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To the Graduate Council:

I am submitting herewith a thesis written by Cherné La-Toi Wallace entitled "Evaluation of pepper heat intensity and textural parameters of cheese biscuits formulated at 3 fat and 4 cayenne pepper levels." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Science and Technology.

Majorie P. Penfield, Major Professor

We have read this thesis and recommend its acceptance:

Clark Brekke, Sharon Melton

Accepted for the Council: Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

To the Graduate Council:

I am submitting herewith a thesis written by Cherné La-Toi Wallace entitled "Evaluation of Pepper Heat Intensity and Textural Parameters of Cheese Biscuits Formulated at 3 Fat and 4 Cayenne Pepper Levels." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Science and Technology.

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We have read this thesis and recommend its acceptance:

Accepted for Council:

Vice Provost and Dean of Graduate Studies

Evaluation of Pepper Heat Intensity and Textural Parameters of Cheese Biscuits Formulated at 3 Fat and 4 Cayenne Pepper Levels

A Thesis

Presented for the Master of Science Degree

The University of Tennessee, Knoxville

Cherné La-Toi Wallace

December 2001

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#### Abstract

Time-intensity profiles were utilized to study the perceived heat intensity of cheese biscuits formulated at 3 fat reduction levels (0, 25, and 30%) and 4 cayenne pepper levels (0.0, 0.1, 0.2, and 0.4%). The design used for the experiment was an incomplete block with a block on panelists and days. Trained panelists evaluated the heat intensity of 3 cheese biscuits per testing session at 5, 15, 30, 45, 60, 90, 120, 150, 180, 210, 240, 270, and 300 s using a 150-point unstructured line scale. On a scorecard, panelists also evaluated the hardness. Sensory data were collected 9 times per wk for 4 wk. Four testing sessions were held within each of 3 replications. Percentages of moisture and fat, hardness, and brittleness were also measured.

Based on the parameters measured on the time-intensity curves, total heat intensity and maximum heat intensity increased as the cayenne pepper level increased from 0.0 to 0.4% in the cheese biscuits regardless of the fat level (p<0.0001). Panelists found cheese biscuits formulated with 0.4% cayenne pepper to be more intense in heat than those made with 0.0, 0.1, and 0.2% pepper. The time it took in seconds to reach the maximum heat measurement for cheese biscuits made with 0.1, 0.2, and 0.4% cayenne pepper was less than the time it took for cheese biscuits made with no cayenne pepper (p<0.0001).

Full-fat cheese biscuits (0% reduction) contained less moisture than did the 25 and 30% reduced-fat cheese biscuits (p<0.0001). As the fat reduction in the cheese biscuits increased from 0 to 30% reduced-fat, the percentage of fat

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decreased from 33.07 to 23.99% (p<0.0001).

Based on the textural analysis of hardness and brittleness, the 25 and 30% reduced-fat cheese biscuits were harder and more brittle than were the fullfat cheese biscuits. Cheese biscuits formulated with 0.2% cayenne pepper were not as hard as those containing 0.0, 0.1, and 0.4% cayenne pepper. Panelists perceived full-fat cheese biscuits to be less hard than those containing 25 and 30% reduced-fat. A strong, positive correlation, 0.92, (n=12) existed between the sensory evaluation and the instrumental analysis of hardness (p>0.0001).

In a low water activity food such as cheese biscuits, the perception of heat caused by capsaicin is dependent upon the concentration. Reducing the fat in cheese biscuits affected the textural parameters. Because of mastication differences among individuals, textural parameters such as hardness and brittleness can influence the rate at which the heat induced by capsaicin is perceived by trigeminal nerve endings.

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#### Chapter 1

#### Introduction

According to LaBell and O'Donnell (1997), Americans are eating more even though their lifestyles are becoming increasingly sedentary. As a result, more than 50% of adults are overweight, 25% obese, and 11% of children and adolescents are overweight (Weaver 2000). Obesity causes approximately 280,000 deaths per year and results in numerous cases of Type II diabetes (Weaver 2000). According to Sloan (1998), 4 in 10 children and 96 million adults have high cholesterol levels and 50 million are plagued with coronary heart disease. Thus, consumers are changing their eating habits to include foods without fat and cholesterol for weight-loss and preventative reasons (Horton 1987; Sloan 1996). However, there is a poor compliance by individuals to low-fat diets (Bruhn and others 1992). Fats in foods provide a wide variety of oral sensations influencing texture, lubricity, and flavor (Glueck and others 1994). On the other hand, when fat is removed from a food system, the perception of fat is altered along with other organoleptic properties (Glueck and others 1994). Since taste is a major factor that influences the food choices of people, the use of capsicum peppers in low-fat versions of original products has increased the acceptance of such products by consumers (Horton 1987; Bruhn and others 1992).

America has become a spicier country over the past 2 decades because of the emergence of ethnic foods containing capsicum peppers and other spices compared to 20 yr ago when foods consumed contained far fewer spices than they do today (LaBell 1997). Growing multicultural influences from an increase in the Hispanic and Asian populations, in which spices are a fundamental part, have shifted the American palate to prefer more intensely flavored and spiced products (Lawless 1989; Sloan 1996). An increase in population diversity along with increased travel, improved communication, and an increase in the elderly population are attributable for an increased consumption of spices (Horton 1987; Hamel and Schreiner 1988; Lawless 1989; Uhl 1996; Bruss 2000). According to Sloan (1996), America's per capita consumption of spices and seasonings is a pound higher than it was a decade ago. Furthermore, the demand for intensely flavored foods is confirmed by restaurant trend data, in which one-half of adults preferred spicy dishes to mild ones and one-third preferred intensely spiced foods (Sloan 1996). Food companies have responded in order to satisfy the public's established taste for spicy foods by producing line extensions, sauces, marinades, condiments, snacks, dry seasoning blends, and other new products (LaBell 1997).

Columbus has been credited with discovering and introducing capsicums to Europe, Asia, and Africa after his voyage in 1492. However, evidence from 7000 BC reveals that Indians once cultivated capsicum plants, and within the same year of Columbus' voyage, capsicum plants were widely used in the

Caribbean, South and Central America, and Mexico (Purseglove and others 1981). The use of capsicums resulted in enhanced flavors in foods and beverages and served as a medicinal combatant against ailments. Today, uses are much the same. The genus *Capsicums*, which belongs to the family Solanaceae, contains 20-22 wild species and 5 domesticated species such as C. annum, C. baccatum, C. chinense, C. frutescens, and C. pubescens (Purseglove and others 1981; Andrews 1995; Bosland 1996). Red pepper, cavenne, green and red bell pepper, jalapeno, and paprika, which are the most commercially cultivated pepper cultivars in the world, belong to C. annum. Tabasco and habanero peppers belong to C. frutescens and C. chinense, respectively (Palevitch and Craker 1995; Bosland 1996). They are grown in warm temperate regions in a variety of soils resulting in numerous varieties that differ in size. color, flavor, and pungency of fruit (Parry 1962; Purseglove and others 1981; Sato and others 1999). According to Parry (1962), the pungent principal along with the resin, a volatile oil, a fixed oil, carotenoid pigments, protein, cellulose, pentosans, and mineral elements are found in the fruits of capsicums. Capsaicinoids, located in the placenta of the fruit, are responsible for the pain and irritation evoked during and after consumption of capsicum peppers.

Allison and others (1999), Lawless (1984), and Lawless and Stevens (1988) found the tongue to perceive the most burn caused by capsaicin. The pattern or duration of burn caused by capsaicin is unclear since contradictory results were derived in studies conducted by Cliff and Heymann (1992, 1993).

Saliva, tap water, sucrose, and milk temporarily decrease the irritation caused by capsaicin when held in the mouth. After expectoration, the burn rebounds (Sizer and Harris 1985; Green 1986; Lawless and Stevens 1986; Cowart 1987; Nasrawi and Pangborn 1989, 1990; Hutchinson and others 1990; Cliff and Heymann 1993; Allison and others 1999). Capsaicin has the ability to mask other flavors and inhibit the intensities of other tastants (Lawless and Stevens 1984). In food systems, increasing the amount of capsaicin present increased the intensity perceived in the mouth (Baron and Penfield 1996). When altering the fat in water, cheese sauce, and starch paste containing various concentrations of capsaicin, a more intense burn was perceived in the low-fat versions compared to the full-fat versions (Baron and Penfield 1996). Similarly, in another study, a less intense burn was perceived from the reduced- and fullfat cheese sauces than the low-fat cheese sauces made with various amounts of capsaicin (Carden and others 1999). However, contradictory results were observed in chicken patties formulated with various fat and cayenne pepper levels. A more intense burn was perceived as the fat level in the chicken patties increased (Keller 2000).

Based on the results of previous research, the perception of the intensity of heat caused by capsaicin is dependent upon the food system in which it is incorporated. Food structure and composition especially moisture content can affect mastication behavior. Differences in mastication behavior can alter the rate at which oral receptors are stimulated by capsaicin. Previous researchers

have yet to fully explain how the intensity of heat caused by capsaicin is perceived in other food products such as snack foods. The market for snack foods will be \$24.2 billion by the year 2005 (Anon 2001). Thus, more research is needed to determine the effects of low water activity foods on perceived pungency induced by capsaicin. In order to better understand the effect of fat content on the perception of heat intensity caused by capsaicin, various amounts of cayenne pepper were incorporated into cheese biscuits. This study was conducted to evaluate the heat intensity of capsaicin in cheese biscuits made at 3 fat reduction levels (0, 25, and 30%) and 4 levels (0.0, 0.1, 0.2, and 0.4%) of cayenne pepper. Twelve trained panelists evaluated the cheese biscuits. The objectives of the study were to...

- Determine the heat intensity of capsaicin in the cheese biscuits as perceived by sensory panelists using a time-intensity procedure,
- 2. Determine the textural parameters of the cheese biscuits,
- Determine, if any, the relationships between or among fat content and pepper concentration in the cheese biscuits, and
- Determine, if any, a correlation between the sensory evaluation and instrumental analysis of the characteristic hardness of the cheese biscuits.

#### Chapter 2

#### **Review of Literature**

Busy lifestyles, more women working away from home, single-parent families, and singles, as well as the emergence of home meal replacements, and grazing or eating on the run have become a way of life for many people (Hollingsworth 1998; Sloan 1998). A movement away from bland foods to rich and spicy foods has been influenced by the exotic tastes, variety, and health benefits of ethnic foods (Horton 1987; Williams and Brown 1987; Hollingsworth 1998). Thus, the production of capsicum peppers has increased by 125% since the late 1970's in order to satisfy the public's taste for edible heat (Anon 1999). By 2020, years of ethnic mixing will have created an insatiable demand for more flavorful and unique foods (Sloan 1998). In order to meet the needs and desires of the public, understanding how the burn or pungency caused by capsicum peppers is perceived in various food matrices is important in the success of food products.

### Pungency

The heat associated with capsicums is caused by a chemical sensation mediated by the stimulation of the trigeminal nerve endings and other gustatory senses or capsaicin-sensitive neural pathways (Green 1991), which are involved in taste sensations (Lawless 1989). The trigeminal nerve system is a distinct

sensory system unlike the senses of taste, touch, and smell (Lawless 1989; Prescott 1994). The trigeminal nerve system is an important contribution to flavor perception, which is defined as the combination of the gustatory, olfactory, and trigeminal sensations perceived when a food or beverage is placed in the mouth (Guinard and others 1997). The trigeminal nerve system or fifth cranial nerve receptors are mostly located on the tip and anterior part of the tongue (Prescott 1994). When the trigeminal nerve system is stimulated by capsaicin, Substance P, a primary neurotransmitter, aids in inducing the sensation of heat (Prescott 1994). This heat, unlike thermal heat, has been described as burning, stinging, painful, long-lasting, tingling, and numbing (Lawless 1989; Green 1991; Cliff and Heymann 1992). In addition, Farrell (1990) describes the heat as being hot, sharp, and cumulative to the point of being overwhelming with a long, lingering effect deep in the throat but not too perceptible in the front of the mouth.

The degree of pungency or heat exhibited in very hot to mild varieties of capsicums is produced by capsaicinoids, alkaloid compounds that are found only in the genus *Capsicum* (Purseglove and others 1981; Farrell 1990; Bosland 1996). Capsaicinoids are produced in the placenta of the pepper and are controlled genetically and environmentally (Palevitch and Craker 1995; Bosland 1996). The 7 capsaicinoids responsible for pungency are capsaicin, the most prevalent and pungent; dihydrocapsaicin, the second most prevalent; norcapsaicin; nordihydrocapsaicin; nornordihydrocapsaicin; homocapsaicin; and

homodihydrocapsaicin (Bosland 1996). Capsaicinoid content of capsicum peppers ranges from 0.1-1.0% depending on the cultivar (Govindarajan and Sathyanarayana 1991). Most (80-90%) of the capsaicinoids found in capsicum peppers are capsaicin and dihydrocapsaicin in a 1:1 or 2:1 ratio (Purseglove and others 1981; Govindarajan and Sathyanarayana 1991). Synthetic capsaicin, C<sub>18</sub>H<sub>27</sub>NO<sub>3</sub>, contains white crystals possessing a burning taste in the pure form and in solutions of 10 parts per million can be detected by human taste buds (Bosland 1996; Purseglove and others 1981). It is fat-soluble, insoluble in water, tasteless, and odorless (Purseglove and others 1981; Lawless 1989; Andrews 1995).

#### Measurements of Pungency

In 1912, Wilbur Scoville developed the Scoville Organoleptic Test to determine the pungency exhibited by peppers (Purseglove and others 1981; Bosland 1996). A small number of trained panelists tasted the diluted samples and recorded the heat level. The samples were increasingly diluted until no further heat was perceived. A number assigned to the samples was based on the number of dilutions needed for the heat to be undetectable called the Scoville Heat Unit (Bosland 1996). However, this test has limitations and has been scrutinized by other researchers because it causes rapid taste fatigue and sensitization, lacks statistical validity, and has a long extraction time and poor reproducibility (Gillette and others 1984). Thus, other tests, chemical and

instrumental, have been sought to combat these problems resulting in a more accurate and reliable measurement of pungency. Gillette and others (1984) devised a new method to sensorily evaluate red pepper heat, which combats the problems associated with the Scoville Organoleptic Test. The chemical and instrumental methods reduce the extraction time from 20 to 16 h, minimize heat build up and taste fatigue by utilizing a standardized initial sample and timed rinsing between samples, and produce consistent results (Gillette and others 1984). In addition, the chemical and instrumental methods are statistically valid (Gillette and others 1984). In addition, High Performance Liquid Chromatography, Capillary Gas Chromatography, and Gas Chromatography with Mass Spectrometry have been successful analytical tools for quantifying the pungency of capsaicinoids found in peppers (Lego 1984; Hawer and others 1994; Manirakiza and others 1999).

#### Effects of Food Composition on Perceived Pungency

Several researchers have investigated the influence of tastants on the perceived burn caused by capsaicin. Sizer and Harris (1985) found that sucrose tended to mask the capsaicin burn when added in increasing amounts to levels of capsaicin ranging from 0.06 to 0.70 mg/L in ethanol and water solutions while sodium chloride and citric acid had no effect. Similar results of sucrose concealing mouth burn were found in studies conducted by Lawless and Stevens (1986) and Nasrawi and Pangborn (1989, 1990).

Sizer and Harris (1985) also investigated the influence of temperature on perceived capsaicin burn. Results revealed that increasing the temperature of the solution could increase one's threshold for pungency. Baron and Penfield (1996) discovered that the rate at which the maximum intensity of capsaicin burn is perceived is likely dependent upon the serving temperature.

Lawless and others (1985) investigated the influence of oral capsaicin irritation on gustatory and olfactory senses. They found that oral irritation had some masking effect on gustatory and olfactory senses, which differ among humans. Capsaicin also has the ability to mask other tastes. Lawless and Stevens (1984) found similar results in an earlier study. Panelists evaluated the taste intensities of various amounts of sucrose, sodium chloride, citric acid, and quinine in solution followed by rinses of capsaicin or piperine in various amounts of polysorbate or water, the control. Capsaicin was able to significantly inhibit intensities of citric acid and quinine; however, the intensities of sucrose and salt were unaffected.

Other investigators have examined the effects of capsaicin in the oral cavity. Green (1986) examined the effects of capsaicin burn on thermal sensations. She discovered that capsaicin enhanced perceived warmness in the mouth from solutions with temperatures ranging from 39°C to 45°C as well as reduced perceived coldness at 13, 17, 21, 25, and 29°C. In addition, the intensity of the burn caused by capsaicin increased after expectoration. An increase in capsaicin burn after expectoration was also observed in studies

conducted by Lawless and Stevens (1986), Cowart (1987), Nasrawi and Pangborn (1989), and Cliff and Heymann (1993). Cliff and Heymann (1993) used time-intensity profiles to accurately account for the oral irritation induced by capsaicin, piperine, and cinnamaldehyde from onset to obliteration. Capsaicin's pattern of burn tended to have a slow onset that gradually increased then slowly decayed resulting in a long duration overall while the opposite was observed for cinnamaldehyde. However, in a previous study by Cliff and Heymann (1992), capsaicin was observed to have a long lag phase with a shorter duration overall.

Other researchers have explored where the burn of capsaicin is most perceived in the oral cavity. In low concentrations, capsaicin stimulated the midmouth and mid-palate along with the throat and back of the tongue in a study conducted by Krajewska and Powers (1988). In another study by Cliff and Heymann (1992), the pungency of capsaicin along with ginger seemed to be perceived in the back of the tongue only. Similarly, in a study done by Allison and others (1999), the tongue perceived most of the heat followed by the oral cavity and the throat. The same stimulation area, the tongue, was observed to perceive the most burn in studies conducted by Lawless (1984) and Lawless and Stevens (1988).

Lawless (1984), Krajewska and Powers (1988), and Baron and Penfield (1996) found that increasing the concentration of irritants could cause an increase in the intensities perceived in the mouth. Because of this increase in intensity, salivation increased (Lawless 1984). Salivation has been found to be

a good inhibitor of the burn induced by capsaicin (Allison and others 1999). Other inhibitors include rinsing with or consuming tap water, rice, unsalted crackers, pineapple, beer, milk, butter, and sugar (Green 1986; Lawless and Stevens 1986; Hutchinson and others 1990; Allison and others 1999). Although, these foods and liquids do decrease the burn while held in the mouth, once expectorated, the burn rebounds (Hutchinson and others 1990).

Baron and Penfield (1996), Carden and others (1999), and Keller (2000) have examined the perceived heat intensity of capsaicin in complex food systems. Carden and others (1999) studied the perceived heat intensity of 5 capsaicin concentrations (0, 0.4, 0.8, 1.2, and 1.6 ppm) in cheese sauces with various fat levels (full, reduced, and low). The reduced-fat cheese sauce contained 25% less fat than the full-fat cheese sauce and the low-fat cheese sauce contained no more than 3 g of fat per serving (Carden and others 1999). A more intense burn was perceived from the low-fat cheese sauces than the reduced- or full-fat cheese sauces. Because less of the hydrophobic capsaicin reached the trigeminal receptors in the mouth, a less intense burn was perceived in the reduced- or full-fat cheese sauces (Lawless and others 2000). In addition, the heat perceived in the low-fat cheese sauces with lower capsaicin concentrations resembled the heat perceived in the full-fat cheese sauces containing higher concentrations of capsaicin. Baron and Penfield (1996) assessed the pungency perceived by capsaicin in various concentrations in water, cheese sauce, and starch paste with various fat levels served at

temperatures of 25 and 38°C. Because capsaicin is insoluble in water, the intensity of heat was more strongly perceived in water than in cheese sauce and in starch paste containing the same capsaicin level. In addition, the temperature of the samples affected the rate at which the burn of capsaicin intensified. A more intense burn was perceived at the lower fat levels than at the higher fat levels in the cheese sauce and starch paste due to an increased release of capsaicin to the oral receptors in the mouth (Lawless and others 2000). However, the opposite effect on perceived heat intensity was observed when chicken patties at various fat levels were formulated with various amounts of cayenne pepper (Keller 2000). The perceived heat intensity increased as the fat level increased. Thus, the food system affects the perceived heat intensity of capsaicin.

#### **Dietary Fat**

The use of fat replacers has made it possible for many people to achieve an intake of 30% or less of their total daily calories from fat, which is recommended by various health organizations to promote better health (Schneeman 1986; Mattes 1998). However, compliance to low-fat diets can be very difficult since fats and oils are greatly responsible for the functional and organoleptic properties of foods that influence palatability; thus, making the diet bland and monotonous when they are removed (Giese 1996; Roller and Jones 1996). Since fats and oils enhance and release the flavors of other ingredients, the incorporation of flavorings and spices into low-fat foods can greatly improve the acceptance and compliance of such foods (Giese 1996).

Fats are chemical compounds made of fatty acids and glycerol and supply 9 kcal/g of energy (Schneeman 1986). Thus, it is an important source of energy especially when other energy sources are unavailable. Fats and oils are derived from plant and animal sources (Schneeman 1986; Dziezak 1989). They serve many functions in the body. Essential fatty acids, from plant sources, make hormone-like substances to aid in the regulation of physiological functions (Dziezak 1989). Fats also serve as vehicles for the delivery and absorption of the fat-soluble vitamins A,D,E, and K (Mattes 1998). In addition, fats contribute to the feeling of fullness or satiety after meals. The chemical nature of fats can greatly determine the physical and chemical properties of foods from post processing to storage (Roller and Jones 1996; Giese 1996). They are a major determinate of food texture including mouthfeel and the overall sensation of smoothness and softness, chewiness, and creaminess, which are determined by the melting point of the fat (Giese 1996; Akoh 1998). In addition, fat can affect the sheen, gloss, and overall surface appearance of foods (Forss 1969; Bennett 1992; Giese 1996; Roller and Jones 1996). In a food system, fats also aid in absorbing moisture, aerating batters, transferring heat, carrying pigments, and distributing and releasing flavors (Mattes 1998; Roller and Jones 1996).

Many factors influence why individuals prefer foods containing fats. Drewnowski (1990) and Mela (1991) suggest that fat preferences are triggered

by physiological and metabolic events. Drewnowski (1990) further states that during childhood this preference for fat-containing foods is acquired. Because of this preference, an increase of women into the work force, a casual lifestyle, or higher disposable incomes, the fat intake of many people is well above the recommended levels (Glueck and others 1994; Mattes 1998). Excesses of fats in the diet are associated with increased incidences of obesity, hypertension, certain cancers, insulin resistance, gall bladder disease, and coronary heart disease (Glueck and others 1994; Giese 1996; Mattes 1998). In order to decrease the incidences of such diseases, various agencies and organizations such as the US Department of Health and Human Services, US Department of Agriculture, National Cancer Institute, American Heart Association, Food Nutrition Board of the National Academy of Sciences, American Medical Association, American Dietetic Association, and American Diabetes Association have made recommendations concerning diet and health consistent with the Dietary Guidelines (Schneeman 1986; Mattes 1998). The guidelines suggest that individuals over the age of 2 should have a total fat intake daily of no more than 30% of calories from fat (Glueck and others 1994). This can be achieved by modifying diets to include fewer fat-containing foods. The food industry has met this demand by developing foods and beverages lower in fat with the aid of fat replacers and other additional ingredients. According to Bruhn and others (1992), consumers desire the incorporation of fat replacers into products that are comparable in taste, flavor, and texture with the full-fat counterpart. Thus, from

1995 to 1997, 73-75% of individuals consumed foods labeled as low-fat or nonfat because of health concerns associated with a high-fat intake and dieting (Katz 1998).

#### **Fat Replacers and Mimetics**

Fat replacers are able to replace fats and fulfill at least some of the functional properties associated with them in the original product (Roller and Jones 1996). According to Katz (1998), fat replacers may chemically resemble fats, proteins, or carbohydrates. A fat-based fat replacer, derived from conventional fats and oils by enzymatic modification or chemically synthesized, is designed to replace fat on a weight-by-weight basis and is resistant to hydrolysis by digestive enzymes (Roller and Jones 1996). Fat replacers can be classified into 4 general categories: carbohydrate-based, protein-based, fat-based, and mixed blends (Glueck and others 1994; Akoh 1998; Mattes 1998).

Fat mimetics cannot replace fat on a weight-by-weight basis. However, fat mimetics can partially replicate the chemical and physical functions of fats they are designed to replace (Akoh 1998). Fat mimetics carry only water-soluble flavors (Mattes 1998). According to Akoh (1998), the caloric values of fat mimetics range from 0 to 4 kcal/g and a high water content is needed for functionality, thus they are unstable at frying temperatures (Roller and Jones 1996; Akoh 1998). Whether a fat substitute or a fat mimetic is used in a food system to replace the fat, other functional ingredients such as proteins, starches, thickeners, gums, stabilizers, flavoring ingredients, sweeteners, emulsifiers, and fibers are needed in order to produce a product organoleptically similar to the original one (Roller and Jones 1996).

#### Protein-based Fat Mimetics

Protein-based fat mimetics are made from egg, milk, whey, soy, gelatin, and wheat gluten (Akoh 1998). Preparation of such proteins includes physical aggregation excluding chemical breakdown; thus, the nutritional value is unchanged (Glueck and others 1994). In food systems, protein-based fat mimetics can exhibit a more characteristic flavor profile similar to fat-containing foods, which is beneficial in product reformulation (Schirle-Keller and others 1992). They can replace 75-100% of the fat; therefore, contributing only 1.3-4 kcal/g to products such as salad dressings, frozen desserts, and margarines (Glueck and others 1994; Mattes 1998; Akoh 1998). These fat mimetics will coagulate and become rubbery in food systems that require frying temperatures; however, they are appropriate for use in products that undergo cooking, retorting and ultra-high temperature processing (Mahan and Escott-Stump 1996; Akoh 1998). Microparticulated proteins are obtained from a process called microparticulation. This process involves heating and blending the proteins, thus causing them to coagulate into large particles which are divided into smaller particles, 1-1.5 µm in diameter, by shearing (Glueck and others 1994; Mattes 1998). These small particles allow the fat mimetic to simulate the feel of fat in

the mouth. An example of a microparticulated protein is Simplesse<sup>®</sup>, which is made from egg white or milk protein (Mahan and Escott-Stump 1996). Simplesse<sup>®</sup> is flavorless and masks flavors (Karkeck 1988; Akoh 1998). It provides 4 kcal/g on a dry basis and in the hydrated form contributes 1-2 kcal/g in such products as yogurts, cheese spreads, baked goods, frostings, sauces, and soups (Akoh 1998).

#### Carbohydrate-based Fat Mimetics

Carbohydrate-based fat mimetics, such as polydextrose, can be made from rice, wheat, corn, oats, tapioca, or potato (Glueck and others 1994). Polydextrose was approved as a food additive by the Food and Drug Administration in 1981 (Glueck and others 1994). Polydextrose, a bulking agent, is made up of randomly bonded glucose polymers, sorbitol, and citric acid (Glueck and others 1994; Akoh 1998). Polydextrose is only partially metabolized providing only 1 kcal/g and does not interfere with the absorption of vitamins, minerals, or amino acids (Gillatt and Lee 1991; Akoh 1998). The carbohydrate-based fat mimetic is used in baked goods, mixes, chewing gum, confections, salad dressings, frozen dairy desserts, puddings, and candies (Glueck and others 1994; Akoh 1998). Polydextrose contributes mouthfeel such as slight smoothness in high-moisture formulations by serving as a humectant and body by replacing solids such as fat and sugar (Glueck and others 1994; Akoh 1998).

#### Effects of Fat-reduced Foods and Water on Trigeminal Stimulation

Trigeminal irritation is produced by a great variety of substances, including chilli, pepper, mustard, ginger, menthol, carbon dioxide, and alcohol (Prescott 1994). Capsaicin produces a chemical heat that contributes much to the overall flavor of foods; however, the mechanism by which it contributes to produce the overall flavor of foods is unknown (Prescott 1994). In a food product, fat develops and stabilizes flavors and serves as a carrier for lipophilic ingredients (Leland 1997; de Roos 1997). Capsaicin is soluble in fat. When fat is not present to mediate the balance of flavor ingredients, a decrease in the chemical stability of the ingredient can occur resulting in a greater and quicker perception of sensations in the mouth (Leland 1997; de Roos 1997). A similar observation was noted when a trained expert panel was used to establish intensity versus time curves for the perceived flavors such as sweet, cream, vanillin, milk, caramel, and butter in full- and reduced-fat vanilla ice creams. The full-fat vanilla ice cream contained 10% milkfat while the reduced-fat vanilla ice cream contained 3% milkfat (Bennett 1992). The reduced-fat vanilla ice cream was found to have a high initial impact of flavors that guickly disappeared while the flavors perceived in the full-fat vanilla ice cream gradually intensified and then slowly dissipated (Bennett 1992).

In a more complex medium, single-point and time-intensity measurements were used to evaluate the flavor intensity of garlic, pepper, and their combinations in beef broth and fat-containing and fat-free mashed potatoes

(Rosin and Tuorila 1992). In all medias, the flavor intensity of the garlic was perceived to be the same. The intensities of pepper and pepper along with garlic were perceived to be higher in the fat-free mashed potato than in the fatcontaining mashed potato. Lastly, the intensity of the pepper was perceived to be most intense in the beef broth than in the mashed potatoes. On the other hand, this pattern of perceived heat intensity by capsaicin in fat-altered products is not predictable as shown in the study conducted by Keller (2000). Clearly, a food's structure and composition can greatly influence the rate and intensity at which chemical receptors are stimulated by capsaicin. In reduced-fat foods, other ingredients such as fat replacers are added to replace the functions of fat in the product. Additional water in the formula is needed to aid in the functionality of the fat replacer. However, water, added or present in the food. and saliva can influence the rate or intensity of heat perceived in the mouth. Saliva acts as a lubricant between the food and the mouth by forming a thin aqueous layer and serves as an intermediate medium facilitating the contact of ingredients with nerve endings on the tongue (Harrison and others 1998). Altering the fat and water content in a food system may influence the rate at which the trigeminal nerve system is stimulated by irritants such as capsaicin, thus affecting the overall perception of flavor in the food product.

#### Chapter 3

#### Materials and Methods

#### **Experimental Design**

An Incomplete Block Design with a block on days and panelists was utilized for the experiment. The cheese biscuits were formulated at 3 fat reduction levels and 4 cayenne pepper levels for a total of 12 treatments. The experiment was replicated 3 times. For each replication, 4 sessions were held. Panelists (12) received 3 random samples per testing session.

#### **Preparation of Cheese Biscuits**

Cheese biscuit formulations and pepper levels as shown in Table 1, and mixing times were chosen based upon preliminary work and feasibility. The cheese biscuits were formulated at a full-fat level (0% reduction) and 2 fat-reduced levels (25 and 30%) and 4 levels (0.0, 0.1, 0.2, and 0.4%) of cayenne pepper. The cheeses used were shredded with a Rival Electric Food Grinder (Model 2250, Kansas City, MO) utilizing a fine grinding disc. All ingredients needed for the entire experiment were weighed out and placed in a blast freezer (-18°C) until further use. All ingredients were set out at the same time at room temperature until needed to make each dough for each treatment. The doughs were made randomly the day before each testing session.

For the 0% reduced-fat cheese biscuit, the flour and the desired amount

	Fat reductions (%)			
Ingredients	0	25	30	
	Formula percent			
Flour <sup>a</sup>	37.5	37.5	37.5	
Polydextrose <sup>b</sup>	0.0	3.9	4.6	
Simplesse <sup>® c</sup>	0.0	1.3	1.5	
Distilled water	0.0	2.6	3.1	
Shortening⁴	15.6	7.8	6.3	
Butter	5.2	5.2	5.2	
2%-Fat cheddar cheese <sup>f</sup>	15.8	15.8	15.8	
Cheddar cheese <sup>g</sup>	26.0	26.0	26.0	
Cayenne pepper <sup>hi</sup>	variable	variable	variable	
Total	100.0	100.0	100.0	

Table 1–Formulations of cheese biscuits made at 3 fat levels and 4 cayenne pepper levels

<sup>a</sup> Gold Medal All Purpose Flour, Minneapolis, MN

<sup>b</sup> Sta-Lite<sup>®</sup> III Polydextrose, A.E. Staley Manufacturing Co., Decatur, IL

<sup>c</sup> Kelco Biopolymers, San Diego, CA

<sup>d</sup> Crisco<sup>®</sup> All-Vegetable Shortening, Cincinnati, OH

\* Kroger<sup>®</sup> Sweet Cream Salted Butter, Cincinnati, OH

f Kraft<sup>®</sup> Natural Reduced Fat Sharp Cheddar Cheese, Cincinnati, OH

<sup>9</sup> Kroger<sup>®</sup> Classic Natural Cheddar Cheese, Cincinnati, OH

<sup>h</sup> Wild Flavors Inc., Mississauga, Ontario, CAN; 32,500 Scoville Heat Units (Warren Analytical Laboratory, Downers Grove, IL), Scoville Heat Units range from 0 to 1,500,000.

<sup>i</sup> 0.0, 0.1, 0.2, or 0.4%

of cayenne pepper were mixed with a Kitchen Aid<sup>®</sup> mixer (Model K45SS, St. Joseph, MI) on speed 1 for 25 s. Next, the shortening, butter, 2%-fat cheddar cheese, and cheddar cheese (30%-fat) were mixed with the mixer for 40 s on speed 2. Lastly, the flour mixture was combined with the cheese mixture with the mixer for 30 s on speed 2.

The 25 and 30% reduced-fat cheese biscuits were made by mixing the flour, the desired amount of cayenne pepper, and gradually the Polydextrose with the mixer on speed 1 for 25 s. Second, the water and Simplesse<sup>®</sup> were mixed with the mixer on speed 2 for 40 s. Third, the liquid mixture was combined with the shortening, butter, 2%-fat cheddar cheese, and cheddar cheese and mixed on speed 2 for 40 s for the 25% reduced-fat cheese biscuit and for 35 s for the 30% reduced-fat cheese biscuit. Lastly, the respective flour mixtures were combined with the respective cheese mixtures with the mixer on speed 2 for 30 s. Each dough was manually packed into a 31.9 X 4.8 cm Ultra-High Molecular Weight plastic rectangular mold (Commercial Plastics, Knoxville, TN), which contained 6 subdivisions. Flat, rectangular plastic pieces, 33.2 X 6.2 X 0.7 cm, were placed on the top and bottom of each mold with rubber bands in order to keep the dough in place. The molds were stored at refrigerated temperature (4.6°C) overnight.

Prior to sensory testing, the doughs were cut into the desired shape and width by using the wire section of an egg slicer (Ecko, Franklin Park, IL) making

the dimensions of the cheese biscuits 4.1 X 3.7 cm and the thickness 0.6 cm. To prepare samples for hardness testing, pieces, 4.1 X 2.7 X 0.6 cm, were cut before baking. The cheese biscuits were placed onto parchment paper on pizza pans and stored at refrigerated temperatures (4.6°C) for 45 min. Next, the pans were placed in a rotary hearth oven (Despatch Oven Co., Minneapolis, MN) at 191°C for 10 min. The cheese biscuits were allowed to cool for 5 min on the pizza pans. Once removed from the pizza pans, an additional 25 min of cooling on wire racks occurred before the cheese biscuits were stored in plastic containers (Glad<sup>®</sup> Ovenware, Oakland, CA) at room temperature.

#### Sensory Evaluation

Twelve trained panelists (3 males, 9 females) evaluated the perceived heat intensity in cheese biscuits in 12 treatments by utilizing time-intensity techniques over 4 wk.

#### Stock Solution Preparation of Synthetic Capsaicin

According to ASTM (1996), a stock solution (6.0 ppm) was made by diluting 0.6 g of synthetic capsaicin, N-vanillyl-n-nonamide, (Sigma Chemical Co., St. Louis, MO) with 20 g of Polysorbate-80 (Loders Croklaan, Channahon, IL). The mixture was heated and mixed in a 50-mL beaker on a hot plate at a setting of 3 for approximately 10-15 min (Keller 2000). After the synthetic capsaicin was completely dissolved, it was transferred quantitatively to a
1000-mL mixing cylinder with heated (70°C) spring water (Mountain Valley, Hot Springs, AR) and diluted to a volume of 1000 mL with room temperature (20°C) spring water (ASTM 1996). The mixture was cooled to room temperature (ASTM 1996). After mixing, 10 g of the solution was put into another 1000-mL mixing cylinder and diluted with spring water to a volume of 1000 mL. The stock solution was kept sealed and refrigerated until further use. Standard solutions were made by diluting the stock solution as shown in Table 2.

## Heat Intensity Training

Methods followed in ASTM Method E1083-88 (ASTM 1996) were utilized in training the 12 panelists (Appendix, page 62). On the first day of training, panelists standardized their tongues and mouths to the reference standards and were instructed on the use of a 15-cm line scale (ASTM 1996). The 15-cm line scale was anchored with the terms none and strong describing the heat level. The points for threshold, slight, and moderate were also marked on the line scale. Panelists received a scorecard (Appendix, page 63) and 4 other samples containing various amounts (0.0-1.3 ppm) of capsaicin to evaluate and "memorize" mentally. Each panelist rinsed with room temperature spring water and unsalted crackers (Kroger<sup>®</sup> Oyster Crackers, Cincinnati, OH) before putting the first reference sample containing 0.4 ppm of capsaicin into the mouth. Once in the mouth, each panelist held the sample for 5 s, swallowed slowly, waited 30 s, and then was instructed to rate the sample as "slight " on the line scale. It

Concentration (ppm)	Stock solution (mL)	Diluted volume (mL)	
0.4	13.4	200	
0.8	26.8	200	
1.3	43.3	200	

 Table 2–Concentrations of reference standards made from dilutions of stock

 solution as stated in ASTM (1996) Method E1083-88

was stressed to all panelists that any of the infinite number points on the line scale could be used to describe the heat intensity of each sample (ASTM 1996). Before proceeding to the next sample, each panelist rinsed with room temperature spring water and ate unsalted crackers for 2 min (ASTM 1996). The same procedure was repeated for the next 3 samples. Discussion of the terms and the correct ratings of the samples indicative of those terms on the line scale followed after completion of the evaluations.

On the second day, panelists were oriented on how to evaluate test samples containing various amounts of capsaicin diluted with spring water (Appendix, page 64). Panelists were also instructed on how to properly evaluate the hardness of cheese biscuits (Appendix, page 64). In addition, proper rinsing techniques were also demonstrated to the panelists. Each panelist was presented with 4 10-mL samples to evaluate and a scorecard (Appendix, page 63). The reference sample (0.4 ppm capsaicin) was given first followed by samples containing 0.4 ppm capsaicin, 0.8 ppm capsaicin, and between 0.4 and 0.8 ppm capsaicin. The correct ratings were discussed after the evaluation of the second and fourth samples. Practice responses from panelists were checked to see if answers were within 2 cm of the correct response according to ASTM (1996). To continue on as a panelist, this level of performance was essential. Next, the panelists were instructed on how to evaluate the characteristic hardness for each sample. The characteristic, hardness, was defined as the force required to completely bite through the sample that was

placed between the molars (Armbrister and Setser 1994). Each panelist was presented with 0, 25, and 30% reduced-fat cheese biscuits formulated with no cayenne pepper. Each panelist placed each cheese biscuit between the molars, determined through one bite the force required to shatter the biscuit completely, and indicated it on a paper ballot (Appendix, page 65) by marking a vertical line across a 15-cm line scale. The line scale was anchored at one end with "not at all hard" and the other with "extremely hard." Discussion of the answers followed after completion of each sample evaluation for the characteristic of hardness. In addition, panelists commented that the size of the cheese biscuits was too large and cumbersome to fit into the mouth to accurately evaluate the characteristic hardness. In actual testing sessions, smaller cheese biscuits were presented to panelists for evaluation of hardness.

On the third day, panelists were oriented to the actual procedures for evaluating the heat intensity on a computer sensory program developed at the Agricultural Experiment Station, University of Georgia, Griffin (Resurreccion 1993) and hardness of the cheese biscuits on paper ballot (Appendix, page 65, 66). Panelists received 2 samples, one sized for heat evaluation and one sized for hardness evaluation, together in a Snap N' Seal Snack Bag (Kroger<sup>®</sup>, Cincinnati, OH) coded with a 3-digit code. First, panelists were asked to evaluate the heat intensity of the 3 cheese biscuits made at various fat and cayenne pepper levels according to instructions outlined on an evaluation checklist (Appendix, page 67, 68). Afterwards, panelists evaluated the hardness

of the samples on a scorecard (Appendix, page 65). On the scorecard, each panelist also had to indicate which side of the mouth would be utilized to evaluate the characteristic hardness throughout the experiment. Panelists noted after the testing session that the spring water was not sufficient at eliminating the burn caused by the cayenne pepper. As a result, lemon water and apple slices (Granny Smith) were used in future testing sessions as rinsing aids. Afterwards, those panelists who needed to retaste samples from the previous training sessions were encouraged to do so.

## Preparation of Lemon Water

Lemon water was made by discarding 10 mL from a 1500-mL bottle of spring water (Mountain Valley, Hot Springs, AR) and adding 10 mL of lemon juice (Kroger<sup>®</sup>, Cincinnati, OH). The solution was mixed by shaking the bottle vigorously.

#### Sensory Panels

Sensory panels were conducted on Monday, Wednesday, and Friday for 4 wk as described in the Experimental Design section (page 21). Each panelist received the reference sample (0.4 ppm capsaicin), samples for which to evaluate the heat intensity and the hardness, and the checklist of evaluation instructions (Appendix, page 67, 68). During a 3-min waiting period after mentally rating the reference sample as slight heat intensity, each panelist rinsed with room temperature, lemon spring water, unsalted crackers, and/or 4 apple slices. Each panelist then placed a cheese biscuit into the mouth, chewed for 30 s and then swallowed it. Heat intensity was evaluated and recorded at 5,15, 30, 45, 60, 90, 120, 150, 180, 210, 240, 270, and 300 s. Each panelist waited for 5 min before repeating the procedure for samples 2 and 3 (ASTM 1996). Afterwards, panelists were given a scorecard (Appendix, page 69) on which to evaluate the hardness of the samples on the left or right side of the mouth.

Data were collected on a computer program (Resurreccion 1993). The evaluation checklist contained the reference standard values as indicated on a 150-point line scale. The heat intensity of none was 0, threshold was 12, slight was indicated by 50, moderate was 100, and strong was 150 (ASTM 1996; Keller 2000). Moving the cursor along the line scale by manipulating the left and right arrow keys allowed each panelist to record his/her judgement of heat intensity for each sample at each time period.

#### **Chemical and Instrumental Analysis**

## Percentage of Moisture

Samples of cheese biscuits from each treatment were ground with a mortar and pestle. Three 2-g samples per treatment were placed in pre-weighed metal dishes. The sample was weighed again before being placed in the oven

(100°C) and dried overnight. Lastly, the dried sample was weighed in the metal dish. The percentage of moisture was obtained from the following equation: [(weight of wet sample - weight of dry sample)/weight of the wet sample] x 100.

# Percentage of Fat

One piece of No.1 filter paper and two staples were weighed on an analytical balance. Duplicate dried samples previously used for moisture analysis were then placed in the weighed No. 1 filter papers, stapled, weighed, and put into the blast freezer (-18°C) until needed for further analysis. The Soxhlet method utilizing petroleum ether (b.p. 35-38°C, Fisher Scientific, Fair Lawn, NJ) was used to determine the fat content in the final product (Min 1994). A final weight was also obtained after the fat extraction process. The percentage of fat on a wet-basis was determined from the following equations: % fat on dry basis = (g of fat in sample/g of dried sample) X 100; % fat on wet basis = [% fat on dry basis (100 - % moisture)]/100.

## **Texture Analysis**

The hardness (peak force) and brittleness (Young's modulus, kg/min) of the cheese biscuits were evaluated by the 3-point break test (Gaines 1991; Swanson and others 1999) on a TA.XT2 Texture Analyzer utilizing the XT.RA Dimension Software Package (Texture Technologies Corp., Scarsdale, NY). The settings on the TA.XT2 Texture Analyzer for force versus time were as follows: autoscaling, on; peak confirmation, off; force threshold, 20 g; file type, Lotus 1-2-3; display and export, plotted points; acquisition rate, 200 pps; results file, closed; force units, g; contact area, 1.00 mm<sup>2</sup>; and contact force, 5.0 g. The settings on the TA.XT2 Texture Analyzer for force in compression are as follows: options, return to start; force units, g; distance format, mm; pre-speed and postspeed, 6mm/s; distance, 30 mm; and trigger type, auto 20 g. The cheese biscuit was supported by 2 adjustable beams spaced 2.2 cm apart. A rounded-edged knife was driven down at a speed of 5 mm/s until the cheese biscuit broke. Fifteen cheese biscuits per treatment were randomly selected for texture analysis.

#### Data Analysis

Parameters for heat measurement including total heat intensity or total pungency, the area under the time-intensity curve; maximum heat intensity, the highest point on the time-intensity curve; and time to reach maximum heat intensity were calculated (Appendix, page 70) from time-intensity curves (Carden and others 1999; Liu and MacFie 1990).

All values were tested for equal variance (PROC MEANS, SAS Institute, Inc. 2000). PROC UNIVARIATE (SAS Institute, Inc. 2000) was used to test for normality of all data. Log transformations of brittleness and time to reach maximum heat helped to meet requirements of normality. The main effects of fat, pepper, and their interaction (fat X pepper) were tested utilizing PROC MIXED (SAS Institute, Inc. 2000)(Appendix, page 71). Judge was included as the random factor in the model. Least-squares means were generated for all data and the PDIFF function tested for significant differences. The leastsquares means generated by PROC GLM (SAS Institute, Inc. 2000) were used to graph time-intensity curves over time. Sensory and instrumental data (means for each treatment) for hardness were analyzed for correlation (PROC CORR, SAS Institute, Inc. 2000).

## Chapter 4

# **Results and Discussion**

## **Sensory Evaluation**

## Perception of Heat

The heat caused by capsaicin is a sensory response that displays a unique course of perception from onset through maximum intensity to extinction (van Buuren 1992). A time-intensity procedure is the best technique for studying dynamic, time-related aspects in flavor perception of foods and beverages (van Buuren 1992). A time-intensity curve displays a succession of intensity measurements on an attribute that can be averaged over judges and replications (van Buuren 1992). Theoretically, a traditional time-intensity curve has somewhat of a behaved, bell shape that starts at 0 heat intensity; ascends to a maximum point, which may plateau; and then declines. The decline will eventually end at 0 heat intensity (Liu and MacFie 1990). In order to study heat perception over time from onset, intensity, and duration, a time-intensity technique was utilized for the experiment. ANOVA tables from Fixed Effects tests are in the Appendix (page 73).

The time-intensity curves in Fig. 1-3 exhibit how panelists perceived the intensity of heat caused by capsaicin over time. All of the curves start at an initial point that is higher than traditional time-intensity curves because panelists were asked to chew for 30 s and start recording their perceptions of heat 5 s



Fig.1–Time intensity curves of full-fat cheese biscuits formulated at 4 cayenne pepper levels as evaluated by 12 panelists. Maximum heat values; none = 0, strong = 150.



Fig.2–Time intensity curves of 25% reduced-fat cheese biscuits formulated at 4 cayenne pepper levels as evaluated by 12 panelists. Maximum heat values; none = 0, strong = 150.





after swallowing. Fig. 1-3 exhibit time-intensity curves that have a characteristic bell shape only at 0.2 and 0.4% cayenne pepper levels. Curves at 0.2 and 0.4% cayenne pepper levels. Curves at 0.2 and 0.4% cayenne pepper levels gradually increase over time to a maximum measurement and slowly decrease toward 0 heat intensity. In Fig.1 and 3, the curves at the highest cayenne pepper level reach a maximum point at 45 s while the curves at 0.2% cayenne pepper reach a maximum point at 30 s. In Fig. 2, panelists perceived the greatest amount of heat at 45 s for cheese biscuits containing 0.2 and 0.4% cayenne pepper. Curves at 0.0 and 0.1% cayenne pepper levels regardless of the fat level do not demonstrate traditional time-intensity curve behavior. The heat profiles of 0.0 and 0.1% cayenne pepper in cheese biscuits regardless of the fat level do not change much over time. Thus, a 0.1% cayenne pepper level is too low in such a food product to display a heat profile that varies as a function of time.

## Total Heat Intensity

Total heat intensity is represented by the area under the time-intensity curve and is a measure of the pungency as perceived by panelists for cheese biscuits formulated at 3 fat and 4 cayenne pepper levels (Keller 2000). Fat level did not have a significant effect on total heat intensity. The area under the timeintensity curve or total heat intensity increased (p<0.0001) as the cayenne pepper level increased regardless of the fat level in the cheese biscuits as shown in Table 3. A higher concentration of pepper was available to stimulate

Pepper (%)	Total heat intensity <sup>de</sup>	Maximum heat intensity <sup>d</sup>	Log of time to reach maximum heat intensity <sup>d</sup>	Time to reach maximum heat intensity (sec) <sup>d</sup>
0.0	1314d	10d	3.82a	136
0.1	6960c	40c	3.22b	56
0.2	14015b	72b	3.04b	37
0.4	22803a	109a	3.07b	34
0.51.02	540			
SEM®	± 512	±2	± 0.14	± 10

Table 3–Total heat intensity<sup>a</sup>, maximum heat intensity<sup>b</sup>, and time to reach maximum heat intensity<sup>c</sup> as perceived by 12 panelists in cheese biscuits formulated at 4 cayenne pepper levels across 3 fat levels

<sup>a</sup> Defined as the area under the time-intensity curve.

<sup>b</sup> Defined as the maximum point on the time-intensity curve at which panelists perceive the hottest amount of heat. Maximum heat values; none = 0, strong = 150.

<sup>c</sup> Defined as the ascension of measurements away from 0 heat intensity to the maximum heat intensity.

<sup>d</sup> Least-squares means followed by unlike letters within columns are significantly different (p<0.0001).

\* Standard error of the mean.

the trigeminal nerve endings found on the tongue. In contrast, in reformed chicken patties formulated at 3 fat and 3 cayenne pepper levels, an increased area under the time-intensity curve was related to a higher fat level in the product. According to Keller (2000), a more intense heat is perceived in meat products with a higher fat level. However, contradictory results were observed in a study in which cheese sauces were formulated at 3 fat levels, 5 capsaicin concentrations, and 4 mimetics. An increased area under the time-intensity curve was observed for reduced- and low-fat cheese sauces (Carden and others 1999). Carden and others (1999) reasoned that the chemical stimulation of trigeminal nerve endings in the mouth by capsaicin was slowed by fat acting as an insulator on the tongue in the full-fat cheese sauces. The fat in chicken patties did not act as an insulator on the tongue because the water content in chicken patties compared to cheese sauces is more loosely bound. The loosely bound waters aids in the breakdown of the matrix of the chicken patty and removes the bolus quickly from the oral cavity. However, in a more viscous food system such as cheese sauce, the surface area is increased in the mouth; thus, coating the oral cavity. In order to remove the cheese sauce from the oral cavity, more saliva is needed. Mastication, saliva flow rate, chewing frequency and swallowing thresholds differ for chicken patties, cheese sauces, and cheese biscuits because the food compositions and structures differ (Harrison and others 1998). Differences in food compositions and structures and in the eating behaviors of these food products influence the rate at which oral receptors are

stimulated by capsaicin. In cheese biscuits, fat does not inhibit, decrease, or promote the chemical stimulation of capsaicin of the trigeminal nerve endings in the mouth.

## Maximum Heat Intensity

Maximum heat intensity is the point at which the intensity of heat is perceived to be the most intense. In cheese biscuits, the maximum heat intensity increased (p<0.0001) as the cayenne pepper level increased as shown in Table 3. Exercising inadequate rinsing techniques, thus perceiving residual heat from the reference sample or cheese biscuits containing pepper and receiving samples before the 5-min waiting period between samples may have caused panelists to perceive heat from cheese biscuits containing no cayenne pepper. Even though the main effect, fat, had no significant effect on the maximum heat intensity, the p-value was very close to 0.05 (p = 0.0669). Because the p-value is so close to 0.05, one could reason that a higher maximum heat intensity could be perceived from the reduced-fat cheese biscuits (means for 25 and 30% reduced-fat cheese biscuits were 61 and 58, respectively) than the full-fat cheese biscuits (mean=54). The fat in the cheese biscuits acts as a carrier for the cavenne pepper causing the heat profile to be balanced across time during mastication. However, when fat is reduced in a system, the chemical stability of the pepper is decreased causing the heat of the pepper to be perceived as intense (Leland 1997). In contrast to the findings of

previous studies conducted by Baron and Penfield (1996) and Carden and others (1999), a more intense burn was perceived from the lower fat versions than the full- or reduced-fat versions of starch pastes and cheese sauces. On the other hand, opposite results were observed in a study by Keller (2000). As the fat level in the chicken patties increased, the maximum heat intensity increased. The effect of fat on heat perception over time differs with food system and how the food is manipulated in the mouth. Increased saliva production, increased moisture content, and increased concentration of capsaicin, aided the contact of capsaicin to trigeminal nerve endings. A higher concentration of capsaicin enriched the saliva, which served as a carrier, causing the heat intensity to be perceived as more intense. The perception of the intensity of heat caused by capsaicin in various food systems, in which the amount of fat is manipulated, cannot be predicted in general but must be specific to the food system.

# Time to Maximum Heat

Time to maximum heat intensity is the time in seconds to reach the maximum heat intensity. Based on the log of time to reach maximum heat intensity (Table 3), the time it took in seconds to reach the maximum heat measurement for cheese biscuits made with 0.1, 0.2, and 0.4% cayenne pepper was less (p<0.0001) than the time it took for cheese biscuits made with no cayenne pepper. Based on Table 3, a longer time for cheese biscuits with no

cayenne pepper to reach the maximum heat measurement was expected because at a concentration of 0.0% cayenne pepper in the cheese biscuits, no pepper was present to be perceived by panelists. Similarly, higher cayenne pepper levels of 0.2 and 0.4% in reformed chicken patties took less time to reach maximum heat compared to reformed chicken patties containing no cayenne pepper (Keller 2000). On the other hand, it took less time for cheese biscuits containing 0.1-0.4% cayenne pepper to reach the maximum heat intensity than the time it took chicken patties to reach the maximum heat intensity. Thus, a combination of the differences in the composition and structure of cheese biscuits and chicken patties, the differences in the breakdown of their matrices in the mouth, and their effects on saliva production influence the rate at which capsaicin stimulates trigeminal nerve receptors in the mouth. Less lipid release into the saliva caused the saliva to be less viscous, thus resulting in a heat profile that had a quick onset. In the present study, fat had no influence in the cheese biscuits formulated at 4 cayenne pepper levels on the time it took for measurements away from 0 heat intensity to reach the maximum heat measurement. However, fat did influence the time it took for measurements away from 0 heat intensity to reach maximum heat in cheese sauces in a previous study conducted by Carden and others (1999).

## Perception of Hardness

Sensory hardness was defined as the force required to completely bite

through the sample when placed between the molars (Armbrister and Setser 1994). Based on data in Table 4, panelists found the 25 and 30% reduced-fat cheese biscuits to be harder than the full-fat cheese biscuits (p<0.0001). In the mixing process when fat interacts with the flour, fat interferes with gluten development causing the product to be tender (Penfield and Campbell 1990). When some of the fat is removed from the product, the product is perceived as less tender because less fat is present to interfere with gluten development.

## **Chemical and Instrumental Analysis**

# Percentage of Moisture and Fat

Percentages of fat and moisture were determined for cheese biscuits formulated at 3 fat levels and 4 cayenne pepper levels as shown in Table 5. Moisture for the highest fat level was significantly lower than the percentages of moisture for the lower fat levels (p<0.0001). A higher percentage of moisture was expected in the lower fat levels than in the high-fat level because additional water was added to their formulas. Additional water was added to facilitate the functionality of the fat replacer, which mimicked the functions of fat in the product. Additional water added to aid the functionality of a fat replacer can affect the shelf-life, textural properties, baking time, staling rate, and the rate or intensity of how flavor ingredients are perceived by oral receptors.

The percentage of fat was determined to see how close or different actual

Table 4–Least-squares means<sup>a</sup> and standard errors for the characteristic hardness of cheese biscuits formulated at 3 fat levels across 4 cayenne pepper levels as evaluated by 12 panelists

Fat level	Hardness⁵		
Full-fat	25.89b ± 4.62		
25% reduced-fat	67.63a ± 4.47		
30% reduced-fat	65.74a ± 4.52		
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<sup>a</sup> p<0.0001, least-squares means followed by unlike letters are significantly different. Measurements taken 9 x per wk for 4wk.

<sup>b</sup> Defined as the force required to completely bite through the sample when placed between the molars (Armbrister and Setser 1994).

Table 5–Least-squares means<sup>a</sup> and standard errors for fat and moisture of cheese biscuits formulated at 3 fat levels and 4 cayenne pepper levels

Fat levels	Predicted fat (%) <sup>b</sup> Actual fat (%		%) <sup>c</sup> Moisture (%) <sup>d</sup>		
Full-fat	31.41	33.08a	11.05b		
25% reduced-fat	24.80	25.03b	14.00a		
30% reduced-fat	22.13	23.99c	14.13a		

<sup>a</sup> Least-squares means followed by unlike letters are significantly different (p<0.0001).

<sup>b</sup> Based on formula.

<sup>c</sup> Standard error of the mean =  $\pm 0.28$ .

<sup>d</sup> Standard error of the mean =  $\pm 0.56$ .

values were from values predicted based on formula calculations. As shown in Table 5, as the fat level increased from 30% reduced-fat to full-fat, the percentages of actual fat significantly increased (p<0.0001). The predicted values for the percentages of fat did not vary much from the actual percentages of fat for all 3 fat reduction levels. However, a fat reduction of 27% for the lowest fat level contradicts the expected fat reduction of 30%, which explains why the percentages of moisture for the lower fat levels are the same (p<0.0001). This variation could be the result of errors in weighing ingredients, errors within the preparation of the method, and differences in loss of fat during baking. A limitation of the Soxhlet process is that it does not extract bound lipids.

#### Hardness and Brittleness

The values for hardness and brittleness as measured with the TA.XT2 Texture Analyzer are shown in Table 6. The interaction of fat X pepper had a significant effect on the hardness of the cheese biscuits as well as fat and pepper, which were dependent upon the interaction (p=0.0065). The full-fat cheese biscuits were less hard than the 25 and 30% reduced-fat cheese biscuits (p<0.0001). This result was expected since fat acts as a tenderizer and imparts lubricity to foods. Proteins in flour must be hydrated in order for gluten development to occur. Increased gluten development occurs when less fat is present to interfere with it and more water is present. Increased gluten development is attributable to a product being less tender. The effect of pepper

Fat level	Pepper (%)	Hardness (g)⁵		Log of brittleness <sup>bc</sup>	Brittleness (kg/min)⁵	
Full-fat	0.0	504e	± 58	4.63cde	122.00	± 21.25
	0.1	479ef	± 58	4.53e	113.84	± 21.21
	0.2	451f	± 57	4.59de	119.32	± 21.13
	0.4	518e	± 57	4.64cde	129.05	±21.13
25% reduced- fat	0.0	660ab	± 57	4.87ab	150.07	± 21.08
	0.1	633bc	± 57	4.88ab	153.16	±21.13
	0.2	610cd	± 57	4.85ab	151.56	± 21.07
	0.4	594d	± 57	4.70cd	132.15	± 21.03
30%	0.0	645abc	± 57	4.87ab	150.27	± 21.08
reduced- fat	0.1	677a	± 57	4.90a	153.97	± 21.05
	0.2	626bcd	± 57	4.76bc	136.02	± 21.05
	0.4	642abc	± 57	4.90a	155.96	± 21.08

Table 6–Textural attributes<sup>a</sup> of cheese biscuits formulated at 3 fat and 4 cayenne pepper levels

<sup>a</sup> Determined with TA.XT2 Texture Analyzer affixed with a 3-point break attachment.

<sup>b</sup> Least-squares means and standard errors for 15 cheese biscuits per treatment x 3 replications. Least-squares means within a column followed by different letters are significantly different; p=0.0065 for hardness and p=0.0085 for log of brittleness.

<sup>c</sup> Standard error of the mean =  $\pm 0.13$ .

on the hardness of the cheese biscuits was unexpected as pepper has no functional role in the structure of the product. Full-fat cheese biscuits containing 0.2% cayenne pepper were not as hard as those containing 0.0, 0.1, and 0.4% pepper. In general, as the cayenne pepper level in the 25% reduced-fat cheese biscuits increased, the hardness decreased. However, 25% reduced-fat cheese biscuits containing no cayenne pepper were similar in hardness to those containing 0.1% pepper. Hardness of cheese biscuits with 25% reduced-fat made with 0.1% cayenne pepper was similar in value to those made with 0.2% pepper. The higher cayenne pepper levels in the 25% reduced-fat cheese biscuits were identical in hardness as well. Cheese biscuits made with 30% reduced-fat containing 0.1% cayenne pepper were significantly harder than those containing 0.2% pepper (p=0.0065). The hardness of the 25% reduced-fat cheese biscuits formulated with 0.0 and 0.1% cavenne pepper was similar in hardness to the 25% reduced-fat cheese biscuits formulated with 0.2% pepper and 30% reduced-fat cheese biscuits made with 0.0, 0.2, and 0.4% pepper. Cheese biscuits made with 25% reduced-fat with no cavenne pepper were similar in hardness to the 30% reduced-fat cheese biscuits formulated with 0.0, 0.1, and 0.4% cayenne pepper.

The interaction of fat X pepper (p=0.0085) had a significant effect on the log of brittleness (Table 6) of the cheese biscuits while the main effect of fat also was significant. Based on the interaction of fat X pepper, the brittleness of cheese biscuits made with full-fat were similar regardless of the cayenne pepper

level. Cheese biscuits formulated with 25% reduced-fat varied. Those made with 0.4% cayenne pepper were less brittle than those made with 0.0, 0.1, and 0.2% pepper. Cheese biscuits containing the lowest level of fat and 0.2% cayenne pepper were less brittle (p=0.0085) than those containing 0.1 and 0.4% pepper. Because the 25 and 30% reduced-fat cheese biscuits were more brittle (p<0.0001) than the full-fat cheese biscuits, less force was needed to break apart the food system; therefore less time was needed for oral receptors to be stimulated by capsaicin.

The instrumental analysis of hardness and brittleness was much more sensitive in detecting the effect of the interaction of fat X pepper. Utilizing this method is advantageous for determining the rheological properties of cheese biscuits. In contrast, a significant interaction was not shown in panel data for hardness. On the other hand, there are limitations to using instrumental methods to determine the texture of foods. Food texture measurements do not take in consideration the various changes a food product goes through during mastication. Once the food enters the mouth, the food is broken down at a variable rate during the chewing cycle into various size particles and undergoes deformations because of temperature changes. The transformation of the food product into a bolus imparts overall textural sensations that an instrument cannot measure (Bourne 1977; Harrison and others 1998). In addition, instrumental textural measurements do not involve the effects of saliva on the textural parameters sensed.

## **Correlation of the Sensory and Instrumental Values of Hardness**

Correlating the results from an instrumental method with those from sensory testing is ideal to validate the instrumental testing method (Gaines 1991). In order to determine the relationship between the instrumental method for determining hardness and the sensory testing of hardness, crackers were broken similarly by the instrument and panelists. Sensory panelists were asked to place the cheese biscuit between the molars and determine through one bite the amount of force required to shatter it. Instrumentally, a cheese biscuit was supported in place by 2 beams spaced an equal distance apart. A roundededged knife, equidistant from the supporting beams, was driven down at a constant speed until the cheese biscuit broke. A strong, positive correlation, 0.92, (n=12; p<0.0001) was found between the instrumental method and the sensory testing method used to determine the hardness of the cheese biscuits formulated at 3 fat and 4 cayenne pepper levels. Thus, the panelists' perception of hardness and the instrumental method used to determine hardness are equally meaningful. The instrumental method utilizing the 3-point break apparatus is valid for assessing the hardness of cheese biscuits.

# Chapter 5

## Summary, Implications, and Recommendations

The objectives of the study were to determine how the heat intensity caused by capsaicin was perceived by panelists in a snack food, cheese biscuits, formulated at 3 fat reduction (0, 25, and 30%) levels and 4 levels of cayenne pepper (0.0, 0.1, 0.2, and 0.4%) using a time-intensity procedure to determine interactions among or between fat content and pepper concentration, to determine the textural parameters of the cheese biscuits, and to determine a correlation between the sensory evaluation of hardness and the instrumental analysis of hardness. The time intensity procedure was effective in showing how panelists perceived the intensity of heat over a 300-s duration. Biscuits with 0.4% cayenne pepper were perceived to be more intense as indicated by the parameters of total heat intensity and maximum heat intensity. Cheese biscuits containing 25 and 30% reduced-fat were found to be harder and more brittle than the full-fat cheese biscuits. Also, the reduced-fat cheese biscuits had a greater percentage of moisture than the full-fat cheese biscuits. It is likely that the rate at which saliva flowed differed among the reduced-fat and full-fat cheese biscuits because the compositions and structures differed. Therefore, the rate at which trigeminal nerve endings were stimulated by capsaicin also likely differed.

Panelist perception of hardness was similar to the results obtained with

the instrumental method. Further analysis identified a strong and positive correlation, 0.92, between the instrumental method and the sensory evaluation of hardness (p<0.0001). Thus, the sensory and instrumental methods for evaluating the characteristic hardness are valid. However, there are advantages and disadvantages to using each method to determine the rheological parameters of cheese biscuits. Sensory evaluation is a subjective measure of a product's characteristics and qualities utilizing all senses. Sensory evaluation is quite essential for food companies and product developers since the purchasing power of individuals is fueled by flavor. Among panelists great variability exists in eating behaviors that cannot be controlled. In addition, from the panel data, the effect of the interaction between fat X pepper was not significant. Instrumental methods are very sensitive and the interaction of fat X pepper for the textural parameters of hardness (p=0.0065) and brittleness (p=0.0085) was significant.

Replication of the current study should include incorporating a better method for handling ingredients and utilizing the same mixing times between formulas. These modifications will minimize variations in quality and texture between the treatments. Before the doughs were made for each cheese biscuit, ingredients were taken out of the blast freezer at the same time and set out for unequal periods of time before being used. This thawing process was not ideal since ingredients thawed for unequal amounts of time. This caused the texture and the quality of the cheese biscuits to differ between treatments and between

days on which the doughs were made. Ingredients should have been taken out of the blast freezer as needed and allowed to thaw for the same amount of time or allowed to temper in the refrigerator until needed to minimize variations among the treatments and days. Lastly, the mixing times for formulas made at 25 and 30% reduced-fat should have been the same. The same mixing times will allow one to make better assessments of differences among the treatments.

Panelists commented throughout the study that they could visually tell which cheese biscuits contained pepper and which ones did not. The use of red lights in the sensory booths would have minimized this observation and problem.

Previous research has yet to give insight on how the mechanism of pungency is perceived by oral receptors in other complex food systems besides chicken patties. However, previous research has revealed that the food system in which capsaicin is incorporated does affect the rate and intensity of its pungency. From the current study, one gets a better understanding of how the pungency of cayenne pepper over time is perceived orally in a lower-moisture food compared to chicken patties. Further research is needed to explore the parameters lag time and rate of release derived from the analysis of a timeintensity study, to determine the effects of other low-moisture foods or meat products on the perception of heat caused by capsaicin, to determine how saliva flow affects the heat intensity of capsaicin over time, and to determine the effect of textural parameters on the perception of heat caused by capsaicin.



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# APPENDICES

Training Session Instructions (Session I)

Objective: Standardize the tongue and palate to reference standards and introduce the practice using the 15-cm line scale

- I. The line scale is unstructured and can be divided into any number of points or categories. You may choose any point on the line to represent your description of the sample's heat intensity.
- II. Taste the first sample; remember to concentrate on the intensity of burn. Instructions:
  - a. Rinse your mouth with water and crackers.
  - b. Take the entire sample into your mouth and hold it for 5 s, slowly swallow the sample, and wait 30 s.
  - c. Rate the heat intensity of the sample on your ballot
  - d. Rinse your mouth with water and crackers. Wait 2 min before tasting the next sample.
  - e. Discuss correct rating.
- III. Repeat step 2 with the 3 remaining samples.

Definitions:

- 1. Threshold heat: the point where the panelist barely senses burn or heat. On the line scale, threshold= 1.25 cm.
- 2. Slight heat: 0.40 p.m., a "slight" amount of heat is sensed by the panelist. On the line scale, slight= 5 cm.
- 3. Moderate heat: 0.80 p.m., panelist refers to this as "moderate" heat. On the line scale, moderate= 10 cm.
- 4. Approaching strong heat: 1.3 p.m., close to the heat of ground red pepper. On the line scale, approaching strong heat= 13 cm.
- 5. Strong heat: greater than 1.3 p.m., extremely hot. On the line scale, strong heat= 15 cm.

Solutions are prepared with N-vanillynonanamide, synthetic capsaicin. Solutions are equal to the concentration given above.

#### **Training Heat Intensity Scorecard**

Judge \_\_\_\_\_

You will receive four samples to evaluate. Take the whole sample marked C into your mouth and hold for 5 s, swallow slowly, and wait 30 s before evaluating the intensity of heat as slight on the line scale. Rinse your mouth for 2 min with water and crackers. Take the entire test sample into your mouth and hold for 5 s, swallow slowly, and wait 30 s before evaluating the intensity of heat. Place a mark across the line to indicate intensity of sensory heat using any point on the line. Rinse with water and crackers and wait 2 min before proceeding to the next sample.

Sample <u>C</u> threshold 0 slight moderate strong Sample 0 threshold slight moderate strong Sample threshold 0 slight moderate strong Sample \_\_\_\_\_ 0 threshold slight moderate strong

Training Session Instructions (Session II)

Objective: Orient the panelists on how they will be evaluating actual red pepper test samples.

- I. The line scale is unstructured and can be divided into any number of points or categories. You may choose any point on the line to represent your description of the sample's heat intensity.
- II. Taste the first sample coded with "C" (or reference); remember to concentrate on the intensity of burn.
   Instructions:
  - a. Rinse your mouth with water and crackers. (Make sure rinsing methods are correct.)
  - b. Take the entire sample "C" into your mouth and hold it for 5 s, slowly swallow the sample, and wait 30 s.
  - c. Rate the heat intensity of the sample as "slight" on your ballot.
  - d. Rinse your mouth with water and crackers. Wait 2 min before tasting the next sample.
  - e. Next Sample -Take the entire sample into your mouth and hold it for 5 s, slowly swallow the sample, and wait 30 s.
  - f. Rate the heat intensity of the sample on your ballot. Rinse your mouth with water and crackers. Wait 2 min before tasting the next sample.
  - g. Discuss correct rating.
- III. Repeat steps e-f with samples 3 and 4. Discuss correct ratings after the evaluation of samples 3 and 4.
- IV. Evaluation of Hardness, the force required to completely bite through the sample that is placed between the molars
  - a. Place each cheese biscuit between the molars, determine through one bite the force required to shatter the biscuit completely
  - b. Rate the hardness of the sample on your ballot

### Hardness Scorecard<sup>a</sup>

Panelist	

Sample \_\_\_\_\_

Evaluate on the scale below the hardness of the cheese biscuit. Place the cheese biscuit between the molars and rate the amount of force required to break it.

#### Hardness

Not at all	Extremely
hard	hard

<sup>a</sup> Actual length of line (15 cm) was reduced for margin requirements.

Training Session Instructions (Session III)

Objective: Orient the panelists on how they will be evaluating actual red pepper test samples and the characteristic hardness with the computer and paper ballot, respectively.

- I. The line scale is unstructured and can be divided into any number of points or categories. You may choose any point on the line to represent your description of the sample's heat intensity.
- II. Taste the first sample coded with "C"; remember to concentrate on the intensity of burn. Instructions:
  - a. Rinse your mouth with water and crackers.
  - b. Take the entire sample into your mouth and hold it for 30 s, expectorate the sample, and wait 30 s.
  - c. Acclimate your mouth to the "slight" heat intensity
  - d. Rinse your mouth with water and crackers. Wait 3 min before tasting the next sample.
- III. Evaluation of heat intensity
  - a. Continue chewing for 30 s.
  - b. 5 s after swallowing, rate the heat intensity according to times noted on checklist
  - c. Rinse your mouth with water and crackers. Wait 5 min before tasting the next sample.
- IV. Evaluation of hardness
  - a. Place sample between molars.
  - b. Indicate on paper ballot the force required to shatter biscuit.
  - c. You may expectorate the sample.
- V. Repeat step 3-4 with the 2 remaining samples.
- VI. Ending session
  - a. Press [Enter]
  - b. [Control-Break] bringing you to [A:]

## **EVALUATION CHECK LIST**

- 1. A:panel2f
- 2. Type judge number where it asks for "Name."
- 3. Rinse mouth thoroughly with water.
- 4. Take reference water sample into your mouth and hold for 30 s.
- 5. Expectorate reference sample and wait 30 s, while acclimating your mouth to the "slight" heat.
- 6. Rinse mouth with at least one small glass of water and crackers or apple slice(s). You may expectorate the rinse water. Wait 3 min.
- 7. Pull hatch towards you.
- 8. Take sample into your mouth and chew for 30 s and swallow.

#### 9. Begin timing after the sample is swallowed.

5 s after swallowing, evaluate the heat intensity of the sample by moving the cursor to the position on the line which represents the intensity and hit return.

Continue to evaluate the heat after:

15	S
30	S
45	S
60	S
90	S
120	S
150	S
180	S
210	S
240	S
270	S
300	S

#### Reference Standards As Represented on the 150-point Line Scale

None	=	0
Threshold	=	12
Slight	=	50
Moderate	=	100
Strong	=	150

10. Rinse with at least one small glass of water along with crackers or apple slice(s). You may expectorate the rinse water. Wait 5 min.

- **11**. At the end of 5 min, pull hatch to indicate you are ready for the next sample.
- 12. Evaluate sample 2 by repeating steps 8-11. Repeat the same procedure for sample 3.
- 13. After the 5 min evaluation of your third sample, press ENTER, you must see the message **"Thank you for attending, have a nice day!"** If you do not see this message, <u>ask for help</u>.

To finish your session press Control- Break bringing you to A: Pop your disk out of the drive and turn it in after completion of the test.

## **Evaluation of Hardness**

- 14. Take the sample into your mouth and place it between the molars. (Always utilize the same side for evaluating the characteristic.) On the paper ballot, mark a vertical line across the line indicating the amount of force required to completely shatter or break the sample within one bite. You may expectorate the sample.
- 15. Repeat same procedures for samples 2 and 3.

#### Hardness Scorecard

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Г	an	CII	SL	_		

Sample \_\_\_\_\_

Evaluate on the scale below the hardness of the cheese biscuit. Place the cheese biscuit between the molars and rate the amount of force required to break it. Please remember to utilize the <u>a</u> side of the mouth when evaluating this characteristic.

Hardness

Not at all hard

Extremely hard

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

Evaluate on the scale below the hardness of the cheese biscuit. Place the cheese biscuit between the molars and rate the amount of force required to break it. Please remember to utilize the \_\_\_\_\_ a side of the mouth when evaluating this characteristic.

#### Hardness

Not at all hard

Extremely hard

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

Evaluate on the scale below the hardness of the cheese biscuit. Place the cheese biscuit between the molars and rate the amount of force required to break it. Please remember to utilize the \_\_\_\_\_ a side of the mouth when evaluating this characteristic.

#### Hardness

Not at all hard

Extremely hard

<sup>a</sup> Left or Right, based on the side of the mouth used to evaluate the characteristic.

#### Time Intensity Data Analysis

A. Creation of maximum heat, time to reach maximum heat, rate of release, and lag

```
proc sort data = heat: by code: run:
proc sort data = codes; by code; run;
data maxheat;
merge heat codes; by code;
if judge =. then delete;
array ttt (13) t5--t300;
array sss (13) s1-s13;
drop s1-s13;
s1=5; s2=15; s3=30; s4=45; s5=60; s6=90; s7=120;
s8=150; s9=180; s10=210; s11=240; s12=270; s13=300;
maxheat=0;
base=ttt{1}; lag=5;
do ii=1 to 13:
if ttt{ii} > base then do;
if lag =0 then lag=sss{ii};
end:
if maxheat<ttt{ii} then do;
maxheat=ttt{ii};
tmxheat=sss{ii};
end:
end:
if tmxheat=. then tmxheat=300;
rrel=maxheat/tmxheat:
vheat=var(of t5--t300);
if t5<5 and vheat=0 then lag=300;
B. Calculation of area under the curve
retain area ; drop dim switch ii hta kk jj htb base check;
```

dim=13; array hhh ht1-ht13; array xxx x1-x13; drop ht1-ht13 x1-x13; ht1=0; x1=0; do ii=2 to 13; hhh $\{ii\}$ =ttt $\{ii-1\}$ ; xxx $\{ii\}$ =sss $\{ii-1\}$ ; end; switch='P'; area=0; if ht1=. then area=.; else do; do ii=2 to dim;

```
hta=hhh{ii-1}-ht1;
do kk=ii to dim;
ii=kk:
htb=hhh{ii}-ht1;
if htb ne . then kk=dim;
end:
if htb ne . then do;
base=xxx{ii}-xxx{ii-1};
ii=ji: ** set for next loop:
check=hta*htb;
if check <0 then do;
** if segments are on opposite sides, need to
add two triangle areas;
if switch='B' then area=area+ .5*( hta*(-hta*base)
+ htb*htb*base) / (htb-hta);
if hta > 0 then check=1:
if htb > 0 then check=0:
if switch='N' then do:
if check=0 then area=area+ .5* hta*(-hta*base)/(htb-hta);
          area=area+ .5*htb*htb*base / (htb-hta);
else
end;
if switch='P' then do;
if check=1 then area=area+ .5* hta*(-hta*base)/(htb-hta);
else
           area=area+ .5*htb*htb*base / (htb-hta);
end:
end:
else do:
check=hta+htb; **which side of t1 are we on?;
if (switch='P' and check<0 ) or (switch='N' and check>0) then
area=area+0; ** area on wrong side;
else area=area + .5*base*(htb-hta) + hta*base;
end:
end:
end:
end:
run:
proc print; run;
run;
```

C. Transformation of data and PROC MIXED programming for variables

data temp; set maxheat; ltmxheat=log(tmxheat); stmxheat=sqrt(tmxheat); run; proc rank data=temp out =temp; var tmxheat; ranks tmxheat; run: %include 'a:\pdmix800.sas': %macro dm(fat,cap,temp); Proc Mixed data=temp; title2 "Mixed analysis of ltmxheat"; Class FAT CAP rep; model ltmxheat= fat cap fat\*cap/outp=rrr; random rep: Ismeans FAT cap fat\*cap/pdiff; ods listing exclude Ismeans diffs: ods output Ismeans=mmm diffs=ppp; run: %pdmix800(ppp,mmm); proc univariate plot normal data=rrr; var resid: run: proc sort data=temp; by cap fat; proc means noprint; by cap fat; var Itmxheat; output out=mmm mean=rawmean std=stddev; run: proc print data=mmm; title2 "Check on equal variance for ltmxheat"; run;

%mend; %dm(tmxheat,maxheat); %dm(itmxheat,temp); %dm(llag,temp); %dm(rlag,temp); %dm(area,maxheat); %dm(area,maxheat); %dm(maxheat); %dm(rrel,maxheat); %dm(lrrel,temp) %dm(lag,maxheat); %dm(slag,temp); \*\*%dm(vheat,maxheat);

### **Tests of Fixed Effects**

Total Heat Inter	nsity			
Source	Num DF	Den DF	F Value	Pr > F
Fat	2	418	2.18	0.1138
Pepper	3	418	328.55	< 0.0001
Fat*Pepper	6	418	0.66	0.6851
Log of Time to	Maximum Heat			
Source	Num DF	Den DF	F Value	Pr > F
Fat	2	418	1.17	0.3113
Pepper	3	418	8.13	<0.0001
Fat*Pepper	6	418	0.27	0.9488
Maximum Heat	Intensity			
Source	Num DF	Den DF	F Value	Pr > F
Fat	2	418	2.72	0.0669
Pepper	3	418	386.45	< 0.0001
Fat*Pepper	6	418	0.70	0.6488
Perception of H	ardness			
Source	Num DF	Den DF	F Value	Pr > F
Fat	2	130	25.50	<0.0001
Pepper	3	130	0.59	0.6216
Fat*Pepper	6	130	0.47	0.8327
Instrumental An	alysis of Hardne	ess (Peak height)		
Source	Num DF	Den DF	F Value	Pr > F
Fat	2	1468	142.51	< 0.0001
Pepper	3	1468	4.76	0.0026
Fat*Pepper	6	1468	3.00	0.0065
Log of Brittlene	ss Num DF	Den DF	F Value	Pr > F
Fat	2	1468	35.97	<0.0001
Penner	3	1468	0.85	0 4680
Fat*Penner	6	1468	2.88	0.0085
i al i oppoi	V		2.00	0.0000

### Percentage of Moisture

Source	Num DF	Den DF	F Value	Pr > F
Fat	2	394	110.15	< 0.0001
Pepper	3	394	1.98	0.1163
Fat*Pepper	6	394	1.73	0.1127

## Percentage of Fat

Source	Num DF	Den DF	F Value	Pr > F
Fat	2	274	822.13	< 0.0001
Pepper	3	274	1.99	0.1158
Fat*Pepper	6	274	1.50	0.1776

#### VITA

Cherné La-Toi Wallace was born in Murfreesboro, Tennessee. She is the daughter of Clarance and Alice Wallace. She received her Bachelor of Science degree in 1999 from Middle Tennessee State University with an emphasis in Nutrition and Food Science and was a member of the Kappa Omicron Nu Honor Society. In August of 1999, upon receiving a Graduate fellowship, she continued her education in the Masters program at The University of Tennessee, Knoxville in Food Science and Technology. As a Graduate Research Assistant in the Tennessee Agricultural Experiment Station, she assisted with the laboratory section of the Sensory Evaluation course and worked in the Sensory Lab. She is currently a member of the Gamma Sigma Delta Honor Society and the Institute of Food Technologists.

