

THE INFLUENCE OF SELECTED BACTERIAL AND FUNGAL  
ENZYMES ON THE BAKING AND KEEPING QUALITY  
OF A FAT SUBSTITUTED MUFFIN

by

Robin L. Canterella

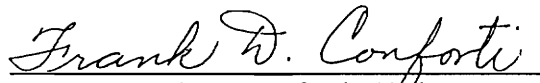
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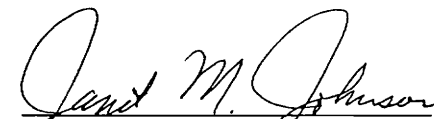
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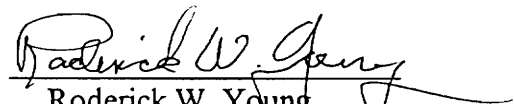
IN

HUMAN NUTRITION AND FOODS

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(ABSTRACT)

Utilization of a fat substitute (100% replacement) with and without added fungal protease, fungal amylase, and bacterial amylase in a muffin was compared to a full fat counterpart. The enzymes were evaluated independently and in combination with each other. Physical and sensory data were reported with a  $p < 0.05$  significance level.

The physical tests indicated that there were no significant differences ( $p > 0.05$ ) among any of the variations in volume, water activity (freshly baked, and after 24 and 48 hours storage), crumb L values and crust L and b values. The full fat muffin (control) was significantly ( $p < 0.05$ ) more tender than all formulations. In addition, the control had a significantly ( $p < 0.05$ ) lower moisture content and a significantly ( $p < 0.05$ ) more yellow crumb color than all the other variations. The 100% fat substituted muffins with enzymes, generally, had lower moisture contents, lower volumes, decreased staling rates, and an increased crumb tenderness when compared to the 100% fat substituted muffin without any enzymes. The 100% fat substituted muffins containing bacterial amylase or fungal protease alone had a significantly ( $p < 0.05$ ) lower staling rate than a 100% fat substituted muffin with a combination of bacterial amylase and fungal protease.

The QDA results indicated that the full fat muffin was perceived to be significantly ( $p < 0.05$ ) different in yellow crumb color, cell size, sweetness, tenderness, moistness, and adhesiveness when compared to 100% fat substituted muffins with and without enzyme. All 100% fat substituted muffins with and without enzyme were perceived to be significantly ( $p < 0.05$ ) more cohesive than the full fat muffin. There were no significant ( $p > 0.05$ ) differences in perceived aftertaste among any of the formulations.

The consumer test indicated that the 100% fat substituted muffin with the combination of fungal amylase and bacterial amylase was liked significantly ( $p < 0.05$ ) more than the full fat muffin.

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## Chapter 1

### Introduction

In today's society, consumers are bombarded with many foods from which to choose. Food manufacturers compete with one another to create the most desirable product. Desirability in a product is comprised of many different aspects. Taste(84%), product safety(71%), and nutrition(69%) were rated in that order as the most important factors to consider when buying foods. In this study by Bruhn et al. , (1992), 78% of respondents avoided certain foods due to nutritive content. The constituents of the most concern were total fat, saturated to unsaturated fat ratio, and cholesterol.

People are more aware of what they are eating and what effect it will have on their body. This increased interest in nutrition has led to a widespread "natural and healthy" phenomenon across the country. Consumers are actually looking for lower fat and nutritious food. Food processors and manufacturers realize this need and are developing such products to satisfy the general public.

Cardiovascular disease(CVD) and coronary heart disease(CHD) currently account for a large proportion of the morbidity and mortality in industrialized countries. CHD has many risk factors including fat intake which is closely linked to the development of atherosclerosis which in turn leads to blockage of coronary arteries and possibly a heart attack (Gurr, 1992). High intake of total dietary fat is also associated with an increased risk for obesity, some types of cancer, and possibly gall bladder disease (Hunter, 1992). Over the last ten years, the percentage of overweight American adults has ballooned from 58 to 66%. However, nine out of ten shoppers say they've changed their eating habits to

ensure a healthier diet. In addition, it was reported that Americans ate an average of 63 lbs. of fats and oils in 1990, up 10 lbs. since 1970 (Glenn, 1994).

It is a well documented fact that people in the industrialized world are in general eating too much. Working conditions have changed, leaving most people with less labor intensive work and consequently with a much reduced need for a high calorie diet. During the same time that these changes have taken place, living conditions have improved and thus made it possible for the majority of people in the industrialized countries to eat more than they can use. As a result, presently, one person out of three is overweight, and more and more people find themselves forced into some type of slimming operation from fasting to just temporarily cutting the daily intake of sweets and other foodstuffs they think that they can do without for the moment (Hageman, 1990).

Fat is vital to proper human growth and development. It provides essential fatty acids necessary to the structure of cell membranes and prostaglandins and also serves as a carrier for fat soluble vitamins A, D, E, and K. Although some dietary fat is necessary to health, too much saturated fat and total fat have been linked to an increased risk for CHD and obesity (Mahan and Arlin, 1992). By using fat substitutes, many of these fat soluble nutrients, such as the fat soluble vitamins and the essential fatty acids can be reduced. Consumers, especially females, on low fat dietary regimes need to be aware of risks (Strugnell, 1993). Dietary factors appear to be one controllable component in the multifactorial etiology of these diseases. The public is beginning to realize this and as a result are changing their habits and behaviors. Approximately 90% of women and 75% of men have expressed a concern about what they eat not only affects their future health but

they are also concerned about the fat and cholesterol in the foods they consume. The use of fat substitutes may facilitate the development of lower fat foods that provide sensory pleasure similar to those of traditional products, however, consumer perceptions of the quality and safety of modified foods could affect marketing success (Bruhn *et al.* , 1992).

These trends open an enormous hole for food manufacturers and processors to develop fat free products. With the advent of new fat substitutes, the possibilities seem exhilarating. A food with the nutritional label as the prime objective has missed the mark; the typical dieter wants to decrease calories without decreasing former eating pleasures. Taste is a very sharp concern. There is a great willingness on behalf of the consumer to spend for flavor. In most foods, when ingredient levels are altered to reduce calories satisfactorily, a loss of appearance, texture, and mouthfeel is noted readily by the consumer (Neville and Setser, 1986).

Fats are responsible for the texture, mouthfeel, and flavor of many foods. They also play a major role in determining the palatability of the diet (Drewnowski, 1992). Consumers want foods with less fat, but they do not want to sacrifice these aforementioned properties of fat. Companies across the United States and even around the world are searching for ways to reduce the level of fat in their products, but differences are obvious as fat is lowered. This puts a tremendous burden on researchers to "mimic" the functionality of fats using fat substitutes and other added ingredients. One area of special interest is baked products because this is an area of food where fat affects the eating and keeping quality of the product.

Cakes and muffins are high in fat and in calories. Consumers do not seem to be as interested in low fat derivatives of these products because the taste and mouthfeel drop considerably. Fat replacers have much potential in baked goods where they mimic the qualities of the fat, however, their acceptability does seem to drop. The product still does not have the same sensory qualities as one with real fat. The use of enzymes in fat substituted products is a novel idea of which there is little research. The hypothesis is that the enzymes (amylases and proteases) will help improve the overall acceptability of the baked product by breaking down some of the linkages between proteins and carbohydrates. Fat substituted products tend to be more rubbery, paler, and have less taste than their full fat counterparts. The use of enzymes will help improve the fat substitute's performance by adding color, flavor, and will also help improve the crust and crumb structure of the baked product.

Muffins were chosen due to the increased demand for fast, convenient foods. Muffins are also available to consumers from a broad spectrum of companies. In addition, while there are many studies of fat substitutes in baked products, there are few if any studies available dealing with a fat substitute and enzyme working together. Amylases hydrolyze starch while proteases break down gluten and peptide bonds. Amylases and proteases have been reported to improve the texture and grain of bread. In addition, the use of fungal amylases and fungal proteases together has been shown to soften a bread product. Amylases have also been shown to decrease the staling rate of bread products (Dziezak, 1991).

Therefore, the main objectives of this study were:

1. To study the application of a protein-based fat substitute in a fat free muffin recipe.
2. To study the interactive action of a fungal protease, fungal amylase, or bacterial amylase enzymes on the baking and keeping quality of a fat substituted muffin.

## Chapter 2

### Review of Literature

#### 2.1 Functionality of Fat and its Role in Water Activity

The typical American diet derives 37% of total daily energy intake from fat, but the current national guidelines recommend reducing fat consumption to 30% or less of total daily energy intake. In addition, high fat foods are among the core items of the American diet. Many obese patients show elevated sensory responses to those sweet foods that are rich in fat (Drewnowski, 1992). Americans are starting to realize their high fat intake and are now searching for new healthy, low fat products. The problem is that these low fat products are not sensory satisfying to consumers. Many healthy foods sold in the past were not tasty and they were hard to prepare. There was a tendency to use high fat foods because of things like superior mouthfeel, but the fat substitutes available today make healthy much more palatable (Glenn, 1994).

Fat provides flavor, a desirable mouthfeel and contributes to satiety following meals; therefore, it can be challenging for an individual to change to a low fat diet (Anon., 1991a). Fat replacements have the potential to be incorporated into food systems and act functionally as fat, and at the same time, reduce the actual fat and calories in the foods. In order to reduce fat in baked products it is necessary to determine what role the fat actually does have in the product.

The major role of fat in baked products such as muffins, biscuits, or cakes is to tenderize the strands of gluten and create a moist, flavorful and tender product. The fat interferes with the gluten and actually coats the flour particles and prevents them from

coming together. This prevention or retardation of gluten by fats is termed "shortening power". Various fats have different shortening abilities but it has been suggested that the fat covering the largest surface area has the greater shortening power. The higher the fat content the greater is the shortening power(Bennion, 1972). The contributions of fat to food properties are related to the chemical nature of lipids and their resulting physical properties. The effects of fats in foods are largely textural, but to a lesser extent also involve color and flavor (Strugnell, 1993). The mouthfeel properties of oils or fats are perhaps the most important characteristics to consider when replacing or reducing fat in a formula. Mouthfeel, or the fatty, oily perception of food oils, results from a combination of several parameters including viscosity, lubricity, absorption, cohesiveness, adhesiveness, and waxiness. Fats also tend to mask certain flavors such as acidity (Anon., 1992b).

Water binding properties of fat are also responsible for the sensation of freshness and moistness in a baked product (Drewnowski, 1992). It has long been recognized that a relationship, although imperfect, exists between the water content of food and its perishability. It has been observed that various foods with the same water content differ significantly in perishability. The term "water activity" is a far better indicator of perishability. In foods, water is not just a medium for reaction, but is also an active ingredient used to control gelatinization and leavening reactions. It is not the total amount of water that is important, but rather the availability of water or water activity (Fennema, 1985).

The water absorption of a wheat flour dough is governed, in practice, by the protein content and quality and by the extent to which the starch is damaged mechanically (the

greater the damage, the greater the absorption) (Czuchajowska *et al.*, 1989). Muffins have a higher moisture content than bread which, in turn, has a higher moisture content than biscuits at water activities above 0.80. Differences in moisture retention between muffin and bread indicate that muffin solids bind more water than bread (Christenson *et al.*, 1989). Moisture content drops rapidly during the first minutes of baking, and then the rate of moisture loss slows down (Bakshi and Yoon, 1984).

Water activity within a product determines its microbiological stability. When carbohydrate based fat mimetics are applied to food systems, the water activity of these systems is altered, and steps must be taken to counter the effects of increased water activity (Yackel and Cox, 1992). Lowering the fat content of a product, in most cases, will result in the water content increasing. This resultant increase in water activity can have an effect on shelf stability and, thus, on the packaging and storage of the product (Strugnell, 1993).

## **2.2 Reducing Fat Levels in Baked Products**

The desire to decrease fat in baked products is not a novel idea. This trend has increased tremendously in current years due to the growing interest in health and nutrition. Back in the late 1970's, the idea of low fat was just beginning to be examined. Physicians recommended lowering fat in order to lower serum lipids. The main idea was to try and substitute hydrogenated vegetable shortening with oil. This would have increased the polyunsaturated to saturated fat ratio and helped to lower serum lipids. Shortened cakes and muffins were made with oil in place of shortening with little change in the quality of

the product (Fulton and Davis, 1978). The next step was to reduce the amount of oil in these baked products and determine what effects it would have in the product. Reduction of the polyunsaturated oils yielded a number of acceptable baked cakes and pie crusts. However, all levels of oil were not as acceptable in muffins or sugar cookies. Muffins made with the lower oil content were less desirable (Berglund and Hertsgaard, 1986).

Reduced-fat products are appearing in increasing numbers on supermarket shelves. Many of these products are the result of commercial techniques of heating, acidifying, and blending common food ingredients such as carbohydrates, milk, egg proteins, and/or water, to replicate the textural properties of fat (Anon. , 1991a).

### **2.3 Advent of Fat Replacers**

Fat reduction has captured the interest of the public and; therefore, the attention of the food processing industry. With the advent of new fat replacers in the past two years which mimic the qualities of fat, consumers are excited and eager to acquire low fat foods that actually have the same qualities of full fat foods. These influences have made calorie reduction agents the single largest segment of the \$3.8 billion market for food additives; accounting for 30% of the total in 1991. This figure is expected to increase and the market value of fat replacers themselves are expected to reach \$300 million by 1996 (Thayer, 1992). Fat substitutes are a booming industry and many food companies are striving to get ahead of the game. A 1991 study by the Food Marketing Institute showed that 42% of all shoppers ranked fat as their number one dietary concern. In 1993, 54% of approximately 1000 shoppers polled indicated fat as their most important concern. The

Calorie Control Council (CCC) is convinced that US consumers remain concerned about eating a healthful diet. The CCC polled 1500 individuals, 18 years old and older, to project that 73% of US adults in 1993 consumed low-fat, reduced-fat, and fat-free foods and beverages. This survey showed that people are using these reduced-fat products and the market for these products is predicted to grow. Since the implementation of nutritional labeling requirements in May 1994, this trend will be promoted because products high in fat will have no place to hide (Haumann, 1994). This message of decreased fat intake has also traveled overseas. The United Kingdom market is paralleling the US in its growth of low fat products, and France and Germany are not far behind which indicates the immense global possibilities for fat substitutes (Wagner, 1992). Bakery products are listed by 42% of processors as having the most potential for fat reduction systems (Anon. , 1992a).

A recent study (Ranhortra et al.,1992) has been completed just at the brink of introducing fat substitutes. Sixty bakery goods consisting of pies, cakes, cupcakes, cookies, and brownies were analyzed that were lower in fat. Modified starches (potato, wheat, corn, and tapioca), emulsifiers (including mono- and diglycerides, sucrose esters, and sodium stearoyl lactylate), gums (carob, locust bean, guar, xanthan, cellulose, and gelatin), fiber (pea, wheat, rice, rye, corn, sucrose polyester, and cellulose), and egg whites were the main ingredients used to reduce the fat content. The products were "successfully" lowered in fat but no sensory panel was involved.

Another study (Anon., 1992b) utilized a hydrated emulsifier mix which replaced the shortening in a reduced calorie cake mix by a stepwise reduction of the shortening level.

In the evaluation there was little difference in the cake batter or the baked cake with a 33% reduction in shortening level. Further reductions resulted in decreased density of the batter, an increased volume, and a decreased cake density. Increasing the amount of water added to the dough resulted in higher batter densities and more desirable cakes. Optimum results occurred at 110-120% hydration even with only one-sixth the shortening level (Anon. , 1992b).

## **2.4 Fat Replacers**

There are several approaches for replacing fats in baked products that companies are undertaking. These fat replacements are based in one of three areas: protein based, carbohydrate based, or lipid based substitutes. Protein based substitutes such as Simplese (The NutraSweet Co., Deerfield, IL) are produced by microparticulation of milk and egg white proteins. In this process a premix of raw egg white, or whey protein concentrate is dissolved with other ingredients, deaerated, and heated to a temperature just below coagulation. This mixture then flows into a homogenizing pasteurizer and heated 20 degrees further in less than 10 seconds. It is also exposed to a uniformly turbulent field of homogenizing force. Some of the protein denatures as a gel and forms beadlets about 1  $\mu\text{m}$ . in diameter. At this particle size the particles are separate, spheroidal, and hydrated, but they are not perceived as individual particles. Upon cooling it becomes creamy, smooth and opaque (Singer and Dunn, 1990).

Carbohydrate based fat substitutes include dextrans, modified food starches, and polydextrose. They are heat stable and can be used in baking. These replacements

incorporate water into a gel type structure resulting in lubricant of flow properties similar to those of fats in some food systems. It has been postulated that the 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). When starch is gelatinized, it has a smooth, creamy, spreadable, elastic gel structure which has fat-like characteristics, together with a bland flavor, making starch-based products ideal for fat substitution in many product formulations (Strugnell, 1993). Most of the carbohydrate and protein based substitutes are either approved or near approval (Anon., 1991b).

Lipid based substitutes have functional and sensory properties similar to those of the fats they are intended to replace. These substitutes are made from chemically altered fatty acids. Their reduced calorie functionality is achieved primarily by resistance to digestive lipases, which makes these compounds unavailable for absorption. The end result is that the quantity of lipid based fat substitutes consumed is excreted, providing a hydrophobic environment that potentially reduces the absorption of fat soluble nutrients. Given the composition of the typical American diet, the small health risks posed by reduced absorption of fat soluble vitamins may be more than offset by the reduced cholesterol absorption efficiency and consequent beneficial effects on plasma cholesterol levels (Hassel, 1993).

Gums or soluble hydrocolloids are not usually used directly as fat substitutes but are used in formulating low fat products. Gums have been used since the 1980's and their use does not require FDA approval. They are used in combination with fat substitutes to provide extra smoothness or creaminess normally associated with fat (Artz and Hansen, 1994). Gums have a variety of functions in foods, including binding, coating, suspending, stabilizing, gelling, emulsifying, and thickening. These functions are used in such applications as frozen desserts, beverages, dressings, sauces, dairy products, and bakery products.

Compared to other food manufacturers in the U.S., the baking industry uses relatively small amounts of gums. However, gums perform an indispensable function in bakery related applications. Gums were originally added to cake batters to increase moisture retention during baking and to prevent staling. Other advantages include increases in volume and texture changes (Miller and Hosney, 1993). Xanthan gum is a microbial polysaccharide produced by aerobic fermentation of Xanthomonas campestris. It is commonly added to commercial cake mixes and has been reported to increase moisture retention and shelf life, and to improve volume and crumb structure (Miller and Hosney, 1993).

Guar gum is a high molecular weight, non-ionic galactomannan obtained from the seed cotyledon of the cluster bean Cyamopsis tetragonolobus. The backbone of the gum is a linear chain of mannose units with single galactose units attached as side chains in the ratio of one galactose per every two mannose units (Meer et al. , 1973). Guar gum tends to produce cakes with greater moisture retention, increased shelf life and reduced crumbling

tendency. Guar gum has also been shown to cause a fall in serum cholesterol, as well as to reduce postprandial insulin and glucose concentrations. Evidence also has been presented to show that guar gum may help treat diabetes and celiac disease (Dogra *et al.* , 1989).

## **2.5 Research on Fat Replacers in Baked Products**

Cakes made with three different starch based fat replacers were examined and compared with a control. Satisfactory white cakes were made with complete replacement of fat with the fat replacers. Although most of the cakes prepared with fat replacers had lower total volume indices and flatter profiles than their full fat counterparts; their internal crumb and grain characteristics were fairly similar (Bath *et al.* , 1992). However, there was no sensory evaluation done to determine actual consumer preference or satisfaction with these substitutes.

In another study, the American Institute of Baking compared the baking and organoleptic properties of shortening free muffins containing Kel Lite CM (a carbohydrate based replacement) against muffins containing 7.5% shortening. The two variations had identical baking and sensory scores. The shortening free muffin also had significantly higher sensory scores even after seven days (Duxbury, 1993). This study does indicate that substitution is possible as far as consumer preference is concerned.

Satisfactory cakes were made with complete shortening replacement by potato maltodextrin (carbohydrate based replacement)- emulsifier combinations. Most of the chocolate layer cakes made with potato maltodextrin and emulsifiers replacing 50-100%

of the shortening were comparable to the control (100% shortening) cake. In the fat reduced cakes the role of maltodextrin in water binding was critical. The emulsifier type and method of incorporation influenced volume of the cakes, and also maintained moisture in the cake (Sobczynska and Setser, 1991). These results demonstrate the water binding properties of the fat replacements, and that additional ingredients that act as emulsifiers are needed to increase the volume of the cakes.

In another study (Pong *et al.*, 1991), alternative fat and sweetener systems were studied in cupcakes. N-Flate, an emulsified blend of mono and diglycerides was used as the fat substitute. These substituted cupcakes were found less tender, had less cell uniformity, and also had lower standing heights than those prepared with shortening. Although the study only examined one fat based substitute, a sensory panel was used for evaluating the product. This is one of the only studies that reports a consumers' evaluation of these new products. In addition, the authors suggested that in order to maximize the volume and to improve the texture of the cupcakes prepared with N-Flate, altering the procedures and the relative proportions of the ingredients may be necessary.

One important consideration is that the different substitutes will affect the baked product in a variety of ways and the replacement value may not be on a one to one basis with fat (Waring, 1988). Therefore, it is necessary to conduct extended research in order to reformulate the original recipe to incorporate and optimize the fat substitute's effectiveness. As the research with these fat substitutes increases, consumers and companies alike will begin to understand what possible role these replacements play and the potential they may have in baked products.

## **2.6 Safety of Fat Replacers**

Although some fat substitutes have been approved, consumers may still be wary of their actual safety. Regulatory approval processes and consequent consumer demand are the major forces that will shape the prominence of fat substitutes in the food supply. Consumer demand is expected to continue to grow as health care professionals extol the virtues of low fat diets (Hassel, 1993). The American Dietetic Association also supports the use of fat modified foods when they are included as part of a well balanced, nutritious diet and contribute to positive health outcomes (Stern and Hermann-Zaidins, 1992). It also appears that consumers have learned to avoid overt sources of fat (meat and butter), replacing them with food products in which the presence of fat is either less obvious or more difficult to detect by sensory means (Drewnowski, 1992). This brings into light the idea of the energy content of lipids and fat. Some questions surrounding fat replacements need to be addressed such as their role in satisfying cravings for food or fat and the fact that people may over- indulge to compensate for less energy (Mela, 1992).

## **2.7 General Applications of Enzymes in Foods**

All living organisms produce enzymes and depend on them for the provision of necessary nutrients. All plant and animal foods contain enzymes which have both a beneficial and detrimental impact on the food themselves. Many manufactured foods; particularly cheeses, baked goods, and yogurt, depend on controlled application of enzymes. The most dynamic expansion in the use of enzymes has occurred over the last

20 years. Their role has been expanding rapidly to provide new and exciting developments with predictions of many more to come (Blenford, 1991).

Enzymes are biocatalysts; they are found in a wide variety in all living organisms and they catalyze all the numerous reactions that occur in nature. Enzymes are protein molecules; they are composed of long chains of amino acids which are arranged in a strictly ordered sequence. This sequence ultimately determines the very specific and complex structure which enables the enzyme to exert a specific and powerful catalytic action (Poldermans, 1990).

There are three advantages associated with enzyme use: reaction specificity, mild operating conditions, and high effectiveness. First, unlike inorganic catalysts, enzymes are highly specific, reacting with only one particular class of compounds such as carbohydrates, fats, or proteins. As a result of this specificity, high volumes of compounds can be broken down or synthesized with a minimum of by-product production. Secondly, enzymes permit reactions to take place under mild conditions of temperature, pressure, and pH (most enzymes work best at temperatures of up to 100 degrees C and pH 3-10). Thus, enzymes may be used to specifically modify selected food components without causing drastic changes in nutrients vulnerable to high temperatures or high/low pH processing. Furthermore, low usage levels at 0.1 to 0.5% of the substrate being processed make enzymes economical as well as practical to use (Dziezak, 1991).

Enzymes have been involved in food processing for many thousands of years, and for at least two thousand years some enzymes have been deliberately, if unknowingly, used in food production. However, it is only in the last century that scientific study of enzymes

has enabled man to understand the functioning of enzymes and, more recently, to develop the science of their application (Cowan, 1983).

### **2.7.1 Sources of Enzymes**

Enzymes may be obtained from animals (lipase, pepsin, rennin, trypsin), plants (bromelain, ficin, papain), and microorganisms (including amylases and proteases); and a wide range of different activities are available. By far the largest range of enzymes are obtained from microbial sources, where fermentation is the production system used. Microbial enzymes from bacteria, fungi, and yeasts account for about 80% of the total industrial enzyme production (Dordick, 1991). The properties of a microbially produced enzyme preparation are dependent upon the type and strain of the microorganism, the different conditions for growth of the microorganisms (pH, temperature, and nutrients) and the finishings and purification of the enzyme extracts. These variables demonstrate the great assortment of enzyme properties and types (Himmelstein, 1984).

### **2.7.2 Complications of Enzyme Use**

While enzymes are an essential part of many food and food ingredient processes, there are still some complications with enzyme use that should be mentioned. First of all, there are almost no standard enzyme assays, and even where there are, enzyme companies will often use their own technique. As a consequence, the enzyme activities quoted by different companies cannot be compared. Whether this is done deliberately or not is open to debate. Another problem is the biological source of an enzyme. Two enzymes from

different suppliers but which come from the same organism and even have the same quoted activity can behave differently in a certain process. This is because the source organism could easily be two different variants. Temperature and pH profiles from the same enzyme of the same species will also differ, depending on the variant used for production. Often this will be reflected not by a difference in the pH or temperature optimum, but by how the enzyme performs away from the optimum (West, 1988).

## **2.8 Enzymes in Food Processing**

Enzymes are classified as food substances, and with the exception of those obtained from edible plant and animal tissues, all new enzyme preparations must undergo toxicological testing to demonstrate their safety. Classification of enzymes is difficult, but in the simplest form they may be divided into proteases, pectinases, lipases, and carbohydrases.

Because of the increased tendency toward processing convenience foods, the use of exogenous enzymes is assuming major significance, made possible by advances in enzyme technology (Fox and Mulvihill, 1982). The largest industrial use of enzymes in the production of food takes place in the dairy, brewing, and baking industries. The functions of enzymes in baking and cereals involve: antistaling, dough improvement, gluten weakening, flour supplementation, improved crust color, and chilled and frozen dough. A new development in the area of baked goods processing is an antistaling enzyme which is produced by fermentation and is considered a natural alternative to mono- and diglycerides commonly used for this purpose (Shallenberger, 1971).

### 2.8.1 Amylases

Amylases are enzymes which catalyze the hydrolysis of starches. They are widely distributed, and occur in cereal grains, fungi, and bacteria. Amylases are often added directly to flour, and are effective both in converting starch into maltose for yeast fermentation, and in retarding staling. They have also been shown to improve crust color and crumb structure.

Amylases are classified into two types according to their action. Alpha amylases are endoenzymes and hydrolyze the alpha 1,4 glycosidic linkages within the starch molecule. They tend to liquify starch and produce sticky dextrans. Beta amylases are exoenzymes and act only on the end of the molecule. They cleave off two glucose units (maltose) without markedly altering the rest of the starch molecule (Dziezak, 1991).

High amylase content leads to high dextrin production with low water retention within the dough, which results in considerable "loaf stickiness", open crumb structure with little strength and high crust color. Low amylase content leads to low dextrin production and results in poor gas production, which results in inferior quality bread of small size and low crust color (Reichelt, 1983).

Although alpha amylases have the same bond splitting action there are marked differences in the temperatures at which they are inactivated. Fungal amylases exhibit a much lower temperature optimum of 50-55 degrees C followed by a rapid loss in activity at higher temperatures. Thus, the heat sensitive fungal amylases have no opportunity to act on the gelatinized starch. The most heat stable is the bacterial amylases which are still

active above 90 degrees C. Thus, the bacterial amylase is active throughout gelatinization which tends to result in an over production of dextrans and hence a gummy, sticky baked product. Cereal amylases have intermediate heat stability and are active above the gelatinization temperature range of starch; and also tend to produce sticky products. This over production of dextrans could be desirable as in the case of fruit cake, but for the most part it is undesirable in the baking industry. A combination of alpha amylases such as bacterial with fungal alpha amylases have been tested to decrease the amount of dextrinization and thus produce a desirable product (Haseborg, 1981).

Research (Asp et al., 1985) has indicated that the alpha amylases from three different sources could be expected to react differently to heat during baking. However, the study investigated enzyme activity in enzyme-starch-water, enzyme buffer or enzyme-flour-buffer systems heated in an amylograph or water bath rather than in a bread dough baked in an oven. Therefore, the researchers looked at the differences in the activity of added enzymes on yeast dough during baking. Their results show that changes in activity of cereal (malted barley flour) and fungal (Aspergillus oryzae) alpha amylases were not significantly different, indicating that fungal alpha amylases may not be less thermostable than cereal sources. The authors also found that there was a decrease and then an increase in the activity of bacterial alpha amylase (Bacillus subtilis) which had not been previously reported. The data suggest that since enzymatic activity continues after baking, enzyme supplementation could be lower than that believed to be needed to achieve the desired crumb softening effects.

In another study by Kuracina et al. (1987), the bread baking quality of starch was analyzed using alpha amylases from the three different sources. As in most baking applications the three enzymes used were bacterial from Bacillus subtilis, fungal from Aspergillus oryzae, and cereal from Hordeum vulgare which is from barley malt. Low levels of enzyme supplementation of flour yielded increased total scores (which included improvements of grain, texture, and total bread quality) when compared to the unsupplemented flour control bread for all enzyme sources. Therefore, there were no significant differences found between the three different alpha amylases. In addition, high levels of enzyme treatment showed detrimental effects on bread baking performance. Perhaps, it is not quite the source that is most important, but rather the amount.

It is also important to consider that while a study may be looking at one particular class of enzyme such as amylase; there may also be some amounts of protease included because preparing chemically pure enzymes for industrial use has been too expensive. Therefore, sometimes it may be hard to prove what kind of enzyme is causing a certain effect. Possibly, combinations of enzymes produce the best effect (Loney and Crawford, 1977).

### **2.8.2 Proteases**

The rheological characteristics of wheat flour dough or batter depend on the state of the gluten protein. The more sulfur bridges present, the stronger and more intractable is the behavior of the gluten. The protease break down the gluten network and the peptide bonds (Haseborg, 1981). These proteases tend to increase machinability of dough, and also create a better bread quality. Soft wheat flours have a low protein content and have

less gluten development, while hard wheat flours enhance gluten development. Bacterial proteases which are endoenzymes, weaken the gluten without affecting the nutritional quality of the flour. The enzymes act on the inner peptide bonds of gluten and result in reduced elasticity and improved extensibility of the dough. Fungal proteases are either endo- or exoenzymes and are added to bread to improve loaf symmetry and uniformity, to improve grain and texture, and to provide a softer crumb (Dziezak, 1991).

In a study done by Moss (1989) fungal enzymes having different amylase:protease ratios were studied in bread baking. The results indicated that all the fungal enzyme preparations improved volume and crumb softness. Although the magnitude of the improvement varied, it was not related to the amylase:protease ratio. However, protease used alone had lower loaf scores than a protease:amylase blend (Moss, 1989). This suggested that possibly proteases should only be used in combination with other enzymes.

In another study (Gaines and Finney, 1989) conducted on cookies, the protease papain was the most effective at increasing cookie spread and top grain. These cookies were made from hard red winter wheat flour. The implication derived from the study was that possibly baked goods could be made from hard wheat flours as long as proteases were added to break down the gluten. The use of bacterial proteases in the manufacturing of crackers, biscuits, and cookie dough has become widespread as the amylase contained in them presents no problems as the baking temperature rises very rapidly and reaches higher levels than those in the bread baking process. The amylase is quickly inactivated and the "sticky crumb" problem is eliminated. Bacterial proteases are usually obtained from

B.subtilis; fungal proteases from A.oryzae; and plant proteases from papain are also used frequently (Reichelt, 1983).

### **2.8.3 Effect of Alpha-Amylase on Bread Staling**

The staling of bread has been attributed mainly to retrogradation of starch, and is caused by changes in the crystallinity of the amylopectin on cooling below 60 degreesC. It should be mentioned that carefully controlled addition of relatively thermostable bacterial alpha-amylase results in a softer crumb and the staling is significantly delayed (Reed, 1966).

The alpha-amylases hydrolyze linkages within the amorphous regions of the starch matrix and reduce staling. It seems as though both cereal and fungal alpha-amylases do not exhibit significant anti-staling activity. However, there is a procedure that uses fungal alpha amylases with a sugar solution that protects the enzyme from thermal denaturation, thus, it can retain its activity after baking. Bacterial alpha-amylases are able to inhibit staling by hydrolyzing glycosidic linkages within the amorphous areas of gelatinized starch but even with close monitoring of the dosage the bacterial alpha- amylases have the potential of producing an unacceptable product (Hebeda et al. , 1990).

A recent development is the use of "intermediate temperature stability" (ITS) amylases. They are more stable than fungal alpha-amylases but less than conventional bacterial alpha-amylases with an optimum temperature range of 65-75 degrees C. Because these enzymes are active above the gelatinization temperature, they sufficiently hydrolyze the

amylopectin fraction for effective anti-staling. Since they are inactivated in the oven, they do not cause stickiness or gumminess in the finished product (Boyle and Hebeda. , 1990).

The ITS fungal enzyme was derived from an Aspergillus sp. and exhibits traditional hydrolytic properties. The ITS bacterial amylase was derived from Bacillus megaterium and cloned into Bacillus subtilis. During a typical baking cycle, oven temperature increases rapidly passing through the starch gelatinization range of about 65-70 degreesC and leveling off near 100 degreesC for the remainder of the bake. The ITS enzymes have optimum activities above the gelatinization temperature of starch and continue to hydrolyze starch well into the baking cycle before inactivation occurs. The Aspergillus ITS enzyme exhibits at least 60% activity at pH 2.5-6, while the B. megaterium ITS enzyme retains 60% or more activity over a pH range of 5-7. Both enzymes are suitable for most types of baked goods although the B. megaterium enzyme may be a better choice for processes at higher pH. When using the Aspergillus ITS enzyme, the shelf life of the bread was increased by 38-75% depending on the amount added. Similarly, the use of the B. megaterium ITS enzyme in the same matter increased shelf life 15-33%. ITS enzymes have also been shown to improve the shelf life of other baked goods such as buns, rolls, and muffins (Hebeda et al. , 1991).

## **2.9 Muffin Ingredient Functionality**

The term quick bread is commonly used to distinguish from yeast breads as a group of relatively quickly prepared flour mixtures that are leavened primarily by chemical agents,

steam, and/or air. It includes muffins, biscuits, popovers, griddle cakes, waffles, fritters, dumplings, and a variety of coffee cakes and nut or fruit breads (Bennion, 1980).

### **2.9.1 Flour**

White wheat flour is milled from wheat kernels after the outer covering called bran and the germ have been removed. Wheat flour contains 63-73% starch and 7-15% protein. The balance is moisture, sugar, minerals, and a low percentage of fat(1-2%). Flour is mostly starch, but it is the protein or gluten content that concerns the baker. Without gluten proteins to give structure, baked goods would not hold together. In order for gluten to be developed, the proteins must first absorb water. Then, as the dough or batter is mixed or kneaded, the gluten forms long, elastic strands. As the dough or batter is leavened, these strands capture the gases in tiny pockets or cells, and the product "rises". When the product is baked the gluten coagulates or solidifies and contributes structure to the product (Gisslen, 1989a). Flour from hard wheats with relatively high protein content (12-14%) is generally used to produce bread with large loaf volume and fine, even texture. Flour from soft wheat, which is lower in protein content (8-10%) and thus has less gluten formation is used to make cakes, pastry and crackers. The greatest production of soft wheat flour takes place east of the Missouri and Mississippi rivers below the Great Lakes. Flour provides the basic structure for quick breads. The relatively high proportion of liquid to flour in many batters limits the development of gluten during mixing (Bennion, 1980).

### 2.9.2 Sugar

Sugar adds sweetness and also has an important effect on texture and volume. It interferes with gluten development from the flour and has a weakening effect on the structure of the quickbread. It also competes with the starch for water and thus interferes with gelatinization. Sugar also adds crust color, and increases the keeping quality by retaining moisture (Bennion, 1985b). Sugar added in increasing amounts up to an optimum quantity decreases the cohesive forces and allows batter to move more freely and also increases volume. Increasing sugar too much may produce a coarse texture, thick cell walls, a gummy product, and an overly browned crust (Bennion, 1990b).

### 2.9.3 Fat

Fat is a tenderizing agent. Products containing fat break apart more easily than those with no fat. Fat is insoluble in water and spreads over the moistened flour particles, whereby, interfering with the development of gluten. A softer crumb structure will result in the quick bread (Bennion, 1980).

The finished quality of baked products is influenced by the fat used- the kind, the amount, and the physical form. In muffins, the fat is a liquid at the time it is mixed with the other ingredients instead of a solid or semi-solid as in pastry, baking powder biscuits, or cake. Because muffin batter is mixed only long enough to moisten the dry ingredients and not until it is smooth, the fat remains in pools rather than uniformly distributed throughout. Oils are extremely plastic and therefore can coat and surround the different strands of gluten and prevent them from coming together (Matthews *et al.* , 1965).

Matthews *et al.* , (1965) evaluated the performance of various fats at different levels in muffins. The researchers found that all the samples became softer as the fat concentration increased, regardless of the type of fat. Muffins decreased in volume as the amount of fat was increased with the lowest volume resulting from muffins made with butter. The investigators attributed the weakening effect of the fat on the power of gluten to resist the pressure of expanding gases during baking. With increasing amounts of fat, muffins became more symmetrical in shape, more pebbly in crust surface, more tender, and more flavorful.

#### **2.9.4 Secondary Ingredients**

The coagulation of egg proteins during baking makes an important contribution to the structure of many quick breads. This is especially important because gluten structure is weak in these products. Egg whites also add moisture and aeration. Egg yolk serves as a source of fat; it also contains lipoproteins which act as emulsifying agents and also affect color and flavor (Bennion, 1990a).

The liquid used in quick breads is usually milk, which adds important nutrients and aids in browning. Liquids also help dissolve the sugar and salt. It also disperses the flour particles and hydrates the starch and protein in the flour, allowing for starch gelatinization and gluten development. Milk also adds sweetness and has some effect in keeping baked goods moist (Bennion , 1980).

#### **2.9.5 Leavening Agent**

To leaven means to "make light and porous." Baking powders were developed as one of the first "convenience" foods. They contain mixtures of dry acid or acid salts and baking soda with starch added to standardize the mixture, and at the same time to help stabilize the components so that they do not react prematurely (Bennion, 1990a). A double acting baking powder means that the baking powder reacts to release carbon dioxide gas at room temperature when the dry ingredients are moistened and it reacts again when heat is applied in the process of baking. This carbon dioxide gas helps provide the volume increase observed in baking (Bennion, 1985a).

### **2.10 Muffin Manipulation**

The mixing method for muffins is also used for pancakes, waffles, quick loaf breads, and coffee cakes; it is termed the "muffin method". It involves combining dry ingredients and combining liquid ingredients. The liquid ingredients are then added to the dry ingredients and mixed just until blended. Muffin batters are mixed as little as possible, only until the dry ingredients are moistened. This, plus the presence of fat and sugar, keeps gluten development low. Overmixing muffins produces not only toughness, but also irregular shapes and large, elongated holes inside the product (Gisslen, 1989b). The proportion of liquid to flour determines the consistency of a flour mixture. A pour batter is quite fluid in consistency and has a ratio of liquid to flour of approximately 1:1. A popover batter is an example. A drop batter has a thicker consistency with a ratio of about 1:2 liquid to flour. Muffin batter is an example of a drop batter. Properly mixed muffins have an even texture although the grain is somewhat open and more coarse than

that of a shortened cake. The surface is symmetrical and has a slightly rough appearance (Bennion, 1980).

## **2.11 Sensory Evaluation**

Sensory analysis is a very useful tool in product development and optimization. Sensory evaluation can also be used to support customer requests (Duxbury, 1993). "Sensory evaluation has been defined as a scientific discipline used to evoke , measure, analyze, and interpret reactions to those characteristics of foods and materials as they are perceived by the senses of sight, smell, taste, touch, and hearing (The Institute of Food Technology, 1981)."

Sensory evaluation as a science plays a critical central role in determining consumers' acceptance limits and the attributes which influence acceptance. Consumers know which products they like and dislike, but usually have a difficult time describing the reasons why, in terms of specific sensory attributes. Precise qualification and quantification of a product's sensory properties are provided by laboratory descriptive panels. It is the combination of these two sensory truths, the affective (consumer) and analytical (descriptive) responses, which determine those characteristics which define acceptable products (Civille, 1990).

### **2.11.1 Quantitative Descriptive Analysis**

Qualitative Descriptive Analysis (QDA) is a technique in which trained individuals identify and quantify, in order of occurrence, the sensory properties of a product or an

ingredient. These data are used to develop the appropriate product multidimensional models in a quantitative form that is readily understood in both marketing and research and development environments (Stone *et al.*, 1974). QDA embodies a formal screening and training procedure, development and use of a sensory language, and the scoring of products on repeated trials to obtain a complete quantitative description (Stone, 1992).

The keys to an effective instrument for sensory evaluation are orienting the panelists to become a valid instrument, choosing appropriate terms and reference material, and selecting an adequate measurement instrument that does not confound attributes or types of test methods (Setser, 1994). The difficulties of using the QDA technique center around the time involved in screening and training the judges. Because of the time involved, the technique is also fairly costly. Turn around time for data can also be a problem if the system is not set up efficiently (Zook and Pearce, 1988).

#### **2.11.1.1 Panelist Training**

Trained panelists provide analytical information that other instruments cannot measure regarding attributes such as sweetness and cohesiveness during mastication. A cognizance of the difficulties in assessing sensory versus chemical or physical characteristics is essential for making appropriate, accurate, and precise measurements. Effective descriptive analysis requires a well trained panel; using an inadequately trained panel to obtain descriptive data is one of the most common mistakes in sensory studies (Setser, 1994).

In QDA 10-12 judges are selected for training to develop terminology which describe the sensory attributes of several samples during approximately ten one hour training sessions (ASTM, 1981). Panelists, as a group, meet with the panel leader (who does not participate as a panelist) and develop a common language that describes their perceptions of the products. Panelists also develop the order of occurrence for the attributes, that is, what is perceived first, second, and so forth. In later sessions, the panelists practice scoring products to familiarize themselves with scale use and to build confidence in their individual and collective judgments (Stone, 1992).

Product evaluation is conducted in standard sensory test booths, with proper lighting and environmental control such that panelists are free from external distractions. A balanced block design is used such that each product is evaluated equally often by each panelist. All products are 3-digit coded and each session should involve enough products to enable data collection but sensory fatigue should be minimized. To record the intensity for each attribute, panelists make a vertical mark on a 6 in. horizontal line at that point that represents the intensity. The line has two word anchors, placed 0.5in. from each end (Stone, 1992). The results of a QDA test are analyzed statistically, and the report generally contains a graphic representation of the data in the form of a "spider web" with a branch or spoke from a central point for each attribute (Meilgaard *et al.*, 1987a).

### **2.11.2 Central Location Tests**

The collection of food acceptance data is a key ingredient in studies of product development, quality control, food product acceptance in the market place and food

service evaluation. A consumer panel must represent the ultimate consumer to be maximally effective. The people who develop food products are not necessarily the best representatives of the consumers who will ultimately consume the product (Meiselman, 1984). Laboratory taste panel tests on foods generate many useful data which are used and interpreted in a number of ways. One use is to get preliminary information on the possible appeal or level of acceptability of a particular food or food product to consumers. The reliability of such data is open to debate and it can be argued that the only way to get information on consumer acceptability is by doing a full scale consumer panel (Gormley, 1989).

A central location test requires a minimum of 50 to 100 consumers per location test. Specific product attributes and overall acceptability can be determined by using hedonic ratings, usually on 9 point scales. A questionnaire should contain a rating scale which is adequately long, which focuses on one root or concept, and which is balanced in number and description of scale points about its neutral point. The nine point hedonic scale satisfies all of these requirements and thus is a good instrument to use for food preference and food acceptance information (Meiselman, 1984). Testing against a benchmark is advisable so a basis acceptability can be established with a known (Katz, 1994). Central location tests are usually conducted in an area where many potential purchasers congregate or can be assembled such as a shopping mall, fair, or grocery store. Respondents are intercepted and screened in the open. One advantage of this type of testing would be that the products are tested by the end users themselves which assures

the validity of the results. A disadvantage could be that the number of questions obtained may be limited (Meilgaard et al. , 1987b).

## Chapter 3

### Materials and Methodology

#### 3.1 Experimental Design

A 2x2x2 augmented factorial block design was used in this study. The presence or absence of the three different enzymes (2x2x2) was analyzed along with the extra control which makes this an augmented factorial. The study took six weeks to complete.

A basic muffin formula derived from The Pillsbury Cookbook (1989) was used as the control (Appendix A). The control muffin and a 100% fat substituted muffin with or without the three different enzymes fungal amylase, fungal protease, and bacterial amylase were used as treatments in this study (Appendix A).

Testing took place twice a week with three muffin variations produced each testing day. Each variation was replicated four times throughout the study (Appendix B).

#### 3.2 Ingredients for Muffin Formulation

A low protein soft wheat flour (Southern Biscuit All-Purpose Flour) was sifted with granulated sugar, baking powder (Calumet Double-Acting Baking Powder), baking soda (Arm and Hammer Natural Baking Soda), salt (Morton Iodized Salt), and if applicable a fat replacer (Simplese 710 Bakery Blend), an enzyme or combinations of enzymes (Enzyme Development Corporation Fresh-N, Enzeco fungal alpha amylase, or/and Enzobake concentrate), and gums (TICAXAN Xanthan and Guar gum). Vegetable Oil (Hunt-Wesson Vegetable Oil), skim milk, buttermilk, egg or egg white, and vanilla were

added to the respective formulas. All ingredients except for the fat replacer, enzymes and gums were purchased locally (Appendix C).

### **3.3 Manipulation of the Batter**

The muffins were produced in a climate controlled laboratory of approximately 70 °F at Virginia Polytechnic Institute and State University, Blacksburg, VA. All formulations were manipulated according to the “muffin method” (Bennion, 1985a). All ingredients were weighed using a top load balance (Fisher Scientific XL-500 Top Load Balance #13028824, Denver Instrument Company, Arvada, CO) except for the enzymes which were weighed using a Mettler AE 260 Top Load Balance (Mettler Instrument Corporation, Hightstown, NJ). The flour, sugar, baking powder, baking soda, salt, fat replacer, gums, and enzymes were sifted together using a Bromwell's measuring sifter (Michigan City, Indiana) into a pyrex mixing bowl (Pyrex, Corning, NY). The eggs, skim milk, buttermilk, vanilla, and oil (if added) were whisked together. The wet ingredients were then added to the sifted dry ingredients and the mixture was folded together using a rubber spatula (RubberMaid Inc., Wooster, OH) just until moistened or about 20 strokes. The muffin tin (Ekco, Franklin Park, Illinois) was sprayed with Pam™ no stick cooking spray (American Home Food Products, New York, NY). Muffin paper bake cups (Reynolds Metal Company, Richmond, VA) were then put into the tin and also sprayed with the PAM™. Fifty grams of batter were placed into each muffin bake cup. The oven was preheated to 400 °F. The muffins were baked on the middle rack of a Frigidaire

electric range (PC No. 1126638, Dayton, Ohio) for 17 minutes. The muffins were removed immediately from the muffin tin and cooled on a wire rack.

### 3.4 Physical Properties

The physical properties that were measured in this study were volume, tenderness or degree of softness, percent moisture, crust color, crumb color, water activity (freshly baked, after 24 hours and after 48 hours storage), and staling rate after 24 hours and after 48 hours storage. All physical measurements were taken after the muffins had cooled for one hour.

#### 3.4.1 Volume

The volume of each muffin was determined by measurements conducted on the baked muffin (Appendix D). A dial caliper (Switzerland), was used to measure the height and diameter of the muffin (Appendix D). The formula used to calculate the volume was:

$$\text{Volume} = \frac{22}{7} r^2 h$$

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

#### 3.4.2 Texture

Texture of foods is regarded by the processing industry as a key quality parameter in the development and acceptance of new food products and "fabricated" goods, as well as in the grading and quality control of traditional food products (Timbers and Voisey, 1987). The advantages for using the Instron include universality of attachments, high

degree of flexibility of operation, and uniform compression at a constant rate. Many independent factors may cause inconsistencies in the dependent factor compression force including thickness of sample, geometry of plunger, and degree of compression (Baker et al., 1986). The Universal Instron machine model 1011 (Canton, M.A.) was used to evaluate crumb firmness (Appendix E).

### **3.4.3 Moisture**

Moisture content of the muffin sample was determined with a moisture tester (C.W. Brabender Instruments, Hackensack, New Jersey)(Appendix F). The machine was preheated for one hour and then ten grams of the crumb was dried at a temperature of 130 degrees F over a period of one and a half hours or until equilibrium was reached. Samples were weighed using a top load balance (Salorius Portable Top Load Balance PT1200, Bohemia, NY). The percent moisture in the muffin was then determined (Appendix F).

### **3.4.4 Degree of Staling**

There is extensive work on the physical and chemical changes that occur in starch during baking and staling. Differential scanning calorimetry (DSC) in particular has proven to be a valuable tool to quantify crystallinity, both in native starch and the retrograded starch of bread crumb and aged gels. The DSC also enables the researcher to directly measure the influence of various factors on the thermal properties of starch during gelatinization and recrystallization. Enthalpy, the energy required to melt the crystalline material is used as the index of crystallinity in bread and gel samples (Zeleznaek and

Hoseney, 1986). Starch has a gelatinization or retrogradation peak at about 55 degrees C and a second peak at over 100 degrees C due to melting of an amylose-lipid crystallite complex (Czudrajowska and Pomeranz, 1989). A Perkin-Elmer Differential Scanning Calorimeter (Perkin-Elmer, Norwalk, CT) was used (Appendix G). The DSC was connected to a Perkin-Elmer Thermal Analysis Data Station (Perkin-Elmer, Norwalk, CT) for data analysis. An indium standard was used to calibrate the machine prior to each test session. Approximately, a forty milligram sample was taken from the muffin crumb and enclosed in a sample pan. The enthalpies measuring the dissociation of the amylopectin bonds formed upon retrogradation were determined by the area of the first endotherm peak. This area is obtained by constructing a baseline, “a smooth line from beginning to end of the endotherm, “ and calculating the area between the baseline to the endotherm’s peak ( Kugimiya and Donovan, 1981). The muffins were stored at room temperature in Tupperware™ containers (RubberMaid, Inc., Wooster, OH) for the duration of the analysis period (24 hours and 48 hours).

#### **3.4.5 Color**

A Hunter Lab L Optical Sensor D25, Hunter Lab (Reston, Virginia) connected to a Toshiba T1000 System Unit (Tokyo, Japan, #PA 7027U) was used to measure the crust and crumb color of the muffin (Appendix H). The L and b values were calculated. The L value measures lightness from a value of 0 (black) to 100 (white). The b values measure positive b (yellowness) to negative b (blueness) (Francis and Clydesdale, 1975a).

### **3.4.6. Water Activity**

Water activity can serve to determine the degree of “free” water available for microbial growth in a food system. Water activity was measured by a Decagon Devices Inc. Water Activity Meter (Pullman, WA) at the Food Science and Technology Department at Virginia Polytechnic Institute and State University, Blacksburg, VA(Appendix I).

## **3.5 Sensory Evaluation**

### **3.5.1 Modified Quantitative Descriptive Analysis**

Quantitative descriptive analysis(QDA) is based on the principle of a panelist’s ability to verbalize perceptions of a product in a reliable manner. Panelists become parts of sensitive instruments that provide quantitative, accurate, and objective analytical assessments of sensory characteristics of the product (Setser, 1994). Twelve graduate students from Virginia Polytechnic Institute and State University served as panelists for the QDA panel.

#### **3.5.1.1 Training**

Panel member training familiarizes an individual with test procedures, improves an individuals’ ability to recognize and identify sensory attributes in complex food systems so he or she can provide precise, consistent, and standardized sensory measurements which can be reproduced (ASTM, 1981). The procedures used to train the twelve panelists are

located in Appendix J. Training consisted of three one hour sessions that introduced the panelists to the control muffin. At the training sessions the attributes that were studied were developed by the panelists. The panelists also determined the order and a definition for each of the attributes. The panelists then did three pre-trials of scoring various muffin formulations in order to demonstrate they could produce reliable and true responses. The principal investigator served strictly as the panel leader to record all responses, and did not participate in the study.

### **3.5.1.2 Testing Procedures**

The scale used for each attribute was an unstructured interval scale. The scale was six inches in length with anchors 0.5 inches from each end. The anchors were words describing extremes of the attributes as developed by the panelists. An example of the scorecard is located in Appendix K. The testing procedures used at each test day are described in Appendix L.

### **3.5.2 Consumer Acceptance**

A consumer survey was completed at a shopping mall (New River Valley Mall, Christiansburg, VA) on one occasion with a sampling quota of one hundred. Since this mall is located in Christiansburg about eight miles away from Blacksburg which has predominately college inhabitants, a more true representation of the public was obtained. The sampling method helped provide a diverse representation of the population. Although

there were a range of respondents, it was not a true representation of the population because the surveying was only done in one area of the United States.

### **3.5.2.1 Testing Procedure**

A control muffin, a 100% fat substituted muffin with no enzymes added, and a 100% fat substituted muffin with the enzymes fungal amylase and bacterial amylase added were the formulations used at the central location test. The testing procedures used for this consumer test are found in Appendix M. Each consumer surveyed was asked to taste one muffin sample. The panelist was given a scorecard with a nine point hedonic scale going from 1= like extremely to 9= dislike extremely (Appendix N). This scale determined their degree of likeness for the muffin sample. A short questionnaire followed to obtain some general information about the panelist (Appendix N). Information on sex, age, education, frequency of eating muffins, and opinions about fat-free muffins was obtained.

### **3.6 Statistical Analysis**

Statistical analysis was conducted using the Statistical Analysis System (SAS Institute Incorporated, SAS Circle, Box 8000, Cary, NC) (Appendix O). For both physical and sensory data, a 2x2x2 augmented factorial design was used. ANOVA and contrast analysis were used. A p-value of <0.05 was used when determining significance levels.

The consumer acceptance results were converted into numerical values with 1.0= 'like extremely' and 9= 'dislike extremely'. A non parametric analysis of variance test was used because the response variable was nominal in nature. The Krustal-Wallis rank sum test

with associated multiple comparisons was used. Again,  $p < 0.05$  was used to determine significant differences.

## Chapter 4

### Results and Discussion

#### 4.1 Rheology of the baked product

When the baking process is considered, rheological properties are important on two premises; firstly, rheological properties change dramatically as a result of the baking process; secondly, the rheological properties of the batter affect transformations during baking. The batter expands in the oven until a structure is fixed. The expansion of gas cells is accompanied by a flow of material between them; this materials' resistance to viscous flow affects oven rise (Bloksma, 1986). Upon hydration and manipulation, gluten is formed which upon heating will form a starch-protein matrix. The degree of toughness of this structure is dependent on the type of flour used and the amount of manipulation. The expansion in muffins is caused mainly by the production of gas by chemical leavening agents and by the evaporation of water in the batter (Bennion, 1985a). The formation of a more or less solid matrix appears to occur at 80 degrees C owing to gelatinization of starch and formation of a protein gel (Ablett *et al.*, 1986).

One of the principal aims of the baking process is to produce a food with desirable textural attributes. These may vary from hard and crisp to soft and springy. Muffin texture is related to the number of air bubbles that are present in the aerated batter which are enlarged during leavening (Tamstorf *et al.*, 1986). The degree of tenderness appears to result from the opposing action of toughening (flour, egg whites) and tenderizing (sugar, oil) ingredients (Paton *et al.*, 1981). In muffins texture is also dependent on the development of the gluten network. Tenderization by sugar is accomplished by increasing

the gelatinization temperature of starch and the coagulation temperature of proteins, allowing longer time for leavening gases to expand the structure. Sugar also retards and restricts gluten formation (Neville and Setser, 1986). Fat lubricates the structure by being dispersed in the batter during mixing and helps prevent the starch and protein from forming a strong gluten network (Sanchez *et al.*, 1995).

#### **4.1.1 Volume**

Table 1 represents the mean volumes obtained from all variations of muffins after baking. There were no significant differences ( $p>0.05$ ) between any of the muffin formulations. However, the 100% fat substituted muffin did have the highest volume. This is most likely due to the addition of hydrocolloids to the batter. Both xanthan gum and guar gum are hydrocolloids which compete with sugar for available water. Research (Miller and Hosney, 1993) has shown that xanthan gum improves cake and muffin volume by increasing batter viscosity which aids in the retention of air cells in the batter. The air cells are released during the baking process and a higher volumed product is the result.

The control muffin was lowest in volume because it had no added hydrocolloids to bind water and trap air to be used for expansion during baking. In addition, the fat would also interfere with the gluten formation which may also explain why the control muffin did not expand as much. The muffins with enzymes did have higher volumes than the control which is due to the addition of gums, however these muffins were lower in volume than the 100% fat substituted muffin. This suggests

**Table 1:** Mean volumes of control and fat substituted muffins.

| Treatment   | Mean Volume(cm) $\pm$ SD # |
|---|----------------------------|
| Control   | 86.250a $\pm$ 6.702        |
| 100% fat substituted                                  | 92.000a $\pm$ 11.165       |
| Bacterial amylase*                                    | 89.500a $\pm$ 5.196        |
| Fungal amylase*                                       | 89.000a $\pm$ 6.055        |
| Fungal protease*                                      | 86.500a $\pm$ 4.123        |
| Bacterial amylase + fungal amylase*                   | 90.750a $\pm$ 6.397        |
| Bacterial amylase + fungal protease*                  | 90.750a $\pm$ 10.436       |
| Fungal amylase + fungal protease*                     | 89.500a $\pm$ 6.557        |
| Bacterial amylase + fungal amylase + fungal protease* | 87.250a $\pm$ 5.679        |

n=4

\* All enzyme muffins are also 100% fat substituted.

# Mean scores with the same letter denote no significant differences at  $p > 0.05$ .

that the enzymes tend to slightly decrease volume. Possibly the use of enzymes decrease batter viscosity and increase specific gravity slightly more than with the use of a fat substitute alone. This would cause a decrease in the retention of air cells and hence a lower volume.

The muffins with amylases, particularly bacterial amylase, did not decrease as much as the muffins with fungal protease or all three enzymes combined. As compared to fungal protease, the amylases had higher volumes suggesting that amylases do not have as much of an affect on volume as do the proteases (Kuracina et al., 1987). Possibly the amylases which break down starch to sugar cause less water to be imbibed by the gums due to increased competition for water by the newly formed sugars. Hence a lower volume may result. However, sugar in itself tends to increase volume because it dilutes the concentration of protein in the batter which elevates the temperature at which protein coagulation occurs during baking. The high coagulation temperature permits the cell structure to expand for a longer period of time (Medved, 1978a). Possibly the fungal protease's action increases the concentration of protein and leads to a decreased coagulation temperature. The fungal protease most likely weakened the gluten structure by breaking peptide bonds.

#### **4.1.2 Crumb Tenderness**

Table 2 shows the effect of the various treatments on muffin crumb tenderness. All the muffins were made from soft wheat flour which has a low protein content (8-8.5%) and

**Table 2:** Mean force deformation values to determine crumb tenderness of control and fat substituted muffins.

| Treatment   | Crumb Tenderness (Force/gm) $\pm$ SD# |
|---|---------------------------------------|
| Control   | 0.3672a $\pm$ 0.0366                  |
| 100% fat substituted                                  | 0.7235b $\pm$ 0.0871                  |
| Bacterial amylase*                                    | 0.6172b $\pm$ 0.0383                  |
| Fungal amylase*                                       | 0.6594b $\pm$ 0.2300                  |
| Fungal protease*                                      | 0.6016b $\pm$ 0.1260                  |
| Bacterial amylase + fungal amylase*                   | 0.5813b $\pm$ 0.0894                  |
| Bacterial amylase + fungal protease*                  | 0.6579b $\pm$ 0.0355                  |
| Fungal amylase + fungal protease*                     | 0.6250b $\pm$ 0.1547                  |
| Bacterial amylase + fungal amylase + fungal protease* | 0.5828b $\pm$ 0.0850                  |

n=4

\* All enzyme muffins are also 100% fat substituted.

# Mean scores with the same letter denote no significant differences at  $p > 0.05$ .

hence will contribute to tenderness because less gluten will be developed. The control muffin was found to be significantly ( $p < 0.05$ ) more tender than all other variations. This would be expected due to the shortening power of fat in a baked product. Concurrent with other research associated with fat substitutes, (Pong *et al.*, 1991), the 100% fat substituted muffin was found to be the least tender.

In the 100% fat substituted muffin, the gluten-starch complex is not retarded by fat and therefore will be relatively strong. While hydrocolloids have been found to help increase tenderness (Miller and Hosoney, 1993), in this case, they did not have this enhancing effect. A possibility could exist that due to the competition for water in this batter system, the gums might have needed more water to be fully hydrated or maybe they were over hydrated leading to a firmer crumb. The fat substitute used was protein based and the increased protein content could have had a strengthening effect on gluten development in the muffin. The fat substitute used also contained emulsifiers which should have acted as a crumb softener, however, this effect was not observed. Another explanation could be the use of egg whites and no egg yolks in the fat substituted muffins. The coagulation of egg white protein during baking adds strength to the cell walls and contributes to the structure of baked products (Medved, 1978a). Egg yolks contain fat in the form of phospholipids and lipoproteins which act as tenderizing and emulsifying agents in baked products (Bennion, 1990a).

The muffins made with fat substitute and enzyme were found to be softer than the 100% fat substituted muffin. Doughs treated with amylases have been shown to produce slightly softer doughs than a control. Crumb grain in breads made with higher levels of

amylases were slightly open which would explain the softness (Valjakka *et al.*, 1994). A more tender muffin was observed when a combination of: (a) all three enzymes and (b) fungal amylase and bacterial amylase were utilized in the formulation. This could be due to an interactive effect of proteases and amylases as well as an interactive effect of amylases from different sources. Proteases have been shown to partially digest gluten which may cause a more tender muffin (Dziezak, 1991). Amylases act to break down starch to maltose and increase the amount of sugars. This increase in sugar may also help to soften the crumb by interfering with gluten development. The interactive effect of fungal amylase with bacterial amylase may have to do with the extent of their activity level. Bacterial amylase is excessively thermostable while fungal amylase may be insufficiently thermostable (Hebeda *et al.*, 1991). A combination of the two may produce a desirable softening effect. While it does seem that enzymes may help to soften the muffin crumb, there does not seem to be a definite trend within the enzymes themselves.

#### **4.2 The Effects of Water in the Baked Product**

In the baked product water plays a dual role. First, when mixed with flour, gelatinization occurs during baking and forms a material whose whole mechanical behavior allows the formation of desirable structures. Secondly, after baking, there is more or less water remaining in the product which will play a major role in determining the texture. During the baking process, the starch gelatinizes and the water content is reduced. The quality and stability of the final product are known to be affected by the

final moisture content. Therefore, the most significant change during starch gelatinization must be a redistribution of water within the dough matrix (Ablett et al., 1986).

The proteins of the flour permit the absorption of large quantities of water to form an elastic dough. The hydration capacity of flour is related to the yield of bread from a specific quantity of flour (Medved, 1978b). Soft wheat flour has a lower protein content and will absorb less water and form less gluten upon manipulation. This is conducive to a softer texture and structure as seen in a muffin product. Using a soft wheat flour would also leave more free water to be involved in other processes. Fats have been found to decrease the hydration capacity of the system.

Water content, water activity and its distribution affect starch gelatinization, protein denaturation, flavor and color formation, and shelf life. Research has been shown that it is water activity rather than water content that governs microbial spoilage of foods (Czuchajowska et al., 1989). Water controls reactions, texture, and general physical and biological behavior. The availability of water or water activity is influenced by salts, sugars, and other strong water-binding agents. If these are present in large amounts, water activity will be low and gelatinization will occur to a limited extent (Whistler and Daniel, 1985). Specific changes in quality and stability occur over a relatively narrow water activity range (Ablett et al., 1986).

Water content and its distribution also govern the shelf life of bread, which is influenced by incidence of microbial damage, and life of bread. The life of bread is influenced by softness of crumb, crispness of crust, crumb hardening, crumbliness, and

many other changes associated with overall staling and lowered consumer acceptance (Czuchajowska *et al.*, 1989).

Staling of baked goods is generally defined as an increase in crumb firmness and a corresponding loss in product freshness, flavor, aroma, texture, and perceived moisture level (Hebeda *et al.*, 1990). Starch retrogradation has been defined as “a process which occurs when the molecules comprising gelatinized starch begin to reassociate in an ordered structure” (Ward *et al.*, 1994).

Although both amylose and amylopectin are subject to retrogradation, the amylopectin component appears to be more responsible for long term quality changes in foods. It has also been suggested that the rate of retrogradation is controlled by the amount of water contained in the system. Other factors that have been considered to be involved in bread staling include starch and protein interactions, and interactions between swollen starch granules and the gluten matrix (Valjakka *et al.*, 1994).

#### **4.2.1 Moisture Content**

Table 3 represents the moisture percentages in each muffin variation after baking. The moisture content was significantly lower in the control as compared to all fat substituted muffins ( $p < 0.05$ ). In the fat substituted muffins less water was able to evaporate due to the action of gums to bind water. The gums imbibe the water and trap it during baking.

The most important property of galactomannans is their high water binding capacity which is dependent on the galactose content. Guar gum has a high galactose content and hence imbibes a great deal of water (Whistler and Daniel, 1985). The monoglycerides and

**Table 3:** Mean percent moisture content of control and fat substituted muffins.

| <b>Treatment</b>   | <b>Percent Moisture <math>\pm</math> SD#</b> |
|--|--|
| Control  | 39.5a $\pm$ 0.577                            |
| 100% fat substituted                                     | 45.0b $\pm$ 1.154                            |
| Bacterial amylase*                                       | 43.8b $\pm$ 0.957                            |
| Fungal amylase*  | 45.8b $\pm$ 1.258                            |
| Fungal protease*   | 45.5b $\pm$ 1.291                            |
| Bacterial amylase + fungal amylase*                      | 44.8b $\pm$ 0.500                            |
| Bacterial amylase + fungal protease*                     | 43.5b $\pm$ 0.577                            |
| Fungal amylase + fungal protease*                        | 44.3b $\pm$ 0.500                            |
| Bacterial amylase + fungal amylase +<br>fungal protease* | 44.3b $\pm$ 0.500                            |

n=4

\* All enzyme muffins are also 100% fat substituted.

# Mean scores with the same letter denote no significant differences at  $p>0.05$ .

sodium steryl lactylate present in the fat substitute have also been shown to absorb water and maintain moisture in bakery products (Sobczynska and Setser, 1991). The enzymes really did not have much effect on the moisture percentage of the fat substituted muffin. In most of the enzyme muffins, a slightly lower moisture percentage was found than in the 100% fat substituted muffin. Possibly the amylases created excess sugar which competed for water, and therefore, the gums had less available water for complete absorption.

#### **4.2.2 Water Activity**

The water activities of the different muffin formulations freshly baked, and after 24 and 48 hours storage are presented in Table 4. There were no significant differences ( $p>0.05$ ) noted among the among the three handling variations. However, the control muffin had the lowest water activity values immediately after baking and after 24 hours of storage. The water activity readings were fairly consistent throughout the testing period. Muffins prepared with fat substitutes and enzymes showed a greater variation in their water activity over the testing period. The 100% fat substituted muffin and the majority of the enzyme muffins decreased in water activity over the 48 hour storage period. The fungal protease and bacterial amylase combination muffin had an initial water activity of 0.927 that went to 0.916. The results do suggest that muffins lose free water upon storage, however, data indicated that with the addition of fat substitutes and enzymes the water system became slightly unstable as evidenced by the varied trends. These water activity levels, though are fairly high and microbial growth will not really

**Table 4:** Water Activity of control and fat substituted muffins freshly baked and after 24 and 48 hours storage.

| Treatment   | Aw Freshly baked $\pm$ SD# | Aw 24 hrs $\pm$ SD# | Aw 48hrs $\pm$ SD# |
|---|----------------------------|---------------------|--------------------|
| Control   | .9233a $\pm$ .004          | .9233a $\pm$ .001   | .9210a $\pm$ .002  |
| 100% fat substituted                                  | .9287a $\pm$ .001          | .9250a $\pm$ .004   | .9243a $\pm$ .005  |
| Bacterial amylase*                                    | .9267a $\pm$ .002          | .9270a $\pm$ .003   | .9220a $\pm$ .002  |
| Fungal amylase*                                       | .9297a $\pm$ .001          | .9270a $\pm$ .005   | .9193a $\pm$ .006  |
| Fungal protease*                                      | .9287a $\pm$ .006          | .9257a $\pm$ .006   | .9233a $\pm$ .003  |
| Bacterial amylase + fungal amylase*                   | .9277a $\pm$ .006          | .9253a $\pm$ .002   | .9253a $\pm$ .005  |
| Bacterial amylase + fungal protease*                  | .9270a $\pm$ .004          | .9253 $\pm$ .006    | .9167a $\pm$ .005  |
| Fungal amylase + fungal protease*                     | .9253a $\pm$ .006          | .9270a $\pm$ .003   | .9220a $\pm$ .003  |
| Bacterial amylase + fungal amylase + fungal protease* | .9267 $\pm$ .003           | .9243a $\pm$ .001   | .9183a $\pm$ .002  |

n=3

\* All enzyme muffins are also 100% fat substituted.

# Mean scores within the same column with the same letter denote no significant differences at  $p > 0.05$ .

decrease at these levels. All water activity values were within the range for microbial growth of yeast and molds (Czuchajowska *et al.*, 1989). Hippleheuser *et al.*, (1995) observed the water activity of low fat muffins substituted with pregelatinized dull waxy corn starches. Their water activity values were generally lower (as low as 0.840 was reported) than the values in this present study. The most likely explanation is the amount of liquid added to the batter. The lower water activity found for the control would be concurrent with the lower percent of moisture also found in the control. However, there were no significant differences ( $p>0.05$ ) between water activities which suggests that while the control had significantly less ( $p<0.05$ ) percent moisture it may have the same amount of free water.

#### 4.2.3 Staling

Table 5 shows the degree of staling in the muffin variations 24 and 48 hours after baking. Figure 1 illustrates the differences between the variations. After 24 hours the only significant difference was found in the fat substituted muffin containing bacterial amylase and fungal protease(BAFP). This combination of enzymes produced significantly higher ( $p<0.05$ ) staling rates than did bacterial amylase or fungal protease alone. Alpha amylases in general are very effective at decreasing staling rates. During baking, amylases partially hydrolyze starch molecules to small dextrans. These dextrans interfere with starch protein interactions and thus retard bread firming. Another proposed mechanism of staling is that alpha amylases cleave linkages in the amorphous regions of starch. This gives the crystallites greater freedom to move and results in a decreased rigidity of the

**Table 5:** Mean peak amylopectin areas (degree of staling) of control and fat substituted muffins after 24 and 48 hours storage.

| <b>Treatment</b>                                      | <b>Mean Staling after 24 hours <math>\pm</math> SD#</b> | <b>Mean Staling after 48 hours <math>\pm</math> SD#</b> |
|---|---|---|
| Control   | 5.662ab $\pm$ 1.316                                     | 11.131ab $\pm$ 1.644                                    |
| 100% fat substituted                                  | 6.012ab $\pm$ 1.046                                     | 9.568ab $\pm$ 1.905                                     |
| Bacterial amylase*                                    | 5.024a $\pm$ 0.650                                      | 7.947a $\pm$ 0.921                                      |
| Fungal amylase*                                       | 6.838ab $\pm$ 1.020                                     | 9.450ab $\pm$ 2.575                                     |
| Fungal protease*                                      | 4.993a $\pm$ 0.598                                      | 8.643a $\pm$ 1.884                                      |
| Bacterial amylase + fungal amylase*                   | 5.067ab $\pm$ 0.750                                     | 8.920ab $\pm$ 1.303                                     |
| Bacterial amylase + fungal protease*                  | 5.720b $\pm$ 1.138                                      | 11.056b $\pm$ 1.911                                     |
| Fungal amylase + fungal protease*                     | 5.380ab $\pm$ 1.918                                     | 9.027ab $\pm$ 1.422                                     |
| Bacterial amylase + fungal amylase + fungal protease* | 6.335ab $\pm$ 0.500                                     | 11.184ab $\pm$ 1.644                                    |

n=3

\* All enzyme muffins are also 100% fat substituted.

# Mean scores with the same letter denote no significant differences at  $p>0.05$ .

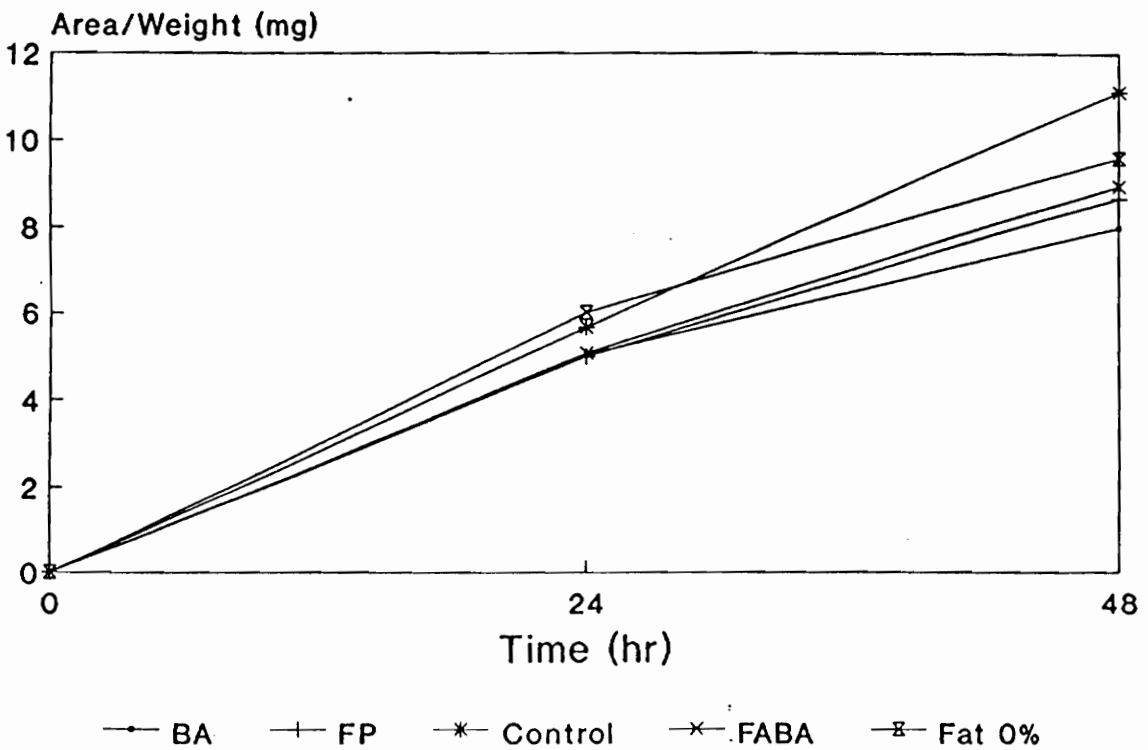
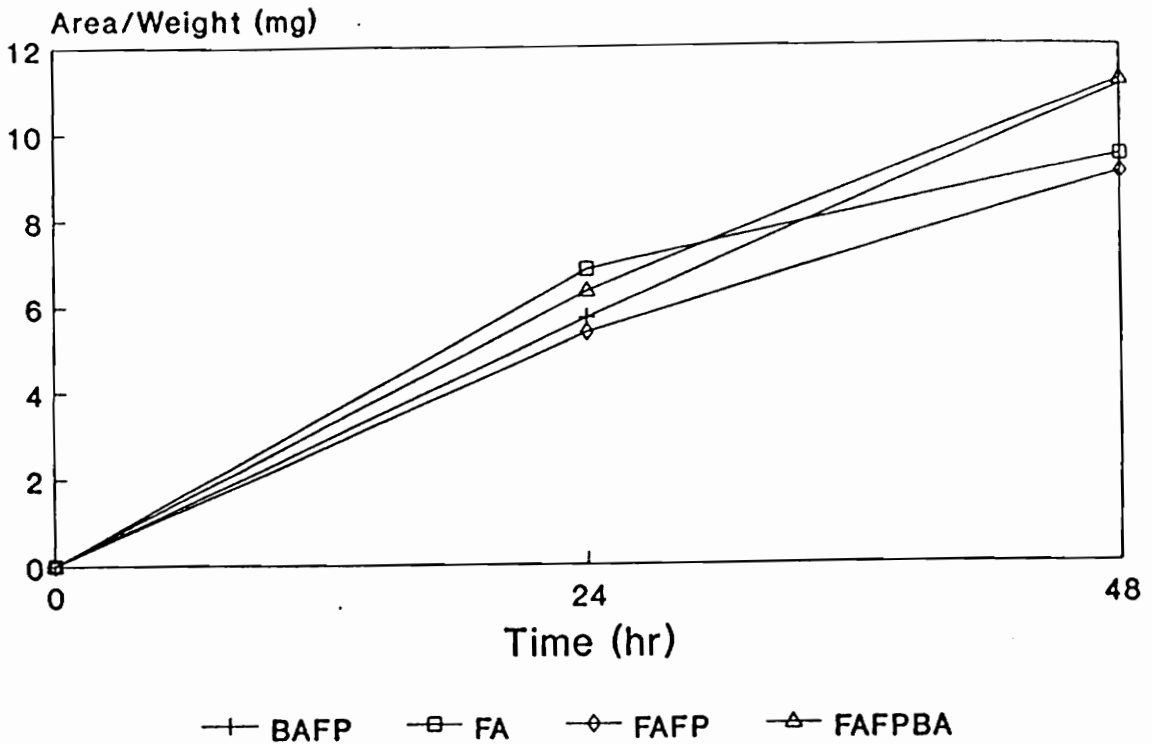


Figure 1. Staling rates of muffins after 24 hours and 48 hours storage time. Control (100% fat), Fat0% (100% fat substituted), BA (bacterial amylase), FA(fungal amylase), FP(fungal protease), FABA(fungal amylase+bacterial amylase), FAFP(fungal amylase+fungal protease), BAFP(bacterial amylase+fungal protease), FAFPBA(fungal amylase+fungal protease+bacterial amylase).

system (Valjakka *et al.*, 1994). A period of amylase activity exists between the temperature at which amylases are denatured. During this period of activity, amylases degrade starch. The bacterial amylase is inactivated at the highest temperature, therefore, a high number of dextrans are produced. This accounts for the low staling rate of the bacterial alpha amylase.

Fungal proteases have not been shown to effect bread firming, rather the proteases are used more to improve grain and texture (Haseborg, 1981). The results (Table 5) obtained after 24 hours suggest that protease alone does help reduce staling in a muffin possibly by interfering with the gluten matrix. However, a combination of fungal protease and bacterial amylase appears to increase staling significantly ( $p < 0.05$ ). This suggests that amylase and protease together in a product may interfere with each enzyme's independent role to prevent staling. Possibly the fungal protease and bacterial amylase compete for some of the same complexes within the muffin and become deactivated before they can work on the starch crystallites. However, this effect was not apparent in the fungal protease and fungal amylase muffin which suggests that bacterial sources of amylases must exhibit some unique property which makes them incompatible with fungal protease in preventing staling. An interesting observation made thus far is that the bacterial amylase and fungal protease combination had the lowest moisture percentage (Table 3) of all the 100% fat substituted variations. This same combination also had the lowest water activity (Table 4) after 48 hours, also possibly contributing to a high staling rate.

The muffin with fungal amylase alone produced the highest staling which suggests it is inactivated before it really can act on the starch. Bacterial amylases are inactivated at a

very high temperature, while fungal amylases are inactivated at a lower temperature. A combination of fungal amylase and bacterial amylase produces an inactivation temperature that is between the two amylases which may be optimal at preventing staling and also from preventing a sticky, gummy crust seen with excessive dextrinization. In fact, some of the newer intermediate temperature stability enzymes designed to decrease staling contain mixtures of fungal and bacterial amylases (Hebeda *et al.*, 1990). After 24 hours the control and the 100% fat substituted muffin had intermediate levels of staling as compared to the enzyme muffins.

After 48 hours there was significantly greater ( $p < 0.05$ ) staling in the fat substituted muffin with the combination of fungal protease and bacterial amylase than when each of the enzymes were used independently (Table 5). The control muffin had a fairly consistent staling rate over the 48 hour period (Figure 1). Whereas in the fat substituted muffins except for the one with fungal protease and bacterial amylase each seemed to stale to a certain degree over the 24 hours and then the staling rate began to level off (Figure 1). This suggests that an enzyme's full effect on reducing staling may not be perceived for many days. Also, another suggestion may be that any of these enzyme combinations may in fact retard staling as compared to the control over time. The control muffin also had the least amount of water as determined by the percent moisture (Table 3) which may also contribute to increased staling rates. The 100% fat substituted muffin had intermediate staling rates suggesting that the increase in water in these products contributed to a slight decrease in staling as compared to the control. After 48 hours the bacterial amylase staled the least which does confirm other research (Valjakka *et al.*, 1994) in that the bacterial

amylase remains active at a high temperature, and therefore, can produce the most dextrins to interfere with starch protein complexes.

### **4.3 Crust and Crumb Color**

Quality factors in food can be divided into three major areas: color, flavor, and texture. Each can be handled in an objective manner, but if color is unacceptable there may be little point in considering the flavor or texture (Francis and Clydesdale, 1975b).

Crust color of a baked product is often associated with the pH of the batter and Maillard Browning. Maillard Browning takes place in the presence of an amino acid bearing compound usually a protein and a reducing sugar and some water (Whistler and Daniel, 1985).

Tables 6 and 7 show the effect of the treatments on the crust and crumb L and b values, respectively. There were no significant differences in the crust L and b values (Table 6) among any of the treatments ( $p > 0.05$ ). This may suggest that the fat substitutes and enzymes were able to mimic the crust color of a fat containing muffin. However, when examining the means, the control muffin did have the highest L value and the lowest b value. Apparently, the control crust was whiter to a certain degree than the other muffins. This could be due to the fact that fat substituted muffins contain a protein based fat substitute which may have contributed to Maillard Browning. Previous research (Pong *et al.*, 1991, Berglund and Hertsgaard, 1986) on fat reduced bakery products has found the crust color to increase in lightness with an increased level of fat substitution. The results of this study are opposite quite possibly because those previous reports used

**Table 6:** Mean crust color values of control and fat substituted muffins.

| <b>Treatment</b>   | <b>L value<sup>^</sup> ± SD#</b> | <b>b value<sup>^</sup> ± SD#</b> |
|--|----------------------------------|----------------------------------|
| Control  | 77.28a ± 2.961                   | 41.20a ± 3.209                   |
| 100% fat substituted                                     | 73.08a ± 3.024                   | 45.78a ± 1.429                   |
| Bacterial amylase*                                       | 73.80a ± 3.830                   | 44.53a ± 3.299                   |
| Fungal amylase*  | 76.80a ± 4.190                   | 43.48a ± 3.566                   |
| Fungal protease*   | 74.03a ± 5.125                   | 43.34a ± 4.790                   |
| Bacterial amylase + fungal amylase*                      | 75.55a ± 2.675                   | 44.08a ± 3.730                   |
| Bacterial amylase + fungal protease*                     | 75.60a ± 2.198                   | 44.83a ± 2.988                   |
| Fungal amylase + fungal protease*                        | 71.78a ± 5.048                   | 44.03a ± 0.981                   |
| Bacterial amylase + fungal amylase +<br>fungal protease* | 74.78a ± 2.890                   | 44.48a ± 2.027                   |

n=4

\* All enzyme muffins are also 100% fat substituted.

# Mean scores within the same column with the same letter denote no significant differences at  $p > 0.05$ .

<sup>^</sup>L= lightness(100) and darkness (0); b= yellow (+70) and blue (-80)

**Table 7:** Mean crumb color values of control and fat substituted muffins.

| <b>Treatment</b>   | <b>L value<sup>^</sup> ± SD#</b> | <b>b value<sup>^</sup> ± SD#</b> |
|--|----------------------------------|----------------------------------|
| Control  | 85.33a ± 1.012                   | 26.28b ± 1.863                   |
| 100% fat substituted                                     | 86.00a ± 0.294                   | 24.28a ± 0.519                   |
| Bacterial amylase*                                       | 85.75a ± 0.580                   | 24.22a ± 0.929                   |
| Fungal amylase*  | 85.58a ± 0.330                   | 24.62a ± 0.591                   |
| Fungal protease*   | 85.82a ± 0.457                   | 24.60a ± 1.055                   |
| Bacterial amylase + fungal amylase*                      | 85.40a ± 0.440                   | 25.72a ± 1.170                   |
| Bacterial amylase + fungal protease*                     | 85.30a ± 0.500                   | 24.32a ± 0.946                   |
| Fungal amylase + fungal protease*                        | 85.28a ± 1.844                   | 24.65a ± 1.330                   |
| Bacterial amylase + fungal amylase +<br>fungal protease* | 85.62a ± 0.960                   | 24.30a ± 1.863                   |

n=4

\* All enzyme muffins are also 100% fat substituted.

# Mean scores within the same column with the same letter denote no significant differences at  $p > 0.05$ .

<sup>^</sup>L= lightness(100) and darkness (0); b= yellow (+70) and blue (-80)

different fat substitutes or none at all. Early research (Maninden and Jorgensen, 1983) has shown that alpha amylases increase crust color, but more recent studies (Valjakka *et al.*, 1994, Kuracina *et al.*, 1987) have shown that alpha amylase has no effect on crust color. The results presented here demonstrate that amylases and proteases have a contributory effect on crust color.

The crumb lightness values (L) (Table 7) were extremely similar ( $p>0.05$ ) for all variations, however the crumb b value for the control muffin was significantly higher ( $p<0.05$ ) than all other variations. This would be opposite the trend observed in the crust color where the b value was lower for the control. The decreased alkalinity of the batter possibly caused by the fat substitute and/or hydrocolloid could have altered the yellow color of the crumb. The color of the fat substitute, the gums, or the enzymes could also have had a contributory effect. Similar results were reported by Pong *et al.*, (1991) when shortening was replaced by a fat substitute in a cupcake.

The enzymes had little effect on the crumb color. However, the data indicated that the combination of bacterial amylase and fungal amylase did produce a slightly more yellow crumb color when compared to the other enzyme preparations. When the bacterial amylase and fungal amylase were used independently, the b values were similar to the 100% fat substituted muffin. Again, this could be due to an interactive role of the amylases from different sources being inactivated at a temperature conducive to producing a slightly yellower crumb color.

#### **4.4 Sensory Evaluation: Quantitative Descriptive Analysis**

#### **4.4.1 Appearance**

Vision is often overlooked as an important component in food perception and appreciation. Anticipatory physiological responses might not occur as they do when we see a food without smelling, tasting, or touching it. Of the different visual characteristics of foods, color is probably the most important (Moskowitz, 1983).

##### **4.4.1.1 Perceived Crumb Color**

Color and appearance are usually the primary basis of acceptance or rejection of a food; yet, important as this may be, there is a surprisingly wide variation in color which fits within preconceived notions of what the acceptable color of a food should be. Color control in foods is employed for many reasons. One is standardization of the product from a quality control point of view. A consumer expects all units of a certain brand of food to be the same color. If one is different it is immediately suspect and probably will be left on the shelves (Francis and Clydesdale, 1975a).

Table 8 shows the mean scores of perceived crumb color. The control muffin had a significantly higher score ( $p < 0.05$ ) than all other variations. A higher score indicates a more yellow color. These results are consistent with the physical measurements in which the crumb in the control was also found to be significantly ( $p < 0.05$ ) more yellow than all other variations (Table 7). A lighter crumb in the fat substituted variations was most likely due to the decreased alkalinity of the batter caused by the addition of the fat substitute, gums, or enzymes. The results agree with Bollonger and Freund (1992), who found that calorie reduced sponge cakes were lighter in color than their full fat counterparts.

**Table 8:** Mean perceived sensory scores of crumb color, cell size and cohesiveness of control and fat substituted muffins.

| Treatment   | Perceived Crumb Color<br># / ** | Perceived Cell Size<br># / ** | Perceived Cohesiveness<br># / ** |
|---|---------------------------------|-------------------------------|----------------------------------|
| Control   | 3.147b ± .190                   | 3.315b ± .151                 | 2.868b ± .106                    |
| 100% fat substituted                                  | 2.444a ± .119                   | 2.553a ± .510                 | 3.962a ± .136                    |
| Bacterial amylase*                                    | 2.395a ± .227                   | 2.937a ± .350                 | 3.915a ± .131                    |
| Fungal amylase*                                       | 2.390a ± .091                   | 2.553a ± .371                 | 3.886a ± .209                    |
| Fungal protease*                                      | 2.370a ± .117                   | 2.840a ± .534                 | 3.867a ± .339                    |
| Bacterial amylase + fungal amylase*                   | 2.439a ± .238                   | 2.970a ± .329                 | 3.941a ± .299                    |
| Bacterial amylase + fungal protease*                  | 2.385a ± .229                   | 2.875a ± .196                 | 3.938a ± .335                    |
| Fungal amylase + fungal protease*                     | 2.662a ± .239                   | 2.936a ± .492                 | 3.977a ± .123                    |
| Bacterial amylase + fungal amylase + fungal protease* | 2.466a ± .229                   | 2.814a ± .241                 | 3.765a ± .115                    |

n=4

\* All enzyme muffins are also 100% fat substituted.

# Mean scores within the same column with the same letter denote no significant differences at  $p > 0.05$ .

\*\* **Crumb Color:** The degree of yellowness in the crumb of the muffin (0=not yellow and 6=very yellow).

\*\* **Cell Size:** The size of cells located in the crumb of the muffin (0=small cells and 6=large cells).

\*\* **Cohesiveness:** The ability of the muffin to stay together (no crumbling) as it is broken in half on the plate (0=not cohesive and 6=very cohesive).

The fungal amylase fungal protease combination had a higher mean score in both the physical and sensory measurements (Tables 7 and 8). Possibly fungal enzymes in general produce a more yellow crumb color, however, muffins with fungal amylase or fungal protease alone were not found to be more yellow. Kuracina *et al.* (1987), found that different amylases had no effect on crumb color of breads which would suggest that the lighter color may be attributed to the fat substitute or gums.

#### 4.4.1.2 Perceived Cell Size

The dimensions, and geometry of a product may influence its acceptance to consumers. A well-made muffin is uniform in texture, however, the grain is usually not very fine and the cell walls are of medium thickness (Bennion, 1990a). The breaking strength of the batter films between gas cells determines the extent to which gas cells combine; this affects the degree of expansion and the fineness and homogeneity of the crumb structure (Bloksma, 1986).

Table 8 shows the mean cell size sensory scores for the muffin variations. The control muffin was found to have significantly larger cells ( $p < 0.05$ ) than the other variations. This is probably due to the action of the gums in the 100% fat substituted muffins to coat gas bubbles and thus retain more air cells and produce a finer crumb structure. Miller and Setser (1982) reported that a xanthan gum -starch complex may form in a baked product and cause the product to retain more gas during baking. Miller and Hosenev (1993) found that xanthan gum slows the rate of gas diffusion and possibly this may lead to a firmer crumb with increased air cells.

When the enzymes were used alone except for fungal amylase or in combination, the cell size appeared to increase when compared to the 100% fat substituted muffin. Research (Dziezak, 1991) has shown the protease's ability to hydrolyze gluten and form a more open crumb. Fungal and bacterial amylases were also shown to produce a more open crumb (Valjakka *et al.*, 1994).

The results appear to be in accordance with the tenderness data in Table 2. The control muffin was significantly tender ( $p < 0.05$ ) than the fat substituted muffins. A product with a more open grain would be easily deformed, than with a more compact grain (Conforti *et al.*, 1993).

#### **4.4.1.3 Perceived Cohesiveness**

Information concerning the textural characteristics of foods may be obtained prior to mastication (Brennan, 1984). Cohesiveness is used as a term to describe interactions among pieces or particles. It is related to the mechanical properties of a product (reaction to stress) (Meilgaard *et al.*, 1987a).

Table 8 shows the mean sensory scores for cohesiveness of the muffin variations. The control muffin was found to be significantly less cohesive ( $p < 0.05$ ) than all other variations. This would be expected due to fats' shortening power. Paton *et al.* (1981) reported that increased oil levels produced less cohesive cakes. The added hydrocolloids in the fat substituted muffins imbibe water, increase batter viscosity and cause a more cohesive structure. Neville and Setser (1986) reported that increased water lead to increased cohesiveness and gumminess in cakes. As evidenced in the percent moisture

results (Table 3), the 100% fat substituted muffins were significantly moister ( $p < 0.05$ ). Civelle (1990) also found that products prepared with microparticulates in place of fat are less oily in feel and more cohesive/gummy in consistency. Other studies (Bollinger and Freund, 1992, Bath *et al.*, 1992) have reported that fat substituted cakes produce a more spongy, dense and cohesive structure.

The muffins with enzymes, generally were less moist than the 100% fat substituted muffin (Table 3) and also produced less cohesiveness (Table 8). Data suggest that the moisture content does affect cohesiveness. Some of the muffins with fungal enzymes were moister and, higher in cohesiveness than the 100% fat substituted muffin which suggests that fungal enzymes hydrolyze protein and thus more water may be needed to form a gluten starch network leading to increased moisture percentages and increased cohesiveness. Cohesiveness seems to result in products which are lower in tenderness but higher in moisture.

#### **4.4.2 Textural Properties**

During the past two decades the importance of texture as an attribute of the sensory quality of food has been recognized. Very often sensory assessments of texture are made on the basis of the sensations perceived when the food sample is manipulated in the mouth, i.e., when it is bitten, masticated, and swallowed. During such manipulation there is a reciprocal interaction between the texture of the food and the buccal work acting to change the texture to a state suitable for swallowing (Brennan, 1984).

#### 4.4.2.1 Perceived Tenderness

While cohesiveness in this study dealt with the non-oral representation of deformation, tenderness deals with the oral characteristics of this mechanical parameter. Table 9 shows the mean sensory scores for tenderness for all muffin variations. The control muffin was found to be significantly tender ( $p < 0.05$ ) than all other formulations. The sensory panel linked visual texture properties (cohesiveness) with oral textural properties (tenderness). Similar results were obtained for the physical measurements for texture (Table 2). The quantity of fat in the control muffin was able to coat the strands of gluten and produce a tender muffin. The water binding property of the gums probably contributed to the decreased perceived tenderness of the fat substituted muffins. Pong *et al.* (1991) also reported significantly less tender ( $p < 0.05$ ) sensory scores for fat replaced cupcakes when compared to a full fat prototype.

According to physical data (Table 2), the enzymes produced an increase in tenderness as compared to the 100% fat substituted muffin, however, this trend was not so pronounced with the sensory results. Bacterial amylase, fungal amylase, and a combination of bacterial amylase and fungal amylase were the only enzyme variations to be more tender than the 100% fat substituted muffin. A conjecture arises that amylases in general produce more of a softening effect than do proteases. Fungal protease produced a less tender muffin in all variations which was not observed in the physical data (Table 2). Dziezak (1991) reported that proteases increase softness which was in direct contrast to the results.

**Table 9:** Mean perceived sensory scores of tenderness, moisture and adhesiveness of control and fat substituted muffins.

| Treatment   | Perceived Tenderness # / ** | Perceived Moisture # / ** | Perceived Adhesiveness # / ** |
|---|-----------------------------|---------------------------|-------------------------------|
| Control   | 3.229b ± .257               | 3.045b ± .167             | 3.083b ± .131                 |
| 100% fat substituted                                  | 2.369a ± .056               | 2.559a ± .159             | 2.756a ± .144                 |
| Bacterial amylase*                                    | 2.389a ± .106               | 2.482a ± .132             | 2.741a ± .101                 |
| Fungal amylase*                                       | 2.440a ± .246               | 2.710a ± .196             | 2.927a ± .153                 |
| Fungal protease*                                      | 2.312a ± .234               | 2.325c ± .190             | 2.745a ± .059                 |
| Bacterial amylase + fungal amylase*                   | 2.450a ± .145               | 2.536a ± .150             | 2.818a ± .206                 |
| Bacterial amylase + fungal protease*                  | 2.156a ± .261               | 2.405a ± .148             | 2.818a ± .162                 |
| Fungal amylase + fungal protease*                     | 2.241a ± .133               | 2.458a ± .259             | 2.906a ± .136                 |
| Bacterial amylase + fungal amylase + fungal protease* | 2.335a ± .264               | 2.444a ± .190             | 2.879a ± .266                 |

n=4

\* All enzyme muffins are also 100% fat substituted.

# Mean scores within the same column with the same letter denote no significant differences at  $p > 0.05$ .

\*\* **Tenderness:** The ease with which the muffin is penetrated with one's teeth upon chewing (0=not tender and 6=very tender).

\*\* **Moisture:** The amount of moisture perceived within the mouth (0=not moist and 6=very moist).

\*\* **Adhesiveness:** The ability of the muffin to stick to the roof of one's mouth (0=not adhesive and 6=very adhesive).

#### 4.4.2.2 Perceived Moisture

Fats endow foods with so many taste and textural qualities that oro-sensory assessment of fat content can sometimes be unreliable. Mouthfeel characteristics are related to the perception of moisture or fat in a product (Drewnowski, 1992). The control muffin was found to be significantly more moist ( $p < 0.05$ ) than all other variations (Table 9). Opposite results were observed in the physical data (Table 3) in which moisture content was significantly less ( $p < 0.05$ ) in the control muffin. Possibly the sensation of perceived moisture is related to the presence of fat and water. The panelists may not have been able to separate textural properties of moistness and oiliness. Perhaps, the fat substitute and gums bind water so tightly that is not able to be perceived in the mouth. Bath *et al.* (1992) reported that fat does impart a moist mouthfeel to a product. Therefore, the fat may have been perceived as moistness.

While Pong *et al* (1991) found no significant differences ( $p > 0.05$ ) in sensory scores for moistness, the fat substituted cupcake did have a higher overall moisture content than the control. This would be in opposition to the results found here which suggests the increased moistness may be due to either the added gums or a protein based fat substitute which were not used in the aforementioned study.

The physical results (Table 3) indicated that all the enzymes caused a decrease in the moisture content. This same trend was detected in the sensory results (Table 9) for all the enzymes except for the fungal amylase. In fact the fungal protease produced a significantly less moist ( $p < 0.05$ ) muffin than all the variations. This would indicate that

amylases and proteases possibly weaken the gluten structure or cause the gums and fat substitute to imbibe less water resulting in a less moist product.

#### **4.4.2.3 Perceived Adhesiveness**

Adhesiveness deals with the work necessary to overcome the attractive forces between the surface of the food and the surface of other materials with which the food comes in contact (e.g. tongue, teeth) (Brennan, 1984). The control was found to be significantly more adhesive ( $p < 0.05$ ) than all other variations. Oil is more easily dispersed or emulsified because it is fluid and exhibits mobility (Birnbaum, 1978). The increased adhesiveness may be due to the oil's ability to spread over a wider surface area and cause a fuller sensation in the mouth. This effect may also be interrelated with the higher moisture level perceived in the control muffin (Table 9). It appears that adhesiveness is a product of interactions between fat and moisture.

The 100% fat substituted muffin had a low adhesiveness score possibly because the addition of hydrocolloids bind water and create a more cohesive, gummy structure. It appears that as cohesiveness increases (Table 8), adhesiveness decreases. In general, the enzymes increased adhesiveness which is due to their action to weaken the gluten starch network and also possibly due to their ability to decrease the gums' functionality.

#### **4.4.3 Flavor Characteristics**

The components of flavor are chemically stimulated. They are often blended and complex and thus more difficult to separate and describe than the physically more discrete

texture or appearance properties. Flavor is the combined effects of the aromatics, tastes, and chemical feelings stimulated by a substance in the mouth (Civelle, 1990). A spectrum of characteristics are involved in the total flavor impact and instruments will detect only a portion of that spectrum (Setser, 1994).

#### 4.4.3.1 Perceived Sweetness

The sweetness that a person perceives does not relate directly to the amount of sugar as measured by chemical methods, because some chemicals suppress the taste of others, and some chemicals are synergistic with each other (Setser, 1994). The perceived sweetness scores for all muffin variations are presented in Table 10. The control muffin was found to be significantly sweeter ( $p < 0.05$ ) than all other variations. The fat in the control muffin probably had an interactive effect with the sugar and produced a sweeter muffin. Fats and oils themselves have low flavor intensity (Civelle, 1990). In many fat-based products the fat contributes to flavor as a carrier of the lipid-soluble flavor fractions. Therefore, less sweetness in a low fat product will be detected because there is little or no fat to act as a flavor carrier. Armbrister and Setser (1994) reported that reducing fat levels in shortbread cookies caused the vanilla-like and related sweet aromatics to be more volatile. Lipophilic flavorings can be poorly bound to the food matrix in the absence of fat.

Armbrister and Setser (1994) also found that all fat reduced (75% level) variations of shortbread cookies were significantly less sweet ( $p < 0.05$ ) than the control. Pong *et al.* (1991) also found that the use of a fat substitute decreased the mean sensory scores for

**Table 10:** Mean perceived sensory scores for sweetness and aftertaste of control and fat substituted muffins.

| Treatment   | Perceived Sweetness / ** # | Perceived Aftertaste / ** # |
|---|----------------------------|-----------------------------|
| Control   | 3.095b ± .150              | 2.922a ± .350               |
| 100% fat substituted                                  | 2.702a ± .232              | 2.953a ± .259               |
| Bacterial amylase*                                    | 2.470a ± .153              | 3.108a ± .154               |
| Fungal amylase*                                       | 2.612a ± .162              | 3.006a ± .156               |
| Fungal protease*                                      | 2.689a ± .105              | 3.102a ± .049               |
| Bacterial amylase + fungal amylase*                   | 2.825a ± .162              | 3.153a ± .250               |
| Bacterial amylase + fungal protease*                  | 2.583a ± .217              | 2.955a ± .146               |
| Fungal amylase + fungal protease*                     | 2.671a ± .207              | 2.907a ± .243               |
| Bacterial amylase + fungal amylase + fungal protease* | 2.596a ± .165              | 3.155a ± .095               |

n=4

\* All enzyme muffins are also 100% fat substituted.

# Mean scores within the same column with the same letter denote no significant differences at  $p > 0.05$ .

\*\* **Sweetness:** The amount of sweetness detected in the mouth (0=not sweet and 6=very sweet).

\*\* **Aftertaste:** The degree to which a bitter and/or undesirable taste is perceived in the mouth after ten chews (0=not detected and 6=very much detected).

sweetness. Possibly the fat substitute masks the perception of sweetness. Moskowitz (1983) reported that viscosity plays a definite, but not the entire, role as a blocking agent to reduce flavor. Possibly since hydrocolloids increase viscosity of the batter they also cause a decreased perception of sweetness. All the enzyme muffins except for the variation with a combination of fungal amylase and bacterial amylase were found to be less sweet than the 100% fat substituted muffin. Possibly the enzymes mask the sweetness perception or maybe they produce other products during the baking process which interfere with sweetness perception. The muffin with both fungal amylase and bacterial amylase was sweeter than the muffins prepared with the other enzymes which could be due to the amylases breaking down the starch to sugar. This effect was not seen with fungal amylase or bacterial amylase independently. An interactive effect between the types of amylase could be the cause. Previous research (Kuracina *et al.*, 1987) reported that amylases had no effect on flavor of breads.

#### **4.4.3.2 Perceived Aftertaste**

Aftertaste was defined as an undesirable and/or bitter taste after ten chews (Appendix J). Some individuals have a difficult time distinguishing sourness from bitterness or bitterness from astringency. Confusion exists between designating a sensation as bitter (Powers, 1984). There were no significant differences among any of the variations in regard to aftertaste (Table 10). However, the control muffin and the 100% fat substituted muffin had low scores suggesting that the enzymes may contribute a bitter or undesirable aftertaste to the muffin. The muffin with both fungal amylase and bacterial amylase had

one of the highest aftertaste scores which also was perceived to be the sweetest among the fat substituted muffins. Possibly the panelists had difficulty in differentiating between sweetness and bitterness. While Kuracina *et al.* (1987) found that amylases had no effect on flavor in breads, possibly, these enzymes may have had more of an effect in a batter system. The bitter taste could also have been attributed to the presence of baking powder, xanthan gum or possibly due to the absence of fat which has been observed to dissolve flavor components and mask the bitterness (Medved, 1978a).

#### **4.5 Sensory Evaluation: Consumer Tests**

The primary purpose of affective tests is to assess the personal response (preference and/or acceptance) by current or potential customers of a product, product idea, or specific product characterization. An acceptance scale is used when the “affective status” of a product, i.e. how well it is liked by consumers needs to be determined. The product is compared to a competitor and a hedonic scale is used to indicate degrees of acceptability or likeness. From relative acceptance scores one can infer preference, the sample with the highest score is preferred (Meilgaard *et al.*, 1987b). It is also important to remember that differences from the control do not automatically imply that the product would be unacceptable to consumers (Armbrister and Setser, 1994).

##### **4.5.1 Acceptance Levels of the Three Muffin Variations**

The control, 100% fat substituted, and 100% fat substituted with fungal amylase and bacterial amylase were used as the muffins to ascertain preference levels. The combination

of fungal amylase and bacterial amylase was used based on results from the physical data and preliminary preference tests. The mean scores of the three variations are represented in Table 11. The 100% fat substituted muffin with fungal amylase and bacterial amylase was preferred significantly more ( $p < 0.05$ ) than the control muffin. The control muffin was liked slightly less than “like moderately” while the 100% fat substituted with fungal amylase and bacterial amylase muffin was liked in between “like moderately” and “like very much”. The 100% fat substituted muffin was liked slightly more than “like moderately”.

#### **4.5.1.1 Demographic Information**

Of the 84 panelists, 58% were female and 42% were male. Forty percent of the consumers who tasted the muffins were under age 25; 30% were between 25 and 44 and 30% were over the age of 44. The high percentage of respondents under 25 is probably due to the proximity of Virginia Tech University to the testing site. Eighteen percent of the respondents had less than a 6th grade education; 32% had received a high school education; 29% had reached a college level of education; and 21% had received an advanced degree education. The percentage of respondents who tasted each variation was evenly distributed throughout the different demographic questions.

#### **4.5.1.2 Opinions on Fat Free Muffins**

Twenty-three percent of respondents reported to consume muffins every few days.

**Table 11:** The mean hedonic scores on the acceptability/preference of three muffin variations.

| Treatment  | Mean Hedonic Score # * |
|--|------------------------|
| Control  | 3.179b $\pm$ 0.31      |
| 100% fat substituted   | 2.964ab $\pm$ 0.02     |
| 100% fat substituted with fungal amylase and bacterial amylase | 2.536a $\pm$ 0.29      |

n=1

# Mean scores with the same letter denote no significant differences at  $p > 0.05$ .

\* Hedonic scale value 1="like extremely" and 9="dislike extremely". (Appendix N)

Twenty-four percent consumed at least one muffin per week, while 26% reported to consume about one muffin a month. Twenty-seven percent of the respondents claimed they hardly ever ate muffins. Possibly these consumers do not recall their actual intake very well. Ninety-six percent of respondents reported that they would be interested in purchasing a fat free muffin if it tasted the same as a full fat muffin. This suggests that consumers are becoming more aware of the role of dietary fats in baked products and they are interested in decreasing their fat intake as long as taste is not sacrificed. Eighty-nine percent of the consumers would purchase the 100% fat substituted muffin with fungal amylase and bacterial amylase. Seventy-five percent of the consumers claimed they would purchase the 100% fat substituted muffin. Only 71% of the consumers indicated that they would purchase the control muffin if it were fat free. This suggests that consumers do not like the full fat muffin as well as fat reduced products. Possibly consumers are beginning to adjust to lower fat products and they are beginning to incorporate these products into their daily diet.

## Chapter 5

### Summary, Conclusions and Recommendations for Future Research

American consumers are realizing the importance of restricting their fat intake to promote their well being, and at the same time to decrease their incidence of various diseases. Consumers are not so willing to give up the taste of full fat foods, therefore, many food companies are competing to develop fat free products with the same attributes. Fat has a multifunctional role in many different food systems and mimicking it is often quite difficult. Fat substitutes from various sources are now being incorporated into fat free foods.

The main objective of this study was to develop a completely fat free muffin with similar properties to a full fat (control) muffin that would be accepted by consumers. A protein based fat substitute along with xanthan and guar gums was employed to mimic the functionality of fat. Amylases and proteases were added to these formulations to hydrolyze peptide and starch complexes in order improve the quality of the fat substituted muffin.

There were no significant differences ( $p>0.05$ ) in volume among any of the variations. The fat substituted muffins were able to produce similarly sized muffins to the control. However, the fat substituted muffins were higher in volume due to the gums' ability to bind water and trap air. The enzymes decreased the volume of the muffins to a level similar to the control due to their weakening effect on starch, protein and gum matrixes.

Tenderness was significantly greater ( $p<0.05$ ) in the control muffin which is consistent with the function of fat. The gums in the fat substituted muffins bound water and created

a rigid structure. The enzymes contributed to a weakening of this structure which may have been due to the breaking down of some of the starch-protein complexes and a softer crumb was produced. The muffin with the lowest volume score (control) was also the most tender suggesting that increased air retention contributed to a tough crumb.

Moisture content was significantly higher ( $p < 0.05$ ) for the fat substituted variations which was consistent with the theory that gum hydration influenced an increase in moisture percentage. The bacterial enzymes were able to produce moisture levels similar to the control suggesting that these enzymes hydrolyze starch complexes that are holding water. As the water content increased, tenderness decreased and volume increased indicating that the water bound within the gums created a less tender and higher volumed product.

There were no significant differences ( $p > 0.05$ ) in water activity among any of the muffins. This demonstrates that while the fat substituted muffins have a significantly higher moisture percentage, this water is not “free” but is “bound” water. The control did, however, have the lowest water activity when freshly baked and after 24 hours storage which is consistent with the decreased moisture content found in the control. After 48 hours storage the water activities in the fat substituted muffins decreased to levels which were similar to the control. Possibly the gums lose water after storage. The enzymes decreased water activity levels to a greater extent as compared to the 100% fat substituted muffin.

After 24 and 48 hours storage, the fat substituted muffin with a combination of fungal protease and bacterial amylase had a significantly greater ( $p < 0.05$ ) staling rate than fat

substituted muffins made with either fungal protease or bacterial amylase. Bacterial amylases have high inactivation temperatures and, therefore produce dextrans to decrease the staling rate. On the contrary, fungal protease must weaken starch-protein complexes leading to a decreased staling rate. However, these enzymes in combination do not work synergistically on staling rates possibly due to their competition for the same complexes. A special point should be made that the control muffin and 100% fat substituted muffins had relatively higher staling rates suggesting that the use of some enzyme preparations will effectively decrease staling rates.

There were no significant differences ( $p>0.05$ ) in crust L and b color values suggesting that the extent of Maillard Browning was similar in all variations. The control muffin did have a significantly higher crumb color b value than the fat substituted formulations. The fat substitute, enzymes, or gums probably lowered the pH of the batter or their inherent colors could also have contributed to the variation in color.

Quantitative descriptive analysis (QDA) and a consumer acceptance/preference test served as tools for sensory evaluation. Appearance characteristics indicated that the QDA panelists found cell size and perceived yellow crumb color to be significantly greater ( $p<0.05$ ) in the control. The panelists also found that the control was significantly ( $p<0.05$ ) less cohesive than the fat substituted variations. The gums entrapped water, increased air retention, and increased batter viscosity which affected a decrease in cell size, and an increase in cohesiveness. The enzymes increased cell size and decreased cohesiveness due to their ability to hydrolyze proteins and starch. The yellow crumb color

of the control was consistent with the physical data, and could be attributed to the decreased alkalinity of the system.

The control muffin was significantly ( $p < 0.05$ ) different in tenderness, moisture, and adhesiveness when compared to the other muffins variations. The oil in the control muffin surrounded gluten strands and produced a tender crumb. The results showed that fat and moisture interact with each other and cause a full bodied mouthfeel sensation that was perceived as moisture and adhesiveness. The gums and fat substitute bound water which produced a tougher crumb. However, this water was not perceived as moisture. The addition of enzymes in the fat substituted muffins suggest that amylases in particular are effective at increasing tenderness by weakening starch complexes. Both amylases and proteases were found to decrease perceived moisture and increase perceived adhesiveness due to the loss of water from the fat substitute and gums.

Sweetness was found to be significantly greater ( $p < 0.05$ ) in the control muffin due to the ability of fat to act as a flavor carrier. Enzymes, particularly amylases, produced a sweeter flavor in the muffin suggesting that the amylases are breaking down the starch to sugars. The QDA panelists found no significant differences ( $p > 0.05$ ) in aftertaste among the variations. Nevertheless, aftertaste scores were higher in the muffins containing enzymes probably due to either interactions of enzymes with food ingredients or an inherent flavor characteristic.

The consumer central location test indicated that among respondents in southwestern Virginia a fat substituted muffin was preferred over a full fat muffin. In fact, the fat substituted muffin with the combination of fungal amylase and bacterial amylase had a

significantly higher ( $p < 0.05$ ) acceptance rate with this population. A 100% fat free baked product was developed that consumers liked and also would consider to purchase.

The results indicated that there is considerable potential in the food industry for fat substituted products. While physical and QDA scores indicated that fat substituted products are different from full fat products, consumers are less concerned about certain attributes but more concerned with taste. It is evident that the enzymes particularly the fungal and bacterial amylases did improve many of the attributes of the fat substituted muffins.

In order to produce acceptable fat free baked products it is necessary to completely understand the role of water in these products. Almost all of the muffin qualities that were evaluated seemed to be related to water content and its availability. The addition of gums and fat substitutes to a baked product altered the functionality of the water. Additional research is needed to produce fat free baked products with similar moisture contents and water activities comparable to a full fat baked product.

Research also needs to be done on different enzymes, enzyme usage levels and their incorporation into fat free products. The results in this study indicated that there is potential for enzyme and enzyme combinations in this area. Possibly higher amounts of amylases would have more of a weakening effect on the rigid structure attributed by the gums and fat substitute. In order to use these enzymes effectively, it appears research needs to be done on the enzyme's side effects on flavor and coloring in the food item. Decreased levels of fat substitutes and/or gums may also enhance the effect of enzymes.

In addition the combined use of various emulsifiers may also be beneficial to enhance enzyme activity and product quality.

The results indicated that the fat's ability to coat gluten strands is not mimicked with these fat substitutes due to the fact they are a dry powder. Perhaps a more liquid, viscous substance would better mimic fat's functionality.

While the results from the preference test are only representative of a sample of consumers in one area of the United States, there is strong evidence that consumers actually have become accustomed to the taste of certain fat free products. Possibly more studies should be aimed at fulfilling consumers' desires rather than mimicking the functionality of fat. Younger generations are being brought up on fat reduced products and this is affecting their preferences and at the same time their selection of a particular food item. Consumers from different geographic areas will most likely have different food preferences. Therefore, broader based sensory studies need to be conducted on fat substitutes. Also, in order to decrease standard error in QDA testing, training and screening of the panelists is of prime importance.

Consumer surveys find Americans reading the new food labels more and using the information in their purchase decisions. The emergence of low fat and no fat food alternatives is at an all time high. Therefore, it is up to the researcher and manufacturer to attempt to maintain quality standards in these products, and at the same time to insure good nutrition is being maintained with the addition of sugar and salts to these foods (Hollingsworth, 1995).

Research must be conducted on the effect that fat substitutes have on macronutrient absorption. There is also a question of satiety. Will people who consume fat substitutes compensate by eating more calories in other ways? While the current trend in the United States is fat reduced foods; there seems to be an issue of who should consume fat substitutes. Children and lean consumers are choosing these fat substituted products, but it is important to remember that 30% of calories should still come from fat. The quality of fat is important to contribute the essential fatty acids that are required to maintain good individual health.

Considering the results of this research, there are some recommendations that can be offered for future development of fat free baked products. The use of either a carbohydrate based or protein based fat replacer should be employed. The results also demonstrate that amylases both bacterial and fungal would have the most potential in a fat free baked product. Possibly the use of bacterial or fungal amylase would work synergistically with the carbohydrate based fat replacer to produce a desirable product. Another suggestion would be to lower the amounts of gums and fat substitute added to the product.

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**Appendix A**  
**Muffin Formulations**

**Table 12:** Formulation of regular and fat-free muffins using a recipe derived from The Pillsbury Cookbook,(1989).

| <b>Ingredients</b> | <b>Control</b> | <b>100% Reduced</b> | <b>100% Reduced with enzymes</b> |
|--------------------|----------------|---------------------|----------------------------------|
| Flour, all-purpose | 225g           | 225g                | 225g                             |
| Sugar, granulated  | 100g           | 100g                | 100g                             |
| Baking Soda        | 2.0g           | 2.0g                | 2.0g                             |
| Baking Powder      | 11.4g          | 11.4g               | 11.4g                            |
| Salt               | 2.5g           | 2.5g                | 2.5g                             |
| Eggs               | 43g            | 43g egg whites      | 43g egg whites                   |
| Nonfat Buttermilk  | 125ml          | 125ml               | 125ml                            |
| Skim Milk          | 125ml          | 125ml               | 125ml                            |
| Vegetable Oil      | 73ml           | 0ml                 | 0ml                              |
| Vanilla            | 5ml            | 5ml                 | 5ml                              |
| Xanthan Gum        | 0.125g         | 0.125g              | 0.125g                           |
| Guar Gum           | 0.125g         | 0.125g              | 0.125g                           |
| Fat Replacer       | 0g             | 1.00g               | 1.00g                            |
| Enzymes            | 0g             | 0g                  | .003g                            |

**Appendix B**  
**Experimental Design**

**Table 13:** Experimental Design for 2x2x2 augmented factorial block design\*\*

| Day | Variation A | Variation B | Variation C |
|-----|-------------|-------------|-------------|
| 1   | 1           | 2           | 3           |
| 2   | 4           | 5           | 6           |
| 3   | 7           | 8           | 9           |
| 4   | 1           | 4           | 7           |
| 5   | 2           | 5           | 8           |
| 6   | 3           | 6           | 9           |
| 7   | 1           | 5           | 9           |
| 8   | 7           | 2           | 6           |
| 9   | 4           | 8           | 3           |
| 10  | 1           | 8           | 6           |
| 11  | 4           | 2           | 9           |
| 12  | 7           | 5           | 3           |

\*\*  $t=9$   $k=3$   $r=4$   $b=12$   $\lambda=1$

For the purpose of randomly assigning the muffin formulations to specific days, numbers were assigned to each variation\*: 1=FP+BA; 2=FA+BA; 3=100% Fat Reduced; 4=FP+FA+BA; 5=FP; 6=Control; 7=FA; 8=FP+FA; 9=BA.

\*All muffins made with enzymes are 100% fat reduced. FP=fungal protease; FA=fungal amylase; BA= bacterial amylase

**Appendix C**  
**Ingredients**

1. Southern Biscuit All-Purpose Flour (Midstate Mills, Inc., Newton, North Carolina)
2. Granulated sugar
3. Calumet Double-Acting Baking Powder (Kraft General Foods, Inc., White Plains, New York)
4. Arm and Hammer Natural Baking Soda (Arm and Hammer, Princeton, New Jersey)
5. Morton Iodized Salt (Morton International, Inc., Chicago, Illinois)
6. Eggs (Grade A large eggs)
7. Skim Milk (less than 0.5% fat, Pasteurized and Homogenized)
8. Non fat Buttermilk (Cultured and Pasteurized)
9. Vegetable Oil (Hunt-Wesson, Inc., Fullerton, California)
10. Vanilla Extract (McCormick) containing vanilla bean extract in water, alcohol (35%) and corn syrup.
11. Ticaxan xanthan and guar gums (TIC Gums Inc., Belcamp, Maryland)

The fat substitute used was:

Simplesse Bakery Blend 710 (NutraSweet Co., Deerfield, Illinois)

The enzymes used were::

1. Fresh-N (bacterial amylase) (Enzyme Development Corporation, New York, NY)
2. Enzeco fungal alpha amylase 5000 (Enzyme Development Corp., New York, NY)
3. Enzobake concentrate (fungal protease) (Enzyme Development Corp., New York, NY)

**Appendix D**

**Volume**

### Sample Analysis

1. Two random muffins from each pan were taken and the average of the two volume measurements was used for analysis.
2. The muffins were cooled for one hour before the readings were taken.
3. A caliper was used to measure the height and the diameter of the muffin.
4. For the height measurement, a reading was taken of the middle or highest part of the muffin and it was averaged with the height of the end or lowest part of the muffin.
5. For the diameter measurement, a reading was taken at the low base or smaller part of the muffin and it was averaged with a reading taken at the top base or larger part of the muffin.
6. All measurements were taken in centimeters.
7. The measurements were put in the following formula which is the volume of a cylinder.

$$\text{Volume} = \left(\frac{22}{7}\right) r^2 h$$

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

**Appendix E**  
**Texture Analysis**

### Instron Preparation

1. The Universal Instron Machine Model 1011 ( Universal Instron, Canton, MA) was turned on along with the computer monitor and the printer.
2. The GPIB button should be pushed so the red light is on. This enables the Instron to interface with the computer.
3. Click on (TEST) Test a sample. Enter the operator's name, sample ID, compressive method, and the method #.
4. Click F10 and Start Specimen will appear on the screen. The specimen gauge length was set at 6.75 mm and the diameter of the muffin was 65 mm. (F10-ok) was then pressed.

### Sample Analysis

1. Each muffin to be tested was cut in cross section so that the top part of each muffin was cut off.
2. The sample was placed under the compression probe assembly. The distance between the muffin and the probe should be 5mm. This was adjusted using the up and down buttons on the Instron.
3. Press enter to begin acquisition. The probe will move downward. When the screen reads: Remove the sample and press enter to return the crosshead, press enter again.
4. Put the next sample under the compression probe and press (F10=Continue) and then (F10=OK). Press enter to begin the acquisition.
5. When all the samples are run, press ESC, and then (F10 if OK). The data will automatically be printed on the printer.

**Appendix F**  
**Moisture Content**

### Sample Preparation

1. A muffin was cooled at room temperature for two hours.
2. The crumb was then torn into small pieces, placed in a teflon-lined metal pan (that was preweighed) and weighed to 10 grams (Sartorius portable top load balance, PT 1200-OUR, Bohemia, NY).

### Brabender Moisture Tester Preparation

1. A Brabender Moisture Tester (C.W. Brabender Instruments, Inc., South Hackensack, NJ) SAS 692 was switched on one hour prior to testing.
2. Once heated to 130 °F , the sample pans were placed into the Brabender and the door was latched.

### Sample Analysis

1. The samples were dried for one and a half hours until the moisture content readings reached an equilibrium.
2. After drying, each sample was reweighed on the top load balance.
3. The percent moisture was determined using the following formula:

$$\% \text{moisture} = [(\text{wt. of sample+pan} - \text{wt. of sample+pan after drying}) / 10\text{g}] \times 100$$

**Appendix G**  
**Differential Scanning Calorimeter**

### Sample Preparation

1. A sample of the crumb approximately 40.0 mgs was removed from the muffin.
2. A Perkin AD-6 Autobalance (Perkin-Elmer, Norwalk, CT) was calibrated and the sample was weighed in an aluminum pan.
3. The sample was encapsulated in the pan and a lid with an O-ring was crimped into place (Perkin-Elmer, Norwalk, CT).

### Differential Scanning Calorimeter Calibration

1. The system TADS, DSC program disk was put into drive 0.
2. The DSC start-up data disk was put into drive 1.
3. TADS was typed and Go to Set Up was pressed.
4. "Indium standard" and the operator was entered.
5. The parameters were modified for the indium standard.
6. Set Up was pressed on the microprocessor controller and parameters of temperature minimum, temperature maximum, heat rate, cool rate, and temperature span were entered (Table 14).
7. The load temperature was modified to that of the temperature minimum.
8. Enter was pressed and the Go to Load and Reset buttons were pressed at the same time.
9. An indium standard weighing 4.36 grams, and a reference pan were placed in the sample compartment head and the lever was pushed down to close the compartment head.
10. The compartment head temperature was equilibrated to the appropriate minimum temperature.

**Table 14:** Parameters for Indium Standard and Muffin Samples

| <b>Parameter</b>          | <b>Indium Standard</b> | <b>Muffin Sample</b> |
|---------------------------|------------------------|----------------------|
| Final Temperature (°C)    | 175                    | 140                  |
| Minimum Temperature (°C)  | 50                     | 15                   |
| Temperature Increase (°C) | 15                     | 10                   |
| Initial Temperature (°C)  | 50                     | 15                   |
| Y Range                   | 5                      | 5                    |
| Heating Rate (°C/Minute)  | 10                     | 10                   |
| Cool Rate (°C/Minute)     | 320                    | 320                  |
| Sample Weight (mgs)       | 4.36                   | 37.0-43.0            |
| Temperature Span (°C)     | 400                    | 400                  |

11. Zero was pressed and the Y range was stabilized between 0.5 and 1.00 mcal/second.
12. Ready and Start were then pressed.
13. Once the run was finished, Go to Load and Reset were pressed together and then Go to Analysis was pressed.
14. The temperature scale was rescaled to 50 to 175 degrees C.
15. If needed the slope was adjusted to a slope=0 position and the Y scale was rescaled.
16. The Peak was then set between 155 and 165 degrees C.
17. For calibration, the indium standard had to have a melting point of 156.6 +/- 0.2 degrees C.

#### Sample Analysis

1. Ice was placed in the sample compartment in front of the sample compartment head.
2. The sample pan and a reference pan were placed in the sample compartment head once it reached minimum temperature.
3. The compartment head was sealed and equilibrated to the minimum temperature.
4. The sample ID and operator were entered.
5. The parameters of the system controller and the microprocessor controller were modified.
6. The Y scale was zeroed and the Ready and Start buttons were pressed.
7. Once the run was finished, the Go to Load and Reset buttons were pressed together to reset the load temperature. Go to Analysis was then pressed.
8. The temperature scale was rescaled to 30 to 140 degrees centigrade and the Y scale was rescaled to 1. If needed the slope was also adjusted.

9. The peak was determined and the thermogram was saved and plotted.
10. The peak area (height x length of the peak) divided by the sample weight in mgs was calculated and displayed graphically for correlations.
11. For each sample a DSC run was done 24 and 48 hours after baking.

**Appendix H**  
**Colorimeter**

### Sample Preparation

1. The sample was cooled to room temperature for about one hour prior to testing.
2. Both crust and crumb values were measured so after an intact muffin's crust was analyzed, the muffin was then cut cross-sectioned to measure the crumb color.

### Colorimeter Preparation and Standardization

1. The Hunter Lab L Optical Sensor 45/0 D25-PC2 (Hunter lab, Reston, VA) was placed on stand-by along with a laptop PC for at least a half an hour prior to testing.
2. The PC, printer, and machine were then switched to operate and a white uncalibrated tile was removed from the specimen port.
3. A black standard glass tile was placed on the specimen port, shiny side toward the port.
4. <F1> was pressed and the bottom of the scale was standardized or zeroed.
5. The black tile was removed and a white standard glass tile was placed on the specimen port.
6. <F1> was pressed and the top of the scale was standardized or zeroed.
7. <F1> was pressed again to place the measurement mode into delta E values of L, a, and b.
8. <F8>, #1, and <F2> were pressed in that order to place the standard values into L, a, and b.

### Sample Analysis

1. The muffin sample was placed on the sample port and positioned so that no light was able to shine through.

2. <F1> was pressed and the L, a, b, and delta E values were calculated. <F5> was then pressed to print the data.

**Appendix I**  
**Water Activity**

### Sample Preparation

1. Muffin crumb was put in a disposable sample cup so that the bottom of the cup was covered.
2. The temperature of the sample should be within 2-3 degrees centigrade of the CX-1.
3. Samples were taken freshly baked, after 24 hours and after 48 hours of baking.

### Operation of the CX-1 Water Activity System

1. Plug in the CX-1 (Decagon Devices, Inc, Pullman, WA) and turn on the power switch. The CX-1 is ready for measuring Aw's when the display shows all zeroes. Warm up time is about 15 seconds.

### Sample Analysis

1. Pull out the sample drawer located on the lower right of the front panel.
2. Place the sample cup in the sample drawer.
3. Close the drawer and turn the knob counterclockwise from "load" to "read". A beep will indicate that the CX-1 has started measuring.
4. The display of the CX-1 will indicate the Aw and the temperature of the food sample as it nears equilibrium. The CX-1 will beep and the display will stop flashing when the reading is complete.

**Appendix J**

**Modified Quantitative Descriptive Analysis Panel Training**

1. Due to time restrictions, the recruited panelists participated in three training sessions.
2. The investigator of this study acted as a facilitator for the training sessions.
3. Sessions were conducted as focus groups.

#### First Training Session

1. This session was used for the panelists to develop terminology and definitions to a wide variety of muffin references.
2. The muffin references used were the control muffin, a fat-free muffin, and a store bought regular muffin.
3. Each of the twelve panelists wrote down every perceived attribute of each muffin.
4. The group narrowed the list of attributes to the eight that would be used throughout the study. The panelists then defined and ordered each attribute in the way in which they were perceived by the senses.
5. The attributes were in order: crumb color, cell size, cohesiveness, tenderness, sweetness, moistness, adhesiveness, and aftertaste.
6. The panelists also signed informed consent forms at this time.

#### Second and third training sessions

1. The panelists evaluated four muffins per session to demonstrate their ability to evaluate reference standards and extremes of the defined attributes.
2. For the second training session the control, the control plus 20 grams of baking powder, and the control with no baking powder but 3 grams of baking soda were used to evaluate the attribute of aftertaste.

3. In the third training session the control, the control with 200 grams of sugar, and the control with 350 grams of liquid was used to evaluate sweetness, tenderness, and moistness, and cohesiveness.

#### Training Location

1. The training was conducted in 336 Wallace Hall and sensory booths were located in 339 Wallace Hall, VPI and SU, Blacksburg, VA.
2. The panelists were in partitioned booths in a laboratory.
3. The lighting was white fluorescent lights.
4. The environment was at room temperature, approximately 72 ° F.
5. Panelists received training material through booth doors.

#### Sample Preparation

1. The muffins were cooled to room temperature and cut into four pieces, put on each panelists plate which was divided into fourths and then wrapped in plastic wrap.
2. The samples were randomly coded with three digit numbers.

#### Testing procedures

1. Panelists received a tray with the scorecard, definitions, a pencil, napkin, a cup of room temperature tap water, an expectorate cup, and a paper plate with the four samples on it.
2. The panelist placed a vertical mark on the line scale at the point at which they believed best represented the perceived intensity of the attribute.
3. Panelists were asked if they understood the scorecard, attributes and definitions at each session.
4. The scorecard was adjusted according to their suggestions

QDA Attribute Definitions:

1. CRUMB COLOR: The degree of yellowness in the crumb of the muffin.
2. CELL SIZE: The size of cells located in the crumb of the muffin.
3. COHESIVENESS: The ability of the muffin to stay together (no crumbling) as it is broken in half on the plate.
4. TENDERNESS: The ease with which the muffin is penetrated with one's teeth upon chewing.
5. SWEETNESS: The amount of sweetness detected in the mouth.
6. MOISTNESS: The amount of moisture perceived within the mouth.
7. ADHESIVENESS: The ability of the muffin to stick to the roof of one's mouth.
8. AFTERTASTE: The degree to which a bitter and/or undesirable taste is perceived in the mouth after ten chews.

**Appendix K**

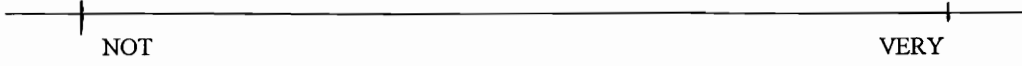
**Modified Quantitative Descriptive Analysis Scorecard**

Panelist # \_\_\_\_\_

Sample # \_\_\_\_\_

Date \_\_\_\_\_

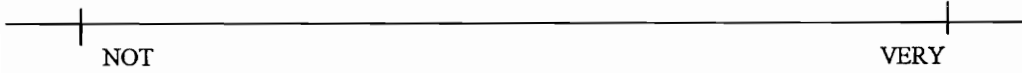
CRUMB COLOR:



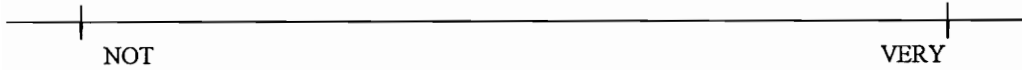
CELL SIZE:



COHESIVENESS:



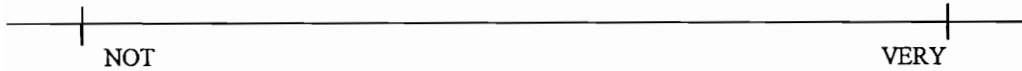
TENDERNESS:



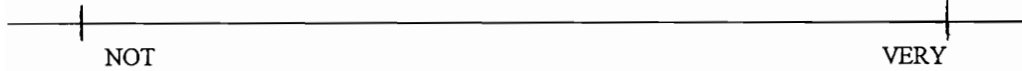
SWEETNESS:



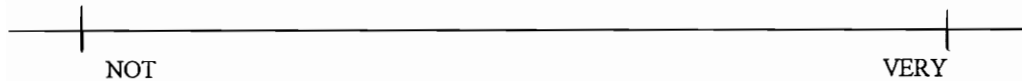
MOISTNESS:



ADHESIVENESS:



AFTERTASTE:



**Appendix L**

**Modified Quantitative Descriptive Analysis Testing Procedures**

### Panelists

1. The panelists who were trained were recruited graduate students from the Department of Human Nutrition and Foods, VPI & SU, Blacksburg, VA.

### Testing Location

1. Each panelist tested samples in a partitioned booth located in 339 Wallace Hall, VPI & SU, Blacksburg, VA.
2. The booths had fluorescent white lighting and were at approximately 72 °F and at relative humidity.

### Sample Preparation

1. The muffins were cooled for one hour after baking.
2. The muffins were cut into fourths and four different muffin samples were put on each panelist's plate. The plate was then wrapped in plastic wrap.
3. Each sample was assigned a randomly selected three digit code.

### Testing Procedures

1. Each panelist received a tray with a scorecard, definitions of attributes, a pencil, a napkin, a cup of room temperature water, an expectorate cup, and a plate with four coded samples on it.
2. Each panelist received four coded samples at each testing session.
3. The four muffin samples were placed on the plate in a random fashion. They included three testing samples and one reference sample which was the control.
4. Between each sample, the panelists took a sip of water and waited 30 seconds before testing the next sample.

5. Panelists evaluated the samples according to the order of the attributes.
6. Panelists were told to rate each sample as it compared to the reference sample which would be in the middle of all attribute scales.
7. Panelists placed a vertical mark on the line scale at the point at which best represented their perceived intensities of the attributes.

#### Statistical Analysis

1. For analysis, the location of the marks were measured from the extreme left end of the scale in inches.
2. A completely randomized 2x2x2 augmented factorial was used to show the presence or absence of the three enzymes and the extra control.
3. Analysis of Variance (ANOVA) using General Linear Models procedure in SAS was used to determine where significant differences existed among overall attribute means at  $p < 0.05$ .
4. Contrast analysis was done only when significant differences were found in the overall ANOVA.
5. Residuals were all checked to examine if variables were from a normal distribution. They were all found to be normal.

**Appendix M**  
**Central Location Testing**

### Testing Location

1. A table was set up in the atrium area or center of the New River Valley Mall, Christiansburg, VA, on Saturday March 11, 1995, from 10:00 AM to 4:00 PM.
2. The table was under fluorescent white lighting and the environment was at room temperature with a relative humidity.

### Panelists

1. Eighty-four untrained panelists who were shoppers at the mall participated.

### Sample Preparation

1. The control, the 100% fat substituted, and the 100% fat substituted with fungal amylase and bacterial amylase formulations were chosen as the samples to be tested based on preliminary results.
2. The muffins were made one and a half hours prior to testing in 339 Wallace Hall, VPI & SU, Blacksburg, VA, and transported by car to the testing location.
3. After cooling, the muffins were cut into fourths and wrapped in plastic wrap. Each variation had a random three digit code.

### Testing Procedures

1. Each shopper received a scorecard with an informed consent paragraph, a piece of muffin, a napkin, and a scorecard.
2. The samples were given to the shoppers by alternating the samples.
3. The shopper tasted the muffin and rated their degree of liking on a 9-point hedonic scale.
4. The shopper then filled out a questionnaire (Appendix N).

**Appendix N**  
**Central Location Testing Scorecard**

# \_\_\_\_\_

### MUFFIN TASTE TEST

These samples are regular or fat reduced muffins which I formulated in my master's thesis in the Human Nutrition and Foods Department at Virginia Tech. The muffins may contain a fat replacer and different enzyme preparations. All of the ingredients used are FDA approved. You may decline participation if you choose. I thank you for your time and participation in my research.

Please taste this sample and indicate on the scale below how well you like it.

- Like extremely
- Like very much
- Like moderately
- Like slightly
- Neither like nor dislike
- Dislike slightly
- Dislike moderately
- Dislike very much
- Dislike extremely

Thank you for your response and please fill out the short questionnaire on the opposite side.

To assist in the analysis of the data, I need to get some additional information from you.

1.  Female  Male

2. Age  Less than 18  
 18-24  
 25-34  
 35-44  
 45-54  
 55-65  
 65 or over

3. Educational Background

Sixth grade or less  
 High School  
 College  
 Advance Degree

4. How often do you eat muffins?

One a day  
 One every few days  
 One every week  
 One every month  
 Hardly ever

5. Would you be interested in purchasing a fat-free muffin if it tasted the same as a regular muffin?

Yes  No

6. Would you be willing to buy a fat-free muffin if it had similar attributes to the muffin you tasted?

Yes  No

Do you have any comments about this research?

Thank you again for your time.

**Appendix O**  
**SAS Program**

```

data one;
input fa 1 ba 2 fp 3 trt 4 t 8-9 vol 10-12 tex1 13-17 tex2 18-20 moi 21-22 crtl 23-26 crtb
27-30 crbl 31-34 crbb 35-38 wa1 39-41 wa2 43-45 wa3 47-49 dsc1 50-55 dsc2 57-62
/*Fabafp T voltex1tex2moicrtlcrtbcrblcrbbwa1wa2wa3 dsc1 dsc2*/
cards;
proc print;
run;
proc glm;
classes trt t;
title1 'analysis of physical data with trial used as block, and trt compared';
title2 'to trt*trial interaction';
model vol tex1 tex2 moi crtl crtb crbl crbb wa1 wa2 wa3 dsc1 dsc2= trt t;
output out=residata r=rvol rtex1 rtex2 rmoi rcrtl rcrtb rcrbl rcrbb rwa1 rwa2 rwa3 rdsc1
rdsc2 p=pvol ptex1 ptex2 pmoi pcrtl pcrtb pcrbl pcrbb pwa1 pwa2 pwa3 pdsc1 pdsc2;
contrast 'fa - yes or no' trt 1 1 1 1 -1 -1 -1 -1 0;
contrast 'ba - yes or no' trt 1 -1 1 -1 1 -1 1 -1 0;
contrast 'fp - yes or no' trt 1 1 -1 -1 1 1 -1 -1 0;
contrast 'fa*ba' trt 1 -1 1 -1 -1 1 -1 1 0;
contrast 'ba*fp' trt 1 -1 -1 1 1 -1 -1 1 0;
contrast 'fa*fp' trt 1 1 -1 -1 -1 -1 1 1 0;
contrast 'all vs control' trt 1 1 1 1 1 1 1 1 -8
constrast '3 way interaction' trt 1 -1 -1 1 -1 1 1 -1 0;
means trt;
run;
proc univariate data=residata plot normal;
var rvol rtex1 rtex2 rmoi rcrtl rcrtb rcrbl rcrbb rwa1 rwa2 rwa3 rdsc1 rdsc2;
run;
proc plot data=residata;
plot rvol*pvol rtex1*ptex1 rtex2*ptex2 rmoi*pmoi rcrtl*pcrtl rcrtb*pcrtb rcrbl*pcrbl
rcrbb*pcrbb rwa1*pwa1 rwa2*pwa2 rwa3*pwa3 rdsc1*pdsc1 rdsc2*pdsc2 / vref=0;
run;
quit;
data one;
title 'sensory data analysis';
input ID fa 6 ba 7 fp 8 t col siz coh ten swe moi adh aft;
if fa=0 and ba=0 and fp=0 then trt=1;
if fa=0 and ba=1 and fp=0 then trt=2;
if fa=0 and ba=0 and fp=1 then trt=3;
if fa=0 and ba=1 and fp=1 then trt=4;
if fa=1 and ba=0 and fp=0 then trt=5;
if fa=1 and ba=1 and fp=0 then trt=6;
if fa=1 and ba=0 and fp=1 then trt=7;
if fa=1 and ba=1 and fp=1 then trt=8;
if fa=2 and ba=2 and fp=2 then trt =9;

```

```

cards;
proc print data=one;
run;
proc sort; by t trt;
run;
proc means data=one mean no print;
by t trt;
var col siz coh ten swe moi adh aft;
output out=meandata mean= ;
run;
proc print data=meandata;
run;
proc glm fata=meandata;
classes trt t;
model col siz coh ten swe moi adh aft =trt t;
output out=residata r=rcol rsiz rcoh rten rswe rmoi radh raft
      p=pcol psiz pcoh pten pswe pmoi padh paft;
contrast 'fa - yes or no' trt 1 1 1 1 -1 -1 -1 -1 0;
contrast 'ba - yes or no' trt 1 -1 1 -1 1 -1 1 -1 0;
contrast 'fp - yes or no' trt 1 1 -1 -1 1 1 -1 -1 0;
contrast 'fa*ba' trt 1 -1 1 -1 -1 1 -1 1 0;
contrast 'ba*fp' trt 1 -1 -1 1 1 -1 -1 1 0;
contrast 'fa*fp' trt 1 1 -1 -1 -1 -1 1 1 0;
contrast 'all vs control' trt 1 1 1 1 1 1 1 1 -8
contrast '3 way interaction' trt 1 -1 -1 1 -1 1 1 -1 0;
means trt;
run;
proc univariate data=residata plot normal;
var rcol rsiz rcoh rten rswe rmoi radh raft;
run;
proc plot data=residata;
plot rcol*pcol rsiz*psiz rcoh*pcoh rten*pten rswe*pswe rmoi*pmoi radh*padh raft* paft /
vref=0;
run;
quit;

```

**VITA**

Robin Canterella was born on October 30, 1971 in Mastic Beach, New York. She received her Bachelor of Science Degree in Nutrition and Foods at Cornell University in Ithaca, New York. She attended graduate school at Virginia Polytechnic Institute and State University in Blacksburg, Virginia where she received a Master of Science Degree in Foods. She completed the requirements for this degree in May, 1995.