CHAPTER 1:

Introduction

Data from the NHANES-II study revealed that 24.2% of men and 27.1% of women, aged 20-74 years of age were overweight. This equates to 34 million people in the United States that were considered overweight (Anon., 1994). Overweight is defined as having an excess of body weight (Bray, 1992). A more appropriate term used to describe the unhealthy population with an excess of body weight is the term obese. Obesity is defined as an overabundance of fat tissue, and is classified as a person whose weight is 20% higher than ideal body weight (Anon., 1994).

Excess body fat is a risk factor for many illnesses that can have a profound effect on our quality of life and reduce our chances for living productively into old age. Studies (Stern et al., 1979; Warner and Garrett, 1968) indicate that people who are 20% or more over desirable body weight are more likely to develop:

- Coronary Artery Disease (heart attack and angina)
- Hypertension
- Type II diabetes
- Hyperlipidemia
- Respiratory dysfunction
- Gallstones
- Abnormal liver function
- Skin disorders
- Certain cancers, particularly of the cervix (women), colon, rectum and prostrate (men)

Therefore, during the past 10 to 15 years, the American Heart Association , the Surgeon General, and other health organizations have called for a reduction in total dietary fat to

30% of kilocalories for most people to help combat obesity and the illnesses associated with obesity in the United States (Anon., 1988, Anon., 1991).

Public awareness about the health risks of dietary fat has increased dramatically over the past two decades. Data from various surveys show that public awareness about the relationship between dietary fat and coronary heart disease grew from 8% of Americans in 1970, to 29% in 1983 (Putler and Frazoa,1991). In 1988, 55% were aware of the link, and in 1991, fat content topped the list of nutritional concerns of food shoppers.

In many ways, fat equals taste in foods. Primarily, fat is said to accentuate flavors. This is because when eating a food, the fat and the flavor compound stays on the tongue longer, and therefore the flavor has more time to stimulate the taste bud, therefore accentuating all flavors associated with the product. Fat adds lubricity as well as texture to a product, and therefore directly affects the mouthfeel of the product. Also, fat contributes to the sheen or gloss of the surface of the product, therefore affecting the visual perception of the product.

Besides affecting our sensory perceptions, fat also contributes to the physical properties of the product. Fat adds structure by trapping air during creaming (Pyler, 1973). These air cells collect water vapor and carbon dioxide during baking, and therefore expand to provide the increased volume of a cake batter (Vetter et al., 1984). Fat also decreases gluten interaction, which ultimately leads a more tender product with a finer crumb (Bennion, 1990a).

Food manufacturer's have not ignored the consumer's growing interest in reduced-fat and nonfat foods. According to *New Product News* (Akoh and Swanson, 1994), 1198 new products that were either low-fat or nonfat were introduced in 1991. This is a big difference from the 38 new products introduced in 1981 with the claim low-fat or nonfat.

A muffin is a quick bread that contains flour, leavening agent(s), salt, sugar, fat, egg, and liquid. The baked product is leavened by carbon dioxide produced by the baking powder, while the structural component is provided by gluten in the flour and coagulation of egg proteins. In this type of batter system, fat gives the product lubricity, mouthfeel, and since fat interferes with gluten development, a tender crumb and increased volume is produced. Sugar in this system has similar functions as to the fat. An increased amount of sugar decreases gluten development and therefore an increase in tenderness is the result. Sugar adds flavor and color to the product, however, the addition of monosaccharides would decrease the gelatinization of the starch and therefore will cause a decrease in volume. When fat is eliminated or reduced in a product, many of these qualities are affected. The food researcher, then, is on an ever continuing quest to develop a reduced fat product that still maintains the qualities of its full fat counterpart.

In muffins and other fat reduced and nonfat products, fat substitutes have been developed to help mimic the functions of fat. Ideally, a single ingredient substitution is desired to make a fat free product, however, this has yet to be done with any success. However, these products have fallen short of the consumer's demand for a product that not only tastes like it has fat in it, but is also perceived as having the fat in it. Therefore,

emulsifiers and enzymes have also been experimented with in conjunction with fat substitutes (Mason et al., 1996) to produce an acceptable reduced/nonfat product. This demonstrates the direction in the research of low fat and nonfat food development.

CHAPTER 2:

Review of Literature:

2.1. Functionality of Fat in Bakery Products

Lipids, most often referred to as fat, are classified as either triglycerides, fatty acids, phospholipids, or sterols. Triglycerides comprise the largest portion of fat found naturally in foods, as well as the more purified fats (Bennion, 1990a). Consisting of one molecule of glycerol and three fatty acids, many variations exist based on the make-up of the fatty acids that comprise the triglyceride

Lipids, most often referred to as fat, are naturally found in almost all food products. Derived from several sources, fats exhibit a wide variety of functional products and therefore play very prominent roles in food systems. In baked products, fats and oils have been important ingredients for centuries. Fat in a bakery item "shortens" (tenderizes) the texture of the finished product (Stauffer, 1996a). The primary properties of a fat or oil that determine its ability to carry out these functions are: its ratio of solid to liquid phase, its oxidative stability, and the plasticity of the fat or oil (Stauffer, 1996a).

The contributions that oil makes to the final quality of the baked product are: tenderness, moist mouthfeel, lubricity, flavor, and structure (Stauffer, 1996a). Tender, according to The Random House College Dictionary is something that is soft or delicate in substance: not hard and tough (Stein, 1980). A moist mouthfeel is achieved when the addition of oil gives the impression of a moister interior. Lubricity is described as a desirable slippery sensation in the mouth that is achieved by the addition of fat.

Fat contributes flavor to baked products, especially apparent in fried foods. This flavor imparted by the fat is attributable to the many products formed by reactions of the triglycerides and fatty acids with proteins, sugars, air, and moisture in the food. At high concentrations, these compounds become objectionable, but at trace levels, these compounds give the appetizing flavor notes associated with deep-fried foods. In baked products, such as muffins and cakes, fat is involved with flavor enhancement. When the product is consumed, the fat stays on the tongue longer, thus promoting enhanced recognition at the site of the taste buds.

Structure is affected tremendously in baked products due to the presence of fat. In muffins, the closeness of the internal grain, and to some extent, the final volume, are strongly influenced by the characteristics of the fat used (Stauffer, 1996a). In the finished product, a high percentage of the total volume is open space, present as finally divided cells. These spaces are created by carbon dioxide (from the leavening system) and steam (formed during baking). When these gases are generated by heat, they migrate to the nearest air bubbles, which were incorporated into the cake batter during mixing. If there are many small air bubbles in the batter, the leavening gases are distributed widely. Each one of the bubbles is small and so does not rise rapidly to the surface of the cake. The leavening gases are retained in the cake and contribute to the final volume (Stauffer, 1996a). Therefore, if the original batter contains many small air cells, the final cake will have a larger volume and a fine crumb. If the original air bubbles are fewer and larger, the final cake will have less volume and a coarse grain. Conversely, fat interferes with gluten

structure by decreasing cohesive forces present in baked products, thus, when fat is added above and beyond optimum levels, volume decreases (Paton et al., 1981). However, when used at the proper level, fats (particularly plastic fats) have the ability to hold and incorporate air and thus increase volume.

2.2. Honey

Honey, by definition, is a sweet viscous substance elaborated by the honeybee from the nectar of plants (White, 1978). It is the only sweetening material that can be stored and used exactly as it was produced in nature, with no refining or processing necessary. As produced however, it is highly variable in color, flavor, moisture content, and sugar composition. These attributes depend on the climate, the floral type, and of course, individual bee keeping practices.

2.2 A. Sources of Honey

As mentioned above, honey comes specifically from honeybees, however, the plants that bees retrieve nectar from to make honey varies greatly. Since bees are kept in all 50 of the United States and in every country of the world, then it is easy to understand the vast number of plants that can be affected by bees to make honey. In fact, more than 300 floral sources have been identified in the United States (NHB, 1988). To appreciate this, Table 1 lists the commercially significant plant sources of honey in the United States alone:

Table 1. “Commercially Significant Plant Sources for Honey in the United States and Their Primary Area of Production.” (White, 1978)

Plant Type	Area of Production
Clover	Central, North-Central, East-Central
Sweet Clover	Central, North-Central, East-Central
Alfalfa	Central and Mountain West, California
Basswood	Mid-Atlantic to Wisconsin
Buckwheat	California
Cotton	Southwest
Fireweed	Oregon
Gallberry	Southeast
Goldenrod	Northeast
Locust, Black	Mid-Atlantic, East Central
Mesquite	Southwest
Orange-Grapefruit	Florida, California
Sage	California
Sourwood	Virginia, Carolinas
Spanish Needle	Central
Star Thistle	California, North Central
Tulip Tree	Mid-Atlantic to Indiana
Tupelo	Florida
Vetch	California, Oregon

2.2 B. World Honey Production and Processing

In 1996, the United States produced 198 million pounds of honey, second only to the Soviet Union (USDA, 1997). This value was down 6 percent from 1995. There were 2.57 million colonies producing honey compared with 2.65 million in 1995 (USDA, 1997). Prices for the 1996 crop averaged a record high 89.4 cents/pound, which was up 31 % from the previous record price of 68.5 cents/pound in 1995 (USDA, 1997). With over 31 countries producing more than 1 million pounds of honey per year, it is easy to understand that honey is a worldwide commodity.

Commercial production of honey was made possible by the invention in the mid-nineteenth century of the mobile frame hive and the centrifugal extractor for removing stored honey without destruction of the comb, allowing its reuse.

It has been estimated that one bee in a lifetime will produce 0.5 teaspoons of ripened honey. In the first stages of honey making, the bee collects the nectar from a plant source and transports it back to the hive. In the hive (specifically the honey bodies or "supers"), the moisture content is decreased from 30-60% to a range of 15-19%. Invertase is added to the moisture reduced honey which inverts much of the sucrose to fructose. Preservation of the honey in the comb is accomplished by the addition of glucose oxidase, which produces a small amount of acidity (acid content of 0.57%) and hydrogen peroxide. The final pH of the honey after being capped off in the honey comb is approximately 3.9 (with a typical range being 3.4 to 6.1 depending on the variety) (NHB, 1988).

During harvesting, the hive bodies containing the combs full of ripened honey, are removed from the colony, freed of bees, and taken to a central location for extraction. The cappings of the cells are removed mechanically and the honey is extracted by centrifugation (White, 1975). From here, the honey may be placed directly into a drum or strained through screens to remove extraneous material.

Honey immediately after extraction is at its best in terms of color and flavor. It is not suitable, however, for large-scale marketing without further treatment, because as extracted, "raw honey" contains pollen, bits of wax, sugar-tolerant yeasts, and crystals of

dextrose hydrate (White, 1978). Therefore, honey is prone to fermentation unless the moisture content is below 17% and is also prone to crystallization. Therefore, the processing of honey includes a controlled heating to destroy yeasts and dissolve dextrose crystals, followed by a fine straining and pressure filtration. Following these processings, drum and or spray drying could be used to produce dry honey or a controlled crystallization technique could be used to produce crystallized honey.

2.2 C. Market Forms of Honey

In the United States, the retail market appears to favor liquid honey (White, 1978), while in many other countries of the world, a solid form is preferred. Since most honeys are supersaturated in dextrose, the most stable form would seem to be biphasic. Although it appears that there is no one completely stable form, the liquid form is sufficiently stable. Honey is also sufficiently shelf - stable for sale in a semisolid form known as “creamed”, “spun”, “churned”, or “recrystallized”. This is a fondant of very small dextrose crystals in a honey matrix. Nothing extraneous has been added in manufacture: the product is a result of a controlled crystallization process which would follow the normal heat treatment to kill off yeast cells.

Honey in the comb is another product consumed. It has virtually disappeared from urban markets, however, being it is difficult and expensive to produce and ship. Market forms that may be available include a section comb, a 4.5 inch square frame , a cut-comb

(piece cut by the beekeeper from a larger comb), or a bulk-comb (piece of sealed honey comb in a container filled with liquid honey).

Dried honey products such as ADM Arkady's Sweet 'N' Neat® 2000, is a honey product made from pure honey that has been converted to a free-flowing powder through special drying processes such as spray-drying, tunnel drying, wiped-film vacuum evaporating, or drum drying (White, 1978). It is used in commercial technologies or other areas where process and product constraints previously prevented the use of liquid honey. This particular dry honey is advantageous because it maintains a full honey flavor; it is a natural sweetener; it has a consistent uniform flavor; it complements other flavors; it has a very low moisture content (not more than 2.5%), and is highly hygroscopic.

2.2 D. Characteristics of Honey

2.2 D1. Composition of Honey

As mentioned earlier, honey produced by honeybees from plant nectars is rather variable in composition reflecting contributions of the plant, climate, environmental conditions and beekeeper skills. However, in general, the composition of United States honey is listed in Table 2.

Table 2. “Composition of United States Honey.” (White, 1978)

Characteristic Measured	Average
Moisture	17.20
Levulose (%) (Fructose)	38.19
Dextrose (%) (Glucose)	31.28
Sucrose (%)	1.31
Maltose (%)	7.31
Higher Sugars (%)	1.50
pH	3.91
Free Acid (meq/kg)	22.03
Lactone (meq/kg)	7.11
Total Acid (meq/kg)	29.12
Ash (%)	0.17
Nitrogen (%)	0.04
Diastase value	20.80

Moisture Content:

The amount of water in honey is of major importance to its stability against fermentation and granulation. Normally ripened honey has a moisture content below 18.6%; honey of higher content does not qualify for the USDA grading classifications (White, 1978). Fermentation of osmophilic yeasts will ensue if the combination of moisture content, temperature and yeast count is favorable (Lochhead, 1933); granulation tendency appears to be fairly predictable by the glucose/water ratio (Hadorn and Zurcher, 1974). In general, normally ripened honey with a moisture content of 17.5-18%, with a water activity of 0.58, requires a natural inoculum of about 1000/gm (Lochhead, 1933).

Carbohydrates:

The largest portion of dry matter in honey consists of sugars. In general, the sugars are responsible for much of the physical nature of honey, its viscosity, hygroscopicity, granulation properties and energy values (Crane, 1975). In nearly all

honeys fructose predominates in the form of levulose, however few varieties contain a higher percent of glucose in the form of dextrose. These two sugars together account for 85-95% of the honey carbohydrates. More complex sugars (oligosaccharides) constitute the remainder except for a trace amount of polysaccharides. Other dissacharides have been identified (besides levulose and dextrose), and include: maltose, isomaltose, nigerose, turanose, maltulose, kojibiose, leucrose, neotrehalose, gentiobiose, and laminaribiose.

Siddiqui and Furgala (1968) identified 11 oligosaccharides, including I-kestose, melezitose, 6-α-glucosylsucrose, panose, iso-maltotriose, erlose, 3α-isomaltosylglucose, isopanose, maltotriose, iso-maltotetraose, isomaltopentaose, and 2 others that could not be identified.

Acids:

The characteristic flavor of honey includes a contribution due to its acidity. Also, the level of active acid probably contributes to the stability of honey against microbial attack.. The total acid content of honey was cited by the NHB (1988) to be 0.57% by weight. Gluconic acid is the principal acid in honey (Stinson et al., 1960). It is produced by the action of the glucose oxidase enzyme found in the honey working on the glucose units. This reaction is extremely slow in full-density honey but rapid when honey is diluted. It has been proposed that the enzyme works on the glucose present in the nectar forming gluconic acid which assists in preserving the nectar from spoilage (Burgett, 1974). Therefore, the amount of gluconic acid in honey should be a reflection of several

contributing factors, the most significant being the time between the collection of the nectar by the bee and the attainment of full density in the comb. This, however, is governed by the sugar content of the nectar, the weather, the strength of the colony, and the quality of the nectar flow. At least ten other organic acids have been identified in honey. They include acetic, butyric, lactic, pyroglutamic, citric, succinic, formic, maleic, malic, and oxalic acids.

Vitamin and Mineral Content:

Honey has measurable amounts of six vitamins: riboflavin, pantothenic acid, niacin, thiamin, pyridoxine, and ascorbic acid. With the exception of ascorbic acid, these vitamins appear at such low levels, that they have relatively no nutritional significance. Ascorbic acid was reported by Rahmanian et al. (1970) at levels of 75-150 mg/100 grams of Iranian honey

The wide variability of honey composition is also reflected in the mineral/ash content. Table 3 demonstrates the average mineral composition of honey.

Table 3 - "Average Mineral Composition of Honey (NHB, 1988)."

Mineral	Amount in 100 g of honey	US RDA
Calcium (mg)	4.4 - 9.9	1000.0
Copper (mg)	0.003 - 0.010	2.0
Iron (mg)	0.06 - 1.5	18.0
Magnesium (mg)	1.2 - 3.50	400.0
Manganese (mg)	0.02 - 0.4	
Phosphorus (mg)	1.9 - 6.3	1000.0
Potassium (mg)	13.2 - 168	
Sodium (mg)	0.0 - 7.60	
Zinc (mg)	0.03 - 0.4	15.0

The predominating mineral element is potassium, which averages about one-third of the total ash content, whereas sodium accounts for one-tenth.

Proteins and Amino Acids:

The nitrogen content of honey is low and variable at approximately 0.041% + 0.026 for United States honey. However, a study conducted by Bergner and Diemair (1975) reported 33-45% of the total nitrogen in honey to be lost by ultrafiltration. The protein content of honey appears to be of approximately 7 different types, 4 of which originate with the bee and three from the plant components.

Paper chromatography brought about interest in honey amino acids; several investigators identified up to 17 amino acids in various samples (Komamine, 1960, Davies, 1975, and Petrov, 1974). Komamine (1960), quantitating paper chromatography, first noted that proline was the main amino acid in honey, representing 50-85% of the total amino acids (Davies, 1975).

Enzymes:

The greatest volume of literature on honey has been with the enzymes present within. Although extensive, the honey enzymes of most direct interest in food applications are amylase, invertase, glucose oxidase (White, 1978).

Invertase, is a sucrose splitting enzyme that is added to the nectar by the honeybee during its harvesting and ripening to honey It continues its activity in extracted honey unless destroyed by heating. During its action on sucrose, six oligosaccharides are

produced, all eventually hydrolyzed to glucose and fructose by the completion of the reaction.

Amylase (diastase) is an enzyme known to be present in honey for well over 120 years, however, very little is known about the enzyme's kinetics, mode of action, and its significance in honey (White, 1978). Since nectar contains no starch or dextrins, the question of amylase's origin has been examined for many years. These years of research have led scientists to believe that a major portion of amylase in honey originates from the food glands of the honeybee, and the variability in amylase content most likely reflect conditions during the gathering and ripening of the nectar.

Glucose oxidase had first been reported in hypopharyngeal glands of honeybees by Gauhe (1941). It was also demonstrated that the enzyme was present in honey and it was responsible for producing gluconic acid and hydrogen peroxide, breakdown products of lactone oxidation. Together, it was thought that gluconic acid and hydrogen peroxide are responsible for honey's "antibiotic" effect. In fact, honey has been thought to have wound-healing and antiseptic properties. It was later discovered that the reason for honey's "wound healing and antiseptic properties" were due to the acid and hydrogen peroxide formed from glucose oxidase activity. A study performed by White and Subers (1964) reported that the sensitivity of this enzyme was maximal at 425-525 nm and a pH of about 3 and was negligible at a about pH of 6-7.

Other enzymes such as catalase and acid phosphatase are the remaining enzymes demonstrated to occur in honey. Catalase has been found to have a pH optimum of 7-8.5

and a optimal substrate concentration of 0.018 M H_2O_2 (Schepartz and Subers, 1966). Phosphatase, on the other hand has optimal activity at a pH of 4.5-6.5 and increased by an increase in magnesium ions.

Flavor, Color and Aroma:

Perhaps the most attractive feature of honey is its characteristic flavor. The flavor complex includes volatile aromatic materials, a dominating sweetness, contributions from acids, polyphenolics, amino acids, and in some cases specific bitter or characteristic nonvolatile notes (White, 1978). Although there does appear to be a distinct honey flavor, the wide variety of flowers attractive to bees overlays a great multiplicity of source-specific flavors and aromas. Color is also variable and strongly influenced by source. Flavors range from desirable to objectionable, but generally the lighter colors are associated with the milder, more pleasant flavors (White, 1978).

Gas-liquid chromatography has been applied to examine the volatile aroma characteristics in honey. Of the over 120 compounds separated by Cremer and Riedmann (1964) in a 1mm x 100m Golay column, the lower aliphatic aldehydes, ketones, alcohols, and esters made up the bulk of the identified compounds. Upon storage, increases in pentanol, 2-mehtyl-1-butanol, 3-methyl-1-butanol, and n-propanol suggested that these compounds corresponded to the amino acids present in the honey.

Milum (1939) ascribed most of the increase in color of honey upon storage to reaction of iron from processing equipment and containers with polyphenols, the browning

reaction of reducing sugars and amino acids, as well as the instability of fructose in the acidic environment of honey.

2.2 D2 Physical Characteristics

The physical characteristics of honey are largely due to the high concentration of sugar that compose the substance. The rheology, or flowability of honey has been found to be quite variable when adjusted to equivalent moisture contents (Lothrop, 1939). Despite this, most honeys, like most sugar syrups are Newtonian liquids, in which viscosity remains constant with changes in shear rate or agitation (Crane, 1975). However, Pryce-Jones (1953) reported non-Newtonian honeys. Pryce-Jones also reported the rheology of Heather honey to be thixotropic in nature, or a fluid with decreasing viscosity at increasing shear rates (Crane, 1975). This type of fluid is also characterized by a tendency to rebuild viscosity upon standing. This property was ascribed to the properties of the proteins present in the Heather honey. Pryce-Jones (1953) went on to describe a Nigerian Eucalyptus honey that exhibited a dilitant property (increase in viscosity as shear rate increases), however this was thought to be related to the presence of a high molecular weight dextran.

Hygroscopicity:

According to White (1978), the ripening of nectar to honey by the bee includes its repeated exposure in a thin film to warm air. The solids content reached is a function of

the extent of moisture saturation of the air in the hive, which is related to temperature and to the external air conditions.

Crystallization:

There are only two sugars that crystallize in honey: glucose and melezitose (White, 1978). Since melezitose is relatively rare to encounter in sufficiently high concentrations it will not be discussed here. The initial texture is influenced by moisture and dextrose content of the honey and by the amount and quality of the seed material. Best (smoothest) texture results from growth of a network of interlaced fine crystals, induced by large numbers of very fine crystals and fragments in the seed material. Natural crystallization of honey before processing is usually is a matrix of glucose hydrate crystals in a syrup, due mostly to the low temperature to which honey is exposed after its removal from the honeycomb. Granulation of glucose in honey is very unlikely to occur in the honeycomb, therefore excessive crystallization of honey most likely occurs due to the extraction process. Granulation occurs generally due to seeding of the honey. The source of the "seed" is usually from equipment, from the air in the extracting plant, and from containers (Crane, 1975). After honey has been processed, seed crystals are no longer present and if proper processing has occurred, coarse granulation will be delayed for many months.

2.2 E. Storage of Honey

Since honey is a concentrated solution of fructose and glucose in an acid solution it is not surprising that honey is susceptible to many physical and chemical changes during storage.

Changes in Color during the Storage of honey:

Color changes that occur during the storage of honey depends upon the fructose, moisture and acid content of the honey which as mentioned earlier is quite variable (Ramsey and Milum, 1933). Most of the darkening of honey is due to Maillard Browning reaction that occurs between amino acids and reducing sugars. In a study conducted by Wootton et al. (1976), the addition of sulfite to honey resulted in a retarding effect of browning which indicated the Maillard reaction to play a major role. The addition of ascorbic acid had little to no effect, which eliminated any oxidative mechanism. Specifically, the most quantitative decrease in amino acid content was that of proline, however, as stated earlier this would seem obvious because proline makes up 80% of the total amino acid content of honey.

Hydroxymethylfurfural (HMF) which is formed from fructose by the action of acid and heat was thought to be a major color producing material in honey, however, different tests (Fiehe test and the Feder test specifically) responded differently for different investigators (Shannon, 1916; Sherwood, 1924; Greenleaf and Browne, 1929; Lampitt et al., 1929; Gautier et al., 1961) thus, judging the effect of invert sugars on color development in storage is somewhat controversial.

Flavor Changes:

As honey is heated or stored for several months at higher temperatures common in the United States, it has been noted by White (1978) that the more delicate aspects of flavor and aroma that can only be detected by comparison with a sample kept at freezer temperatures will change. It is, however, very possible that flavor damage would occur from excessive heating, which can be identified by a darkening of the product.

Enzyme Inactivation:

The effect of storage on enzyme inactivation is of importance largely in honey intended for export to countries with minimum levels for amylase content, the United States being one of those countries. Schade et al. (1958) applied their quantitative procedure for honey diastase activity noted "slight, but not significant in most cases" and was calculated at about 10% in 13-15 months.

Carbohydrate Composition:

The most drastic change in sugar takes place during the ripening stage of honey in the honeycomb, with the inversion of sucrose and the production of the transglycosylation (White, 1978). Enzymic inversion of sucrose into fructose and glucose does continue to occur in full density honey. Although the rate is greatly reduced, enzymic inversion can contribute to error in the analysis of sucrose. Borus et al. (1966) reported an instance in which locust honey of 9.6-12.7% sucrose fell to 1.7-4.3% in a year's storage. This was attributed to the fact that during the relatively warm, dry weather in which the honey was

ripened, the quick and heavy production allowed the honey to reach full density before the sucrose content had the ability to fall within normal limits (<5%).

Besides the inversion sucrose, other changes have been noticed to occur to honey when stored. White et al. (1961) examined the effect of storage of honey on carbohydrate composition. Honey stored at -20°C was compared with samples held up to 2 years at room temperature, with and without heating 30 minutes at 55°C for pasteurization. The results showed a 69% increase in reducing disaccharides, a slight increase in sucrose and other higher sugars, while glucose and fructose fell 13 and 5.5% respectively. White et al. (1961) concluded that the decrease in glucose specifically was the major cause of texture loss and partial liquefaction of finely granulated honey during long-term storage, most likely caused by enzyme activation and acid reversion.

Fermentation:

There are over 14 osmophilic yeasts which can cause fermentation due to the low water activity of honey. Unfortunately, these osmophilic yeasts are nearly ubiquitous on the bodies of bees, in nectar, soil in apiaries, and extracting and storage areas (White, 1978). Thus, with the subsequent granulation that enriches the liquid phase in water will inevitably increase the risk of fermentation. Martin (1958), in his work on hygroscopicity, examined factors leading to yeast growth at the surface and also in the depth of the container. He found that when surface moisture increased above approximately 22%, yeast count increased massively at the surface; although from 2 cm down, counts remained

stable. However, further handling was speculated to redistribute the inoculum throughout the mass of the container allowing for subsequent anaerobic fermentation.

Recommended Storage for Honey:

Problems that have been noticed to occur during the storage of honey are fermentation, granulation, discoloration, flavor damage, destruction of enzymes, and production of HMF (White, 1978). Marvin (1928) stated that the only condition in which all dangers and changes are eliminated is in freezer storage. Since however, this is not completely practical, it is at least vitally important that honey be protected form atmospheric moisture to prevent fermentation. Coarse granulation can occur if temperatures exceed 15°C, therefore, cold storage (below 10°C) is recommended.

2.2 F. Use of Honey in Baked Products

The most significant indirect uses of honey are in the baking, cereal, and confectionery industries however, the following information is a review of the literature present in the area of baked goods.

The use of honey in baking has decreased in the past 20 years because of the run-up in price and the introduction of fructose-containing syrups that approach the functional values of honey. Unduplicated, however, are the flavor advantages conferred by honey and the freedom that exudes from using the word “honey” in the advertising and promotion of a baked product with honey. Proposed advantages for honey-sweetened

baked goods have been noted in moisture retention, texture, keeping quality, flavor, and "eating quality", all of which will be described below.

Smith and Johnson (1951) examined the effect of honey in white bread and whole wheat bread. Breads were made at two different sugar levels (3% and 6% of flour weight) both with either granulated sucrose or honey. Since honey is approximately 17.5% water, honey was substituted for sucrose at 7.3% to 6% sucrose. Rheological, color of crust and crumb, break and shred, symmetry, evenness of bake, grain, aroma, taste, and texture results were all measured by close observation with trained panelists.

Results did not reveal any significant differences in absorption, mixing, fermentation, machining, proofing, or baking quality in doughs containing honey or sucrose at either replacement level. At the 3% level, a very slight effect on crumb color was noticed, but all other parameters were indistinguishable from those made with sucrose.

Samples of bread crumb were air dried 24 hours after which they were subjected to oven drying at 130°C for one hour. No significant differences were noted between the sucrose or honey breads at either the 3% or 6% level in moisture retention. A Bloom gelometer was employed for tests on crumb softness. Again, no significant differences were noted at either sugar level between those loaves made with honey and those made with sucrose.

Smith and Johnson (1952) continued their work into cakes and sweet doughs. The objective of the research was to determine the maximum amount of honey that could be

substituted for sucrose in white layer cakes, yellow base cakes, chocolate layer cakes, and basic sweet doughs.

Preliminary research that was used to study the effect of varying honey concentration noted that when honey was replaced by sucrose at 100%, cakes of poor quality were produced, due to dense structure, low volume, dark crumb, and an undesirable flavor. It was thought that the low volume was produced due to the low pH of the batter produced by honey (pH = 3.6 - 4.0). To overcome the volume problem, various extra leavening agents were added to the formula. However, as a result of the increased alkalinity, the darkening of the crumb and the production of off flavors and aromas were noted. Also, in the chocolate cake variations, the low pH of the batter led to a poor volume as noted earlier, but also led to a change of color from a light amber, to a dark brown.

In a yellow base cake, it appeared that a replacement of honey at the 40% level produced acceptable cakes, however, the volume was slightly lower than those made with sucrose. Symmetry, grain, and texture were all equally good.

Moisture retention was also measured at 24, 48, and 72 hours in unwrapped cakes left at room temperature. The percent moisture loss at 24 hours appeared to be independent of the type of sweetener used after 24 hours, however, at 48 and 72 hours, it appeared that honey cakes retained moisture slightly better than cakes made with sucrose although statistics were not conducted.

As mentioned earlier, a 40% replacement for sucrose was attained in the yellow cake. It was believed that greater than 40% would be able to be utilized in the chocolate cake, however this was shown not to be true because at amounts greater than 40%, "burnt flavors and aromas" were noted and attributed to the browning reaction.

Smith and Johnson (1953), continued their honey research in the field of cookie production. The objective of their research was to investigate whether or not cookies could be improved by the addition of honey. Sugar cookies, ginger snaps, and vanilla wafers represented the class of cookies that were supposed to be somewhat "dry and brittle", whereas, coconut macaroon chips, fruit bars, and brownies represented the class of cookies with "chewy" properties. Optimum levels of honey were determined by a series of bakes in which honey concentration was varied, and the properties of flavor, aroma, color, character, and eye appeal were observed. Crumbliness, oxidative rancidity, and color were also subject to the study.

Optimum honey concentrations were 5% for the sugar cookies, 30% for ginger snaps, 5% for vanilla wafers, 13.3% for coconut macaroon chips, 66.6% for fruit bars, and 50% for the brownies.

Oxidative rancidity measured by peroxide values after 108 days of storage showed very slight to no rancidity in all samples. It was concluded that honey at optimum levels did not affect the development of rancidity in cookies.

Demetriades et al. (1995) evaluated the role of honey in an on-going effort to develop healthy snack foods, in this case potato chips. Honey was chosen because its high

fructose content would enhance browning reactions, a component missing from previous fat free potato chips produced in microwaves. Honey had also been known to intensify the flavors of other food components, as well as enhance crispness by a process that is not well understood.

In this investigation, honey solids were placed in a brine solution of which potatoes were soaked before they entered a microwave tunnel. The color and texture of the chips were then compared to those of a chip that had been microwaved and soaked in a brine solution containing no honey.

There were three variables used in the study: type of honey (dry, liquid, or dry and liquid at a 1:1 ratio), levels of honey (4, 5, or 6%), and the microwave tunnel residence time (4.45, 9.86, and 12.59 minutes).

Results on chip color demonstrated, that the brine solution containing 6% honey solids (as opposed to 4% and 5% in other trials) exhibited the darkest color. Dry honey caused the darkest chip (when compared to liquid honey and a 1:1 ratio of liquid to dry honey). The slowest belt speed, resulted in the darkest chip. No mention was made as to which combination produced the chip closest to the color of a full fat potato chip.

Crispness was evaluated by the amount of force required to break the chip. Dry honey resulted in a potato chip requiring the highest force to break, whereas a combination of honey required the least amount of honey to break. The sample made with 5% honey required slightly more force to break than did the 6% sample, and 9.86 minutes in the microwave tunnel resulted in a sample with the highest force.

Addo (1997) investigated the effects of honey type (liquid vs. dry) and level (4, 6, 8, 10, and 12%) on the baking properties of frozen wheat flour doughs, prepared using a short-time dough method. Doughs were sheeted, molded, and panned. Proof time was defined as the amount of the time for each dough to rise to a predetermined height. Loaf volume, bread firmness, and crust and crumb color were also measured. Following initial measurements, other doughs produced were immediately placed in a blast freezer at -50°C for 15 minutes and stored until they were utilized, whereas the doughs were thawed, proofed, baked, and cooled so that loaf volume measurements could be taken.

The results indicated that addition of 8% or more liquid honey resulted in significant ($p<0.05$) improvements in baking properties of the frozen doughs: lower proof times, higher loaf volumes, and lower crumb firmness than the frozen control dough containing 6% sugar. Loaf volumes from frozen doughs containing 10% or more liquid honey were also significantly higher than the nonfrozen control dough containing 6% sugar. Although the exact mechanism could not be addressed in this study, Addo believed that the honey may have protected the dough structure from damage commonly associated with freezing.

Crumb firmness, an indicator of staling, noted a decrease in firmness with increasing amounts of both liquid and dry honey, with dry honey being the most effective. Perhaps this could be attributed to the very hygroscopic nature of honey in general.

2.3 Fat Substitutes

The term “fat substitute” implies that a substance, when used as a replacement for the traditional fat in a food product, will contribute to the desirable physical and organoleptic properties of the fat it replaces (Vanderveen and Glinsmann, 1992). Although many consumers tend to consider fat substitutes as comprising a single entity (Hassel, 1993) they do comprise 3 major categories: lipid based, protein based, and carbohydrate based.

2.3. A. Lipid-Based Fat Substitutes

Lipid-based fat substitutes consist of lipids that possess functional and sensory properties similar to the fats they tend to replace. Unfortunately, these compounds have recently come under much scrutiny due to toxicity issues. *Caprenin,* is an example of a lipid based fat substitute and is Generally Recognized As Safe (GRAS). It is a reduced calorie triglyceride formed by the esterification of caprylic acid (C8:0), capric acid (C10:0), and behenic acid (C22:0). When metabolized in the mitochondria, *Caprenin* produces 5 kilocalories/gram as opposed to the 9 kilocalories/gram of other triglycerides. This fat substitute which shares similar functions to those fats found in cocoa butter, can replace the fat in products using cocoa butter, such as soft candy and confectionery coatings.

Olestra is another example of a lipid based fat substitute. It has been researched extensively and is produced by the esterification of sucrose with 6 to 8 long chain fatty

acids, making it a sucrose polyester. It is similar to fat in appearance, taste, texture, and function in food, and can therefore be used in practically all applications, such as frying and baking (Anon., 1991). In the case of olestra, the shear size of the molecule makes it impossible for lipase enzyme to attack and break it down, therefore, the compound passes unabsorbed through the intestines and into the colon for excretion. However, this can possibly lead to a reduction in the absorption of essential fat soluble vitamins (Hassel, 1993).

2.3. B. Protein-Based Fat Substitutes

Protein-based fat substitutes are derived from proteins typically found in eggs, milk, and other protein sources. *Simplesse* is an example and was GRAS approved in 1988. This compound is produced from milk or egg by a patented process known as microparticulation. The technique shears the protein into small spherical balls. These balls are perceived as creamy fluid when put into ice-creams, because the product "rolls" off the tongue. The use of these substitutes is, however, rather limited due to their lack of thermostability. For baking applications, protein based fat substitutes are not typically used.

2.3. C. Carbohydrate-Based Fat Substitutes

Carbohydrate-based fat substitutes have been incorporated into many products. There are approximately 40 different types of starch based products out on the market,

most of which were introduced in the 1990's. Starches are used for many reasons: 1) they are heat stable and can therefore be used in baking, 2) gelatinized starch takes on a smooth and creamy consistency, and 3) an "elastic-like" gel structure is formed, which results in rheological properties similar to those of fats in some food systems (Hassel, 1993). It has been postulated that fat mimicking properties of carbohydrates result from an association of water with the structure of the carbohydrate particle. The ideal carbohydrate based fat mimetic will likely possess a structure which strongly binds and orients water in such a way as to provide a sensation which is identified with the rheology of fat in the oral cavity (Yackel and Cox, 1992).

2.3. C1. METHOCEL Food Gum

Methocel, a food gum produced by Dow Chemical, has been approved for use in a variety of baked goods such as cakes, muffins, and yeast products. Methocel is derived from cellulose, the most abundant polymer in nature. Two different forms are available: methylcellulose and hydroxypropyl methylcellulose. Although both have a polymeric backbone of cellulose, each form has different substitutions which have a profound effect on the properties of the different products.

Both METHOCEL products are accepted food additives by the USDA and FDA. They have approved GRAS use in a variety of baked goods such as cakes, muffins, and yeast products. In reduced fat baked products, METHOCEL gums have contributed to improved baked structure, texture, and appearance (Busetti, 1995; Anon., 1993).

Structural improvements in baked goods can be attributed to these gums, which act as surfactants (surface active agents) that entrap and stabilize air or carbon dioxide. They can retain moisture in baked goods since they are hydrophilic and have even been noted to prevent phase separation even under freeze/thaw conditions (Anon., 1993).

METHOCEL food gums are non-ionic and therefore, will not complex with minerals or other components in food systems. Flavor is not adversely affected since these gums are virtually tasteless, odorless, and colorless. Cellulose is also a fiber and can replace fat and will not supply any calories to the finished product (Anon., 1993). Many consumers have begun to realize that just because a product is low in fat, does not mean the product is low in kilocalories. Having a product that is fat-free, yet containing the same amount of kilocalories as its full-fat counterpart is a gimmick to the general consumer (Busetti, 1995).

2.4. Enzymes

A variety of enzymes - proteases, lipoxygenases, and carbohydrases are used in the production of bakery products (Stauffer, 1994). Proteases hydrolyze peptide bonds, and therefore may be used to soften the product. Hydrolysis of the gluten molecule gives an irreversible peptide cleavage, and therefore, once weakened, the gluten structure remains weak through the rest of the production cycle (Stauffer, 1994). Proteases are not dose dependent, meaning that a given amount will react with a given amount of substrate. Instead, protease activity is dependent on the type and amount of protease used, and the

length of time by which the protease is allowed to react. In general, proteases will continue to act until they are denatured (usually by heat in the oven).

Proteases affect doughs in two ways: they reduce mix time and enhance dough flow. To reduce mix time, enough time must be allowed for the protease to hydrolyze a limited number of gluten peptide bonds. Extensive hydrolysis would weaken the dough and give poor loaf volume.

Lipoxygenases affect doughs by increasing the mixing tolerance and gluten strength in much the same way that sodium stearoyl lactylate (SSL), DATEM, and other anionic emulsifiers increase dough strength. For a description of how this occurs, please refer to 2.6. A. Functions of Emulsifiers. In the case of lipoxygenases, a chemical bond is formed between hydroperoxy-linoleic acid (one of the reaction intermediates) and a gluten side chain (Stauffer, 1994). This added hydrophobicity produces the desired gluten strengthening.

The carbohydrase class includes several different enzymes, most with substrate specificity. Of theses, amylases are the most widely used.

2.4. A. Amylases

Enzymes are present in many flours, with the most important being the carbohydrases α- and β-amylases. These enzymes are also found in different cereals, fungi bacteria, and mammals. Barley malt (a cereal source of amylases) is often added directly to wheat flour at the mill to standardize alpha-amylase activity. This process enhances

production of fermentable sugars from damaged starch, increases yeast growth and gas production and improves dough handling and proofing (Hebeda et al., 1990). It also improves color, grain, texture, and flavor, although shelf-life is not improved.

Fungal amylases are effective in partially hydrolyzing damaged starch and are often added to flour to develop desirable properties for baking. Fungal amylases, however, do demonstrate limited thermostability, and are for the most part, inactivated prior to the onset of starch gelatinization during baking since their optimum temperature range is only 50-55°C (Hebeda et al., 1990). As a result, fungal amylases have little effect on amylopectin hydrolysis and do not exhibit significant anti-staling properties. Cole (1982) was able to successfully protect fungal alpha-amylase from thermal destruction by mixing sugar in solution with the enzyme.

Bacterial alpha-amylases are able to inhibit staling by hydrolyzing glycosidic linkages within the amorphous areas of gelatinized starch. However, due to a high degree of thermostability (80-89°C), active enzyme can persist throughout baking and produce excessive levels of dextrins. As a result, the product is often gummy or sticky which may lead to problems during slicing (Hebeda et al., 1990). This problem can be further exacerbated if complete inactivation does not occur during the baking process. Therefore, to achieve an antistaling effect, dose level and process parameters must be carefully controlled.

A recent development in this area has been the development of amylases that exhibit thermostability properties between those of traditional fungal and bacterial

enzymes. These new “intermediate stability” enzymes have optimal reaction rates at 65-75°C, and as a result provide an anti-staling effect in baked goods without adversely affecting product quality.

2.4. B. Effect of Amylases on Staling

The staling mechanism has been studied extensively, and investigators have presented a variety of theories in an attempt to completely describe the phenomenon. It is generally accepted that staling is due to a gradual transition of starch from an amorphous structure to a partially crystalline state (Hebeda et al., 1990). Moisture may be redistributed within the baked good during this transitional period, although water loss is not a requirement for staling.

The increase in starch crystallinity is caused by an intermolecular or intramolecular association of starch molecules via hydrogen bonding that is known as retrogradation. Amylose and amylopectin both retrograde, although the rates of association differ due to differences in polymer structure, such that amylose is more rapid due to the ease of alignment of the linear molecules (Hebeda et al., 1990).

During baking, starch granules swell and absorb moisture. As the granules swell, amylose diffuses from the granules into the interstitial volume. The solubilized linear molecules retrograde rapidly and form a crystalline network. The characteristic structure of baked goods formed during cooling is caused by amylose association (Schoch, 1965) .

Amylopectin remains in the starch granule and retrogrades slowly during product storage, due to the presence of non-parallel branched chains. Eventually, retrogradation occurs by intermolecular and intramolecular association of linear segments. As amylopectin retrogradation proceeds, a three-dimensional crystalline structure is formed slowly, causing an increase in firming.

Factors that control the rate of staling include time, temperature, moisture level, and the presence of additives such as emulsifiers and enzymes. Amylases from bacterial and fungal sources added to a dough or batter mixture, can delay the onset of retrogradation in baked goods (Mason et al., 1996). Specifically, the alpha-amylases hydrolyze the alpha 1,4 glycosidic linkages in starch at random points within the amylose and amylopectin molecules. Under proper conditions of time and temperature, the limited degree of hydrolysis is sufficient to disrupt the starch network and reduce the rate of staling (Hebeda et al., 1990).

2.4. C. Research on Fungal and Bacterial Amylases

In a study by Valjakka et al. (1994), the effect of raw-starch digesting enzyme (RSDE) on bread firming properties was compared to firming properties of bread containing an emulsifier and bacterial and fungal enzymes. Flour containing 12% protein was used and starch damage was 5.4% of flour. Hydrated monoglycerides served as the emulsifier and a bacterial enzyme, or a fungal enzyme and an RSDE were employed. The bacterial enzyme inhibited the firming rate of the bread. Both the fungal enzyme and the RSDE decreased the initial bread firmness, although the rate of firming was not affected.

When an enzyme was used in conjunction with an emulsifier, the bread was less firm than bread in which these additives were used separately. The results show that the incorporation of enzymes did decrease the rate of staling, although each form affected firmness differently.

Another study by Akers and Hoseney (1994) used high performance anion-exchange chromatography (HPAEC) to separate the water-soluble dextrins extracted from bread (alpha-amylase supplemented and unsupplemented) that had been aged for 5 days. The relationship between the soluble dextrins and the rate of bread firming was the primary objective of the study. Seven alpha-amylases were used. Two fungal amylases were cultured from *A. oryzae*, and four different *Bacillus* amylases from different strains and incorporated at different levels. One enzyme was cereal malt amylase. None of the amylases were classified as intermediate stability amylases. Both of the fungal amylases and one bacterial amylase significantly reduced the rate of firming when compared to the standard bread. Three of the bacterial amylases had a profound effect on inhibiting the firming rate of the bread compared to the other treatments. The peaks obtained in the chromatograms, representing the extracted water-soluble dextrins from the bacterial amylase treated breads, were associated with a reduced rate of firming. The results of this study indicated that all of the bacterial enzymes did not produce the same amount of the similar dextrins, and the extent to which they did reduce the staling rate differed.

2.5. Emulsions

An emulsion is a system consisting of two immiscible substances, one dispersed into the other. There are two phases that are present in an emulsion: the dispersed phase (the substance that is finely distributed throughout the other) and the continuous phase (the substance by which the dispersed phase is distributed throughout, Stauffer, 1996a). The dispersed phase is distributed in droplets ranging form 0.1 to 50 æm. When the two immiscible substances are mixed together, an energy exists at the interface between the two phases. The interfacial tension can be increased due to mixing or breaking up of the dispersed phase into smaller droplets. The result, however, of increasing the interfacial tension, is a destabilization of the emulsion which results in one or a combination of three mechanisms: (1) creaming, (2) flocculation, and (3) coalescence. Creaming results in the separation of the two phases based on density from the action of gravitational force. Flocculation is the movement, or clustering of the dispersed droplets. The main reason for flocculation is due to an inadequate electrostatic charge at the globular surface. Coalescence occurs when the droplets aggregate and rupture the interfacial film, thus breaking the film (Nawar, 1985).

One way to decrease the interfacial tension that exists between two immiscible substances, is by the use of an emulsifier. Emulsifiers, referred to as surfactants (surface active agents), migrate to the interface where they ease interfacial tension, and thus slow the rate of coalescence. Therefore, the stability of an emulsion is dependent upon the

extent of mixing it has been subjected to and the presence of emulsifiers (Mason et al., 1996).

2.5. A. Types of Emulsions

The two most common types of emulsions in the food industry are water in oil (W/O) and oil in water (O/W) emulsions, although other kinds of emulsions such as liquid and gas, liquid and liquid, and liquid and solid do exist. Examples of water in oil emulsions are butter (which contains 80% lipid and 20% water), margarine (which also contains 80% lipid and 20% water), and icings. Oil in water emulsions, in which the oil is dispersed into a water phase, consist of such products as mayonnaise, salad dressings, as well as cake and muffin batters (Anon., 1995).

A foam is a mixture of liquid and gas. It involves the same energy considerations (interfacial tension) as an emulsion, and therefore, emulsifiers dissolved in the aqueous phase can promote foaming (Stauffer, 1996a). The lipophilic portion of the surfactant enters the gas phase as opposed to the oil phase of an emulsion. The emulsifier in this situation is usually a protein. When air bubble are introduced into the hydrophilic solution by the action of a mixer, the protein unfolds at the air-water interface . The hydrophobic side chains enter the air phase, and the hydrophilic chains remain in the water phase. The portions of the protein located in the aqueous phase hold water, prevent it from draining, and prevent the air bubbles from coalescing and destabilizing the foam.

2.6. Emulsifiers

In order to maintain a stable emulsion, coalescence must be prevented through the usage of emulsifiers. The functions that emulsifiers perform in food products make them an asset to the food industry. Emulsifiers not only stabilize emulsions, but foams and suspensions as well. The result is a substantial effect on texture by interacting with polymers such as starch and protein. Emulsifiers can also modify the crystallization of lipids (Anon., 1994; Dziezak, 1988, Shepard and Yoell, 1976).

The key molecular characteristic of an emulsifier is that it is "amphiphilic" in nature. This means that the emulsifier possesses both hydrophilic and lipophilic constituents. The lipophilic or hydrophobic part of the molecule prefers to be in a lipid environment and is usually a long-chain fatty acid obtained from a food-grade fat or oil (Stauffer, 1996a). The hydrophilic portion prefers to be in an aqueous environment and is usually nonionic, anionic (negatively charged), or cationic (positively charged). In food systems, cationic emulsifiers are not used because they are derived from bacterial sources and may be toxic as food additives.

Hundreds of emulsifiers are available in the food industry today, and can be classified by their Hydrophilic/Lipophilic Balance (HLB). This values range from 1-20. Those emulsifiers with an HLB value between 1-5 are commonly used in water in oil systems and are characterized as having a strong affinity for oil. Examples of these types of emulsifiers are propylene glycol monoester (1.8), glycerol monostearate (3.7), and succinylated monoglyceride (5.3). Emulsifiers with an HLB value between 6-12 are

emulsifiers than can be used in any application because neither constituent predominates (they are amphiphilic). Examples of these types of emulsifiers are triglycerol monostearate (7.2), sucrose distearate (8.9), diacetyl tartaric esters of monoglycerides (DATEM, 9.2), and sucrose monostearate (12.0). Finally, emulsifiers with an HLB value from 13-20 are commonly used in oil in water emulsion applications due to their strong affinity for water portion of the emulsion. Examples of these types of emulsifiers are Polysorbate 60 (14.4), Polysorbate 80 (15.4), and sodium stearoyl lactylate (SSL, 21.0).

2.6. A. Functions of Emulsifiers

Emulsifiers can perform a number of functions that can be attributed to their differences in chemical structure and the types of fatty acids used for the lipophilic portion of the substance. For those emulsifiers with an HLB value between 6-12, the lipophilic and hydrophilic tendencies are evenly balanced, and can promote the spreading of a liquid phase onto a solid surface. This function is known as wetting and occurs because the emulsifier increases the rate at which water displaces air from the surface of the solid, enhancing the dispersion of the solid phase into the water (Stauffer, 1996a).

Starch molecules are polymers composed of glucose units. In solution, the starch chain coils to form a helix, with about six residues per turn (Stauffer, 1996a). The result is a hollow cylinder with a hydrophilic outer surface and a hydrophobic inner surface. The inner diameter of this hollow cylinder in approximately 4.5 Angstroms and is large enough for the straight chain hydrophobic portion of the emulsifier to fit into it. Because of this

phenomenon, emulsifiers can act to reduce the firming of baked products that occurs due to changes after baking (staling). Specifically, the fatty acid part of the emulsifier can form a complex with gelatinized starch, retarding starch crystallization and thus slow the process of staling.

Emulsifiers can also act as protein aggregators or dough strengtheners (Stauffer, 1996a). The hydrophobic portion of the emulsifier can interact with the hydrophobic "patches" on the protein.

Wheat gluten consists of about 40% hydrophobic amino acids which interacts with lipid soluble substances such as emulsifiers. The addition of an acid to the wheat flour dough solubilizes some of the protein. As the pH lowers, many of the organic acid groups on the amino acid are protonated (becoming neutral) and the protein molecule takes on a net positive charge (Stauffer, 1996a). This net positive charge causes the molecules to repel each other, creating a dispersion. If an emulsifier is added (preferentially an anionic surfactant) the protein molecule aggregates even at an acidic pH and the integrity of the dough is strengthened.

Some other properties of emulsifiers include 1. aeration, 2. defoaming, and 3. lubrication (Stampfli and Nersten, 1995; Anon., 1992; Dziezak, 1988; Schuster and Adams, 1984; Garti et al., 1980; Shepard and Yoell, 1976; Nash and Brickman, 1972).

Since emulsifiers contribute many of the attributes to foods that fat does (aeration, starch complexing, lubrication) then emulsifiers can be used to reduce or eliminate the fat

in baked goods. Any given emulsifier might perform one or a number of these traits (Krog, 1981).

2.6. B. Crystal Form and Mesomorphic Behavior of Emulsifiers

Emulsifiers are fat derivatives, therefore, they exhibit polymorphism and different crystalline forms such as α, β, and β´. In order to understand how emulsifiers function in food, it is important to be familiar with these crystal forms.

In addition to the crystal form, the size of the crystals have a significant effect in the action of water-free emulsifier proportions in food systems. Schuster and Adams (1984) demonstrated that the interaction of MG's with amylose depended upon the physical state of the MG and especially upon its crystal form. Results have shown that the α-form of emulsifiers exhibits particular advantages over the other forms by increasing emulsifying activity, facilitating dispersability, and enhancing aerating properties (Schuster and Adams, 1984).

Mesomorphism is the ability of substances to form mesophases, or liquid-crystal phases. Lyotropic mesomorphism exists as substances form a liquid-crystalline phase when treated with a certain concentration of water and align themselves in such a way as to minimize the repulsion that is present. Emulsifiers have the ability to form such lyotropic mesophases due to their amphiphilic nature, although the specific structure does not dictate what type of mesophase is formed (Krog et al., 1985). Many structural types

of mesophases exist, but the only phases that are of concern to the baker are the lamellar, hexagonal, or cubic structures (Birnbaum, 1981).

When a food system exists in a lyotropic mesophase, the emulsifiers are capable of interacting with flour components on a molecular level. Effective dough conditioners and starch complexing agents are dispersible in highly polar water because they form lyotropic mesophases with liquid crystalline properties (Birnbaum, 1981). For instance, concentrated MG's, which are normally insoluble in water, are able to disperse in water at a temperature in the vicinity of their melting point. When this occurs, lamellar structures are formed which consist of double layers of MG's alternating with layers of water. This type of arrangement allows the saturated MG's to react optimally with amylose. Of all the food grade emulsifiers, the distilled saturated MG's are the most efficient starch complexing agents and a good correlation between the amylose complexing index and crumb softness has been observed. The anionic emulsifiers such as DATEM and SSL, also form lamellar phases. Due to their strong hydrophilic character, both of these emulsifiers can be easily dispersed in water. These anionic compounds, in their crystalline state, act as dough conditioners (Birnbaum, 1981).

2.6. C. Diacetyl Tartaric Acid Esters of Monoglycerides (DATEM)

Diacetyl tartaric acid esters of monoglycerides (DATEM) are esters of glycerol with fatty acids and acetylated tartaric acid. Figure 1 shows the chemical structure of DATEM:

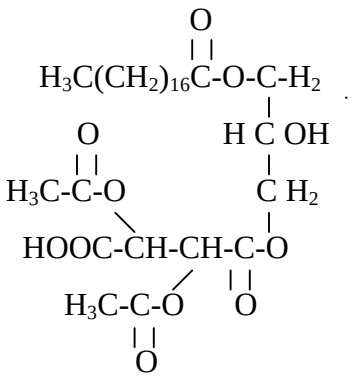

Figure 1: “Chemical Structure of Diacetyl Tartaric Acid Esters of Monoglycerides (DATEM)

DATEM is used in such applications as bakery products, extruded products (such as cereals and pastas), icings, and margarines.

DATEM can be synthesized in one of two ways: (1) by reacting MDG’s with diacetyl tartaric anhydride in the presence of acetic acid or (2) by esterifying the MDG’s with tartaric acid and acetic acid in the presence of acetic anhydride (Lorenz, 1983). The diacetyl tartaric acid portion of the molecule is responsible for an anionic charge to the emulsifier (Hoseney et al., 1976).

Different forms of DATEM exist, therefore, the functions and physical properties differ. Regardless, the anionic nature of the tartaric acid in DATEM makes it a very effective dough strengthener (Stauffer, 1996a) by promoting aggregation of the protein even at pH below the isoelectric point of native proteins. The fatty acid present on the molecule also complexes with the starch and thus helps to retard staling that occurs due to the retrogradation of the starch (Stauffer, 1996a). It has also been demonstrated as a “fair” crumb softener (Kulp and Ponte, 1981).

2.6. D. Research on the Utilization of DATEM in Baked Products

2.6. D1. DATEM and Its Roles in Bread Production

In the industrial production of bread, the rheological properties of the dough are important. The dough is expected not to stick to metal surfaces and to show a good resistance to vibration and mechanical shock. Therefore, emulsifiers are used as dough conditioners to obtain a good machine tolerance (Krog, 1981). Krog (1981) went on to report that bread made with shock-treated doughs without DATEM showed a loss of volume by 45% in comparison with a shock-treated bread made with DATEM.

DATEM, sodium stearoyl-2-lactylate (SSL), calcium stearoyl-2-lactylate (CSL), and polysorbate are the most commonly used dough strengtheners (Stampfli and Nersten, 1995) These particular emulsifiers exert their effect during fermentation, mechanical handling, and transport. They also are effective during the proofing of the first part of the baking period, therefore, the final results being higher volume and improved crumb structure of the finished product (Tamstorf, 1983).

Relatively little has been reported about the effect of emulsifiers on dough mixing characteristics and rheological properties of dough. Water absorption was measured with a research water absorption meter by Tsen and Weber (1981) and found no differences in water absorption using SSL, CSL, DATEM, ethoxylated monoglycerides (EMG), Polysorbate 60, monoglycerides, and sucrose monopalmitate, but did continue to report an increased stability for SSL, CSL, and DATEM. In contrast, Tamstorf (1975) found no effect of DATEM, CSL, and di-monoglycerides (DMG) on farinogram characteristics.

Tsen and Weber (1981) compared proofing times and gassing power of doughs made with and without emulsifiers. The investigators found that DATEM, SSL and CSL shortened proofing times and increased gassing power, thereby resulting in improved loaf volumes when compared to shortened doughs.

Fibers added to doughs tend to have a deleterious effect on loaf volume, and therefore Jodlbauer et al. (1992) demonstrated that that DATEM, EMG, sucrose palmitate, SSL, and lecithin could all be used successfully to minimize volume loss due to fiber.

Mettler et al. (1991a-d, 1992 a-c) conducted a detailed study on the effects of emulsifiers (MDG, DATEM, and lecithin) and hydrocolloids during the entire breadmaking process. The researchers measured dough properties by final proof time, fermentation stability, dough elasticity, and an increase in crumb firmness during storage. DATEM showed a positive influence on the fermentation of the dough measured by maturograph and oven rise recorder. With increasing dosage, the fermentation time and the stability increased and the dough standing was improved. All emulsifiers increased the oven rise during baking, while DATEM specifically showed a strong CO_2 retention coefficient.

2.6. D2. Effect of DATEM on Crumb Softness

Crumb softeners create a longer lasting softness in the crumb of bread by interacting with the flour components as described in 2.6. A. The change in crumb softness is generally called staling. Staling is a phenomenon which describes the deterioration of bread quality during storage. Typical sensory changes that occur with staling are a loss of flavor, loss of crispness in the crust, increased crumbliness, and crumb firmness (Stampfli and Nersten, 1995). The phenomenon of staling is still not completely understood, and therefore, preventing staling through the use of emulsifiers has been a tedious task.

The role of water in the staling of bread is important, however, staling is not due to water loss because crumb moisture during storage can be maintained even though the firming of the crumb cannot be prevented. He and Hoseney (1990) went on to report that the higher the moisture retention in the crumb when measured over time, the slower the firming rate. Pisesookbunterng and D'Appolonia (1983) investigated the effect of emulsifiers on moisture migration from crumb to crust. After storage for 1 to 4 days, bread with emulsifiers had greater moisture migration from the crumb to the crust than the control bread. They suggested that, in bread containing emulsifiers, the adsorption of emulsifiers onto the starch surface might not allow the starch granules to take up water released by gluten to the same extent as the control bread. As a result, this water would be available for migration to the crust.

In spite of all the work performed up to now, it appears that the phenomenon of staling still remains unresolved. Staling appears to be a consequence of many reactions by which emulsifiers can help (Stampfli and Nersten, 1995). Therefore, emulsifiers will remain important additives, and with the synergistic effect of enzymes, perhaps the two additives together will impact staling in other beneficial ways.

2.6. D3. DATEM and Its Role in Cake Production

DATEM and emulsifiers in general, can exert a number of favorable affects in cakes, such as: 1. improved rate of hydration and water absorption, 2. greater tolerance to resting time and shock, 3. improved crumb structure: finer and closer grain, brighter crumb, and increased uniformity of cell size, 4. improved gas retention resulting in lower leavening requirements, better ovenspring, and increased loaf volume, and 5. longer shelf-life (Marnett, 1977; Knightly, 1981; Potgieter 1992; Kamel and Ponte 1993).

A batter containing fat is considered an oil in water emulsion, whereas, the continuous phase is a sugar solution containing protein, and the dispersed phase consists of flour and fat particles. The batter is essentially a foam, and the presence of fat makes it unstable. Since the air is dispersed in the fat particles, a fine distribution of the air is possible only when the fat is finely distributed (Krog et al., 1985; Schuster and Adams, 1984). Therefore, aerating the batter is of critical importance when making cakes. The finely distributed air particles increase the viscosity of the batter, leading to a better volume and texture in the finished product (Krog et al., 1985).

Emulsifiers, such as mono- and diglycerides are incorporated into shortening. They reduce the surface tension of the fat, thus improving the dispersability of the fat phase. This enhances the cake texture, crumb softness, and volume (Bennion, 1990b).

2.6. D4. Emulsifiers and Their Role in Low-Fat and Fat-Free Cakes.

In low fat or fat-free cakes, emulsifiers can be used as aerating agents. It is important, however that the emulsifier is in the correct physical form to obtain an effective contribution to the aeration of the batter and cake. In fat-free cakes, emulsifiers in gel form or α-crystalline powders, often serve as aerating agents (Krog, et al., 1985; Schuster and Adams, 1984; Shepard and Yoell, 1976).

Certain MG derivatives can form α-crystalline films around air bubbles thus entrapping them in a cake batter while at the same time keep them dispersed throughout the batter. By holding the bubbles, the emulsifiers can increase the overall cake volume. The leavening gases can expand the bubbles, which contributes to the development of a very fine crumb structure (Henry, 1995). Some monoglycerides may also react with the starch portion of the batter, forming insoluble amylose complexes. This inevitably decreases the starch gelatinization in cakes, resulting in a better cake structure with improved tenderness (Krog et al., 1985).

A study by Kim and Walker (1992a) assessed the interactions between starches, sugars, and emulsifiers in high-ratio cake model systems. Cake flour was replaced by a blend of commercial wheat starch, vital gluten, and a mixture of the emulsifiers lecithin

and ethoxylated MDG's (EMD-20). The emulsifiers decreased the specific gravity of the batter and were found to increase cake volume. The presence of emulsifiers also decreased the crumb firmness. The researchers concluded that a successful high-ratio cake system could be formulated by using the proper combinations of starch, sugar, water, and emulsifiers.

The relationship among heat transfer, water loss rates, and crumb structure development were studied by Cloke et al. (1984) in model cake systems made with different levels of saturated (SMG) and unsaturated monoglycerides (USMG). In addition to control cakes that were unemulsified, emulsifiers were incorporated into a lean cake formulation. In the unemulsified cakes, the air cells were small, however the crumb appeared very "knobby" and the texture was rubbery. All the cakes made with the emulsifiers were less rubbery. The cakes made with USMG had a better crumb structure than the ones made with SMG. As the concentration of USMG increased, the air cells became progressively larger and tunnels ultimately formed above the 5% level. Higher dosages of emulsifiers have a negative effect on crumb cohesion and texture (Schuster and Adams, 1984). The microscopic studies showed that emulsifiers definitely influenced the air cell stability during baking by irregular pooling of lipids. Crumb air cell structure is also influenced by starch granule swelling. The amount of water loss during the original baking was not affected by the nature of the emulsifier system, however, the measurements of water-loss rates and temperature during baking were related to the

nature of the emulsifier system and could be associated with physiochemical changes during baking and final cake structure.

Del Vecchio (1975) studied the effects of SSL and stearoyl fumarate (SF) on tunneling in cakes. Tunneling is the result of holes that proceed through the center of a cake. A cake was made without emulsifiers. Four cakes were made with emulsifiers, two containing SSL and two with SF, at levels of 0.4 g and 1.0 g, respectively. The cake control developed many tunnels. SSL, at the 0.4 g level, increased volume. When SSL was used at 1.0 g, the volume decreased. The emulsifier at both levels reduced the amount of tunneling when compared to the control cake. SF decreased cake volume, but no tunnels were observed. The data indicated that emulsifiers could positively influence the number of tunnels produced in cakes, although this may not be related to the amount of stabilized air cells which are incorporated into the batter and the relative sizes of these air cells produced by different types of emulsifiers.

CHAPTER 3:

Justification and Purpose of the Study

To maintain growth, food industry must continue to create products that meet consumer's needs. The food industry has responded to the health concerns of the consumer by producing a multitude of fat reduced and nonfat products. Baked goods, including muffins, are high in fat and kilocalories, and therefore, an attempt has been made to produce low fat and nonfat variations.

A fat substitute is a substance that is used as a replacement for the traditional fat in a product. Theoretically, this substance imparts the same desirable physical and sensory properties of the fat in the product. However, up to now, all attempts to substitute a fat solely with a fat substitute in a baked product such as muffins have failed, because the removal of fat and the addition of a substitute produces a product that is tougher, stales quicker, and exhibits a lighter color.

Fat plays an integral role in many aspects of a muffin. It appears that the answer to produce a reduced fat or fat free product that tastes like the full fat counterpart is to utilize a combination of ingredients that synergistically work together to mimic the functionality of the fat in the product. Therefore, the goal of this study is to examine the performance of a combination of ingredient substitutions for fat in a full fat muffin.

Fat replacers represent a wide variety of chemical compounds, and each has been developed for a specific application. Lipid based fat substitutes have been used in the confectionery industry, while protein based fat substitutes have been used in the dairy

industry to mimic a rich and creamy full fat product. A carbohydrate based fat replacer, such as METHOCEL, is the fat replacer of choice to use in baking applications because it is heat stable at temperatures used for baking, and its "elastic-like" gel structure provides flow properties consistent with those of fats in similar applications (Hassel, 1993).

Amylases from bacterial and fungal sources that have been added in correct ratios to baked products help to increase the rate of amylose cleavage (amylolysis). The result is an increase in simple sugars. Simple sugars located in the crumb contribute to the taste and water retaining ability of the muffin resulting in a sweeter product with a lower water activity and therefore, longer shelf life. Simple sugars located in the crumb contribute to the color of the muffin by participating in Maillard Browning.

Emulsifiers, such as DATEM (diacetyl tartaric acid esters of monoglycerides), can exhibit the properties of emulsification, lubrication, starch complexing, protein complexing, aeration, defoaming, and crystallization control (Stampfli and Nersten, 1995; Anon, 1992; Dziezak, 1988; Schuster and Adams, 1984; Garti et. al, 1980; Shepard and Yoell, 1976; Nash and Brickman, 1972). Since fat serves in aerating the batter, tenderizing, and flavor, emulsifiers can be used to help reproduce these effects.

Sugar has a tenderizing effect in a baked product. Specifically, sugar competes for water that is essential for gluten development. Sugar ties up water which becomes less available for gluten and thus interferes with gluten development (Bennion, 1990b). This decrease in gluten development affects muffin volume. Since gluten structure is

decreased, there is less of a pressure exerted on the leavening gases resulting in an increased volume by the leavening agents.

It has been speculated that honey would interfere with gluten development and increase muffin volume due to the high fructose content in honey. However, the added monosaccharides as well as the acidic nature of the honey may also contribute to a decrease in volume. Honey has also been shown to affect crust and crumb color, and also should have an improving effect on the keeping quality of the baked product due to the humectant properties of fructose. The ability of fructose "to hold water" would be essential in preventing staling.

The presence of gliadin and glutenin in flour has been shown to contribute structure to baked products. Together, gliadin and glutenin form a protein structure known as gluten. Therefore, gluten and its development provide the structural integrity of the baked product. In general, systems that employ a high gluten content, result in a more cohesive and less tender product. All-purpose flour is the flour used in most household situations. It is a blend of hard and soft wheats that together yield a protein content that is intermediate to that of bread flour and cake flour (Bennion, 1990b). Biscuit flour is derived from soft wheat flour which contains less protein. Therefore, the use of a soft wheat flour will result in a less developed gluten structure, and therefore, an increase in product tenderness.

Therefore, the purpose of this investigation was to study the effect of a 25%, 35%, and 45% replacement for sucrose with liquid or dry honey on the baking quality of a fat

reduced muffin containing a carbohydrate based fat replacer, fungal and bacterial amylases, and DATEM.

Specifically, the objectives of this study were to:

1. To determine the optimal honey replacement for sucrose (liquid and dry) on maintaining physical and sensory characteristics of fat reduced muffins.
2. To observe the effects of honey on the staling/retrogradation of fat reduced muffins.

CHAPTER 4:

Materials and Methodology

4.1 Experimental Design

This investigation had 8 treatments that collectively investigated the use of dry or liquid honey in conjunction with soft wheat flour, enzymes, emulsifier, and a carbohydrate-based fat substitute in a fat reduced muffin. A standard full fat muffin formula was adapted from The Pillsbury Cookbook (1989). This full fat formula was used as a basis of comparison with the fat reduced muffin (Mason et al., 1996) which contained an enzyme system, an emulsifier system, and a fat replacer, as well as all other formulas containing either liquid or dry honey at various replacement levels for sugar. Since all sugar replacement treatments were based on the Mason fat-reduced formula, this same formula was also used specifically to evaluate the use of honey as a partial replacement for sucrose. Furthermore, it also helped to determine the most effective honey for sucrose replacement (either 25%, 35%, or 45%). Important to note, since the formulas for muffins made with dry honey and liquid honey are slightly different in water content, comparisons between the two cannot be made. Therefore, the study had 8 total treatments: 3 sugar replacement treatments for 2 different types of honey and two control treatments. Since treatments made with different types of honey cannot be compared, the liquid and dry honey replacement treatments were compared solely to the two control treatments. To get 83% efficiency, the five formulas (to compare the three liquid honey treatments or the three dry treatments with the two controls) were repeated six times in

blocks of 3. This incomplete block design (Appendix A) demonstrates how this was achieved. Three treatments took 3 days to complete. The three days were sufficient to perform all sensory work, as well as crumb firmness, crust and crumb color, moistness, water activity, volume and DSC measurements. Six treatments (1 block) were achieved in one week. The data collection period for one type of honey with the two controls took 5 weeks, therefore, a total of 10 weeks was sufficient for data collection involving the two types of honey with the controls. An incomplete block design was used and Type I sums of squares was used to adjust for the block effect.

4.2 Control Treatments

Appendix B represents the two control formulas for the study. One formula is for a standard full fat muffin adapted from The Pillsbury Cookbook (1989). This formula was used to compare the synergistic effect of an enzyme system, an emulsifier system, and honey in a fat free formula. The second formula, is a fat free formula adapted from research performed at Virginia Polytechnic Institute and State University (Mason et al., 1996). Since all sugar replacement treatments are based on this formula, this control formula was used specifically to evaluate the use of honey as a partial replacement for sucrose.

4.3 Experimental Treatments

Appendix C represents the 6 formulas for the 6 experimental treatments used in this investigation. Each treatment is based upon the fat free muffin control formula (Appendix B) in order to evaluate the use of a honey replacement for sucrose in a fat free muffin. Liquid Clover Honey (Kroger, Cincinnatti, OH) was used to replace sucrose at a replacement rate of 25%, 35%, and 45% (w/w). Dry honey ("Sweet'N'Neat 2000",ADM Arkady, Olathe, KS) was used and substituted for sucrose in the second three treatments at the same replacement rate of 25%, 35%, and 45% (w/w). Pilot work indicated that batter prepared with dry honey was thicker than the batter containing liquid honey. The batter made with dry honey was noted as being more "dough like". Therefore, muffins made with dry honey had water added back to the formula at a rate of 1 g water/gram of dry honey used. This addition rate gave the batter the same perceived viscosity and flowabiltiy of treatments made with liquid honey or with straight sucrose (control). For example, in the 25% dry honey replacement, 25 grams of dry honey were used, and therefore 25 ml (grams) of water was added back to the formula. For this reason, dry honey replacement formulas were not compared to the liquid honey replacement formulas.

4.4 Manipulation of the Batter

A majority of the ingredients (flour, eggs, buttermilk, skim milk, vanilla extract, sugar, oil, baking powder, baking soda, and salt) were locally bought (Kroger Supermarket, Blacksburg, VA), with the exception of the dry honey ("Sweet'N'Neat

2000",ADM Arkady, Olathe, KS), fungal amylase (Enzeco 5000, Enzyme Development Corp., New York, NY) and bacterial amylase (Fresh-No, Enzyme Development Corp., New York, NY), DATEM (Panodan SDK, Danisco Ingredients USA, Inc.), and METHOCEL (Dow Chemical Co., Midland, MI).

The muffins were produced in a climate controlled laboratory of approximately 70°F at Virginia Polytechnic Institute and State University, Blacksburg, VA. All formulas were produced according to the "Muffin Method" (Bennion, 1990b). All ingredients were weighed to the nearest hundredth using a top loading balance (Fisher Scientific XL-500 Top Load Balance #13028824, Denver Instrument Company, Arvada, CO), except for the bacterial and fungal enzymes which were measured to the nearest thousandth using a Mettler AE 260 Top Load Balance (Mettler Instrument Corporation, Hightstown, NJ). In all formulas, except those using dry honey, flour, sugar, baking soda, baking powder, salt, fat substitute, and enzymes were sifted together using Bromwell's measuring sifter (Michigan City, IA) into a large Pyrex bowl (Pyrex, Corning, NY). In a smaller bowl, eggs, emulsifier, skim milk, buttermilk, vanilla, oil (full fat control only), and liquid honey were beaten together for a total of 60 seconds with a Hamilton Beach Electric Mixer (speed 2 for 15 seconds and speed 5 for 45 seconds) to ensure dispersion of all ingredients. The wet ingredients were then added (all at once) to the sifted dry ingredients and gently folded together for 25 strokes using a Rubbermaid rubber spatula (Wooster, OH). In formulas utilizing dry honey, the honey was rehydrated with water (at a 1:1 ratio) prior to addition into the wet ingredients. An upright blender (Wear Ever - Proctor

Silex, Baltimore, MD)was used to ensure a homogeneous mixture. Muffin paper baking cups (Reynold's Metal Company, Richmond, VA) were placed into the muffin tin (Ecko, Franklin Park, IL) and sprayed with PAM™. Approximately 50 grams (± 1 gram) of batter were spooned into the muffin cups and placed in a General Electric Electric Range (Louisville, KY) that had been preheated to 400°F for 17 minutes.

4.5 Physical Tests

4.5 A. Moisture Content

Samples were cooled to room temperature for one hour . The crumb of the muffin was torn into small pieces approximately 1/4" x 1/4", placed in a Teflon-lined metal pan with a fork and weighed to 10 grams with a top loading balance (Fisher Scientific XL-500 Top Load Balance #13028824, Denver Instrument Company, Arvada, CO).

A Brabender Moisture Tester SASS 692 (C.W. Brabender Instruments, Inc., South Hackensack, New Jersey) was switched on one hour prior to testing and set to 130°C. Once heated the sample pans were placed into the Brabender and the door latched (Appendix D). The samples were dried for one hour. After drying, each sample was reweighed. The difference between pre and post weights was divided by the original weight (before drying) and multiplied by 100 to determine the percentage of moisture in the product, such that:

$$\% \text{ Moisture} = \frac{\text{weight of sample before heating} - \text{weight of sample after heating}}{\text{weight of sample before heating}} \times 100$$

4.5 B. Water Activity

Water activity is a measure of the amount of water in a product that is available to participate in reactions that cause staling and microbial growth. Therefore, the water activity test is an accurate measure of shelf life of a food product. Samples were cooled to room temperature for 1 hour. The muffin was cut in half and enough crumb was removed from the center of the muffin to fill a water activity cup half way.

The water activity instrument (Decagon CX-2, Aqua Lab, Pullman, WA) was turned on 10 minutes before use. When the LCD display read "0.000", 2 salt solutions of known water activity were inserted and used to calibrate the machine. Once calibrated, prepared samples were placed into the cup holder and the knob on the machine turned to "read". When the LCD display blinked, the analysis was completed and the value recorded (Appendix E).

4.5 C. Colorimetry (crust and crumb)

A Hunter Lab Optical Sensor 45/0 D25-PC2 (HunterLab, Reston, VA) was used to evaluate the change in crust and crumb quality due to the addition of honey. A sample was cooled to room temperature for 1 hour, and cut horizontally such that the dome of the muffin was completely removed.

The machine was switched to "operate" and then calibrated according to the directions in Appendix F, in order to attain L, a, b and Delta E values. The L value distinguishes varying levels of whiteness (perfect white = 100) and blackness (perfect

blackness = 0). The “a” value measures redness when plus, gray when zero, and greenness when minus. The “b” value measures yellowness when plus, gray when zero, and blueness when minus. The delta E (ΔE) value gave the total color difference.

The sample was placed in the Colorimeter such that either crumb or crust was analyzed. Results were printed on a Citizen 120D Dot Matrix Printer #1295120 (Citizen Watch Co, Ltd).

4.5 D. Differential Scanning Calorimeter

A Differential Scanning Calorimeter (Perkin Elmer DSC7 #5021501, Norwalk, CT) that is connected to a Thermal Analysis Controller (Perkin Elmer DSC7/DX, Norwalk, CT) was used to measure the degree of staling after 24 and 48 hours of storage and then after 14 and 28 days in a stand-up freezer. Samples were stored in Rubbermaid Plastic Containers (Wooster, OH) and removed just prior to testing (Appendix G).

A sample was cut down the center vertically while still in the muffin cup. A small piece of crumb was torn from the center using a clean metal fork and pressed together (while wearing rubber gloves) to form a ball. This was done to make the sample easier to place inside the weighing dish. The small ball was placed inside the weighing dish and measured to an approximate weight of 30 milligrams (mg). The sample was pressed down into the dish so that it was evenly distributed. The exact weight of the sample was recorded and the lid, with rubber gasket, placed on the dish. The dish and lid were pressed together to ensure a tight fit.

The sample and reference were placed on a metal heat sink. The difference of heat flowing from the heat sink into the sample and reference substance (indium) was proportional to the temperature difference of the sample and the reference, and therefore heat flow was indirectly obtained by measuring the temperature difference. In the present investigation, and endothermic response ("hump") was noticed and the height of that "hump" recorded (Delta H), such that; the higher the degree of staling the higher the "hump".

4.5 E. Crumb Firmness

Crumb firmness was measured with an Instron Corporation Model 1011 #11281G (Canton, MA). A muffin was placed underneath the probe. Firmness was then analyzed by measuring the force required for the probe to penetrate 10 mm into the product. For the purposes of this study, crumb firmness was evaluated by measuring the peak force required in compressing the muffin to a depth of 10 mm (Appendix H).

A sample was cooled for one hour prior to testing, and cut horizontally so as to remove the dome of the muffin. The paper muffin cup was removed and the bottom of the muffin placed under the probe. A muffin (63 mm in diameter and cylindrical geometry) was placed underneath the probe. The probe was mechanically brought to within 1 mm of the muffin before the test was to occur. The probe moved in a downward direction at a speed of 100 mm/min. The Instron was set to read compression forces at a rate of 10 points/second until either a 45 kg force or 100 mm of penetration had been reached. At this point, a graph of the force measured over the compression period was produced and

printed. The probe was brought back to the starting position, and the next sample measured.

4.5 F. Volume

Two muffins that had been cooled for 1 hour were randomly sampled from each treatment. A dial caliper (Switzerland) was used to measure the height (at the highest point) of the muffin, followed by the diameter of the muffin. Once these measurements had been ascertained, they were incorporated into the following formula:

$$\text{Volume} = (22/7)r^2h$$

In this formula, r is equal to the radius or one half of the diameter of the muffin and h is equal to the standing height of the muffin (Appendix I).

4.6 Sensory Evaluation

Sensory evaluation is a scientific discipline used to evoke, measure, analyze , and interpret reactions to those characteristics of foods and materials as they are perceived by the senses of sight, smell, taste, touch, and hearing. Therefore, sensory evaluation is a way that food scientists use the human body (specifically the 5 senses) as a tool to measure differences in characteristics of food.

4.6 A. Quantitative Descriptive Analysis (QDA)

Quantitative Descriptive Analysis (QDA) is the most sophisticated sensory methodology. The results of QDA are a complete sensory description of the test

treatments (determined by the sensory panel), that provide a basis for relating specific ingredients to specific changes in sensory characteristics of a product. QDA was chosen as the analysis tool for the study since it yields quantitative data that is used for interpretation.

4.6 B. Training

Prior to selection and training of the testing panel, approval was granted on the use of human subjects in the study (Appendix J). Ten people voluntarily (Appendix K) comprised the sensory panel. Each member of the sensory panel underwent 3 two-hour training sessions. At these sessions: 1. panel members verbalized their perceptions of a fat free muffin; 2. descriptive terminology was developed that best identified the observed perception; 3. scales for recording intensities, as well as word/product anchors for each scale category were determined (Appendix L).

Once the panelists had agreed on the meaning of the terms they had chosen to describe the muffin, then a period of training followed. After training, the judges were able to quantify the degree to which the term was perceived in the product they were testing.

4.6 C. Testing Procedure

To be quantitative, a line scale was developed (Appendix M). The scale was a 6 inch horizontal line with word anchors (determined by the sensory panel), always moving from left to right with increasing intensity. The task of the panelist was to mark a vertical line across the horizontal line at the point that best reflects the relative intensity of the

particular term. The mark is converted to a numerical value by measuring the distance from the left end of the line.

4.7 Statistical Analysis

Multiple comparison analysis of variance (ANOVA) was utilized to test for overall differences in all responses that were measured in the present investigation (i.e., moisture, firmness, DSC, color, volume, water activity, and sensory). This test also attempts to explain where significance or insignificance of the model occurs by determining p-values for the: block effect, the treatment effect, the time effect (where applicable), as well as an interaction between treatment and time.

Paired Wise Difference testing was utilized to detect differences between all treatments within all parameters. The significance level was corrected to control for comparison wise error. For example, 10 comparisons were typically made within a given parameter. Therefore, the significance level was corrected by taking 0.05 and dividing it by 10 to get a corrected significance level of 0.005.

The Paired Wise Differences have been designated on the tables that contain the LS Means. In every column designating the LS Means for a given parameter, an “a” has been assigned for all Means that were not significantly different from the full fat control. An “f” has been assigned for all Means that were not significantly different from the fat reduced control. An example of this notation is as follows:

Full cntrl	197 a,g
Red. cntrl	235 b,f
25%	180 b,h
35%	230 b,f
45%	200 a,g

In this example, following the LS Means, are the letter designations that represent the results of the Pair Wise Difference Testing. The first column of letters demonstrates that the 45% honey replacement formula is not significantly different from the full fat control (both are designated with the letter "a"). The first column also demonstrates that the fat reduced control, the 25% and 35% honey replacements are different from the full fat control, but not significantly different from one another (all three designated with the same letter "b"). The second column of letters demonstrates that the 35% honey replacement is not significantly different from the fat reduced control (both designated with the letter "f"). This second column also demonstrates that the full fat control, the 25% and the 45% replacements are all significantly different from the fat reduced control. However, the letter "h" in the second column also suggests that not only is the 25% significantly different from the fat reduced control, but is also significantly different from the full fat control and the 45% honey replacement.

Finally, linear and quadratic trend analyses were performed on the 25%, 35%, and 45% experimental treatments for moisture and firmness (both responses measured over time). Regression was used in place of linear and quadratic trend analysis on DSC measurements because the spacing of the time parameter was not even. The Statistical Analysis System (SAS Institute Incorporated, SAS Circle, Box 8000, Cary, NC) was used

to perform calculations, with an alpha value of $p<0.05$ recognized as significant.