THE INTERACTIVE EFFECTS OF SELECTED EMULSIFIERS, ENZYMES, AND A CARBOHYDRATE BASED FAT SUBSTITUTE IN A LOW FAT MUFFIN

by

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(ABSTRACT)

Selected emulsifiers, enzymes, and a fat substitute were incorporated into a standard muffin recipe, and their effects were compared to a full fat counterpart. Physical and sensory data were reported at the 0.01 significance level.

Physical tests indicated no significant differences ($p>0.01$) among crust "L" and "b" values, specific gravity, water activity, and staling rate after 48 hours storage. The control muffin had a significantly ($p<0.01$) more yellow crumb, was significantly ($p<0.01$) less firm, and contained less moisture ($p<0.01$). The versions containing SSL and DATEM were less firm ($p<0.01$) and retained slightly more moisture ($p<0.01$) than the other reduced fat muffins. The control muffins and the versions containing SSL and DATEM were significantly ($p<0.01$) greater in volume than the other reduced fat muffins. Fat and DATEM were found to prolong retrogradation at a significantly ($p<0.01$) slower rate than in the other versions after 24 hours storage.

Sensory results indicated no significant differences ($p>0.01$) in perceived adhesiveness. The muffins containing only the fat substitute and enzymes were rated with a significantly ($p<0.01$) darker crust color and ($p<0.01$) aftertaste than the other versions. The control muffin was significantly ($p<0.01$) moister, and contained a larger crumb

($p<0.01$) than the other versions. The control muffin was significantly ($p<0.01$) less cohesive. The results obtained from the data indicated that there were similarities and differences between the reduced fat versions and the control.

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Chapter 1

Introduction

According to numerous health professionals, the average fat intake of many Americans continues to exceed what is considered to be a healthy amount. While current nutritional guidelines advise reducing fat consumption to 30% or less of total daily energy intake, the typical American diet consists of approximately 34% (Sanchez et al., 1995). It has been stressed that high levels of saturated fat have been associated with increased risks of coronary heart disease, atherosclerosis, and obesity. Fat continues to be the leading nutritional concern among a majority of people (Busetti, 1995), and there has been a notable change in the dietary habits of consumers over the past several years. Although fats and oils, meat, poultry and fish, and dairy products remain the primary sources of fat in the American diet (Anon., 1991), different selections and proportions of such items are being made with respect to the consumer. For instance, the use of lard and butter has given way to vegetable fats in the forms of margarines and salad and cooking oils. Beef consumption has decreased, while levels of poultry have continued to increase. Even low-fat milk has surpassed whole milk (Anon., 1991).

A national survey sponsored by the American Dietetic Association (ADA) and the International Food Information Council indicates that more than half of Americans derive less pleasure from eating at times due to their deep concern with fat and cholesterol (Anon., 1991). Despite the fact that many Americans are reducing their level of fat intake, this modification may be quite arduous for some individuals. A majority of people like the

taste of fat-containing foods and are only willing to give them up to a certain extent (Drewnowski, 1992). Fats not only provide a concentrated source of energy to the diet, but also contribute essential fatty acids and fat soluble vitamins (A,D,E, and K). While this is of utmost importance, it is the sensory properties of fats that contribute to consumer reluctance. Fats play a major role in determining the palatability and variety of the diet. They impart differences in texture, mouthfeel, and flavor in many foods (Drewnowski, 1992). Given all the functions that fat performs, developing one ingredient that can replace the fat is quite difficult. Food manufacturers have been given the task to develop new foods that will produce the organoleptic characteristics similar to fat in foods without sacrificing quality.

Chapter 2

Review of Literature

2.1 Functionality of Fat

Lipids, more commonly known as fat, are found naturally in a variety of foods. Derived from several sources, ranging from animals to plants, fats exhibit a wide range of functional properties and play prominent roles in food preparation. Their composition, crystalline structure, melting and solidifying behavior, and association with water and other nonlipid molecules are especially important with regard to the various textural properties they impart and their functionality in bakery and confectionery products (Nawar, 1985). With respect to baked products, fat contributes characteristics such as flavor, richness, viscosity, aeration, and tenderness (Drewnowski, 1992; Bennion, 1990b; Cauvain, 1987).

Lipids are classified as triglycerides, fatty acids, phospholipids, and sterols. Triglycerides comprise the major part of fat found naturally in foods, as well as the more purified fats, and are of utmost importance with respect to food preparation (Bennion, 1990a). Consisting of one molecule of glycerol and three fatty acids, many variations can exist. Two interrelated factors affect the ability of fats to produce the desired characteristics in food products: (1) their molecular composition and (2) their crystal habits (size, shape, and form). The many forms of fat, due to differences in molecular structure, affect the crystalline properties of various fats. In turn, the crystalline characteristics determine how the fat will perform in various applications (Anon, 1994b). The crystalline form of a fat is the solid state in which the molecules arrange themselves in a repeatable, highly ordered, three dimensional

pattern (Nawar, 1985). Most fats normally exhibit three crystalline forms: alpha (α), beta-prime (β'), and beta (β). The α-form has a low melting point because the molecules are aligned randomly. There is not a high degree of order. Crystals formed by the α-form are small and smooth, yet a high proportion of them are unstable (Penfield and Campbell, 1990c). Beta-prime crystals have a melting point higher than those crystals of the α-form and are more stable. These crystals are also small. Beta stable glycerides exhibit the highest melting point of all three forms due to the highly ordered lattice that is formed. Beta crystals are larger than the α or β' form. Each crystal form has its own physical properties such as plasticity, hardness, softness, texture, and mouthfeel. It is the properties of the crystalline forms that can have profound effects on the product into which a fat is formulated. For instance, small crystals enhance aeration properties in fats. Therefore, the α-form is desired in fats used in cake batters, whipped toppings, and other products where aeration is important. Small crystals make high volume, fine-grained cakes, whereas larger crystals make flaky pie crusts (Anon., 1994b).

In baked products, one of the most important functions of fat is its ability to tenderize. Fat is capable of interfering with gluten development during mixing. Gluten is predominantly responsible for the unique bread making properties in wheat flour upon hydration (Pyler, 1983). Fat is insoluble in water, and therefore is absorbed on the surface of the gluten proteins, thus interfering with the full hydration capacity of the gluten. As a result, the fat prevents such a cohesive gluten structure from developing, a trait essential in many baked goods (Bennion, 1990b; Penfield and Campbell, 1990c).

Fat also contributes to the incorporation and retention of air in the form of small bubbles throughout a batter. Serving as gas cell nuclei into which carbon dioxide and steam diffuse through during baking, these bubbles have an affect on the volume of a product. With respect to batter viscosity, air finely dispersed by fat enhances the batter viscosity. Increased viscosity, up to a certain point, increases gas retention (Penfield and Campbell, 1990c).

Fat plays different roles in various foods and enables such foods to be flavorful and rich. Due to health concerns, however, the consumption of fat containing products has decreased. The Calorie Control Council (CCC) reported that four out of five Americans (152 million) consumed low-calorie, sugar-free, and/or fat-reduced foods and beverages in 1992. Improving overall health was their main objective, with half of these people choosing these foods for everyday consumption and not just some special diet. The CCC also reported that 519 new low fat/low-cholesterol foods were introduced in 1992, a 39% gain over 1991 (Alexander, 1995). The consumption of low fat foods is definitely on the rise. By developing alternative ingredients such as fat replacements that can produce the organoleptic characteristics similar to real fat, reducing fat intake can be achieved. Fat substitutes can enable people to reduce the amount of high fat foods consumed while preserving basic food selection patterns. Good tasting foods containing fat replacements may promote the gradual acquisition of a preference for lower-fat foods (Anon., 1991).

2.2 Fat Substitutes - Definition

The term *fat substitute* implies that a substance, when used as a replacement for the traditional fat contained in a food, has certain desirable physical or organoleptic properties of

the fat that it replaces while lacking undesirable properties of this fat (Vanderveen and Glinnsman, 1992). Although many consumers tend to consider fat substitutes as comprising a single entity, they clearly represent a wide range of chemical sources with a diverse array of sensory and functional properties (Hassel, 1993). Ingredients that serve as a partial or complete replacement of fat fall into three major categories: lipid based, protein based, and carbohydrate based (Hassel, 1993; Anon., 1991).

2.2A. Lipid Based Fat Substitutes

Lipid based fat substitutes comprise lipids that possess functional and sensory properties similar to those fats they are intended to replace. Ironically, the use of some of these compounds is under considerable scrutiny due to concerns regarding toxicity and metabolism at macronutrient levels. *Caprenin*, which is a Generally Recognized as Safe (GRAS) food product, is an example of a lipid based fat substitute. It is a reduced calorie triglyceride formed by the esterification of glycerol with three naturally occurring fatty acids: caprylic, capric, and behenic. While other dietary fats contain 9 kilocalories per gram, *caprenin* supplies 5 kilocalories per gram. This fat substitute, which shares similar functions to those fats found in cocoa butter, can replace some of the cocoa butter in soft candy and confectionery coatings. *Caprenin* is digested, absorbed, and metabolized by the same pathways in the body as other triglycerides (Anon., 1991). *Olestra* is another example of a lipid based fat substitute that is more common to the public. It has been researched extensively. Although this compound is produced by the esterification of sucrose with six to eight long chain fatty acids from edible oils, it is not perceived as being sweet. *Olestra* is similar to fat in appearance, taste, texture,

and function in foods. It can also be used in frying, cooking, and baking (Anon., 1991). Lipid based fat substitutes are resistant to digestive lipases, thus the compounds are not available for absorption. This is how they acquire their reduced calorie functionality. As a result though, the amount of lipid based fat substitute consumed is excreted, providing a hydrophobic environment. This can possibly reduce the absorption of vital fat-soluble nutrients (Hassel, 1993).

2.2B. Protein Based Fat Substitutes

Protein based fat substitutes are derived from proteins found in eggs, milk, and other foods. *Simplesse* is a popular example, which received GRAS status in 1988. This compound is produced from milk and/or egg protein by a patented process called microparticulation. It is the blending or shearing technique that shapes the protein gel into spheroidal particles to the extent that they are perceived as a rich, creamy fluid similar to fat rather than the rough, large particles normally associated with coagulated proteins (Hassel, 1993).

2.2C. Carbohydrate Based Fat Substitutes

Carbohydrate based fat substitutes have been incorporated into many products for quite some time. There are approximately 40 different starch based products that have been recommended for use as fat substitutes, with most of them being introduced in the early 1990's. Dextrins, modified food starches, polydextrose, and gums are just a few examples. There are several reasons for using starches. They are heat stable, therefore they can be used in baking. When starch is gelatinized, it acquires a smooth and creamy consistency. In addition, an

"elastic-like" gel structure is formed, which results in lubricant or flow properties similar to those of fats in some food systems (Hassel, 1993). These features make starch based formulations ideal candidates for fat substitutes. 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 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).

2.2C1. Carbohydrate Based Fat Substitutes: METHOCEL® Food Gums

An example of a carbohydrate based fat replacer is METHOCEL® food gums. Produced by Dow Chemical, these gums are derived from cellulose, the most abundant polymer in nature. Two different forms are available: methylcellulose (MC) and hydroxypropyl methylcellulose (HPMC). Although both have a polymeric backbone of cellulose, each form has different substitutions, which has a profound effect on the properties of the different products. While methylcellulose contains methoxyl groups, hydroxypropyl methylcellulose has different ratios of hydroxypropyl to methoxyl substitution (Anon., 1993).

Both METHOCEL® products are accepted forms of food additives by the USDA and FDA. Methylcellulose has also received GRAS status from the FDA. These products have approved use in a variety of baked goods such as cakes, muffins, and yeast products. In reduced-fat baked goods, as in higher fat baked products, METHOCEL® gums have

contributed to improved baked structure, texture, and appearance (Busetti, 1995; Anon., 1993).

Improved structure of baked goods can be attributed to these gums, which act as surface active polymers that entrap and stabilize air or carbon dioxide bubbles in foods. They can retain moisture in baked products since they are hydrophilic polymers, and help prevent phase separation even under freeze/thaw conditions. This is very important with respect to the keeping quality (i.e., shelf life) of the product.

METHOCEL® food gums are non-ionic, therefore they will not complex with minerals or other components in food systems. The flavor of baked goods with one of these products is not adversely affected, for these gums are virtually tasteless and odorless. Not only can they replace fat in baked goods, but they do not contribute any calories either because METHOCEL® gums are indigestible (Anon., 1993). This is another reason to use this product as opposed to other fat substitutes, which can add even more calories to a product. Many consumers have begun to realize that just because a product is lower in fat does not necessarily mean that it is lower in calories. The amount of fat *and* calories are two critical components in baked products, and *both* have to be taken into consideration when reformulating baked goods. Having a product that is fat-free, yet containing the same amount of calories as the full-fat version is a gimmick to the general consumer (Busetti, 1995).

2.3 Enzymes

There are several enzymes that are present in flour, with the most important being the carbohydrases, mainly α- and β-amylases. Other flour enzymes include proteolytic enzymes,

oxidative enzymes, lipase, and phytase (Penfield and Campbell, 1990d). Proteases cleave the peptide bonds in proteins, thus breaking them down into amino acids. As a result, proteolysis brings about significant alterations in both the chemical and physical properties in proteins (Drapron and Godon, 1987). In flour, proteases act on gluten, thus increasing the machinability of the dough and the overall bread quality. Other enzymes such as lipoxidase are important because they contribute to flour bleaching, while phytase hydrolyzes phytic acid, preventing its tying up of divalent metallic ions (Penfield and Campbell, 1990d).

2.3A. Amylases

Amylases are found in different cereals, fungi, bacteria, and mammals. Cereal α-amylases possess properties similar to fungal or bacterial sources, however they are quite different. These dissimilarities have a profound effect in baking. For instance, cereal α-amylases are more heat stable than fungal α-amylases, yet they are less heat stable than bacterial α-amylases. Thus, whereas fungal or cereal amylases may be used as a supplement to enhance and sustain gas production, use of bacterial α-amylase can lead to excess dextrinization during the baking process, because of its high thermal stability. As a result, the bread quality will be negatively affected (Drapron and Godon, 1987).

Cereal α-amylases are endoenzymes that cleave the α- (1→ 4) D-glucosidic linkages in native, undamaged starch components (Drapron and Godon, 1987). The amount of this enzyme present varies depending on the growth stage of the cereal; whether it is immature, resting, or germinated. In wheat, α-amylase can be identified shortly after flowering and will

continue to increase until late-maturity. With further maturation and desiccation, the concentration of α-amylase decreases.

Cereal β-amylases, which are exoenzymes, alternate α-(1→ 4) D-glucosidic linkages in starch components in a stepwise fashion from the non-reducing end, resulting in the production of β-maltose (Drapron and Godon, 1987). When the enzyme comes in contact with an α-(1→ 6) region, activity ceases. The amount of β-amylase contained in the endosperm increases throughout wheat kernel development. Upon germination of wheat, the level of β-amylase has proliferated greatly. There are several forms of this enzyme as a result of genetic variation, and due to the fact that multiple forms may associate by disulfide bonds (Drapron and Godon, 1987; Schuster and Adams, 1984). β-amylase attaches only to damaged starch granules and is present is all wheat.

Both α- and β- amylases play a significant role in the overall effect on the outcome and quality of different products. In wheat flour, for instance, a certain amount of both forms of these enzymes are necessary to produce an adequate amount of fermentable sugars needed for gas production in bread making. However, if the amount of enzymes are produced in excess, they have a deleterious effect on bread, yielding a sticky loaf of bread. On the other hand, abundant amounts of carbohydrates are required in the brewing industry in order to ensure the maximum production of fermentable sugars (Drapron and Godon, 1987; Minor, 1984).

With respect to bread making, amylolytic activity begins as soon as it comes in contact with liquid. At this stage of dough making, the temperature is quite low and therefore the rate of enzyme activity is not affected to a great degree. Some of the liquid is associated with the

starch, but the liquid is also distributed among insoluble proteins, pentosans, and yeast (Drapron and Godon, 1987). The α-amylase, although at a low rate, can hydrolyze some of the undamaged starch granules. However, both α- and β-amylase can attack the damaged granules. More granules are damaged in hard wheat flours as opposed to soft wheat flours because more pressure is required to fracture the hard endosperm. Therefore, hard wheat flours used in bread making are more susceptible to amylase attack during dough making. Amylase activity also leads to dextrins, which have an effect on water-holding ability and porosity of the dough as well as on bread softness. An excessive dextrin production, from flour originating from germinated wheat, can result in a sticky dough (Drapron and Godon, 1987).

During the baking process, amylolytic activity continues until the enzymes are thermally deactivated. Both forms of amylase have a synergistic effect on amylolysis, although the rate of hydrolysis depends on the ratio of enzymes. If there is a significant proportion of α- and β-amylase, not all of the oligosaccharides produced are consumed for carbon dioxide production. As a result, some of those enzymes, located within the crumb, contribute to its taste. Enzymes on the surface of the bread participate in Maillard browning reactions, thereby producing color and flavor in the crust. Amylases are incorporated into a flour mixture when amylolysis is deficient. Although sound wheat flours usually contain an adequate level of β-amylase, the level of α-amylase is not sufficient though to produce a highly acceptable bread. As a result, exogenous amylases derived from fungal and bacterial sources, are being used to

supplement flour and improve bread quality (Drapron and Godon, 1987; Schuster and Adams, 1984).

2.3B. Effect of Amylases on Staling

Exogenous amylases, when added to a dough mixture, can delay the onset of retrogradation in baked goods. This occurs when the amylopectin molecules, one of the main components of starch, begin to tightly align themselves. Water is thus forced out, and the product becomes more firm. Prolonging the shelf life of bread and other baked products is very important, especially since a large amount of such goods get thrown away each year as a result of staling. In 1988, it had been estimated that up to 8% of all fresh baked goods were returned as stale and unsalable (Knightly, 1988). Bacterial α-amylase, a heat stable enzyme, has the ability to dextrinize a starch fraction after baking. This amylase breaks down some amorphous sites located between the crystalline areas of the gelatinized starch, separating them from each other and thereby reducing the rigidity of the starch network (Drapron and Godon, 1987). The incorporation of this enzyme can help maintain moisture, improve crumb softness, and reduce crumbling.

2.3C. Research on Fungal and Bacterial Enzymes

Fungal α-amylase can also delay the staling of bread and other leavened cereal products. In 1992, Cole (Drapron and Godon, 1987) discovered that when α-amylase was distributed throughout a concentrated sugar solution, the amylase could delay the onset of

staling because the medium would protect the enzyme against thermal denaturation. In one study by Valjakka et. al. (1994), the effects of raw-starch digesting enzyme (RSDE) on bread firming properties were compared to those of 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, fungal, and a fungal RSDE were employed. The bacterial enzyme inhibited the firming rate of 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 the enzymes did decrease the rate of staling, although each form affected the 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 (α-amylase supplemented and unsupplemented) that had been aged for five days. The relationship between the soluble dextrins and the rate of bread firming was the primary objective of the study. Seven α-amylases were used. Two fungal α-amylases were cultured from *A. oryzae*, and four bacterial α-amylases (*Bacillus* strain) were used in various amounts. One enzyme was a cereal malt amylase. Two of the fungal amylases and one bacterial α-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 and α-amylase treated bread 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 reduced the staling rate differed. However, the dextrins that were produced by the amylases are important in controlling the rate of bread firming.

2.4 Emulsions

An emulsion is a system consisting of two immiscible substances, one finely dispersed into the other. There are two phases that are present in an emulsion: the dispersed phase and the continuous phase. The dispersed phase constitutes the substance which is distributed as droplets ranging from 0.1 to 50 μm (Nawar, 1985). The continuous phase is the medium in which the dispersed phase is suspended. When two immiscible substances are mixed together, the interfacial tension between them becomes incredibly large. Due to the large positive free energy at the interface, emulsions, as a result, are thermodynamically unstable (Nawar, 1985). The stability of an emulsion is therefore dependent upon the extent of mixing it has been subjected to and the presence of emulsifiers (Anon., 1995).

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 exist (Anon., 1995). A W/O emulsion is an emulsion in which the water phase is dispersed into the oil phase. Examples of W/O emulsions are butter, margarine, and icings. In an O/W emulsion, oil is dispersed into a

water phase. Mayonnaise, salad dressings, and cake batters are forms of O/W emulsions (Anon., 1995).

Many emulsions occur naturally in foods such as milk, cream, and egg yolk. The fat in these foods are in the form of droplets throughout the watery portion. Emulsions are also formed during food processing and preparation. A large input of energy is required in the manufacturing of emulsions. Work is necessary to divide the fat into tiny droplets, therefore it is critical that the fat globules, and thus the emulsion, are protected against destabilization (Bennion, 1990a).

Due to the increase in tension that occurs between two immiscible substances when they are mixed together, they are highly unstable. Emulsions tend to destabilize by 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 is the most serious form of destabilization. This occurs when the droplets aggregate and rupture the interfacial film, thus breaking the emulsion (Nawar, 1985).

2.5 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. They not only stabilize emulsions, but foams and

suspensions as well. They have a substantial effect on texture, serving as texture modifiers which interact with polymers such as starch and protein. Emulsifiers can also modify the crystallization of lipids (Anon., 1994a; Dziezak, 1988; Shepard and Yoell, 1976).

Emulsifiers are substances that have the ability to lower the interfacial tension that exists between two normally immiscible phases, allowing them to mix and form an emulsion (Stampfli and Nersten, 1995; Anon., 1994a; Dziezak, 1988). Emulsifiers can exert such behavior because of their molecular structure. Characterized as being "amphiphilic" molecules, emulsifiers possess both hydrophilic and lipophilic moieties. The presence of these two moieties enable emulsifiers to perform such functions. The hydrophilic, or "water-loving" portion of the molecule, consists of various polar groups. A polar group is a functional group containing either a hydroxyl and/or a carboxyl group. The electron distribution that exists among this group causes the molecule to have a considerable dipole moment. This functional group brings about the affinity to other polar liquids, such as water, and thus the hydophilic character of the compound. The lipophilic, or "fat-loving", portion of the molecule consists of hydrocarbons that may be branched, straight-chained, or cyclic. Since no electronegative atoms are part of this portion, the electron distribution present does not contribute to the dipole moment. The nonpolar character of the molecule attracts other nonpolar liquids, especially organic solvents having low polarity such as oil (Dziezak, 1988; Schuster and Adams, 1984; Del Vecchio, 1975).

2.5A. Examples of Emulsifiers

Hundreds of emulsifiers are available in the food industry today. However, there are several forms of emulsifiers that are commonly employed in foods. The most common types of food emulsifiers are:

(1) Mono- and diglycerides (MDG)
  (a) Glycerol esters
  (b) Distilled glycerol esters

(2) Propylene glycol esters

(3) Sorbitan esters

(4) Polyoxyethylene sorbitan esters

(5) Polyglycerol esters

(6) Ethoxylated esters
  (a) Ethoxylated monoglycerides
  (b) Ethoxylated fatty acids

(7) Lactated esters
  (a) Lactylate esters
  (b) Lactic acid derivatives

(8) Lecithin and lecithin derivatives

(9) Miscellaneous esters (Nash and Brickman, 1972)

2.5B. Applications of Mono-and Diglycerides (MDG's), Diacetyl Tartaric Acid Esters of Monoglycerides (DATEM), and Sodium Stearoyl-2-Lactylate (SSL)

2.5B1. MDG's

Mono-and diglycerides and their derivatives are used in a variety of food products including baked goods, dairy products, frostings, and frozen desserts. In baked goods these emulsifiers function as dough conditioners and/or crumb softeners in bread and rolls, and aerating agents in cake mixes (Henry, 1995; Krog, 1981).

2.5B2. DATEM

Diacetyl tartaric acid esters of monoglycerides is used in such applications as bakery products, extruded products (cereals, pastas), icings, and margarines. This emulsifier is an effective dough conditioner, as well as a crumb softener (Henry, 1995; Dziezak, 1988).

2.5B3. SSL

Sodium stearoyl-2-lactylate, the most hydophilic emulsifier in the food industry, is used principally as a dough conditioner in a number of baked products. It is also an effective crumb softener (Dziezak, 1988).

2.5C. 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 in their synthesis. Generally speaking, some properties of emulsifiers include: 1.) Emulsification, 2.) Lubrication, 3.) Starch Complexing, 4.) Protein Complexing, 5.) Aeration, 6.) Defoaming, 7.) 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).

Many baked products are high in fat. In such goods, the fat serves in aerating the mixture, starch complexing, tenderizing, and flavor. Emulsifiers can reduce, or replace the fat in baked goods because they can produce these effects (Anon., 1992). Any given emulsifier might perform one, or a number of these traits, or they may be combined (Krog, 1981).

2.5D. Classification of Emulsifiers

Emulsifiers can be classified according to various characteristics such as origin, either natural or synthetic; the presence of functional groups; and the phase inversion temperature (PIT) system. The ionization potential and the hydrophilic/lipophilic balance (HLB) are two other means of classification (Stampfli and Nersten, 1995; Dziezak, 1988; Nawar, 1985).

Some emulsifiers can be found naturally in foods. Lecithin, or more specifically phospholipids, can be obtained from soybeans and eggs. Synthetic emulsifiers are esters derived from a polyol and a fatty acid or fat. The most commonly used polyols are

glycerine, propylene glycol, and sorbitol. The fats and fatty acids are obtained from various animal and vegetable sources (Anon., 1994a).

The presence of functional groups is also important with respect to different emulsifiers. Substituting such groups on otherwise similar molecules can yield quite different properties in a product. For example, the substitution of glycerol monooleate, an ester with an unsaturated fatty acid, for glycerol monostearate, an ester with a saturated fatty acid, can exert different effects. Since both compounds have different melting points and other properties, they exert different effects such as gloss retention and bloom inhibition (Dziezak, 1988).

The PIT system, designed in 1964, classifies emulsifiers by taking into account the emulsion conditions. Although this method is not used extensively in the food industry, it is useful in relating the complex, temperature-dependent phase behavior of certain emulsifiers, such as nonionic emulsifiers in water. Nonionic emulsifiers become preferentially oil soluble, as opposed to water soluble, as the temperature increases over a small range (Dziezak, 1988).

In practice, the most widely used methods of classification are the ionization potential and the HLB (Stampfli and Nersten, 1995; Anon., 1994a). The ionization potential is based on the electrochemical charge of the emulsifiers in aqueous systems following its disassociation into ions. Emulsifiers can be classified then as being either nonionic, anionic, cationic, or amphoteric. Nonionic emulsifiers do not dissociate in water because their atoms are tied up in strong covalent bonds. They are relatively insensitive to the effects of pH and salt content. Monoglycerides (MG), ethoxylated monoglycerides

(EMG), sorbitan esters (SE), and polysorbates (PS) are a few examples. Anionic emulsifiers possess a negative electrical charge and include DATEM, SSL, calcium stearoyl-2-lactylate (CSL),and succinylated monoglycerides (SMG). Cationic emulsifiers are positively charged. These are not used in foods, but consist of long chain primary, secondary, and tertiary amines, which are water soluble at all pH values (Dziezak, 1988). Amphoteric compounds possess both positive and negative charges. They are pH dependent. Lecithin is an example of an amphoteric molecule. Most commercially available food emulsifiers fall into the nonionic category (Anon., 1995).

The HLB system provides an index of the solubility of an emulsifier in oil or water systems, and whether it is a W/O or an O/W emulsion (Dziezak, 1988). This is based on the relative percentage of hydrophilic to lipophilic groups within the emulsifier molecule (Stampfli and Nersten, 1995; Shepard and Yoell, 1976). When the system was implemented in 1949, emulsifiers were assigned a number from 1 to 20, the HLB scale. Those with hydrophilic characteristics had higher numbers, whereas lipophilic emulsifiers were given lower numbers. The turning point between lipophilic and hydrophilic properties in the HLB values were purely empirical and were determined from a long series of tests (Schuster and Adams, 1984).

The HLB value of nonionic compounds can be calculated based on the following formula:

$$HLB = 20 * [1 - (S/A)]$$

where S is the saponification number of the ester, and A equals the acid number of the acid (Anon., 1995; Nawar, 1985; Del Vecchio, 1975). The value obtained for nonionic

emulsifiers expresses which fraction of the molecule, based upon molecular weight, extends into or interacts with the aqueous phase (Schuster and Adams, 1984).

Propylene glycol esters, sorbitan tristerate, and glycerol monostearate are a couple examples of emulsifiers with low HLB values. These compounds have a HLB value between 1 and 4, thus they are lipophilic in character. DATEM, tetraglycerol monostearate, and Polysorbate 65, have HLB values between 9 and 11, so these compounds are borderline in hydrophilic/lipophilic character, having approximately the same proportion of both polar and nonpolar groups. Polysorbate 80 and SSL range from 15-22, therefore, these emulsifiers contain more hydrophilic groups (Dziezak, 1988).

This system of classification provides useful information on nonionic emulsifiers when a particular formula consists of water, oil, and an emulsifier exclusively. However, it does not work well for ionic emulsifiers, which comprise a large percentage of emulsifiers used in the baking industry. A charge on the hydrophilic portion of a molecule makes it more hydrophilic, and thus the emulsifier will act like it has a higher HLB than the actual calculated value (Rogers and Hoseney, 1983). This method does not account for the presence of other compounds in a system, such as flour, starch, and sugar, which might affect emulsification. These ingredients, in addition to others, can react and interact with each other. Therefore, the HLB system has limited usefulness in complex foods (Anon., 1995).

2.6 Review of Selected Emulsifiers Used in Foods

2.6A. Mono- and Diglycerides (MDG)

Produced as early as 1853, MDG's are the most commonly used form of emulsifiers used in today's food products. The first U.S. patents for MDG's were granted in 1933 (Birnbaum, 1981). Monoglycerides are derived from the esterification of glycerin with fatty acids or the interesterification (i.e., glycerolysis) of glycerin and fat in the form of triglycerides (Henry, 1995; Birnbaum, 1981). In this process, oils or fats, in the form of triglycerides, are treated with an excess of glycerol at elevated temperatures. This is done in the presence of an alkaline catalyst, usually sodium hydroxide. The reaction continues until the fatty acid radicals of the triglycerides are randomly redistributed among the available hydroxyl groups of the glycerol. Considerable quantities of MG's are formed by this process, in addition to diglycerides (DG's) and triglycerides that have been altered or rearranged to some extent. Free glycerol is also produced (Birnbaum, 1981). Three forms of MG's are manufactured: 40-45% alpha mono content, 50-56% alpha mono content, and 90% alpha mono content (distilled monoglycerides, DMG) (Nash and Brickman, 1987). Monoglycerides comprise approximately 40-60% of the reaction product, whereas DG's account for 35-45% (Henry, 1995).

2.6A1. Molecular Distillation

Concentrated (or distilled) MG's {i.e., distilled MG (DMG), when the MG concentration is 90% or higher} can be acquired by molecular distillation (Henry, 1995). Molecular distillation is a form of evaporative distillation, where a compound in the liquid

state evaporates without boiling. This is because the vacuum essentially removes the effect of the external atmosphere pressure. Under these conditions, MG molecules need only minimal energy to escape from their liquid state to the vapor phase, leaving the heavier di- and triglyceride molecules behind in the distillation residue. This residue is then recycled in order to produce more MDG's (Birnbaum, 1981). The molecular distillation process protects the MG's, which are not heat stable. One advantage of using this process is that the product obtained is the actual portion of MG's that serve as the functional emulsifier. Diglycerides tend to have low emulsification properties, and perform more like a fat than an emulsifier (Henry, 1995). Mono-and diglycerides exert different emulsification properties, and can be compared by examining their HLB values. Distilled monoglycerides have a HLB between 3.5 and 4, whereas DG's products have a HLB value of less than 2 (Henry, 1995). A MG consists of one long-chain fatty acid and two hydroxyl groups, whereas a DG contains two fatty acids and one hydroxyl group. As a result, the DG's are very soluble in oil, due to the presence of the hydrocarbon chains, and only slightly soluble in water. Distilled monoglycerides then, because of their structure, are more effective emulsifiers than DG's by having a higher ratio of water-soluble to oil-soluble structures (Henry, 1995). (Figure 1)

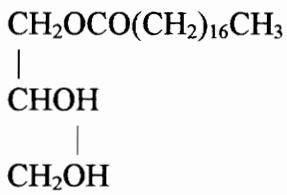

Fig 1: Structural formula of DMG

2.6B. MG Derivatives

The structure of MG's enables them to form several derivatives because one or both of the hydroxyl groups (or their hydrogen groups) can be replaced by different groups, producing esters and ethers (Birnbaum, 1981). Esters vary extensively and are the more prominent form of MDG's. They can form nonionic and ionic (anionic) derivatives. These derivatives can be manufactured by treating the partial glycerides with an acid, acid chloride, acid anhydride, or another ester. Ethers, on the other hand, can be obtained by condensing the partial glycerides with ethylene oxide (Birnbaum, 1981).

Anionic MG derivatives are produced by reacting a MG with the acidic radical portion of a dibasic or polybasic acid (Birnbaum, 1981). Tartaric acid, diacetyl tartaric acid anhydride, succinic acid, and citric acids are examples of polybasic acids that can be used to produce food grade emulsifiers. Each variation serves a different function (Dziezak, 1988).

2.6B1. Synthesis of Diacetyl Tartaric Acid Esters of Monoglycerides (DATEM)

Diacetyl tartaric acid esters of monoglycerides are esters of glycerol with fatty acids and acetylated tartaric acid. They can be synthesized two ways: (1) by reacting MDG's with diacetyl tartaric anhydride in the presence of acetic acid or (2) by esterfying 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). (Figure 2)

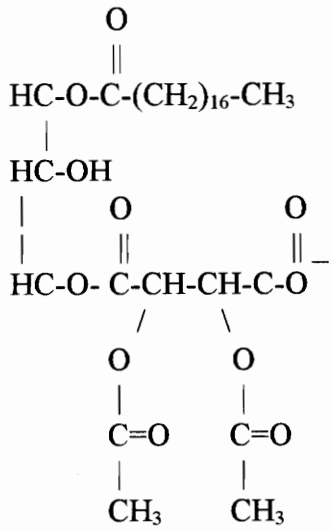

Figure 2: Structural formula of DATEM

Different forms of DATEM exist, therefore the functions and physical properties will differ, depending on the types of fatty acids used and the quantity of esterfied tartaric acid (as compared to esterfied fatty acid) (Shepard and Yoell, 1976).

2.6C. Synthesis of Lactylated Esters

Lactylated esters are other forms of emulsifiers that are commonly used in the food industry. Similar to MG's and DATEM, lactated esters can form an array of derivatives such as lactylate esters and lactic acid derivatives. These variations can be further divided into two classes: nonionic esters and anionic esters (Shepard and Yoell, 1976).

The anionic lactic acid derivatives are characterized as polylactic esters of fatty acids and salts (Shepard and Yoell, 1976). There are two forms of these anionic emulsifiers that are more commonly used in food products, calcium stearoyl- 2- lactylate

(CSL) and SSL. SSL is strongly hydrophilic , therefore it is capable of stabilizing O/W emulsions. SSL can be produced by the reaction of stearic acid, 2 molecules of lactic acid, and sodium hydroxide (Nawar, 1985). (Figure 3)

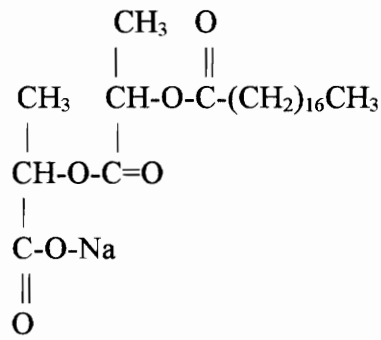

Figure 3: Structural formula of SSL

2.7 Crystal Form and Mesomorphic Behavior of Emulsifiers

Emulsifiers are fat derivatives, therefore they exhibit polymorphism and different crystalline forms (i.e., α- β'-, β-) similar to real fat. In order to understand how emulsifiers function in food, it is important to know such properties. The various configurations affect how the emulsifiers perform in particular products.

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. In 1970, research (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 dispersibility, and enhancing aerating properties (Schuster and Adams, 1984).

Mesomorphism is the ability of substances to form mesophases, or liquid-crystal phases. Lyotropic mesomorphism exists when 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 of the emulsifier does not dictate what type of mesophase is formed (Krog et. al, 1985; Schuster and Adams, 1984; Shepard and Yoell, 1976). 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 consists 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).

2.8 Research on Emulsifiers in Baked Products

2.8A. Emulsifiers and their Roles in Bread Production

Emulsifiers have been used in improving the quality of yeast-raised bakery goods for over 50 years (Krog, 1981). Emulsifiers perform many functions in food, but in bread they serve three main purposes: (1) to strengthen the dough , (2) soften the crumb, and (3) extend the shelf-life, a property critical to other baked products as well (Schuster and Adams, 1984).

Emulsifiers are known as "dough conditioners" or "crumb softeners", while some can be both (Dziezak, 1988; Knightly, 1988; Schuster and Adams, 1984). Emulsifiers that function as dough conditioners interact through hydrophobic and/or hydrophilic binding (electrostatic or hydrogen bonding) with the gluten proteins in dough (Krog, 1981). The association of the two enhances the viscoelasticity of the gluten, producing a dough that is more tolerant to mixing. Apparently, the emulsifier when existing in the lamellar form can establish a liquid film between the gluten strands and the starch. The emulsifier thereby enhances the ability of gluten to form a film which can retain the gas produced by yeast (Stampfli and Nersten, 1995), which positively effects the rate of proof (Knightly, 1988). Not only can dough conditioners improve gas retention, but they can also excel the rate of hydration and water absorption of dough ingredients. After baking, the product with emulsifiers has a higher loaf volume, better crumb texture and cell structure, and greater resistance to staling than a baked product without emulsifiers (Knightly, 1988; Krog, 1977). Emulsifiers used as dough conditioners are usually

anionic compounds such as SSL, DATEM, and succinylated MG (SMG). Nonionic forms such as ethoxylated derivatives of MG and polysorbates can be used as well (Stampfli and Nersten, 1995; Krog, 1977; Newbold, 1976).

Aust and Doerry (1992) demonstrated that when a MG-Lecithin blend was incorporated into dough, the specific volume of the finished bread was greater than in bread without a dough conditioner. Research had shown that bread prepared with DATEM attained a volume that was 45% greater than bread made without emulsifiers (Schuster and Adams, 1984).

A study by Moore and Hoseney (1986) showed that breads made independently with DMG, DATEM, and SSL had better CO_2 retention properties. As a result, the experimental breads had higher volumes than the breads made with shortening alone, although the breads made with DATEM were slightly greater in volume.

2.8B. Effect of Emulsifiers on Crumb Softness

The staling of bread and other baked goods is a complex and gradual process in which undesirable changes occur in the starch and gluten fraction (Flack, 1983), and a "leathery" texture is the result (Knightly, 1988). It is known that the starch and protein fractions, as well as water, play significant roles in this phenomenon (Stampfli and Nersten, 1995).

The starch portion is the principle component responsible for staling, or retrogradation. During baking, starch granules swell and the amylose portion of starch leaches out, while the amylopectin portion becomes dilated. This process essentially begins as soon as the product is removed from the oven. Amylose associates rapidly after baking, and therefore affects only the

initial firmness. Amylopectin, on the other hand, re-associates more slowly because it requires more time for these branched molecules to realign and form hydrogen bonds (Stampfli and Nersten, 1995).

Emulsifiers can function as crumb softeners or "anti-staling agents" (Stampfli and Nersten, 1995; Dziezak, 1988; Newbold, 1976). In the presence of a complexing agent such as emulsifiers, amylose forms a helical structure. This structure can entrap linear substances, such as MG's. The long hydrocarbon chains of MG's stabilize the helix, thus making the complex insoluble in water (Krog, 1981). This emulsifier-complex delays retrogradation. Emulsifiers can also slow down the rate of release of bound moisture from the gelatinized starch as the starch retrogrades (Knightly, 1988). As a result of these two actions, bread can remain softer longer (Stampfli and Nersten, 1995; Krog, 1981). Emulsifiers used for this application include DMG based on fully hydrogenated fats, DATEM, and propylene glycol mono- and diesters of fat-forming fatty acids (Dziezak, 1988).

Rao et. al (1992) quantitatively measured the degree of amylopectin crystallization to bread recoverability as influenced by added emulsifiers. SSL, sucrose esters (SE), and DMG (Dimodan) were used in a straight dough bread formula. Recoverable work, a value that represents any irreversible damage upon compression, was measured. Amylopectin crystallization was analyzed, and specific volume and moisture content were also determined. The data obtained indicated that each individual emulsifier had different effects on the quality of bread in terms of retrogradation, loaf volume, cell structure, and recoverability of bread. However, SSL, DMG, and SE all inhibited the retrogradation process significantly.

Another study by Pisesookbunterng and D'Appolonia (1983) investigated the effect of emulsifiers on moisture migration from the crumb to the crust of bread. SSL (Emplex), Tandem (a soft plastic form of 40% Polysorbate 60 and 60% MDG) were added at 0.5% of the flour weight and compared to a control without emulsifiers. Crumb moisture and firmness values were assessed. A higher percentage of moisture migrated from the crumb to the crust in bread containing the emulsifiers. The firmness values obtained indicated that the emulsifiers did not affect the firmness of fresh bread crumb, but did reduce the firming rate during storage. Among the emulsifiers studied, SSL had the strongest binding ability with the starch molecule.

In a study done by Krog et. al (1989) the effects of MG and DATEM on starch retrogradation in bread were measured by the Instron and DSC. Both emulsifiers reduced the firming rate, although the MG was more effective.

2.8C. Additional Research on Emulsifiers used in Bread Production

An abundant amount of research has been published on the use of emulsifiers, in particular, MG, DATEM, and SSL, in the bread making process. In a study done by Garti et. al (1980), the effects of different emulsifiers in a standard no-fat bread procedure were assessed. Breads made with SSL, calcium stearoyl-2-lactylate (CSL), and isostearoyl-lactylate (ISL), and MG were evaluated on volume, symmetry, general crust characteristics and texture. In addition, farinograph and extensograph studies were carried out. The results obtained indicated that the addition of SSL, CSL, and ISL improved the mixing tolerance of the dough, increased volume by 10-15%, and dramatically improved the shelf-life when compared to the control. The bread loaves incorporated with the different emulsifiers had good symmetry and

sidewalls, with a very uniform and silky texture. The bread containing MG increased in volume and shelf-life when compared to the control, but not to the extent as the other emulsifiers.

In a study by Kamel and Hoover (1992), bread incorporated with different forms of SSL were used as a replacement for shortening. Bread was made according to the straight dough method, with an unemulsified hydrogenated vegetable shortening serving as one control, and loaves made with shortening as another. SSL was added in either a powdered, beaded, or sprayed form to both unshortened and shortened formulas. Physical measurements such as volume, moisture content, and compressibility were assessed. The results indicated that the various forms of SSL did not have a substantial effect on the final products. Bread made with SSL had higher volumes than did both of the controls. The compression data for breads produced with SSL and no shortening showed lower values than the control breads. These results indicated that breads incorporated with SSL may have a longer shelf-life than those breads made with shortening. In addition, the bread baked with SSL (without shortening) had better internal characteristics such as grain, color of crumb, aroma, taste, and texture, than the bread baked with shortening and SSL. These results are due to the fact that more air can be occluded during the mixing of dough containing emulsifiers and that emulsifiers cause smaller cells to form during mixing.

Rogers and Hoseney (1983) studied the effects of DATEM compared to SSL and ethoxylated MG (EMG) in bread. Three samples of DATEM (DATEM-39,-40,-41), SSL, and EMG , all at the 0.5% level, were incorporated into their respective formulas and mixed according to the straight dough method. A control was made, and all treatments were made with and without shortening. Volume, compressibility, and staling measurements were

quantified. DATEM produced loaves higher in volume than SSL and EMG. No significant differences ($p>0.05$) were detected among the emulsifiers with respect to the staling rate. The results implied that DATEM can serve as an effective emulsifier in bread, thereby replacing shortening and producing acceptable doughs and bread loaves. DATEM can also function as an effective anti-staling agent.

2.8D. Emulsifiers and their Roles in Cake Production

Although a wide variety of recipes are included in the class of baked products called *cakes*, they can be classified into two major groups: (1) shortened cakes or cakes containing fat and (2) unshortened cakes or cakes without fat (Bennion, 1990d). Emulsifiers perform important functions in both fat and fat-free cakes.

Emulsifiers can exert a number of favorable affects in cakes such as: (1) greater cake specific volume, (2) improved eating quality due to increased moistness and more rapid flavor release, (3) better crumb structure, (4) greater crumb softness, (5) retardation of staling, (6) improvement in performance of dried egg for use in cake mixes, (7) greater specific cream volume for fat-sugar mixtures, and (8) cost savings by some reduction in expensive cake ingredients (Shepard and Yoell, 1976).

A cake batter containing fat can be called an O/W emulsion. The continuous phase is a sugar solution containing protein, the dispersed phases consists of suspending flour and fat particles. The batter is essentially a foam, and the presence of fat makes it unstable, because the whipped in air is grossly and irregularly distributed. Since the air is dispersed in the fat particles, a fine distribution of the air is possible only when the fat is correspondingly finely

distributed (Krog et. al, 1985; Schuster and Adams, 1984; Shepard and Yoell, 1976). Aerating the batter is of critical importance when making cakes. The more finely distributed air particles increase the viscosity of the batter, leading to a better volume and texture in the finished product (Krog, 1977).

Emulsifiers, such as MDG's, are incorporated into shortening (a plastic fat). They reduce the surface tension of the fat, thus improving the dispersibility of the fat phase. This enhances the cake texture, crumb softness, and volume (Bennion, 1990b; Krog et. al, 1985; Shepard and Yoell, 1976).

2.8E. Emulsifiers and their Roles in Low Fat and Fat-Free Cakes

Plastic fats have the ability to entrap air, a property that is essential in cake making. A reduction in fat then, will result in less air being incorporated into the batter. As a result, a cake would be smaller in volume, uneven in texture, and tough (Cauvain, 1987).

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 powder often serve as aerating agents (Krog et. al, 1985; Schuster and Adams, 1984; Shepard and Yoell, 1976).

Certain MG derivatives, such as propylene glycol monoesters, lactylated MG's, and acetylated MG's, are often called α-tending emulsifiers. These emulsifiers can form an α-crystalline film around air bubbles entrapped in a cake batter, and 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; Schuster and Adams, 1984; Shepard and Yoell, 1976). Some emulsifiers, in particular the MG's, may also react with the starch portion of the batter, forming an insoluble amylose complex. This 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 (1992) 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 relationships among heat transfer, water loss rates, and crumb structure development were studied (Cloke et. al, 1984) in model cake systems made with different levels of saturated (SMG) and unsaturated (USMG) MG's. 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 stearyl 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 0.4g and 1.0g, respectively. The control cake developed many tunnels. SSL, at the 0.4g level, increased volume. When SSL was used at 1.0g, 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.

2.9 The Role of Water in Baked Goods

2.9A. Moisture Content

The moisture content in baked products has a significant influence on several parameters relating to food quality. For instance, a higher moisture content promotes a softer crumb. The degree of moisture contained within a baked product has a profound effect on

retrogradation as well. Although the amount of water controls the rate of retrogradation to a great extent, the changes in baked products such as bread, are not solely due to moisture content. Crumb moisture during storage can be maintained with appropriate packaging, however, firming of the crumb cannot be prevented (Stampfli and Nersten, 1995).

Rogers et al. (1988) assessed the effect of native lipids, shortening, and bread moisture on bread firming. Using a bread formula containing 11.8% protein, breads were made by incrementally shortening the baking time (9 and 12 minutes) to produce loaves of higher moisture. To reduce the moisture content, loaves were baked for 24 minutes, followed by fan-drying for times ranging from 2 to 26 hours. The investigators concluded that the rate of firming was found to be a function of the moisture content. Breads having a low moisture content firmed at a faster rate, yet had a slow rate of starch retrogradation. Breads having a higher moisture content had a delayed rate of firming. Maleki et al. (1980) demonstrated the effects of loaf volume, moisture content, and protein quality on the softness and staling rate of bread. The researchers found that breads with a higher moisture content were initially softer than breads of a lower moisture content, and that they remained softer during a storage time of two days. After three days of storage, however, no significant differences in staling rate were detected.

2.9B. Water Activity

While the moisture content of a baked product can influence perishability, it is not the sole indicator of quality, for various foods with the same water content can differ substantially in perishability (Fennema, 1985). This is because water can exist in many forms. Water can

exist in a "free" form in which it is present within the intergranular spaces and within the pores of a material. "Free" water maintains its usual physical properties, and serves as a dispersing medium for colloidal substances and a solvent for crystallizing compounds (Pomeranz, 1991a). Water can also adhere to the surface of proteins, starches, and cellulose, for example, through hydrogen bond formation, hydrophobic interactions, and Van der Waals forces. "Bound" water has assumed many definitions by various authors and can collectively be described as that water which exists in the vicinity of solutes and other nonaqueous constituents, and exhibits reduced molecular mobility (Fennema, 1985). It is the manner in which water interacts with other components that effects shelf-life.

Chapter 3

Justification and Purpose of Study

Fats are largely responsible for contributing to the texture, flavor, and appearance of many foods, particularly baked products. The water-binding properties of fats in bakery goods provide moistness and a sensation of freshness. Fats also "shorten" the strands of gluten, resulting in a more tender product (LaGuardia, 1994; Bennion, 1990b).

Fat is crucial in foods, yet due to health concerns, consumption of this substance must be reduced. Fat has been linked to heart disease, obesity, some types of cancer, and possibly gallbladder disease (Anon., 1991). Consumers are aware of the need to limit fat-containing foods, and have done so in the last few years. Reducing fat consumption is no easy task. On the one hand, consumers want healthy foods, foods that are lower in fat, sodium, and cholesterol, but they still want foods that are rich, flavorful, and creamy.

In order to meet this demand, the food industry is diligently working to develop foods that are lower in fat, while having the same sensory attributes that are associated with real fat. Given all the functions that fat performs, it is practically impossible to have one ingredient replace the fat and replicate all of the functions of fat (Busetti, 1995). There is a vast array of fat replacers on the market today, so it is important that the substitutes are tailored for specific applications.

Baked products consist of many foods that range from bread and biscuits, cream puffs and popovers, to cakes and muffins. All of these products are complex mixtures in which the interactions between each ingredient can affect the outcome of the product. Reducing the fat

content in such goods while maintaining the sensory characteristics of real fat is of utmost importance.

Carbohydrate fat substitutes can be used in a variety of foods, especially baked products. Not only are they heat stable, but these replacements can incorporate water into a gel type structure resulting in lubricant or flow properties similar to those of fats in some food systems (Yackel and Cox, 1992). Enzymes also have certain roles in baking and function in hydrolyzing starch. Alpha fungal amylase, in conjunction with bacterial amylase, can reduce the amount of dextrinization that occurs in starch, thus producing a favorable product (Drapron and Godon, 1987; Schuster and Adams, 1984). Amylases can also improve crumb structure and crust color (Schuster and Adams, 1984).

Emulsifiers are compounds that can be used in a variety of products. Possessing both hydrophilic and lipophilic moieties, these compounds can reduce the interfacial tension between two immiscible substances, providing stability in a system. Emulsifiers perform a number of other functions such as starch and protein complexing, aeration, and lubrication. Emulsifiers such as MDG's have been incorporated into shortening since the 1930's. In shortening, they enable the fat to disperse better in the mixture, thus less can be used. Since emulsifiers are fat derivatives, they possess many similar properties to fat. They not only have a fatty (or oily) consistency similar to fat, but also possess the texture and cohesiveness of fat as well. Emulsifiers can be used in foods with a reduced fat content and still maintain the desirable attributes of the conventional product (Stampfli and Nersten, 1995; Schuster and Adams, 1984; Birnbaum, 1981; Garti et. al, 1980; Krog, 1977).

A minimal amount of research has focused on the combined use of emulsifiers, a fat substitute, and enzymes in a baked product, particularly quick breads. Quick breads include a variety of products that can be prepared without the rising or proofing time required by yeast breads, hence the term "quick". Muffins, which are available in a variety of flavors, are an example of quick breads (Bennion, 1990c). Muffins serve as a quick convenience food for breakfast, lunch, or dinner.

Therefore, the primary objective of this research was to study the effect of selected emulsifiers in a reduced fat muffin recipe containing a carbohydrate based fat substitute and a combination of fungal and bacterial amylase enzymes. The focus of the research was to determine the effects of the interactions among the emulsifiers, enzymes, and fat substitutes on physical properties such as color, volume, texture, moisture, water activity, and degree of staling. Also, sensory properties such as perceived crust color, moistness, aftertaste, cohesiveness, adhesiveness, and cell size were assessed.

Chapter 4

Materials and Methodology

4.1 Experimental Design

A randomized incomplete block design (Appendix A) was used in this study. There were five variations that were replicated six times in the experiment. Three batches of muffins were made twice a week for 10 weeks for the duration of the study. Physical measurements were made during the first 5 weeks of the experiment. The same variations were repeated again for sensory evaluation.

The muffin recipe that was used was derived from the Pillsbury Cookbook (1989) (Appendix B). The variations were:

(A) Hydroxypropyl Methylcellulose (HPMC) and Enzymes Alone

(B) HPMC, Enzymes, and Sodium Stearoyl-2-Lacylate (SSL)

(C) HPMC, Enzymes, and Distilled Monoglycerides (DMG)

(D) Control (100% Fat Substituted Muffin)

(E) HPMC, Enzymes, and Diacetyl Tartaric Acid Esters of Monoglycerides (DATEM)

4.2 Manipulation of the Ingredients

All of the ingredients (Appendix C) were purchased locally, except for the fat substitute, enzymes, and emulsifiers. All of the muffins were produced in a climate controlled laboratory (Room 337 Wallace) between 70-73° F at Virginia Polytechnic Institute and State University, in Blacksburg, Virginia. The variations were mixed according to the "muffin

method" (Bennion, 1990b). All of the dry ingredients were weighed on a top load balance (OHAUS Top Load Balance, Model CT 1200, Florham Park, NJ), except the HPMC, emulsifiers, and enzymes, which were weighed using a Mettler AE 160 DeltaRange balance. The dry ingredients were first sifted together into a Pyrex mixing bowl (Pyrex, Corning, NY.). The liquid ingredients were mixed together using a wire whisk and then poured into the dry ingredients. Using a rubber spatula (RubberMaid, Inc., Wooster, OH.), the mixture was stirred until the dry ingredients were moistened, approximately 20 strokes. Fifty grams of batter were placed into muffin liners (Reynolds Metal Company, Richmond, VA.) that had been previously sprayed with a non-stick cooking spray (PAM™, American Home Food Products, New York, NY) and placed into a one dozen count muffin tin (Ekco, Franklin Park, IL.). The muffins were placed in a preheated oven (Frigidaire electric range, PC No. 1126638, Dayton, OH.) of 400° F (205° C) on the middle rack and baked for 17 minutes. The muffins were immediately removed from the pan and cooled on a wire rack for 1 hour prior to analysis.

4.3 Physical Properties

The physical properties that were measured in this study were crust and crumb color, volume, crumb firmness, moisture content, water activity (1 hour after baking, and after 24 and 48 hours storage), and staling rate (after 24 and 48 hours storage).

4.3A. Crust and Crumb Color

A Hunter Lab Optical Sensor D25 (Reston, VA.), in conjunction with a Toshiba T1000 System Unit (Tokyo, Japan, No. PA 70276), was used to measure the crust and crumb

color of the muffins (Appendix D). Measurements from this instrument were based on the Hunter Color Solid. Spaces within the solid may be located from the values designated as L, a, and b. L represents a value that measures lightness from 0(black) to 100(white). Red is represented by +a, whereas green is -a. Yellow is +b, and blue is -b. These values are read via the computer (as measured against a standard) and were obtained on a printout (Penfield and Campbell, 1990a).

4.3B. Volume

The volume (Appendix E) of two randomly selected muffins from each batch was quantified using a dial caliper (Switzerland) to measure the standing height and diameter. The values obtained were incorporated into the following formula to assess volume:

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

where r= radius and h= height (Haag et.al, 1970).

4.3C. Crumb Firmness

The method used to determine crumb firmness was based on the AACC method 74-09. The Instron universal testing machine (Universal Instron Machine, Model 1011, Canton, MA.) was used to measure crumb firmness in the muffins (Appendix F). An aluminum plunger, 36-mm in diameter, was used to compress the samples at a rate of compression of approximately 12 mm/minute.

4.3D. Moisture

The Brabender moisture analyzer (C.W. Brabender Instruments, Hackensack, NJ.) was used to measure the percentage of moisture contained within the muffins (Appendix G). After preheating the machine for 1 hour, ten grams of sample were taken from the interior of the muffin and placed in a Teflon coated circular tin pan. The sample was dried in the oven of the machine at a temperature of 130°C for approximately 1 ½ hours or until equilibrium was attained.

4.3E. Water Activity (a_w)

A Decagon AquaLab CX-2 water activity meter (Pullman, WA.) was used to measure the a_w (Appendix H). A small portion of the crumb from the interior of the muffin was pressed into the bottom of a disposable cup (40mm x 13mm), and placed in the drawer of the machine until a reading was obtained (approximately 2 minutes).

4.3F. Degree of Staling

A Perkin-Elmer Differential Scanning Calorimeter (Perkin-Elmer, Norwalk, CT.), connected to a Perkin-Elmer Thermal Analysis Data Station (Perkin-Elmer, Norwalk, CT.) for data analysis, was used to determine degree of staling (Appendix I). A 40 mg sample was obtained from the center of the muffin and enclosed in an aluminum pan. The muffins were stored at room temperature in Tupperware™ (RubberMaid, Inc., Wooster, OH.) containers for the duration of the analysis (24 and 48 hours after baking).

4.4 Sensory Evaluation

4.4A. Quantitative Descriptive Analysis (QDA)

QDA is a method of sensory evaluation used to identify and quantify sensory characteristics of a particular product. Each trait can be graphically and statistically represented by placing all of the traits on a scale (Penfield and Campbell, 1990b). Eleven students (10 females, 1 male) ,ages 23-28 from Virginia Polytechnic Institute and State University, served as panelists.

4.4B. Training

Panelists participated in three training sessions (Appendix J) in order to be familiarized with descriptive terminology and to practice using line scales. Training is done not only to achieve valid and consistent results, but to enable the panelists to gains skills and confidence (Meilgaard et al., 1991). Each session had a duration of approximately 1 hour. During the first session, panelists were presented with background information and the importance of QDA. The panelists generated descriptive terms, defined those terms (Appendix K), and listed them in order of importance. This was done by comparing two muffins and by answering questions concerning characteristics of those muffins asked by the investigator, who remained non-participatory and served only as a facilitator. During this session, the panelists read and signed consent forms (Appendix L). The second and third sessions focused on the panelists sampling different muffin variations. This was done to assess their ability to discriminate, quantify the intensity of the perceived attribute(s) using a line scale, and to determine their consistency.

4.4C. Testing Procedures

The panelists came in twice a week to evaluate the muffins (Appendix M). Each judge individually sampled three muffins in a clockwise fashion that had been assigned a random number. A sip of water was taken between each sample. As a muffin was sampled, the panelist placed a horizontal mark on the scale which represented the perceived intensity of the particular attribute. The scorecard (Appendix N) consisted of scales that used an unstructured 15cm line scale, with anchors that were placed 1.5 cm from the two ends. The anchors, derived by the panelists, are terms that represent two extremes of an attribute.

4.5 Statistical Analysis

The Statistical Analysis System (SAS Institute, Inc., SAS Circle, Box 8000, Cary, NC.) was used to conduct the statistical evaluation. A randomized incomplete block design was used to evaluate both physical and sensory data and reported at the 0.01 significance level. Descriptive statistics for each response variable and descriptive statistics for response for treatment were given in addition to an analysis of variance (ANOVA) table. The Bonferroni approach was used to determine which variations were significantly different, since ordinary multiple comparison procedures (such as Tukey's HSD method or Duncan's multiple range test) could not be used with a randomized incomplete block design. A significance level of 0.10 was used for the experimentwise error rate, however, each test was performed at a significance level of 0.01

(0.10/10), the comparisonwise error rate. With respect to the sensory analysis, the presence of missing data forced the analysis to be performed on the average of the numbers obtained from the eleven panelists rather than the actual responses. As a result, the p-values given in the analysis are approximations. However, these p-values were extremely close to the true p-values and the difference is of no practical concern.

Chapter 5

Results and Discussion

5.1 Physical Measurements

5.1A. Crust and Crumb Color

The appearance of a product largely influences a consumer's decision regarding the purchase of that particular item. The color of a product is an important quality attribute. For example, with respect to bakery products, a cake that is too brown or too light might be rejected by the consumer, leading to a loss of sales.

Tables 1 and 2 show the mean "L" and "b" values for crust and crumb, respectively, for the five muffin variations. The "a" values were not applicable to this product, and were therefore excluded from the study. There were no significant differences ($p>0.01$) found among the various muffin formulas with respect to crust "L" and "b" values (Table 1). Version B, which consisted of HPMC, enzymes, and SSL, produced muffins of a slightly darker crust color with respect to "L" values when compared to the other versions. The control had the lightest crust color, although this was not significant ($p>0.01$).

Crust color can be attributed to two factors: the Maillard reaction and the enzymes. The Maillard reaction results in the surface browning of baked products upon the interaction of the carbonyl group of a reducing sugar and a free amino group of a protein (Whistler and Daniel, 1994). Reducing sugars and free amino groups of proteins found in milk can bind together and induce browning. The caramelization of sugars may also have been involved in

Table 1: Mean crust color values obtained for the five muffin variations

VERSION	TREATMENT◆	L VALUE*,*** ± SD**	b VALUE*,*** ± SD**
A	HPMC and Enzymes Alone	$72.38_a \pm 3.67$	$43.43_a \pm 1.89$
B	HPMC, Enzymes, and SSL	$72.14_a \pm 4.25$	$41.16_a \pm 2.29$
C	HPMC, Enzymes, and DMG	$74.26_a \pm 2.14$	$44.02_a \pm 1.32$
D	Control	$75.38_a \pm 3.49$	$41.72_a \pm 2.98$
E	HPMC, Enzymes, and DATEM	$72.31_a \pm 3.52$	$42.61_a \pm 2.71$

◆ HPMC = Hydroxypropyl Methylcellulose
SSL = Sodium Stearoyl-2-Lactylate
DMG = Distilled Monoglycerides
DATEM = Diacetyl Tartaric Acid Esters of Monoglycerides
* Values within the same column with the same letter denote no significant differences ($p > 0.01$).
** SD = Standard Deviation
*** "L" = lightness (100) to darkness (0)
*** "b" = yellow (+ 70) to blue (- 70)

Table 2: Mean crumb color values obtained for the five muffin variations

VERSION	TREATMENT◆	L VALUE*,*** ± SD**	b VALUE*,*** ± SD**
A	HPMC and Enzymes Alone	$80.19_d \pm 1.26$	$24.22_a \pm 0.90$
B	HPMC, Enzymes, and SSL	$80.83_c \pm 2.01$	$23.87_a \pm 0.76$
C	HPMC, Enzymes, and DMG	$80.69_c \pm 1.15$	$24.64_a \pm 2.43$
D	Control	$77.46_a \pm 1.11$	$28.43_b \pm 1.61$
E	HPMC, Enzymes, and DATEM	$81.85_b \pm 0.63$	$22.77_a \pm 1.21$

◆Refer to Table 1 for definitions.

* Values within the same column with the same letter denote no significant differences ($p > 0.01$).

** SD = Standard Deviation

*** "L" = lightness (100) to darkness (0)

*** "b" = yellow (+ 70) to blue (- 70)

browning. This was seen in all the versions, however it was the use of enzymes that produced the highest degree of browning. The fungal and bacterial α-amylase enzymes were incorporated into all of the muffin formulas except the control. These enzymes hydrolyze starch into dextrins, and the dextrins are hydrolyzed by indogenous β-amylase to maltose or by amyloglucosidase to glucose (Sproessler, 1993). The higher reducing sugar content produced from the action of these enzymes thus contributed to increased Maillard browning.

The positive "b" values shown in Table 1 are indicative of the yellow color that was detected in the crust of the muffins. Although no significant differences ($p>0.01$) were detected in crust color "b" values among the variations, those versions containing enzymes produced crusts that were more yellow in color than the control (except Version B, containing HPMC, enzymes, and SSL). Again, this can be attributed to the increased sugar formation from the enzymes contributing to the browning reactions.

Significant differences ($p<0.01$) in crumb L and b values were observed in the muffin variations (Table 2). The control muffin (Version D) had "L" and "b" values which were significantly different ($p<0.01$) when compared to the other versions. These values were indicative of a darker and more yellow crumb color. The reason for these differences can be ascribed to the use of the whole egg in the control formula. All of the other muffin variations contained only egg whites. Egg yolks contain pigments called carotenoids, namely xanthophyll. It is this pigment that contributed to the darker crumb color. The enzymes produced a lighter crumb and the opposite effect was noted when emulsifiers were added.

5.1B. The Significance of Rheology in Baked Products

Rheology is the study of how materials deform or flow when force is applied (Hoseney, 1994). Attempting to understand the rheological properties of baked products is of critical importance because such characteristics have a significant influence on texture in the final product. Rheological properties are significant because they not only change substantially during the baking process, but also because such properties of the batter or dough affect transformations during baking (Hoseney, 1994).

Many structural changes occur during baking. The most notable change is the gelatinization of starch. It is also the most obvious explanation for the transformation of the viscous dough or batter into a predominantly solid baked product (Bloksma, 1986). The gelatinization process is influenced by other components such as water, sugar, fats, emulsifiers, and enzymes. For example, the temperature at which gelatinization begins increases as the water content increases. Sucrose has been known to delay the onset of gelatinization by binding to water molecules (Whistler and Daniel, 1994). Fats and emulsifiers also retard this process, although the extent to which this is done is influenced by their physical state.

A muffin batter resembles a cake batter because both are basically aqueous systems that contain a number of dispersed phases such as fat, air, and starch granules. The viscosity of such batters is very important because if it is too low, the dispersed phases will separate readily. This will create a product with a tough, rubbery layer, which is the gelatinized starch, at the bottom and a light, open-cell structure (the air) on top (Hoseney, 1994). The proper viscosity will prolong the separation.

The mixing stage is perhaps the most important step when preparing a cake as well as a muffin batter. It is at this stage when the incorporation of sufficient air is vital, because after the batter has been mixed, subsequent leavening and baking can form no new air cells. Air cells can be lost however, by rising to the surface and dissipating, or by the coalescence of air cells (Hoseney, 1994).

5.1C. Specific Gravity

The specific gravity is a measurement which estimates the amount of air incorporated into a batter (Penfield and Campbell, 1990a). A low value indicates a greater amount of air incorporation. The addition of emulsifiers to a formula can increase batter viscosity which is an indication that these compounds can incorporate air into a batter. Table 3 shows the mean specific gravity obtained for each muffin batter variation. No significant differences ($p>0.01$) were found among the variations, however, the version containing HPMC, enzymes, and SSL had the highest value.

5.1D. Volume

The volume achieved in baked products, particularly breads, is of critical importance with respect to texture because it is inversely related to crumb firmness. In quick breads, the volume can be achieved by chemical leavening and through the use of fats and/or emulsifiers. The addition of fat enables the finished product to have a larger volume, thus resulting in a more tender product. The crumb structure becomes both finer and more uniform with thin cell walls (Tamstorf et al, 1986). Many studies have shown that plastic shortenings incorporated

Table 3: Mean specific gravity values of the five muffin batters

VERSION	TREATMENT◆	SPECIFIC GRAVITY (g/ml)* ± SD**
A	HPMC and Enzymes Alone	$0.9784_a \pm 0.08$
B	HPMC, Enzymes, and SSL	$1.0608_a \pm 0.08$
C	HPMC, Enzymes, and DMG	$1.0128_a \pm 0.13$
D	Control	$1.0393_a \pm 0.16$
E	HPMC, Enzymes, and DATEM	$1.0379_a \pm 0.07$

◆Refer to Table 1 for definitions.

* Values with the same letter denote no significant differences ($p > 0.01$).

** SD = Standard Deviation

with emulsifiers such as mono- and diglycerides enables the fat to become more dispersed throughout the batter. The volume and crumb texture are related to the number of air bubbles that are created in the batter, and emulsifiers such as DATEM and SSL have the ability to surround and trap these air bubbles (Krog, 1981).

Table 4 represents the average volume of each muffin variation. Significant differences ($p<0.01$) were found among the different varieties, with Versions A and C (HPMC and enzymes alone and with DMG, respectively) having a volume significantly lower than B, D, and E. Contrary to theory, the control muffin with a high specific gravity (Table 3) produced a high volume. The volume of muffins becomes larger with moderate overmixing. Peaks and tunnels are characteristic of such manipulation due to the overdevelopment of gluten and loss of CO_2 (Penfield and Campbell, 1990e). Perhaps the excess gluten and loss of CO_2 prevented the muffin from rising normally during the early stage of baking. After the crust had formed, steam pressure increased within this structure. The release of this pressure occurs by the steam "pushing" through the batter which leads to tunnel and peak formation.

SSL and DATEM produced volumes comparable to the control. This demonstrates that emulsifiers can enhance volume, although muffins containing DMG produced a low volume product. Due to the fact that SSL and DATEM are highly ionic in structure, the polar portions of these compounds are soluble in the aqueous phase. Therefore, these emulsifiers can surround gas bubbles throughout the batter and can contribute to a higher volume (Krog, 1981). These hydrophilic emulsifiers can also increase the viscosity of the aqueous phase which also aids in gas retention (Flack, 1983). Krog (1977) demonstrated that DATEM and

Table 4: Mean volumes obtained for the five muffin variations

VERSION	TREATMENT◆	VOLUME (cm^3)* ± SD**
A	HPMC and Enzymes Alone	$96,372.56_a$ ± 8418
B	HPMC, Enzymes, and SSL	$104,036.63_b$ ± 7271
C	HPMC, Enzymes, and DMG	$95,706.69_a$ ± 4489
D	Control	$108,760.15_b$ ± 4288
E	HPMC, Enzymes, and DATEM	$103,019.47_b$ ± 3019

◆Refer to Table 1 for definitions.

* Values with the same letter denote no significant differences ($p > 0.01$).

** SD = Standard Deviation

SSL, when compared to a less hydrophilic emulsifier such as glycerol monostearate (GMS), produced a marked increase in volume. When specific gravity was related to volume, Del Vecchio (1975) showed that DMG produced the lowest volume and the highest specific gravity compared to SSL, which is substantially more hydrophilic than DMG.

The version containing HPMC, enzymes, and DMG resulted in muffins with a low volume. The HPMC can bind water and at the same time control batter viscosity and improve gas retention (Anon., 1993). If the gum was not hydrated enough, however, a lower volume would result. Perhaps this occurred in the study. DMG may have competed with HPMC for water as well, thus preventing the full hydration of this compound. Muffins containing the polar emulsifiers increased in volume. This demonstrated that these emulsifiers, due to their polarity, were able to bind to other polar molecules and trap gas cells thereby releasing these gas cells during the baking process.

The fact that the control muffins acquired a high volume can be attributed to emulsifiers present in the egg yolks. These emulsifiers in their natural form are present in the egg yolk as phophatides and membrane-forming lipoproteins. The skim milk used in all variations also has favorable enhancing emulsifying properties. Skim milk has an abundance of proteins which can bind through hydrophobic interactions to fats and the hydrocarbon chains within an emulsifier molecule (Pomeranz, 1991c). This lowers the surface tension throughout the batter, which creates a stable emulsion. A stable emulsion contributes to an increase in viscosity.

Specific gravity is inversely correlated with volume. A lower specific gravity denotes a higher volume (Penfield and Campbell, 1990a). Table 5 compares the specific gravity to

Table 5: Comparison of the mean values obtained for specific gravity (g/ml) and volume (cm^3) among the five muffin variations

VERSION	TREATMENT◆	SPECIFIC GRAVITY (g/ml)* ± SD**	VOLUME (cm^3)* ± SD**
A	HPMC and Enzymes Alone	$0.9784_a \pm 0.08$	$96{,}372.56_a \pm 8418$
B	HPMC, Enzymes, and SSL	$1.0608_a \pm 0.08$	$104{,}036.63_b \pm 7271$
C	HPMC, Enzymes, and DMG	$1.0128_a \pm 0.13$	$95{,}706.69_a \pm 4489$
D	Control	$1.0393_a \pm 0.16$	$108{,}760.15_b \pm 4288$
E	HPMC, Enzymes, and DATEM	$1.0379_a \pm 0.07$	$103{,}019.47_b \pm 3019$

◆Refer to Table 1 for definitions.

* Values within the same column with the same letter denote no significant differences ($p > 0.01$).

** SD = Standard Deviation

volume. Data did not support this inverse relationship. The version containing HPMC and enzymes alone had a low specific gravity, and produced a low volume product. The version containing SSL had a high specific gravity and produced a large volume as well. However, this might be attributed to extraneous variables such as fluctuations in temperature or relative humidity that could have occurred within the laboratory.

5.1E. Crumb Firmness

Table 6 shows the average values obtained for crumb firmness. The control muffin was found to be significantly ($p<0.01$) softer when compared to the other variations. This can be attributed to the oil that was used in the formula. One of the main functions of fat is its ability to impart tenderization in baked products. When oil was incorporated into the formula, it became dispersed throughout the mixture in the form of irregularly shaped droplets. These layers of droplets interrupt the continuity of the gluten chains that form when flour proteins become hydrated. This induces a weakening affect in the structure (Cauvain, 1987).

The variations utilizing DATEM produced a softer muffin when compared to the other versions with emulsifiers. SSL performed comparably to DATEM with some differences. DATEM, when hydrated, has the ability to trap moisture and promote gassing power which contributes to a softer muffin. SSL, which is more hydrophilic than DATEM, complexes with gluten and starch and has the ability to surround and trap gas as well (Tamstorf et al, 1986).

Muffins containing DMG were firmer when compared to the other formulations utilizing emulsifiers. Emulsifiers can be classified as dough conditioners or crumb softeners. DMG is considered to be a crumb softener, because it has the ability to complex with amylose

Table 6: Crumb firmness values obtained for the five muffin variations

VERSION	TREATMENT◆	CRUMB FIRMNESS (kg)* ± SD**
A	HPMC and Enzymes Alone	$1.1044_{c,d} \pm 0.21$
B	HPMC, Enzymes, and SSL	$0.7959_{c} \pm 0.20$
C	HPMC, Enzymes, and DMG	$1.0060_{c,d} \pm 0.11$
D	Control	$0.3343_{b} \pm 0.05$
E	HPMC, Enzymes, and DATEM	$0.6180_{a,c} \pm 0.10$

◆Refer to Table 1 for definitions.
* Values with the same letter denote no significant differences ($p > 0.01$).
** SD = Standard Deviation

within the starch granule thus rendering it water insoluble (Pomeranz, 1991c). This complex prevents the amylose from leaching into the interstitial space. When amylose chains in this space realign through hydrogen bonding, a gel is formed, contributing to a "firming" affect. DMG, because it is lipophilic, as well as saturated, can align itself inside and complex with the hydrocarbon portions of amylose. The less amylose in the interstitial space, the less firming occurs, but only for a very limited time since other factors may affect firming (Pomeranz, 1991b).

The muffin containing only HPMC and enzymes produced a firm texture. Enzymes have been shown to improve loaf volume in a baked product, and thus enhance softness. It is plausible that the extra sugars created by the enzymes resulted in an increased competition for water, thus preventing the HPMC to bind this moisture. The enzymes (fungal vs. bacterial) have different thermostabilities which may be a factor in affecting firmness. The optimum temperature for fungal amylose activity is 55°C. This enzyme decreases in activity between temperatures of 55-80°C. Bacterial amylase has an optimum temperature of 70°C and becomes inactivated around 70-100°C (Drapron and Godon, 1987). Therefore, bacterial amylase has more time to hydrolyze the starch extensively. As a result, a decrease in viscosity occurs which results in a lower volume and a tougher product.

Loaf volume has been found to be inversely related to crumb firmness (Krog, 1977). Table 7 shows the relationship obtained between volume and firmness in the muffin variations. With respect to crumb firmness, the muffin containing HPMC and enzymes alone was significantly different ($p<0.01$) from the control and the polar emulsifiers, SSL and DATEM,

Table 7: Comparison of the mean values obtained for volume (cm^3) and crumb firmness (kg) among the five muffin variations

VERSION	TREATMENT◆	VOLUME (cm^3)* ± SD**	CRUMB FIRMNESS (kg)* ± SD**
A	HPMC and Enzymes Alone	$96{,}372.56_a \pm 8418$	$1.1044_{c,d} \pm 0.21$
B	HPMC, Enzymes, and SSL	$104{,}036.63_b \pm 7271$	$0.7959_c \pm 0.20$
C	HPMC, Enzymes, and DMG	$95{,}706.69_a \pm 4489$	$1.0060_{c,d} \pm 0.11$
D	Control	$108{,}760.15_b \pm 4288$	$0.3343_b \pm 0.05$
E	HPMC, Enzymes, and DATEM	$103{,}019.47_b \pm 3019$	$0.6180_{a,c} \pm 0.10$

◆Refer to Table 1 for definitions.

* Values within the same column with the same letter denote no significant differences ($p > 0.01$).

** SD = Standard Deviation

respectively. However, this same version was not significantly different ($p>0.01$) from the muffins containing DMG. Because these two versions could not retain gas cells to the extent of the control or the polar emulsifiers, they were lower in volume and firmer in texture. If DMG was not hydrated enough, it could not perform adequately, thus it could not entrap gas cells sufficiently. Likewise, if HPMC was not hydrated fully, the same effects could be observed.

5.1F. The Role of Water in Baked Products

Water is a major component in many foods and has a substantial influence on various physical and chemical properties. In baked products, water plays a significant role in the changes that occur during mixing, baking, and storage. Water affects starch gelatinization, protein denaturation, flavor, and color. Water is crucial for the integrity of protein molecules, which can affect texture and other attributes that can be influenced by enzymes, such as Maillard browning. Water also participates in molecular interactions. Water enhances lipid binding to flour proteins, and can also associate with emulsifiers to influence emulsion formation. The moisture content and water activity of a baked product are two key factors that determine the shelf life of bread. These factors are influenced by the incidence of microbial damage, softness of the crumb, crispness of the crust, crumb hardening, crumbliness, and other changes associated with overall staling and lowered consumer acceptance (Pomeranz, 1991a).

5.1G. Moisture Content

Table 8 denotes the percent moisture content obtained in the five muffin variations. Significant differences ($p<0.01$) were found among the versions, with the control retaining the least amount of water. The significant differences that were found might not be due to the actual variations themselves, but extraneous variables such as relative humidity. The five versions were not prepared on the same days throughout the testing period, and differences in the temperature or relative humidity within the laboratory could have accounted for such differences. It is also possible that the moisture contained in the control muffin evaporated during the baking process.

Although the enzymes had a negligible effect on moisture retention, it was apparent that HPMC was able to imbibe a high amount of moisture. HPMC is a highly efficient water retention agent because of its polar character. Water molecules can become tightly bound through hydrogen bonds to the polar groups of this hydrocolloid and therefore, render the water immobile (Ganz, 1977). The amount of water that can be bound by HPMC varies according to the viscosity of this gum. The high viscosity type appears to hold water more effectively than lower viscosity types (Ganz, 1977). METHOCEL® brand HPMC comes in a variety of viscosities that range from 15 to 100,000 mPa's (millipascal seconds) (Anon., 1993). The viscosity of the HPMC used in this study was 4,000 mPa's, a good indicator that this particular form of hydrocolloid was effective in trapping water.

The emulsifiers in conjunction with HPMC retained a high percentage of moisture in the muffins. DATEM and SSL are both hydrophilic emulsifiers which can interact to a greater

Table 8: Mean moisture content values obtained in the five muffin variations

VERSION	TREATMENT◆	% MOISTURE * ± SD**
A	HPMC and Enzymes Alone	$38.75_a \pm 11.15$
B	HPMC, Enzymes, and SSL	$39.42_a \pm 10.51$
C	HPMC, Enzymes, and DMG	$39.33_a \pm 11.27$
D	Control	$38.50_b \pm 0.89$
E	HPMC, Enzymes, and DATEM	$44.33_a \pm 1.21$

◆Refer to Table 1 for definitions.

* Values with the same letter denote no significant differences ($p > 0.01$).

** SD = Standard Deviation

degree with the polar water molecules and retain moisture. However, DMG, which is more hydrophobic in character may not be able to have the same ability to retain moisture efficiently.

5.1H. Water Activity

Water activity is a measurement that takes the interaction of water and other components into account. The a_w of a food is defined as the ratio of p/p_o, in which p denotes the partial pressure of water above the sample and p_o the vapor pressure of pure water at the same temperature (Penfield and Campbell, 1990f). Water activity values are usually lower than 1.0 because the presence of non-aqueous nonvolatile substances lowers vapor pressure (Penfield and Campbell, 1990f).

Table 9 shows the mean a_w values for the muffin variations one hour after baking, and after 24 and 48 hours storage. Although no significant differences ($p>0.01$) were found among the formulations throughout the testing period, the control muffin had the lower a_w when compared to the other variations. The reduced fat muffins had a higher a_w than the control and this was enhanced with the addition of the emulsifiers. DATEM is a very hydrophilic compound and thus can hydrogen bond with water molecules. If more of its hydroxyl groups are "bound" to water molecules, starch, or cellulose, for instance, less "free" water is available for participation in other reactions. This theory however, does not explain why the version containing SSL, an emulsifier as hydrophilic as DATEM, acquired a higher activity.

Most foods have an a_w greater than 0.8 at the time of consumption (Bourne, 1987). The a_w ranges for the muffins are favorable for the product to remain moist and tender.

Table 9: Mean water activity (a_w) values obtained for the five muffin variations 1 hour after baking, and after 24 and 48 hours storage

VERSION	TREATMENT◆	a_w* 1 hr after baking± SD**	a_w* after 24 hr storage ± SD**	a_w* after 48 hr storage ± SD**
A	HPMC and Enzymes Alone	$0.94_a \pm 0.00$	$0.94_a \pm 0.00$	$0.94_a \pm 0.00$
B	HPMC, Enzymes, and SSL	$0.95_a \pm 0.02$	$0.94_a \pm 0.00$	$0.94_a \pm 0.01$
C	HPMC, Enzymes, and DMG	$0.95_a \pm 0.01$	$0.94_a \pm 0.01$	$0.94_a \pm 0.01$
D	Control	$0.94_a \pm 0.01$	$0.94_a \pm 0.01$	$0.93_a \pm 0.01$
E	HPMC, Enzymes, and DATEM	$0.94_a \pm 0.02$	$0.95_a \pm 0.00$	$0.94_a \pm 0.01$

◆Refer to Table 1 for definitions.

* Values within the same column with the same letter denote no significant differences ($p > 0.01$).

** SD = Standard Deviation

As the a_w (and moisture content) decrease, undesirable textural attributes relating to a dry and crumbly product will result. Products with high a_w levels, however, are susceptible to microbial growth. The values obtained were indicative that the muffins could be susceptible to some type of microbial attack with prolonged storage.

5.1 I. The Staling Process

Extending the shelf life of baked products is one of the primary concerns of the manufacturer in the baking industry. As a baked product stales, changes in texture, moisture and flavor are apparent, and this leads to rejection of the product by the consumer. While the stages in the staling process mainly involve the starch fraction, other components such as proteins and water play a significant role.

Differential scanning calorimetry (DSC) is a method used to quantify the extent of starch crystallization in a given sample by recording the energy (ΔH) required to melt the crystals comprising the starch granule (Zeleznak and Hoseney, 1986). Therefore, the enthalpy value is indicative of the degree of retrogradation in a sample. Table 10 shows the mean enthalpy values for the muffin versions after 24 and 48 hours of storage. Significant differences ($p<0.01$) in the degree of staling were found after 24 hours storage. Within the first 24 hours, muffins containing DATEM and the control staled at a significantly ($p<0.01$) slower rate than the other versions (Figure 4). Krog et. al (1989) assessed the retrogradation of the starch fraction in wheat with the separate incorporation of DATEM and DMG in bread formulas. The investigators concluded that while both emulsifiers reduced the extent of retrogradation,

Table 10: Mean enthalpy (ΔH) values of the five muffin variations after 24 and 48 hours of storage

VERSION	TREATMENT◆	ΔH (J/g)* after 24 hours ± SD**	ΔH (J/g)* after 48 hours ± SD**
A	HPMC and Enzymes Alone	$0.450_a \pm 0.08$	$0.760_a \pm 0.19$
B	HPMC, Enzymes, and SSL	$0.485_a \pm 0.11$	$0.761_a \pm 0.12$
C	HPMC, Enzymes, and DMG	$0.510_a \pm 0.07$	$0.785_a \pm 0.24$
D	Control	$0.419_b \pm 0.08$	$0.672_a \pm 0.11$
E	HPMC, Enzymes, and DATEM	$0.331_b \pm 0.08$	$0.610_a \pm 0.08$

◆Refer to Table 1 for definitions.

* Values within each column with the same letter denotes no significant differences ($p > 0.01$).

** SD = Standard Deviation

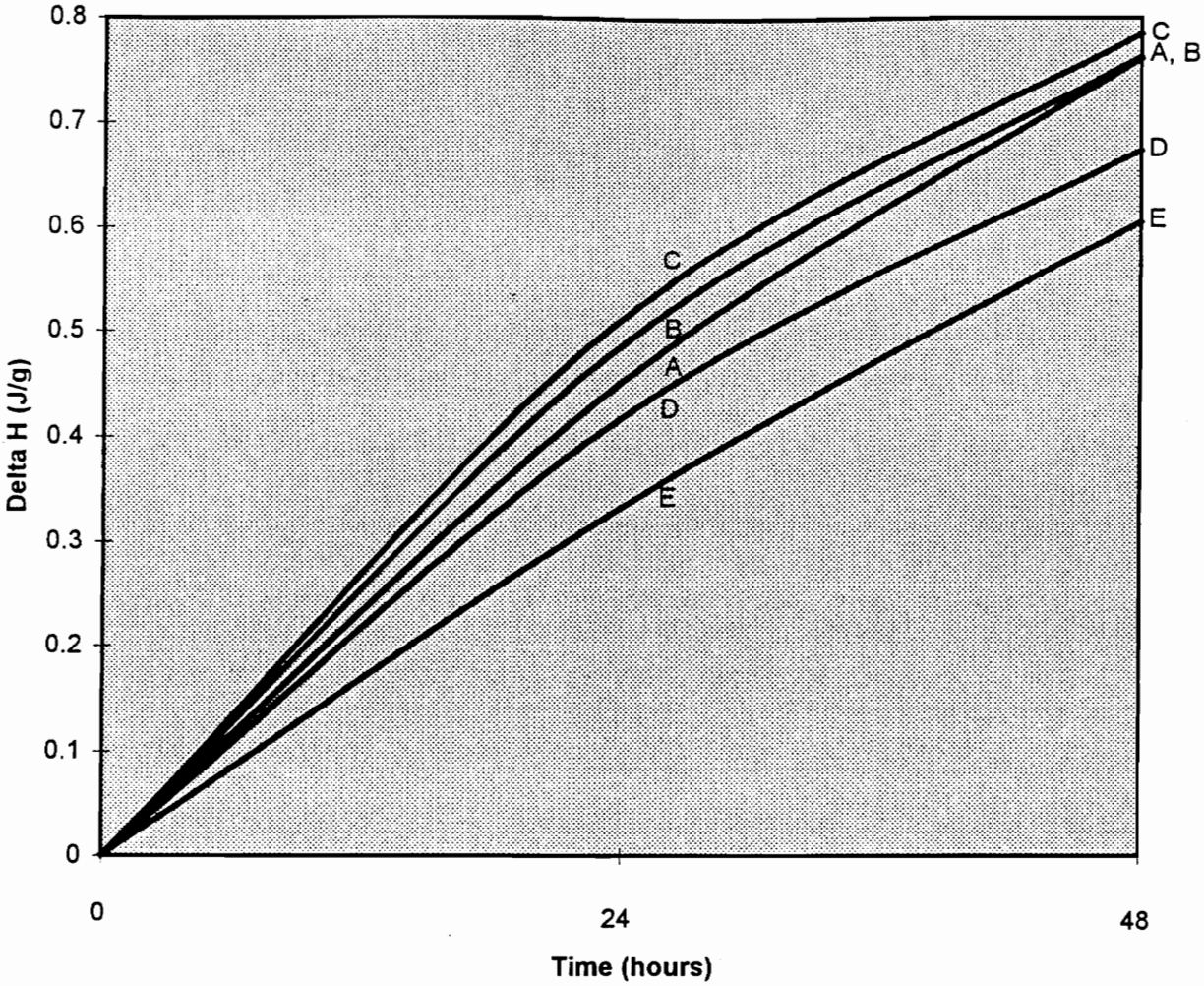

Figure 4: Staling rates of muffins after 24 and 48 hours storage*

*After 24 hours, the control and muffins containing DATEM staled at a significantly ($p<0.01$) slower rate compared to the other versions. After 48 hours, all versions had staled at the same rate.

A = HPMC and Enzymes Alone
B = HPMC, Enzymes, and SSL
C = HPMC, Enzymes, and DMG
D = Control
E = HPMC, Enzymes, and DATEM

DMG was more effective. The researchers attributed this to the principle that DMG can interact with amylose to a greater degree than DATEM because of its chemical structure. Saturated DMG's containing a fatty acid chain ranging from 14 to 18 carbons have the highest amylose complexing index. This is because the long hydrocarbon chain can fit within the helix formed by the amylose and prevent the amylose from leaching into the intergranular space (Knightly, 1988; Krog, 1981).

According to the data (Table 10), DMG was the least effective in inhibiting retrogradation after 24 hours of storage. Saturated DMG, and other emulsifiers, can only exert this effect if they are added in the proper form. For example, some types of this emulsifier are available in beaded, hydrated, or powdered form (Newbold, 1976). The form used in this study was a water dispersible powder. A possibility may be that the emulsifier did not become fully hydrated, and thus it could not exert its optimum effect. Several ingredients in the muffin formulation were competing for water, such as the starch, flour proteins, HPMC, and the sugar. This competition could have prevented the emulsifier from becoming fully hydrated. Since DATEM was used in a hydrated form, it was more dispersible than a powdered form (Newbold, 1976).

Research has shown that the benefits of adding emulsifiers to batters and/or doughs to produce baked products is due to their ability to complex with the starch fraction (Krog, 1981; Pomeranz, 1991c; Hoseney et al., 1976). Different emulsifiers, however, complex with amylose to various degrees. It is this difference and the chemical structure of the emulsifier that predicts the effectiveness as an anti-staling agent. The amylose complexing index for DMG is around 87, DATEM, 49, and SSL, 72 (Flack, 1983). The larger the index, the more

amylose is prevented from leaching from the starch granule. This indicates that DMG should be the most effective at delaying retrogradation. According to theory, SSL is an adequate anti-staling agent not only due to its high amylose complexing index, but also because it can apparently complex with portions of amylopectin as well. The results from this study indicate the opposite trend. DATEM, with the lowest index compared to the other two emulsifiers, was more effective in the prevention of staling. The version containing SSL was more effective than DMG at retarding staling after 24 hours.

Fats in the form of shortenings and oils have been shown to retard staling as well, however, the manner in which they perform is unclear. Penfield and Campbell (1990g) state that lipids bind to the gluten proteins and amylose. Therefore, starch chains that are bound to lipids (or emulsifiers) are not free to participate in starch-starch reassociation. On the contrary, Krog (1981) stated that shortening did not complex with amylose. It is possible that the lipids interact through hydrophobic/electrostatic interactions with the polar flour lipids. Rogers et. al (1988) assessed the effect of native lipids, shortening, and bread moisture on bread firming. After baking bread loaves containing shortening alone and breads with monoglycerides alone, the researchers concluded that breads containing shortening and emulsifiers reduced the rate of firming. However, the breads containing emulsifiers were more effective. Another study conducted by Hoseney et al. (1976) showed that surfactants such as SSL, DATEM, and DMG were more effective at retarding the staling rate when compared to shortening. The researchers concluded that SSL produced the best results, followed by a mixture of DATEM and DMG, and then DATEM alone.

The enzymes used in the formulations, particularly the bacterial amylase, exerted an effect in delaying retrogradation. This was especially evident in the version containing DATEM. Fungal α- amylase has a negligible effect on retrogradation because it has a limited amount of time to act on gelatinized starch before it is inactivated by the heat, which is around 75°C (Sproessler, 1993). Therefore, the anti-staling effects are mainly the action of bacterial amylase. Bacterial amylase is more heat stable and has a longer time to hydrolyze the gelatinized starch. As a result of this longer hydrolyzing time, dextrins are formed. Dextrins of a particular size interfere with the cross-links between starch and protein (Martin and Hoseney, 1991). As a result, the more dextrins that are created, retrogradation is delayed to a greater extent. Rohm Tech (1986) demonstrated that fungal enzymes, when used in conjunction with emulsifiers, could prolong the shelf life of bread by 3-7 days.

Dragsdorf and Varriano-Mornston (1980) tested the effects of barley malt, fungal, and bacterial alpha amylase on starch crystallization. Although all breads increased in firmness during storage, the bread containing bacterial amylase did so at a slower rate. However, starch recrystallization increased at a faster rate and to a greater degree with the bacterial amylase. Therefore, starch crystallization and bread firming are not synonymous. When the investigators determined the starch crystalline organization through X-ray diffraction for starches in the breads, they noticed differences in the structures. Starch from the control bread developed a combination of "V_h" and "B" structures, whereas starch from bacterial alpha amylase supplemented breads exhibited "V_h" and "A" structures. B structures can hold more water than A structures, 36 vs 8 molecules, respectively. During retrogradation, moisture is transferred from gluten to starch. The B and A structures indicate that more water can be

transferred from the gluten to the B starch (i.e., the control) than to the A starch in the enzyme supplemented breads. The gluten in the control breads, as a result, would have less of a moisture content than the breads supplemented with the amylase, which would account for the increased rate of firming for the control.

Perhaps this mechanism of starch crystallization explains how the versions containing bacterial enzymes delayed the staling rate to an extent comparable to the control during the first 24 hours. The binding of DATEM to gluten proteins, in addition to the hydrogen bonding of water molecules and the action of the enzymes, might have trapped the liquid, causing a high moisture content, with a decreased staling rate. Since the a_w slightly decreased from 24 to 48 hours for all the versions (except DMG), this loss of free water could explain why all the versions exhibited no significant differences ($p>0.01$) in the degree of staling after 48 hours storage. DATEM was the most effective at delaying retrogradation during the first 24 hours compared to the other versions. However, as storage time increased, the amount of water that was lost increased as well for all versions. As a result, retrogradation for all versions proceeded at relatively the same rate.

SSL also exhibits a strong "V_h" pattern because the hydrocarbon chains complex with the starch molecules. Breads without this emulsifier tend to have weak "V_h" structures. Apparently this structure prevents the amylose from leaving the granule, thus delaying the firming process. This structure, when bound to the starch, could reduce the redistribution of moisture from gluten to the starch. This could prevent subsequent firming of the gluten phase (Dragsdorf and Varriano-Marston, 1980). Perhaps DATEM has strong "V_h" structures, which might explain how this emulsifier delayed retrogradation within the first 24 hours.

However, this theory does not explain why the version containing SSL had a high degree of staling. This might be attributed to the two different physical forms of these anionic emulsifiers used in the study, a hydrated vs. powdered form, for DATEM and SSL, respectively.

The moisture content of a baked product has shown to have an influence on the rate of retrogradation. Rogers et. al (1988) studied the effects of bread moisture on firming. The investigators concluded that the moisture content was inversely proportional to the firming rate and that the rate of starch retrogradation did not correlate with the rate of firming in the same samples. The bread with the fastest firming rate had the slowest retrogradation rate. For example, the driest bread (22% moisture) had a slower rate of retrogradation than did the samples with 25% and 30% moisture. Maleki et al. (1980) found that increasing the water content of bread resulted in a slower rate of starch retrogradation. Table 11 shows the relationship between moisture content and degree of staling among the muffin variations. Muffins with DATEM contained a high moisture content and a low staling rate after 24 hours.

Water activity, in addition to moisture content, influences starch retrogradation. The role of starch retrogradation increases with decreasing a_w. For example, breads containing 10% sorbitol ($a_w = 0.95$) or 20% sorbitol ($a_w = 0.93$) had a faster rate of starch retrogradation than regular bread (a_w=0.97) during the first week of storage (Leung, 1987). Although no significant differences were found in the muffin variations with respect to a_w immediately after baking, and after 24 and 48 hours (Table 9), muffins containing DATEM had a higher a_w than the control, and these same muffins also had the lower degree of starch retrogradation.

Table 11: Comparison of the mean values obtained for moisture content and ΔH values among the five muffin variations

VERSION	TREATMENT◆	% MOISTURE* ± SD**	ΔH (J/g)* after 24 hours ± SD**	ΔH (J/g)* after 48 hours ± SD**
A	HPMC and Enzymes Alone	$38.75_a \pm 11.15$	$0.450_a \pm 0.08$	$0.760_a \pm 0.19$
B	HPMC, Enzymes, and SSL	$39.42_a \pm 10.51$	$0.485_a \pm 0.11$	$0.761_a \pm 0.12$
C	HPMC, Enzymes, and DMG	$39.33_a \pm 11.27$	$0.510_a \pm 0.07$	$0.785_a \pm 0.24$
D	Control	$38.50_b \pm 0.89$	$0.419_b \pm 0.08$	$0.672_a \pm 0.11$
E	HPMC, Enzymes, and DATEM	$44.33_a \pm 1.21$	$0.331_b \pm 0.08$	$0.610_a \pm 0.08$

◆Refer to Table 1 for definitions.

* Values within each column with the same letter denotes no significant differences ($p > 0.01$).

** SD = Standard Deviation

5.2 Sensory Assessment: Quantitative Descriptive Analysis

5.2A. Significance of Sensory Analysis

The primary goal of any food manufacturer is to develop a product that will be accepted by consumers and will result in sales. Sensory characteristics govern the success of a product. Therefore, in developing products, it is imperative to know what consumers desire. As a result, many food companies have implemented sensory evaluation programs in order to improve quality in products.

Numerous instruments have been designed to analyze various attributes, particularly those regarding texture. While a particular instrument can quantify color or the degree of staling, for instance, no instrument can perceive and characterize food texture (Munoz and Civille, 1987). However, no instrument has been able to imitate the human mouth. Utilizing both means could provide valuable information to be employed by the food manufacturer.

5.2B. Appearance

The appearance of a product is the main attribute which largely influences a consumer's decision to either purchase or consume a product (Meilgaard et al, 1991). Consumers are accustomed to seeing baked products, such as cookies, of all the same color and size. If several of those cookies were black as opposed to light brown, the consumer might reject the product as a whole. The sense of vision can also influence one's perception of texture (Szczesniak, 1981). A flat, concave cake for example, might indicate that the cake is dense and adhesive. An overbaked pie might suggest that the crust is crumbly and fragile.

5.2C. Crust Color

Table 12 depicts the mean crust color scores obtained for the five muffin variations. While all of the muffins containing emulsifiers were perceived to have a color comparable to the control, the version containing HPMC and enzymes was significantly ($p<0.01$) darker. Physical data also established that this same version was darker, although no significant differences ($p>0.01$) were found (Table 1). The mean obtained for the control muffin was slightly lower than the other variations perhaps demonstrating that the incorporation of enzymes into the formula enhanced browning. A typical muffin is expected to be a light brown color (Bennion, 1990c). However, the values obtained for the muffins variation were middle of the rating scale, which was indicative of a muffin that was rated as neither light or dark.

5.2D. Texture in Product Development

Over the past decade, consumers have developed a heightened awareness in texture (Szczesniak, 1981), which can be attributed to the desire to consume "healthier" products. Fat performs several functions which impart different textural properties. The food industry has responded to this need to reduce fat by reformulating products. This reformulation of foods is a challenge to create textures that are comparable to their traditional counterparts (Jack et al., 1995). In order to achieve this, people within the food industry must acquire a full understanding of how consumers perceive texture and how to measure this perception. The term "texture" encompasses several meanings that occur both prior to and during food consumption (Jack et al., 1995). For example, handling or squeezing a muffin can

Table 12: Mean sensory scores for perceived crust color for the five muffin variations

VERSION	TREATMENT◆	PERCEIVED CRUST COLOR ± SD*,**,***
A	HPMC and Enzymes Alone	$6.97_b \pm 0.781$
B	HPMC, Enzymes, and SSL	$6.12_a \pm 0.564$
C	HPMC, Enzymes, and DMG	$5.83_a \pm 0.385$
D	Control	$5.22_a \pm 0.587$
E	HPMC, Enzymes, and DATEM	$5.30_a \pm 0.683$

◆Refer to Table 1 for definitions.
* Values with the same letter denote no significant differences ($p > 0.01$).
** SD = Standard Deviation
*** **Crust Color:** The degree of lightness/darkness perceived on the surface of the muffin (0 = light and 15 = dark)

provide an initial idea as to the toughness or springiness of the muffin. While there does not appear to be an universal definition of texture, many people feel that texture comprises both sensory and physical properties. An appropriate definition of texture is defined by Jowitt (Jack et al., 1995) as being "the attribute of a substance resulting from a combination of physical properties and perceived by the senses of touch (including kinesthesis and mouthfeel), sight, and hearing. Physical properties may include size, shape, number, nature and conformation of constituent structural elements".

It is evident that texture comprises numerous terms, ranging from firmness and cohesiveness, to oily and crunchy. With respect to baked products, some of the various terms used to describe texture are moistness/dryness, springiness, cohesiveness, and oiliness (Meillgaard et al., 1991). In this particular study, the textural attributes that were analyzed in the muffins were: moistness, aftertaste, cohesiveness, adhesiveness, and cell size.

5.2E. Moistness

The control muffin was found to be significantly ($p<0.01$) more moist when compared to the other versions (Table 13). The perception of moistness in the control can be attributed to the oil used in the formula. Oil, as the word implies, sometimes tends to form a light "coating" on the mouth, particularly the lips. Although consumers are generally aware of the physical changes that occur as a food is being masticated, the thermal and salivary changes are less obvious to them, unless there is not enough lubrication provided by these changes to breakdown the product. The presence of oil creates an easier and smoother breakdown of the baked product, therefore, it is easier to swallow (Munoz and Civille, 1987).

Table 13: Mean sensory scores for perceived moistness for the five muffin variations

VERSION	TREATMENT♦	PERCEIVED MOISTNESS ± SD*,**,***
A	HPMC and Enzymes Alone	$5.26_a \pm 0.781$
B	HPMC, Enzymes, and SSL	$5.71_a \pm 0.564$
C	HPMC, Enzymes, and DMG	$5.46_a \pm 0.385$
D	Control	$7.42_b \pm 0.587$
E	HPMC, Enzymes, and DATEM	$5.96_a \pm 0.683$

♦Refer to Table 1 for definitions.
* Values with the same letter denote no significant differences ($p > 0.01$).
** SD = Standard Deviation
*** **Moistness:** The degree of dryness/moistness perceived when the muffin is sampled (0 = dry and 15 = moist)

Comparing the objective moisture content (Table 8) to the perceived moistness (Table 13), it was interesting to note that the control version had a lower percentage of moisture, however the panelists perceived this version to be the most moist. Perhaps the panelists associated the oiliness imparted by the fat as moistness, and therefore were unable to differentiate between the two attributes. Again, with respect to the physical measurements, versions containing DATEM and SSL retained a high amount of moisture, and the panelists rated these two versions as being more moist than the other reduced fat versions. While the version containing HPMC and enzymes alone retained more moisture than those muffins containing DMG (or the control) (Table 8), the panelists perceived the version containing HPMC and enzymes as the least moist. Even though HPMC can bind and retain water molecules, as evident by its high moisture content, the version containing DMG and HPMC could not. Since DMG is hydrophobic, it is possible that it could not retain during baking.

5.2F. Aftertaste

The attribute "aftertaste" was used as a term to represent the degree of either a pleasant (or unpleasant) sensation that remained in the mouth after swallowing the muffin sample. All of the reduced fat muffins were significantly ($p<0.01$) different from the control muffin (Table 14). The version containing HPMC and enzymes alone was perceived as having the most aftertaste. The component partially responsible for this off-taste could be attributed to the enzymes. The extra sugars produced by the enzymes induced flavor and taste compounds through enzymatic browning (Sproessler, 1993). However, these compounds

Table 14: Mean sensory scores for perceived aftertaste for the five muffin variations

VERSION	TREATMENT◆	PERCEIVED AFTERTASTE ± SD*,**,***
A	HPMC and Enzymes Alone	$5.78_a \pm 1.604$
B	HPMC, Enzymes, and SSL	$6.26_a \pm 1.129$
C	HPMC, Enzymes, and DMG	$6.05_a \pm 0.501$
D	Control	$7.54_b \pm 0.415$
E	HPMC, Enzymes, and DATEM	$6.49_a \pm 0.564$

◆Refer to Table 1 for definitions.
* Values with the same letter denote no significant differences ($p > 0.01$).
** SD = Standard Deviation
*** **Aftertaste:** The degree to which either a pleasant/unpleasant taste is perceived in the mouth (0 = unpleasant and 15 = pleasant)

were found unfavorable to the panelists. Perhaps the emulsifiers, which are fat derivatives, imparted a slightly pleasant flavor to the muffins. The use of emulsifiers might have also "diluted" the excess sugar created by the enzymes, thereby masking the aftertaste. While amylases are known for inducing flavor compounds through their amylolytic action, it is possible that the panelists misconceived the aftertaste attribute. Perhaps the panelists were detecting bitterness or sweetness to a certain degree. Although the panelists were asked to refrain from eating 30 minutes prior to testing, some disregarded this request several times throughout this study. This could have exerted a negative influence on how the muffins were perceived. Although the reduced fat muffins were perceived as tasting less pleasant than the control, all values were approximately in the middle of the scale, indicating that neither the reduced fat or the control muffins exhibited a high degree of a pleasant/unpleasant aftertaste.

5.2G. Cohesiveness

Cohesiveness was defined by the panelists as the degree to which the muffin either stayed intact or crumbled as it was pulled apart. A more tender muffin, should be less cohesive and easier to be pulled apart. Table 15 shows the mean cohesiveness scores for the muffin variations.

All of the reduced fat muffins were significantly ($p<0.01$) more cohesive than the control. These findings complement the physical data obtained for assessing firmness (Table 6), which also found the muffins containing HPMC and enzymes alone (and those containing SSL and DMG) to be significantly firmer compared to the control. Although HPMC has the

Table 15: Mean sensory scores for perceived cohesiveness for the five muffin variations

VERSION	TREATMENT◆	PERCEIVED COHESIVENESS ± SD*,**,***
A	HPMC and Enzymes Alone	$9.56_a \pm 0.546$
B	HPMC, Enzymes, and SSL	$7.95_a \pm 2.333$
C	HPMC, Enzymes, and DMG	$9.14_a \pm 0.775$
D	Control	$4.18_b \pm 2.432$
E	HPMC, Enzymes, and DATEM	$7.04_a \pm 0.555$

◆Refer to Table 1 for definitions.
* Values with the same letter denote no significant differences ($p > 0.01$).
** SD = Standard Deviation
*** **Cohesiveness:** The extent to which the muffin either stays intact or crumbles as it is pulled apart (0 = crumbles and 15 = stays intact)

ability to retain water, thus forming a rigid structure, it is possible that this compound was not hydrated enough in the version containing HPMC and enzymes alone. Perhaps the additional sugar molecules created by the enzymes competed with the HPMC for water. This could have led to a more cohesive structure.

Both the sensory and physical data show the significance of the incorporation of polar emulsifiers into the formula. DATEM and SSL contributed to a less cohesive muffin when compared to those versions containing DMG and HPMC and enzymes alone. DATEM and SSL have the ability to bind water and trap gas cells which promote a larger volume. A larger volume was attributed to a softer crumb (Table 7). The control muffin was found to be the least cohesive, and this can be attributed to the oil used in the formula. The oil interferes with protein-starch interaction, thus creating a softer muffin. Fulton and Hogbin (1992) assessed the eating quality of muffins, cake, and cookies prepared with reduced fat and sugar. The investigators, using a sensory panel, found that tenderness decreased as the fat was reduced in the specific product.

5.2H. Adhesiveness

Adhesiveness and cohesiveness appear to be synonymous, however, they are different. While cohesiveness is more related to deformation, adhesiveness was defined as the degree to which the sample adhered to the teeth or palette. No significant differences ($p>0.01$) were found in the muffin variations with respect to adhesiveness (Table 16). All values for adhesiveness were between 5.2-6.0, which indicated that none of the muffins adhered to neither the teeth nor palette to a great extent.

Table 16: Mean sensory scores for perceived adhesiveness for the five muffin variations

VERSION	TREATMENT◆	PERCEIVED ADHESIVENESS ± SD*,**,***
A	HPMC and Enzymes Alone	$5.26_a \pm 0.970$
B	HPMC, Enzymes, and SSL	$5.86_a \pm 0.689$
C	HPMC, Enzymes, and DMG	$5.83_a \pm 0.850$
D	Control	$5.78_a \pm 0.360$
E	HPMC, Enzymes, and DATEM	$6.02_a \pm 0.535$

◆Refer to Table 1 for definitions.
* Values with the same letter denote no significant differences ($p > 0.01$).
** SD = Standard Deviation
*** **Adhesiveness:** The degree to which the muffin adheres to the teeth and palate (0 = not sticky and 15 = very sticky)

5.2I. Cell Size

Another important component of texture is the geometrical characteristics that comprise a product. With respect to baked goods, such characteristics refer to the perception of particles (i.e., size, shape, orientation) measured either by tactile means or through vision (Meillgaard et al., 1991). Geometrical properties can often influence consumer acceptance (Munoz and Civille,1987). For example, the presence of flour clumps or tunnels in a cake can lead to a negative influence about quality. In baked products, the size of the air cells formed in the batter or dough is a good indicator of the grain of the crumb (i.e., coarse, fine, etc.). A well-made muffin is uniform in texture, however, the cell walls are of medium thickness. As a result, the crumb grain is not very fine (Bennion, 1990c).

Table 17 depicts the mean scores obtained by the sensory panelists for cell size. Data did indicate that enzymes had an effect on cell size. The control muffin was perceived as having significantly ($p<0.01$) larger sized cells when compared to muffins containing HPMC and enzymes alone. Through starch modification, amylases can improve gas retention properties, and the addition of fungal amylase had been shown to lead to better machinability and a finer crumb (Sproessler, 1993).

Data also indicated that the emulsifiers was influential in reducing cell size. Muffins with DATEM, SSL, and DMG had significantly ($p<0.01$) smaller cells when compared to the control. DATEM, SSL, and DMG have been found as being very effective at retaining gas, and therefore, can improve loaf volume and yield a resilient and fine grain (Tamstorf et al, 1986).

Table 17: Mean sensory scores for perceived cell size for the five muffin variations

VERSION	TREATMENT◆	PERCEIVED CELL SIZE ± SD*,**,***
A	HPMC and Enzymes Alone	$6.05_b \pm 0.461$
B	HPMC, Enzymes, and SSL	$5.55_a \pm 0.694$
C	HPMC, Enzymes, and DMG	$5.76_a \pm 0.596$
D	Control	$7.41_c \pm 1.458$
E	HPMC, Enzymes, and DATEM	$5.95_a \pm 0.521$

◆Refer to Table 1 for definitions.
* Values with the same letter denote no significant differences ($p > 0.01$).
** SD = Standard Deviation
*** **Cell Size:** The size of the cells in the muffin interior (0 = small and 15 = large)

Chapter 6

Summary, Conclusions, and Suggestions for Future Research

Americans are aware of the potential dangers that could result from the high consumption of fat. There is a strong correlation between the amount of fat consumed and the risk of coronary heart disease, diabetes, and stroke (Hollingsworth, 1996). As a result, Americans are purchasing more reduced/no fat foods. According to a 1993 national survey conducted by the Calorie Control Council (CCC), 136 million American adults consume low fat, reduced fat, or fat-free foods and beverages (Hollingsworth, 1996).

Consumers have high expectations of foods. They want high quality and indulging foods. Consumers want foods such as baked products to taste creamy, rich, and moist. They want to taste the flavor and texture that fat employs, yet they want the fat to be removed. The food industry is working diligently to produce foods that are lower in fat while maintaining the same textural properties that fat employs.

The purpose of this study was to formulate a reduced fat muffin that would yield comparable quality properties to a full fat muffin and would be appealing to consumers as well. The reformulated muffin consisted of a carbohydrate based fat substitute, specifically, hydroxypropyl methlycellulose (HPMC). The emulsifiers SSL, DMG, and DATEM were individually incorporated into the formula, along with a combination of fungal and bacterial α-amylase, in order to assess which ingredient(s) simulated the properties of the fat in the baked muffin. Quantitative descriptive analysis (QDA) was also implemented to assess how the muffins were perceived.

6.1 Summary

No significant differences ($p>0.01$) in crust L and b values were detected instrumentally, indicating that the sugars created by the enzymes participated in the Maillard reaction to produce browning comparable to the control muffin. The sensory panel, however, perceived the version containing only HPMC and enzymes to be significantly ($p<0.01$) darker. The crumb of the control muffin was also found to be significantly ($p<0.01$) darker in color than the other variations. This was attributed to the carotenoids in the egg yolk, a component not included in the other versions. The sensory panel did not assess crumb color.

Specific gravity and volume are two parameters that relate to crumb firmness. A lower specific gravity is indicative of a high degree of air incorporation into a batter, which should result in a high volume (Penfield and Campbell, 1990a). No significant differences ($p>0.01$) in specific gravity were found among the muffin variations, thus indicating equal entrapment of air among the five variations. However, significant differences in volume ($p<0.01$) were found in versions containing HPMC and enzymes alone and HPMC, enzymes, and DMG. The polar emulsifiers, DATEM and SSL, produced volumes comparable to the control. These polar emulsifiers can surround and entrap gas cells, which would contribute to a higher volume. The version containing HPMC, enzymes, and DMG however, had the lowest volume among the variations. DMG probably competed with the HPMC for liquid and could not become hydrated enough, thereby resulting in a lower volume. The fact that DMG is hydrophobic might imply that the water used to hydrate this emulsifier was lost during baking and could not be retained by this compound.

The control muffin was significantly ($p<0.01$) less firm than the other variations. The variations made with DATEM (followed by SSL) produced softer muffins when compared to the other fat reduced versions. DATEM in its hydrated form can exert a softening effect comparable to the control. DATEM cannot only entrap gas cells, but also can bind to polar water molecules. As a result, more water could be retained and impart a softer crumb. The enzymes could also have contributed softness by hydrolyzing the amylose, thus reducing the degree of protein-starch interaction. The control muffin was soft as well, which can be attributed to the oil used in the formula. The oil was dispersed throughout the other ingredients, and therefore could interfere with the interaction between gluten proteins and starch.

The control muffin contained the least amount of moisture, which was significantly different ($p<0.01$) from the other variations, however, this could be due to other extraneous variables such as temperature and relative humidity. The fact that all the other versions retained a higher degree of moisture can be ascribed to the HPMC, a polar compound capable of hydrogen bonding to water molecules. When DATEM and then SSL were used in conjunction with the HPMC, more moisture was retained compared to the version containing HPMC and DMG.

The panel found the muffin containing fat to be significantly ($p>0.01$) more moist than the other versions. The moisture the panelists perceived however, was the oil and not the actual moisture content. Although the other versions retained more moisture, this "bound" water was interacting with other molecules and therefore was not perceived as moisture.

DATEM and SSL produced muffins that were perceived to be nearly as moist as the control, while the versions containing DMG or HPMC and enzymes alone were less moist.

No significant differences ($p>0.01$) were found in water activity among the different muffins 1 hour after baking and 24 and 48 hours after storage. The control muffin had the lowest water activity at each stage, which complies with the decreased moisture content. Although each variation had a water activity greater than 0.9, they did slightly differ in moisture content.

Significant differences ($p<0.01$) in the degree of staling were found after 24 hours storage, although no significant differences ($p>0.01$) were found in the staling rate after 48 hours storage. Although all versions became stale to a certain degree, the control muffin and those muffins containing DATEM staled at a significantly ($p<0.01$) slower rate than the other versions after 24 hours storage. This demonstrated the effectiveness of the enzymes and the hydrated, polar emulsifier. The bacterial enzyme, which is more thermostable than fungal amylase, produces dextrins, thereby delaying the reassociation of amylose. Contrary to other research findings, DATEM inhibited the staling rate most effectively. Although DATEM does not complex with amylose to the extent as DMG, DATEM is more polar and could retain more moisture. Perhaps DMG was not hydrated enough and therefore could not exert its maximum effect. Even though SSL is more polar than DATEM or DMG, a higher degree of staling after 24 hours was observed. The fact that SSL was not hydrated could also explain the slightly higher degree of staling.

All of the reduced fat muffins were perceived as having an aftertaste significantly ($p<0.01$) different when compared to the control muffin. The sugars produced by the enzymes

might have created a slight aftertaste by interacting with other ingredients. The use of emulsifiers might have masked, or "diluted" the perceived aftertaste. However, values obtained by the panelists were middle of the scale which indicated that neither the reduced fat or the control muffins exhibited a high degree of a pleasant/unpleasant aftertaste.

All of the reduced fat muffins were significantly ($p<0.01$) more cohesive than the control. Muffins containing DATEM and SSL were less cohesive than the versions containing DMG and HPMC and enzymes alone. The HPMC could entrap moisture, therefore contributing to a more rigid structure, however, the lack of a polar emulsifier to bind moisture and trap gas cells resulted in increased cohesiveness. The excess dextrinization created by bacterial amylase could have also increased cohesiveness as well. No significant differences ($p>0.01$) in adhesiveness were detected in the muffins, indicating that none of the variations were adherent to the teeth or palette to a great extent.

The control muffin contained significantly ($p<0.01$) larger sized cells than the reduced fat muffins. HPMC, in addition to polar emulsifiers as well, which retain air and increase batter viscosity. It is this action that resulted in smaller cells when compared to the control. The enzymes also contributed to the smaller cells through starch modification during baking.

6.2 Conclusions

The results obtained from the physical and sensory data indicated that there were similarities between the reduced fat muffins containing emulsifiers, particularly DATEM and SSL, and the full fat control. There were some attributes displayed in these reduced fat muffins

that were not as comparable to the full fat muffin such as crumb firmness and moisture content. More research needs to be conducted to reproduce these aforementioned quality attributes.

6.3 Suggestions for Future Research

In this respect, research should be conducted on the incorporation of different forms of emulsifiers into various baked product formulas. DMG might have exerted more of a positive effect if it had been hydrated prior to its addition to the formula. Another area would be to focus on using the emulsifiers in combination. DATEM, in its hydrated form, along with DMG, for example, could be used in conjunction to enhance their effects. The use of a carbohydrate based fat substitute in a prehydrated form should be assessed as well, in order to determine how the textural properties would be affected when combined with a polar emulsifier.

Additional research needs to be conducted on the usage levels of enzymes. In this study, both fungal and bacterial amylase were used at an equal level. Perhaps less bacterial amylase should have been used, while keeping the level of fungal amylase constant. The results indicated that the enzymes, when used with DATEM, had a staling rate comparable to the control. Incorporating enzymes that exhibit stability in between that of fungal and bacterial amylase, (i.e., "intermediate temperature stability enzymes") (Hebeda and Teague, 1992), into a muffin formula might be beneficial in prolonging retrogradation, while at the same time improving loaf volume and a finer crumb without gumminess. Research also should be focused on the use of fungal and bacterial amylase in baked products to assess their affect on flavor.

Another research criteria would be to assess how high fructose corn syrup (HFCS) and DATEM, or another polar emulsifier, would interact in a muffin formula. The HFCS is an extremely effective humectant agent, and therefore might retain more moisture. DATEM, which retains moisture as well, could also entrap gas cells to provide volume (and tenderness) to the product. The two ingredients might work synergistically to provide an acceptable muffin.

There is potential for the use of emulsifiers, enzymes, and a carbohydrate based fat substitute to be employed in muffins in the food industry. Conducting more sensory research would enable food companies to determine how consumers perceive reduced fat muffins. This information could be used to reformulate reduced fat muffins that resemble the full fat version with respect to flavor and textural properties. The researcher, however, should remember when reformulating these products to select ingredients that will maintain quality, but will not increase the caloric content of the product. The product may not be high in fat, but the ingredients used to mimic the functional properties of fat may contribute more calories.

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Appendix A

Experimental Design

Table 18: Experimental Layout for an Incomplete Block Design*

Day	Variations**		
1	A	B	C
2	A	B	E
3	A	D	E
4	B	C	D
5	C	D	E
6	A	B	D
7	A	C	D
8	A	C	E
9	B	C	E
10	B	D	E

* t=5, k=3, r=6, b=10, λ=3

** Variation A= 100% Fat Substituted muffins with enzymes
B= 100% Fat Substituted muffins with enzymes and SSL
C= 100% Fat Substituted muffins with enzymes and DMG
D= Control
E= 100% Fat Substituted muffins with enzymes and DATEM

Appendix B

Muffin Formulations

Table 19: Formulations of regular and reduced fat muffins*

INGREDIENTS	CONTROL	100% REDUCED WITH ENZYMES	100% REDUCED WITH ENZYMES AND SSL	100% REDUCED WITH ENZYMES AND DMG	100% REDUCED WITH ENZYMES AND DATEM
Flour, all-purpose**	225.0g	225.0g	225.0g	225.0g	225.0g
Sugar, granulated	100.0g	105.0g	105.0g	105.0g	100.0g
Baking Soda	2.0g	2.0g	2.0g	2.0g	2.0g
Baking Powder	11.4g	11.4g	11.4g	11.4g	11.4g
Salt	2.5g	2.5g	2.5g	2.5g	2.5g
Eggs	43g	43g (egg whites)	43g (egg whites)	43g (egg whites)	43g (egg whites)
Nonfat Buttermilk	125ml	130ml	130ml	130ml	130ml
Skim Milk	125ml	130ml	130ml	130ml	130ml
Vegetable Oil	73ml	-	-	-	-
Vanilla	5ml	5ml	5ml	5ml	5ml
Fat Substitute	-	0.75g	0.75g	0.75g	0.75g
Enzymes	-	0.006g	0.006g	0.006g	0.006g
Emulsifier	-	-	1.125g	1.125g	1.125g

* The regular (full-fat) muffin contains approximately 10% fat by weight and the reduced fat versions (B, C, and E) contain 0.17% fat by weight.

** Southern Biscuit All-Purpose flour contains 8.3% protein.

Appendix C

Muffin Ingredients

(1) Southern Biscuit All-Purpose Flour (Midstate Mills, Inc., Newton, N.C.)

(2) Granulated Sugar

(3) Arm and Hammer Natural Baking Soda (Arm and Hammer, Princeton, N.J.)

(4) Calumet Double-Acting Baking Powder (Kraft General Foods, Inc., White Plains, N.Y.)

(5) Morton Iodized Salt (Morton International, Inc., Chicago, IL.)

(6) METHOCEL Hydroxypropyl Methylcellulose (F50 Premium, DowChemical Company, Midland, MI.)

(7) Fresh-N Bacterial Amylase and Enzeco Fungal Alpha Amylase 5000 (Enzyme Development Corporation, New York, N.Y.)

(8) STARPLEX 90K (Kosher DMG, American Ingredients Company, Kansas City, MI.)

(9) PANODAN (DATEM, Danisco Ingredients USA, Inc., New Century, KS.)

(10) EMPLEX K (SSL, American Ingredients Company, Kansas City, MI.)

(11) Skim Milk (pasteurized and homogenized)

(12) Nonfat Buttermilk (cultured and pasteurized)

(13) Vegetable Oil (Hunt-Wesson, Inc., Fullerton, CA.)

(14) Pure Vanilla Extract (35% alcohol)

Appendix D

Determination of Crust and Crumb Color

Sample Preparation

(1) Three muffins were randomly selected from each batch that had been cooled for one hour prior to testing.

(2) Crust and crumb color were assessed. After analyzing the crust color, the muffin was sliced horizontally to measure crumb color.

Operating Instructions for the Hunter Colorimeter

(1) A Toshiba T1000 System Unit Laptop PC (Tokyo, Japan, #PA7027U) was connected to a Hunter Lab L Optical Sensor D25 (Reston, VA.) that was placed on "Stand-by" one hour before testing. The printer was also turned on.

(2) Prior to the analysis, the Hunter Lab was switched to the "operate" mode.

(3) The uncalibrated white tile, which remains on the sample port during periods of nonuse, was removed prior to standardization.

(4) To zero the instrument, a calibrated black tile was first placed on the sample port, with the shiny side facing up towards the port.

(5) The F1 button, which designated the "read" function, was pressed to "zero" the instrument.

(6) The black tile was removed and the white calibrated tile was placed on the sample port.

(7) The F1 button was pressed to set the instrument in the standardization mode. As soon as the instrument was in the standardization mode, the white tile was read (F1) and L, a, and

b values appeared on the screen. The white tile is used to standardize the instrument. The tile was then removed.

(8) The F8 button was pressed to display a menu of indices. From this menu, button number 1 was pressed. This sets the instrument in the measurement mode. The F2 key was then pressed to obtain Delta E values. To print the data, F5 was pressed. The instrument had been standardized at this point and was ready for sample analysis.

(9) The muffin sample was placed on the sample port, positioned so that no light could enter.

(10) F1 was pressed and the L, a, b and Delta E values were displayed on the monitor.

(11) F5 was pressed to print the data.

Appendix E

Determination of Volume

(1) The muffins were cooled one hour prior to obtaining the measurements.

(2) Two muffins from each batch were randomly chosen.

(3) A dial caliper was used to ascertain the height and diameter of the muffin in centimeters:

- The height was determined by inserting the probe into the highest point of the muffin until it touched the base of the muffin. A value was then obtained.
- The diameter was assessed by placing the muffin in between the calipers.

(4) After the average of the two muffins were determined for height and diameter, the values obtained were incorporated into the following formula:

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

Where r=radius of the muffin and h=height of the muffin.

Appendix F

Determination of Crumb Firmness

(1) Turn on the Instron Model 1011, (Canton, MA.) and press the "GPIB" button, so the metered light is on, followed by the computer, monitor, and printer.

(2) Highlight "Test a sample", press enter.

(3) Enter your last name beside "Operator".

Enter a sample id for your sample (up to 6 letters).

The method should state "compressive" and the method number is 06.

(4) Press F10, "Continue".

(5) The menu: "Start specimen: 1" will appear.

Enter in the sample number (ex. 1A1), diameter, and the lens gauge (25% of diameter).

Press F10, "Continue".

(6) Remove the top (i.e., crust) off the muffin by making a horizontal incision in order to expose the flat crumb surface.

(7) Place the sample under the compression probe. Check the platen distance, which should be 5mm in this method. If this is not the distance, turn off the GPIB button and use the up and down buttons on the Instron to correct the distance. Turn the GPIB button back on.

(8) Press F10, "Continue".

Press F10, "OK".

Press Enter. This will begin the acquisition.

(9) When the compression is complete, the unit will sound a beep. At this time, press enter.

This will return the crosshead. Remove the sample. Press F10, "Continue".

(10) Follow steps 4-9 to analyze remaining samples.

(11) After all of the samples have been tested, press ESC. This automatically prints the data to the printer. The main menu will appear on the screen.

(12) Highlight "Quit", and press enter to exit to DOS.

(13) Turn off the computer, monitor, printer, and the Instron.

(14) Clear the compression probe and base.

Appendix G

Determination of Moisture Content

(1) Turn on the Brabender Moisture Tester Model SAS (Hackensack, NJ) machine one hour prior to analysis by pressing the switch up on the left hand side of the unit and on the bottom right front of the machine. This is done to enable the machine to reach the desired temperature of 130°C.

(2) The muffins cooled one hour prior to analysis.

(3) Six Teflon lined metal pans were weighed individually (two per batch) on an OHAUS balance Model CT 1200 (Florham Park, NJ). The balance was tared and the weights of the empty pans recorded.

(4) The interior of the muffin was torn into small pieces and ten grams of sample were weighed into the tared pan.

(5) After opening the door on the Brabender by twisting the knob on the oven door, the pan and sample were placed in the oven. Additional samples were placed in the oven by turning the black knob clockwise on the left side of the unit.

(6) After the sample(s) were loaded in the oven, they were dried until they reached equilibrium (approximately 1 1/4 hours).

(7) To determine when equilibrium was attained, quarterly hour measurements were taken by:

(a) Turning off the fan (i.e., by pressing the switch to the left) on the front of the unit.

(b) Opening up the door of the square unit by lifting up on the latch located on the right hand side.

(c) Placing the 2g weight on the center of beneath the samples

the circular piece and closing the door.

(d) Lifting the handle up on the left hand side of the unit. At this time, a scale should appear in the circular window on the right-front of the machine.

(e) Waiting for the scale to stabilize, record the value after adding 20 to this value (sample weight * weight of weight).

(f) The sample reached equilibrium when two quarterly readings had not differed by ± 2.

(8) After the sample reached equilibrium, it was removed from the oven and placed in a desiccator for 24 hours. The sample was reweighed the next morning using the same scale, and this number, along with the original pan and sample weights, were substituted into the following equation:

{[(wt. of empty pan(g) + 10g sample) - (wt. of pan + sample after 24 hours)] / 10} * 100

Appendix H

Determination of Water Activity

(1) Turn on the power switch 30 minutes prior to analysis to allow the Decagon Aqua Lab CX-Z Water Activity Meter (Pullman, WA.) to warm up. Four zeroes will be displayed when ready.

(2) To ensure that the meter is operating correctly, fill the plastic disposable cup (40mm X 13mm) intended for the use of instrument with room temperature distilled water until it is half full.

(3) Turn the knob to "load" and gently pull the drawer out and place the cup in the circular area.

(4) Close the drawer and turn the knob counterclockwise to "read". After the sample has been read, a series of beeps will be sounded and the values will blink. The distilled water should display a a_w of 1.000 $\pm$ 2C. If the unit does not display values within this range, the instrument needs to be re-calibrated.

(5) After the muffins had cooled for one hour, crumbs from the center of the muffins were taken and pressed into the bottom of the sample cup.

(6) Load the sample after removing the distilled water sample, close the drawer, and turn the knob to "read". Within a few minutes, the a_w and temperature will be displayed. When the samples have reached equilibrium, the values will blink and a series of beeps will be sounded. Turning the knob to "open" will discontinue the beeps.

(7) Load the next sample.

(8) After analyzing all of the samples, turn the knob back to load, place an empty disposable cup into the holder within the drawer, and close the drawer.

(9) Turn off the unit.

Appendix I

Determination of Staling Rate

(1) Turn on chiller/circulator (Fisher Scientific Model 9105, Pittsburg, PA) one hour prior to analysis.

(2) Turn on the DSC-7 TAC 7/DX controller (Perkin Elmer, Norwalk, CT.)and the sample unit, followed by the computer (Digital PC), monitor and plotter (Hewlett Packard, Model 7475A, San Diego, CA.).

(3) Place a piece of non-glossy printer paper longways into the printer.

(4) Wait for the system to boot (approximately 60 seconds). Enter the date and time if it is not correct. If these are correct, press ENTER.

(5) At the command prompt "Scosysv!login", type PETA and press ENTER.

(6) The word "PASSWORD" will then appear. Press ENTER.

(7) Highlight "Run 7 Series UNIX System" from the main menu. Press ENTER.

(8) Highlight "*2". Press ENTER. Wait a few seconds until the "Perkin Elmer Thermal Analysis Software - Task 1 - Ready Screen" appears.

(9) Press F2, "Set Up and Run". Press ENTER.

(10) Press F2, "Recall Method". Highlight the last IND method from the hard drive that has already been run and stored. For example, it is usually labeled as IND and the date (i.e., IND109). Press ENTER.

(11) Press F3, "Modify Parameters". Move to the parameter via the arrow keys and then make the change, such as operator name, file name, and weight (in mg). Press F1, "Exit".

(12) Press F11, "Go to Temp". Put in the starting temperature, which should equal the minimum temperature of the run. The starting temperature for the Indium standard is 50.0 °C. The screen should read "Analyzer going to temperature". Press F1, "Exit".

(13) Wait for the milliwatt signal to stabilize (upper left corner) before loading the sample.

(14) When the control light on the DSC-7 controller unit comes on and the milliwatt reading has stabilized, load the sample. This is done by lifting the hinge and gently turning it to the right. This exposes the sample holder and load cells. Place the standard to the left, the reference to the right. Close the lid and gently press the hinge back down.

(15) Wait for the control light to come back on and the milliwatt reading to restabilize. At this time, press F8, "Start Run".

(16) At the end of the run, press ENTER, then press F5, "Optimize Data".

Press F2, "Rescale".

Press F3, "Autorescale".

Press F1, "Exit", twice.

Press F4, "Slope".

Press F3, "Align Endpoints".

Press F1, "Exit".

Press F6, "Select Calculation".

Press F2, "Peak".

Press F4, "Include Onset". At this time, use the left and right buttons on the mouse to define the start and finish of the peak, respectively.

Press F7, "Autocalculation".

Press the "Print Screen" button, and press "Enter" on the plotter once the monitor reads "Plot is queued".

(17) If the onset temperature of the standard is 156.6 ± 0.2 ° C, the instrument is calibrated. If not, calibration of the instrument is necessary.

(18) If the onset temperature is within limits, press F11 and enter 17.0 ° C, so the unit can be cooling down to 17.0 ° C, to run the samples.

(19) Remove the Indium standard only.

(20) Press F2, "Set Up and Run", and then F3. Make the necessary changes. Press F1, "Exit". Wait for the milliwatt reading to stabilize and for the control light to come on before loading a sample. See Sample Preparation.

(21) After the sample has been prepared, load the sample into the left side of the sample holder after the control light is lit and the milliwatt reading has stabilized.

(22) Wait for the control light to come back on and for the milliwatt reading to stabilize. When this occurs, press F8, "start run".

(23) At the end of the run:

Press ENTER.

Press F5, "Optimize Data".

Press F2, "Rescale".

Press F4, "Rescale X axis".

Type in 30, ENTER, 120, ENTER.

Press F2, "Rescale".

Press F1, "Exit".

Press F3, Shift y".

Type in 0, ENTER, 2, ENTER.

Press F1, "Exit".

Press F3, "Shift y".

Press F2, "Shift y".

Type in 0.4, ENTER.

Press F1.

Press F4, "Slope".

Press F3, "Align End points".

Press F1, twice.

Press F6, "Select Calc".

Press F2, "Peak".

Press F4, "Include Onset".

Use the mouse to define the beginning and end of peak.

Press F2, twice.

Press the "Print Screen" button, the Enter on the plotter.

(24) Run remaining samples by following steps 18-23.

Sample Preparation

(1) Calibrate the microbalance (Perkin Elmer, Norwalk, CT.). Gently twist the gray knob on the left hand side to lower the holders.

(2) Press autotare. Zeroes should be displayed.

(3) Twist the gray knob until the holders support the discs.

(4) Place a 20mg weight on the balance, release the supports and make sure the balance displays 20.0. Remove the weight and raise the support. If it does not, the machine needs to be calibrated. Follow in calibration procedure in the manual.

(5) Obtain the top and bottom of the stainless steel large volume pan (top-flat; bottom has a lip) (Part # 0319-0218, Perkin Elmer, Norwalk, CT.) and place the sample pan on the bottom portion on the scale. Lower the supports and press autotare. Raise the supports and remove the pan.

(6) Remove a small portion of crumb from the center of the muffin, roll it into a small ball and place it in the pan. Weigh. The sample should be 40.0 mg $\pm$ 0.3.

(7) Place an "O"-ring in the top portion of the pan and place on top of the bottom pan.

(8) Place the sample pan with lid and "O" ring on top of sample holder and place in the crimper (Perkin-Elmer Norwalk, CT.). Crimp by pressing the handle down.

To Calibrate the DSC

(1) Press CONTROL+S (Shutdown)

(2) Press "Y", yes, to the question, "Do you want to shutdown the system".

(3) Wait approximately 1 minute.

(4) At the main menu, highlight "Calibrate an Analyzer". Press ENTER.

(5) Press F2, "Calibrate Temp/Area".

(6) The screen will display several parameters. In the bottom left hand corner where it reads "Measured Onset Temperature and Measured J/g", enter in those values obtained from the plot of the last run Indium standard.

(7) Press F7 Begin Cal".

(8) Press "Ctrl Shutdown", then "Y".

(9) From the main menu, highlight "Run Series UNIX System". Press ENTER.

(10) Select "*2", ENTER.

(11) Recall the method Calind3S and run the Indium standard again to see if the unit is calibrated.

Shutdown Procedure:

(1) Press CTRL Shutdown (the "zero" button). Answer (y) for yes to shutdown all processes. When the monitor reads, "Are you sure?", type in "yes".

(2) Wait one minute.

(3) Highlight "Shutdown the Unix Operating System".

(4) When the monitor reads "System is now shutting down. Safe to turn power off", turn on the TAC7/DSC-7 Unit, DSC-7 Controller, computer, monitor, and plotter.

(5) Remove the sample and reference pans.

(6) Turn off the chiller/circulator.

Table 20: Parameters for the Indium Standard and Muffin Samples

Parameter	Indium Standard	Muffin Sample
Sample Weight (mg)	4.359	40± 0.3
Initial Temperature (°C)	50	17
Final Temperature (°C)	175	140
Heating Rate (°C/min)	10	10
Cooling Rate (°C/min)	320	320
Temperature Span (°C)	400	400
Y Range	5	5

Appendix J

QDA Training Sessions

The eleven panelists only participated in three training sessions due to time constraints.

First training session

(a) The panelists were given an introduction to QDA through the use of overheads by the investigator in Wallace Hall.

(b) Panelists generated specific terms related to muffins by comparing attributes from two reference standards, a full fat muffin and a reduced fat muffin. To generate these terms, the investigator asked questions relating to the appearance and texture without influencing panelists. The investigator served as a facilitator.

(c) All panelists generated descriptors that were written down on a transparency and shown to the panel by the investigator.

(d) The list of descriptors was condensed to the six terms of greatest importance. These terms would serve as the basis for the scorecard.

(e) Panelists defined these terms and placed them in the order of importance as follows: moistness, aftertaste, cohesiveness, adhesiveness, color, and cell size.

(f) Panelists signed consent forms, and then times were allotted for the remaining sessions.

Second and Third training sessions

(a) The purpose of these sessions was to introduce the panelists to the scaling technique and to assess how the panelist rated the intensity of each attribute. The consistency of the individual panelists and the panel as a whole was ascertained.

(b) The control muffin was manipulated in such as way for the panelists to be able to distinguish among attributes, starting with simple assessments and then to the more complex.

(c) For the second training session, 6 batches of muffins were made. Two batches of muffins were made to reflect different color intensities. One batch remained in the oven for only 14 minutes, while another batch remained in the oven for 22 minutes. Perceived aftertaste was done by comparing the regular control muffin to the control muffin with 4g baking soda. To evaluate perceived moistness, two batches were made. One containing 200g sugar, and the other containing no sugar.

(d) Cohesiveness, adhesiveness, and cell size were the attributes tested in the third training session. Six dozen muffins were made. To evaluate cohesiveness, the control was made, followed by the control without oil, 43g egg white, and 1g HPMC. For adhesiveness, the first batch were the control muffins. They were made with 200 mls of skim milk (and nonfat buttermilk) and 75 mls oil. The second dozen contained only 100 mls of each milk and 50 mls oil. To demonstrate differences in cell size, the control muffin with only 13g egg yolk and 20g egg white (whipped to a foam) was used, followed by the regular control muffin.

(e) Training sessions took place in the sensory evaluation lab (Wallace 339) in partitioned booths under white fluorescent lighting. The temperature remained between 70-72° F.

Sample Preparation

The muffins had cooled 30 minutes prior to sampling and stored in Tupperware™ containers for one hour.

(1) The muffins were assigned geometrical symbols.

(2) Paper plates were divided into 3 sections using a black marker and a symbol was written in each section. An exclamation mark was drawn at the top of each plate.

(3) A whole muffin was placed in its respective place.

(4) The samples and plate were placed on a plastic tray, along with a napkin, pencil, a cup filled with room temperature water, an empty paper cup, a scorecard, and definitions.

(5) Panelists received samples through the swinging doors.

Testing Procedures

(1) As the scorecard indicated, panelists tasted the sample directly underneath the exclamation mark and proceeded clockwise, sampling one muffin at a time. A sip of water was to be taken in between samples.

(2) After sampling a portion of the muffin, the panelist placed a vertical mark on the line scale that best represented their perceived intensity of a particular attribute.

Appendix K

QDA Attribute Definitions

QDA Attribute Definitions

Moistness:

the amount of moisture/dryness perceived in the mouth

Aftertaste:

the degree to which either a pleasant/unpleasant taste is perceived in the mouth

Cohesiveness:

the extent to which the muffin either stays intact or crumbles as it is pulled apart

Adhesiveness:

the degree to which the muffin adheres to the teeth and palate

Color:

the degree of browning observed when examining the crust

Cell Size:

the size of the cells in the muffin interior

Appendix L

Consent Form

Virginia Polytechnic Institute and State University Informed Consent for Participation in Sensory Evaluation

Title of Project: The Interactive Effects of Selected Emulsifiers, Enzymes, and a Carbohydrate Based Fat Substitute in a Low Fat Muffin

Principal Investigator: Pamela Mason

I. PURPOSE OF PROJECT:

You are invited to participate on a sensory evaluation panel that involves assessing the various sensory characteristics pertaining to a particular formulation of low fat muffins. Establishing such information will be correlated with physical measurements to ascertain how the emulsifiers, enzymes, and the carbohydrate based fat substitute interact with one another, and to see how such ingredients can exert the same characteristics observed in a full fat muffin recipe.

II. PROCEDURES:

There will be a total of 12 sessions, with 2 sessions being held each week for 6 weeks. Each session will be approximately 15 minutes, except for the 3 training sessions, which will be 45 minutes each. You will be presented with 3 samples at each session. As a panelist, it is critical to the project that you attend each session. If you can not attend a particular session, please let me know. Other arrangements can be made. Should you find a sample unpalatable or offensive, you may choose to spit it out and continue to other samples.

Certain individuals are sensitive to some foods such as milk, eggs, wheat gluten, strawberries, chocolate, artificial sweeteners, etc. If you are aware of any food or drug allergies, please list them on the following page.

III. BENEFITS/RISKS OF THE PROJECT:

Your participation in the project will provide useful information that can be used in further development of this product. All food additives have been approved by the Food and Drug Administration, so there are no risks involved provided you do not have any unknown food allergies. You may receive the results or summary of the panel when the project is completed.

IV. EXTENT OF ANONYMITY AND CONFIDENTIALITY

The results of your performance as a panelist will be kept strictly confidential. Individual panelists will be referred to by code for analyses and in any publication of the results.

V. FREEDOM TO WITHDRAW

It is essential to sensory evaluation projects that you complete each session in so far as possible. However, there may be conditions preventing your completion of all sessions. If after reading and becoming familiar with the sensory project, you decide not to participate as a panelist, you may withdraw at any time without penalty.

VI. APPROVAL OF RESEARCH

This research project has been approved by the Institutional Review Board for projects involving human subjects at Virginia Polytechnic Institute and State University and by the human subjects review of the Department of Food Science and Technology.

VII. SUBJECT'S RESPONSIBLITIES

I know of no reason I cannot participate in this study which will require meeting twice a week for 6 weeks.

_____

Please provide your address and phone number so I can reach you in case of an emergency or if the schedule should change for some unknown reason.

Address: _____

Phone:_____

Please list any allergies, medications, etc.:_____

VIII. SUBJECTS'S PERMISSION

I have read the information about the conditions of this sensory evaluation project and give my voluntary consent for participation in this project.

I know of no reason I cannot participate in this study which will require meeting twice a week for 6 weeks.

_____

Signature Date

Should I have any questions about this research or its conduct, I should contact:

Pamela Mason	951-1597 (h)	231-5375(lab)
Investigator		
Dr. Frank Conforti	231-8765	
Dr. Janet Johnson	231-6779	
Faculty		
Ernie Stout	231-6077	
Chair, IRB		
Research Division		

Appendix M

QDA Testing Procedures

Sample Preparation

The muffins had cooled 30 minutes prior to sampling and stored in Tupperware™ containers for one hour.

(1) The muffins were randomly assigned 3-digit numbers obtained from an Amtrak® timetable.

(2) Paper plates were divided into 3 sections using a black marker and the numbers corresponding to the muffins were written in each section. An exclamation mark was drawn at the top of each plate.

(3) A whole muffin was placed in its respective place.

(4) The samples and plate were placed on a plastic tray, along with a napkin, pencil, a cup filled with room temperature water, a scorecard, and definitions.

(5) Panelists received samples through the swinging doors.

Testing Procedures

Testing sessions took place in the sensory evaluation lab (Wallace 339) in partitioned booths under white fluorescent lighting. The temperature remained between 70-72° F.

(1) As the scorecard indicated, panelists tasted the sample directly underneath the exclamation mark and proceeded clockwise, sampling one muffin at a time. A sip of water was to be taken in between samples.

(2) After sampling a portion of the muffin, the panelist(s) placed a vertical mark on the line scale that best represented their perceived intensity of a particular attribute.

(3) Upon completion, the panelist(s) passed their tray through the swinging door and the investigator preceded to collect the scorecard and cleaned up.

Appendix N

QDA Scorecard

Sensory Evaluation of Muffins

Panel #:_____ **Date:_____**

Instructions: Please evaluate the samples provided to you in a clockwise fashion (starting at the (!) sign) and place a mark on each scale representing the intensity of each attribute. Please take a sip of water in between samples. Thanks.

Moistness:

Dry — Moist

Aftertaste:

Unpleasant — Pleasant

Cohesiveness:

Crumbles — Stays intact

Adhesiveness:

Not sticky — Very Sticky

Color:

Light — Dark

Cell Size:

Small — Large

VITA

Pamela Mason was born on August 7, 1970 in Newport News, Virginia. Receiving a Bachelor of Science Degree in Biology in 1993 at Virginia Polytechnic Institute and State University in Blacksburg, Virginia, she continued her education at this university and received a Master of Science Degree in Foods from the Department of Human Nutrition and Foods in June, 1996.

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