Capsaicin concentration, fat level, fat mimetic and time: effects on the sensory perception of heat in cheese sauce

Lou Ann. Carden

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To the Graduate Council:

I am submitting herewith a dissertation written by Lou Ann. Carden entitled "Capsaicin concentration, fat level, fat mimetic and time: effects on the sensory perception of heat in cheese sauce." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Food Science and Technology.

Marjorie P. Penfield, Major Professor

We have read this dissertation and recommend its acceptance:

Genevieve Christen, Betsy Haughton, Sharon Melton, Arnold Saxton

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:

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Marjorie P. Penfield, Major Professor

We have read this dissertation and recommend its acceptance:

Sharon L. Melton
Arnold Snyder
Genevieve Christen
Betsy Houghton

Accepted for the Council:

[Signature]

Associate Vice Chancellor and Dean of the Graduate School
Capsaicin Concentration, Fat Level, Fat Mimetic and Time: Effects on the Sensory Perception of Heat in Cheese Sauce

A Dissertation
Presented for the Doctor of Philosophy Degree
The University of Tennessee, Knoxville

Lou Ann Carden
May 1997
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Abstract

Capsaicin (CAP) concentration, fat level and fat mimetic effects on perceived heat intensity over time were studied in a randomized design blocked on panelist. Cheese sauces were formulated with 5 CAP levels—0.0, 0.4, 0.8, 1.2, and 1.6 ppm across 3 fat levels—full, reduced and low. Reduced- and low-fat sauces were formulated with 4 fat mimetics—Dairy Trim, N-Lite L, Paselli Excel, and Simplesse. Measurements of pH and viscosity were recorded on days 1 and 3 of each week. Sensory heat data were collected at one sitting per day, 3 d a week for 5 wk. A trained sensory panel scored heat intensity every 15 sec for 180-sec in 3 samples at each sitting.

Decreasing fat levels and increasing CAP levels led to increased pH. Viscosity increased as fat mimetic within fat level was changed. Neither was correlated with perceived heat intensity.

Pungency in cheese sauces was related to CAP concentration and fat level, but not to fat mimetic. Time-intensity parameters of pungency did not differ among fat levels at low CAP levels (0.0 and 0.4 ppm). At 0.8 ppm CAP, perceived intensity over time, maximum heat and total intensity were higher in reduced- and low-fat sauces. Low-fat sauces were perceived as more pungent over time with greater maximum heat and total intensity than full-fat sauces at 1.2 ppm CAP. Across all fat levels at 1.6 ppm CAP, no differences occurred in perceived heat over time. Maximum heat in full-fat sauces did not differ from that in reduced- or low-fat sauces at 1.6 ppm CAP; reduced-fat sauces had lower maximum heat than low-fat. Total heat intensity differed across all fat levels at 1.6 ppm CAP; low-fat cheese sauces with 1.6 ppm CAP had greater total intensity than either reduced- or full-fat sauces at the same level of CAP. At low fat levels, less capsaicin will impart heat intensity equal to that of moderate to high concentrations of capsaicin in full-fat cheese sauces.
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Chapter I

Introduction

The 1988 release of the Surgeon General’s report (USDHHS, 1988) on the health of Americans created an interest among consumers in reducing fat intake with a resulting trend toward development of reduced-fat products by the food industry. The need for methods of lowering fat in popular foods led to the introduction of a number of fat replacement systems, including hydrocolloids, starch derivatives and microparticulated proteins (Glicksman, 1991). In 1992 California consumers indicated an interest in and willingness to try products formulated with carbohydrate and/or protein-based fat replacers. In addition, the consumers surveyed considered the products containing fat replacers to be healthier than full-fat products (Bruhn et al., 1992). While some 1994 surveys indicated a lessening of concern about fat intake among consumers, sales of products with decreased fat content continue to grow and major snack food companies continue efforts to introduce new “healthier” snacks (Busetti, 1995).

Production of foods containing fat replacers introduces new concerns to manufacturers. Lipid-based flavor volatiles may be affected by the decrease in fat and introduction of carbohydrate and/or protein replacements. Without fat, the lipophilic flavors may not be tightly bound and may be released more quickly and in greater concentration into the oral cavity headspace (Plug and Haring, 1993). Research studies have examined the effects of fat reduction on flavor release and perception. Tuorila et al. (1995) examined the effects on perception of sweetness and sourness when fat is reduced. While they found an enhancement of sweetness, the intensity of the taste was not affected by decreasing fat levels. Shamil et al. (1992) measured flavor release and perception in reduced and full-fat versions of cheddar and Edam cheeses and in salad
dressings formulated with full fat and with 2 fat replacers. They concluded that the fat/water ratio in foods affects bitter tastes which are hydrophobic and as such may be released more easily by the hydrophilic environment created with fat replacers. Rosin and Tuorila (1992) examined the flavor intensity of garlic and pepper in varied foods. While no differences in intensity of garlic flavor among the dispersion media occurred, the perceived intensity of black pepper and black pepper plus garlic was stronger in fat-free mashed potatoes than in those containing fat.

As Americans have revamped their eating styles to reflect concerns about high-fat foods, they have developed an interest in the pungent flavors of ethnic dishes. Foods such as Thai and Indian curries flavored with hot spices are increasingly popular with American diners. Spicy Mexican cuisine is second only to Italian (Sloan, 1994). Mexican salsas have reached sales above those of tomato ketchup in the United States (Testerman, 1995). The spiciness of ethnic dishes is provided by capsicum peppers which originated in the Americas and were carried to Europe by the early explorers of the New World. From Europe, capsicums spread across the continent into Asia and Africa and eventually were carried back to America by early European traders (Andrews, 1995).

Pungency of chilies derives from a group of capsaicinoids; while as many as 7 are present in most peppers, capsaicin (N-vanillyl-8-methyl-6-nonenamide) is predominant (Bosland, 1992). Humans recognize the presence of capsaicin as a sensation of "burning"; when ingested, the alkaloid irritates or stimulates trigeminal nerves in the oral cavity (Prescott et al., 1984).

The mechanism of capsaicin and its interaction with various tastants and temperatures have been studied in depth. A linear relationship between the concentration of capsaicinoids and intensity of pungency was established by Krajewska and Powers (1988). Green (1986) examined the influence of capsaicin on non-painful
thermal perception. He expected the temperature of capsaicin solutions to affect thermal heat perception in the oral cavity and also expected the temperature of the solutions to affect intensity of capsaicin burn; results indicated that solution temperatures between 34 and 45°C increased perception of burn, while capsaicin decreased perception of cold at temperatures below 30°C. Prescott et al. (1984) found that temperature did not influence capsaicin burn intensity. They also examined the interaction of tastants with capsaicin; sweetness of sucrose was suppressed by the heat intensity of capsaicin, but saltiness was unaffected. Sizer and Harris (1985) investigated the interactions of food additives and temperature with threshold perception of capsaicin. Sodium chloride and citric acid did not affect intensity of burn, but sucrose depressed the pungency of capsaicin. Very high solution temperatures (60°C) intensified the burn.

Several studies have examined the ability of tastants and foods to reduce burn after capsaicin is ingested. Stevens and Lawless (1986) determined that sucrose and citric acid would produce a marked decline in intensity of burn; water and sodium chloride depressed burn somewhat, but quinine had little effect. Nasrawi and Pangborn (1990) examined the effects of sucrose, fat level and temperature on mouth-burn, using milk with 2 levels of fat as well as tantant solutions and ethanol. Sucrose was found to be more effective in reducing burn. While results were not statistically significant, the authors suggested that whole milk depressed burn more than skim milk. Fat in the whole milk may have reduced the perception of pungency. Hutchinson et al. (1990) examined foods as vehicles to reduce burn. Rice, butter and pineapple juice as well as water were used to rinse after ingestion of Tabasco sauce. Butter with a high-fat content was expected to be most effective in reducing intensity of burn because of capsaicin's affinity for lipids. While the foods were held in the mouth, burn was reduced by all samples; when foods were expectorated, burn intensity rebounded. Intensity values were slightly lower, but not
different when butter was the coolant. None of the foods were effective in reducing intensity of burn.

While numerous studies have examined the intensity of burn from capsaicin in solution, little sensory research has been done with capsaicin incorporated into food systems. Because capsaicin is a lipophilic compound, reduction of fat in a capsaicin-containing food may alter the perception of the heat of the compound in the oral cavity. Baron (1995) examined the perceived pungency of capsaicin heat in cheese sauces and starch pastes with varied fat levels and at two temperatures. Serving temperature of the sauces/pastes did not affect perceived intensity; perceived pungency increased as fat level decreased.

As fat-reduced, highly pungent foods are developed to meet consumer demands, further study of the interaction of fat and other ingredients with capsaicin will be needed. The current study was designed to examine the effects on sensory heat perception of capsaicin, fat and fat mimetics in cheese sauce formulas. Cheese sauces were formulated at 3 fat levels—full, reduced and low. Shortening was replaced in part or in full with 1 of 4 mimetics—Simplesse, microparticulated protein; Dairy Trim, modified rice and oat carbohydrate; N-lite L, modified waxy corn starch; or Paselli Excel, modified potato starch. Capsaicin was incorporated into the cheese sauces at 5 levels—0.0, 0.4, 0.8, 1.2 and 1.6 ppm. Perceived sensory heat was evaluated using time/intensity methods and a trained sensory panel. The objectives of the study were to determine the effects over time of concentration of capsaicin, fat level, fat mimetic and interactions between and/or among the three factors on perceived pungency.

Physical and chemical characteristics of samples were examined. Viscosity and pH measurements of the cheese sauces were analyzed for correlations among pH, viscosity and pungency. Proximate analysis determined nutrient composition.
Chapter II

Review of Literature

Dietary Fat

Effects on Health

Dietary fat has been identified as a contributing factor in many of the health problems which plague Americans. The Surgeon General's Report on Nutrition and Health (USDHHS, 1988) reported a correlation between high fat intake and coronary heart disease, a number of cancers, gall-bladder disease, diabetes and obesity, diseases which afflict large numbers of Americans and some of which are ranked among the 10 leading causes of death in the United States. When the Surgeon General's Report was released, Americans were consuming 37% of their calories in the form of dietary fat. Evidence of detrimental effects of high dietary fat intake led to recommendations from the Surgeon-General's office, the U. S. Department of Agriculture, the Food and Nutrition Board of the National Research Council, and the American Dietetic Association that Americans reduce their dietary fat intake to 30% or less of total daily caloric intake (USDHHS, 1988; USDA/USDHHS, 1989; NRC, 1989; Hudnall et al., 1991).

As government and health agencies of the United States were linking high fat intake to debilitating diseases, nutrition and obesity scientists were serendipitously discovering a link between dietary fat intake and weight control. While investigating the effect of dietary fat on caloric compensation, Lissner et al. (1987) found that study subjects who reduced fat intake without decreasing caloric intake lost weight. Prewitt et al. (1991) placed subjects on two high-calorie food plans—one high-fat, one low-fat; both body weight and fat decreased on the low-fat regimen although total caloric intake remained equal or higher than that of subjects on the high-fat plan. Sheppard et al.
(1991) reviewed 1- and 2-yr weight changes in a group of 303 women. Among this group of subjects, weight loss and decreased dietary fat intake were more highly correlated than weight loss and reduction of total dietary energy intake.

Consumer Reaction

As the government was warning Americans of the health dangers of a high fat diet, findings from the nutrition studies were made public by the popular press. Americans were suddenly aware that indulging in high-fat foods not only increased fat, but could lead to serious illness. This new awareness created an interest among consumers in reducing fat intake with a resulting trend toward development of reduced-fat products by the food industry. The Calorie Control Council surveyed American adults in 1990; 2 of every 3 respondents consumed some low- or reduced-fat products; 66% of adults surveyed indicated a need for ingredients to replace fat in foods (Nabors, 1992). In a 1992 California survey, consumers indicated an interest in and willingness to try products formulated with carbohydrate and/or protein-based fat replacers. In addition, the consumers surveyed consider products containing fat replacers to be healthier than full-fat products (Bruhn et al., 1992). The American Dietetic Association—recognizing that fat replacement systems are being used increasingly in food products—issued a position statement in which they recommend that consumers incorporate low- and reduced-fat foods into meal plans consistent with the Dietary Guidelines for Americans (Hudnall et al., 1991). While some 1994 surveys indicate a lessening of concern about fat intake among consumers, sales of products with decreased fat content continue to grow and major snack food companies continue efforts to introduce new "healthier" snacks (Busetti, 1995).
Fat Substitutes

Consumer demands for lower fat products led to an industry-wide search for fat substitutes. Originally, the term "fat substitute" defined any ingredient used to replace all or part of the fat in a food product. To serve as an adequate fat substitute, an ingredient is required to produce the sensation of "fattiness"—the fullness of body and the creamy, smooth mouthfeel of a full-fat product. The ideal substitutes are synthetics—ingredients with similar chemical structure and physical properties of fat, but resistant to digestion in the human body. Proctor and Gamble developed Olestra, a sucrose polyester with long-chain fatty acids esterified to a sucrose backbone. The resultant molecule is not digestible, providing no calories (Glicksman, 1991; Jones, 1996). However, digestive difficulties were incurred by some subjects who sampled foods containing Olestra and the Food and Drug Administration (FDA) delayed approval of the substance. The lack of the ideal fat substitute led to the introduction of a number of fat replacement products; currently over 200 ingredients suitable for use as fat replacers are available to food manufacturers.

Fat Replacers

The term "fat replacer" is used to define any ingredient used to take the place of fat in a product. Fat replacers are grouped by the source of the molecule: included among the sources are lipids, carbohydrates and proteins. Lipid-based fat replacers include the modified or synthesized fat analogs such as the previously-discussed Olestra, emulsifiers generally used as part of a replacement system and structured lipids. Structured lipids are triglycerides formed from hydrolysis and interesterification of medium- and long-chain fatty acids. Caprenin, which combines caprylic, capric and
behenic fatty acids, was designed by Proctor and Gamble for use as a confectionery fat. Because behenic—a 22-carbon (C22:0) triglyceride derived from hydrogenated canola oil—is primarily unmetabolized in its passage through the intestinal tract and caprylic (C8:0) and capric (C10:0) are inefficiently metabolized, Caprenin provides only 5 kcal/g of energy. A second type of structured lipid is formed from short-chain (SCT) and long-chain triglycerides (LCT). The Nabisco Food Group designed Salatrim which is comprised of randomly distributed SCT—primarily acetic, propionic and/or butyric—and LCT from stearic acid. This structured lipid also is not metabolized efficiently, providing only 5 kcal/g. The combination of SCT and LCT controls melting point; the ratio of SCT to LCT gives flexibility in the functionality of the lipid (Swanson, 1996).

**Fat Mimetics**

Many of the 200 available fat-replacing ingredients are "fat mimetics," products which require a high water content in order to achieve functionality (Jones, 1996). Glicksman (1991) classified the majority of fat mimetics as hydrocolloids—long-chain polymers which thicken or gel in aqueous systems, giving them the ability to mimic fat. Carbohydrate-based replacers (starch derivatives and glucose polymers) are included in the wider category of hydrocolloids, as are edible gums, hemicelluloses and soluble bulking agents. Egg albumins, caseins and whey proteins have been modified to serve as protein-based fat mimetics (Glicksman, 1991; Setser and Racette, 1992).

**Carbohydrate-based mimetics—modified starches.** Starch-derived fat mimetics are usually maltodextrins, partially hydrolyzed products with low dextrose equivalents (DE). The FDA defines maltodextrins as non-sweet, nutritive saccharide polymers consisting of D-glucose units linked by α-1,4 glycosidic bonds, having a DE of < 20 and prepared by
partial hydrolysis of cornstarch using acids or enzymes. The FDA's definition excludes starches other than corn, but the term maltodextrin as used in the general literature describes modified starches with DE < 20 from any source including tapioca and potato (Roller, 1996).

Two processes are used to produce maltodextrins. The single-stage process involves gelatinization of the starch combined with an acid or enzyme treatment at high temperatures. Dent cornstarch treated with acid is usually heated to 105°C; waxy maize starch treated with bacterial α-amylase or other enzyme is heated to 82-105°C. The dual-stage process utilizes the process above until a DE < 3 is obtained. In the second stage the product is jet-cooked at 110 to 180°C to complete the gelatinization of the starch; the slurry is then cooled to 82-105°C and treated with a fresh batch of bacterial α-amylase. Hydrolysis in both processes is stopped by change in pH or temperature. The resultant product is then spray-dried (Roller, 1996). Low DE maltodextrins are cold-water soluble, have low viscosity in solution and form gels at high concentrations (> 20%w/w). The gels are plastic, spreadable and shortening-like. Maltodextrin fat replacers with DE levels < 10 and produced by the above processes include the N-Lite® and N-Oil® series from National Starch & Company; Amalean® I and II, American Maize Products Co.; the Sta-Slim® group and Stellar®, A.E. Staley Manufacturing Co.; and Paselli SA2® from Avebe. Fat mimetics derived from starches find application in a variety of products from salad dressings and spreads to frozen desserts to baked goods (Glicksman, 1991; Jones, 1995; Harkema, 1996).

**Other carbohydrate-based mimetics.** Several fat mimetics are derived from other carbohydrates: cellulose, fiber and polydextrose products are often used. Cellulose, the most abundant plant polysaccharide, does not contribute food energy; it has little or no
color or flavor. Cellulose is modified to form microcrystalline cellulose by treating it with high intensity shear force which disrupts the native structure. The result is a colloidal form of cellulose (Humphreys, 1996). Treating cellulose with sodium hydroxide swells the cellulose and allows substitution on the molecule. It is then reacted with methyl chloride to produce methyl cellulose. The by-products are removed with hot water and filtration and the resultant product dried, ground and packaged as methyl cellulose (de Mariscal and Bell, 1996). When modified, cellulose-based products will form an aqueous gel network which contributes to emulsion stability and viscosity, controls syneresis, improves texture. Such a gel mimics sensory attributes such as the fullness of body and creamy texture expected when a high fat product is ingested (Setser and Racette, 1992). An additional carbohydrate used as a mimetic is pectin, also a structural component of plants. For use as a fat mimetic (Slendid®, Hercules, Inc.), pectin is produced by traditional methods. It is extracted from citrus peel; if a low-methoxy pectic (LMP) is desired, it is de-esterified to a low-methoxyl state. Different batches of high-methoxy pectin (HMP) or LMP are blended for uniformity and adjusted by addition of sucrose or dextrose to achieve the desired strength (Neilsen, 1996). Pectin-based fat mimetics form a gel which simulates an emulsified fat; they are useful in salad dressings, sauces, frozen desserts and baked products (Setser and Racette, 1992).

β-glucans, glucose polymers containing 1-4 linkages connected to 1-3 linkages are found in all cereal grains, but are more highly concentrated in oats and barley. The presence of the 1-3 linkage contributes to their water solubility and distinguishes β-glucans from cellulose. Enzymatic modification of oat flour or bran produces an ingredient containing 2-10% β-glucans which is cold-water dispersible and forms a gel which functions as a fat mimetic. This product is produced by Rhone-Poulenc under the trade-name Oatrim®. Recently a second oat-based product, Dairytrim®, has been added
to the line; it is modified rice and oat flours and is recommended for formulation of dairy products (Glicksman, 1991; Setser and Racette, 1992; Jones, 1995).

Polydextrose, originally marketed as a bulking agent, is a long-chain polymer; the molecule is randomly bonded with traces of glucose sorbitol (which acts as a plasticizer in the reaction) and of citric acid (the polymerization catalyst) (Jones, 1995; Mitchell, 1996). Polydextrose replaces sugar and fat, acting as an humectant and adding the bulk of sugar. In addition, polydextrose thickens, stabilizes and adds texture to products in which it is used. While not recommended as a full-fat replacement, polydextrose can mimic fat in reduced-fat foods due to its high viscosity. Applications are almost limitless: ice creams, instant pudding mixes, salad dressings, confections, bakery fillings, pastries, jams and jellies (Mitchell, 1996).

**Microparticulated proteins.** Microparticulated proteins used as fat replacers are denatured with heat and agitated with enough force to interrupt the natural aggregation of the proteins. Singer (1996) describes this patented process as simultaneous homogenization and pasteurization. The proteins aggregate in a micrometer size range and maintain a spherical shape. Sizes range from 0.1 to 3 microns; the particles are pH stable in a range from pH 3 to 7 and heat stable between 10 and 95°C. The shape and size of the microparticulates—often described as ball bearings—impart a creamy, rich mouthfeel to foods in which they are used. Egg albumins in combination with casein were used in the early production of microparticulates; currently, ingredients derived totally from whey protein concentrates are available. More recently-developed protein microparticulates withstand UHT, pasteurization and retorting temperatures. The Simplesse® products produced by Nutra-sweet Kelco are microparticulated proteins with
application in dairy products, margarine spreads, salad dressings and mayonnaise (Singer, 1996; Jones, 1996).

Research and Development Concerns

Production of foods containing fat replacers introduces new concerns to the manufacturers. Fat serves multiple functions in a food system, one of which is to carry fat-soluble flavors. It is involved not only in the distribution of flavors within the oral cavity, but also in their balance, intensity and release. Lipid-based flavor volatiles may be affected by the decrease in fat and introduction of carbohydrate and/or protein replacements. Reduction of fat in a food system can result in an increased initial perception of the flavor. Fats allow gradual release of flavor in the oral cavity during mastication of a food. Without fat, the lipophilic flavors may not be tightly bound and may be released more quickly and in greater concentration into the oral cavity headspace. A flavoring such as vanillin will be perceived much stronger in a reduced-fat food, altering the perception of other flavor notes in the same system. While the flavor may be perceived earlier than in a full-fat food, it dissipates more quickly. The vanillin will be perceptible for a shorter time in the reduced-fat product (Bennett, 1992; Setser and Racette, 1992; Plug and Haring, 1993). Fat replacers dependent upon high water content for functionality can affect the strength of flavor impact; the more lipophilic a fatty acid is, the lower its threshold in high water systems. Decenoic acid (C10:0) will be detected at 4 ppm in water; in oil, 200 ppm are required to reach threshold (Bennett, 1992). If the use of a fat mimetic alters the pH of the food system, the equilibrium of the system may shift and the flavor potential of fatty-acid based flavors may change. Fatty-acid based compounds exhibit flavor only in the associated state. If the pH is decreased, the
equilibrium shifts so that more of the fatty acid is associated and the flavor intensity is increased (Bennett, 1992). Protein-based fat replacers may bind water-soluble flavors. Aldehydes or ketones may be held by the protein and no flavor released at all. Lipophilic flavor compounds can react similarly with amylose which is a component of many carbohydrate-based mimetics. In a low-fat system, the flavor may be trapped within a helical coil and be unable to interact with the olfactory receptors (Bennett, 1992).

Researchers have examined the effects of fat reduction on flavor release and perception. Schirle-Keller et al. (1992) examined head-space concentrations of flavor compounds in either oil-based carriers or carriers formulated with protein- and carbohydrate-based fat mimetics. Carbohydrate-based mimetics showed very little reaction with flavor compounds. Neither did a protein-based mimetic comprised of egg proteins. However, the whey-protein mimetic behaved more like the oil-based carrier and showed an increased interaction with non-polar flavor compounds. In a second study, Schirle-Keller et al. (1994) again found that protein-based mimetics showed greater interaction with the flavor compounds. However, none of the replacers in either study interacted with flavors equally to the flavor interaction of the oil-based carrier; therefore, any flavor formulation planned for a reduced- or low-fat food system requires changes to produce a flavor profile like the full-fat product.

Tuorila et al. (1995) examined the effects of fat-reduction on perception of sweetness and sourness. Using strawberry yogurts with 4 fat levels, they asked 14 panelists to rate sweetness and sourness. While they found an enhancement of sweetness and suppression of sourness, the intensity of the water-soluble flavor was not affected by decreasing fat levels. However, the research team acknowledged the possibility of deficiencies in their study methods and recommended additional research. Shamil et al. (1992) measured flavor release and perception in reduced- and full-fat
versions of cheddar and Edam cheeses and in salad dressings formulated with full-fat and with 2 different fat replacers. A 6-member trained sensory panel evaluated sharpness, bitterness and astringency in cheddar cheese; sharpness, astringency and saltiness in Edam; astringency, saltiness and vinegariness in salad dressings. For cheddar cheese, data revealed greater intensity of response for astringency and bitterness; sharpness was not more intense. The "rounded" flavor of the cheese was lost when fat was reduced. Results were similar for Edam; sharpness and astringency were more intense in the reduced-fat version. Sensory differences were found between full- and reduced-fat salad dressings, but no differences were perceived between the mimetics. Differences in perceived bitterness and saltiness in the cheeses and in vinegariness in the salad dressings may be attributed to the fat/water ratio discussed earlier. Bitter taste compounds are hydrophobic and as such are more readily released by the reduced-fat cheeses and salad dressings. The acetic acid of the vinegary dressings may be intensified by a decrease in pH as the water content of the dressing increases.

Rosin and Tuorila (1992) examined the flavor intensity of garlic and pepper in food systems with varied levels of fat. Dispersion media were broth, fat-free mashed potatoes and fat-containing mashed potatoes. Spices were added in amounts equal to no-flavor and intense-flavor. Intensity of flavor in all dispersions containing the spices was perceived as relatively strong. While no differences in intensity of garlic flavor among the dispersion media occurred, the perceived intensity of black pepper and black pepper plus garlic was stronger in broth and in fat-free mashed potatoes than in potatoes containing fat. Time to intensity was also shorter for both pepper and pepper plus garlic. Differences were not attributable to texture or starch content; only the presence of fat altered flavor response in these particular food systems. Rosin and Tuorila concluded that flavor
potency in food systems is difficult to predict due to the multiplicity of possible interactions.

Americans, Ethnic Cuisine and Chile Peppers

The pleasure derived from eating has decreased with the change from high fat cuisine to healthier low-fat foods. Although Americans may suffer guilt when they indulge in old-fashioned high-fat meals, they receive little comfort from bland healthy foods. As a result, the use of intense flavors is increasing as Americans search for better tasting food. Larger numbers of Americans are travelling in countries where native cuisine is more flavorful and are returning home with a taste for hot foods. In addition, Asian and Hispanic Americans, now a large segment of the U.S. population, have grown up with intense spices and flavors (Sloan, 1994; Uhl, 1996). These factors have created a demand for highly spiced ethnic cuisine which is becoming a popular alternative to bland, low-fat foods. Americans are adding flavor to low-fat foods with pungent spices. A marked trend toward use of pungent, flavorful spices became evident in 1994. Ethnic dishes on restaurant menus account for 30% of entrees. Foods such as Thai and Indian curries flavored with hot spices are increasingly popular with American diners. Spicy Mexican cuisine is second only to Italian (Sloan, 1994). The demand for increased flavor continues into 1995-96. Thai cuisine is growing in popularity with an annual growth rate of 25%. Taco Bell serves hot spiced tacos and burritos to 45 million Americans per week (Sloan, 1996). Mexican salsas, tomato-based products containing peppers, onion, cilantro and cumin, have reached sales above those of tomato ketchup in the United States, earning $716.1 million in 1994 (Testerman, 1995).
The pungency of salsas as well as that of Middle-Eastern/Asian cuisine is provided by chili peppers which also are growing in popularity. Chilies not only invigorate bland, low-fat dishes, but in themselves are low in fat, plentiful, inexpensive, high in vitamins A and C as well as a good source of potassium and folic acid. In the United States, more chilies are grown than honeydew melons or celery. Chilies are a $3 billion industry in the U.S.; 60% of the American crop is grown in New Mexico which has designated the chili as its state vegetable and is the home of several festivals which celebrate the colorful peppers (Miller, 1991; Garcia, 1992; Sheridan, 1993).

The peppers known as chile peppers or hot peppers are in reality Capsicum peppers. Capsicum is a small shrub of the Solanaceae genus, not related to the Piper genus from which black pepper is derived; Capsicum's relatives, also members of the Solanaceae family, include the tomato, potato, eggplant, tobacco, petunia and the poisonous nightshade. The fruits of the Capsicum popularly are considered vegetables, but are classified scientifically by fruit characteristics (Cotter, 1980; Bosland, 1992).

Chilies have a history as colorful as they. The oldest known record of chile was found in Tehuacán, Mexico, 150 miles south of Mexico City. Seeds were found on the floor of ancient caves. Human coprolites indicate that natives of the area were ingesting the peppers as early as 7000 BC. The earliest peppers used as food grew wild in the area, but by 5200-3400 BC, people of the region were cultivating them; thus, capsicums were among the first plants domesticated in the Americas (Andrews, 1995). The name "pepper" is a misnomer given to the fruit by Columbus and his ship's crew who were the first Europeans to encounter them. Because chile added a pungency to food similar to that of the highly prized black pepper, Columbus assumed it was black pepper and so called it. Columbus and other early explorers of the New World carried capsicum peppers back to Spain and Portugal; from there, the plants traveled to Africa and Asia.
Eventually, traders carried peppers from Bermuda to the English colonies on the East coast of North America. But their predominant use as a food ingredient has been confined to Latin American/Caribbean countries, Asia and Africa until recently (Miller, 1991; Andrews, 1995; Uhl, 1996).

Pungency

Pungency Defined

Humans recognize the presence of chilies as a sensation of "burning"; when ingested, chilies irritate or stimulate trigeminal nerves in the oral cavity. The name given to this sensation of warmth is "pungency." While pungency has been defined variously as a stinging, irritating or caustic effect, Govindarajan (1979) commented that these meanings are too general and may apply to chemical compounds such as phenols, ammonia and strong acids which humans do not ingest. The terms connote undesirable characteristics. Pungency should be recognized as a gustatory characteristic—a mouth-watering quality which enhances enjoyment of an otherwise bland food (Govindarajan, 1979). Govindarajan and Sathyanarayana (1991) describe the response to a first encounter with a capsicum-containing food as uncomfortable. The mouth and throat burn, the face and neck become flushed, the forehead may sweat. The "victim" of chilies who repeats the experience learns to enjoy the sensory response and will seek it out by preparing and eating chili-spiced foods. Andrew Weil (1976) recounts the story of a 5-year-old Mexican girl whom he saw nibbling a raw chili pepper; fanning her mouth with her hand, the girl squealed with delight, "It bites, it bites!" Weil likens pungency to a "rush," a sensation of pain intertwined with pleasure that builds to a peak before subsiding and leaving the body unharmed. Repeated chili consumption reduces the
perception of pain without decreasing the pleasure. There is no addictive component in capsicum; no withdrawal symptoms occur if a chili-lover must give-up eating spicy foods. But it is the pungency of capsicums which attracts the human to them (Rozin et al., 1981; Govindarajan and Sathyanarayana, 1991).

Capsaicin

Pungency of chilies derives from a group of related compounds called capsaicinoids; while several are present in most capsicum fruits, capsaicin (N-vanillyl-8-methyl-6-nonenamide) is predominant (Bosland, 1992). Capsaicin is synthesized within the fruit (Fig 1), secreted by cells located in the dissepiment along the placenta. Occasional studies have found high levels of capsaicinoids in chili seeds, but it is believed that capsaicin is absorbed by the seeds due to their proximity to the placental wall. The pericarp has virtually no pungency. Capsaicin has been found in the fruit as early as 10 days after flowering of the plant; the secretion of capsaicin plateaued after 4 weeks (Huffman et al., 1978; Cotter, 1980; Suzuki and Iwai, 1984; Bosland, 1992; Andrews, 1995). Pungency levels of the capsicum may be affected by genetic inheritance, temperature, light, fertilization and moisture. The genetic make-up of the capsicum determines the total capacity of the pepper for formation of capsaicin. Different varieties or cultivars have different concentrations of capsaicin. Increasing levels of moisture during the growing season decreases capsaicin concentration. The temperature of the environment also influences the concentration of pungency; capsicum grown at higher temperatures have higher levels of capsaicin. The effects of fertilization and light are less well documented. Results of experiments in which light has been controlled have been mixed. High temperatures combined with no light produced higher
Figure 1—Cross section of a typical Capsicum fruit (Andrews, 1995).
levels of capsaicin, but studies involving light without temperature intervention have not been definitive. The same is true of use of fertilizers; although addition of nitrogen to the soil positively affects growth of the plant, capsaicin concentration does not appear to be effected (Cotter, 1980; Suzuki and Iwai, 1984; Andrews, 1995).

Isolation of Capsaicinoids and Determination of Chemical Structure

Maga (1975) reviewed the early work which identified the properties of the capsaicinoids. The pungent component of peppers was first isolated by Buchholiz (1816) who found that he could extract the pungent compound from peppers by macerating them with organic solvents. Braconnot (1817) expanded Buchholiz' work when he not only isolated the compound but also formed soluble salts with alkalies. Thresh (1846) crystallized the compound and named it capsaicin. Later, Micko (1896) improved Thresh's method of isolating capsaicin and was able to show the presence of both a hydroxyl and a methoxyl group. He proposed that capsaicin is a relative of vanillin.

Perhaps the greatest strides were taken by Nelson (1919) who determined that capsaicin is composed of a base (vanillyamine) and an acid (isomeric decenoic acid). He detected vanillylamine when capsaicin was acid hydrolyzed under pressure; when the compound was alkaline hydrolyzed under pressure, a fatty acid was found. Further, Nelson determined the presence of a double-bond; when the fatty acid was hydrogenated, it absorbed 2 molecules of hydrogen. Later, Nelson and Dawson (1923) was able to synthesize capsaicin by reacting synthetic vanillylamine with decenoic acid extracted from a capsicum. Suzuki and Iwai (1984) report that Spath and Darling (1930) chemically synthesized the compound from zinc iodide and ethyladipyl chloride in cold toluene; the resultant synthetic contained both structural and geometrical isomers and yielded less than 6% capsaicin. It was not until the late 1950's and 60's that the
combination of capsaicinoids contributing pungency was detected. Suzuki and Iwai (1984) summarize the work of Kosuge who, using paper chromatography and subsequent colorimetric determination, separated 2 compounds from capsaicin. The dominant compound was capsaicin; the lesser compound he called dihydrocapsaicin. Bennett and Kirby (1968) using nuclear magnetic resonance, mass spectrometry and radioisotopic techniques were able to detect 5 closely related amides in the pungent compound of peppers (Table 1). Two—capsaicin and dihydrocapsaicin—were the same as those detected by Kosuge; capsaicin was most prominent, comprising ~70% of the compound. Dihydrocapsaicin accounted for another ~30%. The newly discovered compounds, present only in trace amounts, were named nordihydrocapsaicin, homodihydrocapsaicin and homocapsaicin. More recent studies have uncovered a number of analogs; the 5 capsaicinoids elucidated by Bennett and Kirby are most common in the capsicum fruit.

Pungency Evaluation

As capsicums came to be used increasingly in food products, methods of evaluating the pungency of pepper varieties were needed. Early capsicum researchers did not have reliable objective methods available to them. In 1912, Scoville published a subjective method which became widely used in evaluation of pungency. He suggested use of ascending serial dilutions to determine the point at which pungency was just perceptible (threshold level). He recommended defining pungency of capsaicin as the highest dilution at which a burn was perceived. The dilution value, given in milliliters per gram, is termed Scoville Heat Units (SHU); SHU are the current unit of measure of pungency. The Scoville method has been adapted by a number of organizations including the Essential Oil Association (EOA), The British Standards Institution (BSI) and The International Standards Organization (ISO). The EOA specifies the method for
Table 1—Chemical formula, nomenclature and molecular weight of 5 capsaicinoids

<table>
<thead>
<tr>
<th>Capsaicinoid</th>
<th>Chemical formula</th>
<th>Nomenclature</th>
<th>Molecular weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capsaicin</td>
<td>C_{18}H_{27}O_{3}N</td>
<td>N-[(4-hydroxy-3-methoxy-phenyl)methyl]-8-methyl-6-nonenamide</td>
<td>305</td>
</tr>
<tr>
<td>Dihydrocapsaicin</td>
<td>C_{18}H_{29}O_{3}N</td>
<td>N-[(4-hydroxy-3-methoxy-phenyl)methyl]-8-methylnonenamide</td>
<td>307</td>
</tr>
<tr>
<td>Nordihydrocapsaicin</td>
<td>C_{17}H_{27}O_{3}N</td>
<td>N-[(4-hydroxy-3-methoxy-phenyl)methyl]-7-methyl-octanamide</td>
<td>293</td>
</tr>
<tr>
<td>Homodihydrocapsaicin</td>
<td>C_{21}H_{35}O_{3}N</td>
<td>N-[(4-hydroxy-3-methoxy-phenyl)methyl]-8-methyl-9-methyldecenamide</td>
<td>321</td>
</tr>
<tr>
<td>Homocapsaicin</td>
<td>C_{19}H_{29}O_{3}N</td>
<td>N-[(4-hydroxy-3-methoxy-phenyl)methyl]-9-methyl-7-decenamide</td>
<td>319</td>
</tr>
</tbody>
</table>
oleoresins. They recommend dilution of 0.200 g oleoresin in ethanol with 50 to 140 ml of a 5% sucrose solution. Five trained judges swallow 5 mL of the highest dilution and record presence or absence of pungency. If the panel perceives the first dilution as strong, weaker dilutions are made until judges can agree. When 3 of the 5 agree that pungency is perceptible at a specific dilution, that value is called the Scoville value of pungency. Obvious difficulties arise from this method. Tasting from strong to weak solutions can distort perception. The panel, though experienced, receive no instruction prior to beginning the test; each member may have a different concept of pungency. The EOA also does not provide a standardized sample preparation method; extraction differences could result. The ISO in adapting Scoville's methodology addressed some problems by specifying that only weak to strong dilution order should be used and that panelists should wait 5 sec between dilutions and 30 min between repeats.

The American Spice Trade Association (ASTA) (1968) has established an official method for pungency evaluation. This method again is very similar to Scoville's, but incorporates some safeguards. A specific method of extraction requiring 16 h is defined. The panel is trained and undergoes several trials before the actual test begins. Warm water is provided for palate clearing. Each panelist is taught to swallow the sample, wait 30 sec, record his response, wait 5 min before tasting the next sample. Again, 3 of 5 judges must agree on the threshold. Problems encountered with the ASTA method include the long extraction time and a long preparation of dilutions.

Govindarajan et al. (1977), recognizing the difficulties in the Scoville method which could negate reproducibility and validity of the test, designed a new procedure which they believe eliminates the problems of Scoville's method; it provides a dilution table and correlates with objectively determined capsaicin levels. Govindarajan’s method has been adopted by the Indian Standards Institution (1976). Samples for sensory
determination of pungency are prepared from oleoresins and from alcoholic extracts at 1:10,000 and 1:100 respectively in 3% sucrose solutions. Oleoresins are emulsified with glycerol monostearate. A geometric progression of dilutions with a common ratio (such as 1.5) are made. The panel leader uses the dilutions to determine a threshold and from the threshold selects a range of dilutions to present to the judges. Training sessions are designed to educate the panelists about pungency. They are familiarized with the concepts of onset, duration and decay of the sensation. In addition, panelists learn the procedures, the use of the scorecard and are familiarized with various levels of pungency.

A group of 10-12 persons tests 4-7 dilutions (5 mL each) in ascending order; 1-3 blanks are presented prior to the first actual dilution. Puffed rice or another bland food is given with water to cleanse the palate between dilutions. Each panelist continues until he perceives pungency; he then stops. After the training sessions are completed, the panel members are grouped according to low or high sensitivity and 5 persons with low sensitivity are selected to continue. Data from the training session are used to determine an appropriate threshold level for the testing sessions. A range of dilutions is prepared with a difference of 10% of the threshold value between them. The five panel members participate in 3-4 repeats of the test to give 15-20 judgements. When data are compiled, the mean ± one standard deviation is expressed as SHU. Values with standard deviation > 2 are deleted.

A method described as "radical" by Govindarajan et al. (1987) was developed by Gillette et al. (1984). Their purpose also was to replace the Scoville method with a more reproducible and valid test of pungency. In addition, they wished to decrease extraction time and control other problems which frequently occur with the Scoville method. Gillette et al. prepared 15 artificial pepper samples by adding oleoresin capsaicin at known levels to ground paprika. They then added 5 g of the pepper to 1995 g spring water, extracted
the capsaicin, filtered it and diluted it 10-fold (20 g filtrate to 180 g water). Next, a standard for objective testing was prepared from a ground red pepper of known capsaicin concentration (slight heat-20,000 SHU). A sensory control was prepared with 0.6 g N-vanillyl-n-nonamide with 20 g Polysorbate-80 simmered in 1 L water. Ten milliliters were then diluted to 1 L and used to prepare concentrations ranging from 0.11 mg/L to 1.32 mg/L.

Gillette et al. (1984) used a 15-cm line scale anchored at 0 = no heat; 1.25 cm = threshold, 5 cm = slight, 10 cm = moderate and 15 cm = strong. Panelists were instructed carefully in a timed procedure. Two samples were presented—a control of slight heat and an unknown. The entire 15 mL sample was taken into the mouth, held 5 sec, swallowed and after 30 sec, perceived pungency marked as slight on the scale. The palate was cleansed with an unsalted cracker and spring water for 60 sec. The second sample was presented; the procedure was repeated; the panelist rated the unknown. If another sample was to be rated, each panelist was required to wait 5 min; otherwise, the panel was dismissed. Gillette et al. found that sensory heat values determined by their method were highly coordinated with the percentage capsaicin in the set of artificial red pepper samples (r=.92). Among the 15 samples, they found significant differences in sensory heat ratings which clustered into 4 groups—threshold, slight (25,000 SHU), moderate (50,000 SHU) and strong (75,000 SHU). Results of solvent and extraction procedures revealed that the use of Polysorbate-80 at 20 ppm increased the amount of pungency extracted from red pepper. Also, simmering the water increased the extraction rate. Ethanol did not extract greater amounts than hot water or water with Polysorbate-80. This method circumvents many of the problems of the Scoville method. The incorporation of a control or standard solution of known pungency and the use of timed rinsing between samples decreases the likelihood of heat build-up, of taste fatigue and of
increased threshold values. The aqueous extraction eliminates the possibility of an ethanol bite affecting perception of pungency. A major advantage of this method for many laboratories is the decreased extraction time. Gillette et al. required only 20 min to extract heat compared to the 16 h of the Scoville method.

Any newly devised testing method must be scrutinized. Quinones-Seglie et al. (1989) compared pungencies as determined by high-performance liquid chromatography (HPLC), the Scoville method and the Gillette method. Heat components were extracted by Soxhlet method from Jalapeno, Serrano and Chili peppers grown in Texas; diluted with acetonitrile, they were quantified by HPLC methods using a mobile phase composed of water/acetic acid/acetonitrile (1:65:35). Scoville test samples were prepared by ASTA method 21.0 and evaluated as discussed previously. Preparation and evaluation of Gillette method samples followed the procedures just described above. The Gillette method and the Scoville method both correlated well with the objective quantification of pungency (r=.97 and r=.98, respectively). The Gillette method has been adopted by ASTM (1991).

Capsaicin and Sensory Response

Mechanism of Neuroreception

The mechanism of capsaicin and its interaction with various tastants and temperatures have been studied in depth. Extensive research into the mechanism of capsaicin-induced pungency has resulted in a partial understanding of the process. Capsaicin produces the sensation of burning by activation of polymodal nociceptors (pain receptors) in the central nervous system. When capsaicin is detected by the nociceptor, the nerve membrane is depolarized, affecting its permeability. An ion channel is opened
which allows Ca\(^{2+}\) passage across the membrane. At the free nerve ending, the ion initiates the release of the neuropeptide Substance P, giving rise to the sensation of pain. Several studies have suggested that a specific receptor must exist in the membrane to mediate this action; no such receptor has been positively identified (Govindarajan and Sathyanarayana, 1991; Dray, 1992).

**Time-Intensity Evaluation Techniques**

Human sensory response is time related. Foods must enter the mouth where they undergo a process of manipulation and mastication which releases the flavors and textural attributes providing sensorial pleasure. The sensations change in intensity over the time required for the process. Traditional methods of sensory data collection require the judges to give single intensity values for a flavor, a basic taste or a textural characteristic. A single value omits information about the time needed to reach the value, about the duration of the intensity and about the time required for the perception to decay (Lee and Pangborn, 1986). Development of low-fat and/or low-sugar products necessitates an understanding of flavor release over time. Product designers need to understand the human response to traditional foods in order to formulate equivalent products. Time-intensity (T-I) measurements provide a curve (Fig. 2) which describes time to maximum intensity (T\(_{\text{max}}\)), a value for maximum intensity (I\(_{\text{max}}\)) and the duration of the sensation. Additional information may include lag time—time that elapses between entry of the food into the mouth and onset of response; plateau or time the sensation is sustained; and rate of release which is the ratio of I\(_{\text{max}}\) to T\(_{\text{max}}\). Other parameters can be defined and determined as need to meet experiment objectives (Lee and Pangborn, 1986; Anonymous, 1995). All of this information may be of value in new product development or in elucidating the physical/neuromechanisms of sensory response. T-I
Fig. 2—Typical time-intensity curve with defined parameters. T-Max is time to maximum intensity; I-Max is maximum intensity; Lag represents the time from 0 to time of onset of response; RATE is the ratio of I-Max to T-Max and represents the time of release; Duration is the total time a sensory perception endures; AREA is the total area under the curve and can be equated with total intensity.
testing is used in measurement of flavor response, basic tastes and sensory food texture. In the future it may be used in aroma research although measurement of responses to aroma over time will be difficult. T-I can also be used for hedonic measurements; a response of like/dislike or intensity of like/dislike may change over a few seconds time (Lee and Pangborn, 1986).

Methods of collecting T-I data are evolving rapidly. Early efforts involved utilization of chart paper and self-timing by the judges. Later, moving-chart recorders set at known speeds were used. A continuous curve was drawn as the chart paper advanced. The judge moved his/her pen up as intensity increased, down as it decreased. This method is labor-intensive for the experimenter who must spend time training a panel and afterward manually digitize the curve before data can be analyzed. Currently a number of computerized programs are available which utilize light pens, keyboard or the mouse for data collection. The computer programming instructs the judge during the experiment. Judges may be timed either by the computer or by handheld timers. Data are stored on a floppy disc and can be quickly submitted for analysis by a statistical package, requiring little or no manual effort by the scientist (Lee and Pangborn, 1986).

Time-intensity techniques were used to compare bitterness in caffeine and quinine by Leach and Noble (1986) because bitter taste lingers and its contribution to flavor cannot be estimated accurately by a single-point value. Guinard et al. (1995) used T-I methods to describe temporal properties of sweet and bitter tastes in an effort to derive the number of receptors for the two tastes in the oral cavity. Temporal characteristics of capsaicin pungency have been examined by a number of scientists.
Effects of Capsaicin Concentration, Temperature and Tastants

Krajewska and Powers (1988) investigated the contribution of individual capsaicinoids to total pungency of peppers and determined thresholds for four of the five elucidated by Bennett and Kirby (1968)—capsaicin, nordihydrocapsaicin, dihydrocapsaicin and homodihydrocapsaicin. Synthetic capsaicin (vanillylamide of n-nonanoic acid) was also used. Krajewska and Powers used the detection threshold method established by ASTM-practice E679-79 (ASTM, 1982) which they describe as a forced-choice triangle test. Ascending concentrations of capsaicin in solution were prepared by 1:1 serial dilutions of a 2.5 ppm stock solution. The stock solution was ethanol-based with 2.5% sucrose. Six dilutions ranging from 0.019 ppm to 0.625 ppm were used with most panelists. Magnitude estimation was used to establish a relationship between total pungency and pungency of each capsaicinoid. A linear relationship between the concentration of capsaicinoids and intensity of pungency was established. The sum of pungencies of individual capsaicinoids was found to correlate with total pungency. Krajewska and Powers conclude that individual capsaicinoids have an additive effect on total pungency.

Green (1986) examined the influence of capsaicin on non-painful thermal perception. He expected the temperature of capsaicin solutions to affect thermal heat perception in the oral cavity and also expected the temperature of the solutions to affect capsaicin burn. He defined burn as the sensation produced by a moderate concentration of capsaicin. Using 2 ppm capsaicin in ethanol and water solutions at 12 temperatures ranging from 13 to 45°C, Green found that solution temperatures between 34 and 45°C increased the panel's perception of burn. In addition, perception of cold at temperatures below 30°C was depressed in the presence of capsaicin.
Prescott et al. (1984) conducted a series of experiments in which they presented capsaicin in tastant solutions and in tomato soup. The tastant solutions were presented at room temperature (~20°C) and at mouth temperature (~37°C); soup was presented at mouth temperature and at the usual serving temperature of ~60°C. No interaction between temperature and perceived burn was found. As concentration of capsaicin increased, perceived sweetness of sucrose was suppressed; the intensity of burn was not affected by sucrose concentration. In salty tastant solutions prepared with sodium chloride (NaCl), the perception of saltiness increased with NaCl concentration; 8 parts per million (ppm) of capsaicin was required to suppress saltiness. The intensity of burn was increased in solutions containing NaCl and capsaicin. The tomato soup was also rated for sweetness, saltiness and burn. In soup, capsaicin did not affect saltiness, but again sweetness was depressed.

Sizer and Harris (1985) used threshold concentrations of capsaicin to investigate the effects of food additives on perception of pungency among eaters and non-eaters of chili peppers. They defined pungency as a sensation of spicy burning or biting associated with foods containing hot peppers. The additives used in the experiment, NaCl, citric acid and sucrose, were held constant as capsaicin concentration increased from 0.06 to 0.70 mg/L. Samples were presented at 3 temperatures—2, 18 and 60°C. No differences occurred between eaters and non-eaters. Neither NaCl nor citric acid affected pungency perception; however, unlike the results of Prescott et al. (1984), Sizer and Harris found a suppression of perceived pungency in the presence of sucrose. Higher levels of capsaicin were required to recognize the burn. They theorize that sucrose may have had a masking effect; sucrose molecules may have interfered with or bonded the capsaicin molecules. Also, the possibility exists that the increased viscosity produced by addition of sucrose may have interfered with recognition of the capsaicin.
Nasrawi and Pangborn (1989) also examined the effect on perceived heat intensity of tastants prepared with NaCl, sucrose and citric acid. In addition, they investigated the effects of viscosity by using solutions thickened with Xanthan gum. A trained panel rated solutions on a 10-cm scale anchored with the terms "not sweet (salty, sour, hot)" and "extremely sweet (salty, sour, hot)". On 3 test days, panelists used time/intensity methodology to rate the mouth burn from "none" to "extremely hot" over a 5-min period. Nasrawi and Pangborn comment that they obtained more information through time-intensity measurement as they were able to examine perceptions from onset to decay. On the single-point scale, the perceived pungency of solutions containing Xanthan gum and sucrose differed from other tastant solutions. The burn was depressed. NaCl and citric acid again had no effect on perceived burn. Over time, sucrose solutions reached a lower maximum intensity than capsaicin alone, but did not differ from other tastant solutions.

The ability of tastants and foods to reduce burn after capsaicin is ingested has been studied by several sensory scientists. Stevens and Lawless (1986) examined the effectiveness of basic tastants in reducing perceived burn. Solutions of sucrose (sweet), citric acid (sour), NaCl (salty) and quinine (bitter) were prepared as rinses. Ethanol solutions of 1 ppm capsaicin and 100 ppm piperine were prepared as pungency samples. Panelists rinsed with the basic tastants after expectorating the pungent solutions. Irritation was less intense while any of the tastant solutions were held in the mouth, but after expectorating tastants, burn would rebound. Results indicated that sour tastants would somewhat reduce the burn of piperine while sweet tastes reduced capsaicin burn. Nasrawi and Pangborn (1990) examined the effects of sucrose, fat level and temperature on mouth-burn. Panel members ingested 3 ppm capsaicin in 5 mL water, expectorated and followed it with a rinse. Perception of burn was rated continuously from the time the
sample entered the mouth through expectoration for a total of 5 min. A 5 min rest period was observed. The process was repeated with the addition of a 20 mL rinse placed in the mouth for 15 sec after expectoration of the capsaicin solution. Pungency was rated from entry into the mouth through extinction of heat. Rinses included water, water plus sucrose, ethanol, whole milk and skim milk. In a second experiment, sucrose was added to the milks. Temperatures of rinses ranged from 5°C for water and milks to 20°C for the ethanol and sucrose solutions. Nasrawi and Pangborn expected high fat whole milk to reduce burn due to the lipophilic nature of capsaicin. Also, because capsaicin is soluble in alcohol, they expected ethanol solutions to reduce burns. However, ethanol was ineffective in reducing intensity of burn. While results were not statistically significant, whole milk depressed burn more than skim milk. The authors credit the presence of fat in the whole milk as the possible cause of the trend toward reduction of irritation. Again sucrose was found to be an effective rinse for reduction of perceived burn; these researchers theorize that sucrose inhibits binding of the capsaicin molecule to the oral receptors. Sucrose may stimulate release of saliva which coats the tongue, interfering with the perception of burn or inhibiting Substance P which is required for transmission of the signal to the brain.

Hutchinson et al. (1990) examined foods as vehicles to reduce burn. Rice, butter and pineapple juice as well as water were used to rinse after ingestion of a 1% solution of capsaicin-containing Tabasco sauce in spring water. Tabasco samples were held in the mouth for 15 sec, burn recorded, sample expectorated, intensity of burn rated after 30 sec. The process was repeated. After the second Tabasco sample was rated, the food sample was taken into the mouth, held for 15 sec, intensity rated, the food expectorated and after 30 sec, a second intensity rating recorded. The process was repeated. A total of 180 sec elapsed during the procedure. Panelists timed themselves, but were
supervised by an experimenter. Butter with a high-fat content was expected to be most effective in reducing intensity of burn. While the foods were held in the mouth, burn was reduced by all samples; when foods were expectorated, burn intensity rebounded. While butter and rice showed a tendency to lessen intensity slightly more than the other foods, none were significantly effective.

While sensory scientists have examined the intensity of burn from capsaicin in solution, little sensory research has been done with capsaicin incorporated into food systems. Because capsaicin is lipophilic, reduction of fat in a capsaicin-containing food may alter perception of heat in the oral cavity. Baron (1995) examined perceived intensity of capsaicin heat in cheese sauces and starch pastes with varied fat levels and at two temperatures. While serving temperature of the carriers did not affect perceived intensity, pungency increased as fat level decreased. As fat-reduced, pungent foods are developed to meet consumer demands, further study of the interaction of fat with capsaicin will be needed to ensure that appropriate levels of capsaicin are being used in the industry.
Chapter III

Materials and Methods

Experimental Design

A randomized block design with repeated measures was implemented as an incomplete block. In a design produced by a software package (E4®, Evolutionary Software, Inc., 1991), eleven panelists evaluated pungency in 45 treatment combinations over a 15-day data collection period with 33 observations per day. Days were blocked in the incomplete implementation; panelists were blocked over the complete design. The model was:

\[ y_{ijkmn} = \mu + D_i + P_j + D_i P_j + C_k + \text{fatL}_i + C_k \text{fatL}_k + \text{fatR}(\text{fatL})_{mn} + D_i P_j C_k \text{fatL}_k + T_i C_k T_i + \text{fatR}(\text{fatL})_{mn} T_i + \epsilon_{ijkmn} \]

where
- D is day
- P is panelist
- C is capsaicin level
- fatL is fat level
- fatR is fat replacer
- T is time.

Because the treatment combinations used for sensory evaluation were also used for physical tests, the objective part of the study utilized the same design. Sauces were prepared at the beginning of each 3-day period of sensory evaluation. Viscosity measurements were recorded at 5 sec intervals over 60 sec and changes over time were of interest. The model for viscosity analysis is shown below.
where

\[ Y_{ijkm} = \mu + D_i + C_j + \text{fatL}_k + C \cdot \text{fatL}_j + \text{fatR} \cdot (\text{fatL})_k + \]
\[ C \cdot \text{fatR} \cdot (\text{fatL})_{jd} + D \cdot C \cdot \text{fatR} \cdot (\text{fatL})_{jd} + T \cdot T \cdot C \cdot \text{fatR} \cdot (\text{fatL})_{jd} + e_{ijkm} \]

where

- D is day
- C is capsaicin level
- fatL is fat level
- fatR is fat replacer and
- T is Time.

The model for analysis of pH and components determined through proximate analysis follows:

\[ Y_{ijkm} = \mu + D_i + C_j + \text{fatL}_k + C \cdot \text{fatL}_j + \]
\[ \text{fatR}(\text{fatL})_{id} + C \cdot \text{fatR}(\text{fatL})_{jd} + e_{ijkm} \]

where

- D is day
- C is capsaicin level
- fatL is fat level
- and fatR is fat replacer.

**Experimental Material**

The effects of concentration of synthetic capsaicin (N-vanillyl-8-methyl-6-nonamide), fat level, fat mimetic and time on perceived sensory heat were investigated. Cheese sauces were formulated with three levels of fat—full, reduced and low (Table 2). Full-fat sauces with a high proportion of shortening and small amount of modified starch
<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Full</th>
<th>Reduced</th>
<th>Low</th>
</tr>
</thead>
<tbody>
<tr>
<td>Modified food starch®</td>
<td>9.8</td>
<td>17.5</td>
<td>26.2</td>
</tr>
<tr>
<td>Cheese powder, full fat&lt;b&gt;</td>
<td>24.9</td>
<td>24.9</td>
<td>24.9</td>
</tr>
<tr>
<td>Shortening powder&lt;sup&gt;c&lt;/sup&gt;</td>
<td>29.4</td>
<td>15.5</td>
<td>0</td>
</tr>
<tr>
<td>Sweet whey&lt;sup&gt;d&lt;/sup&gt;</td>
<td>12.7</td>
<td>14.8</td>
<td>15.6</td>
</tr>
<tr>
<td>Cheddar powder, low-fat&lt;sup&gt;e&lt;/sup&gt;</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Fat mimetic&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0</td>
<td>3.3</td>
<td>6.6</td>
</tr>
<tr>
<td>NFDM&lt;sup&gt;g&lt;/sup&gt;</td>
<td>6.3</td>
<td>6.1</td>
<td>6.4</td>
</tr>
<tr>
<td>Corn syrup&lt;sup&gt;h&lt;/sup&gt;</td>
<td>0</td>
<td>1</td>
<td>3.4</td>
</tr>
<tr>
<td>Salt&lt;sup&gt;i&lt;/sup&gt;</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Blue cheese powder&lt;sup&gt;j&lt;/sup&gt;</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Lactic acid&lt;sup&gt;k&lt;/sup&gt;</td>
<td>0.4</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

<sup>a</sup>Firmtex<sup>®</sup>, National Starch and Chemical Company, Bridgewater, NJ.
<sup>b</sup>Chez-tone 153 Kosher, Kerry Ingredients, Beloit, WI.
<sup>c</sup>Beatreme<sup>®</sup> 2784, Kerry Ingredients, Beloit, WI.
<sup>d</sup>Extra-grade dry sweet whey, Land O'Lakes, Inc., Minneapolis, MN.
<sup>e</sup>Lowfat cheddar seasoning, Mid-America Farms, Springfield, MO.
<sup>f</sup>Fat substitutes vary by treatment. See Table 3.
<sup>g</sup>Armour<sup>®</sup> Food Ingredients Company, Springfield, KY.
<sup>h</sup>Kroger, Cincinnati, OH.
<sup>i</sup>Cheese-ireme<sup>®</sup> 1923B, Kerry Ingredients, Beloit, WI.
<sup>j</sup>ADM Arkady, Decatur, IL.
were designed to approximate the fat content of commercial cheese sauces and dips—21 g per 100 g serving (Marsh, 1980). Reduced- and low-fat sauces were designed to approximate legal definitions of the terms; reduced-fat sauces have at least 25% less fat than the full-fat product and low-fat sauces have no more than 3 g per serving (FDA, 1995). To control extraneous sources of fat, the cheese powders used in formulating sauces were held constant across treatment combinations. Fat levels of the three cheese powders used in the sauces ranged from 5% in the low-fat cheddar powder to 42% in the blue cheese powder. Reductions in fat were achieved by replacing shortening in part or in full with a fat mimetic. Four commercial fat mimetics were chosen from those available to the food industry; included were a microparticulated protein, a modified waxy maize starch, an oat/rice-derived product and a modified potato starch (Table 3). Five concentrations of capsaicin (0, 0.4, 0.8, 1.2 and 1.6 ppm) were chosen to contribute pungency to the cheese sauces. These concentrations increase in even increments and provide two levels of pungency defined by the ASTM (1991)—slight (0.4 ppm) and moderate (0.8 ppm)—as well as concentrations just below (1.2 ppm) and above (1.6 ppm) the level described as approaching strong (1.3 ppm) in water samples.

Table 3—Fat mimetics replacing shortening in cheese sauce formulas

<table>
<thead>
<tr>
<th>Fat substitute</th>
<th>Manufacturer</th>
<th>Composition derivation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simplesse®</td>
<td>Nutrasweet Kelco Chicago, IL</td>
<td>Microparticulated whey protein</td>
</tr>
<tr>
<td>Dairytrim®</td>
<td>Rhone-Poulenc Cranberry, NJ</td>
<td>Rice and oat flours</td>
</tr>
<tr>
<td>Paselli Excel®</td>
<td>Avebe Princeton, NJ</td>
<td>Enzymatically converted potato starch</td>
</tr>
<tr>
<td>N-lite L®</td>
<td>National Starch and Chemical Company Bridgewater, NJ</td>
<td>Modified waxy maize starch</td>
</tr>
</tbody>
</table>

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Stock solutions were prepared following ASTM method E 1083-88 (ASTM, 1991), using synthetic capsaicin (8-methyl-N-vanillyl-6-nonenamide, ≥98.0%, Sigma Chemical Co., St. Louis, MO). A mixture of 0.60 g of capsaicin and 20.0 g of Polysorbate-80 (Vanden Berg Foods Co., Lisle, IL) was heated in a 50-mL beaker on a stirring hot-plate at heat setting 5 until the capsaicin was dissolved (10-15 min). The mixture was quantitatively transferred into a 1000-mL mixing cylinder using 70°C spring water. The mixture was allowed to come to room temperature, after which it was brought to volume (1000 mL) with spring water and mixed thoroughly. To reach a concentration of 6.0 ppm capsaicin and 20 ppm polysorbate-80, 10 mL of the mixture were transferred to a second 1000-mL mixing cylinder and brought to volume with spring water. After thorough mixing, the stock solution was transferred to glass storage jars and held until needed for preparation of the carrier systems.

Cheese sauces were prepared weekly for sensory evaluation and physical measurement. Cheese sauce powders were prepared in advance by combining all dry ingredients in a Kitchen-Aid® mixer (Model k45ss, St. Joseph, MI). Ingredients were blended at speed 2 for 2 min and the powders transferred to glass containers for storage until needed. On preparation day, the prepared dry mix was blended in a 600-mL beaker with lactic acid, corn syrup, water and stock solution and heated on a Bamstead/Thermolyne Stirring Hot Plate (Dubuque, IA) at heat setting 6 and stirring setting 7-8 for 12-15 min until the mixture thickened. Levels of stock solution were designed to provide specified capsaicin concentrations in the sauces. Water levels varied in proportion to levels of stock solution (Table 4). After cooling, 10 g of each sample were transferred to coded 60-mL glasses, covered and refrigerated until needed for sensory testing. The remaining sauce (~85-100 g) was transferred to a 100-mL beaker, covered and refrigerated for objective analysis.
Table 4—Dry cheese powder, water and stock solution levels in cheese sauce formulas

<table>
<thead>
<tr>
<th>Fat level</th>
<th>Capsaicin (ppm)</th>
<th>Powder (g)</th>
<th>Water (g)</th>
<th>Stock solution (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full</td>
<td>0.0</td>
<td>85</td>
<td>215</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>85</td>
<td>195</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>85</td>
<td>175</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>1.2</td>
<td>85</td>
<td>155</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>1.6</td>
<td>85</td>
<td>135</td>
<td>80</td>
</tr>
<tr>
<td>Reduced</td>
<td>0.0</td>
<td>60</td>
<td>240</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>60</td>
<td>220</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>60</td>
<td>200</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>1.2</td>
<td>60</td>
<td>180</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>1.6</td>
<td>60</td>
<td>160</td>
<td>80</td>
</tr>
<tr>
<td>Low</td>
<td>0.0</td>
<td>50</td>
<td>250</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>50</td>
<td>230</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>50</td>
<td>210</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>1.2</td>
<td>50</td>
<td>190</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>1.6</td>
<td>50</td>
<td>170</td>
<td>80</td>
</tr>
</tbody>
</table>

* Capsaicin concentration = 6 ppm.
Physical and Chemical Tests

Proximate Analysis

Moisture. Frozen samples (~ 85-95 g) were transferred to pre-weighed bowls (Coming Glass Works, Coming, NY) and weighed to the nearest 0.01 g. Bowls were then placed on trays to be freeze-dried (Virtis Co. Inc., Model FDD-15-ws, Gardiner, NY) under the following parameters: condenser temperature, -60°C; shelf temperature, 38°C; vacuum, ≤ 100 μm Hg. Samples remained in the freeze-drier until a constant weight was reached (~48 h). Moisture was then determined and samples were powdered, transferred to Whirl-pak® bags (Nasco, Fort Atkinson, WI) and stored in desiccators until needed for further analysis.

Crude Fat. Dry sample (~2 g) was placed on a Number 1 filter; the filter paper folded and stapled and the packet weighed and transferred to a desiccator until fat analysis could be performed. Soxhlet extraction was used to determine fat content. A 5000-mL round bottom flask containing ~4000 mL petroleum ether was placed in a Glas-Col heating unit at heat setting 30. Samples were allowed to extract for 8 h, removed from the Soxhlet, dried and placed in a desiccator until weighed to determine fat lost.

Ash. AOAC (1990) method 942.05 was used to determine ash. Dry sample (~2 g) was weighed into a pre-weighed porcelain crucible. Samples were placed in a cold Sybron Muffle Furnace Model F-A1730 (Dubuque, IA) and brought to a temperature of 600°C. Crucibles remained in the furnace for a minimum of 12 h until ashed to a constant weight.
The muffle furnace was allowed to cool to room temperature and the crucibles removed to a desiccator. Samples were weighed as soon as crucibles were cool.

**Protein.** Percent protein was projected by calculation. Protein content of formula ingredients were obtained from manufacturers and used to determine expected protein levels for each treatment combination.

**Viscosity and pH**

On days 1 and 3 of sensory evaluation, samples in 100-mL beakers were removed from the refrigerator and allowed to come to room temperature. Viscosity of the cheese sauces was measured with the Brookfield Digital Viscometer Model DV-II+ (Brookfield Engineering Laboratories, Stoughton, MA) interfaced with a Zenith 344D+ PC. Sauces were stirred with a glass stirring rod prior to inserting spindle number 6 into the sample and setting rotation at 10 rpm. Measurements (centiPoise (cP)) were taken at 5-sec intervals over a 60-sec period for a total of 12 measurements. Alternate measurements beginning with the first reading and ending with the eleventh were recorded. The pH levels were measured on the same 2 d of each of 4 wk of sensory evaluation, using a Corning pH Meter 215 equipped with a general purpose combination electrode (Corning Glass Works, Coming, NY). A slurry composed of a 10-g sample diluted with 50 mL of deionized water was prepared from each treatment combination and 2 pH readings taken. Sample remaining after viscosity and pH measurements were completed on day 3 was transferred to pre-weighed plastic containers, covered and stored at -20°C until needed for proximate analysis.
Methods outlined in ASTM Method E 1083-88 § 9.1-9.5 (1991) were used to train and select 13 panelists from among 14 volunteers. Training/selection took place on 4 consecutive days. On day 1, the panel were instructed in use of line scaling for recording sensory perception of heat. A hand-out containing instructions for the session and definitions of the terms involved in sensory heat evaluation was distributed (Appendix A). They also were given a 15-cm line scale (Appendix B) anchored with the terms none (0) and strong (150) indicating sensory heat. The terms threshold, slight and moderate were also indicated on the line scale. The possibility of using any point on the line to indicate a perception was emphasized to the panel. The panel received a set of water samples ranging in intensity from 0.0 to 1.3 ppm capsaicin. A separate sample of 0.4 ppm concentration was given as a reference. Before evaluating the samples, the panel were instructed to concentrate carefully on the perception of heat and to memorize the heat levels of each sample as they experienced them. They then took the reference sample into the mouth, held it for 5 sec, swallowed slowly, waited 30 sec and rated the sample as "slight" on the line scale. After a 60-sec rinse with room temperature water and unsalted crackers, the panel continued with the 4 standard samples, evaluating them with the same procedure. Correct ratings and definitions were given to the panel after completion of the evaluation. The term threshold was explained as the point at which the panelist just notices a burning sensation from the sample. Other terms were explained in reference to the appropriate point on the line scale—slight (0.4 ppm), 5 cm; moderate (0.8 ppm), 10 cm; strong (>1.3 ppm) 15 cm. Again it was emphasized that the individual panelist should use the point which he/she recognized as corresponding to the sensation being experienced. Questions were answered and panelists dismissed. One prospective
Panelist withdrew after day 1 due to stomach discomfort. On days 2-4, panelists were given a control and 2 experimental (unknown) samples. They were reminded of the procedures used the day before and were instructed to rate the intensity of burn as they had in the previous session. Samples were 0.8 ppm which should have been rated as moderate and 0.4 ppm, slight. Sample identities and individual scores were discussed with each panelist. After day 4, 13 panelists were asked to continue with the study.

Over the next 5 days, panelists were introduced to the cheese sauce carriers, instructed in time-intensity techniques and computerized data collection. At each sitting, panel members received four samples—a control and three experimental—to evaluate in individual booths under red lights which adjusted for color variation in the sauces. Each panelist was given a hand-held timer with his/her tray. The tray also held a glass of room temperature spring water, 4-5 unsalted crackers, a spoon for each sample and a napkin.

**Time-Intensity Procedures**

Panelists were instructed to cleanse their palates with room temperature spring water and unsalted soda crackers before sampling; they then placed the entire reference sample (0.4 ppm CAP in spring water) into the mouth and held it for 5 sec before swallowing. Thirty seconds after swallowing, they mentally rated the intensity as being "slight" on the 150-point line scale. They cleansed with unsalted soda cracker and spring water. The entire first experimental sample was spooned into the mouth, held for 5 sec, intensity evaluated and sample swallowed slowly. Intensity was indicated at 15-sec intervals for 3 min. The mouth was rinsed and the procedure repeated with samples 2 and 3.
Computerized Data Collection

A computer sensory program developed at the Agricultural Experiment Station, University of Georgia, Griffin (Resurreccion, 1993) was used to record panelists' responses. The program provides a line scaled to 150 points. For purposes of evaluating pungency, the line was augmented with a scale containing the pungency values with which the panel were trained. The scale was anchored with none at 0 and strong at 150 points. Additionally, the scale contained threshold at 25 points, slight at 50 and moderate at 100. Judgements were recorded by moving a cursor along the scale with right or left arrow keys to the desired point and pressing enter on the keyboard. The 5-day training period allowed panelists to become adjusted to moving the cursor, entering their judgements and timing themselves while concentrating on perceived pungency. Each panelist's data were recorded in ASCII text on a floppy disc; at the end of each training session, the data were checked by the experimenter for evidence that each panelist perceived heat in that day's samples. At the end of the training period, data were evaluated for consistency in heat perception as evidenced by normal appearance of time-intensity curves and the 13 prospective judges were asked to continue with the study.

Panelists evaluated heat in cheese sauces once a day, 3 d a week for 5 wk. They were offered the option of coming into the lab an additional day each week to sample the 5 capsaicin levels in water, but declined. They were comfortable with their abilities to accurately perceive heat in the carriers.
Statistical Analysis

After completion of sensory data collection, each panelist's data were examined for consistency of heat perception over time across the complete design. Perceptions of 2 judges were found to be erratic. One judge exhibited evidence of desensitization to capsaicin; she seldom perceived heat in any sample. The second judge developed an aversion to the cheese sauces in the latter days of the study and failed to consume an adequate amount of sample to accurately judge perception of heat. Values of these 2 panelists were dropped from the analysis. Equal variance within treatments in sensory and physical test data was verified (PROC MEANS, SAS Institute Inc., 1989). PROC UNIVARIATE (SAS Institute Inc., 1989) was used to produce normality plots for the purpose of verifying normal distribution of the data. If data lacked either equal variance or normality, it was transformed to meet necessary assumptions for valid analysis (Cochran and Cox, 1980). Following the statistical models designed for each section of the study, all data were analyzed for differences due to main effects and interactions with PROC MIXED (SAS Institute, Inc., 1996) (Appendix C); Least-squares mean (LSmeans) estimates were generated for each and the PDIFF function used to determine sources of differences.

Viscosity data were analyzed for differences across time due to day, capsaicin concentration (CAP), fat level (FAT), and fat replacer nested in fat level (R(fat)). Viscosity at 5 sec was then analyzed for main effect and interaction differences. Data from pH measurements were analyzed for effects of day, CAP, FAT, R(fat) and interactions using the same statistical procedures. Data from pH and viscosity measurements were analyzed for correlation with perception of heat (PROC CORR, SAS Institute, Inc., 1989).
Analysis of sensory data collected through time-intensity techniques required programming to create the dependent variables. In order to analyze data for the components of a time-intensity curve, a SAS program (Appendix C) was used to calculate the variables of interest—lag, rate of release, maximum intensity, time to maximum intensity, perceived heat at 180 sec and area under the time-intensity curve as well as heat intensity over time (Saxton, 1996). All dependent variables were then analyzed with PROC MIXED (SAS Institute, Inc., 1996) for differences and sources of differences due to CAP, FAT, R(fat) and time as well as the possible interactions between and among them, following the statistical model planned for testing sensory data. PROC GPLOT (SAS Institute, Inc. 1996) was used to graph relationships between design factors and predicted perceived heat.
Chapter IV
Results and Discussion
Physical and Chemical Tests

Proximate Analysis

Moisture, fat and ash composition of cheese sauces was determined by proximate analysis (Table 5). Ash percentages were within expected ranges. As fat was reduced in sauce formulas, water was increased; low- and reduced-fat sauces were expected to have higher moisture levels than full-fat sauces. Moisture levels in sauces ranged from 71% in full-fat sauces to 84% in low-fat. Based on amounts of water added during preparation, these levels were within expectations.

Table 5—Mean proximate composition of cheese sauces formulated at 3 fat levels

<table>
<thead>
<tr>
<th>Component (%)</th>
<th>Fat Level</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Full n</td>
<td>Reduced n</td>
<td>Reduced n</td>
<td>Low n</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moisture²</td>
<td>70.88 24</td>
<td>79.70 95</td>
<td>83.36 95</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat³</td>
<td>8.4 24</td>
<td>3.73 95</td>
<td>1.00 95</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ash⁴</td>
<td>2.6 23</td>
<td>1.87 91</td>
<td>1.55 89</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

²Means across 5 capsaicin levels; reduced- and low-fat across 4 mimetics.

The purpose of characterizing nutrient composition was to determine actual percentage of fat at each formulated fat level. Sauces were designed to have levels of fat defined as reduced- and low-fat. To be labeled reduced-fat, a product must have 25% less fat than its full-fat counterpart. A low-fat product can have only 3 g fat per serving.
Fat levels in the sauces decreased across decreasing fat level from 8.4% to 1.0%. The reduced- and low-fat sauces met federal labeling requirements. Full-fat sauces had fat levels lower than commercial sauces which may have resulted from the use of one low-fat cheese powder in the sauce formulas.

Protein levels were projected by calculation from manufacturers' ingredient specification sheets. Based upon the percentage of each protein-containing ingredient used at each fat level, full-fat sauces were projected to contain ~3.4 % protein; reduced-fat should be ~2.4 % protein and low-fat, ~2 %.

**pH**

Formulating reduced- or low-fat foods with fat mimetics necessitates attention to chemical interactions which occur in food products. One chemical characteristic which should be monitored is pH which can affect sensory qualities of foods. The increase in water content required when replacing fat with mimetics can result in pH changes which may affect the textural attributes and potential shelf life of the product (Bennett, 1995). In addition, pH should be monitored to ensure that ingredient limitations are not exceeded. Mimetics may have pH limitations which if exceeded affect gelation; proteins may denature and precipitate if acidity in the product is too high (Hegenbart, 1994; Bennett, 1995). Replacing fat in cheese sauces affects pH (Appendix D); decreasing fat levels were related to increases in pH levels. The LSmean estimate of pH in full-fat (FF) sauces was 5.91, lower than in reduced- (pH 6.08) or low- (pH 6.09) fat sauces (p<0.0001).

Similarly, addition of capsaicin increased pH; sauces with no capsaicin (CAP) had an LSmean pH of 5.96, lower than those of sauces containing CAP (p<0.02). Sauces with CAP ranging from 0.4 to 1.6 ppm had LSmean pH levels which ranged from 6.03 to 6.05.
and did not differ (p>0.05). However differences in pH did occur as a result of an interaction between fat level and CAP concentration (Table 6). Sauces with lower fat levels exhibited increases in pH at all levels of CAP except 1.2 ppm. Full-fat sauces with 0.0 ppm CAP differed from all other combinations (p<0.01). Addition of capsaicin to the full-fat combination increased pH (p<0.05). While pH levels of FF sauces with CAP were higher than FF 0.0 CAP, LSmean pH levels of FF 0.4, 0.8 and 1.6 ppm sauces were lower than all reduced- (RF) and low-fat (LF) sauces. However, the LSmean pH of FF 1.2 CAP differed only from LF 0.0 and RF 1.6 CAP sauces (p<0.05). Based on these results, it appears that pH levels in cheese sauces are related to fat level more than to added capsaicin. Full-fat sauces at all levels of CAP except 1.2 ppm were clustered at the lower end of the pH range of all treatments.

LSmean estimates of pH also differed due to the effect of fat mimetic nested in fat level (Table 7). Again full-fat combinations had pH levels lower than all combinations of fat level and mimetic (p<0.0001). At the reduced-fat level, no differences occurred among mimetics (p<0.05). At the low-fat level, several differences were found. Dairy Trim (DT) and Paselli Excel (PE) effected lower pH levels than did N-Lite L (NL) and Simplesse (S) (p<0.05). Within a mimetic across reduced- and low-fat sauces, pH did not differ (p>0.05).

Active acidity (pH) of cheese sauces was measured on the first and third day of each week of the sensory study. No differences occurred between days 1 and 3; however the day x R(fat) interaction did result in differences (Table 7). Day 1 full-fat and day 3 full-fat LSmeans were lower than those of all other day x R(fat) combinations (p<0.0001). The FF sauces did not change across days (p=1.0). No differences between days 1 and 3 were found among the reduced-fat sauces. Between day 1 and day 3, a sharp decline occurred in the LSmean estimate of pH in the low-fat S combination.
Table 6—Least-squares means \(^a\) and std err of pH levels in cheese sauces as effected by capsaicin and fat level

<table>
<thead>
<tr>
<th>Capsaicin (ppm)</th>
<th>Fat level</th>
<th>Full(^b)</th>
<th>Reduced (^c)</th>
<th>Low (^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5.72a ± 0.42</td>
<td>6.06cd ± 0.21</td>
<td>6.10 d ± 0.24</td>
<td></td>
</tr>
<tr>
<td>0.4</td>
<td>5.91b ± 0.42</td>
<td>6.08cd ± 0.21</td>
<td>6.08 cd ± 0.22</td>
<td></td>
</tr>
<tr>
<td>0.8</td>
<td>5.94b ± 0.42</td>
<td>6.08 cd ± 0.23</td>
<td>6.08 cd ± 0.21</td>
<td></td>
</tr>
<tr>
<td>1.2</td>
<td>5.99bc ± 0.42</td>
<td>6.08 cd ± 0.21</td>
<td>6.09 cd ± 0.22</td>
<td></td>
</tr>
<tr>
<td>1.6</td>
<td>5.95b ± 0.42</td>
<td>6.10 d ± 0.21</td>
<td>6.08 cd ± 0.22</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Values followed by unlike letters differ at \(p< 0.005\).

\(^b\) Values taken over 4 weeks, 2 times per week.

\(^c\) Values taken over 4 weeks, 2 times per week across 4 mimetics.
Table 7—Effects of fat replacer(fat level) and fat replacer(fat level) by day interaction on pH measurements in cheese sauces formulated with 9 combinations of fat mimetic and fat level

<table>
<thead>
<tr>
<th>Fat Level Replacer</th>
<th>Replacer (fat level)</th>
<th>Std. err</th>
<th>Days&lt;sup&gt;b&lt;/sup&gt;</th>
<th>1</th>
<th>3</th>
<th>Std. err</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full Fat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reduced</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dairy Trim</td>
<td>6.11cd</td>
<td>0.020</td>
<td>6.10ce</td>
<td>6.13ef</td>
<td>0.027</td>
<td></td>
</tr>
<tr>
<td>N-Lite L</td>
<td>6.07bcd</td>
<td>0.019</td>
<td>6.09ce</td>
<td>6.05bc</td>
<td>0.026</td>
<td></td>
</tr>
<tr>
<td>Paselli Excel</td>
<td>6.08bcd</td>
<td>0.019</td>
<td>6.09ce</td>
<td>6.07bce</td>
<td>0.026</td>
<td></td>
</tr>
<tr>
<td>Simplesse</td>
<td>6.06bce</td>
<td>0.020</td>
<td>6.07bce</td>
<td>6.05bcd</td>
<td>0.027</td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dairy Trim</td>
<td>6.06bc</td>
<td>0.021</td>
<td>6.09ce</td>
<td>6.11cef</td>
<td>0.028&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>N-Lite L</td>
<td>6.12d</td>
<td>0.020</td>
<td>6.01b</td>
<td>6.14ef</td>
<td>0.027</td>
<td></td>
</tr>
<tr>
<td>Paselli Excel</td>
<td>6.05b</td>
<td>0.020</td>
<td>6.04bc</td>
<td>6.05bcd</td>
<td>0.027</td>
<td></td>
</tr>
<tr>
<td>Simplesse</td>
<td>6.12de</td>
<td>0.021</td>
<td>6.19f</td>
<td>6.05bcd</td>
<td>0.028&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>LSmeans ± std errs of averages of 2 values across 5 capsaicin levels and 4-5 measurements; Values within the column with unlike letters differ at p<0.05.

<sup>b</sup>LSmeans ± std errs of averages of 2 values across 5 capsaicin levels and 4-5 measurements; values with unlike letters differ at p<0.05.

<sup>c</sup>Day 3 Std err = 0.029.
(p<.01); low-fat NL increased from day to day (p<0.05). Thus, the only changes in pH LSmeans from day 1 to day 3 were among low-fat sauces, leading to the conclusion that substitution of mimetics for fat may influence levels of molecular dissociation in cheese sauces. Heat data were analyzed for correlation with pH data to verify any effects of pH changes on perception of heat. Heat perception was not correlated with pH (r = 0.06).

Viscosity

For purposes of this study, the possible effect of viscosity on perception of heat intensity was of interest. Viscosity may be defined as resistance to movement due to intermolecular forces within a food system. The magnitude of that resistance is related to the concentration of macromolecules interacting with other solutes or the solvent (Zapsalis and Beck, 1985). Glicksman (1991) relates viscosity to the sensory properties of thickness, body and fullness which are associated with high quality fat-containing foods. Thus viscosity is a textural characteristic which should be monitored when fat levels are altered. Apparent viscosity was measured in sauces which were held at refrigerated temperature over a 4-d period. Sauces were allowed to come to room temperature and viscosity measured on days 1 and 3 of each 3-d period of sensory evaluation. Sauce viscosity values were recorded across a 60-sec period and measurements taken at 5, 15 and 55 sec were analyzed for changes over time. Preliminary data analysis indicated that differences occurred across time, fat level, and day of measurement (Appendix D); viscosity decreased from day 1 to day 3 and across the 5-, 15- and 55-sec measures (Table 8). LSmean viscosity was greater at 5 sec than at 15 or 55 sec; viscosity at 55 sec differed from 15 sec (p<0.05). The continued motion of the spindle within the sauces decreased resistance to movement. Apparent viscosity is time dependent; food systems often are subject to shear thinning, exhibiting decreased
<table>
<thead>
<tr>
<th>Day</th>
<th>Time (sec)</th>
<th>Fat level (^b)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Full</td>
<td>Reduced</td>
<td>Low</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>cP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td>23272c</td>
<td>25473f</td>
<td>30838c</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>20740a</td>
<td>24192e</td>
<td>31141c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>15</td>
<td>22424b</td>
<td>24851e</td>
<td>29314d</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>20492a</td>
<td>23519d</td>
<td>29565d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>55</td>
<td>22404b</td>
<td>24831e</td>
<td>28594e</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>20572a</td>
<td>23619d</td>
<td>28904e</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Std Err</td>
<td></td>
<td>± 989</td>
<td>± 644</td>
<td>± 648</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>± 989</td>
<td>± 647</td>
<td>± 648</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Values with unlike letters differ at p<0.05.

\(^b\) Values across 5 capsaicin concentrations; reduced- and low-fat values are across 4 mimetics.
viscosity with continued shearing (Bourne, 1982). The shear thinning of the sauces interacted with fat level and day of measurement to effect changes in viscosity.

Because sauces exhibited Non-Newtonian behavior, averaging of values across time was not appropriate. Additionally, panelists recorded their first perception of heat at 5 sec and immediately swallowed their samples; thus, only viscosity at 5 sec had the potential of impacting perceived heat. Changes in viscosity at 5-sec from day 1 to day 3 of sensory data collection also had the potential of affecting heat perception; therefore, determination of any relationship between viscosity and heat perception was important. The viscosity measurements recorded at 5 sec on days 1 and 3 were analyzed for differences due to main effects. Capsaicin concentration did not affect viscosity at 5 sec. Changes in viscosity occurred due to fat level and day of measurement (Appendix D). Full-fat sauces were less viscous than reduced- or low-fat sauces and reduced-fat were less viscous than low-fat sauces (p<0.05). From day 1 to day 3, viscosity decreased in full- and reduced-fat sauces (p<0.05), but remained stable in low-fat sauces (p>0.05). Interactions among these effects and fat replacer(fat level) produced some variation in changes (Table 9). While full-fat sauces were less viscous than low-fat sauces (p<0.05), reduced-fat PE sauces had viscosity equal to viscosity in full-fat sauces on day 1 (p>0.05). On day 3, full-fat sauces were less viscous than all other sauces. Reduced-fat sauces made with Dairy Trim and Paselli Excel maintained viscosity over the 3-d period; N-Lite L and Simplesse at reduced-fat levels exhibited decreased viscosity from day 1 to day 3. At low-fat levels, Paselli Excel increased in viscosity (p<0.05) over the 3-d period while other low-fat sauces did not change (P>0.05). All low-fat sauces were more viscous than either reduced- or full-fat sauces and with the exception of Paselli Excel, held viscosity at a stable level across the 3-d holding time. Viscosity in low-fat sauces is apparently more stable than in reduced- or full-fat sauces. This property of low-fat
Table 9—Effects of fat replacer (fat level) and day of measurement on viscosity at 5 sec in cheese sauces formulated with 9 combinations of fat level and mimetic®

<table>
<thead>
<tr>
<th>Fat level Replacer</th>
<th>Days</th>
<th>Std err</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Full-fat</td>
<td>23272b</td>
<td>20740a</td>
</tr>
<tr>
<td>Reduced-fat</td>
<td>25146df</td>
<td>25108df</td>
</tr>
<tr>
<td>Dairy Trim</td>
<td>25066f</td>
<td>25088de</td>
</tr>
<tr>
<td>N-Lite L</td>
<td>23694bc</td>
<td>23549bc</td>
</tr>
<tr>
<td>Paselli Excel</td>
<td>26986ce</td>
<td>23028b</td>
</tr>
<tr>
<td>Simplesse</td>
<td>30696g</td>
<td>31047g</td>
</tr>
<tr>
<td>Low-fat</td>
<td>31032gh</td>
<td>30202g</td>
</tr>
<tr>
<td>Dairy Trim</td>
<td>32074g</td>
<td>33561h</td>
</tr>
<tr>
<td>N-Lite L</td>
<td>29538g</td>
<td>29744g</td>
</tr>
</tbody>
</table>

aLSmeans followed by unlike letters differ at p <.05. Values across 5 capsaicin levels prepared and measured 4-5 times.

bStd err on day 3 = 1080.53.
sauces is probably related to the water-binding properties of fat mimetics. Modified starches, regardless of source of the starch, are designed to form an association between water and the carbohydrate particle (Yackel and Cox, 1992). Microparticulates absorb water to swell and mimic thickness of fats (Glicksman, 1991). Increasing levels of the mimetic probably strengthens the association of mimetic with water, stabilizing the viscosity of the system.

In a previous study of viscosity in capsaicin-containing cheese sauces with varied fat levels, it was found that medium- and high-fat sauces were similar in viscosity and low-fat sauces were less viscous and did not change in viscosity over days of holding (Baron, 1995). Sauces were not formulated with different fat mimetics. The fat mimetics selected for this study were either low-DE modified starches or microparticulated protein. All are intended to replace fat, mimicking its sensory properties, providing thickness and creaminess associated with high-fat products. Those which originate from starches have the ability to associate with water, binding it within the product to produce the mouthfeel of fat. Microparticulates will swell in the presence of water, providing similar properties (Glicksman, 1991; Yackel and Cox, 1992). Low-fat sauces contained higher levels of fat mimetic than RF sauces which may have increased their initial viscosity as well as their stability.

Differences among fat replacers nested in fat level were not as distinct. They reflect the trend of increasing viscosity with decreasing fat level; all combinations at the low-fat level were more viscous than other fat levels. Differences occurred at all levels of mimetic use and differed from mimetic to mimetic, but without forming a discernible pattern. Additional research into effects of mimetics on viscosity of spoonable sauces should clarify differences among the mimetics. Also, effects of longer holding times should be investigated. Consumers may refrigerate spoonable sauces for longer periods
of time than sauces were held for this study.

Effects of viscosity on perceived heat were also of interest in this study. The role of viscosity in heat perception has been investigated. Sizer and Harris (1985) suggested that viscosity related to concentration of solute in sugar solutions influenced perception of capsaicin heat. Nasrawi and Pangborn (1989) subsequently investigated effects on perceived heat of solutions thickened with Xanthan gum and concluded that viscosity in the gum solutions masked heat. Low DE fat mimetics have some characteristics in common with hydrocolloids such as Xanthan gum (Glicksman, 1991). In this study, viscosity and sensory perception of time-intensity heat parameters were not correlated ($r = .003$) Although differences in viscosity occurred, the texture of the sauces apparently did not affect perception of pungency.

Sensory Evaluation

Perception of heat intensity resulting from oral ingestion of capsicums is temporal—over the passage of time, perceived heat may intensify and/or decrease in relation to the pungency component of the pepper and the chemical composition of the food mixture in which it is found. To obtain a more comprehensive picture of the role of capsaicin concentration, fat level, fat mimetic nested in fat level and interactions between/among the three, time/intensity techniques were used to investigate the perception of pungency in cheese sauces. By analyzing measures of perceived intensity recorded over a period of 180 sec, a number of parameters including heat intensity over time ($\Delta$ Heat), lag, maximum intensity (IMAX), time to maximum intensity (TMAX), rate of release (RATE), perceived heat at 180 sec and total intensity as area under the time/intensity curve (AREA) were examined. No differences attributable to fat mimetic
were found. Means for two dependent variables (maximum heat and total intensity) are reported in Appendix E.

**Heat Intensity over Time**

Analysis of perceptions of heat over the 180 sec in which each sauce was evaluated indicated that heat intensity was affected by capsaicin concentration, fat level and time as well as by the interactions of CAP with time and CAP with fat level (Appendix D). As CAP increased from 0.0 ppm to 1.6 ppm, heat also increased incrementally from a low LSmean intensity of 8.2 at 0.0 ppm CAP to 30.5 at 0.8 ppm CAP to the highest level of 58.2 at 1.6 ppm (p<0.0001). Changes also occurred in perceived heat as a function of time (p<0.05). LSmean intensity at 5 sec was 28.2; perceived intensity LSmeans peaked at 30 sec and began decaying at 45 sec to a low pungency LSmean rating of 17.1 at 180 sec.

Differences resulting from the time x CAP interaction reflect those observed in other parameters and indicate the influence of CAP and time on perceived intensity (p<0.05). Time intensity curves depict differences across time among levels of CAP (Fig. 3). At 5 sec, sauces containing 0.8, 1.2 and 1.6 ppm CAP were perceived as more pungent than those with 0.0 and 0.4 ppm CAP (p<0.05). Sauces with 1.2 and 1.6 ppm did not differ (p>0.05), but both had LSmean intensity ratings higher than sauces containing 0.8 ppm CAP (p<0.05). Panelists perceived intensity at highest levels earlier in sauces containing 0.0 or 0.4 ppm CAP than in other sauces and indicated that the intensity decayed more quickly (p<0.05). At like times, levels of intensity perceived by panelists in cheese sauces with 0.8 ppm CAP differed from intensities of other sauces. Differences did not occur between 1.2 and 1.6 ppm CAP-containing sauces until panelists passed the 45-sec rating.
Fig. 3—Perceived heat intensity as a function of time x capsaicin concentration. Cheese sauces formulated with 5 levels of capsaicin across 3 fat levels and 4 fat mimetics were evaluated by 11 panelists at 15 sec intervals over a period of 180 sec on a rating scale anchored by the terms none=0 and strong=150.
Fat levels also affected perception of heat over time. Differences attributable to fat were related to capsaicin concentration as shown in Fig. 4. While no differences in LSmeans occurred in perceived heat among fat levels at 0.0 and 0.4 ppm CAP, RF and LF sauces with 0.8 ppm were perceived as more pungent than FF 0.8 CAP sauce (p<0.001). The FF sauces at 0.8 ppm CAP were perceived at the same intensity level as RF and FF sauces with 0.4 ppm CAP (p>0.05). Heat in LF sauces at 1.2 ppm CAP was perceived at a higher level than in FF sauces (p<0.0001). Perceived heat in FF sauces at 1.6 ppm CAP equalled that in both RF and LF sauces (p>0.05). The influence of fat level on perceived heat is clearly depicted graphically in Fig. 5. With quadratic relationships included in the model (Appendix F), RF and LF sauces increase linearly in perceived heat as CAP increases. However, FF sauces exhibit a curvilinear depression between 0.4 and 1.2 ppm CAP, supporting the theory that the higher-fat level depresses the perception of heat intensity. This differs from the findings of Baron (1995). He reported no effect of fat level on heat perception over time in cheese carriers.

Lag

Lag is defined as the period of time that elapses between introduction of the stimuli into the oral cavity and recognition of sensory response. Among the cheese sauces evaluated in this study, neither capsaicin concentration, fat level nor fat replacer nested in fat level produced differences in lag (Appendix D). The time between introduction of the sauce into the mouth and recognition of heat was equal among all treatment combinations (p>0.05). Recognition of heat apparently and logically occurred at the 5-sec point at which panelists were directed to record their first response just before swallowing the sample.
Fig. 4—Perceived heat intensity as a function of fat level and capsaicin concentration across 13 time periods. Sauces were formulated with 3 levels of fat and 5 levels of capsaicin. Eleven panelists evaluated sauces on a rating scale anchored with the terms none=0 and strong=150. Bars with unlike letters differ at p<0.05.
Fig. 5—Predicted perceived heat as a function of fat level x capsaicin. The statistical model included linear and quadratic functions. Sauces formulated with 3 fat levels and 5 capsaicin concentrations were evaluated by 11 panelists on a rating scale anchored by the terms none=0 and strong=150.
Maximum Heat

Maximum heat (IMAX) is the highest intensity perceived by panelists over the 180 sec during which they recorded their impressions of pungency in cheese sauces. As expected, panelists rated IMAX higher as levels of capsaicin increased in the sauces (Appendix D). Differences were found at all levels of capsaicin (p<0.0001).

Because capsaicin is a fat-soluble compound, fat may influence the sensory perception of maximum heat. Least-squares analysis of perceived intensity ratings support that supposition (Appendix D). Panelists perceived higher maximum heat in low-fat cheese sauces than in full-fat (p<0.001). They also rated reduced-fat sauces as having higher IMAX than full-fat (p<0.05) and as imparting a less intense burn than low-fat (p<0.05). LSmean ratings of perceived heat increased as fat level decreased, but the pattern was related to CAP concentration (Table 10). With no capsaicin in the sauces, panelists rated maximum heat at a level below "slight" (25) on the scale. No difference in IMAX across fat levels was indicated by LSmeans at 0.0 or 0.4 ppm CAP (p>0.05). At 0.8 ppm CAP, LSmean estimates of perceived maximum heat differed among the three fat levels; FF sauces had an LSmean maximum heat lower than either RF or LF sauces (p<0.05); at a CAP level of 1.2 ppm, FF sauces were perceived as having less maximum heat than LF (p<0.0001), but did not differ from RF (p>0.05). With a concentration of 1.6 ppm CAP, FF sauce LSmeans differed from neither RF or LF (p>0.05). Between RF and LF sauces at equal CAP concentrations, differences in IMAX occurred only at 1.6 ppm CAP (p<0.0001). The graphical representation of the LSmeans clarifies the interactions of CAP and fat level (Fig. 6). Sensory perception of maximum heat intensity increased gradually in full-fat sauces as CAP concentration increased, but increased more sharply when fat level was decreased. As CAP concentration increased above the 0.8 ppm level, LSmean estimates indicated that panelists perceived IMAX in full-fat sauces at an
Table 10—Sensory perception of maximum heat intensity in cheese sauces formulated with 3 fat levels and 5 capsaicin levels

<table>
<thead>
<tr>
<th>Capsaicin (ppm)</th>
<th>Full</th>
<th>Reduced</th>
<th>Low</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>23.3ab ± 7.1</td>
<td>20.1a ± 4.5</td>
<td>19.5a ± 4.5</td>
</tr>
<tr>
<td>0.4</td>
<td>37.6bc ± 7.1</td>
<td>38.2c ± 4.5</td>
<td>40.1c ± 4.5</td>
</tr>
<tr>
<td>0.8</td>
<td>41.3c ± 7.1</td>
<td>66.7d ± 4.5</td>
<td>71.1d ± 4.5</td>
</tr>
<tr>
<td>1.2</td>
<td>74.7de ± 7.1</td>
<td>88.0ef ± 4.5</td>
<td>92.7fg ± 4.5</td>
</tr>
<tr>
<td>1.6</td>
<td>101.7ghi ± 7.1</td>
<td>102.3hi ± 4.5</td>
<td>112.1i ± 4.5</td>
</tr>
</tbody>
</table>

LSmean ± std err of the maximum values of perceived heat; none=0, strong=150; values followed by unlike letters differ at p<.05.

*Values for 11 panelists.

Values for 11 panelists across 4 fat mimetics.
Fig. 6—Effects of capsaicin concentration and fat level on sensory perception of maximum heat intensity. Eleven panelists evaluated heat intensity in 45 cheese sauces at 15 sec intervals over 180 sec on a scale anchored by none=0 and strong=150. Reduced-fat and low-fat LSmeans across 4 mimetics.
intensity equal to IMAX in RF sauces; at 1.6 ppm, IMAX in FF sauces did not differ from either RF or LF sauces. In RF and LF sauces, moderate CAP concentrations (0.8 ppm) were perceived at a maximum level of heat equal to that assigned by panelists to higher concentrations (1.2 ppm) in FF sauces.

Including quadratics in the statistical model to predict the relationship between fat and CAP clarifies the influence of fat in the interaction (Fig. 7) (Appendix F). In RF and LF sauces, a slight trend toward a positive curvilinear relationship between fat level and capsaicin concentration is predicted as CAP increases to 0.8 and 1.2 ppm CAP. In FF sauces, the opposite trend is seen; the effect of CAP x fat level at CAP levels between 0.4 and 1.2 ppm is shown by the negative response of the quadratic component in the formula. At the full-fat level, lower levels of maximum heat are predicted at 0.8 ppm CAP than at reduced- and low-fat levels; at 1.2 ppm CAP, maximum heat is lower in FF sauces than in low-fat. The interaction of fat with capsaicin is predicted to decrease perception of heat at moderate concentrations of capsaicin.

**Time to Maximum Heat**

Time to maximum heat (TMAX) is the number of seconds which pass before panelists note the highest level of heat intensity in a food system. Both fat level and capsaicin concentration have the potential of affecting TMAX. Least-squares analysis of sensory heat perception data finds no indication that CAP level or fat mimetic affected TMAX (p>0.05) (Appendix D), but supports the possibility that TMAX is influenced by fat level (Table 11). Time to sensory perception of maximum heat intensity of full-fat sauces did not differ from that required for reduced and low-fat sauces (p>0.05). The occurrence of a wider standard error of FF LSmeans due to the lower number of full-fat samples influenced the differences. However, the LSmean estimate of TMAX in RF sauce differed
Fig. 7—Predicted perceived maximum heat as a function of fat level x capsaicin. Model included linear and quadratic functions. Sauces formulated with 3 fat levels and 5 capsaicin concentrations were evaluated by 11 panelists on a rating scale anchored by the terms none=0 and strong=150. Reduced-fat and low-fat means across 4 fat mimetics.
from that of LF sauce (p<0.02). Panelists recorded maximum heat a few seconds earlier for RF sauces than for LF sauces.

Table 11—Time to perceived maximum heat in cheese sauces

<table>
<thead>
<tr>
<th>Fat level</th>
<th>Time to maximum heat (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full</td>
<td>38.7ab ± 7.6</td>
</tr>
<tr>
<td>Reduced</td>
<td>29.6a ± 6.2</td>
</tr>
<tr>
<td>Low</td>
<td>37.6b ± 6.2</td>
</tr>
</tbody>
</table>

*LSmean ± std err across 5 capsaicin levels; reduced- and low-fat LSmeans across 4 mimetics; evaluated by 11 panelists on a scale anchored 0=none, strong=150; values followed by unlike letters differ at p<0.05.

Rate of Release

Rate of release (RATE) is the ratio of IMAX to TMAX; it indicates the rate at which perception increases from onset to IMAX. Theoretically, the presence of fat in a cheese sauce could affect the interaction of lipophilic capsaicin with nociceptors in the oral cavity. However, tests of fixed effects indicated differences in RATE only among CAP levels (p<0.0001) (Appendix D). Neither fat level nor replacer nested in fat affected RATE; nor did differences occur as a function of interactions (p>0.05). RATE varied from 3.2 with no CAP in the sauce to 5.6 with 1.6 ppm CAP (Table 12). As concentration increased, more capsaicin was available to contact pain receptors, increasing the rate of heat perception.
Table 12—Rate of release of perceived heat in cheese sauces containing 5 concentrations of capsaicin®

<table>
<thead>
<tr>
<th>Capsaicin ppm</th>
<th>Rate of release</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>3.2b ± 0.8</td>
</tr>
<tr>
<td>0.4</td>
<td>3.1a ± 0.8</td>
</tr>
<tr>
<td>0.8</td>
<td>3.6c ± 0.8</td>
</tr>
<tr>
<td>1.2</td>
<td>4.9d ± 0.8</td>
</tr>
<tr>
<td>1.6</td>
<td>5.6d ± 0.8</td>
</tr>
</tbody>
</table>

*LSmeans ± std err across 3 fat levels and 4 fat mimetics; 11 panelists evaluated sauces on a scale anchored by 0=none, 150=strong; values with unlike letters differ at p<.05.

Perceived Heat at 180 sec

Heat perception at 180 sec was the last response recorded by panelists. At 180 sec, LSmean values of perceived heat reflect the perceptions of heat in other parameters. Differences in the LSmeans occurred among CAP concentrations. At a CAP level of 0.0, perceived heat at 180 sec was near 0; intensity increased 3 fold at 0.4 ppm CAP and continued increasing sharply across higher levels of CAP (p<0.001). Levels of fat in the cheese sauces also affected perceived heat at 180 sec (Table 13). While perceived heat in RF and FF sauces did not differ at 3 min, both were perceived as having less heat intensity than low-fat sauces (p<0.05). Perceived heat remains greater in LF sauces at 180 sec.
Total Intensity

The area under a time-intensity curve (AREA) represents total perceived intensity. The area under a time-intensity curve (AREA) represents total perceived intensity. In this study, AREA is the total pungency of capsaicin perceived over a period of 180 sec of measurement. Differences were effected in total intensity by fat level (p<0.0001), capsaicin concentration (p<0.0001) and an interaction between fat and CAP (p<0.05) (Appendix D). Total perceived heat differed as expected across CAP concentrations. Increasing levels of CAP increased total intensity (p<0.0001) as higher concentrations of CAP contacted more receptors within the oral cavity. The fat-solubility of capsaicin may affect total perceived heat in food systems; inclusion of capsaicin in a fat-containing food may result in a failure of the capsaicin molecule to adhere completely to receptors. The fat may serve as a buffer between receptors and CAP. Analysis of the data support this possibility. Total intensity increased as fat decreased. The total heat perceived by panelists in full-fat sauces was lower than in either RF (p<0.01) or LF sauces (p<0.0001). Total perception of heat in RF sauces also was lower than in LF sauces (p<0.01). When fat level and CAP concentration were examined in concert, the differences were more subtle (Table 14). In FF sauces, AREA did not differ across the three lower capsaicin concentrations—0.0, 0.4 and 0.8 ppm (p>0.05). A sharp increase at 1.2 ppm CAP and

Table 13—Sensory perception of heat at 180 sec as affected by fat levels in cheese sauces

<table>
<thead>
<tr>
<th>Fat level</th>
<th>Perceived heat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full</td>
<td>14.0 ± 5.9</td>
</tr>
<tr>
<td>Reduced</td>
<td>17.0 ± 5.7</td>
</tr>
<tr>
<td>Low</td>
<td>20.4 ± 5.7</td>
</tr>
</tbody>
</table>

Values are LSmeans ± std err of perceived heat across 5 CAP levels rated by 11 panelists on a scale anchored by 0=none and 150=strong; values with unlike letters differ at p<.02.
Table 14—Sensory perception of total heat intensity in cheese sauces prepared with 3 fat levels and 5 capsaicin levels.

<table>
<thead>
<tr>
<th>Capsaicin —ppm—</th>
<th>Full</th>
<th>Reduced</th>
<th>Low</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1617.1ab ± 1063.5</td>
<td>1400.6a ± 859.9</td>
<td>1206.6a ± 859.1</td>
</tr>
<tr>
<td>0.4</td>
<td>2759.1abc ± 1063.1</td>
<td>3040.3bc ± 859.2</td>
<td>3574.6c ± 859.0</td>
</tr>
<tr>
<td>0.8</td>
<td>3564.7bc ± 1064.3</td>
<td>6116.3d ± 859.0</td>
<td>6855.0d ± 858.4</td>
</tr>
<tr>
<td>1.2</td>
<td>6757.1d ± 1063.2</td>
<td>8394.8e ± 858.5</td>
<td>9222.7e ± 858.2</td>
</tr>
<tr>
<td>1.6</td>
<td>9519.5ef ± 1063.1</td>
<td>10349.2f ± 859.0</td>
<td>11845.2g ± 859.5</td>
</tr>
</tbody>
</table>

*aTotal heat intensity as area under the curve.

*b n=11; values are LSmeans ± std err over 45 treatment combinations rated on a scale anchored by the terms none=0 and strong=150; values with unlike letters differ at p<0.05.
again at 1.6 ppm produced changes in FF sauces at those CAP levels (p<0.05). This effect of increasing AREA with increasing CAP was greater in RF and LF sauces which increased with each increase in capsaicin concentration (p<0.05). Among fat levels across CAP concentrations, total intensity did not differ at 0.0 or 0.4 ppm CAP, but a difference occurred between FF and the two sauces with decreased fat content at 0.8 and 1.2 ppm CAP (p<0.0001); at 1.6 ppm CAP, total intensity of FF sauces did not differ from RF (p>0.05), but was less than that of LF sauces (p<0.05). Reduced fat sauces differed from LF sauces only at 1.6 ppm CAP (p<0.01). A distinctive pattern of increased perceived heat developed as fat level decreased and CAP concentration increased. The influence of fat in the effect of the interaction between fat level and CAP on total intensity of perceived heat in cheese sauces can be predicted graphically using regression analysis (Fig. 8) (Appendix F). While the difference may appear to be attributable to the increasing capsaicin concentrations, the presence of the quadratic in the statistical model reveals a similarity of total intensity to the graphical representation of the predicted fat x CAP interaction in heat perceived over time and maximum heat. Between CAP levels 0.4 and 1.2, a slight curvilinear depression in total intensity occurs in the FF sauces; this negative curve does not appear in LF and RF sauces which appear to increase linearly in total intensity of perceived heat. This indicates that full-fat content decreases perception of heat intensity at lower levels of capsaicin. The combination of fat and capsaicin at 0.4 and 0.8 ppm decreases the ability of the capsaicin molecule to contact pain receptors, reducing the total intensity of heat perceived by panelists. High concentrations of capsaicin—1.2 and 1.6 ppm—at the full-fat level are able to stimulate receptors responsible for pungency perception.
Fig. 8—Predicted area under the time intensity curve as a function of fat level x capsaicin. Statistical model included linear and quadratic functions. Sauces formulated with 3 fat levels and 5 capsaicin concentrations were evaluated by 11 panelists on a rating scale anchored by the terms none=0 and strong=150. Reduced-fat and low-fat LSmeans across 4 fat mimetics.
Psychological Biases in Evaluation of Heat

Trained panelists may be subject to errors of central tendency, the reluctance to assign extreme values on a rating scale to the product being evaluated. The use of a reference sample has been shown to increase susceptibility to errors of central tendency. When the panel receive a reference of known value, they are more likely to avoid the extremes of the rating scale (Stone and Sidel, 1993). The trained panel in this study exhibited a reluctance to use values at either end of the scale. The use of a food system as the carrier of capsaicin was expected to decrease the use of extreme high values as the perception of heat was expected to be lessened by the carriers. Baron and Penfield (1996) demonstrated that perceived heat is decreased in food systems as compared to water carriers. Conversely, the food system has the potential of decreasing initial perception of heat. In this study, the LSmean value at 5 sec of perceived heat across time, the first judgement made by panelists, was above the slight rating (25) assigned to the reference sample. Among the 5 capsaicin levels, initial responses for perceived heat across time ranged from 16.76 at 0.0 ppm CAP to 43.16 for 1.6 ppm. The panel recognized that sauces with 0.0 ppm capsaicin carried less heat than the reference (0.4 ppm), but avoided the 0 on the scale.
Chapter V
Conclusions and Implications

Few sensory researchers have investigated the oral neuroresponse to capsaicin in food systems. Oral irritation related to capsaicin was characterized by Lawless (1984) in emulsions. Nasrawi and Pangborn (1988) and Cliff and Heyman (1993) presented water solutions of capsaicin to sensory panelists. Baron (1995) examined perceived heat intensity of capsaicin in water and in starch pastes and cheese sauces with varied fat levels. Authors report that increasing concentrations of capsaicin increased intensity parameters. Baron (1995) determined that fat level in carriers affected maximum heat perception. Both starch pastes and cheese sauces exhibited higher maximum heat with decreasing fat levels. However, only starch paste fat levels affected heat intensity over time; fat levels in cheese sauce had no impact on heat over time.

In the present study, the roles of capsaicin concentration, fat level and fat mimetic in perception of sensory heat in cheese sauces were examined. Capsaicin at 0.0, 0.4, 0.8, 1.2 and 1.6 ppm was added to cheese sauces with full-, reduced- or low-fat levels. Reduced- and low-fat sauces were formulated with 4 fat mimetics—Dairy Trim, N-Lite L, Paselli Excel and Simplesse. Reflecting results of previous studies (Lawless, 1984; Baron, 1995), heat intensity over time, maximum heat and total intensity increased as capsaicin concentration increased. Sauces with the high-fat level exhibited lower heat intensities over time at concentrations of capsaicin between 0.4 and 1.2 ppm. Maximum heat was similarly affected. Decreasing fat also increased maximum heat. Perception of maximum heat in reduced- and low-fat sauces at 0.8 ppm capsaicin and in low-fat sauces at 1.2 ppm capsaicin was greater than in full-fat sauces. The curvilinear depression of maximum heat intensity at full-fat levels was similar to the decrease in perceived heat
over time; heat was lower than expected between 0.4 and 1.2 ppm capsaicin. The trend toward lower perception of heat at mid-range capsaicin concentrations in full-fat sauces was also exhibited in area under the curve which represents total heat intensity. In cheese sauces with full-fat levels, perception of total heat intensity increased at 1.2 ppm capsaicin; in reduced- and low-fat sauces, total intensity increased with the first increase in capsaicin concentration and with each successive change. Total heat intensity in full-fat sauces exhibited a depression similar to that found in heat over time and maximum heat; at 0.8 and 1.2 ppm capsaicin, less heat is perceived in full-fat sauces than in reduced- and low-fat sauces. At 1.6 ppm capsaicin, low-fat sauces were perceived as having greater total heat intensity than reduced- or full-fat sauces.

Cliff and Heyman (1994) postulated that at low capsaicin concentrations, perceived intensity is proportional to concentration, dependent upon molecule diffusion across the epithelia layer and adsorption onto a receptor. Because capsaicin is lipophilic, it was expected in this study that its inclusion in a full-fat cheese sauce would result in the adherence of capsaicin to fat molecules which would either carry the capsaicin past the oral pain receptors without stimulating them or act as a buffer, protecting the receptors from the capsaicin. Data support the expectation. The proportion of fat in the sauces affected perception of heat as capsaicin increased. Reducing fat level intensified perception. Perceived heat intensity in sauces made at a full-fat level increased at a slower rate than in reduced- and low-fat sauces. The inclusion of fat delayed increases in perception until concentration reached the mid-range level (in this study) of 0.8 ppm. In reduced- and low-fat sauces, intensity increased with the first increase in capsaicin. At low concentrations, capsaicin was bound by fat, interfering with stimulation of receptors. As concentration increased, fat no longer separated capsaicin from receptors, allowing stimulation of the nociceptors and greater perception of heat.
Data were examined for effects on heat perception related to the mimetic included in the sauce. The fat mimetic nested in fat level did not affect pungency; no differences in perceived heat were found among the mimetics. Manufacturers of capsaicin-containing reduced- and low-fat sauces can choose starch- or protein-based mimetics based upon needed functional properties rather than effects on perceived heat. While carbohydrate- and protein-based mimetics did not affect pungency in reduced- and low-fat cheese sauces, lipid-based replacers have a greater potential to do so. Their effects on perception of heat should be characterized in future research.

Functional properties of mimetics require additional study also. Attention should be given to the rheological properties and active acidity of sauces made with mimetics. Differences in viscosity of cheese sauces were related to mimetic nested in fat level as were pH levels. In some instances, changes in viscosity and pH occurred over a 3-d holding period. Longer holding times could affect perception of pungency as well as stability of the sauce. In development of cheese or other sauces containing mimetics, data should be collected over a longer holding period to better examine sauce stability.

Future researchers should investigate further the role of food systems in perception of pungency. This study has confirmed that capsaicin pungency is influenced by fat level. Other pungent compounds such as those in cinnamon and ginger are used extensively in the food industry and may also be affected by fat levels in food systems. While water may be an appropriate medium for establishment of thresholds, it does not adequately characterize the effects of food ingredients on pungency. Attempts should be made to do so.
List of References
List of References


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Appendix A

Instructions to Panel at Initial Training Session
Initial Training Session

Purpose: to standardize the tongue and mouth to reference standards as measured on a 15-cm line scale

1. The line scale can be divided into an infinite number of points. You may choose any point on the line which to you describes the heat of the sample being evaluated.

2. Taste the sample presented and evaluate its heat or burn; concentrate upon the individual heat level and try to memorize it.
   a. Eat at least part of an unsalted soda cracker and rinse your mouth with water
   b. Take the entire first sample into your mouth and hold it there for 5 sec. Swallow slowly. Wait 30 sec.
   c. Rate the sample as slight on your ballot.
   d. Again rinse the mouth with cracker and water. Wait at least two minutes before tasting the next sample.

3. Repeat #2 with the remaining 3 samples.

4. Compare your evaluation with the actual concentration of capsaicin.

Definitions:

1. Threshold heat—that point where the panelist just barely senses burn or heat. On the line scale, threshold = 1.25 cm.

2. Slight heat—0.40 ppm—a "slight" amount of heat is sensed by the panelist. It is 5 cm on the line scale.

3. Moderate heat—0.80 ppm—10 cm on the line scale—panelist refers to this as "moderate" heat.

4. Approaching strong heat—Close to the heat of ground red pepper, this is 1.3 ppm and is equal to 13 cm on the line scale.

5. Strong heat—Extremely hot, sensed as hotter than the hottest sample presented. 15 cm on the line scale. Greater than 1.3 ppm.

The solutions are prepared with N-vanillyl-n-nonamide, synthetic capsaicin, and are equal to the concentrations given in the definitions above—0.4, 0.8, and 1.3 ppm. You will learn to recognize the burn which equals those concentrations as slight, moderate, and approaching strong. When evaluating a sample, use your judgement in selecting a heat intensity score; if you sense the intensity of the burn at a lower or higher position on the line scale than the anchor point, mark that position.
Appendix B

Sample Rating Scale
Sample Intensity Rating Scale

Judge ___________

You will receive 2 sets of 2 samples to evaluate. Take the whole sample into your mouth and hold for 5 secs. Swallow and wait 30 secs before evaluating the intensity of heat. Place a mark across the line to indicate intensity of sensory heat. Use any point on the line. Please rinse for 2 minutes between samples.

Sample __C__

Heat
0 threshold slight moderate strong

Sample ______

0 threshold slight moderate strong

Sample __C__

Heat
0 threshold slight moderate strong

Sample ______

0 threshold slight moderate strong
Appendix C

SAS Programming for Analysis of Data
I. pH data

proc sort data = vise; by point Fat Replacer Cap; run;
proc means noprint; by point Fat Replacer Cap;
  var pH;
  output out = mmm mean = mpH
       std = spH;
run;
proc print data = mmm;
proc sort; by day week point Fat Replacer Cap; run;
  proc print; var day fat replacer cap ph; run;
run;

Proc mixed data = vise;
title2 "Mixed analysis for pH";
classes week point Fat Replacer Cap;
model pH = point|fat|caplreplacer(fat)/predicted;
random week week*Replacer*cap(fat);
lsmeans Fat Cap Replacer(Fat) fat*cap
  point*Replacer(Fat)/pdiff;
run;
make 'predicted' out = rrr noprint; run;
proc univariate plot normal; var resid;
run;

II. Viscosity data

A. Transformation and analysis of viscosity data at discrete times

proc sort data = vise; by point Fat Replacer Cap; run;
proc means noprint; by point Fat Replacer Cap;
  var vis5 vis15 vis55;
  output out = mmm mean = mvis5 mfis15 mvis55
       std =svis5 sfis15 svis55; run;
proc print data = mmm;
proc rank data =visc out =rvisc;
  var vis5 vis15 vis55;
  ranks vis5r vis15r vis55r; run;
%mend;
%dmrep(visc);
%macro dm(var);
Proc mixed data = rvisc;
title2 "Mixed analysis for &var";
classes week point Fat Replacer Cap;
model &var = point|fat|cap Replacer(Fat) Cap*Replacer(Fat)
point*replacer(fat) point*cap*replacer(fat);
model &var = point cap Replacer(Fat) Cap*Replacer(Fat)
point*cap point*replacer(fat) point*cap*replacer(fat);
random week week*replacer*cap(fat);
ismeans point Fat Replacer(Fat)
point*fat point*replacer(fat)/pdiff;

MAKE 'ismeans' out=mmm noprint;
MAKE 'difs' out=ppp noprint;
make 'predicted' out=rrr noprint;
run;
%pdiffmix(ppp,mmm);

%mend;
%dm(Vis5r);
%dm(Vis15r);
%dm(Vis55r);

B. Transformation and analysis of viscosity data over time

data long; set vise;
time=5; visc=vis5; output;
time=15; visc=vis15; output;
time=55; visc=vis55; output;
run;
proc rank data=long out=rlong;
var visc;
ranks viscr;
run;
Proc sort data=rlong; by point Fat Replacer Cap time;
run;
proc means noprint; by point Fat Replacer Cap;
var viscr;
output out=mmm mean=mviscr
       std=sviscr var=vviscr; run;
proc print data=mmm; run;
Proc univariate data = rlong plot;
var viscr;
title 'normality test for ranked viscosity data';
run;
%macro dmrep(var);
Proc mixed data=long;
title2 "Mixed analysis for &var;"
classes week point Fat Replacer Cap time;
model &var = point|fat|time Replacer(Fat) Cap*Replacer(Fat)
   point*replacer(fat) point*cap*replacer(fat)
   time*Replacer(Fat) time*Cap*Replacer(Fat)
   time*point*replacer(fat) time*point*cap*replacer(fat);
random week week*Replacer*cap(fat) week*point*cap*replacer(fat);
""repeated time /subject=week*point*replacer*cap(fat) type=vc;
*/
lsmeans point|fat|cap|time Replacer(Fat) Cap*Replacer(Fat)
   point*replacer(fat) point*cap*replacer(fat)
   time*Replacer(Fat) time*Cap*Replacer(Fat)/pdiff;
***lsmeans time*point*replacer(fat) time*point*cap*replacer(fat)/pdiff; ***see note above
MAKE 'lsmeans' out=mmm noprint;
MAKE 'diffs' out=ppp noprint;
make 'predicted' out=rrr noprint; run;
%dendiffmix(ppp,mmm);
run;
%mend;
%dmrep(viscr);

C. Transformation and analysis of viscosity data at 5 sec

proc sort data = visc; by point Fat Replacer Cap ; run;
proc means noprint; by point Fat Replacer Cap;
   var visS ;
   output out =mmm mean = mvis5 std=svis5 ; run;
proc print data =mmm;
proc rank data =visc out =rvisc;
   var vis5 ;
   ranks vis5r ;
run;
Proc mixed data =rvisc;
title2 "Mixed analysis for vis5r;"
classes week point Fat Replacer Cap;
model vis5r = point|fat|cap Replacer(Fat) Cap*Replacer(Fat)
   point*replacer(fat) point*cap*replacer(fat);
model vis5r = point |cap Replacer(Fat) Cap*Replacer(Fat)
   point|cap point*replacer(fat) point|cap*replacer(fat);
random week week*Replacer*cap(fat);
lsmeans point Fat Replacer(Fat)
   point|fat point*replacer(fat)/pdiff;
MAKE 'lsmeans' out=mmm noprint;
MAKE 'diffs' out=ppp noprint;
make 'predicted' out=rrr noprint; run;
III. Time-intensity data

A. Creation of the variables—maximum heat, time to maximum heat, rate of release and lag

PROC SORT DATA=HEAT; BY sample;
PROC SORT DATA=CODE; BY sample;
data CAP; merge HEAT CODE; by sample;
  if pan =. then delete;
array ttt (13) t05-t3;
array sss (13) s1-s13;
drop s1-s13;
s1=5; s2=15; s3=30; s4=45; s5=60; s6=75; s7=90;
s8=105; s9=120; s10=135; s11=150; s12=165; s13=180;
maxheat=0;
base=ttt{1}; lag=0;
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=180;
rrel=maxheat/tmxheat;
vheat=var(of t05-t3);
if t05<5 and vheat=0 then lag=180;

B. Calculation of the variable—area under the curve

/* This data step calculates area under the curve described by various heights h1-ath7 at various x values x1-x7. The h1 value is taken as the base, and area above and below this are added or subtracted to get a total. Algorithm: For each interval between two x values calculate hta and htb - heights above the h1 baseline. base - x distance in the interval. Then if both heights are on the same side of h1, the area added is the square area of hta*base plus the triangle of hta to htb. If the heights are on opposite sides, there is a positive and a negative area. First the point at which the curve crosses h1 is obtained by regression 0 = hta + [(htb-hta)/base] *point point = -hta*base/(htb-hta). Then the first area is the triangle .5 * hta * point, which has the sign of hta since point is positive, and the second area is the triangle .5 * htb * (base - point) = .5 * htb * base/(htb-hta), which has the sign of htb. The positive or negative or total area is controlled by SWITCH being given the value P, N or B, respectively. */
retain area; drop dim switch ii hta kk jj htb base check;

**** user set following to problem size;
dim=14;
array hhh h0-h14;
array xxxx x1-x14;
drop h0-h14 x1-x14;
ht0=0; x1=0;
do ii=2 to 14;
  hhh[ii]=ttt[i-1];
  xxxx[ii]=sss[i-1];
end;
switch='P';
area=0;
if ht0= then area=.;
else do;
do ii=2 to dim;
  hta=hhh[ii-1]-ht0;
do jj=ii to dim;
    htb=hhh[jj]>ht0;
    if htb ne . then do;
      base=xxxx[jj]-xxxx[ii-1];
      ii=jj; ** 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);
          else area=area+ .5*htb*htb*base / (htb-hta);
        end;
      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;
end;
run;
***proc print; run; run;
C. Transformation of data and PROC MIXED programming for variables created above

data temp; set cap;
  ltmx = log(tmxheat);
  lt3 = log(t3);
  lrrel = sqrt(rrel);
run;

proc rank data=temp out=temp;
  var lag;
  ranks llag;
run;

%macro dm(var,dname);
  Proc Mixed data=&dname;
  title2 "Mixed analysis of &var";
  Class FAT Replacer CAP pan Day;
  model &var= FAT Replacer(FAT) CAP cap*fat CAP*Replacer(FAT);
  random day pan day*pan;
  lsmeans FAT Replacer(FAT) CAP cap*fat CAP*Replacer(FAT)/pdiff run;
  make ‘predicted’ out=rrr noprint;
  run;
  proc univariate plot normal;
  var resid;
run;
%mend;

%dm(area,cap);
%dm(rrel,cap);
%dm(maxheat,cap);
%dm(ltmx,temp);
%dm(lrrel,temp);
%dm(llag,temp);
%dm(lt3,temp);
%dm(lag,cap);
%dm(t3,cap);
D. Calculation and PROC MIXED analysis of the variable—heat over time

```sas
proc mixed data=long;
  title2 'heat analysis over time';
  class FAT Replacer CAP pan Day time;
  model heat= FAT Replacer(FAT) CAP cap*fat CAP*Replacer(FAT)
          time time*fat time*replacer(fat) time*cap time*fat
          time*cap*replacer(fat) /predicted;
  random day pan day*pan day*pan*cap*replacer(fat);
  repeated /group=cap;
  parms (2.29638202) (183.24629691) (3.25863003)
       (136.67638652) (253.57666640);
  lsmeans FAT Replacer(FAT) CAP cap*fat CAP*Replacer(FAT);
  lsmeans time time*fat time*replacer(fat) time*cap time*fat;
  lsmeans time*cap*replacer(fat);
run;
```

V. Programming to produce slope, intercept and $R^2$ for regression plots

```sas
*proc sort data=long; by nfat;
proc sort data=cap; by nfat;
proc glm; by nfat;
* model heat= cap cap*cap;
  model area maxheat= cap cap*cap;
run;
proc glm; by nfat;
* model heat= cap;
  model area maxheat= cap;
run;
```
IV. Programming for regression plots

data full; ***time-intensity data sets were used for regression plots;
do nfat = 3 to 12 by .5;
do cap = 0 to 1.6 by .05;
   area = .; maxheat = .; heat = .; output;
end; end;
run;
data full;
set full cap;
set full long; *** use this for heat;
run;
proc mixed;
id nfat cap;
***Replace heat with the appropriate dependent variable in the model below;
model heat = nfat cap nfat*cap nfat*cap cap*cap*cap*cap nfat
   cap*cap*cap*cap predicted;
make 'predicted' out = ppp noprint;
run;
data ppp; set ppp;
format pred 7.0;
run;
goptions ftext = swissx device = hpljs S hsize = 6 vsize = 7;
****for response surface plots
/*proc g3d data = ppp;
plot nfat*cap = pred / grid side;
run;*/
proc gplot; where nfat in(3,6,12);
title1 ‘’;
title2 ‘’;
title3 ‘’;
footnote1 ‘’;
footnote2 ‘’;
**axis1 width = 3 minor = none major = (width = 3) value = (font = swissx)
   label = (a = 90 font = swissx h = 1.5 "Area Under Curve");
**axis1 width = 3 minor = none major = (width = 3) value = (font = swissx)
   label = (a = 90 font = swissx h = 1.5 "Maximum Heat");
axis1 width = 3 minor = none major = (width = 3) value = (font = swissx)
   label = (a = 90 font = swissx h = 1.5 "Heat Over Time");
axis2 width = 3 minor = none major = (width = 3) value = (font = swissx)
   label = (a = 90 font = swissx h = 1.5 "Capsaicin Level (ppm)");
legend1 mode = share across = 1 position = (top left inside) value = (h = .75 'L' 'R' 'F')
frame label = (font = swissx h = .75 "Fat Level");
symbol1 i = ri v = square e = black w = 3;
symbol2 i = ri v = star c = black w = 3;
symbol3 i = ri v = circle c = black w = 3;
plot pred*cap = nfat/vaxis = axis1 haxis = axis2 legend = legend1;
run;
quit;
Appendix D

Tests of Fixed Effects
## Tests of Fixed Effects

### Physical and Chemical Tests

#### pH

<table>
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#### Viscosity at 5 seconds

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Tests of Fixed Effects
Viscosity over Time

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### Heat Analysis over Time

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### Lag

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### Maximum Heat

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</tr>
<tr>
<td>Replacer(Fat) x Cap</td>
<td>24</td>
<td>286</td>
<td>0.51</td>
<td>0.9734</td>
</tr>
</tbody>
</table>
### Time to Maximum Heat

<table>
<thead>
<tr>
<th>Source</th>
<th>NDF</th>
<th>DDF</th>
<th>Type III F</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat</td>
<td>2</td>
<td>286</td>
<td>3.44</td>
<td>0.0335</td>
</tr>
<tr>
<td>Replacer(Fat)</td>
<td>6</td>
<td>286</td>
<td>0.40</td>
<td>0.8783</td>
</tr>
<tr>
<td>Cap</td>
<td>4</td>
<td>286</td>
<td>56.98</td>
<td>0.0001</td>
</tr>
<tr>
<td>Fat x Cap</td>
<td>8</td>
<td>286</td>
<td>0.54</td>
<td>0.8254</td>
</tr>
<tr>
<td>Replacer(Fat) x Cap</td>
<td>24</td>
<td>286</td>
<td>0.38</td>
<td>0.9967</td>
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</tbody>
</table>

### Rate of Release

<table>
<thead>
<tr>
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<th>NDF</th>
<th>DDF</th>
<th>Type III F</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat</td>
<td>2</td>
<td>286</td>
<td>0.88</td>
<td>0.4169</td>
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<tr>
<td>Replacer(Fat)</td>
<td>6</td>
<td>286</td>
<td>0.69</td>
<td>0.6616</td>
</tr>
<tr>
<td>Cap</td>
<td>4</td>
<td>286</td>
<td>23.26</td>
<td>0.0001</td>
</tr>
<tr>
<td>Fat x Cap</td>
<td>8</td>
<td>286</td>
<td>0.78</td>
<td>0.6218</td>
</tr>
<tr>
<td>Replacer(Fat) x Cap</td>
<td>24</td>
<td>286</td>
<td>0.8</td>
<td>0.6979</td>
</tr>
</tbody>
</table>

### Total Intensity (Area under the Curve)

<table>
<thead>
<tr>
<th>Source</th>
<th>NDF</th>
<th>DDF</th>
<th>Type III F</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat</td>
<td>2</td>
<td>286</td>
<td>12.46</td>
<td>0.0001</td>
</tr>
<tr>
<td>Replacer(Fat)</td>
<td>6</td>
<td>286</td>
<td>0.25</td>
<td>0.9608</td>
</tr>
<tr>
<td>Cap</td>
<td>4</td>
<td>286</td>
<td>211.72</td>
<td>0.0001</td>
</tr>
<tr>
<td>Fat x Cap</td>
<td>8</td>
<td>286</td>
<td>2.07</td>
<td>0.0386</td>
</tr>
<tr>
<td>Replacer(Fat) x Cap</td>
<td>24</td>
<td>286</td>
<td>0.34</td>
<td>0.9988</td>
</tr>
</tbody>
</table>
Appendix E

Tables of LSmean Values for Replacer(Fat Level)
Table E-1—Least-squares mean estimates of perceived maximum sensory heat intensity in cheese sauces as a function of capsaicin concentration and fat replacer (fat level)

<table>
<thead>
<tr>
<th>Capsaicin concentration (ppm)</th>
<th>Fat level</th>
<th>Fat replacer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.0</td>
</tr>
<tr>
<td>Full</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>23.3</td>
</tr>
<tr>
<td>Reduced</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dairy Trim</td>
<td></td>
<td>19.1</td>
</tr>
<tr>
<td>N-Lite L</td>
<td></td>
<td>17.3</td>
</tr>
<tr>
<td>Paselli Excel</td>
<td></td>
<td>24.2</td>
</tr>
<tr>
<td>Simplesse</td>
<td></td>
<td>19.7</td>
</tr>
<tr>
<td>Low</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dairy Trim</td>
<td></td>
<td>13.7</td>
</tr>
<tr>
<td>N-Lite L</td>
<td></td>
<td>24.0</td>
</tr>
<tr>
<td>Paselli Excel</td>
<td></td>
<td>16.8</td>
</tr>
<tr>
<td>Simplesse</td>
<td></td>
<td>23.4</td>
</tr>
</tbody>
</table>
Table E-2—Least-squares mean estimates ± standard error of perceived total heat intensity in cheese sauces as a function of capsaicin concentration and fat replacer (fat level)

<table>
<thead>
<tr>
<th>Fat level</th>
<th>Capsaicin concentration (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat replacer</td>
<td>0.0</td>
</tr>
<tr>
<td>Full</td>
<td>1617.1 ± 1063.5</td>
</tr>
<tr>
<td>Reduced</td>
<td>Dairy Trim</td>
</tr>
<tr>
<td></td>
<td>N-Lite L</td>
</tr>
<tr>
<td></td>
<td>Paselli Excel</td>
</tr>
<tr>
<td></td>
<td>Simplesse</td>
</tr>
<tr>
<td>Low</td>
<td>Dairy Trim</td>
</tr>
<tr>
<td></td>
<td>N-Lite L</td>
</tr>
<tr>
<td></td>
<td>Paselli Excel</td>
</tr>
<tr>
<td></td>
<td>Simplesse</td>
</tr>
</tbody>
</table>
Appendix F
Regression Equations
Regression Equations

Low fat

Linear:

Heat = 6.9916 + (36.4003 * CAP) \[ R^2 = 0.336 \]
Maximum heat = 20.0273 + (58.8523 * CAP) \[ R^2 = 0.650 \]
Total intensity = 1192.5000 + (6693.1534 * CAP) \[ R^2 = 0.525 \]

Quadratic:

Heat = 6.4841 + (38.9378 * CAP) - (1.5859 * CAP \times CAP) \[ R^2 = 0.336 \]
Maximum heat = 17.9299 + (69.3393 * CAP) - (6.5544 * CAP \times CAP) \[ R^2 = 0.651 \]
Total intensity = 1104.7240 + (7132.0333 * CAP) - (274.2999 * CAP \times CAP) \[ R^2 = 0.526 \]

Reduced fat

Linear:

Heat = 7.3021 + (31.4038 * CAP) \[ R^2 = 0.299 \]
Maximum heat = 20.7364 + (52.8750 * CAP) \[ R^2 = 0.653 \]
Total intensity = 1247.2273 + (5759.2329 * CAP) \[ R^2 = 0.486 \]

Quadratic:

Heat = 7.1732 + (32.0482 * CAP) - (0.4027 * CAP \times CAP) \[ R^2 = 0.299 \]
Maximum heat = 18.8078 + (62.5179 * CAP) - (6.0268 * CAP \times CAP) \[ R^2 = 0.655 \]
Total intensity = 1222.4708 + (5883.0154 * CAP) - (77.3640 * CAP \times CAP) \[ R^2 = 0.486 \]

Full fat

Linear:

Heat = 5.1580 + (27.3706 * CAP) \[ R^2 = 0.295 \]
Maximum heat = 17.0182 + (48.2727 * CAP) \[ R^2 = 0.589 \]
Total intensity = 837.3636 + (5004.7727 * CAP) \[ R^2 = 0.508 \]

Quadratic:

Heat = 9.9413 + (3.4545 \times CAP) + (14.9476 \times CAP \times CAP) \[ R^2 = 0.315 \]
Maximum heat = 25.0701 + (8.0130 \times CAP) + (25.1623 \times CAP \times CAP) \[ R^2 = 0.625 \]
Total intensity = 1702.0390 + (681.3961 \times CAP) + (2702.1104 \times CAP \times CAP) \[ R^2 = 0.541 \]
Lou Ann Carden was born in Sylva, North Carolina, the daughter of Mr. and Mrs. Asbury Carden. She was educated in the public school system, graduating with honors from Sylva-Webster High School. She earned the B.S. in Education degree with a major in English at Western Carolina University and taught English at the senior high school level. In 1973, she earned the M.A. in English from the University of Tennessee, Knoxville. For a number of years, she supervised the Developmental Reading Laboratory at Cherokee High School, Cherokee, N.C. For 18 years, Lou Ann owned and managed Jay Gee's Crafts, a retail outlet for art and craft supplies in Sylva, and taught a variety of crafts to people throughout Western North Carolina. In 1990, she entered the Nutrition/Dietetics program at Western Carolina, earning the M.S. in Human Environmental Science and becoming a Registered Dietitian in 1993, shortly after entering the doctoral program in Food Science and Technology at the University of Tennessee, Knoxville. Lou Ann has been a graduate research assistant in both the Nutrition/Dietetics program at Western Carolina University and the Department of Food Science and Technology at the University of Tennessee, Knoxville. She is a member of the American Dietetic Association, International Food Technologists, the American Association of Cereal Chemists and Gamma Sigma Delta and Sigma Xi honor societies.