) had the lowest score at the level of 10g for locust bean gum and 40g for modified starch when xanthan gum was at the level of 25g. Stickiness of the non-gluten pasta was also the highest when there was 40g of each (xanthan gum, modified, and locust bean gum). Off-flavor had its lowest score with the level of locust bean gum 40g, modified starch 30g,

and xanthan gum at the level of 40g. The micrographic images showed that the matrix of non-gluten pasta was different from that of the control pasta. However, the non-gluten pasta had similar sensory attributes to the control pasta. In general, at higher levels of xanthan gum, modified starch, and locust bean gum, non-gluten pasta had a more compact matrix structure and had sensory characteristics more similar to the control pasta than those of the lower levels.

INTRODUCTION

Non-gluten products are essential for celiac patients and others who are gluten sensitive to replace their gluten-containing foods. However, sensory characteristics of non-gluten foods such as non-gluten pasta are generally less acceptable compared to control pasta. A variety of starch and non-starch polysaccharides have been used in many non-gluten products to replace wheat flour. Researchers reported that non-gluten flour products were less desirable in taste, texture, color, and product variety because they lack gluten (Ylimaki, 1989). For example, non-gluten bread without wheat flour had less volume and was tougher in texture. Cakes had a heavy layer on the bottom with less volume and less tenderness (Ylimaki et al., 1988). These reactions were explained by Abecassis et al. (1989) who stated that gluten in wheat flour plays a major role in sensory characteristics of wheat flour products. Gluten, a protein complex, contributes viscoelasticity that can entrap the CO_2 in baked products and bind the starch structure making it more cohesive. For years, researchers applied different starches, flours, and non-starch polysaccharides to non-gluten products seeking to improve the functional properties and sensory characteristics. Toufeili et al. (1994) used methylcelluloses, gum Arabic, and egg albumen to improve gluten-free pocket-type flat breads. Methylcelluloses and egg albumen significantly improved sensory acceptability.

Pasta is an ancient food, made from wheat flour or durum wheat flour. The durum wheat flour gives pasta a light yellow color and contains a very high proportion of gluten, compared to most other wheats. The gluten formation contributes two main functions: (a) dough development during mixing and extrusion; (b) prevention of disintegration of pasta during drying and boiling (Feillet, 1984). Dick (1985) reported

that a high gluten content provided a higher quality of pasta with a rubbery (slightly elastic) structure and less cooking loss. Gluten in pasta contributes the desired textural characteristics. Nevertheless, celiac patients can not ingest gluten-containing products. Therefore, wheat or durum wheat can not be used as a ingredient for pasta for these people. But non-gluten pasta has a less rubber texture in sensory characteristics. Over the years researchers reported that non-starch polysaccharide polymers were successfully added to replace gluten to produce non-gluten products such as breads or cakes (Ylimaki et al., 1991). Edwards et al. (1995) reported that xanthan gum can be used in whole-wheat pasta to enhance the pasta texture. Gums mixed with other non-gluten starches and flours affected gelatinization in cereal-based products (Ferrero et al., 1993). Non-starch polysaccharides such as xanthan gum and locust bean gum have very significant viscoelastic properties and perhaps could be used to mimic the properties of gluten to form a rubbery texture of pasta.

Response surface methodology (RSM) is a collection of statistical and mathematical techniques which are useful for developing, improving, and optimizing processes. This methodology has important applications in the design, development, and formulation of new products, as well as in the improvement of current products (Myers and Montgomery, 1995). The formulation of a new product or the improvement of an old one, and the development of a new process or the optimization of an existing one; can be better understood using response surface methodology (Floros and Chinnan, 1988). For food scientists, response surface methodology provides many benefits to different fields in food science. Ylimaki et al. (1991) used response surface methodology to

develop new gluten-free breads and to optimize the formula for gluten-free breads based on sensory qualities.

A mixture experiment is a special type of response surface experiment in which the factors are the ingredients or components of a mixture, and the response is a function of the proportions of each factor (Myers and Montgomery, 1995). The development of new products involving more than one ingredient (factors) requires the design of a mixture experiment, as opposed to a factorial experiment (Hare, 1974). Because the total amount of a food product is fixed, each factor is not independent; if one of the components changes, the others will change. In a factorial experiment, each factor is independent. As one of factors changes, the others will not be affected. In the mixture experiment, the total amount of product is held constant (Cornell, 1990), so the value of the response changes are made in the relative proportions of those ingredients in the combination. However, in a factorial experiment, the change in the response is measured when the level of one or more of the factors are changed while holding the levels of the other factors fixed. The change in the response is affected not only by the levels of factors but also by the total amount. Prinyawiwatkul et al., (1997) applied response surface methodology in a mixture experiment to investigate physicochemical properties such as fat content, moisture loss, color changes, and sensory properties of flavor and texture for chicken nuggets extended with fermented cowpea and peanut flours.

The objective of this study was to use surface response methodology in a mixture experiment to investigate sensory characteristics and microstructure of non-gluten pasta.

MATERIALS AND METHODS

Non-gluten flours, starches, and gums

The non-gluten pasta formulas contained seven different polysaccharides (Table 1); five were the independent variables in the research: locust bean gum (TIC GUMS, Inc.), xanthan gum (Kelco, Inc.), modified potato starch (Staley, Inc.), tapioca starch (Staley, Inc.), and potato starch (Staley, Inc.). The other two ingredients, yellow corn flour (Shawnee Milling, Co.) and rice flour (Erawan, Co.), were the fixed variables.

Experimental design

This research employed a mixture experiment with five components. The mixture components were locust bean gum (X_1), xanthan gum (X_2), modified starch (X_3), tapioca starch (X_4), and potato starch (X_5) with corn and rice flours. According to preliminary tests, each independent variable had these constraints: locust bean gum (X_1): 10, 25, and 40g; xanthan gum (X_2): 25 and 40g; modified starch (X_3): 30, 35, and 40g; tapioca starch (X_4): 63.35, 66.65, 70.00, 73.35, 76.50, 80.00, 83.40, 86.50, and 90.00g; potato starch (X_5): 31.67, 33.35, 35.00, 36.67, 38.35, 40.00, 41.67, 43.35, and 45.00g. Fixed variables are: corn flour: 250g and rice flour: 50g where $X_1+X_2+X_3+X_4+X_5$ + corn flour + rice flour = 500g (100%).

Multiple regression analysis was used to fit the model:

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

where y is a measured response.

The treatment structure consisted of 15 combinations. The treatment combinations are shown in Table 1. Each treatment was replicated twice except for Treatments 8 and 11 which were replicated four times (Table 2).

Panel selection and training

The sensory panel was selected from Oklahoma State University students, staff, faculty, and other Stillwater residents. Before they became actual panelists, each person was tested for the ability to identify the four basic tastes, sweet, sour, salt, and bitter. Twenty-one panelists, after screening, were trained for three hours to identify the sensory attributes of non-gluten pasta: smoothness of surface, hardness of first bite, adhesiveness of chew down, cohesiveness of chew down, and off-flavor. First the panelists assigned intensity values to the reference standards and control pasta (regular pasta, gluten-containing) through discussion and consensus. The intensity values were assigned to the reference standard and the control pasta by making a horizontal line on a numerical scale (0-10). Second, the panelists practiced evaluating sample intensity against reference standards and the control pasta. A control pasta was used as the comparison for each attribute. After training, the panel evaluated the samples.

The definition of each attribute and the evaluation procedure is provided. Some reference standards were obtained from Spectrum Intensity Scales (Meilgaard et al., 1991). Jello brand gelatin (Kraft Foods, Inc.) and cereal-Fiber One (General Mills Sales, Inc.) were used as reference standards for smoothness of surface. Cream cheese (Kraft, Inc.) and carrot (Fresh 1 Marketing, Inc.) were used for hardness of first bite. Tomato (Del Cabo, Inc.) and Rice Krispies (Kellogg's, Co.) were used for adhesiveness of chew down. Muffin and chewing gum (Warner-Lambert, Co.) were used for cohesiveness of

chew down. The control pasta (Barilla, Co.) and a non-gluten slurry were used for off-flavor.

Sensory evaluation form

The panel used a 10-cm scale (0-10) line scale to evaluate non-gluten pasta smoothness of surface, hardness of first bite, adhesiveness of chew down, cohesiveness of chew down, and off-flavor against reference standards and control pasta.

Sample preparation and testing

Each pasta formula was blended with 330-350g distilled water in a single screw pasta mixer/extruder for 15 min. Pasta was extruded through a 1.5-mm noodle shape die (ABC, Inc., Model D-45 S.H.). Fresh pasta was dried at a controlled temperature of 90°C for 5 hours in a food dehydrator (Alternative Pioneering Systems, Inc., Model FD-300T). Dried pasta was boiled in 1000g tap water for 13 min. Panelists judged the pasta samples made from the formulas given in Table 2. Testing sessions took place over six days. Sessions were held in a room with ambient temperature and lighting with environmental sounds and odors minimized. Panelists were apprised of terminology definitions and procedure at each session. Fresh reference standards and control pasta were prepared for each session. In the first and fourth blocks, each panelist received six samples (Treatments 1, 6, 8, 9, 11, and 14). In the second and fifth blocks, each panelist received 5 samples (Treatments 2, 4, 7, 12, and 15). In the third and sixth blocks, each panelist received 6 samples (Treatments 3, 5, 8, 10, 11, and 13). Panelists rated each of the tested samples against reference standards and a control pasta. While testing, they were requested to refrain from discussion and to remain within their individual booths.

The panelists had an unlimited supply of distilled water and unsalted crackers to rinse their palates between the samples.

Specimen preparation for scanning electron microscope

Fixation. Cooked pasta was cut into 1 cm^2 for a surface view and 1x 0.5 cm^2 for a cross-section view. Samples were fixed with 1.6% glutaraldehyde in 0.1M cacodylate buffer at 25°C for 2 hrs and rinsed three times in phosphate buffers (20 min/rinse). After the third rinse the buffer was removed and replaced with 1% osmium in 0.1M cacodylate buffers at 25°C for 2 hrs to fix the samples. The samples were then rinsed three more times in phosphate buffer (20min/rinse). The samples were allowed to stand in the phosphate buffer after the last rinse and then stored at 4°C overnight.

Dehydration. Before dehydration, the phosphate buffer was removed. The samples were dehydrated in ethanol at concentration: 50%, 70%, 90%, 95%, and 100%. The ethanol (100%) dehydration was repeated three times.

Critical point drying. After dehydration, the samples were critical-point-dried (DENTON DCP-1). This technique allowed sample drying without the surface damage that accompanies air drying. The critical point of a substance is the specific temperature and pressure where the densities of its liquid and vapor phase are equal, resulting in zero surface tension. The gaseous substance can be released from the sample without surface damage. Liquid carbon dioxide is commonly used because its critical point (36.5°C and 1080 p.s.i.) can be conveniently reached with a single apparatus to dry samples. Dried samples were mounted on stubs and put into the desiccator before gold coating.

Gold coating. Samples were placed into a Hummer II (Technics, Inc., Alexandria, VA) machine for gold coating. Each sample was coated for three minutes

and thirty seconds. After coating, the gold-coated specimens were placed in a desiccator to prevent moisture absorption.

Sample scanning

Samples were observed in a JEOL (JSM-35, JEOL LTD., Japan) scanning electron microscope at an acceleration voltage of 25 KeV. Micrographs of the surface and the cross-sections of each sample were taken at magnification of 50X and 1000X.

RESULTS AND DISCUSSION

The ranges of the panelists' sensory response scores for non-gluten and control pastas are shown in Table 3. Off-flavor has a narrow range among the five attributes. In fifteen treatments, means for hardness of first bite, adhesiveness of chew down, cohesiveness of chew down, and off-flavor are significant ($p<0.05$), but smoothness of surface is not significant (Table 4).

Choice of model selection

Hardness of first bite, adhesiveness of chew down, cohesiveness of chew down, and off-flavor are selected as model selection responses. Using the Model Selection Procedure (SAS), the selected model is in Table 5. The independent variables X_1, X_2, and X_3 were selected with interaction terms X_1X_2, X_1X_3, and X_2X_3 in the model. Coefficients of determination (R^2) indicate that the regression equation explains 22~75% of total variation (Table 5). Five independent variables were introduced so the adjusted R-square (R_a^2) was used rather than R^2 (Xi, i =1, 2, 3,….). The regression model for hardness of first bite, adhesiveness of chew down, and cohesiveness of chew down are significant ($P<0.05$), but the regression model for off-flavor is not significant (Table 5).

Surface and contour plots

Hardness of first bite. Hardness of first bite for the control pasta has a score of 3.5. Hardness of first bite of non-gluten pasta had the highest scores at the level of 40g for locust bean gum and 40g for modified starch when xanthan gum is at the level of 40g (Figures 1 and 2). Hardness of first bite at this combination has a higher score (4.3) than that of the control pasta (3.5). At the charted level of locust bean gum greater than 27g and modified starch greater than 34g, hardness of first bite can reach scores greater than

3.5 but only when xanthan gum is at the level of 40g (Figures 3 and 4). Modified starch and locust bean gum improved hardness of first bite when xanthan gum was at the high level; xanthan gum apparently interacted with locust bean gum to increase strength of gel (Zan et al., 1993). Moreover, hardness of first bite can obtain scores of more than 4 when the level of locust bean gum was greater than 35g, with modified starch greater than 38g and xanthan gum at the level of 40g. At a combination of 10g for locust bean gum and 30g for modified starch, hardness of first bite has a lower score at the high level (40g) of xanthan gum than at the low level (30g) of xanthan gum (Figures 1 and 2). It seems that when locust bean gum is low, xanthan should be low as well, since a higher proportion of xanthan gum does not enhance the non-gluten pasta structure and may even weaken the matrix structure. At the level of 40g for xanthan gum, hardness of first bite scores fall to less than 2 when the charted level of locust bean gum is less than 12g and charted modified starch is less than 31g (Figure 4). Low levels of modified starch and locust bean gum do not provide enough structure for non-gluten pasta. Further, locust bean and xanthan gums should be present in about the same level from the analysis of hardness of first bite alone.

Adhesiveness of chew down. Xanthan gum not only provided a firm structure but also increased stickiness for non-gluten pasta. The panelists gave a rating of 2 for adhesiveness of the control pasta. Therefore, a lower score (closer to 2) for adhesiveness is a good property since that is nearest to the control pasta. Adhesiveness of chew down of non-gluten pasta decreased when xanthan gum was increased at the level of 10g for locust bean gum and 30g for modified starch (Figures 5 and 6). Adhesiveness of chew down has the lowest score at the level of 10g for locust bean gum and 40g for modified

starch when xanthan gum is at level of 25g (Figure 5). At the level of 25g for xanthan, adhesiveness of chew down was decreased by modified starch, but was increased by locust bean gum. The plot illustrated that a combination of locust bean gum less than 13g and modified starch greater than 35g can lower the score to below 1.75 at the level of 25g for xanthan gum (Figure 7). This is lower than adhesiveness of chew down of the control pasta (2), and at any level of locust bean gum and modified starch, the score of adhesiveness of chew down was over 1.75 when xanthan is at the level of 40g (Figure 8).

Cohesiveness of chew down. The panelists rated this attribute for the control pasta at 4. Cohesiveness of chew down of non-gluten pasta gave similar results as hardness of first bite. Toufeili (1994) found non-wheat starch and non-starch polysaccharides interacted and increased cohesiveness in non-gluten bread. Cohesiveness of chew down of non-gluten pasta had the highest score at the level of 40g for locust bean gum and 40g for modified starch when xanthan is at the level of 40g (Figures 9 and 10). But the lowest score of cohesiveness of chew down is at the level of 10g for locust bean gum and 30g for modified starch when xanthan gum is at the level of 40g. This may be caused by extra xanthan gum and not enough locust bean gum or modified starch to interact with. Locust bean gum increased cohesiveness of chew down as illustrated at levels of either 25g or 40g of xanthan gum with modified starch at 30g (Figures 9 and 10). However, modified starch decreased cohesiveness of chew down at 25g of xanthan gum, but increased cohesiveness of chew down at the level of 40g of xanthan gum. Cohesiveness of chew down can reach scores of greater than 4 (control pasta) with the plot level of locust bean gum greater than 32.5g, modified starch greater than 37g, and xanthan gum at the level of 40g (Figures 11 and 12).

Off-flavor. The off-flavor of all non-gluten pastas had a mean score of (0.92) compared to the control pasta rating (0). Off-flavor had the lowest score at the level of 40g for locust bean gum and 30g for modified starch when xanthan gum was at the level of 40g (Figures 13 and 14). Modified starch may contribute more off-flavor for non-gluten pasta at the level of 40g of xanthan gum than at 25g. Since off-flavor has a negative impact on sensory acceptability of non-gluten pasta, low off-flavor scores were desired since the panel had reported no off-flavor in the control pasta. The plots showed that off-flavor of non-gluten pasta can score less than 0.7 at the level of locust bean gum greater than 33g, modified starch less than 33.5g, and xanthan gum at the level of 40g (Figures 15 and 16).

Optimum regions. Optimum regions of xanthan gum, modified starch, and locust bean gum were selected by overlapping the contour plots of sensory properties of non-gluten pasta as compared to the control pasta. At xanthan gum level of 25g, the optimum region was: hardness > 3.5, cohesiveness > 3.5, adhesiveness < 0.8, and off-flavor < 0.8. At xanthan gum level of 40g, the optimum region was: hardness > 4, cohesiveness > 4, adhesiveness < 2, and off-flavor < 0.7. Possible formulas were selected from optimum regions by calculating the functions and responses of each property. Three predicted formulas were selected from the possible formulas by choosing ranges: hardness 3.4~3.6; adhesiveness 1.8 ~ 2.2; off-flavor <0.9; cohesiveness 3.7 ~ 4.0. Three formulas were replicated three times and tested for validation (consistency) by a small research panel that compared to sensory characteristics and physical properties. Each formula was consistent among the three replications. This final formula of non-gluten pasta that possessed the most desirable properties among the three formulas was xanthan

gum at 40g, modified starch at 35g, locust bean gum at 40g, tapioca starch at 113g, potato starch at 57g, corn flour at 250g, and rice flour at 50g.

Scanning electronic microscopy

The SEM pictures of the microstructure provided images of non-gluten and control pastas for comparison. The control pasta had a compact gluten-protein matrix entrapping starch granules (Figures 17 and 18). In Treatments 1 and 3, the matrices of the surface and cross-section of non-gluten pasta were more compact when modified starch was increased, and locust bean gum and xanthan gum were fixed. (Figures 19, 20, 21, and 22). In Treatments 8 and 11, xanthan gum was increased while modified starch and locust bean gum remained fixed; surface structure and cross-section of non-gluten pasta were more compact (Figures 23, 24, 25, and 26). At higher levels of xanthan gum, modified starch, and locust bean gum, non-gluten pasta had a more compact matrix structure than that of lower levels, which is similar to the results of Chinnaswamy and Hanna (1991). The long length of non-starch polysaccharides such as xanthan and locust bean gums can interact with starch to strength the structure of the extruded products. Nevertheless, we have to consider other views on sensory characteristics. Some combinations at higher levels of xanthan gum, modified starch, and locust bean gum increase off-flavor and also increase adhesiveness beyond that of the control pasta. Although images of non-gluten pasta showed a different matrix structure from the control pasta, the new matrix structure that was created by non-wheat flour may provide similar sensory characteristics to the gluten matrix.

CONCLUSIONS

This study showed that quality of non-gluten pasta could be improved by using different levels of non-gluten starches and flours, and non-starch polysaccharides by following a mixture experiment and surface response methodology. Xanthan gum, modified starch, and locust bean gum showed significant effects on sensory characteristics and scanning electron microstructure. Values most similar to the control pasta were obtained at higher levels of xanthan gum, modified starch, and locust bean gum for the sensory characteristics: hardness of first bite and cohesiveness. The micrographs of non-gluten pastas also showed that higher levels of locust bean gum, modified starch, and xanthan gum had more compact matrix structures similar to the control pasta. However, highest levels of these caused more adhesiveness (stickiness) on non-gluten pasta surface than the control pasta. By following mixture experiment, the effect of almost infinite levels of several ingredients were evaluated and successful ratios of these ingredients postulated.

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Table 1. Experimental treatment structure of the 15 formulas (grams)

Trt	LBG	XG	MS	TS	PS	CF	RF
1	10	25	30	90.00	45.00	250	50
2	10	25	35	86.50	43.35	250	50
3	10	25	40	83.40	41.67	250	50
4	10	40	30	80.00	40.00	250	50
5	10	40	35	76.50	38.35	250	50
6	10	40	40	73.35	36.67	250	50
7	25	25	30	80.00	40.00	250	50
8	25	25	35	76.50	38.35	250	50
9	25	25	40	73.35	36.67	250	50
10	25	40	30	70.00	35.00	250	50
11	25	40	35	66.65	33.35	250	50
12	25	40	40	63.35	31.67	250	50
13	40	25	30	70.00	35.00	250	50
14	40	25	35	66.65	33.35	250	50
15	40	25	40	63.35	31.67	250	50

Trt-treatment, LBG-locust bean gum, XG-xanthan gum, MS-modified starch, TS-tapioca starch, PS-potato starch, CF-corn flour, RF-rice flour

Table 2. Experimental design structure of 15 treatments in 6 incomplete blocks (grams)

Variable		X1 LBG	X2 XG	X3 MS	X4 TS	X5 PS	--- CF	--- RF
Block 1	Trt 1	10	25	30	90.00	250	50	45.00
	Trt 6	10	40	40	73.35	250	50	36.67
	Trt 8	25	25	35	76.50	250	50	38.35
	Trt 9	25	25	40	73.35	250	50	36.67
	Trt 11	25	40	35	66.65	250	50	33.35
	Trt14	40	25	35	66.65	250	50	33.35
Block 2	Trt 2	10	25	35	86.50	250	50	43.35
	Trt 4	10	40	30	80.00	250	50	40.00
	Trt 7	25	25	30	80.00	250	50	40.00
	Trt 12	25	40	40	63.35	250	50	31.67
	Trt 15	40	25	40	63.35	250	50	31.67
Block 3	Trt 3	10	25	40	83.40	250	50	41.67
	Trt 5	10	25	40	83.40	250	50	41.67
	Trt 8	25	25	35	76.50	250	50	38.35
	Trt 10	25	40	30	70.00	250	50	35.00
	Trt 11	25	40	35	66.65	250	50	33.35
	Trt13	40	25	30	70.00	250	50	35.00
Block 4	Trt 1	10	25	30	90.00	250	50	45.00
	Trt 6	10	40	40	73.35	250	50	36.67
	Trt 8	25	25	35	76.50	250	50	38.35
	Trt 9	25	25	40	73.35	250	50	36.67
	Trt 11	25	40	35	66.65	250	50	33.35
	Trt14	40	25	35	66.65	250	50	33.35
Block 5	Trt 2	10	25	35	86.50	250	50	43.35
	Trt 4	10	40	30	80.00	250	50	40.00
	Trt 7	25	25	30	80.00	250	50	40.00
	Trt 12	25	40	40	63.35	250	50	31.67
	Trt 15	40	25	40	63.35	250	50	31.67
Block 6	Trt 3	10	25	40	83.40	250	50	41.67
	Trt 5	10	25	40	83.40	250	50	41.67
	Trt 8	25	25	35	76.50	250	50	38.35
	Trt 10	25	40	30	70.00	250	50	35.00
	Trt 11	25	40	35	66.65	250	50	33.35
	Trt13	40	25	30	70.00	250	50	35.00

LBG-locust bean gum, XG-xanthan gum, MS-modified starch, TS-tapioca starch PS-potato starch, CF-corn flour, RF-rice flour.

Table 3. Range of sensory response scores of 15 non-gluten pasta treatments and the control pasta

Response	Range of non-gluten pasta	Control pasta
Smoothness of surface	6.50 ~ 0.50	2.00
Hardness of first bite	6.50 ~ 0.50	3.50
Adhesiveness of chew down	6.00 ~ 0.00.	2.00
Cohesiveness of chew down	6.00 ~ 0.00	4.00
Off-flavor	4.00 ~ 0.00	0.00

Table 4. Sensory response score means of 15 non-gluten pasta treatments and the control pasta

Response	Mean of non-gluten pasta	Control pasta
Surface of smoothness	2.96 (F=0.87, P=0.5963)	2.00
Hardness of first bite	2.79[a] (F=8.90, P<0.0001)	3.50
Adhesiveness of chew down	2.01[a] (F=4.57, P=0.0032)	2.00
Cohesiveness of chew down	3.04[a] (F=5.57, P=0.0011)	4.00
Off-flavor	0.92[a] (F=3.28, P=0.0154)	0.00

[a] indicates significant difference among the 15 treatment means ($P < 0.05$).

Table 5. Regression analysis of sensory evaluation of non-gluten pasta

	Hardness of first bite	Adhesiveness of chew down	Cohesiveness of chew down	Off-flavor
Coefficient				
b_0	6.5418	3.8811	10.0502	0.6724
b_1	-0.0934	-0.0446	-0.0908	-0.0288
b_2	-0.1377	-0.0350	-0.2161	-0.0294
b_3	-0.1094	-0.0770	-0.1981	0.0020
b_{11}	0.0009	-0.0007	0.0026	0.0013
b_{12}	0.0029	0.0023	0.0055	-0.0005
b_{15}	0.0032	0.0014	0.0009	-0.0006
Coefficient of Determination				
R^2	0.7588	0.5188	0.6212	0.2238
R^2_a	0.7052	0.4118	0.5370	0.0513
F-test	$P < 0.0001$	$P = 0.0018$	$P < 0.0001$	$P = 0.2920$

$E(y)= b_0+b_1X_1+b_2X_2+b_3X_3+b_{11}X_1X_2+b_{12}X_1X_3+b_{15}X_2X_3$, X_1=locust bean gum, X_2=xanthan gum, X_3=modified starch.

Figure 1. Surface plot for hardness of first bite at xanthan gum = 25g

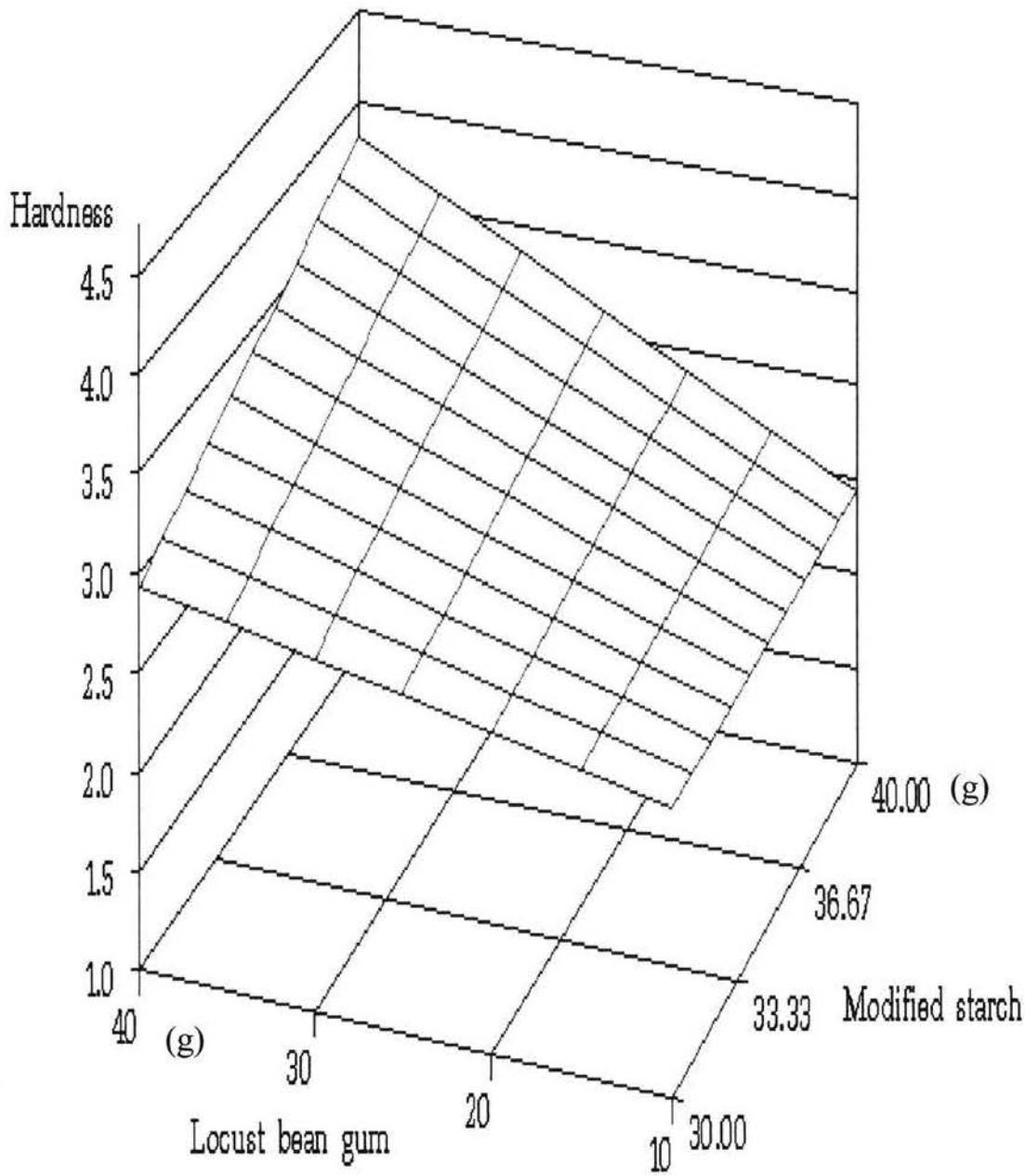


Figure 2. Surface plot for hardness of first bite at xanthan gum = 40g

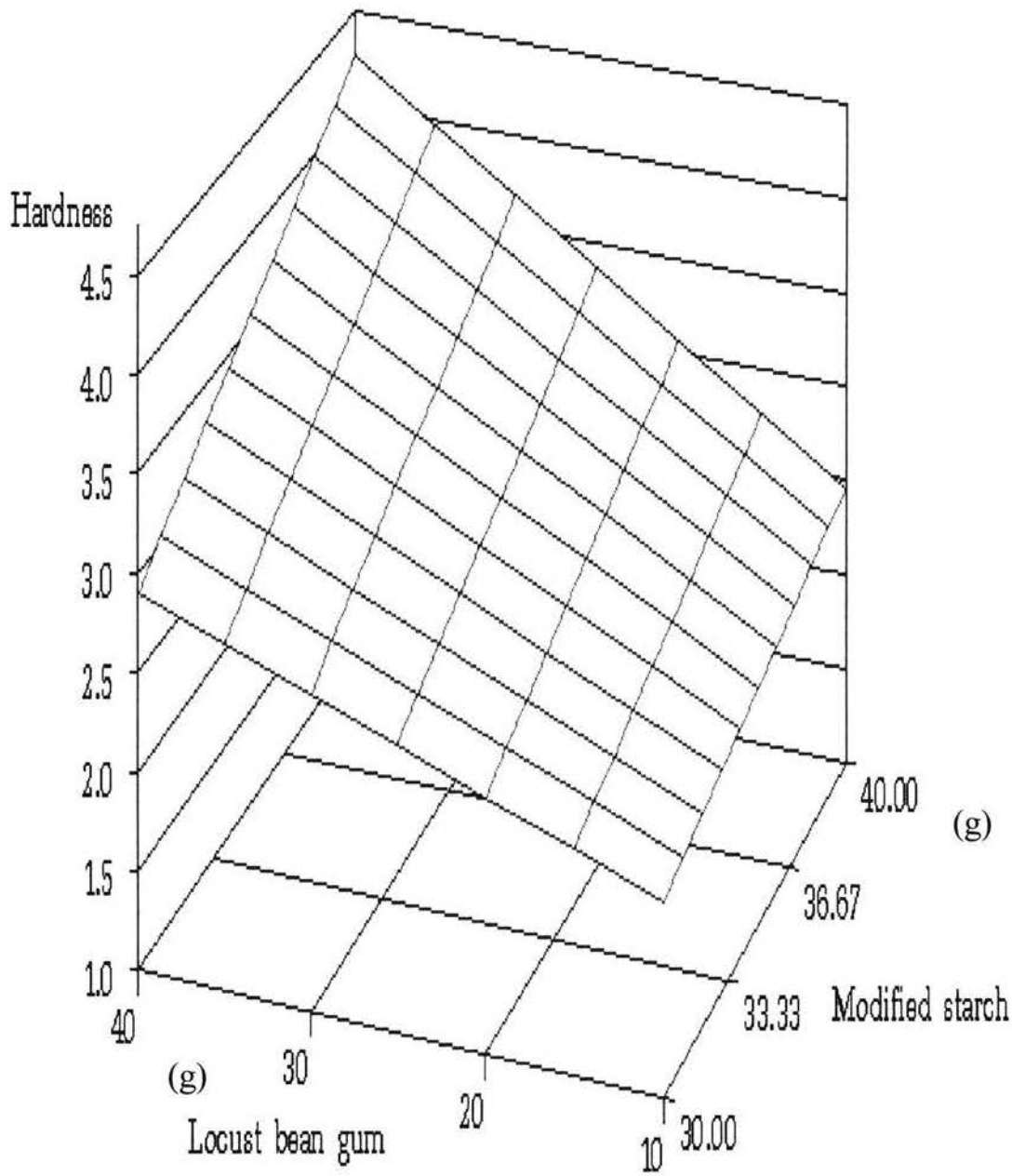


Figure 3. Contour plot for hardness of first bite at xanthan gum = 25g

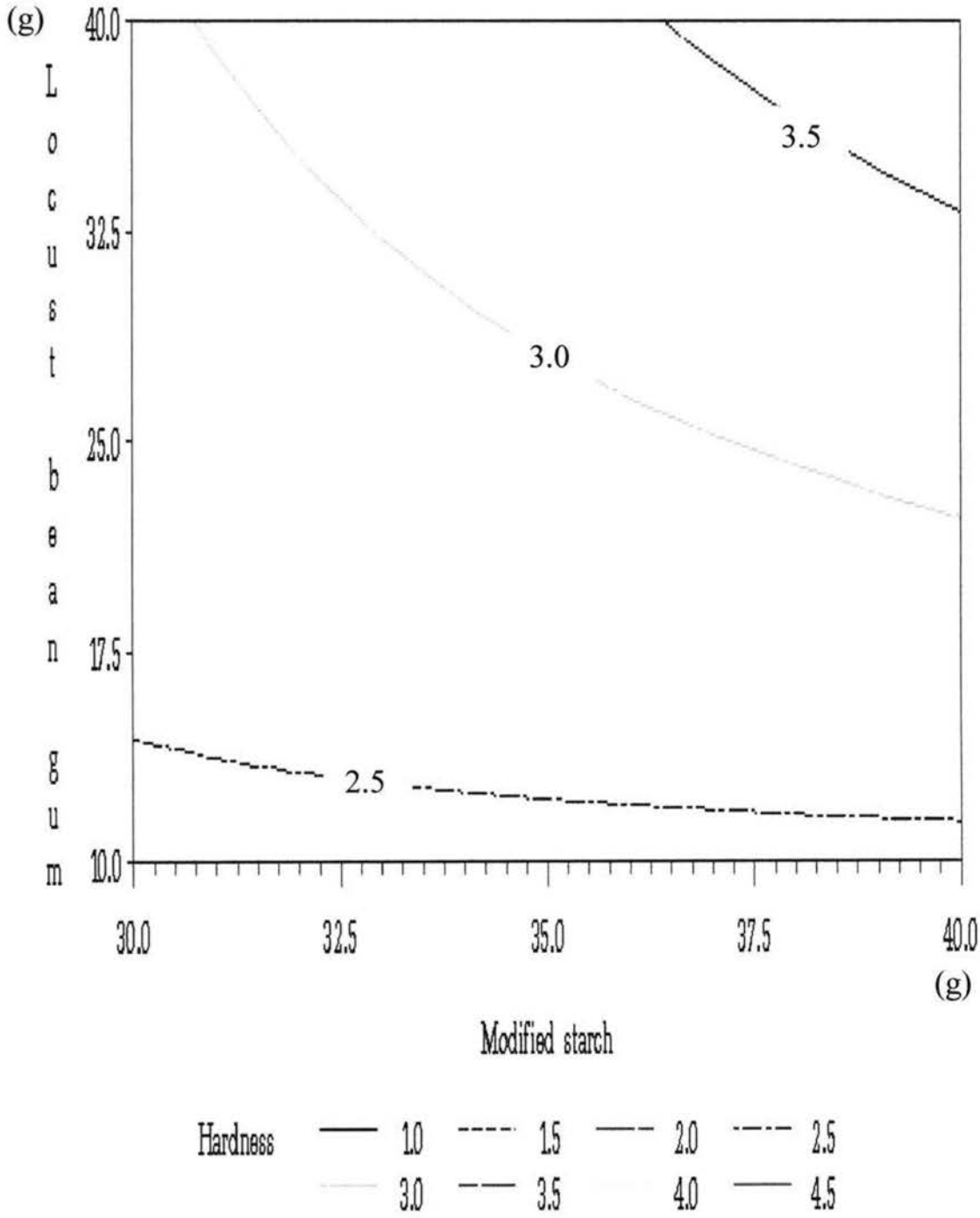


Figure 4. Contour plot for hardness of first bite at xanthan gum = 40g

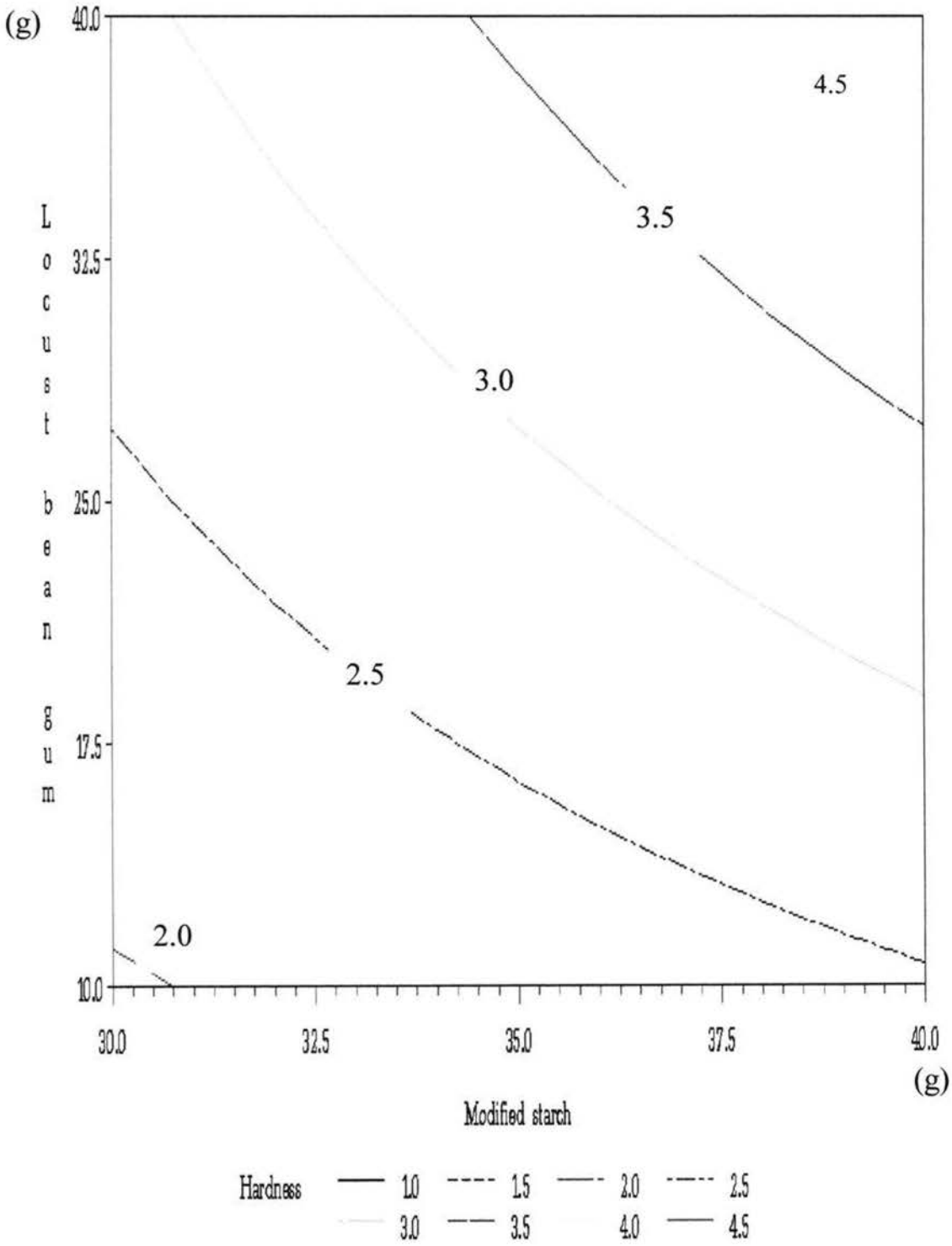


Figure 5. Surface plot for adhesiveness of chew down at xanthan gum = 25g

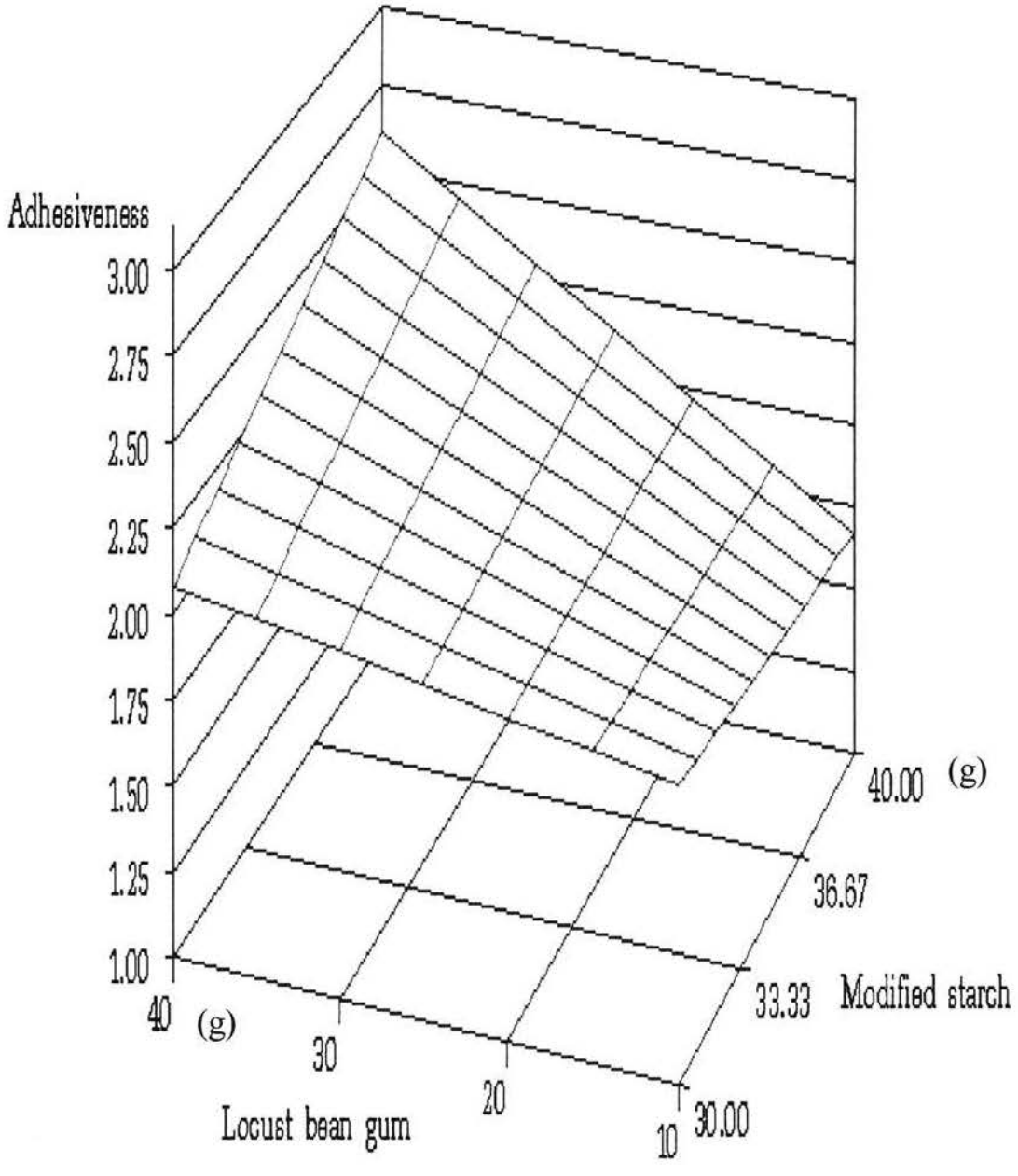


Figure 6. Surface plot for adhesiveness of chew down at xanthan gum = 40g

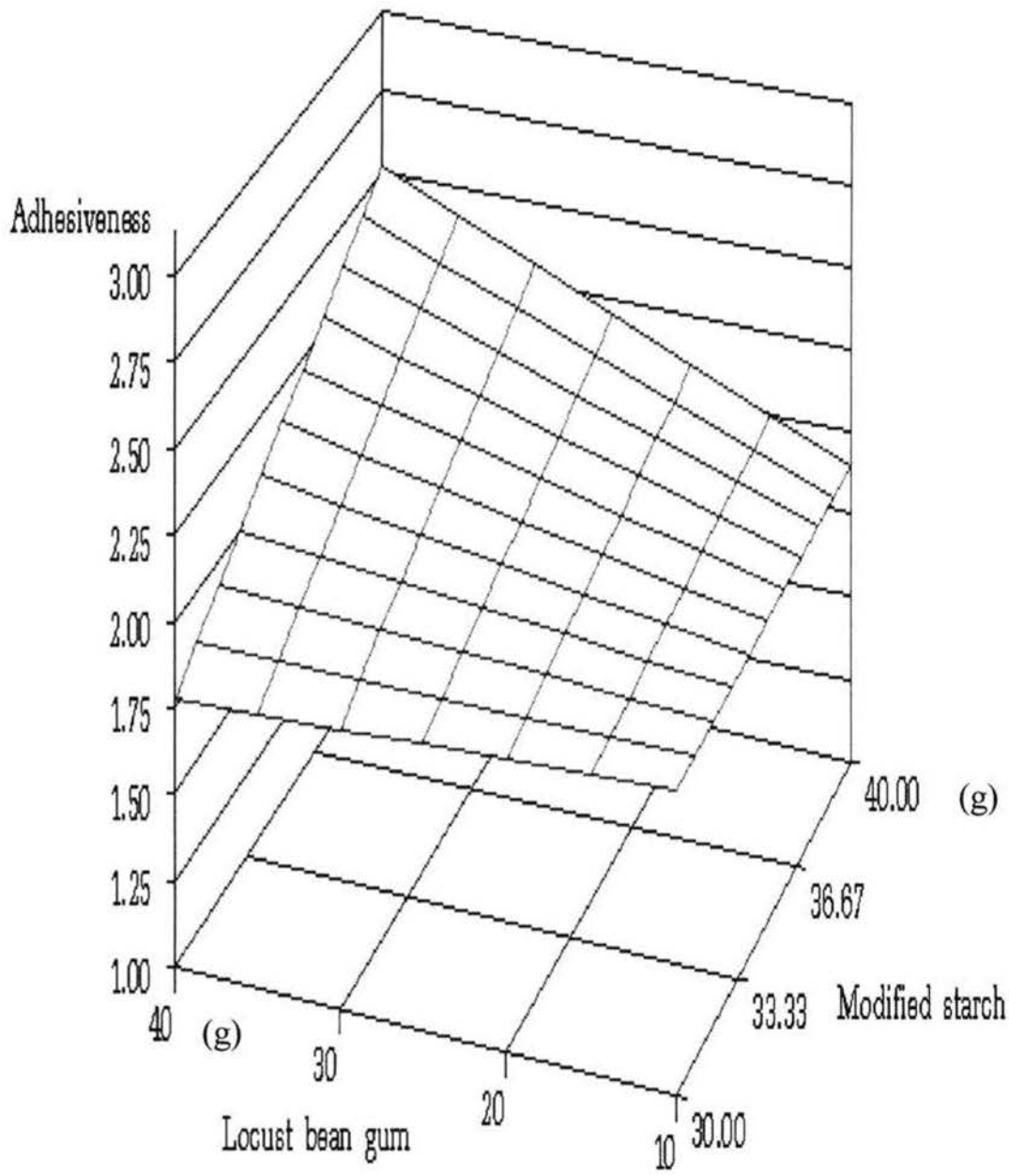


Figure 7. Contour plot for adhesiveness of chew down at xanthan gum =25g

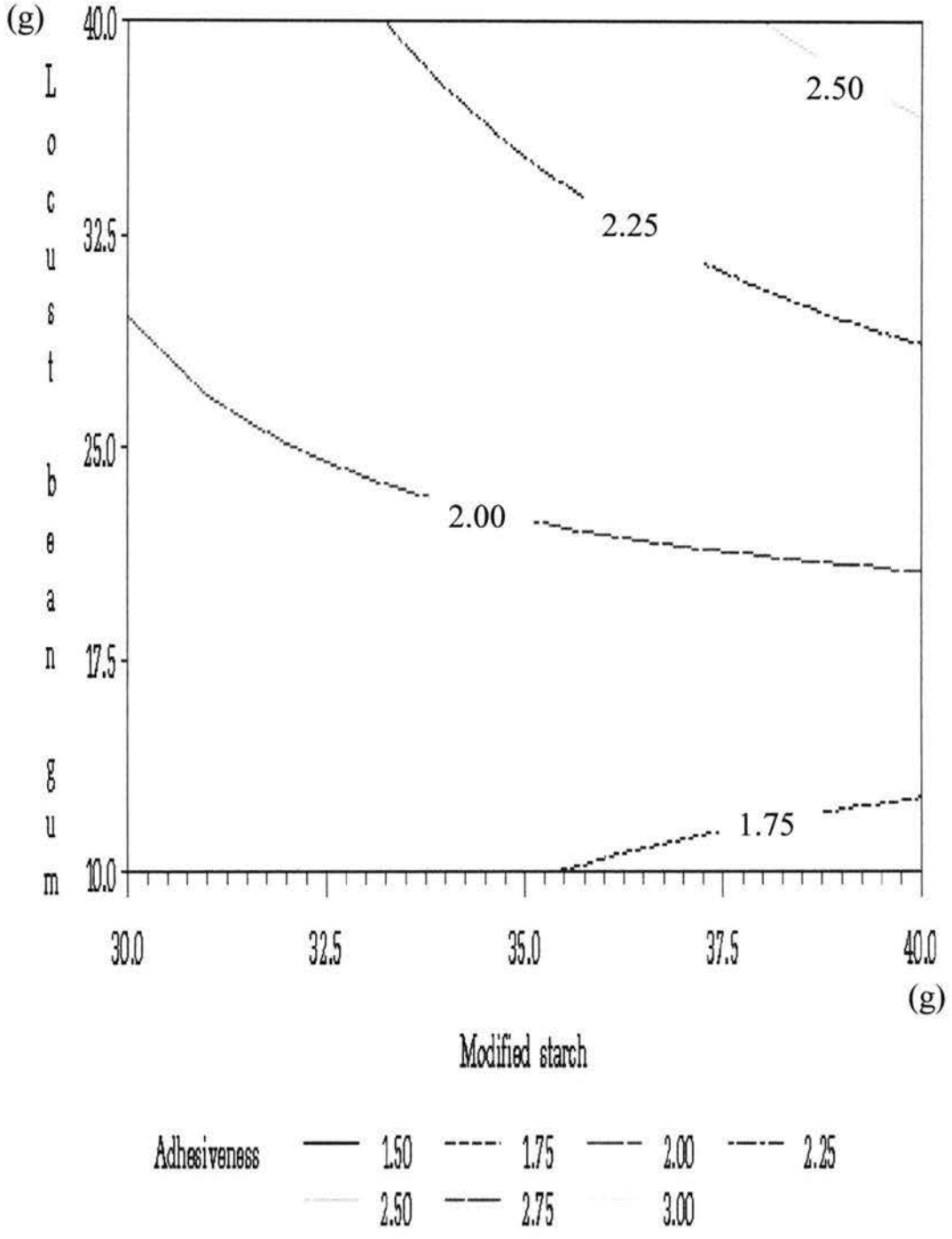


Figure 8. Contour plot for adhesiveness of chew down at xanthan gum =40g

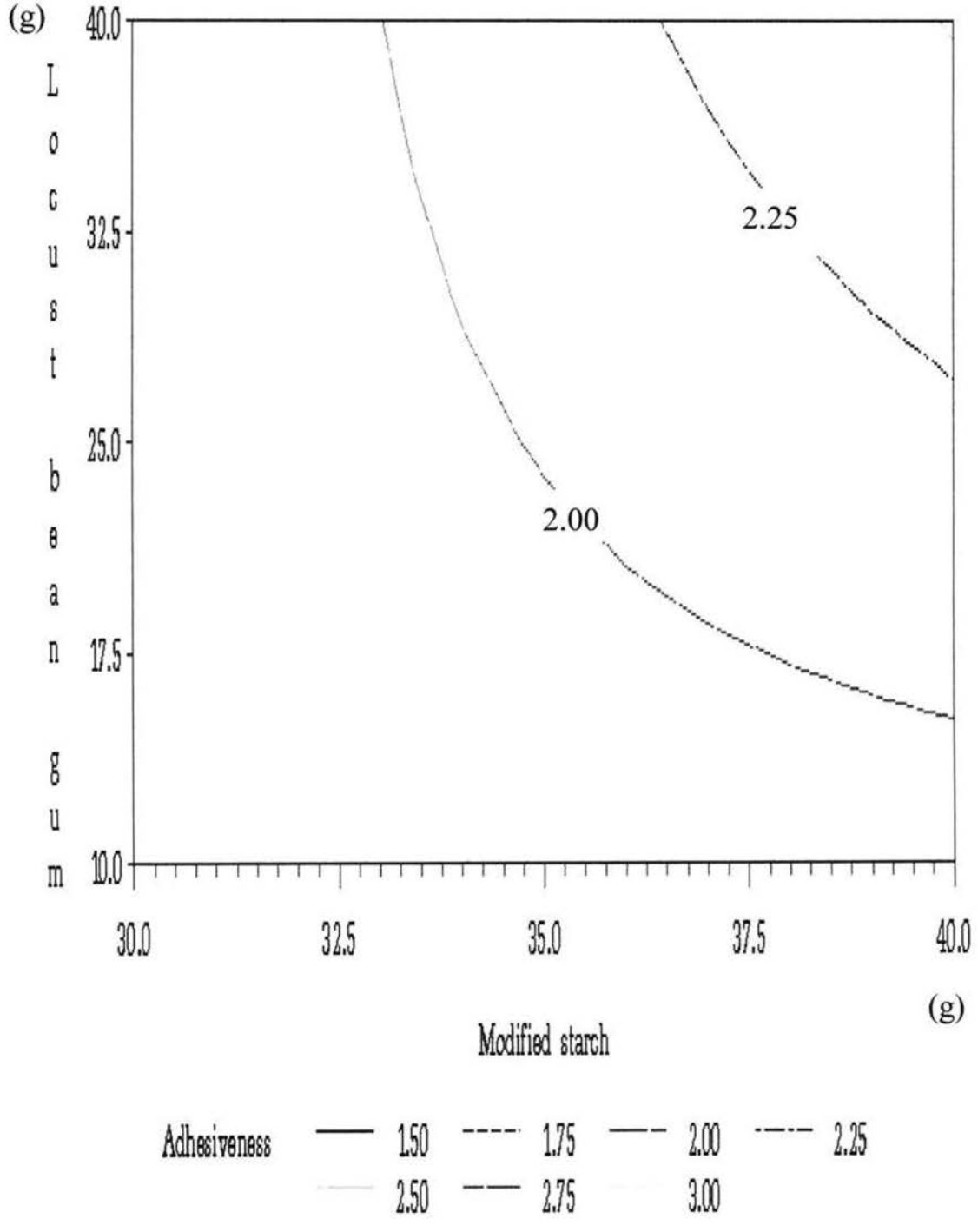


Figure 9. Surface plot for cohesiveness of chew down at xanthan gum = 25g

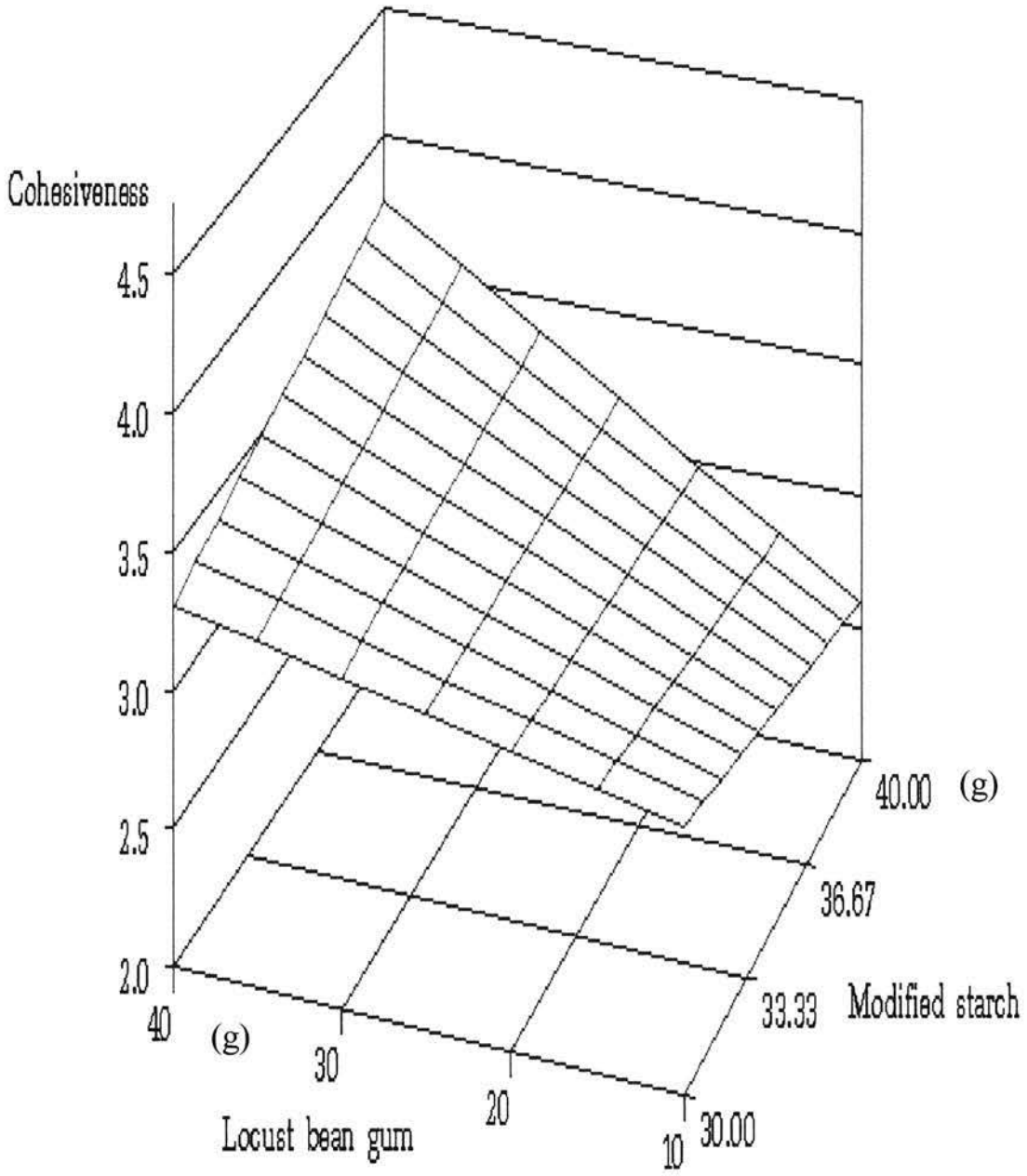


Figure 10. Surface plot for cohesiveness of chew down at xanthan gum = 40g

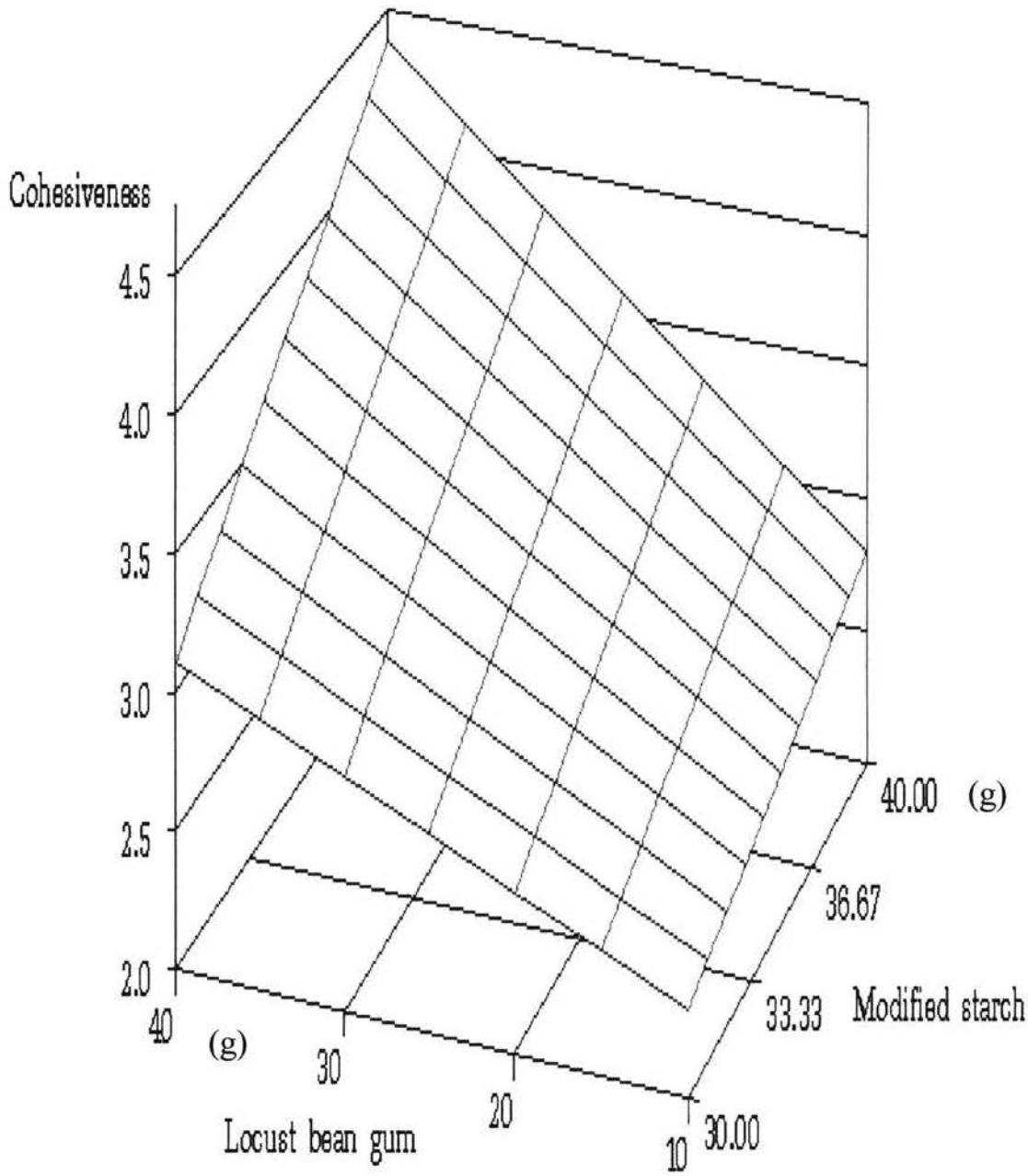


Figure 11. Contour plot for cohesiveness of chew down at xanthan gum = 25g

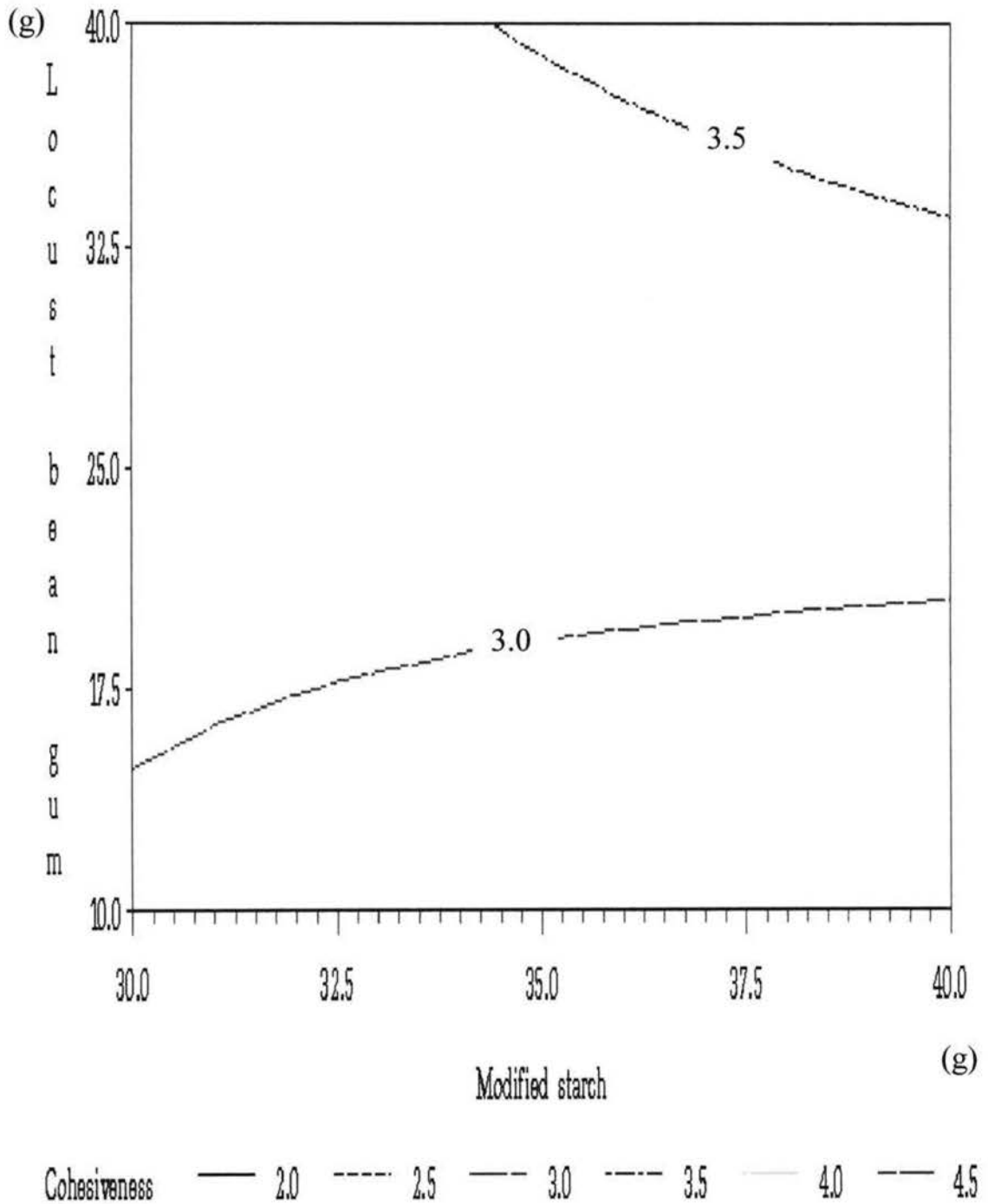


Figure 12. Contour plot for cohesiveness of chew down at xanthan gum =40g

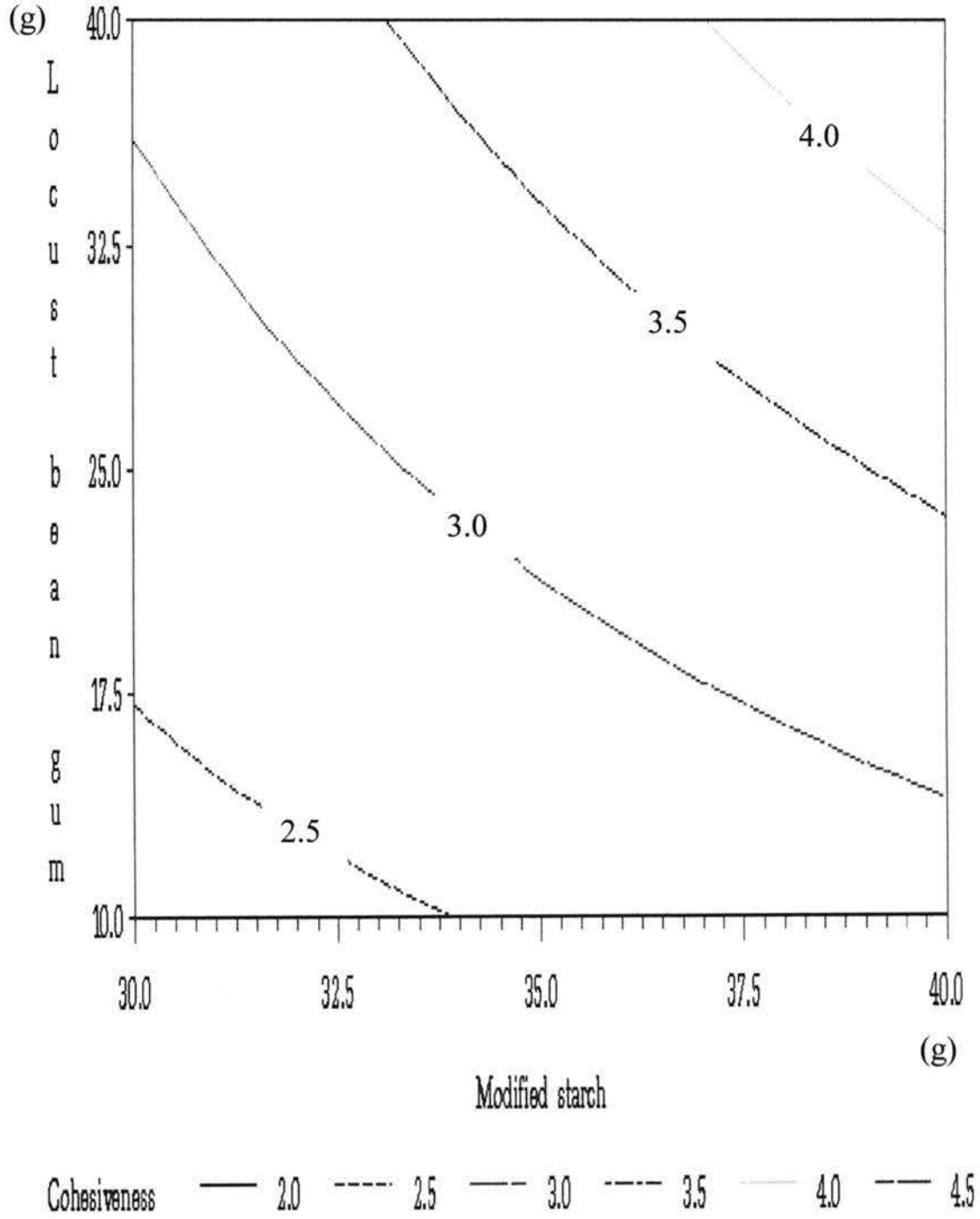


Figure 13. Surface plot for off-flavor at xanthan gum = 25g

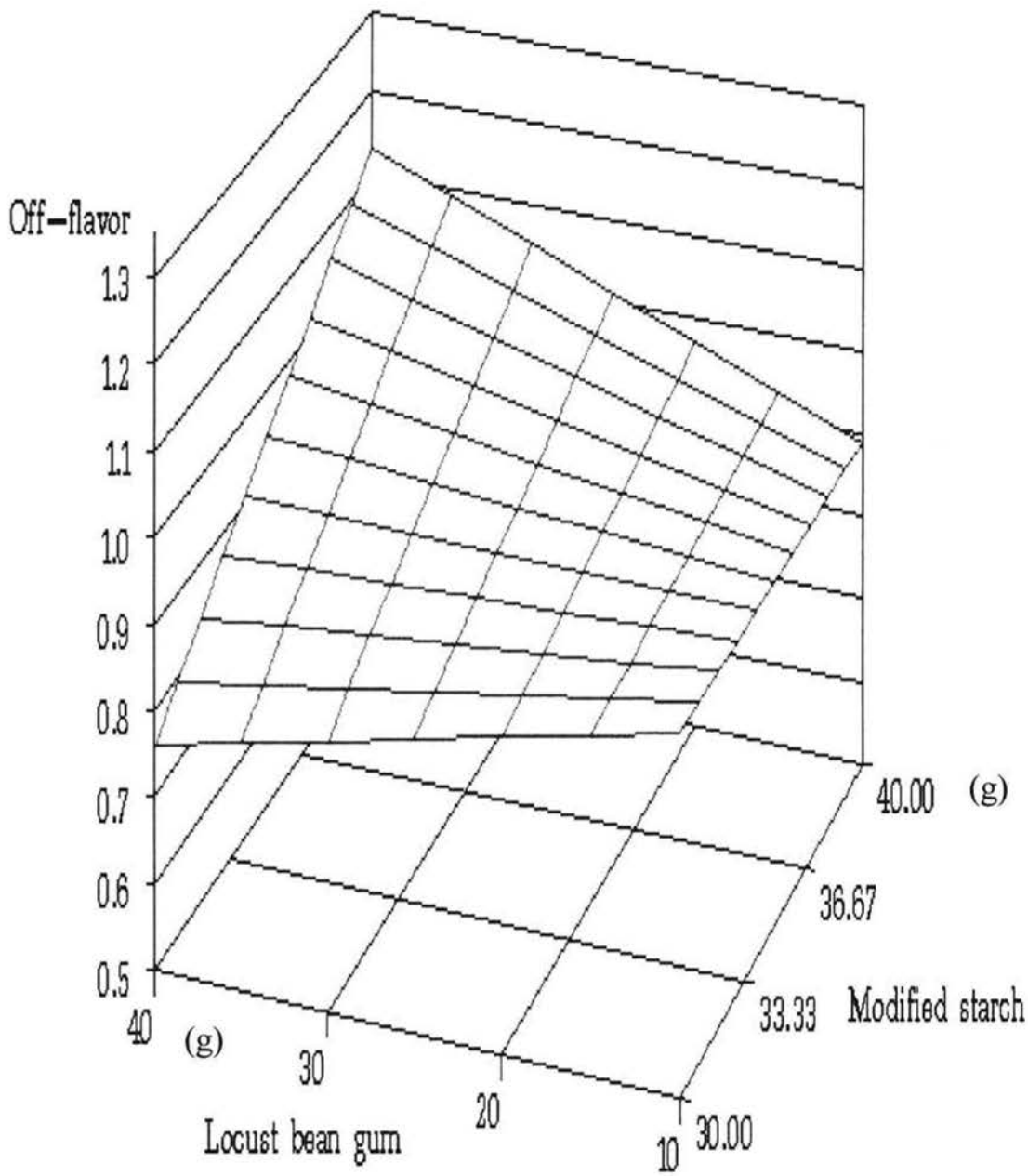


Figure 14. Surface plot for off-flavor at xanthan gum = 40g

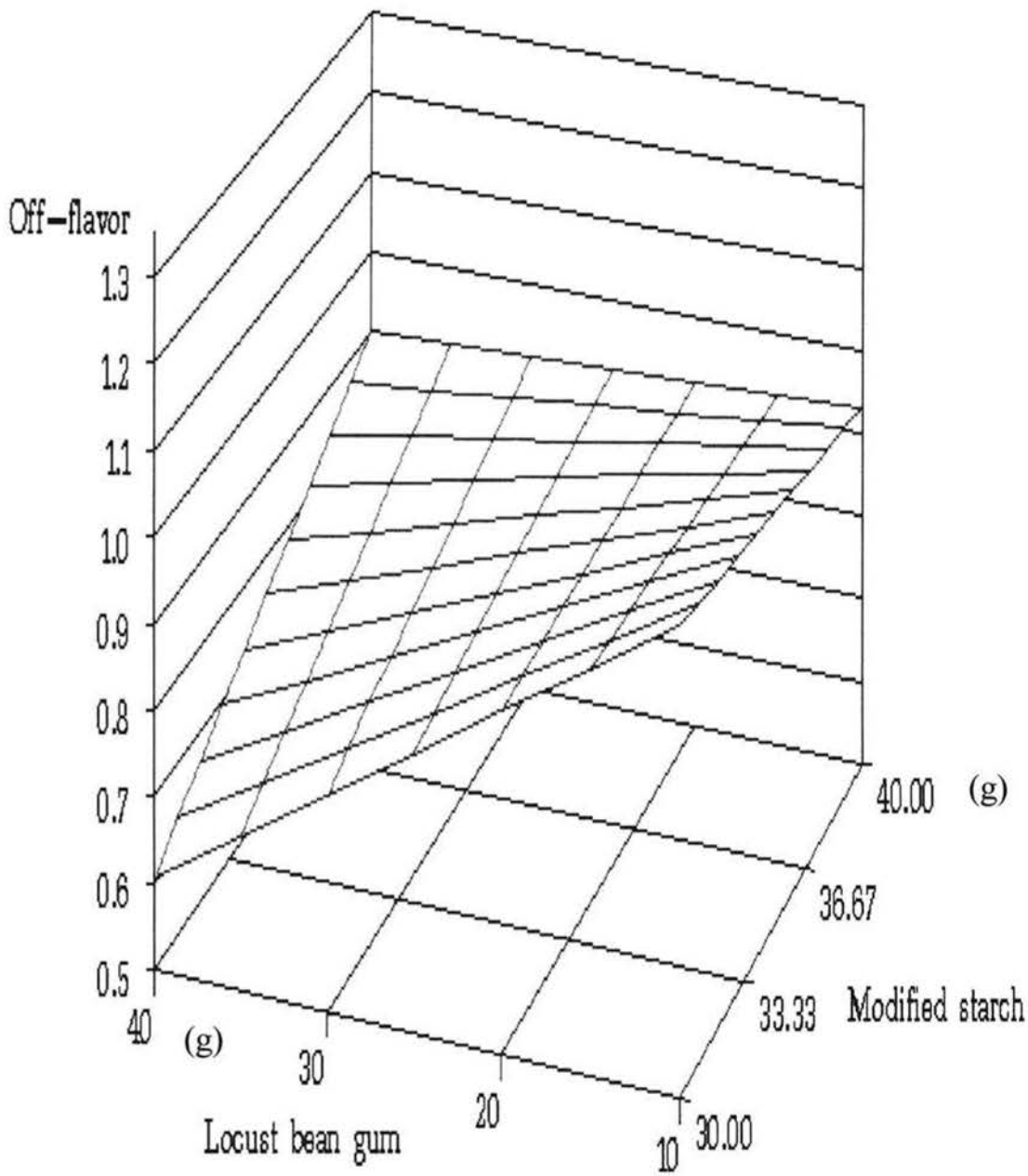


Figure 15. Contour plot for off-flavor at xanthan gum = 25g

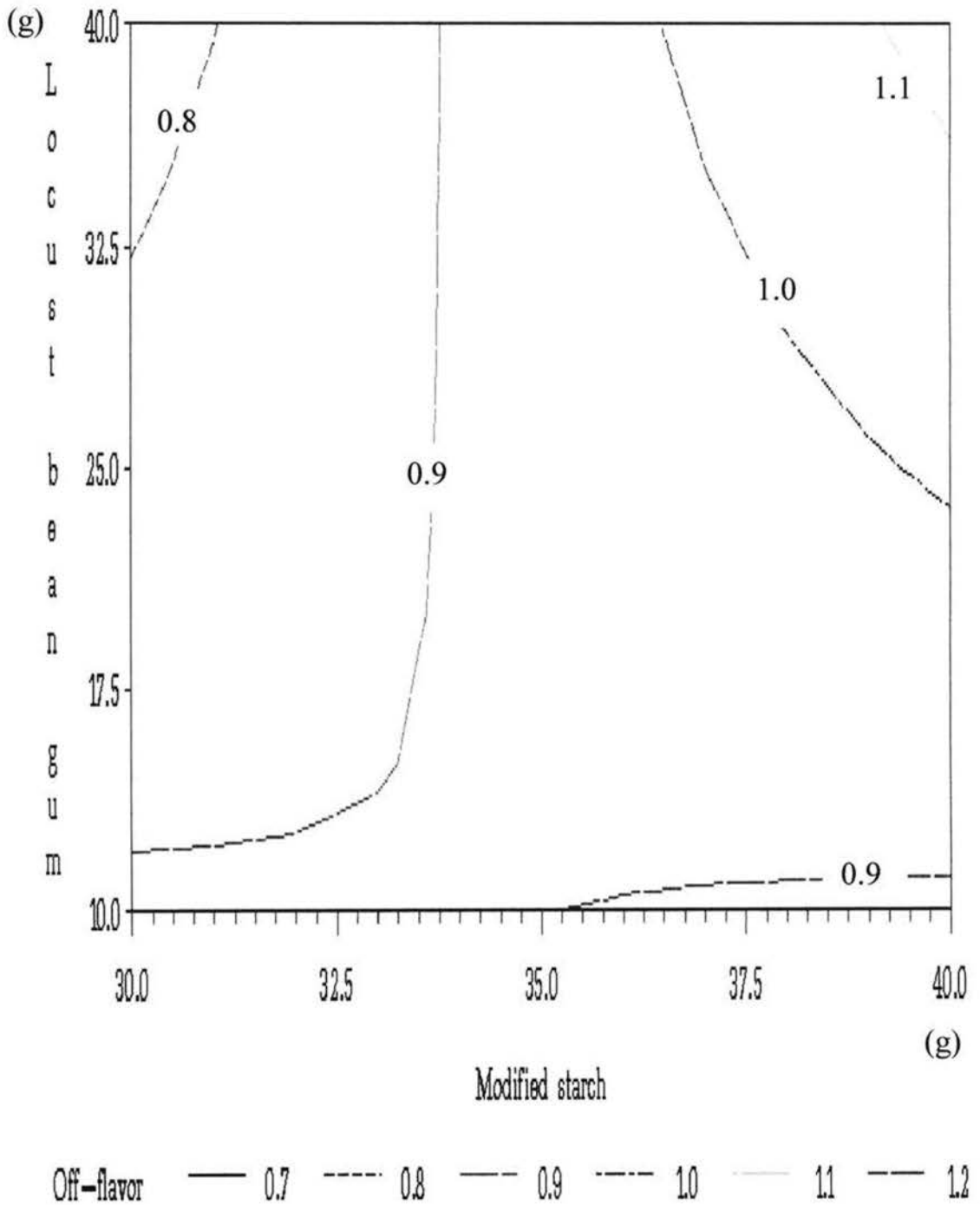


Figure 16. contour plot for off-flavor at xanthan gum = 40g

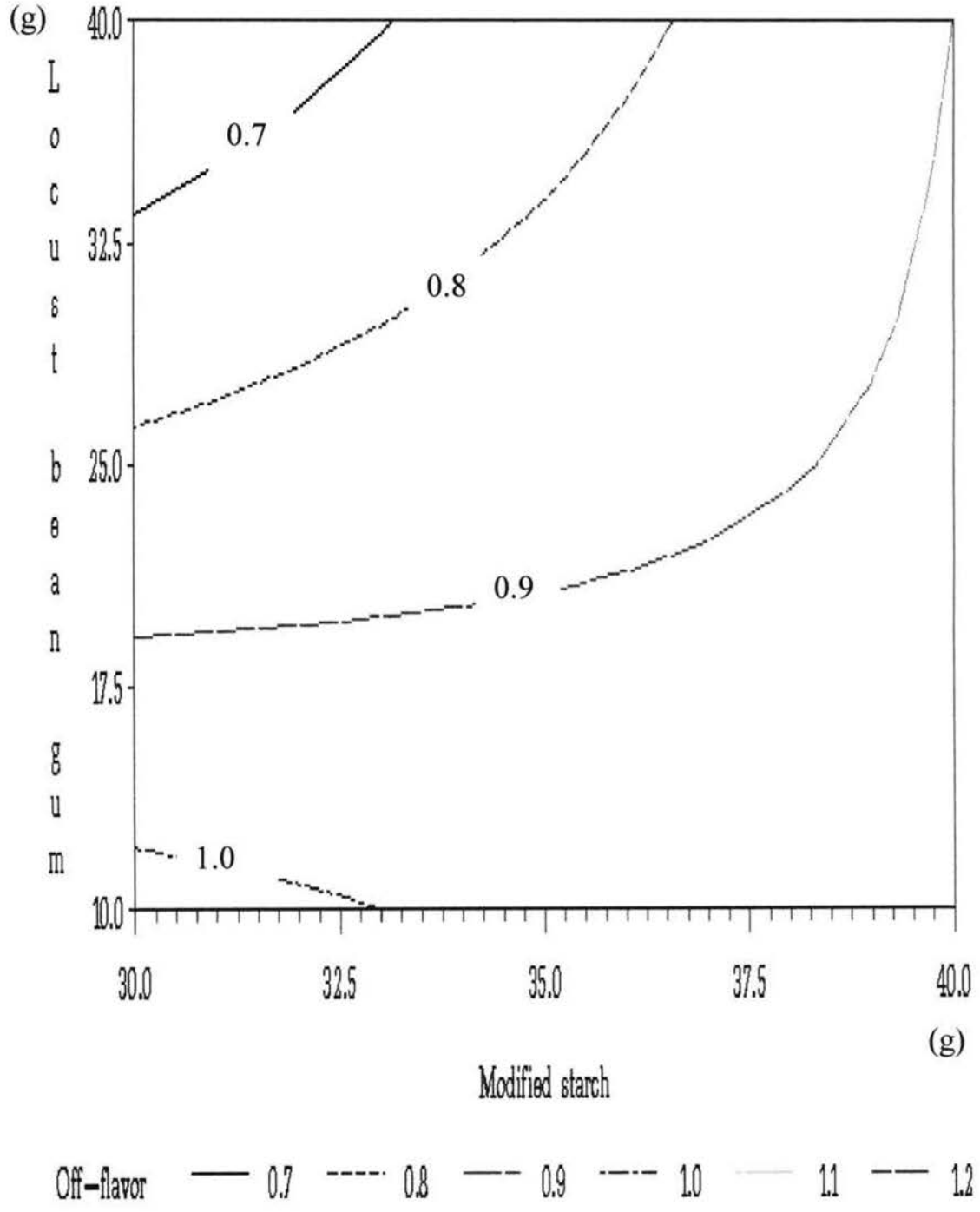
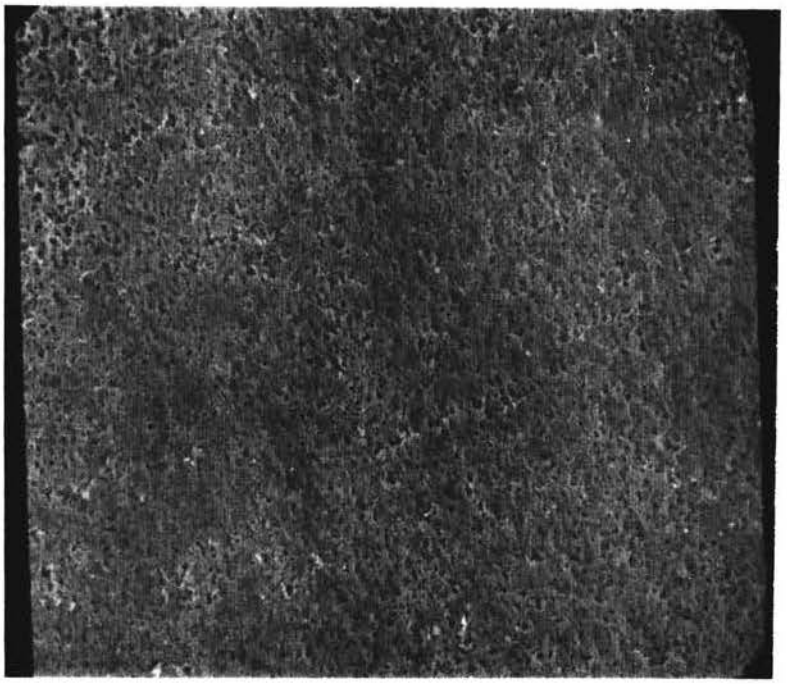
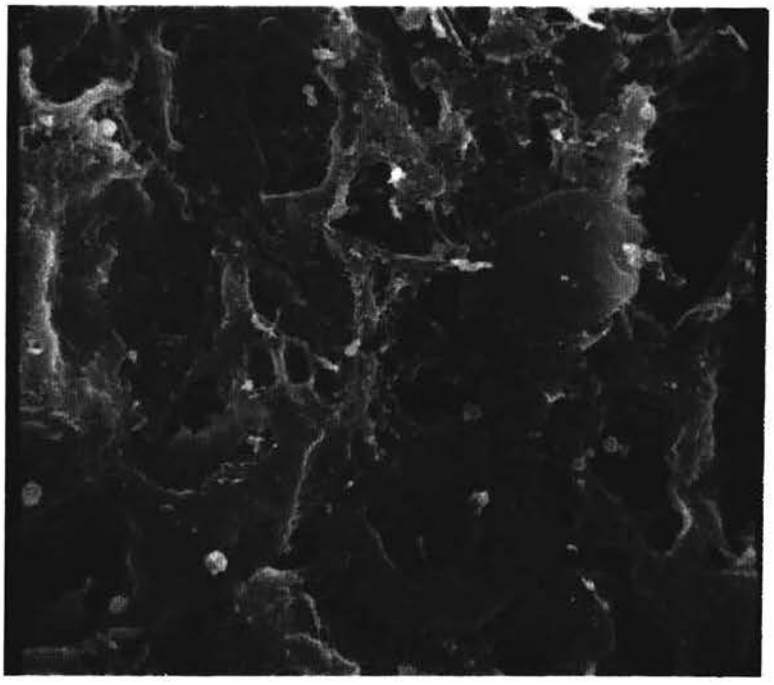


Figure 17. Control pasta (surface)

a



b

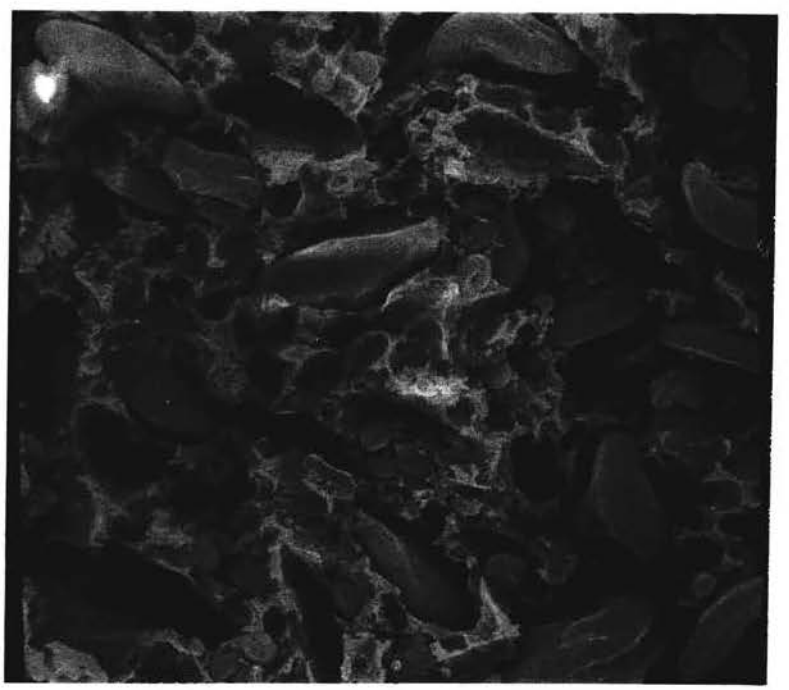


Surface of control pasta. a) 50X; b) 1000X

Figure 18. Control pasta (cross-section)

a

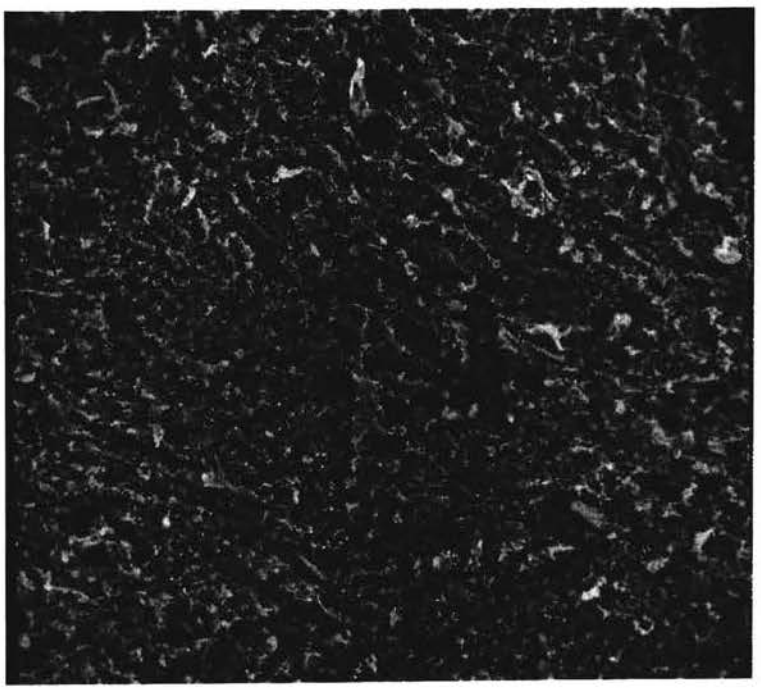
b



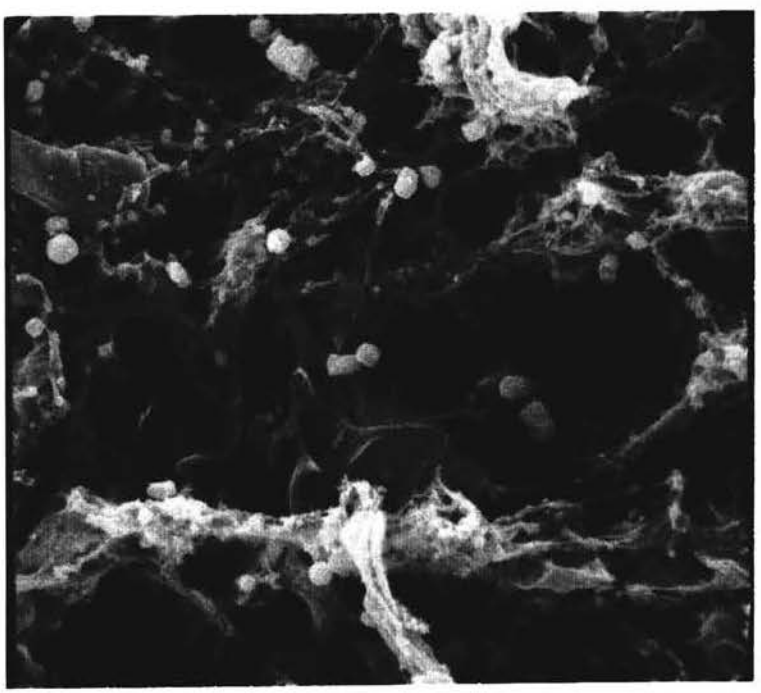
Cross-section of control pasta. a) 50X; b) 1000X

Figure 19. Treatment 1 at modified starch = 30g (surface)

a



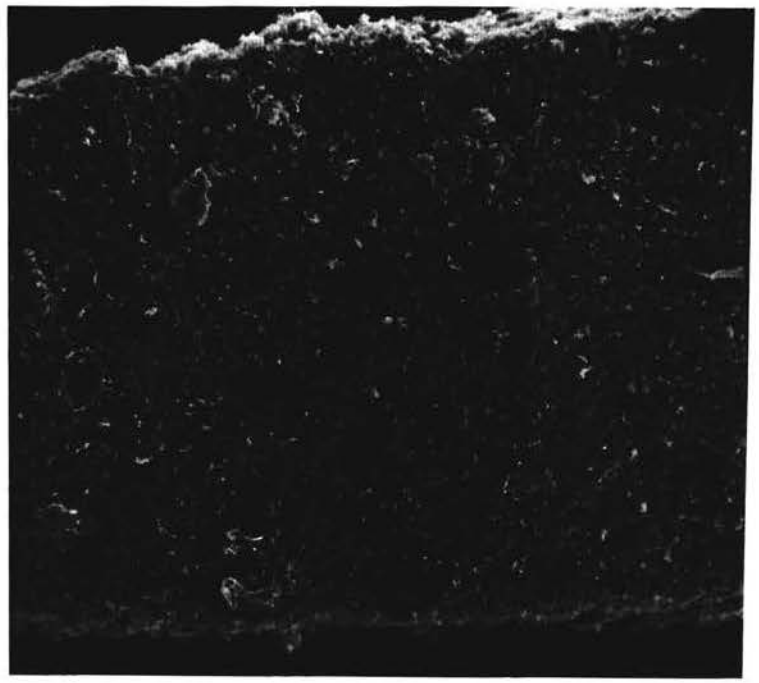
b



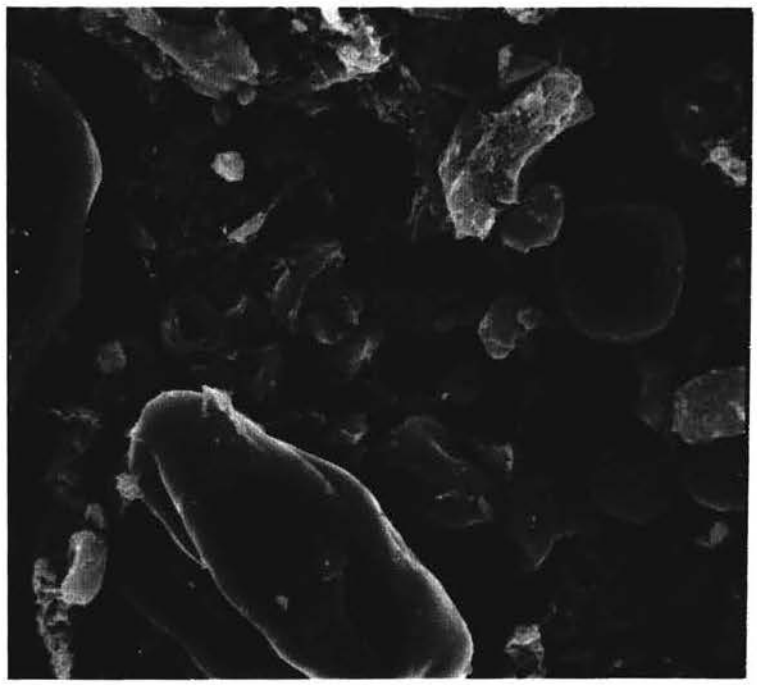
Surface of non-gluten pasta. a) 50X; b) 1000X

Figure 20. Treatment 1 at modified starch = 30g (cross-section)

a



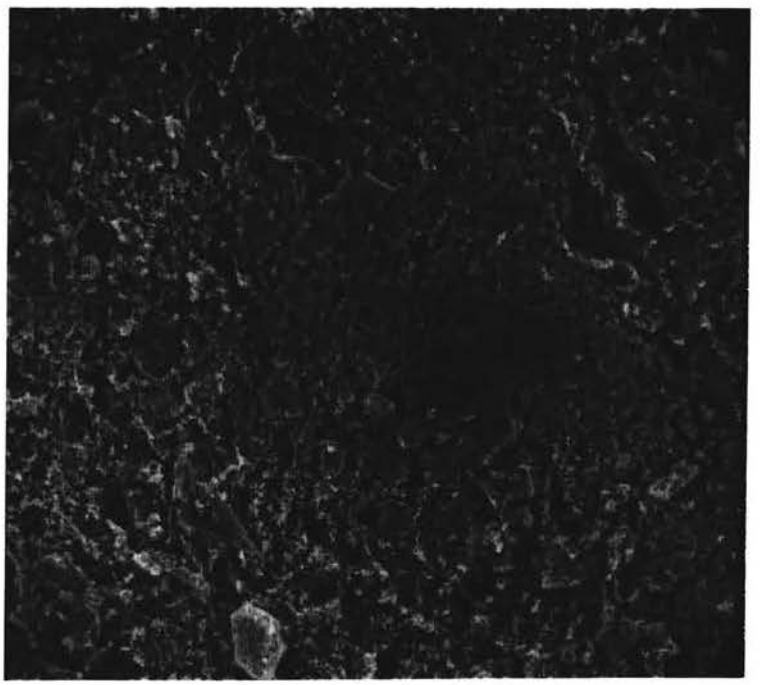
b



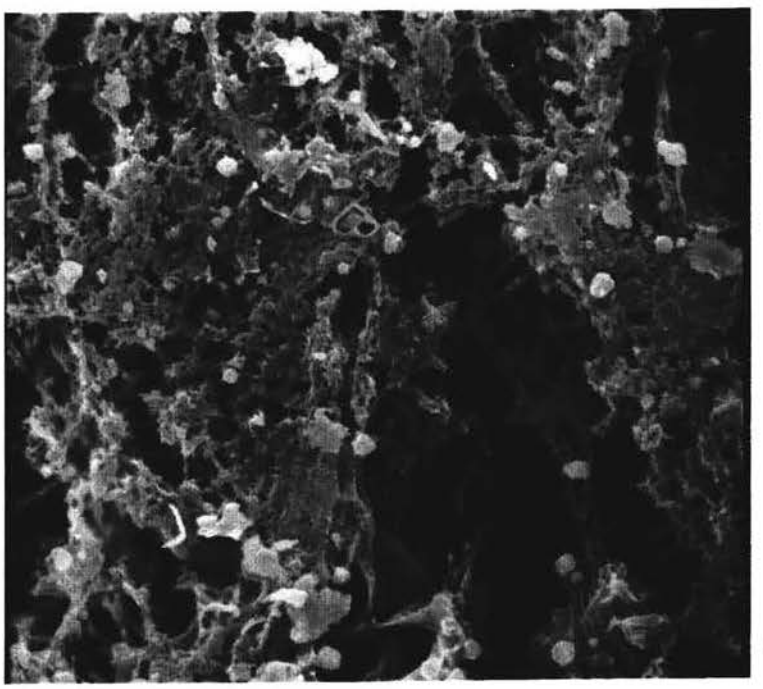
Cross-section of non-gluten pasta. a) 50X; b) 1000X

Figure 21. Treatment 3 at modified starch = 40g (surface)

a



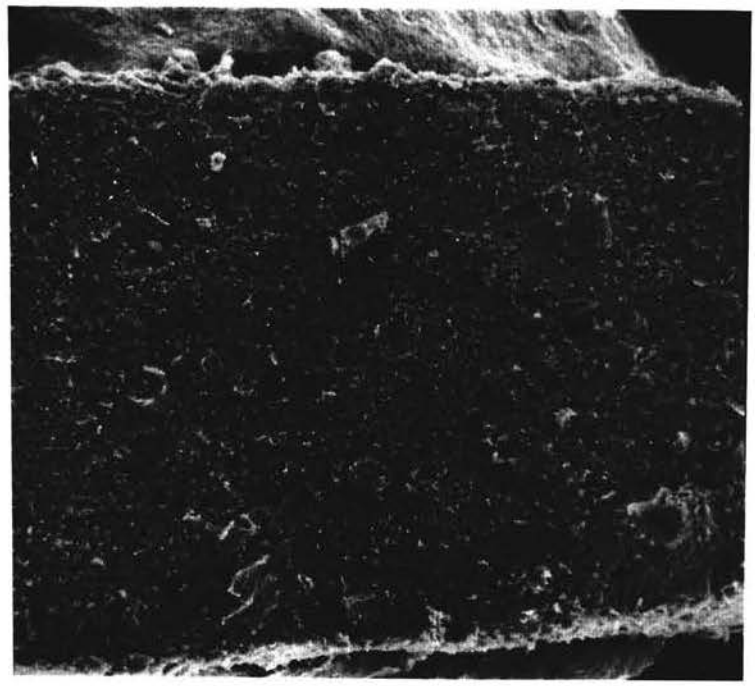
b



Surface of non-gluten pasta. a) 50X; b) 1000X

Figure 22. Treatment 3 at modified starch = 40g (cross-section)

a

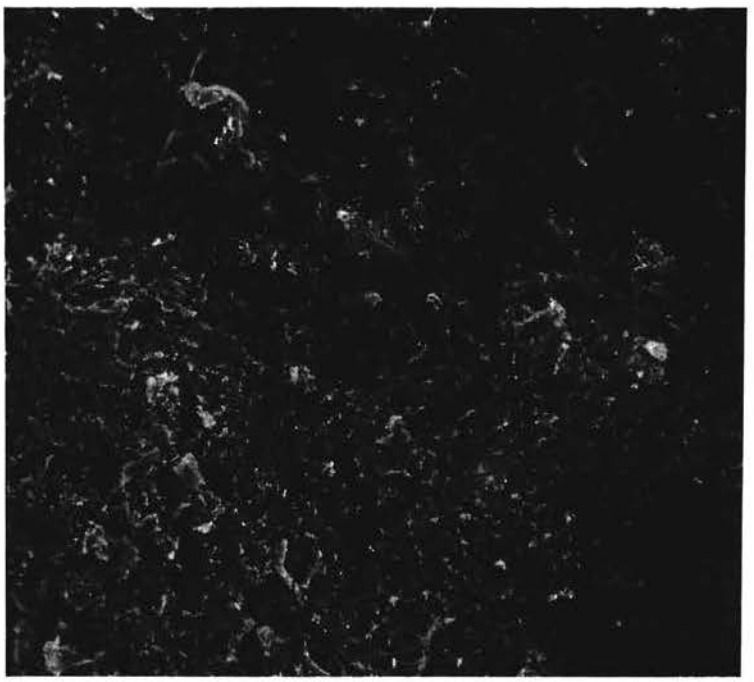


b

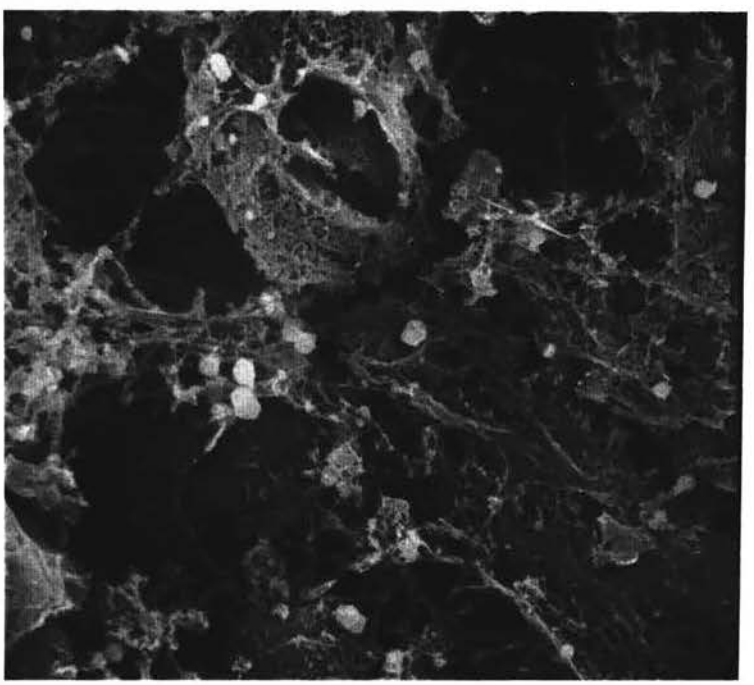
Cross-section of non-gluten pasta. a) 50X; b) 1000X

Figure 23. Treatment 8 at xanthan gum =25g (surface)

a



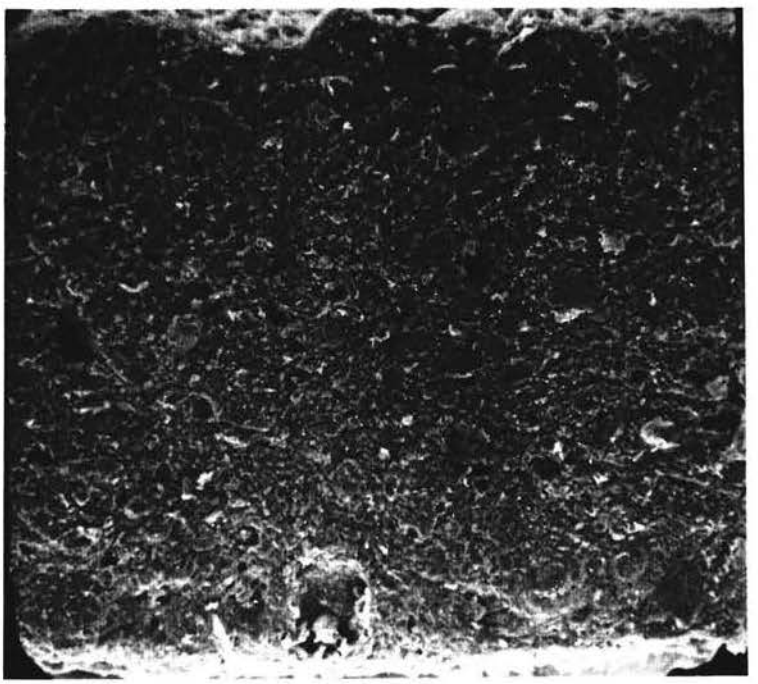
b



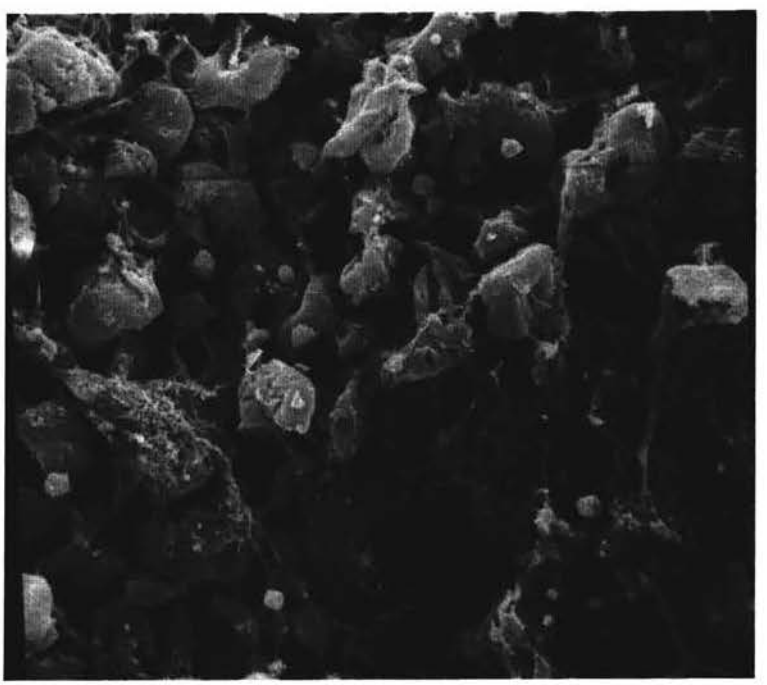
Surface of non-gluten pasta. a) 50X; b) 1000X

Figure 24. Treatment 8 at xanthan gum = 25g (cross-section)

a



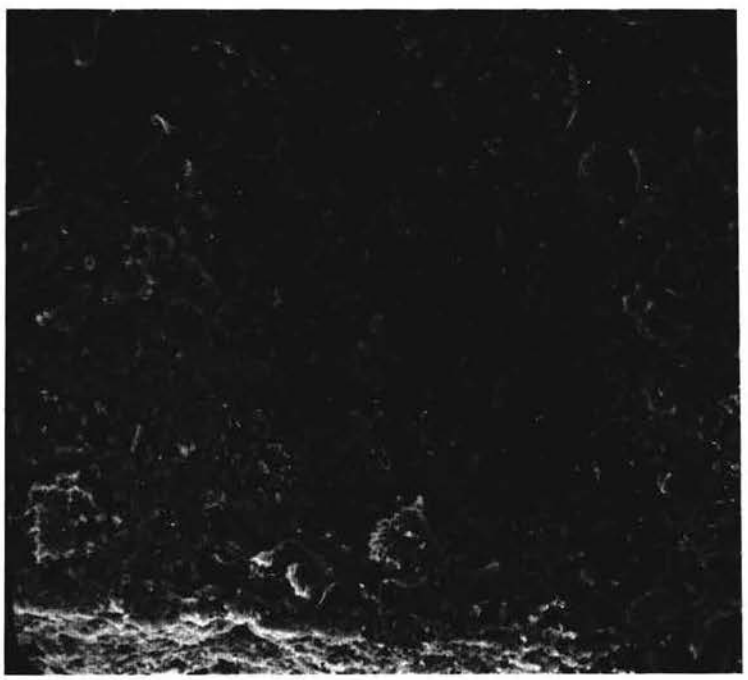
b



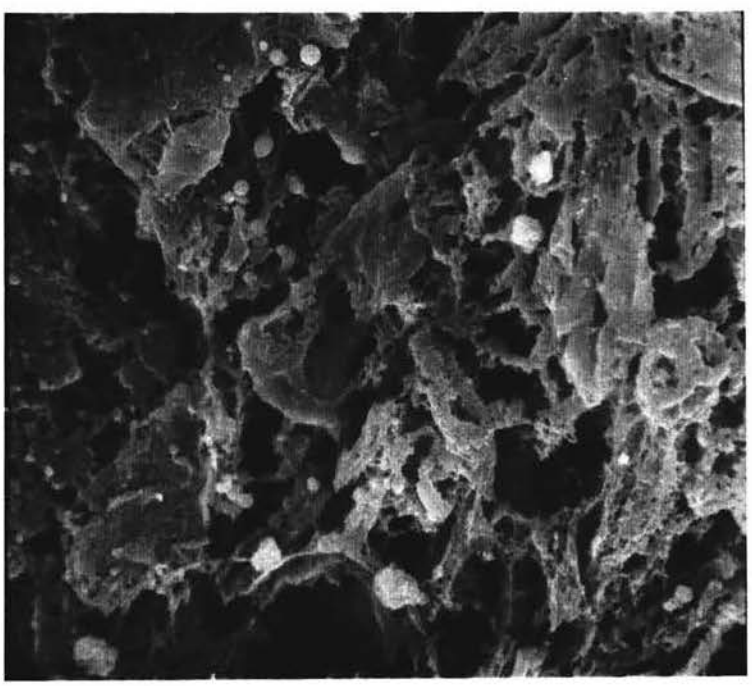
Cross-section of non-gluten pasta. a) 50X; b) 1000X

Figure 25. Treatment 11 at xanthan gum = 40g (surface)

a



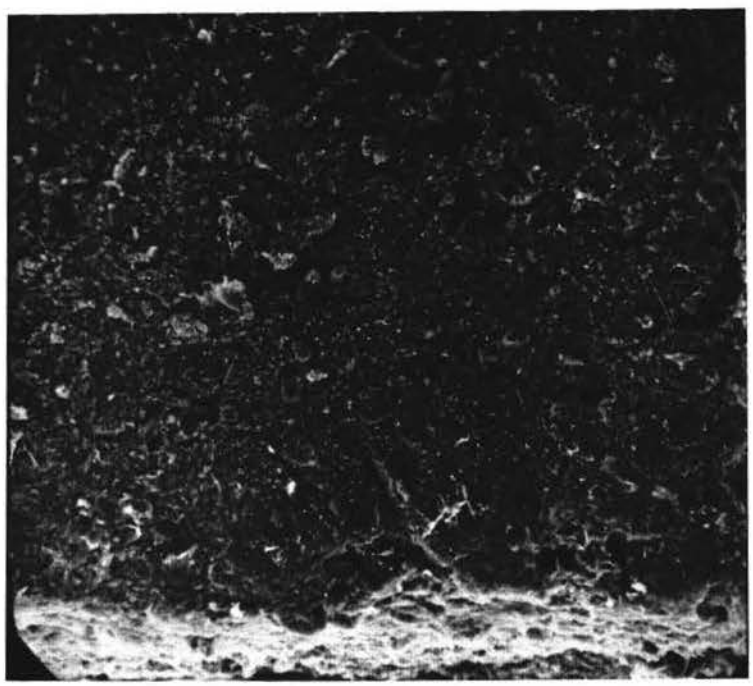
b



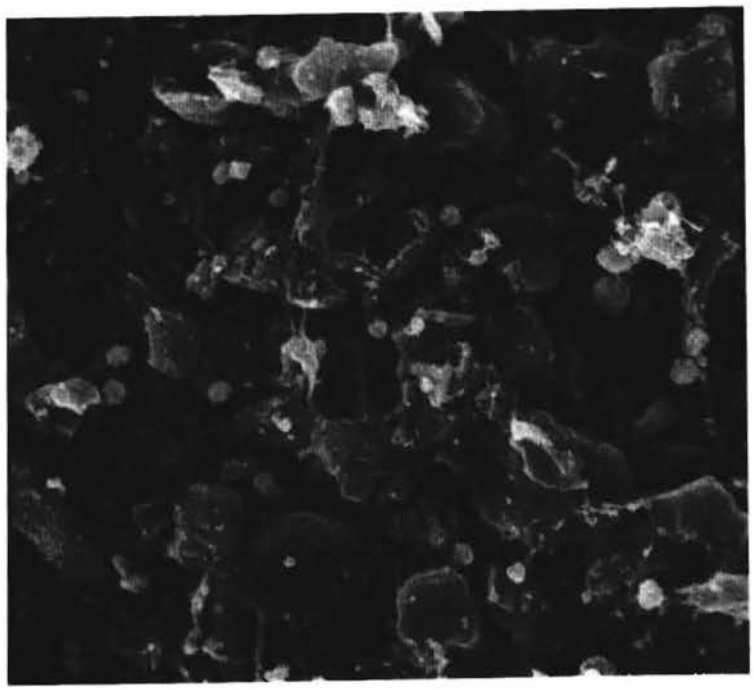
Surface of non-gluten pasta. a) 50X; b) 1000X

Figure 26. Treatment 11 at xanthan gum = 40g (cross-section)

a



b



Cross-section of non-gluten pasta. a) 50X; b) 1000X

CHAPTER VI

CONCLUSIONS

Response surface methodology combined with physical measurements and sensory panel evaluations provided a clear view of effects of different ingredients in various levels on quality of non-gluten pasta. The mixture experiment investigated the effects of factors on response more precisely and excluded the amount different among treatments. These procedures showed how the ingredients interacted with each other at different levels of combinations. The selected regression model predicted the mean response of the combination. This method helps optimize the ingredients' ranges and improve product quality.

Locust bean and xanthan gums increased sensory and functional properties of non-gluten pasta. The gums interacted not only each other but also with the starch polysaccharides to enhance the matrix structures and increase matrix gel firmness. Locust bean and xanthan gums also showed high water absorption ability. In general, cooking gain and peak force were higher at higher levels of locust bean gum, modified starch, and xanthan gum which was the same for the sensory qualities of hardness of first bite and cohesiveness of chew down. However, higher levels of these caused extra stickiness on the non-gluten pasta surface and in the cooking water.

Micrographs also showed that non-gluten pasta at higher levels of these polysaccharides had a better, more compact matrix structure. Xanthan gum, modified starch, and locust bean gum showed significant effect on sensory characteristics, physical properties, and microstructure.

Overall, compared to control pasta, non-gluten pasta had light yellow color, little or no off-flavor, and light stickiness. Nevertheless, non-gluten pasta could replace control pasta because it had very similar sensory characteristics and functional properties of control pasta. A non-gluten pasta formula predicted by the response surface methodology with mixture experiment was prepared and rated for acceptability by a consumer panel. It was rated as significantly better than a non-gluten pasta formula attempted before the design experiment and not significantly different from the control pasta (Appendix F).

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Appendix A

Preliminary tests

First preliminary test

According to the information on literature, researchers have applied different flours, starch, and non-starch polysaccharides instead of wheat flours in non-gluten products such as non-gluten cakes and breads. Pasta, compared to bread and cake, needs a more firm structure. Therefore, different flours and starch/non-starch polysaccharides were tested in preliminary work. In the first preliminary test, xanthan gum was selected as non-starch polysaccharide; modified starch, tapioca starch, potato starch were selected with yellow corn and rice flour as non-wheat starches and flours. There were thirty-six treatments in this experiment. Sensory evaluation tests were used to choose the constraint for each component. Results showed that yellow corn and rice flour did not affect sensory characteristics, so their levels could be fixed in formula. All treatments of non-gluten pasta had higher stickiness ratings than that of control pasta.

Session 1						
Treatments	**1**	**2**	**3**	**4**	**5**	**6**
XG	5	5	5	7.5	7.5	7.5
MS	2.5	2.5	2.5	5	5	5
TS	2/3 X	1/2 X	1/3 X	2/3 X	1/2 X	1/3 X
PS	1/3 X	1/2 X	2/3 X	1/3 X	1/2 X	2/3 X
CF	20	25	30	25	30	20
RF	5	5	5	5	5	5
Session 2						
Treatments	**7**	**8**	**9**	**10**	**11**	**12**
XG	5	5	5	7.5	7.5	10
MS	5	5	5	2.5	2.5	2.5
TS	2/3 X	1/2 X	1/3 X	2/3 X	1/2 X	1/3 X
PS	1/3 X	1/2 X	2/3 X	1/3 X	1/2 X	2/3 X
CF	30	20	25	30	20	25
RF	5	5	5	5	5	5
Session 3						
Treatments	**13**	**14**	**15**	**16**	**17**	**18**
XG	5	5	5	6	6	6
MS	5	5	5	2.5	2.5	2.5
TS	2/3 X	1/2 X	1/3 X	2/3 X	1/2 X	1/3 X
PS	1/3 X	1/2 X	2/3 X	1/3 X	1/2 X	2/3 X
CF	20	25	30	20	25	30
RF	5	5	5	5	5	5

Second preliminary test

In the second preliminary test, locust bean gum was introduced into the formula and xanthan gum was reduced. Results showed that stickiness decreased and firmness increased for non-gluten pasta. The final experiment followed a mixture experiment with five-component constraints, so that the mixture components were locust bean gum (X_1), xanthan gum (X_2), modified starch (X_3), tapioca starch (X_4), and potato starch (X_5) with the corn and rice flours. According to preliminary tests (Appendix A), each independent variable had these constraints:

Independent variables:

locust bean gum (X_1): 10, 25, and 40g ($10g \leq X_1 \leq 40g$)

xanthan gum (X_2): 25 and 40g ($25g \leq X_2 \leq 40g$)

modified starch (X_3): 30, 35, and 40g ($30g \leq X_3 \leq 40g$)

tapioca starch (X_4): 63.35, 66.65, 70.00, 73.35, 76.50, 80.00, 83.40, 86.50, and 90.00g ($63.35g \leq X_4 \leq 90.00g$)

potato starch (X_5): 31.67, 33.35, 35.00, 36.67, 38.35, 40.00, 41.67, 43.35, and 45.00g ($31.67 \leq X_5 \leq 45.00g$)

Fixed variables:

corn flour: 250g

rice flour: 50g

where $X_1+X_2+X_3+ X_4+X_5$ + corn flour + rice flour = 500g (100%). The ratio of tapioca starch and potato starch is 2 to 1.

Session 4						
Treatments	**19**	**20**	**21**	**22**	**23**	**24**
XG	5	5	5	6	6	6
MS	2.5	2.5	2.5	5	5	5
TS	2/3 X	1/2 X	1/3 X	2/3 X	1/2 X	1/3 X
PS	1/3 X	1/2 X	2/3 X	1/3 X	1/2 X	2/3 X
CF	25	30	20	30	20	25
RF	5	5	5	5	5	5
Session 5						
Treatments	**25**	**26**	**27**	**28**	**29**	**30**
XG	4	4	4	4	4	4
MS	2.5	2.5	2.5	5	5	5
TS	2/3 X	1/2 X	1/3 X	2/3 X	1/2 X	1/3 X
PS	1/3 X	1/2 X	2/3 X	1/3 X	1/2 X	2/3 X
CF	25	30	20	20	25	30
RF	5	5	5	5	5	5
Session 6						
Treatments	**31**	**32**	**33**	**34**	**35**	**36**
XG	6	6	6	6	6	6
MS	2.5	2.5	2.5	5	5	5
TS	2/3 X	1/2 X	1/3 X	2/3 X	1/2 X	1/3 X
PS	1/3 X	1/2 X	2/3 X	1/3 X	1/2 X	2/3 X
CF	25	30	20	20	25	30
RF	5	5	5	5	5	5

1. XG-xanthan gum, MS-modified starch, TS-tapioca starch, PS-potato starch, CF-corn flour, and RF-rice flour.
2. XG + MS + TS + PS + CF + RF = 50g; X = 50g - (XG + MS + CF + RF).

Appendix B

Consent to Participate in Research
Sensory Evaluation of Non-Gluten Pasta

I, _____, voluntarily agree to participate in the above titled research that is sponsored by the College of Human Environmental Sciences at Oklahoma State University.

I understand that:

(1) I will be participating in research to test the sensory qualities Non-Gluten Pasta

(2) the sensory panel will be drawn from faculty, staff and students of Oklahoma State University.

(3) This study will take place during the 1998 school year.

(4) participation or non-participation in this study will in no way affect my grade or performance rating; but by participating in this research I will see how sensory evaluation can contribute to scientific research designed to help Celiac patients.

(5) I will be informed of all foods and ingredients that I will be asked to evaluate. If I know or suspect that I am allergic to any of them, I will withdraw myself from testing that product.

(6) all results obtained from my participation in this research will be recorded by code number; my identity will be kept confidential, and I will not be identified as an individual or by response in any presentation of the results.

(7) my participation is voluntary, and I have the right to withdraw from this study at any time with no penalty by contacting the principal investigators;

(8) I have not waived any of my legal rights or released this institution from liability for negligence.

I may contact Dr. Sue Knight at (405)744-5043 or Jen-Chieh Huang at (405) 744-2298 should I wish further information. I may also contact Gay Clarkson in the office of University Research Services, 305 Whitehurst, Oklahoma State University, Stillwater, OK 74078 at (405) 744-5700.

I have read and fully understand this consent form. I sign it freely and voluntarily. A copy has been given to me.

Date_____ Time _____(am/pm)

Signed _____

I certify that I have personally explained all elements of this form to the subject before requesting the subject to sign it.

Signed _____
(project director or her authorized representative)

Printed name Dr. Sue Knight
(project director or her authorized representative)

Appendix C

Sensory panel training: basic taste

The purpose of this exercise is to familiarize you with the four basic tastes and give you an opportunity to attempt to identify the four basic tastes: salt, sweet, sour, and bitter, from unknown samples. You have been given a cup of water so you can rinse your mouth between samples.

1. Place the four named samples in a row before you. Place the coded samples in a separate row. Taste each of the named samples and familiarize yourself with each taste.

2. Taste each of the coded samples in the second row and determine which basic taste is present in each cup. Put your answer in the space beside each code number. Before evaluating next sample, **Please rinse your mouth with distilled water.**

765 _____

319 _____

026 _____

994 _____

571 _____

Appendix D

Sensory panel training: terminology and procedure

1. This is designed to help each panelist familiarize the terminology of each attribute of sample and how to proceed the sensory evaluation of sample. Therefore, each panelist can evaluate different attributes of the sample accurately.

2. **Use spoon or fork to pick up sample. Do not use your fingers.**

3. Before evaluating next sample, **please rinse your mouth with distilled water.**

***Surface:**

Definition- Degree to which sample surface is smooth to rough.

Method- 1) Determined by a single piece of sample drawn across the lips and tongue.
2) Feel the degree of smothness and record it.

***Hardness of first bite:**

Definition- Force required to bite through the sample.

Method- 1) Place a single piece of sample between incisors, and bite down.
2) Feel the degree of hardness and record it.

***Adhesiveness of chew down:**

Definition- Degree to which mass sticks to the roof of mouth or teeth.

Method- 1) Place 2 pieces of sample between molars, chew 10 times, then press mass against the roof of mouth and release.
2) Feel the force when you opened your mouth and record it.

***Cohesiveness of chew down:**

Definition- Degree to which sample holds together in a mass.

Method- 1) Place 2 pieces samples between molars, chew 10 times, then chew one more time.
2) Feel the force when you break the mass and record it.

***Off flavors:**

Definition- Flavors are not found in the control sample.

Method- 1) Place pasta sample into mouth and chew.
2) Identify intensity of off-flavors and record it.

Appendix E

Sensory Evaluation Form

Panelist code: _____

Product: **Non-Gluten Pasta**

- **Smoothness of surface**

0 (Smoothness) 5 (Roughness) 10

- **Hardness of first bite**

0 (Softness) 5 (Hardness) 10

- **Adhesiveness of chew down**

0 (No Stickiness) 5 (Very stickiness) 10

- **Cohesiveness of chew down**

0 (Loose mass) 5 (Compact mass) 10

- **Off-flavor**

0 (None) 5 (Very strong) 10

Appendix F

Acceptance test

The final formula of non-gluten pasta that possessed the most desirable properties of the three formulas was chosen for a consumer acceptance test. This final formula of non-gluten pasta was xanthan gum at 40g, modified starch at 35g, locust bean gum at 40g, tapioca starch at 113g, potato starch 57g, corn flour at 250g, and rice flour at 50g. Three different pastas were used for the test: (1) a non-gluten pasta formula developed prior to the mixture experiment; (2) the final non-gluten pasta formula; (3) control (gluten) pasta. A thirty-six consumer panelists conducted this test. The panel rated on a hedonic scale where one was the least acceptable and seven the most acceptable. Three attributes were tested: appearance, texture, and overall acceptability.

Results and discussion

The appearance of the final non-gluten pasta formula was different ($P< 0.05$) from the previous non-gluten pasta, but not different from the control pasta. Texture results were that both the final non-gluten formula and the control pasta had better texture than the old non-gluten formula. For overall acceptance, the final non-gluten pasta had the highest mean ratings among the three and was significantly better than the previous formula.

A non-gluten pasta formula, chosen from overlapping optimum regions predicted by the response surface methodology, had similar or even better sensory attributes to the control pasta (regular pasta, gluten-containing) and was significantly better than a previous formula.

Appearance, texture, and overall acceptance of non-gluten and control pastas.

	Appearance	Texture	Overall
Previous non-gluten formula	4.00[a]	4.03[a]	3.92[a]
Final non-gluten formula	5.87[b]	5.00[b]	5.00[b]
Control pasta	4.46[ab]	4.77[b]	4.54[ab]

[a, b] means in the same column with different superscripts are significantly different ($P<0.05$).

The seven-point hedonic scales

Appearance

	246	735	081
Like extremely	❒	❒	❒
Like very much	❒	❒	❒
Like moderately	❒	❒	❒
Neither dislike or like	❒	❒	❒
Dislike moderately	❒	❒	❒
Dislike very much	❒	❒	❒
Dislike extremely	❒	❒	❒

Comment:_____

Overall

	246	735	081
Like extremely	❒	❒	❒
Like very much	❒	❒	❒
Like moderately	❒	❒	❒
Neither dislike or like	❒	❒	❒
Dislike moderately	❒	❒	❒
Dislike very much	❒	❒	❒
Dislike extremely	❒	❒	❒

comment:_____

Texture

	246	735	081
like extremely	❒	❒	❒
like very much	❒	❒	❒
like moderately	❒	❒	❒
Neither dislike Or like	❒	❒	❒
Dislike Moderately	❒	❒	❒
Dislike Very much	❒	❒	❒
Dislike Extremely	❒	❒	❒

Comment:_____

Appendix G

OKLAHOMA STATE UNIVERSITY
INSTITUTIONAL REVIEW BOARD
HUMAN SUBJECTS REVIEW

Date: 04-24-98 ERB iN: HE-99-093

Proposal Title: SENSORY EVALUATION OF NON-GLLITEN PASTA

Principal Investigator(s)- Sue Knight, Jen-Chich Huang

Rcvlcwcd smd Processed as: Expedited

Approval Status Recommended by Reviewer(s): Approved

ALL APPROVALS MAY BE SUBJECT TO REVIEW BY FULL INSTTRLMONAL REVIEW BOARD AT NEXT 1,,EETING, AS WELL AS ARE SUBJECT TO MONITORING AT ANY TIME DURING THE APPROVAL PERIOD.

APPROVAL STATUS PERIOD VALID FOR DATA COLLECTION FOR A ONE CALENDAR YEAR PERIOD AFTER WMCH 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 DisApprovAl are as follows:

Signature:

Chair of Institutional Review Board

Date: June 9. 1998

c,c: Jen-Chich Huang

OKLAHOMA STATE UNIVEP-SITY
IN,STITUTIONAL REVIEW 130APD

Date: **April 27,** 1999 IRB #: BE-98-093

Proposal Title: "SENSORY EVALUATION OF NON-GLUTEN PASTA"

Principal **Investigator(s):** **Sue Ki@ght** len-Chich **Huang**

Reviewed and Processed is: Continuation

Approval Status Reconunended by Reviewer(s): Approved

Signature:

Carol Olson, Director of University Research Compliance

Ap@l 27- 1999
Date

Approvals are valid for one calendar year, after which time a request for continuation must be subrr@t,,cd. Any modification to the research project approved by the IRB must be submitted for approval. Approved projects are subject to monitoring by the IPB. Expedited and exempt projects may bc reviewed by the full Institutional Review Board.

VITA

JEN-CHIEH HUANG

Candidate for the degree of

Doctor of Philosophy

Thesis: RESPONSE SURFACE METHODOLOGY WITH MIXTURE EXPERIMENT FOR NON-GLUTEN PASTA DEVELOPMENT

Major Field: Food Science

Biographical:

Personal Data: Born in Taipei, Taiwan, on September 20, 1967, the oldest-child of the family.

Education: Internship at Provincial Taichung Hospital, Taiwan, in July 1990; received Bachelor of Science degree with major in Nutritional Science from Chung Shan Medical & Dental College, Taiwan, in May, 1991; received Master of Science degree with major in Food Science from Kansas State University, May, 1996; completed requirements for Doctor of Philosophy with major in Food Science and minor in Statistics, December, 1999.

Experience: Research Assistant, Department of Foods and Nutrition, Manhattan, KS, August, 1995-1996. Research Associate, Department of Nutritional Science, August, 1996-1997. Research Associate and Teaching Assistant, Department of Nutritional Science, August, 1997-1999.

Membership: Member of Institute of Food Technologists
Member of Chinese American Food Society