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Influence of solvent extraction, maturity stage, and thermal treatment on the determination of capsaicin in capsicums (*Capsicum annuum* spp.) and their products

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Influence of solvent extraction, maturity stage, and thermal
treatment on the determination of capsaicin in capsicums
(*Capsicum annuum* spp.) and their products

by

Alfonso Rafael Rocha-Herrera

A dissertation submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of
DOCTOR OF PHILOSOPHY

Major: Food Science and Technology

Major Professor: Lester A. Wilson

Iowa State University

Ames, Iowa

1997

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**To my loving wife, Irma,
and my three hearts, Alfonso Salvador,
Javier, and Sandra Mireille**

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ABSTRACT

The rapid increase and consumption of ethnic foods has motivated the search for improved methods to evaluate the quality and pungent characteristics of capsicum products. In addition, little information is available about pungency changes during the ripening stage of capsicums, as well as changes during their heating. Accordingly, the objectives were: a) to evaluate different organic solvents on the extraction efficiency of capsaicinoids in hot salsas; b) to evaluate the composition, aroma, and pungency in commercial serrano peppers (*Capsicum annuum*) at three maturity stages: green, yellow, and red; and c) to evaluate the thermal degradation of capsaicin in different matrixes under conventional and microwave heating.

Magnetic stirring for 10 min extracted 95% of capsaicin in Cayenne pepper compared with a 1-h Goldfish extraction using 95% ethanol. No difference was found among different solvents on the extraction efficiency of dry Cayenne pepper, but diverse results were obtained from hot salsas. Extraction efficiency was affected by texture, capsaicin content, and sample preparation. Salsas labeled as "mild", "medium", and "hot" covered a broad range of pungency levels with some of them overlapping independent of the label

In serrano peppers, the red stage contained more soluble solids.

The sugar/acid ratio was higher for the yellow and red stages. No difference was found in titratable acidity or the capsaicin content in different maturity stages. The three stages showed differences in the electronic nose mapping. Untrained panelists were unable to identify different stages and discriminate which sample had the highest intensity in aroma.

Thermal degradation of capsaicin was evaluated in different matrixes (pure capsaicin, palmitic acid, soybean oil, a mixture of palmitic acid-soybean oil, and Cayenne pepper in soybean oil) heated in conventional and microwave ovens. Degradation of pure capsaicin and capsaicin in palmitic acid during conventional heating followed a pseudo first-order reaction rate. Activation energy was calculated as 9.3 Kcal/mol. No degradation was observed during the conventional heating of capsaicin and Cayenne pepper in a soybean oil matrix. In the mixed palmitic acid-soybean oil, capsaicin degradation was observed at high concentrations of palmitic acid. No degradation was detected in any matrix during microwave heating.

GENERAL INTRODUCTION

Introduction

The production and consumption of capsicums, chiles, or hot peppers (*Capsicum annuum* spp.) has increased tremendously during the last 6 years in the U.S. Two reasons exist for this: consumers are adapting to new types of low-calorie diets, where spices are added to overcome the loss of flavor because of the reduction in fat content. The other reason is related to the success of new ethnic cuisines, like Asian or Mexican. The important characteristic of these foods is the spicy notes and, as in Mexican foods, the hotness or pungency.

Another important trend in the U.S. is the fact that many people spend less time cooking their own meals. This has promoted the development of ready-to-cook products. With 95% of the households having a microwave oven, the trend is to design products with a dual-cooking characteristic: they can be cooked in either a conventional oven or a microwave oven.

Many studies have been made to evaluate changes in food flavors due to microwave or conventional heating. However, information about the effects of these processes on the compounds responsible for the pungency in capsicums is very limited. In fact, no previous reports exist about the effect of microwave heating on these compounds, called capsaicinoids.

The initial goal of this work was to evaluate the effect of conventional and microwave cooking on capsaicinoids. However, soon we realized that capsicums and their products, in most of the cases, present a great variability in pungency levels. No precise data has been reported about how the variety and maturation stage, to cite some examples, affect the content of capsaicinoids. Besides this, no general agreement exists about how to extract these compounds for further analysis. Numerous authors have reported different methodologies for this extraction, with very variable results. This information is, at present, very important for the food industries who are developing capsicum products. A precise quantification of the pungency is vital to keep good quality control practices, as well as to produce an homogeneous product which could satisfy the consumer demand.

The scientific literature has reported very little information about capsicums other than bell peppers. Therefore, many other varieties need to be characterized to get a better understanding of their properties and changes during processing.

Accordingly, this study was initiated to investigate: (1) the evaluation of different organic solvents on the extraction efficiency of capsaicinoids in hot salsas; (2) the composition, aroma, and pungency in commercial serrano peppers (*Capsicum annuum*) at three maturity stages: green, yellow, and red; and (3) the evaluation of the thermal

degradation of capsaicin in different matrixes under conventional and microwave heating.

Dissertation Organization

This dissertation contains three manuscripts which will be submitted for publication, and it is organized in the following manner: the general introduction is followed by a literature review which discusses the previous information to support the manuscripts. The first manuscript: "Evaluation of different organic solvents on the extraction efficiency of capsaicinoids in hot salsas", was partially presented during the poster session of the Institute of Food Technologists Annual Meeting, June 4, 1995, in Anaheim, California. This paper was authored by the degree candidate and Dr. Lester A. Wilson and written according to the Journal of Food Science style and sent to this journal for publication. The second manuscript: "Evaluation of composition, aroma, and pungency in commercial serrano peppers (*Capsicum annuum*) at three maturity stages: green, yellow, and red" was partially presented during the poster session at the Institute of Food Technologists Annual Meeting, June 23, 1996, in New Orleans, Louisiana and at the 50th Annual Conference, Midwest Food Processing Conference, September 30, 1996, LaCrosse, Wisconsin. This paper was authored by the degree candidate, Roger G.

Fuentes-Granados, and Dr. Lester A. Wilson. R. G. Fuentes-Granados' participation was related with support in the gas chromatography analysis. The manuscript was written according the Journal of Food Science style (abstract, introduction, materials and methods, results and discussion, conclusions, and references) and submitted for publication to this journal. The last paper: "Thermal degradation of capsaicin in different matrixes under conventional and microwave heating", was partially presented at the 208th American Chemical Society Annual Meeting, August 22, 1994, Washington, DC and at the poster session of the Institute of Food Technologists Annual Meeting, June 15, 1997, in Orlando Florida. This paper was authored by the degree candidate and Dr. Lester A. Wilson. This manuscript was also written according to the Journal of Food Science style and sent to this journal for publication. Following the manuscripts are the concluding remarks, research recommendations, and acknowledgments.

Literature Review

Capsicums - Introduction

Capsicum is a genus of the family *Solanaceae* and is closely related to another genus, "*Solanum*", which covers many economically important plants such as potato (*Solanum tuberosum* L.), tomato (*Lycopersicon esculentum* Mill.), and tobacco (*Nicotiana tabacum* L.)

(Govindarajan, 1985). The term "capsicum" actually refers to a fruit of numerous species of the solanaceous genus, *Capsicum* (Maga, 1975).

The name capsicum may have been derived from the Greek "*Kapso*" meaning to bite, in reference to its pungency, or from the Latin "*Capsa*" or box referring to the fruit pod (Govindarajan, 1985; Maga, 1975).

In spite of the fact that early taxonomists used the term *Capsicum* for the genus, the trade and scientific literature have continued to use the term "pepper" derived from the term "red pepper". This term was used by Columbus to describe a colorful red fruit called "aji" by the natives of the New World. This product was found to be much stronger than the black pepper of Asia, in search of which he had undertaken his expedition. He took samples of this fruit back to Spain and named it "red pepper" (Govindarajan, 1985).

The term "pepper" is actually used with various prefixes -red pepper, bird pepper, chili pepper, chilli pepper, bell pepper, Cayenne pepper- to include color, shape, size, pungency, or source of the spice. The usage of this term has created much confusion in agricultural and trade statistics for black pepper (*Piper nigrum* L.) with red pepper, chilli pepper, paprika, capsicums (all *Capsicum* spp.), and sometimes, even pimento (*Pimenta dioica* L.). Even in the scientific literature the word "pepper" is used to refer to the *Capsicum* spp. as well as terms like

"chillies" and "paprika" (Govindarajan, 1985).

According to Govindarajan (1985), different terms have been used in different countries and even in the same country. He mentioned that the term "chilli" is not generally used in United States (probably because of the connotation with the Texas official state dish made with beans, meat, and hot pepper). Bosland (1994) mentioned that the Spanish word "chile" is a variation of "chil" derived from the Nahuatl (Aztec) dialect which referred to plants now known as *Capsicums*, whereas "aji" is a variation of "axi" from the extinct Arawak dialect of the Caribbean. In Mexico, Central America, and the southwestern United States, capsicum is called "chile". In fact, the review written by Bosland, from New Mexico State University, is titled: "CHILES: History, Cultivation, and Uses" (Bosland, 1994). In this review, the term "capsicum" is used throughout to refer generally to the fruits of the plants of the genus *Capsicum*. The terms of fresh fruits and processed capsicum products will be referred to by including the suffix "pepper".

Consumption of chile products

The United States is the world's largest consumer and importer of spices. A growing population, a trend toward using spices to compensate for less salt and fat in food, and a heightened popularity of ethnic foods have pushed U.S. demand for spices to record levels.

Rapid expansion of eating away from home in recent years has increased the commercial use of spices. U.S. spice consumption averaged an estimated 815 million pounds in 1990-1994, compared with 541 million in 1980-1984. Per capita, spice consumption increased nearly 1 pound from a decade ago to 3.1 pounds in 1990-1994. Imports and domestic production increased over the past decade in response to greater U.S. spice demand (Buzanell and Lipton, 1995).

Buzanell and Lipton (1995) reported that the United States produces more than one-third of its annual spice needs. Domestic production accounted for 310 million pounds of U.S. consumption in 1990-1994, up from 195.8 million pounds in 1980-1984. Domestic production consists of four major spice types. Dehydrated garlic and onions represent nearly two-thirds of U.S. spice production, capsicums (Cayenne or red) represent about 30 percent, and mustard seed and various herbs account for the remainder. New Mexico, California, and Arizona are important producers of capsicums. U.S. production of dried capsicum totaled 160 million pounds in 1994, nearly double the amount produced in 1985.

The food processing and foodservice industries are the major customers for spices, accounting for an estimated 60 percent of the amount sold. Use of spices by these sectors has expanded because of population growth, the greater popularity of ethnic foods and prepared

meals, and increased consumption of food away from home (Buzzanell and Lipton, 1995).

The *Capsicum* genus produces the most consumed spice in the world. Capsicum is used for flavoring in food manufacturing, coloring for cosmetics, and for imparting heat to medicines. Besides pungency, they are used to give taste, aroma, texture, color, and visual appeal to ethnic foods (Uhl, 1996). This genus provides many species and varieties used in flavoring foods popular in cuisines of many parts of the world. Today, approximately three-fourths of the world's population use capsicums regularly in their diets (Govindarajan, 1985). From a nutritional perspective, capsicums are good sources of vitamins, particularly vitamin C in green fruits and vitamin A in mature red fruits (Bosland, 1994).

Most countries in the world grow capsicums. In the United States, New Mexico is the leading state in pungent capsicum production. There, this production has increased from 20 to 45 million kgs from 1976 to 1990, which is indicative of the increasing popularity of capsicums. In 1991, Pakistan, México, India, China, and Chile were the principal sources of U.S. capsicum imports, while most paprika imports were from Spain, Morocco, and Hungary. Canada, México, and Germany are the leading importers of U.S. capsicum (Bosland, 1994).

The consumption of spice ethnic foods has increased dramatically during recent years (e.g., salsa has surpassed ketchup in U.S sales). Traditionally, foods from Latin America, India, Indonesia, China, and Africa have contained many more spice ingredients than did food from Northern Europe and North America. *Capsicum* species, the major flavor component of such foods, led all spices in consumption gain during 1992. Between imports and domestic production, this family of spice products, ranging from mild-sweet paprika to the hottest of habanero pepper increased 25% to 165.5 million pounds (Finne, 1994).

Capsicums are widely used in the preparation of curries, hot sauces, and Mexican and Asian cuisines. Recently, the popularity of these cuisines has increased. This is due in part to the desire to reduce fats in the diet by substituting foods that do not depend upon fat for their flavor and appeal (Henderson and Henderson, 1992).

Recently, Sloan (1996) reported the trend of the American diet for the future. She mentioned that growing multi-cultural influences and a gradual migration to lower fat and vegetable-rich diets will continue to accelerate the fundamental shift in American taste preferences to more highly flavored and often intensely spiced products. Salsa -America's #1 condiment and the barometer of the spicy foods market, according to Sloan- nearly doubled to \$448 million in supermarket sales since 1991. Use of "hot spices," such as black

pepper, red pepper, mustard, and ginger, jumped 73% during the past 20 years and now represent 41% of U.S. spice consumption. It has been estimated that combined retail sales of all channels of hot and spicy foods to be \$1.03 billion and it is projected an annual growth rate of 10%. Restaurant trend data confirms that while one-half of adults prefer spicy dishes to mild ones, one-third prefer intensely spicy meals. Southern males are the demographic group most apt to prefer intensely spicy foods.

Sloan (1996) also mentioned that ethnic food trends indicate that the desire for hot and spicy foods will increase. One important indicator, Mexican foods, is projected to explode from \$2.5 billion and to nearly \$3.5 billion by 1999. Thai food is the fastest growing segment of the \$1.05 billion Asian food category, exhibiting an annual growth rate of about 25%. It is reported that foodservice professionals predict that, next to traditional leaders like Italian (15%) and Mexican (14%), Caribbean (14%), Thai (8%) and Indonesian (6%) will lead the ethnic food trend.

Classification of capsicums

There is a debate in the total number of *Capsicum* species, however, according to Bosland (1994), most investigators agree on 27 species. From these, *C. annuum*, *C. frutescens*, *C. chinense*, *C. baccatum*, and *C. pubescens* are considered the five domesticated

species. Capsicum fruits are considered vegetables, but are berries botanically. Capsicum types are usually classified by fruit characteristics, i.e. pungency, color, shape, flavor, size, and their use. Despite their vast trait differences, virtually all capsicum cultivars commercially cultivated in the world belong to the specie *C. annuum*.

Among the important varieties from this species, it is possible to mention (Bosland, 1994; Govindarajan, 1985):

C. annuum : Sweet or mild types: Hungarian paprika, Spanish pimientos, bell pepper. Pungent types: Jalapeño, serrano, ancho, New Mexican, Cayenne, pasilla, piquín, de árbol, Poblano.

C. frutescens : Tabasco and malagueta.

C. baccatum: aji.

C. pubescens : rocoto, manzano o perón, chamberoto.

Composition of capsicums

The proximate chemical composition of capsicums from different sources and one paprika is given in Table 1. Carbohydrates, protein, fat, and fiber are the major components which increase rapidly from the green stage to the ripe stage. The fiber content varies greatly between sources. The volatile ether extract values are low, as capsicums are generally poor in volatiles, although the trace of volatiles have a great sensory impact in some varieties. Fructose, glucose, galactose, and

Table 1. Composition of Capsicum fruits, (%) - Different types and sources

	U.S. Cayenne	Hungary, paprika	Indian chillies		Nigerian chillies	
			Green	Dry	Green (<i>C. annuum</i>)	Ripe fresh (<i>C. frutescens</i>)
Moisture	3.7 - 9.0	7.0 - 9.5	82.6	10.0	91.8	63.5
Carbohydrate	54.0 - 58.0	58 - 60	6.1	31.6	0.98	-
Starch	0.5 - 1.5	-	-	-	3.0	-
Protein	11.2 - 14.4	13.8 - 17.5	2.9	15.9	0.5	5.42
Fiber, crude	17.5 - 25.0	14 - 20	6.8	30.2		11.0
Ash, total	5.1 - 6.4	5.6 - 7.6	1.1	6.1		2.75
Ash, water soluble	2.8 - 4.9	4.7 - 5.5				
Ash, acid insoluble	0.05 - 0.25	0.0 - 0.35				
Ether extract, volatile	0.7 - 2.6	0.3 - 1.5				
Ether extract, nonvolatile	15.5 - 22.0	7.5 - 12				
Ascorbic acid (mg/100 g)	29.4 - 63.7	48.6 - 58.8	111	50	110	263

(Govindarajan, 1985a).

sucrose have been identified. Fructose is the major sugar and with glucose amounts to about 70% of total reducing sugars. Citric acid was the major acid, the others being succinic, fumaric, malic, and quinic acids. Of nutritional importance is the vitamin C content, vitamin A and the vitamin B complex (Govindarajan, 1985).

The three functionally important components for the use of capsicum as spice are principally the red color and capsaicinoids, the pungency stimulant and to a lesser degree, the characteristic aroma of some varieties. These components are discussed next.

Functional properties

Capsicums are valued for uniformity of shape, size, minimum broken, and the defined color and pungency combination of the trade types. The sweet or mildly pungent paprika are valued for their bright color and, to a minor extent, for their aroma while other capsicums are valuable for their pungency and color. As the pungency decreases, generally there is an increase in size and fleshy nature of the pericarp. The group paprika contains less than 0.1% of capsaicinoids, the pungent stimulant: the best grade of Spanish sweet paprika, 0 to 0.003%, and the pungent grade, a maximum of 0.05%. Other varieties of capsicums vary considerably in their pungency. The capsaicinoids content cover a range of values evaluated as mild, 0.1 to 0.2%; medium, 0.2 to 0.4%; hot or strong, 0.4 to 0.6%; and very pungent, 0.6 to 1.4%.

The valued red pigment content, expressed as total extractable color, varies according to the grades and quality of paprika from 0.3 to 0.8%, except for the pungent "ordinary" grades where color is lower. These grades are used mainly for the pungent and characteristic paprika flavor (Govindarajan, 1985).

Color components

Color is localized in the outer pericarp tissues. With storage, the color could spread to other tissues due to breakup of the cells or due to the fat component solubilizing and distributing the color. The varying shades of color of capsicums are derived from a number of related carotenoids, identified as capsanthin, capsorubin, cryptoxanthin, and zeaxanthin. Of these, capsanthin is the major component, accounting for 30 to 60% of the pigments in the different varieties of red-colored capsicums. Capsorubin, also contributing to the red color, is present in the range of 6 to 18% of the total (Govindarajan, 1985).

Deep red color varieties are composed of up to 70% of red pigments, while the components contributing yellow to orange colors form the rest. In other capsicums, such as the green bell peppers and the green stage of red peppers, capsanthin and capsorubin are absent and the composition is dominated by lutein (40%), beta-carotene (13 to 20%), and additionally, chlorophyll a and b in the immature green

stage. In the mature stage, the yellow fruits contain the pigments lutein, violaxanthin and neoxanthin (Govindarajan, 1985).

An extensive review on carotenoid composition, chemical properties, and color degradation was reported by Govindarajan (1985, 1986a, 1986b).

Volatiles

Starting with the work of Buttery et al. (1969), numerous publications have reported volatile identification, quantitation, and correlation with sensory characteristics in bell peppers (Wu and Liou, 1986; Luning et al., 1994a, 1994b, 1994c; van Ruth and Roozen 1994; Yuksel et al., 1994; Luning, 1995). Few studies have reported volatile analysis in different varieties of capsicums: in Tabasco and red pepper, Keller et al., (1981) and Haymon and Aurand (1971); in Jalapeño, Huffman et al. (1978); in Jalapeño, Anaheim, and Fresno, Chitwood, et al. (1983).

Most data found in the literature is related with bell peppers. Steam volatile components in bell peppers were qualitatively analyzed by using conventional and capillary gas-liquid chromatography with characterization by mass, infrared, ultraviolet, and proton magnetic resonance spectra (Buttery et al., 1969). In the volatiles of the green bell pepper, the character impact component was identified as 2-methoxy-3-isobutylpyrazine. According to Buttery et al. (1969), this

component, which has a strong odor, has one of the lowest odor thresholds, 2 parts in 10^{12} in water. They found a total of 23 compounds in the volatile oil of uncooked bell peppers, the principal classes of compounds being aliphatic alcohols and carbonyl compounds (11), aromatic compounds (6), and monoterpenes (3). They reported the presence of trans-beta-ocimene, methyl salicylate, limonene, linalool, cis-3-hexenol, trans,cis-2,6-nonadienal, cis-cis-2,4-decadienal, and trans-2-nonen-4-one.

Haymon and Aurand (1971) isolated volatile constituents of Tabasco peppers by steam distillation and lyophilization. The resolved compounds totaled 125, of which 24 were identified by infrared and mass spectrometry. The major components identified were 4-methyl-1-pentyl-2-methyl butyrate, 3-methyl-1-pentyl-3-methyl butyrate, and isoheptyl-isocaproate.

Volatiles from *C. annuum* and *C. frutescens* were isolated and identified by Keller et al. (1981). The identified compounds were: 17 alcohols, 20 carbonyl compounds, 14 carboxylic acids, 21 esters, 6 pyrazines, 14 terpene hydrocarbons, and 10 miscellaneous compounds. The compound characteristic in bell peppers, 2-methoxy-3-isobutylpyrazine, was found in both species.

Chitwood et al. (1983) suggested the relation between the volatile constituents 2-isobutyl-3-methoxypyrazine, 2-sec-butyl-3-methoxy-

pyrazine, cis-3-hexenol and some sensory-perceived "green" aroma characteristics of three chiles grown in California. Wu and Liou (1986) indicated that tissue disruption stimulated enzymatic formation of hexenal, trans-2-hexenal, hexanol, cis-3-hexen-1-ol and trans-2-hexen-1-ol, while the content of 2-methoxy-3-isobutylpyrazine, linalool, trans-beta-ocimene, and benzaldehyde were similar before and after disruption.

Using dynamic headspace gas chromatography, mass spectrometry, and sniffing port detection, Luning et al. (1994b) analyzed the volatile compounds of a Dutch commercial bell pepper cv. Mazurka at the ripening stages green, turning, and red. They identified 64 compounds and reported that different bell pepper samples had several odor compounds in common: 2,3-butanedione, 1-penten-3-one, hexanal, 3-carene, trans-beta-ocimene, octanal, and 2-isobutyl-3-methoxypyrazine. They mentioned that disruption of the cell structure favored lipid oxidation and the formation of related alcohols, aldehydes, and ketones.

Huffman et al. (1978) attributed the Jalapeño flavor to 2-methoxy-3-isobutylpyrazine. Although the concentration of volatiles was low, with selected ion monitoring, concentrated Jalapeño aroma gave an identical ion chromatogram as the aroma concentrated from bell pepper under the same conditions. They mentioned that the odor

was so potent, that it was easily discernible at the effluent port of the gas chromatograph-mass spectrometer, however they were not able to identify this compound.

Pungency

Flavor is a complex combination of taste, aroma, and irritation. In the oral cavity, the taste buds respond to sweet, sour, salt, and bitter. In the nasal cavity, the olfactory epithelium responds to odorants. When foods are ingested, aromas are transferred from the oral cavity to the nasal cavity via the nasopharyngeal region. As humans we are unaware of this retronasal transfer, thereby confusing taste and smell. However, there is another component of "flavor" which has been very much overlooked and that is irritation or the "common chemical sense" (Cliff, 1992).

Irritation or common chemical sense stimulates the free nerve endings of the trigeminal nerve, which is distinct from the nerves that make contact with the taste buds and are involved in taste sensations. Lawless (1989) mentioned that, although the trigeminal nerve is also responsible for touch, heat, cold, and pain sensations in the mouth, the capsicum sensations cannot be classified as touch either, since they involve no physical deformation of touch receptors.

According to Govindarajan (1979), "pungency" is a term which connotes some undesirable characteristics and is used as a synonym

of "hot" or "irritant". However, this author mentions that with respect to food, for which the term "pungency" should really be used, it assumes a desirable quality. The "piquant" and "mouth-watering" characteristic of some spices lead to a greater acceptance and higher intake of foods. He mentions that these characteristics identify "pungency" as different from the other descriptors used to define the attribute, such as irritant, stinging, caustic, etc., all of which have undesirable connotations.

Lawless (1989) reported that there are about two dozen common spices and more than 40 flavor compounds that have irritation or astringent properties. Capsicums have a group of naturally occurring pungent compounds called capsaicinoids, which will be considered later. The principal irritant of black pepper (*Piper nigrum* L.) is piperine, and it is 150 times less pungent than capsaicin, one of the most pungent compounds found in capsicums. The principal irritants of fresh ginger (*Zingiber officinale*, Roscoe) is a group of phenylalkyl ketones called gingerols. During drying and storage, gingerols dehydrate resulting in a group of compounds known as shogaols, which are even more potent than the gingerols. During heating of ginger, gingerols are cleaved producing a methyl ketone, zingerone, which exhibits only mild pungency. However, the relative pungency of these compounds is still less than capsaicin. The principal irritants for other spices such as

cinnamon, clove/nutmeg, cumin, mustard, and horseradish are cinnamaldehyde, eugenol, cumin aldehyde, butenyl isothiocyanate, and allyl isothiocyanate, respectively (Cliff, 1992; Lindsay, 1996).

Much research has been directed to understanding the sensory reactions of capsicum compounds under controlled conditions.

Psychophysical studies, like the relationship between intensity and concentration of irritant, sensitization-desensitization, effects of external factors -like time and temperature- on the perception of pungency, and influence of tastants and food additives on the oral irritation of capsaicinoids, have been reported (Rozin and Schiller, 1980; Lawless, 1984; Sizer and Harris, 1985; Nasrawi and Pangborn, 1898; Hutchinson et al., 1990; Green, 1991 and 1991b).

Capsaicinoids

Apart from color, which is important in paprika, pungency is the most important attribute of capsicums used in foods. Although capsicum products have been widely accepted for centuries, it was not until the last part of the 19th Century that researchers began to investigate the composition and properties of capsicums. In 1846, Thresh was the first to crystallize the primary pungent principle and he named it capsaicin. Two decades later, Nelson made the significant contribution to the structure of capsaicin by identifying the compounds formed by hydrolysis. He demonstrated that capsaicin was composed of

a basic unit (vanillylamine) and an acid component (an isomeric decenoic acid). Further, Nelson and Dawson established the structure of capsaicin as 8-methyl-6-nonenoyl vanillylamide (Maga, 1975; Govindarajan, 1986b).

Kosuge and Inagaki in 1962 showed that two related components are found in capsicum extracts, both of which stimulate pungency. They showed that the ratio of the two components did not vary with the varieties they studied or the degree of maturity at harvest, and they suggested the term "capsaicinoids" for the mixture of these components (Govindarajan, 1985).

The nature of the capsaicinoids has been established as a mixture of several homologous branched-chain alkyl vanillylamides or, as reported by others, vanillylamides of fatty acids. These compounds differ in the length of the aliphatic side chain, the presence or absence of a double bond, the branching point, and by their relative pungency. (Krajewska and Powers, 1988a). They often are called capsaicin after the most prevalent one (Figure 1).

At present, according to some authors, there are five closely related compounds: capsaicin, dihydrocapsaicin (vanillylamide of 8-methylnonanoic acid), nordihydrocapsaicin (vanillylamide of 7-methyloctanoic acid), homocapsaicin (vanillylamide of 9-methylen-trans-7-enoic acid), and homodihydrocapsaicin (vanillylamide of 9-

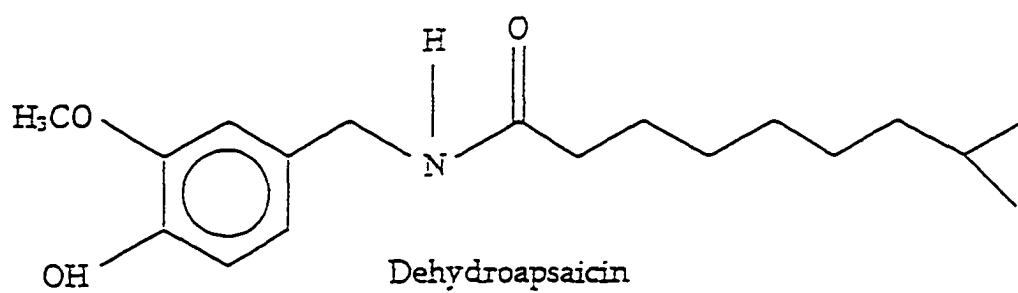
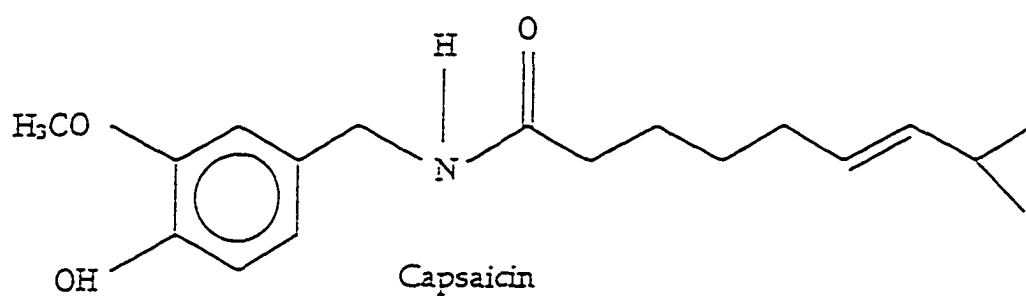
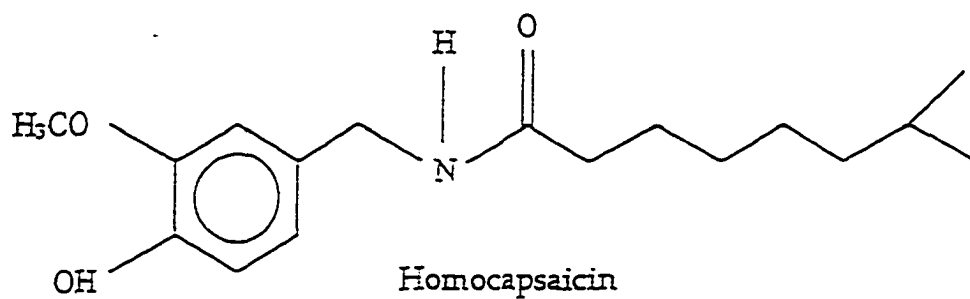


Figure 1. Structure of major naturally-occurring capsaicinoids.

methyldecanoic acid) (Suzuki, et al., 1980; Hoffman, et al., 1983; Chiang, 1986). Only trans-isomers of capsaicinoids with a double bond in a side chain were found in extracts of capsicums. No cis-isomers were found in these extracts (Krajewska and Powers, 1988a).

The capsaicinoids are produced in glands on the placenta of the fruit. While seeds are not the source of pungency, they occasionally absorb capsaicin because of the proximity to the placenta (Bosland, 1994).

The capsaicinoids content is affected by the genetic make-up of the capsicum, weather conditions, growing conditions, and fruit age. Plant breeders can selectively develop cultivars with varying degrees of pungency. Stress also plays an important role. Capsicum is hottest after it has survived a stressful growing and adverse weather conditions (Bosland, 1994). Several authors have mentioned that the variability of pungency is very important in the selection of the variety for processed products. Bajaj (1980) mentioned that it is possible to find a great variability in the same plant.

The average capsaicinoids content of some capsicum varieties is presented in Table 2. The composition of capsaicinoids of natural origin, is generally: capsaicin (69%); dehydrocapsaicin (22%); nordihydrocapsaicin (7%); homocapsaicin and homodihydrocapsaicin (1% each). However it has been demonstrated that large variation exists

Table 2. Capsaicinoids content and pungency in some varieties of capsicums.

Type of capsicum	CSDS ^a	CAP ^b	CAPC ^c	SHU ^d
Cayenne red pepper	0.2360 ^e			40,000 ^e
Red pepper	0.0588 ^e			10,000 ^e
Chilli	0.0058 ^e			900 ^e
Mombasa (Africa)	0.800 ^e			120,000 ^e
Uganda (Africa)	0.850 ^e			127,000 ^e
Mexican pequinios	0.260 ^e			40,000 ^e
Abyssinian	0.075 ^e			11,000 ^e
Bahamian (Bahamas)	0.5100 ^e			75,000 ^e
Santaka (Japan)	0.3000 ^e			55,000 ^e
Sannam (India)	0.3300 ^e			49,000 ^e
Bird chilli (India)	0.3600 ^e			42,000 ^e
Jalapeño ^g		1.58 ^f 1.307 ⁱ	20.42 ^f 20.81 ^k	2,300 - 6,500 ⁱ
Green finger hot ^g		2.17 ^f	41.08 ^f	
Red finger hot		1.22 ^f	32.62 ^f 28.08 ^k	
Tabasco ^j		4.11 ^k	65.41 ^k	30,000 - 50,000 ^l

^a Capsaicinoids content (% w/w); ^b Capsaicin (mg/g dry wt.)

^c Capsaicin (mg/100 g original wt.); ^d Scoville Heat Units

^e Govindarajan, 1979; ^f Krajewska and Powers, 1987a

^g Canned peppers; ^h Collins et al., 1995; ⁱ Quinones-Siegle et al., 1989; ^j Fresh peppers; ^k Krajewska and Powers, 1988b; ^l DeWitt and Gerlach, 1990

Table 2. (Continued)

Type of capsicum	CSDS ^a	CAP ^b	CAPC ^c	SHU ^d
Hot banana ^j		0.33 ^k	3.07 ^k	
Pasilla		0.195 ^h		1,000 - 1,500 ^l
Cascabel		0.088 ^h		1,500 - 2,500 ^l
Cubanella		0.834 ^h		
New Mexican		0.039 ^h		
Yellow mushroom		1.196 ^h		
<i>C. baccatum</i>		0.558 ^h		
<i>C. cardenasii</i>		0.984 ^h		
Habanero		10.95 ^{hk}		100,000 - 300,000 ^l
<i>C. pubescens</i>		0.40 ^{hk}		
Serrano				6,000 - 8,000 ⁱ

among many varieties of capsicums from different growing regions (Govindarajan, 1985). In another study, Govindarajan (1986b) mentioned that the average composition of capsaicinoids in *C. annuum* var. *annuum* varieties is capsaicin, 33 to 59%; dihydrocapsaicin, 30 to 51%; nordihydrocapsaicin, 7 to 15%; and others, in the range of 0 to 5% each. Fruits of the species of *C. frutescens* have higher capsaicin (63 to 77%) and dehydrocapsaicin (20 to 32%) content, with other homologs and analogs making up around 10%.

Govindarajan (1985) reported that the capsaicinoids content of 14 cultivars of chiles from different sources determined by thin-layer

chromatography varied between 0.05 to 0.33% in *C. annuum* L. and 0.35 to 0.85% in *C. frutescens*. This author stated that the range for capsaicinoids content for *C. annuum* L. var. *annuum* cultivars vary widely from 0.07 to 0.63%, and a number of selections have shown higher values, from 0.6 to 1.5% covering the range generally attributed to the small-fruited cultivars *C. frutescens*. In spite of large variations in the total capsaicinoids between and within cultivars, the ratio of the principal components, capsaicin and dehydrocapsaicin remained the same, around 1:1 for *C. annuum* and 2:1 for *C. frutescens*.

Sensory evaluation

Capsaicin itself is practically devoided of odor and flavor.

Nelson, in 1910, determined than one drop of a solution containing 1 part in 100,000 causes a persistent burning on the tongue while 1 drop of a solution of 1 part in a million imparts a perceptible warmth.

Gathercoal and Terry, in 1921 and Munch, in 1930 determined that a dilution of 1 part in 10 million is the threshold of detectable pungency of capsaicin. Other values, as these authors state, have been reported as the limiting dilution for capsaicin detection: 1 in 15 million by Suzuki et al. in 1957; 1 in 17 million by Todd in 1958; and 1 in 30 million by an anonymous author (Krajewska and Powers, 1988a).

A scientific approach using sensory evaluation to measure the pungency of capsicums was first reported by Wilbur L. Scoville in 1912

and described as the Scoville Heat Test. This test relied on trained judges. The basics of the test are: the sample to be tested is steeped overnight (or 16 hours) in ethanol. The extract is filtered and serially diluted with a 5% sugar solution. The samples are tested by five trained testers who are asked to identify the weakest dilution at which the hotness sensation is detectable. The reciprocal of the dilution at which three of the five panelists last perceive pungency upon swallowing 5 ml of the dilution, establishes the numerical Scoville Heat value or the Scoville Heat Unit (SHU) for that sample (Finne, 1994; Bosland, 1994).

Scoville Heat units for several chiles are: sweet bell peppers: 0 heat units, the Jalapeño usually ranges from 2,500 to 10,000, and the habanero in excess of 200,000 (Bosland, 1994).

Although seemingly simple and straight forward, the Scoville Heat test has its limitations and has been criticized by many researchers. Some disadvantages can be mentioned (Govindarajan, 1979; Maga, 1975; Govindarajan et al., 1977; Rhyu, 1978; Todd et al., 1977):

- It is time consuming because of the long extraction time and the need for accurate preparation of numerous dilutions.
- By its nature, it is subjective.

- Reproducibility among different samples is also a problem that can result in misunderstanding between customers using different panels.
- Variation of acuity of panelists.
- Aroma lowering the thresholds and thereby biasing the judgement to higher values than the real.
- Carry-over of pungency and adaptation effects biasing judgement of subsequent samples to lower values.
- Other psychological anticipatory errors now recognized in sensory testing.
- Non-standardization of procedures and preparation of samples for dilution testing.

A more recent method designed to replace the Scoville Heat Test was reported by Gillette et al. (1984). In this procedure, ground peppers are stepped in water with polysorbate at 90°C for 20 min, filtered and the filtrate diluted in 20°C water. Trained panelists compared the pungency of pepper extracts with a known concentration of a standard, synthetic capsaicin, (N-vanillyl-n-nonenamide). A high correlation between the sensory evaluation and the analytical method (HPLC) was found. According to the authors, this method avoids some problems inherent to the Scoville test. Heat build up, taste fatigue and increased threshold are accounted for by use of a standardized initial sample, as

well as timed rinsing between samples. Ethanol bite is avoided by use of an aqueous extraction. The panel data may be manipulated statistically due to the linearity of the scale and the number of panelists. Reference standards are included. Extraction time is reduced from 16 hours to 20 minutes. Reproducibility of results was demonstrated. This method is more comparable to normal food usage as it uses an aqueous rather ethanol extraction. This method was evaluated and approved as a standard method by the ASTM (Finne, 1994).

Instrumental analysis

In order to overcome the subjective nature of sensory quantitation of pungency, numerous studies have reported instrumental methods for the determination of capsaicinoids in capsicums. Colorimetric methods (Bajaj, 1980), spectrometry (Trejo-Gonzalez and Wild-Altamirano, 1973; Rymal et al., 1984; Ogbadu et al., 1989), thin-layer and paper chromatography (Rajpoot and Govindarajan, 1981; Pankar and Magar, 1977) have been used for the analysis of total capsaicinoids. Other techniques like gas chromatography and high-performance liquid chromatography (HPLC) are used for identification and quantitation of individual capsaicinoids with varying degrees of sensitivity. Gas chromatography analysis has been reported by Krajewska and Powers (1987a, 1987b) and Hawer et al. (1993); gas-

liquid chromatography by Todd et al. (1977) and gas chromatography-mass spectrometry by Iwai et al. (1979). With the development of HPLC, the instrumental analysis of capsicums was further improved and simplified. Lee et al. (1976) first reported separation by HPLC of capsaicin and dehydrocapsaicin from commercial capsicums. The HPLC method most commonly used is described in the Official Analytical Method of the American Spice Trade Association (ASTA, 1985).

However, numerous investigators have reported analysis by using different HPLC techniques: Sticher et al., 1978; Saria et al., 1981; Law, 1983; Hoffman et al., 1983; Chiang, 1986; Weaver and Awde, 1986; Woodbury, 1980; Quinones-Siegle et al., 1989; Collins et al., 1995; Cooper et al., 1991). Some variations from these methods are: gas chromatography with electron-capture detection (Krajewska and Powers, 1988b); reverse-phase low-pressure chromatography (Krajewska and Powers, 1986); HPLC-chemiluminescent nitrogen detector (Fujinari, 1994).

Gas chromatography and HPLC are the most used techniques to separate and quantitate capsaicinoids. Some researches have mentioned several drawbacks in the gas chromatography analysis, the most important being the derivatization procedure required for capsaicin before analysis to form compounds that are sufficiently volatile for the analysis (Hoffman, et al., 1983; Cooper, 1991). Sticher et

al. (1978) and Woodbury (1980) mention that HPLC analysis, which does not require derivatization is, therefore, simple and direct. Capsaicinoids analysis can be done by both normal and reverse-phase chromatography, however reverse-phase is preferred to normal-phase chromatography because the capsaicinoids differ only in their fatty acid side chains and can be better separated by a non-polar support in the column (Cooper, 1991). Collins et al. (1995) reported that techniques using HPLC provide accurate and efficient analysis of content and type of capsaicinoids present in a capsicum samples.

Hawer et al. (1993) reported that even sample preparation for HPLC analysis may be simple and convenient, the interfering substances extracted with capsaicinoids interfere with the chromatogram and can easily be absorbed to the column-packing materials, increasing pressure in the system and shortening the column life.

Correlation between analytical and sensory analysis

Although a number of early instrumental methods were successful in arriving at a relative quantitation of capsaicinoids, none of them could translate the results of the assays into numbers equivalent to Scoville units.

The research of Todd et al. (1977) changed this concept. Using gas-liquid chromatography, they were initially able to separate and

quantitate the individual capsaicinoids in capsicums. These capsaicinoids were later synthesized and the threshold pungency determined for each through a series of controlled triangular tests. These values are, expressed in SHU $\times 10^6$, 9.3 for nordihydrocapsaicin; 16.1 for capsaicin; 16.1 for dehydrocapsaicin; 6.9 for homocapsaicin; and 8.1 for homodihydrocapsaicin. These investigators showed that the total pungency calculated using these values and the concentration of each compound as determined by gas chromatography was highly correlated (>0.95) with pungency determined sensorially.

Krajewska and Powers (1988a), using an American Society of Testing Materials (ASTM) method, determined that sensory evaluation of isolated natural capsaicinoids showed a significant linear relationship between pungency and concentration. They also found how capsaicinoids are perceived in the oral cavity. Nordihydrocapsaicin was found to be the least irritating and described with a fruity, sweet, and spicy character. The biting sensation developed immediately and it was located in the front of the mouth and palate. Capsaicin and dehydrocapsaicin turned out to be the more irritating, causing a sharp and stinging bite. It was described as a "typical" biting sensation. Both compounds produced sensations located in the mid-mouth and mid-palate as well as the throat and back of the tongue. The sensation developed rapidly and lasted longer than the pungency of

nordihydrocapsaicin. Pungency of homodihydrocapsaicin was very irritating, harsh and very sharp. The authors mentioned that despite the general opinion that capsaicinoids do not have flavor, homodihydrocapsaicin was observed to have a peppery smell and slightly sour taste making it easily distinguishable from the other capsaicinoids. Its pungency did not develop immediately after swallowing; it affected the throat and back of the tongue and palate and was prolonged and difficult to rinse out. These differences were observed only at low concentration solutions. At higher concentrations, the pungency of all capsaicinoids developed rapidly all over the mouth and throat and was too strong to detect any differences in perception.

The pungency thresholds of capsaicinoids were also determined by Krajewska and Powers (1988a). These results are, in SHU $\times 10^6$: nordihydrocapsaicin, 9.38; capsaicin, 16.35; dihydrocapsaicin, 16.3; homodihydrocapsaicin, 14.04. With these values, the total pungency value expressed in SHU can be calculated from a unknown capsicum sample. Pungency of each compound can be calculated by multiplying its threshold value expressed in SHU by its concentration, obtained from gas chromatography or HPLC.

van Germet et al. (1983) found a SHU of 25×10^6 for natural capsaicin. They reported that the Dutch panel used in the test were more sensitive because of their low frequency of capsicum

consumption.

Extraction of capsaicinoids

Several solvents have been used for the extraction of capsaicinoids from capsicums. Isopropanol was used in ground fresh serranos (Trejo-Gonzalez and Wild-Altamirano, 1973). Carbon tetrachloride in green paprika (Govindarajan, 1986b). Bajaj (1980) and Suzuki et al. (1980) used ethyl acetate to produce a nearly colorless extract from dried immature green peppers. Nagin Chand and Govindarajan (1985) found that acetone extraction of fresh fruit resulted in poor extraction and messy layer formation. However, drying of cut sections of green fruits allowed a clean solvent extraction.

Capsaicinoids from ground red pepper were extracted with 95% ethanol (Hoffman et al., 1983), however other solvents were examined in order to determine the most suitable medium for isolating the capsaicinoids. The solvents tested included acetonitrile, acetone, methylene chloride, and ethanol. Acetonitrile required a lengthy extraction time and carried with it extraneous interfering compounds. Acetone, although an excellent solvent for removing the capsaicinoids, carry some undesirable components which adversely affected the resolution of the individual compounds during the HPLC analysis. Methylene chloride extracted the materials in a reasonable time but was impractical because it carried residues not totally miscible with

the mobile phase (acetonitrile-water (1% acetic acid), 40:60 v/v).

Ethanol extracted greater than 98% of the capsaicinoids after 5 hours.

Ethanol saturated with sodium acetate was used by Woodbury (1980). Methanol was used by Chiang (1986) to extract capsaicinoids from different samples such as spicy cheese sauce, salsa, picante sauce, spicy mix, and spicy tomato sauce.

Collins et al. (1995) extracted oven-dried capsicum fruits with acetonitrile. These authors also evaluated other solvents, including 95% ethanol, 95% ethanol saturated with sodium acetate, chloroform, and ethyl acetate, for the extraction of capsaicinoids from the same capsicum sample for varied lengths of time. They found that acetonitrile yielded the highest amount most quickly, although they did not report any data.

Iwai et al. (1979) used acetone and/or ethyl acetate in dried capsicums and Weaver and Awde (1986) refluxed samples of ground pepper with acetone, drying and redissolving in denatured ethanol. Ogbadu et al. (1989) used methanol to extract capsaicinoids from ground, fresh capsicums.

Whole capsicum fruits were cut finely and blended with acetone and extracted in a Soxhlet apparatus with the same solvent. Sauces were extracted with acetone as well (Edwards, et al., 1990).

Attuquayefio and Buckle (1987) evaluated different solvents on the extraction of capsaicinoids from dehydrated ground pepper and fresh capsicum. They reported that acetone gave the highest yield, however also extracted significant levels of pigments and lipids. Chloroform gave reasonable yields but also extracted red pigments, interfering in subsequent HPLC analysis. Methanol extracted less capsaicinoids than either acetone or chloroform but also extracted less pigments and produced extracts in which particulates settled to the bottom of the clear extract. Extraction of capsaicinoids with acetonitrile gave a clear, light-colored extract with particulates that settle well, a yield of capsaicinoids comparable to those of the other solvents, and an extract that was easily and effectively cleaned by Sep-pak filtration. Acetonitrile also enabled uniform extraction conditions for both capsicums and oleoresins. Capsaicinoid extracts were obtained by Soxhlet extraction of freeze-dried capsicums in acetone (Quinones-Siegle et al., 1989; Hawer et al., 1993).

Yao et al. (1994) reported extraction of capsaicinoids with supercritical CO₂ and organic solvents. They reported that supercritical CO₂ extraction was a superior solvent to extract capsaicin and dehydrocapsaicin from Scotch Bonnet peppers.

Evaluacion of pungency - Importance

Because of the contribution to pungency, color, and flavor, food producers use capsicums in a variety of different products including meats, cheeses, sauces, candy, beverages, and baked goods. In order to correctly formulate a consistent product for the consumer, it is important for the industry to have an accurate reproducible method of quantitating pungency (Finne, 1994).

Collins et al. (1995) reported that accurate measures of pungency have become important because of the increased demand by consumers for southwestern foods; moreover, accurate determination of levels of various capsaicinoids is also needed due to their increased use in pharmaceuticals. Food industry researchers need reliable, safe, and standard analytical procedures that are useful for comparing pungency levels among different samples. Therefore, the Scoville Organoleptic test has been replaced with instrumental methods.

Changes in functional components with maturity and ripening

Very limited information exists relating changes in volatile content and pungency at recognizable stages of maturity for different cultivars of capsicums.

Govindarajan (1986b) evaluated two small capsicums with high content in capsaicinoids and observed that these compounds were not

found until 21 days after the fruit set. Capsaicinoids rapidly increased up to 49 days 50- to 70-fold and were predominantly found in the dissepiment rather than in the outer pericarp. In another study, also reported by Govindarajan (1986b), he reported that capsaicinoids content in a high-pungent capsicum, were first detected around 20 days after flowering and rose sharply up to 40 days. However, there was a large reduction of total capsaicinoids (around 60%) between 40 and 50 days.

According to Luning et al. (1994b), changes in volatile bell pepper constituents during maturation had not been investigated. They reported changes of different constituents in bell peppers at the ripening stages green, turning, and red. Fresh weight did not change during maturation, while dry matter content increased gradually. The chlorophyll a content was maximal at the green stage and decreased during ripening. In contrast, the carotenoid content increased upon maturation. Evaluation of gas chromatograms obtained from samples prepared by homogenization and cutting, showed that the volatile compounds decreased or disappeared and only a few compounds increased upon ripening. In other words, the total peak areas decreased during maturation. They mentioned that, as stated for tomatoes, the activities of several enzymes seem to be changed during

ripening of bell peppers, especially the ones involved in the formation of lipid degraded products.

Changes in functional properties during processing

Very limited information was found about changes in functional properties in capsicums during processing. Capsicums are available as ripe fruits, dried whole, ground, frozen, and pickled. Several types like Jalapeño and serrano are canned whole and, as in the case of Jalapeño, it is possible to find canned slices and dices for use in specific foods. However, the varieties of canned peppers are increasing because of changes in the consumer patterns.

Govindarajan (1985, 1986b) reviewed changes in capsicum components during drying, with special attention to carotenoids. Limited reports were found about changes in volatiles and pungency. The aroma of fresh bell peppers, mainly due to 2-methoxy-3-isobutyl-pyrazine, is modified to a cooked bell pepper aroma by the increased amounts of C₉-ketones (Govindarajan, 1986b). van Ruth and Roozen (1994) reported that the flavor of rehydrated bell pepper cuttings is due to compounds formed during processing and rehydration. They also showed that rehydrated dried Chilean, Turkish, and Hungarian bell peppers were more different in taste attributes like sour, bitter, and pungency than in aroma attributes and mentioned that drying of fresh bell peppers greatly changed the composition of volatile compounds;

most volatiles evaporated and new volatile odor compounds were formed by chemical reactions.

Luning et al. (1995) evaluated the aroma of fresh and hot-air dried bell peppers by sensory and instrumental methods. They reported that hot-air drying decreased levels of the odor compounds cis-3-hexenal, 2-heptanone, cis-2-hexenal, trans-2-hexenal, hexanol, cis-3-hexanol, trans-2-hexenol, and linalool, which have green, vegetable-like, fruity, and floral notes, while intensity scores of corresponding sensory aroma attributes also decreased. The aroma of rehydrated dried samples was mainly characterized as savory, rancid/sweet, sweet/sickly, hay-like, cacao, caramel, and nutty. Drying increased the levels of 2-methylpropanal, 2- and 3-methylbutanal, which have cacao, spicy, and rancid/sweet odor notes, respectively. These volatiles may be correlated with the corresponding aroma attributes in the dried fruits.

Huffman et al. (1978) reported that after thermal treatment, Jalapeño peppers exhibited an altered or "cooked" flavor. These peppers exhibited a low concentration of the original flavor compound and this decrease was attributed to the thermal treatment. They mentioned that 2-isobutyl-3-methoxypyrazine is converted into a different, as yet not identified, component or is partially destroyed.

Information reporting the evaluation of capsaicinoids loss during processing has been very limited. Evaluating the amount of capsaicinoids in fresh and thermal processed Jalapeños (100°C for 50 min), Hoffman et al. (1978) reported that heated Jalapeños contained much higher capsaicin levels than do the raw. They attributed this result to the thermal treatment. During heating, capsaicin is volatilized and many cells are lysed. This allows the capsaicin to spread freely through the fruit. They also mentioned that any complexing agent present might be split off, leaving free capsaicin. Thus capsaicin is more readily available for analysis and higher readings result.

Harrison and Harris (1985) evaluated the capsaicin content in frozen, cooked, and canned Jalapeño peppers by using gas-liquid chromatography and compared it to the capsaicin content in raw capsicums. They reported a decrease in capsaicinoids in the canned Jalapeños compared with those in the raw product, result which contradicts that one from Huffman et al. (1978), who used the same processing conditions. Harrison and Harris (1985) assumed that the contact between the blanched peppers (100°C for 3 min in water) and the surrounding water during cooling after blanching could have resulted in leaching of the capsaicinoids. During thermal treatment, cells are lysed allowing the capsaicin to spread from the pericarp

throughout the pepper. During the 4-day holding time, capsaicin may have leached into the brine. They mentioned that other chemical reactions could occur during boiling involving shortening of the fatty acid chain, hydrolysis of the phenolic hydroxy group, and loss of nitrogen. Any of these reactions could have decomposed the capsaicinoid molecule. A slight increase in capsaicin was reported after cooking in boiling water for 10 min. Frozen Jalapeños were reported to retain the smallest concentration of capsaicin. These capsicums were blanched, but the treatment may not have completely inactivated important enzymes, and cooling in water may have led to leaching. In addition to this, the freezing treatment may have ruptured cells which could have led to capsaicin loss.

Srinivasan et al. (1992) reported loss of active principles of spices during domestic cooking (boiling of mixed spices -turmeric, black pepper, and red pepper-, with food ingredients for 15-30 min). At the normal pH of 6.1, capsaicin loss was negligible during 15 min cooking while at pH 5.1, the loss was around 19%. Slightly higher losses (19-33%) of capsaicin were recorded when the duration of cooking was increased to 30 min in either pH.

Henderson and Henderson (1992) evaluated the chemical changes that occurred during the process of cooking with capsaicin. They studied the decomposition at high temperatures of capsaicin

alone and in mixtures with oleic acid in presence of air. The decomposition products were determined by coupled gas chromatography-mass spectrometry. These authors mentioned that, previous to this work, no studies were found reporting the effects of heat on capsaicin. Heating conditions were carried out at 200°C for 2 h. They found that in thermal degradation of capsaicin, the primary route for the formation of products is by cleavage of the bond between the amide group and the vanillin moiety. This leads to the production of vanillin and 8-methyl-6-nonenamide. The free acid, methylnonenoic acid was also identified. The unique products formed as a result of heating capsaicin with oleic acid were amides. The principal product was cis-9-octadecenamide. It was also suggested that the oxidation of oleic acid appears to be inhibited by the presence of capsaicin. The oxidation of capsaicin appears to be preferred over the oxidation of oleic acid, and thus capsaicin may serve as an antioxidant in nonaqueous systems.

Conventional vs. microwave cooking

The worldwide consumer desire for a rapid and convenient food preparation method has, in recent years, provided the driving force behind the rapidly increasing ownership of domestic microwaves (George, 1993). The percentage of U.S. households with at least one microwave oven was 1.2% in 1973, 40% in 1983, 76% in 1987, 89% in

1991, and 93% in 1993 (Anonyme, 1994). Although there was an increase in microwave-specific foods, their sales in 1994 were at their lowest point. However, certain microwave foods, like popcorn, pizza and potatoes, keep a high sales rate. Indeed, 82.9% of all popcorn sold in 1993 was destined for a microwave; in the same way, 14.8% of all frozen pizzas and about 10% of prepared potatoes are cooked in microwave oven. This treatment is the preferred cooking method for 99.9% of ready-to-eat shelf-stable entrees. It also significantly penetrates the shelf-stable category for stews (40%), macaroni products (26%) and chicken (16%). It is dominant for refrigerated and frozen sandwiches (79.9%) and canned lasagna (75.4), and accounts for 30.6% in non-specific frozen entrees (Anonyme, 1994).

Among the main advantages of microwave heating compared with conventional treatment, it is possible to mention: convenience, fast heating rate, efficiency, and cost (Katz, 1992). However, since 1990, sales of microwave product have declined. Schiffmann (1992) mentioned that:

...while microwave manufacturers were selling these appliances as cooking devices, most consumers saw them simply as convenient food reheaters. Despite the often well written and beautifully illustrated cookbooks provided with the ovens, offers of free cooking classes, and growth of special microwave cooking

schools, consumers resisted cooking in the microwave oven. If they did try roasting meat, for example, they came away confused and dissatisfied. The roast looked different, and the wonderful aroma of roasting meat didn't come across, and so it was hard to tell when it was done. The cooking of many items required following recipes with care, doing some things that were unusual and then frequently having to accept food that just didn't appear or taste right. Anyone who has ever tried to bake a microwave cake may end up with something that doesn't brown, may be too moist or collapse, or may be overheated and turn dark brown or black in the inside, while the surface still looks golden yellow (p.386).

Currently, consumers continue to want increased quality and convenience with microwave products. However, cost is often increased, and the flavor and textural characteristics do not compare to those of a conventionally prepared product (Whorton and Reineccius, 1990). Comparing sensory properties of microwave and conventionally cooked foods, lower hedonic ratings are readily assigned to flavor, texture and color of many of the microwave cooked foods by the consumer. The most important problems found during microwave cooking, are:

- **No flavors from Maillard reaction.** In the conventional oven, the high temperature of the cooking environment results in the surface of the product reaching high temperature over a long time period, causing dehydration and browning. When the moisture content of the food ranges from intermediate to low, surface browning and flavor production result from complex Maillard reaction; in microwave heating, on the other hand, the short term exposure to temperatures does not allow for the flavors to develop by the Maillard reaction.
- **Water pumping.** In conventionally heated foods moisture migrates to the surface in a somewhat passive manner. Water evaporates in the surface, usually through capillary action, in order to maintain a moisture equilibrium between a drying surface and a wet interior. The rate at which water is transported is diffusion rate dependent. Such movement can be slow enough to permit the water activity of the surface to decrease below 1.0. This allows the surface temperature to climb above 100°C and the heat plus the concentration of Maillard reactants promotes browning with its associated aroma, flavor and color effects.
- **In a microwave oven, water movement is due to the generation of an internal vapor pressure which actively pumps water to the surface.** No longer is water movement diffusion rate limited, but may

continue at such a rate to maintain the surface at a saturated condition, and evaporative cooling will maintain the temperature at or below 100°C. Water pumping acts to defeat crisping as well as Maillard browning. This condition also cause distillation effects, resulting in the loss of volatile flavor compounds, specifically those with polar characteristics.

- **Uniformity of heating.** From an oven perspective, the uniformity of temperature or lack of thereof, in foods is due to several factors. First, all ovens have hot and cold zones, that is areas of high or low electric field, as a result of standing wave patterns. These may, in turn, produce areas of high and low heat within a food. The electric fields also can cause edge heating effects and concentrations of energy in corners. With this uneven heating we can expect to find uneven development of aromas. (Katz, 1994; Schiffmann, 1994).

According to Hassel (1989), practical experiences have led to the conclusion that a product seasoned with ground spices and cooked in a microwave oven will not taste the same as when it is cooked in a conventional matter. The less intense flavor developed by spicy foods during microwave treatment, is due to the short cooking time used in this process, and this is the reason why ground seasonings are not efficient in developing a food flavor profile in a microwave oven. The flavor components in the spice or herb are not permitted time to

migrate through the cell wall and distribute throughout the product, with the net result being an overall less intense flavor. The same product exposed to conventional cooking times and temperature, typically develops a more intense flavor profile. The flavor components of spices and herbs are able to slowly steam distill out of the spice into the food. Other important reason why foods heated in conventional and microwave oven often deliver significantly different flavor profiles, is that individually flavor components are often lost at variable rates through volatilization or degradation, leading to loss of top-notes or unbalancing and distortion of the desired flavor profile (Stanford and McGorin, 1994).

No reports were found in the literature relating the effect of microwave cooking with changes in the capsaicinoids content or pungent characteristics in foods containing this compounds.

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**EVALUATION OF DIFFERENT ORGANIC SOLVENTS ON THE
EXTRACTION EFFICIENCY OF CAPSAICINOIDS IN HOT
SALSAS**

A paper to be submitted for publication to the Journal of Food Science

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ABSTRACT

Acetone, acetonitrile, methanol, 95% ethanol, and chloroform were used for the extraction of capsaicin from ground Cayenne peppers and commercial hot salsas. Quantification of capsaicin was made by HPLC. Magnetic stirring for 10 min extracted 95% of capsaicin in Cayenne pepper compared with 1-h extraction in the Goldfish apparatus, using 95% ethanol. No difference was found among the solvents on the extraction efficiency in Cayenne pepper. In hot salsas, different solvents produced diverse results. Efficiency was affected by texture, capsaicin content, and type preparation of the sample. Salsas labeled as "mild", "medium", and "hot" presented a broad range of pungency levels with some overlapping among each other, independent of the type of designation.

INTRODUCTION

The American diet is gradually shifting to lower fat and vegetable-rich diets, which require the use of spices to intensify the flavor perceived in these meals. Besides this, the growing of multicultural influences have accelerated the preferences for more flavored and often intensely spiced products (Sloan, 1996). Salsa -which has surpassed ketchup in U.S. sales (Finne, 1994)- doubled to \$448 million in supermarket sales since 1991 (Sloan, 1996). Because of this increased demand, accurate determination of the pungency level has become important to evaluate the quality characteristics of raw and processed materials as well as the formulation of a consistent product. The substances responsible for the pungency in capsicums are a group of related compounds called capsaicinoids which consist of several vanillylamides of fatty acids (Krajewska and Powers, 1987). The three major capsaicinoids are nordihydrocapsaicin, capsaicin, and dehydrocapsaicin. They constitute about 95% of the total capsaicinoids in capsicums (Krajewska and Powers, 1987b). The other naturally occurring capsaicinoids are homocapsaicin and homodihydrocapsaicin. Numerous authors have reported a variety of instrumental methods to identify and quantitate these products (Iwai et al., 1979; Hoffman et al., 1983; Chiang, 1986; Quinones-Siegle et al., 1989; Cooper et al., 1991; Hawer et al., 1993; Collins et al., 1995).

Currently, gas chromatography and high-performance liquid chromatography (HPLC) are the most suitable methods for the analysis of capsaicinoids. From these, HPLC provides accurate and efficient results with the advantage that it does not require derivatization and, therefore, is simple and direct (Hoffman et al., 1983; Collins et al., 1995). Extraction of capsaicinoids is the preliminary step before HPLC analysis. Different extraction methods have been used in capsicums and salsas. These techniques range from the simplest one, stirring the dried chile with the solvent for 2 min (Attuquayefio and Buckle, 1987; Chiang, 1986) at room temperature, to stirring for 4-5 h at 60-80°C (Collins et al., 1995; Hoffman et al., 1983; Woodbury, 1980). Some authors have reported using the Soxhlet extractor with different solvents (Sankarikutty et al., 1978; Huffman et al., 1978; Harrison and Harris, 1985; Ogbadu et al., 1989; Quinones-Siegle et al., 1989; Hawer et al., 1993). The type of solvent is another important factor affecting the extraction efficiency. A variety of solvents have been used for this extraction: isopropanol (Trejo-Gonzalez and Wild-Altamirano, 1973); carbon tetrachloride (Govindarajan, 1986); ethyl acetate (Bajaj, 1980; Iwai et al., 1980; Collins et al., 1995); acetone (Sankarikutty et al., 1978; Iwai et al., 1979; Hoffman et al., 1983; Nagin-Chad and Govindarajan, 1985; Weaver and Awde, 1986; Attuquayefio and Buckle, 1987; Quinones-Siegle et al., 1989; Edwards et al., 1990; Hawer et al.,

1993); 95% ethanol (Sankarikutty et al., 1978; Hoffman et al., 1983; Collins et al., 1995); acetonitrile (Hoffman et al., 1983; Attuquayefio and Buckle, 1987; Collins et al., 1995); methylene chloride (Sankarikutty et al., 1978; Hoffman et al., 1983); ethylene dichloride (Sankarikutty et al., 1978); chloroform (Sankarikutty et al., 1978; Attuquayefio and Buckle, 1987; Collins et al., 1995); methanol (Attuquayefio and Buckle, 1987; Ogbadu et al., 1989); ethanol saturated with ethyl acetate (Woodbury, 1980; Collins et al., 1995); and supercritical CO₂ (Yao et al., 1994).

No consensus exists about the best solvent for extraction of capsaicinoids, and limited reports were found about extraction of these compounds in hot salsas. Accordingly, our objective was to evaluate the extraction efficiency of capsaicin using different organic solvents in ground Cayenne pepper and salsas with different ranges of texture and pungency content.

MATERIALS AND METHODS

Materials

Capsaicin (8-methyl-N-vanillyl-6-nonenamide; approximately 60%) was purchased from Sigma Chemical Co. (St. Louis, MO). Standard capsaicinoid solutions were prepared with methanol. HPLC-grade methanol, 95% ethanol, HPLC-grade acetone, HPLC-grade

acetonitrile, HPLC-grade chloroform, and acetic acid were purchased from Fisher Scientific (Fair Lawn, NJ). Ground Cayenne pepper was donated by Burns Philp Food Inc. (Ankeny, IA). Commercial salsas (Chi-chi's[®], Zapata[®], Frank's[®], Tabasco[®], and La Victoria[®]) were purchased from a grocery store in Ames, IA.

Extraction procedures

a) Cayenne pepper

Two methods for the extraction of capsaicinoids from ground Cayenne pepper were evaluated. In the first one, 5 g of the sample was extracted in a Goldfish apparatus for 1, 2, 3, and 4 h with 95% ethanol. The solution was filtered through a 0.45- μ m disposable filter (PVDF type, Alltech Associates, Inc., Deerfield, IL) and transferred to a 15-mL Teflon-lined screw-cap vial and stored at 4.4°C until injection into the HPLC system. In the second method, 10 mL of 95% ethanol was added to 2 g of the Cayenne powder and stirred in a magnetic stirrer for 5, 10, 15, and 20 min. The suspended material was allowed to settle and the solution was filtered and stored under the same conditions as the Goldfish extracts and injected into the HPLC. This method was also carried out with methanol, acetonitrile, acetone, and chloroform.

b) Salsas

A sample of each salsa (300 g) was mixed in a Waring blender for

three periods of 1 min each at maximum rate. Extractions from these "blended" salsas was carried out by weighing 3 g of each sample in a beaker and adding 10 mL of 95% ethanol. After 10-min stirring in a magnetic stirrer, the solid material was allowed to settle and the solution was filtered through a 0.45- μ m disposable filter (PVDF type, Alltech Associates, Inc., Deerfield, IL), transferred to a Teflon-lined screw-cap vial and stored at 4.4°C until injection into the HPLC.

Extraction of blended salsas with the remaining solvents, followed the same procedure as described for 95% ethanol.

For the "blended and dried" salsas extraction, approximately 40 g of each "blended" salsa was dried in a food dehydrator (Snackmaster® Dehydrator, Mod. FD 50/30, Alternative Pioneering Systems, Inc. Chaska, MN) at 70-72°C for 8 h or until they reached a constant weight. Dried samples were ground in a coffee mill (Mr. Coffee, Inc., Mod. 1DS50, Bredford Heights, OH), screened through a No. 25 mesh, and stored in Ziploc® bags in the dark, at room temperature, until extraction. Extraction with 95% ethanol was accomplished by weighing 1 g of the dried salsa, adding 10 mL of ethanol and stirring for 10 min with a magnetic stirrer. Filtration and storage followed the same procedure used by the blended salsa. Extraction of the blended and blended and dried salsas with different solvents, followed the same conditions described for ethanol.

Viscosity evaluation

The viscosity of the previously blended salsas, was measured with a Brookfield Digital Viscometer, Mod. DV-II+ with a No.1 spindle at shear rates from 10 to 90 rpm (Brookfield Engineering Laboratories, Inc., Strughton, MA), at 20.4°C. Viscosity values were reported in mPa.s.

HPLC analysis

Capsaicinoids separation was accomplished using a Beckman System Gold liquid chromatograph (Beckman Instruments, Inc., San Ramon, CA) equipped with a Model 110 pump and a Beckman UV detector set at 280 nm. The reverse-phase separations were carried out in a Supelco C₁₈ column (Supelco Inc., Bellefonte, PA) 25 x 0.46 cm i.d., with a 5- μ m particle size. A mobile phase of 55:45:1 (v/v) water: acetonitrile:acetic acid at a flow rate of 1.5 mL/min was previously determined to give good base line separations of the major capsaicinoids. The injection volume was 20 μ L.

Statistical evaluation

Viscosity data (10 replicates per sample) were analyzed by one-factor completely randomized analysis of variance. Evaluation of different solvents on the extraction efficiency from blended and blended and dried salsas, was performed through a 2 x 4 (type of salsa

x type of solvent) factorial experiment with a completely randomized design. Three replicates were used per treatment in every case.

Analysis of variance (ANOVA) and Least Significant Difference (LSD) test were carried out in the SAS Statistical Analysis Program (SAS Institute, Inc., 1985) with significance established at $p < 0.05$.

RESULTS AND DISCUSSION

Extraction of Cayenne pepper with 95% ethanol

Different methodologies have been used for capsaicinoids extraction. They range from a simple stirring for a short period of time (Chiang, 1986; Attuquayefio and Buckle, 1987) to the use of a Soxhlet apparatus (Sankarikutty et al., 1978; Ogbadu et al., 1989; Quinones-Siegle et al., 1989) for several hours. In this study, we conducted two preliminary tests to evaluate the efficiency of 95% ethanol on the extraction of capsaicinoids from ground Cayenne pepper. One test used the Goldfish apparatus and the other one used magnetic stirring at room temperature. Both tests were conducted for different time periods. Results from these tests (Table 1) showed that no significant difference ($p < 0.05$) was found in the Goldfish extraction from 1 to 4 h, which means that 1-h extraction is sufficient to extract an adequate amount of capsaicin. Hawer et al. (1993) determined that 1-h extraction with acetone, in a Soxhlet apparatus, extracted 99% of capsaicinoids present in ground red peppers. Although the Goldfish and Soxhlet

extractions are not exactly the same procedures, some comparisons can be made because of their similarities.

The major capsaicinoids peaks in the Cayenne pepper extracts were identified by their retention times as compared with those from the standard solution. Nordihydrocapsaicin, capsaicin, and dehydrocapsaicin were the major peaks (Figure 1). Homocapsaicin and homodihydrocapsaicin peaks were observed, however they were present only in trace quantities. This confirms earlier reports from Sticher et al. (1978) and Cooper et al. (1991) who observed that these two peaks are present in only low levels.

Results of the extraction with magnetic stirring (Table 1) showed that stirring for 10-min was sufficient to extract a reproducible amount of capsaicin. Similar extraction procedures are reported in the literature, which range from 2-min extraction (Chiang, 1986) to 5-h extraction (Hoffman et al., 1983). In our study, the extraction efficiency of magnetic stirring compared with that in the Goldfish apparatus was about 95%. This difference is reasonable considering the saving in extraction time, preparation procedures, materials, and costs between both procedures.

HPLC chromatograms of Cayenne pepper extracts obtained by the procedures mentioned earlier, showed that the capsaicin represented 63% of the total area of the detected capsaicinoids. This result is very

close to that one reported by Govindarajan (1985) who reported a content of 69% of capsaicin in capsicums. The total content of capsaicinoids in Cayenne ground pepper, as reported by our method, ranged from 0.23 to 0.25%. This result is similar to that found by Govindarajan (1979) where he reported a capsaicinoids concentration of 0.236% in the same pepper.

Recovery studies were conducted for the stirring extraction of Cayenne pepper. Samples were spiked with standard capsaicinoids and their extraction was performed as previously described for Cayenne pepper. Based on the peak area calculations, good recoveries at or above 88% were obtained (Table 2). Chiang (1988) suggested that the calculations be based on the peak areas rather than peak heights, to reduce discrepancies in the results.

Extraction of Cayenne pepper with different solvents

Stirring extraction of ground Cayenne pepper was also evaluated using different organic solvents: 95% ethanol, methanol, chloroform, acetone, and acetonitrile. Results of the extracted capsaicinoids are presented in Table 3. No significant difference ($p < 0.05$) was found using the indicated solvents on the recovery of capsaicin. In other studies, Attuquayefio and Buckle (1978), Sankarikutty et al. (1978), and Hoffman, et al. (1983), also evaluated different solvents in the extraction efficiency of capsaicinoids, however none of them reported

statistical difference in their results. In our study, methanol extracted the highest amount of nordihydrocapsaicin. Acetone has been reported to produce the highest yields in capsaicin extraction, compared with solvents like methanol, chloroform, methylene chloride, and ethylene dichloride (Sankarikutty et al., 1987). In our study, acetone affected the resolution of individual capsaicinoids during the HPLC analysis, by showing a wide peak appearing between those peaks corresponding to nordihydrocapsaicin and capsaicin. This observation was also reported by Hoffman et al. (1983). Chloroform, although an excellent solvent for capsaicinoids, did not show any peak corresponding to the elution of these compounds. A solution prepared with pure capsaicinoids and chloroform, did fail in showing any peaks as well. This result contradicts reports from different authors, who have been successful in the extraction of capsaicinoids using this solvent (Sankarikutty et al., 1978; Attuquayefio and Buckle, 1987). Similar results were obtained with different lots of chloroform.

Although acetonitrile produced the highest yield on the extraction of capsaicin, it was only 1.7% higher than the ethanol extraction. It also produced the lowest extraction yield for dehydrocapsaicin. In addition, acetonitrile carried with it some extraneous materials that did not dissolve well. This was also observed by Hoffman et al. (1983). According to these results, 95%

ethanol was initially selected for the extraction of capsaicinoids from different types of salsas. Salsas were chosen trying to include a broad range of textures and, at the same time, different pungency content. The selected salsas are listed in Table 4.

Extraction of salsas with 95% ethanol

Results from the extraction of capsaicin, dry solids content, and viscosity in the hot salsas, are presented in Table 5. The sample preparation followed two procedures: in one case, salsas were blended to homogenize the particle size as well as the pungency content, which is essential for chunky-style salsas. The second procedure was carried out by blending and drying. Capsaicin content is reported by comparing this two types of preparation.

In the blended samples, the content of capsaicin is reported on a dry basis (db) by correcting the results according the percentage of dry matter in each sample. In the blended and dried samples, this concentration is obtained directly. This was done to facilitate the comparison of the results between the blended samples and the blended and dried ones. Initially, we realized that the texture *per se*, was not a good indicator of the general characteristics of the salsas. For instance, we found that those salsas initially considered as "liquids" (Frank's and Zapata), had a higher content of solids than those found in the chunky salsas, considered as a more "solid" salsas.

Accordingly, the viscosity of the salsas was evaluated to define a better indicator of their texture. Results in Table 5 show that there exists a variability in capsaicin content present in the same salsas labeled as "hot". For instance, Frank's has a content of 0.09 mg of capsaicin/g db, compared with Chi-chi's hot, which has a content of 1.28 mg of capsaicin/g db. Higher differences were found among the salsas labeled as "mild", like La Victoria, Chi-chi's, and Enchilada Sauce (no detection, 0.41, and 1.40 mg of capsaicin/g salsa db, respectively).

Significant difference ($p < 0.05$) in capsaicin content between the blended and blended and dried samples, was found in those salsas with the lowest viscosity values: Frank's, Zapata liquid, and Tabasco (179.2, 92.2, and 67.6 mPa.s, respectively). In addition to this, it can be observed that Frank's and Zapata liquid salsas resulted in less capsaicin content when blended and dried compared with the blended type ($p < 0.05$). The opposite was obtained for the Tabasco salsa. These results show that, in these types of salsas, viscosity and capsaicin content have an important effect on the extraction of the principal pungent compound. It is also possible that some ingredients present in the salsas, like some gums or stabilizers, could be an important factor on the extraction efficiency. In the case of Tabasco sauce, the high content of moisture in the blended salsa, could play an important role in the extraction efficiency. The high water content could dilute

the solvent in such way that it became less nonpolar, reducing the solubility of capsaicin.

In salsas with higher viscosity values, no difference ($p < 0.05$) was found between blended and blended and dried salsas, which means that evaluation of pungency in these type of products, can be done avoiding the drying process.

Comparison of the means for the blended salsas by the least significant difference test ($p < 0.05$), indicated that those salsas that did not show significant difference were Zapata liquid (0.13 mg capsaicin/g db) with La Victoria Hot (0.11 mg capsaicin/g db). In the same way, Frank's (0.09 mg capsaicin/g db) was not different from La Victoria Hot. The rest of the salsas differed in the content of capsaicin. Among the blended and dried salsas, extraction with ethanol did not show significant difference ($p < 0.05$) for Enchilada Sauce (mild) and Chi-chi's (medium). The rest of the salsas differed in their capsaicin content. These results confirm that salsas labeled with the same level of pungency, differ statistically in their capsaicinoids content evaluated through HPLC analysis. In the same way, salsas labeled as "mild" (like Zapata Enchilada) have higher content of pungent compounds than those labeled as "hot" (like La Victoria) and viceversa. These results does not necessarily mean that these same differences could be found in the sensory evaluation of these products, but they are an example of

the variability in these products. Although every salsa processor can label its product according to established rules, the consumption of these type of products with similar pungency level in the label but different sensation, can produce confusion among consumers.

Extraction of salsas with different solvents

In order to compare the extraction efficiency of different solvents (methanol, acetonitrile, acetone, and chloroform) to 95% ethanol in commercial salsas, we decided to choose those salsas that would be representative of a wide range in both pungency and viscosity. The results are presented in Table 6. Tabasco was the only salsa that showed significant difference ($p < 0.05$) between blended and blended and dried by using methanol and ethanol. Higher extraction was observed with the dried samples. No difference was detected for these alcohols with the rest of the salsas. Acetonitrile produced opposite results compared with those ones from ethanol and methanol. In fact, apart from Tabasco, acetonitrile extracted a higher capsaicinoids content from the blended samples. However, it was observed that the extraction with this solvent produced agglomeration of solid residues derived from the salsa. This same effect was observed in the Enchilada Sauce, Chi-chi's, and La Victoria, all of them with tomato as is, puree, or paste, as one of the principal ingredients. The same result was observed when tomato puree was mixed with acetonitrile in a collateral

study. Unlike the salsas with high pungency level, like Tabasco, it is likely that the high-moisture content of the blended salsas could help to distribute the solvent, resulting in a more homogeneous extraction. In the case of the dried samples, the high concentration of other components different from the capsaicinoids, could affect the extraction efficiency. Acetone resulted in the production of a messy layer formation, specifically in Enchilada Sauce, Chi-chi's, and La Victoria. This problem was also reported by Nagin Chad and Govindarajan (1985). In addition, acetone showed some interfering peaks, which in the case of the Enchilada Sauce resulted in a higher capsaicin response. Chloroform did not show any peak corresponding to capsaicinoids in the HPLC chromatogram, problem which was also observed in the Cayenne pepper extraction.

Comparison of the differences in extraction between the blended and the blended and dried salsas by using different solvents, is presented in Table 6. In Enchilada Sauce, Chi-chi's and La Victoria, ethanol did not show extraction differences for blended and blended and dried salsas ($p < 0.05$). This is advantageous because this solvent is safe and its cost is lower compared with methanol and acetonitrile. It was interesting to note that differences in extraction capacity was found for every solvent in the Enchilada Sauce, in both blended and blended and dried salsa.

Results from the factorial analysis design in Tabasco, Enchilada Sauce, and La Victoria, indicated that the factors evaluated (type of solvent and nature of the sample: blended and blended and dried) act independently each other. This means that there is no interaction between these factors on the extraction efficiency. However, this was not the case with Chi-chi's Chunky Salsa. We found an statistical difference ($p < 0.05$) in the interaction between both factors. In other words, the effect of the solvent on the extraction efficiency depends on the nature of the salsa. This case is clearly exemplified with acetonitrile in the chunky salsa extraction (Table 6). This solvent produced high extraction effectiveness with blended salsa, but the lowest value in the blended and dried sample.

These results give an idea about the complexity of the evaluation of capsaicinoids in salsas. Depending of the salsa characteristics, we found that in some instances it is possible to avoid some previous preparation, like drying, to extract a representative amount of capsaicinoids.

CONCLUSIONS

Magnetic stirring of ground Cayenne pepper for 10 min, extracted 95% of the major capsaicinoids compared with the extraction in the Goldfish apparatus. Methanol, 95% ethanol, acetone, and acetonitrile did not show differences in the extraction efficiency of capsaicin from

Cayenne pepper. 95% ethanol was found as the most convenient solvent for the extraction of capsaicin from the high-viscosity salsas. Results showed that salsas labeled with a specific pungency label, differed statistically in their pungency content, therefore we found "mild" salsas with a higher content of capsaicin than some salsas labeled as "hot". Determination of capsaicinoids in salsas depended on the procedure of the sample preparation (blended or blended and dried), the capsaicinoids concentration, viscosity, and likely, on the type of ingredients present. In salsas with a high viscosity values, extraction and evaluation of pungency can be done avoiding the drying process.

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TABLE CAPTIONS

- Table 1.** Extraction of capsaicin (mg/g Cayenne pepper) with 95% ethanol using Goldfish and magnetic stirrer extractions.
- Table 2.** Recovery of capsaicin from spiked ground Cayenne pepper samples.
- Table 3.** Extraction of capsaicinoids from ground Cayenne pepper using different organic solvents.
- Table 4.** Pungency level and consistency of commercial salsas selected for the extraction of capsaicin.
- Table 5.** Solids percentage, capsaicin content, and viscosity evaluation of commercial salsas.
- Table 6.** Capsaicin content extracted with different solvents from selected commercial salsas.

Table 1

Goldfish				Magnetic stirring			
1 h	2 h	3 h	4 h	5 min	10 min	15 min	20 min
1.55	1.55	1.57	1.56	1.36	1.48	1.49	1.49
(0.05) ^a	(0.06) ^a	(0.05) ^a	(0.04) ^a	(0.08) ^b	(0.02) ^a	(0.03) ^a	(0.02) ^a

^{a,b} Means of three determinations. Values in the same extraction procedure followed by different letters are significantly different ($p < 0.05$). Standard deviations are indicated in brackets.

Table 2

Original amount (mg/g)	Spiked level (mg/g)	After spiked (mg/g)	Recovery ^a (%)	CV ^b (%)
1.48 (0.9)	0.9	2.28	88.3 (2.4)	2.7
1.48 (0.9)	1.4	2.85	97.5 (1.7)	1.7
1.48 (0.9)	1.9	3.28	92.3 (2.1)	2.3
1.48 (0.9)	2.7	4.57	108.1 (6.3)	5.8

^a Average of three determinations. Standard deviations are indicated in brackets.

^b Coefficient of variation.

Table 3

Solvent	Capsaicinoids (mg/g of Cayenne pepper)		
	Nordihydro-capsaicin	Capsaicin	Dehydrocapsaicin
95% ethanol	0.18 (0.01) ^a	1.47 (0.03) ^a	0.74 (0.02) ^a
methanol	0.21 (0.01) ^b	1.42 (0.03) ^a	0.75 (0.07) ^a
acetone	0.18 (0.02) ^a	1.43 (0.09) ^a	0.73 (0.01) ^a
acetonitrile	0.18 (0.01) ^a	1.50 (0.03) ^a	0.68 (0.06) ^b
chloroform	nd ^c	nd	nd

^{a,b} Means of three determinations. Values in the same column with different letter significantly differ ($p < 0.05$). Standard deviations are indicated in brackets.

^c Not detected

Table 4

Brand	Pungency level ^a	Type of salsa
Tabasco®	-	liquid
La Victoria®	mild	red taco sauce, thick
La Victoria®	hot	red taco sauce, thick
Zapata®	mild	enchilada sauce, semi-liquid
Zapata®	hot	liquid
Frank's®	hot	liquid
Chi-chi's®	mild	chunky salsa
Chi-chi's®	medium	chunky salsa
Chi-chi's®	hot	chunky salsa

^a As indicated in the label.

Table 5

Salsa	Solids (%)	Capsaicin content (mg/g of salsa db) blended	Capsaicin content (mg/g of salsa db) blended and dried	Viscosity (mPa.s)
Chi-chi's mild	8.2 (0.28)	0.41 (0.01) ^{aA}	0.48 (0.02) ^{aA}	1,569.6 (30.65)
Chi-chi's medium	8.5 (0.02)	0.86 (0.03) ^{aB}	0.88 (0.02) ^{aB}	1,744.4 (9.67)
Chi-chi's hot	8.8 (0.04)	1.28 (0.03) ^{aC}	1.23 (0.19) ^{aC}	1,824.1 (15.40)
Frank's liquid	14.1 (0.02)	0.09 (0.01) ^{aD}	0.08 (0.01) ^{bD}	179.2 (0.56)
Tabasco	4.2 (0.04)	3.34 (0.04) ^{aE}	3.67 (0.02) ^{bE}	67.6 (0.12)
La Victoria mild	11.3 (0.01)	nd ^I	0.04 (0.01) ^F	2,505.2 (34.60)
La Victoria hot	11.8 (0.12)	0.11 (0.01) ^{aDF}	0.09 (0.2) ^{aD}	2,513.4 (15.66)
Zapata enchilada	11.8 (0.05)	1.40 (0.05) ^{aG}	1.32 (0.03) ^{aC}	3,046.2 (27.35)
Zapata liquid	10.9 (0.02)	0.13 (0.03) ^{aF}	0.12 (0.01) ^{bG}	92.2 (0.14)

^{a,b} Means of three replicates. Values in the same row with different lowercase letters are significantly different ($p < 0.05$). Standard deviations are indicated in brackets.

^{A-H} Values in the same column with different uppercase letters are significantly different ($p < 0.05$).

^I Not detected.

Table 6

Capsaicin content (mg/g db)								
Solvent	Tabasco		Enchilada		Chi-chi's (medium)		La Victoria	
	blended	blended and dried	blended	blended and dried	blended	blended and dried	blended	blended and dried
ethanol	3.34 (0.04) ^{aA}	3.67 (0.01) ^{bA}	1.40 (0.05) ^{aA}	1.32 (0.03) ^{aA}	0.86 (0.03) ^{aA}	0.88 (0.02) ^{aA}	0.11 (0.01) ^{aA}	0.11 (0.01) ^{aA}
methanol	3.32 (0.03) ^{aA}	3.66 (0.06) ^{bA}	1.53 (0.06) ^{aB}	1.47 (0.02) ^{aB}	0.90 (0.12) ^{aA}	0.93 (0.01) ^{aA}	0.09 (0.02) ^{aB}	0.09 (0.01) ^{aB}
acetoni- trile	3.16 (0.14) ^{aB}	3.23 (0.41) ^{aB}	1.08 (0.16) ^{aC}	0.86 (0.07) ^{bC}	0.95 (0.02) ^{aB}	0.43 (0.01) ^{bB}	0.11 (0.01) ^{aA}	0.10 (0.01) ^{bC}
acetone	2.93 (0.11) ^{aC}	2.92 (0.21) ^{aC}	2.11 (0.06) ^{aD}	1.19 (0.08) ^{bD}	0.94 (0.06) ^{aB}	0.51 (0.06) ^{bC}	nd ^c	0.07 (0.01) ^D
chloro- form	nd	nd	nd	nd	nd	nd	nd	nd

^{a,b} Means of three replicates. Values in the same row with different lower case letters are significantly different ($p < 0.05$). Standard deviations are indicated in brackets.

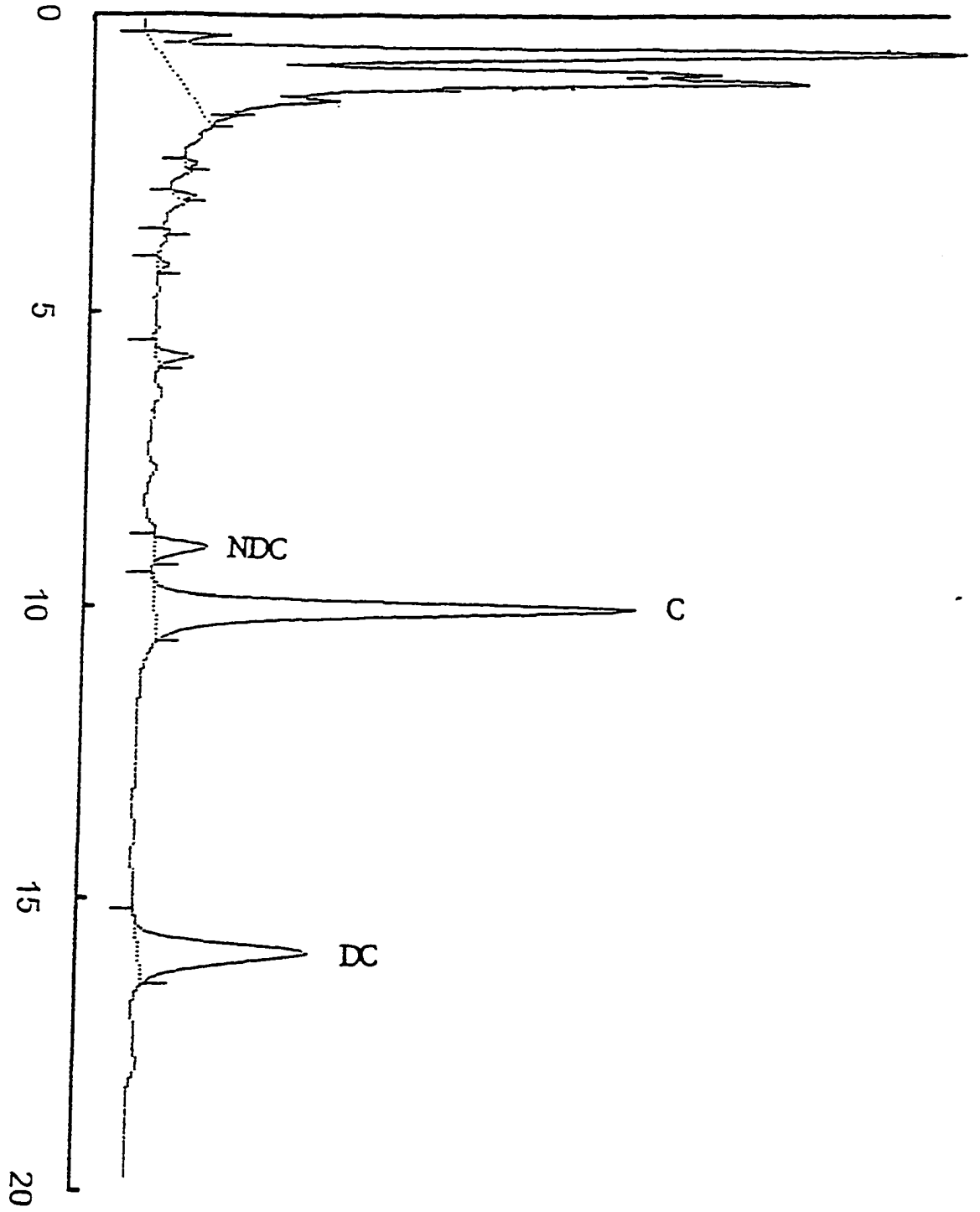
^c Not detected.

A-D Values in the same column with different uppercase letters are significantly different ($p < 0.05$).

FIGURE CAPTIONS

FIGURE 1. HPLC chromatogram of capsaicinoids extracted from ground Cayenne pepper with 95% ethanol. (NDC: nordihydrocapsaicin; C: capsaicin; DC: dehydrocapsaicin).

Detector Response



Time (min)
Figure 1

**EVALUATION OF COMPOSITION, AROMA, AND PUNGENCY
IN COMMERCIAL SERRANO PEPPERS (*CAPSICUM
ANNUUM*) AT THREE MATURITY STAGES: GREEN,
YELLOW, AND RED**

A paper to be submitted for publication to the Journal of Food Science

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ABSTRACT

Dry matter, soluble solids, titratable acidity, pungency level, volatile concentration, and aroma mapping were used to evaluate serrano peppers at three maturity stages: green, yellow, and red. The red stage was higher in the soluble solids content. No difference was found in titratable acidity or capsaicin content. The sugar/acid ratio was higher for the yellow and red stages. The three stages showed differences in the aroma mapping as evaluated by electronic nose. Untrained panelists were unable to identify different stages and discriminate which sample has the highest intensity in aroma.

INTRODUCTION

Capsicum (*Capsicum annuum*) cultivars have the special characteristic, not found in other fruits, that they can be consumed

in immature and mature stages (Luning, et al. 1994a). For instance, Yuksel et al. (1994) reported that bell peppers are eaten both as unripe and ripe fruits because of their different flavor.

Limited information was found reporting changes in chemical characteristics in chiles at different ripening stages. As the fruit ripens, changes in color are more perceptible. This change is often the major criterion used by consumers to determine whether the fruit is ripe or unripe (Wills, et al., 1989). The most common change is the loss of the green color associated with the synthesis and/or revelation of pigments ranging from yellow to red. Some chiles change directly from green to red, like Jalapeño, while others change from green to yellow and then to red, like serrano.

The substances responsible for the pungency in capsicums are a group of related compounds called capsaicinoids. Nordihydrocapsaicin, capsaicin, and dehydrocapsaicin constitute about the 95% of the total and they have the most important role on the pungency sensation. Capsaicin is found in higher concentration than the other two components (Krajewska and Powers, 1987). Factors such as variety, geographical location, climatic condition, stage of maturity, and location within the fruit have been reported to influence capsaicin content. Maga (1975) cited some reports where the concentration of capsaicinoids varied in a wide range among varieties and also among

plants within the same variety. Govindarajan (1986) reported that capsaicinoids were not found in high-pungency capsicums until 21 days after the fruit set and they rapidly increased from 50- to 70-fold up to 49 days after this point.

Luning et al. (1994b) evaluated the flavor of fresh bell peppers at different ripening stages perceived while eating. Sweetness, sourness, and red bell pepper aroma were characteristic attributes for ripe red stages, and bitterness and "green" flavor notes, such as grassy, herbal, cucumber, and green bell pepper aroma, characterized immature green stages. Luning et al. (1994c) studied the composition of volatile compounds of a Dutch bell pepper (*Capsicum annuum* cv. Mazurka) at three different ripening stages. Results showed that different bell peppers samples had several odor compounds in common: 2-3, butanedione, 1-penten-3-one, hexanal, 3-carene, (Z)- β -ocimene, octanal, and 2-isobutyl-3-methoxypyrazine. This authors also found that during bell pepper maturation, the majority of volatile compounds decreased or even disappeared. Chitwood et al. (1983) suggested a relation between the sensory-perceived "green" aroma and the concentration of 2-isobutyl-3-methoxy-pyrazine, 2-sec-butyl-3-methoxypyrazine, and (Z)-3-hexenol on three cultivars of *Capsicum annuum*: Jalapeño, Anaheim, and Fresno. Haymon and Aurand (1971)

found 125 components in the neutral volatile fraction of Tabasco peppers (*C. frutescens*). Huffman et al. (1978), found that the flavor of fresh Jalapeño pepper is primarily due to 2-isobutyl-3-methoxy-pyrazine.

Govindarajan (1985) reported that citric acid is the major acid in chiles, the others being succinic, fumaric, malic, and quinic acid. Carbohydrates increase rapidly from the green stage to the ripe stage.

In Spanish, *serrano* is an adjective meaning "from the mountains". This chile was first grown in the mountains of northern Puebla and Hidalgo, Mexico. Immature fruits are dark green in color and ripen to red or sometimes brown, orange, or yellow. DeWitt and Gerlach (1990) reported that serranos vary in heat between 10,000 and 23,000 Scoville Units. Among the more known varieties of serranos, the most important are Long, Balin, Tipico, Altamira, Panuco, Tampiqueño, and Hidalgo. The most common use of serranos is in fresh salads where the chile is minced and combined with a variety of vegetables (DeWitt and Gerlach, 1990).

In the food market it is possible to find serrano peppers at different ripening stages (green, yellow, and red). However, both consumer and food processor could be confused about the properties and uses of these peppers because of the lack of information about the specific characteristics on each of the maturity stages. No previous

reports were found in the literature evaluating the changes in serrano pepper characteristics at different ripening stages. Accordingly, our objective was to evaluate differences in composition, aroma, and pungency in commercial serrano peppers (*Capsicum annuum*) at three maturity stages: green, yellow, and red.

MATERIALS AND METHODS

Materials

Serrano peppers selected at different ripening stages, namely "green, yellow, and red" according to surface color, were purchased from a Mexican-products grocery store in Des Moines, IA. It was not possible to determine the origin of these peppers. Different types of capsicums: Jalapeño, habanero, Poblano, chile guero, Anaheim, and bell pepper (green, yellow, orange, and red) were purchased from a grocery store in Ames, IA.

Capsaicin (8-methyl-N-vanillyl-6-nonenamide, 60% pure), was obtained from Sigma Chemical Co. (St. Louis, MO). Different concentrations were prepared in HPLC-grade methanol and used for retention-time verification and instrument calibration. HPLC-grade methanol, HPLC-grade acetonitrile, and HPLC-grade acetic acid were purchased from Fisher Scientific (Fair Lawn, NJ).

Dry matter content

Whole sliced serrano peppers were dried in a food dehydrator (American Harvest™ Snackmaster® Dehydrator, Mod. FD 50/30, Alternative Pioneering Systems, Chaska, MN) at 70-72°C for 4 h, or until a constant weight was reached.

Total soluble solids content

Serrano peppers were hand-cut in slices and the placenta and seeds were removed. The pericarp was hand-sliced into fine pieces and ground in a mortar. The sample was manually pressed in a cheese cloth and the filtrate was measured in an Abbe-3L Refractometer (Milton Roy, Co., Rochester, NY).

Titrateable acidity

Serrano pepper preparation was similar to the procedure for the soluble solids. The pepper was ground in a mortar and the extract was placed in a 100-mL volumetric flask. Purified water (50 mL, reagent grade water, Milli-Q Reagent Water System, Millipore Corp., Bedford, MA) was added to the flask and it was swirled for 3 min and filled with purified water to the mark. The sample was filtered through several layers of cheese cloth and 40 mL of the filtrate was used titrated with 0.1025N NaOH. Titration was carried-out until a pH of 8.2 was reached (Corning pHmeter, Mod. 340, Corning, NY). Acidity was expressed as

citric acid.

HPLC analysis

Sample preparation

Dry peppers prepared under the same conditions as for dry matter content, were ground in a Moulinex® spice mill Mod. 505 (Mam, France) and screened through a 1-mm screen, and stored in 20-mL capped glass vials at 20-23°C. All samples were analyzed within 7 days of drying.

Capsaicin extraction

For capsaicin analysis, a 1:10 (gram: milliliter) ratio of dried chile powder to HPLC-grade acetonitrile was placed in 120-mL glass bottles, sealed with Teflon-coated rubber septa and aluminum crimp caps. Bottles were placed in a 70°C oven for 4 h; they were swirled manually every hour. Samples were removed from the oven and allowed to cool at room temperature. Three to 4 mL of the supernatant was extracted and filtered in a 10-mL disposable syringe attached with a disposable 0.45- μ m nylon 66 membrane (Alltech Assoc., Deerfield, IL) and 2 mL of the filtrate was transferred into a 2-mL vial, capped, and stored at 4.4°C until analyzed. A 20- μ L aliquot was used for each HPLC injection.

Instrument

A Beckman System Gold® HPLC (Beckman Instruments, Inc., San Ramon, CA) instrument equipped with a Model 110 pump and a Beckman UV detector set at 280 nm was used. The reverse-phase separations were carried out in a Supelco C-18 column (Supelco, Inc., Bellefonte, PA), 25 x 0.46-cm i.d. and 5- μ m particle size. The isocratic mobile phase consisted of water:HPLC-grade acetonitrile:acetic acid (55:45:1). The flow rate was set at 1.5 mL/min.

GC analysis**Sample preparation**

Serrano pepper slices (2 g) of each of the three ripening stages were placed in a 20-mL vial sealed with Teflon rubber septa and aluminum caps. Samples were heated at 70°C in the oven for 10 min, allowed to cool at room temperature for 10 min and then a headspace sample was injected into the gas chromatograph.

Instrument

Headspace samples were analyzed with a Varian 3700 gas chromatograph (Palo Alto, CA) equipped with a flame ionization detector. The column was a 30-m fused silica DB-5 capillary column (J&W Scientific, Folsom, CA) with a 1.0- μ m film thickness and 0.25 mm i.d. The oven temperature was initially held at 50°C for 2 min and then

programmed at 10°C/min to 220°C and held at this temperature. The injector and detector temperatures were 110°C and 230°C, respectively. The column flow rate was 1 mL/min of nitrogen. Total nitrogen flow at the detector was 30 mL/min. Air and hydrogen flow rates were 300 mL/min and 30 mL/min, respectively. Detector sensitivity was set at 1×10^{12} amp/sec. A variation of the on-column cryofocusing technique of Wilson et al., (1992) was used for injection of the sample. A loop of the column was placed in liquid nitrogen while the sample was obtained and injected. A 5-mL gas tight Hamilton syringe (Hamilton Co., Reno, NE) was used to withdraw a 3-mL sample of the headspace vial. Expelling 1 mL, the remaining 2 mL was injected into the chromatograph at a 1 mL/min rate. The split ratio was 20:1. After 30 sec, the splitter was turned on, and after the next 30 sec the liquid nitrogen was removed and the temperature program started.

GC-MS analysis

Isolation of volatiles

Serrano pepper samples in the red stage were prepared by slicing 5 g of the sample and transferred it to a glass-volatiles stripping apparatus (Lee et al., 1995), for dynamic headspace isolation of volatile compounds. The apparatus was immersed in a 50°C water bath and flushed with helium at 75 mL/min for 30 min. The volatiles stripped

from the pepper were trapped in a 3-mm o.d. x 72-mm glass tube filled with Tenax TA (Alltech Assoc., Deerfield, IL) as an adsorbent.

Desorption of volatiles and GC-MS detection

Volatile compounds were desorbed from the Tenax packed tubes, located inside the injector port of a Hewlett-Packard Model 5890A gas chromatograph (Hewlett-Packard Co., Avondale, PA), at 230°C, transferred in helium at 1.5 mL/min onto a SPB-1 fused-silica capillary column (30 m, 0.25mm i.d., 0.25- μ m film thickness) (Supelco, Bellefonte, PA) and condensed in the first loop of the column, which was cooled in a dry ice bath. After exactly 5 min of desorption and transfer, the dry ice bath was removed and the temperature was held for 3 min at 40°C and raised from 40° to 250°C at 10°C/min. Mass spectra were recorded in the electron impact mode in a Hewlett-Packard 5970 mass selective detector (Hewlett-Packard, Co., Avonlea, PA).

Electronic nose analysis

Serrano peppers (green, yellow, and red stages), bell peppers (green, yellow, orange, and red stages), Jalapeño, Anaheim, chile guero, Habanero, and Poblano peppers (*Capsicum annuum*, spp.) were visually selected cut in 2-mm slices. Three grams of slices from each pepper were transferred into 500-ml taint-free barrier plastic pouches with a

special connector for attachment to the electronic nose (AromaScan®, Mod. A32S, AromaScan, Inc., Hollis, NH). The pouches were filled with filtered air at 30°C and 45% relative humidity, and allowed to equilibrate at 30°C for 15 min. During this period, volatiles were allowed to accumulate in the headspace. After equilibration, headspace-air was pulled across the sensors. The sampling procedure for the electronic nose was: reference (30 sec), sampling (120 sec), washing (90 sec), and final calibration (30 sec). Measurement interval for each sample was 1 sec.

Sensory evaluation

Aroma evaluation was carried out using 20 untrained panelists, with ages between 20 and 45 years-old all, of them graduate students and staff from the Dept. of Food Science & Human Nutrition, Iowa State University. Sliced serrano peppers were placed in 15-mL glass vials, capped, and wrapped with aluminum foil. Vials were placed at 40°C in the oven for 10 min to facilitate the release of aroma compounds. After that, they were allowed to cool to room temperature and tested. The samples were presented labeled with random three-digit numbers. Three vials containing each of the three maturity stages were presented to the judges, who were instructed to uncap and sniff each sample and indicate the intensity of the aroma on a semi-

structured 15-cm line scale anchored with "low intensity" on the left and "high intensity" on the right side. Scores were determined by the simple ranking test procedure (Meilgaard, et al., 1987). A difference test was carried out by using a triangular test. Equal numbers of the six possible combinations were prepared as described in the aroma test, and presented at random to the panel members. They were asked to identify the odd sample. Every member carried out two different triangular tests. Analysis of results was evaluated according to the method described by Meilgaard et al. (1987). Evaluations were conducted in partitioned booths illuminated by red lights. Scores from the ranking tests were analyzed by using the Friedman analysis (Meilgaard, et al., 1987).

Statistical Analysis

All data was analyzed by one-factor completely randomized analysis of variance. Means from the soluble solids content, titratable acidity, and dry matter represent an average of ten replicates. Average values from the electronic nose response for serrano peppers, represented data obtained from 20 replicates for each maturity stage. Sensor-average responses for the other chile varieties represented 5 replicates. Euclidean distance measurement was used to evaluate differences among means in serrano peppers and among other chile varieties. Analysis of Variance (ANOVA) and Least Significant

Difference (LSD) test were carried out in the SAS Statistical Analysis Program (SAS Institute, Inc., 1985) with significance established at $p < 0.05$.

RESULTS AND DISCUSSION

Govindarajan (1985) reported that the composition of carbohydrates, organic acids, volatile compounds, and color components changes during maturation of capsicums. Color changes usually go from green to red. In serrano peppers, an intermediate stage (yellow color) is observed between the green and the red stage. Dry matter, total soluble solids, and titratable acidity data from the serrano peppers are showed in Table 1. Dry matter increased ($p < 0.05$) gradually with the ripening stage. Because the peppers were not stored in hermetic packages, loss of water could explain this increase in solids. This result was highly correlated with reduction in the average fresh weight of these peppers (data not shown).

A gradual increase in soluble solids content was observed among the three stage, although no difference ($p < 0.05$) was detected between the green and yellow stages. Many consumers relate the red stage with a sweeter perception compared with the others. This phenomena results from the breakdown of starch into sugars, which produces an increase in the soluble solids content (Wills et al., 1989). The percentage of acidity, based on titration of citric acid, was not

significantly different in the three ripening stages. Few references related with capsicums were found that mentioned changes in composition in relation to different maturity stages. In our case, we expected to detect a decrease in % acidity and, at the same time, an increase in soluble solids, according to the normal pattern observed in the majority of fruits during ripening (Reid, 1992; Wills, et al., 1989). Although the sugar/acid ratio is used as the legal maturity index for citrus (Reid, 1992), under the same assumptions we expected to detect an increase in this ratio as the pepper ripened. We found an increase in this ratio (Table 1) from the green stage to the yellow and red stages. However, no apparent difference occurred between the yellow and red stages.

HPLC results

Identification and quantification of capsaicin was accomplished by comparing the peak retention times with those of the external standard. The elution peaks agreed with that reported by Collins et al. (1995). Although several authors have reported the concentration of capsaicinoids in different varieties of chiles (Krajewska and Powers, 1987; Collins, et al., 1995; Edwards et al., 1990; Govindarajan, 1985; Govindarajan 1979), a limited number have showed results on serrano peppers. Quinones-Siegle et al. (1989) reported the percentage of

capsaicin and dehydrocapsaicin in different varieties of serranos: TAM Hidalgo, Panuco, and Tampiqueño.

Quantification of capsaicin in green, yellow, and red serranos is presented Table 2. No significant difference ($p < 0.05$) was found among the three ripening stages. This result contradicts the popular belief, at least for this pepper, that red peppers are "hotter" or more pungent. It is possible that the consumer creates a psychological relation between color and pungency level.

The high values obtained in the standard deviations suggested a relatively high variation in capsaicin content among the same fruit at identical ripening stage. These results corroborate the citation made by Maga (1975) that capsaicinoids are present in a great variability in fruits from the same plant. Purseglove et al. (1981) reported a gradual increase in the capsaicin levels as the chile matured.

Although no data has been previously reported for serrano peppers at different maturity stages, our calculated range of capsaicin concentration agreed with those data reported for different varieties of *C. annuum* (Collins, et al., 1995; Edwards, et al., 1990). The ratio of capsaicin to dehydrocapsaicin in the three ripening stages of serrano peppers averaged 1.4:1, compared with 1.6:1 in *C. frutescens* (Tabasco pepper) and 1.4:1 in *C. baccatum* var. *pendulum* (green pepper), reported by Quinones-Siegle, et al. (1989).

Scoville Heat Units (SHU) were calculated based on the quantification of ppm of capsaicin according the equation from the Method 21.1 of the American Spice Trade Association (ASTA,1985). Conversion to Scoville Heat Units can be made by multiplying the ppm of capsaicin by a factor of 15. According to the results shown in Table 2, no significant difference ($p<0.05$) was observed among the Scoville Units in the three ripening stages. The average value of the Scoville Units for the three stages (23,233 SHU) is higher than the range reported by DeWitt and Gerlach in 1990 (5,000-15,000 SHU) as well as the SHU reported by Quinones-Siegle et al. (1989) for serrano peppers cultivars: TAM Hidalgo, Panuco, and Tampiqueño with 6 000, 7 500, and 8 000 SHU respectively.

GC-MS results

Although Wu and Liou (1986) observed that disruption of the cell structure affected the composition of volatile compounds of bell peppers, we found in previous work with serrano peppers, that the same peaks extracted in the whole pepper appeared in the sliced chiles (data not shown). In our case, the difference was a higher peak areas from the cut pepper. Figure 1 illustrates the gas chromatograms of the headspace of sliced serrano peppers in the maturation stages green, yellow, and red. Comparison of these stages shows that the total area peaks increased during maturation. A significant difference ($p<0.05$)

was found between the total area peak of the red stage compared with both green and yellow stages. No difference was found between green and yellow stages. In another study, Luning et al. (1994c) reported that total peak areas decreased during maturation of bell peppers (*C. annuum* cv. Mazurka). Identification of the different compounds from the red serrano headspace concentrates was based upon mass spectral matching with the NBS mass spectral data bases. The identified volatiles are listed in Table 3. Compounds that we identified and have been previously identified in other capsicum varieties (Luning et al., 1994b; Keller et al., 1981; Chitwood et al., 1983; Haymon and Aurand, 1971; van Ruth et al., 1994; Buttery et al., 1969; Luning et al., 1995) were: alpha-pinene, beta-pinene, 3-carene, limonene, pentadecane, and heptadecane.

Electronic nose results

The average response for each of the 32 sensors from the electronic nose is shown in Table 4. A significant difference ($p < 0.05$) was found by comparing the average results for each sensor in the green, yellow, and red stages. The relative responses of individual sensors reflect the range of volatiles given off by the sample. The amplitude of the response is determined by the number of volatiles competing for adsorption, their concentration, their diffusion coefficients, and their polarity (AromaScan, 1995). The higher response

obtained from the red stage reflects, in part, a higher concentration of volatiles compared with the responses from the green and yellow stages. This result agrees with those obtained from the gas chromatograph, where the higher area peaks, related directly with concentration, were observed for the red stage.

Measurement of different aromas by the electronic nose produce different patterns that are projected into a multidimensional space (Sammon, 1969). Figure 2 shows this response in a two-dimensional space, representing the equipment detection for each sample (20 replicates each) in the three maturity stages. We observed that several samples from one stage overlap or are very close to a different cluster. Although we tried to analyze representative samples from each stage, the lack of availability of the product and natural differences present in the samples, resulted in a diverse range of physiological states with diverse characteristics among them. However, in Figure 2 it is possible to identify three separate clusters which represent the maturity stages of the sample. The yellow stage, which represents an intermediate step between the green and red stage, presented the most diverse range of distribution.

Comparing the average values for the three stages and using the Euclidean distance to measure similarities between samples, we observed that the aroma of the yellow sample is more similar to the

aroma of the red one (Figure 3). Comparing the electronic nose response of different varieties of chiles, we observed a great variability in responses. Each pepper type produced a different response suggesting an specific aroma pattern in every one of these products. Responses from the bell pepper produced different patterns in each maturity stage: green, yellow, orange, and red.

Sensory evaluation results

There was no significant differences ($p < 0.05$) found in the triangular test or in the ranking test. This means that the panelists were unable to differentiate among the three maturity stages by smelling and, in the same way, they were unable to determine what maturity stage had a more intense aroma. Although the GC analysis showed that the red stage had more amount of volatiles than the other stages, they apparently did not influence the panelists perception of intensity. Besides this, it is likely that common compounds present in the three stages, although not in high concentrations, could play an important function on the perception of the intensity.

CONCLUSIONS

A higher content of soluble solids resulted in the red stage. This stage also presented the higher concentration of volatiles, although panelists were unable to detect differences and intensities among the

three maturity stages. The capsaicin content was similar in any ripening stage. Differences in aroma patterns among the serrano peppers and different capsicum types were detected by the electronic nose.

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TABLE CAPTIONS

- Table 1.** Dry matter, soluble solids, % acidity, and sugar/acid ratio in serrano peppers at three different ripening stages: green, yellow, and red.
- Table 2.** Capsaicin content and Scoville Heat Units (SHU) of serrano peppers at three ripening stages: green, yellow, and red.
- Table 3.** Identified volatiles by gas chromatography-mass spectrometry in serrano peppers in the red stage.
- Table 4.** Sensor responses of the electronic nose detection of aroma in serrano pepper.

Table 1

Analysis	Green	Yellow	Red
Dry matter (%)	12.2 (0.8) ^a	14.5 (1.5) ^b	15.5 (1.4) ^b
Total soluble solids (%)	5.6 (0.7) ^a	6.8 (1.8) ^a	8.3 (0.7) ^b
% acidity	1.7 (0.8) ^a	1.4 (0.4) ^a	1.8 (0.6) ^a
Sugar/acid ratio	3.3 ^a	4.9 ^b	4.6 ^b

^{a,b} Means (n=10) within a row having different superscripts are significantly different (p<0.05)
Standard deviations are presented in brackets.

Table 2

	Green	Yellow	Red
Capsaicin content (mg capsaicin/g pepper d.b.)	0.94 (0.48) ^a	0.97 (0.66) ^a	0.99 (0.70) ^a
Range of capsaicin content	0.41-1.70	0.23-1.87	0.23-2.24
SHU	21,945 ^a	23,407 ^a	24,348 ^a

^a Means (n=10) in the same row having different superscript are significantly different ($p < 0.05$). Standard deviations are presented in brackets.

Table 3

alpha-pinene	beta-pinene	3-carene
1-methyl-2-(methylethyl) benzene	limonene	nonanol
1-methoxy-4-(2-propenyl)-benzene	butyl-2-methyl butanoate	cis-2-methyl-octadecene
3-methyl-dodecane	tetradecane	4-methyl-tetradecane
pentadecane	(Z)-3-hexadecene	1,15-pentadecadienol
8-heptadecene	heptadecane	(E)-3-octadecene
2-methyl-heptadecane	(E)-9-octadecene	cyclopentadecane
1-tetradeceno	9-nonadecene	nonadecane
hexadecanoic acid	3-methyl-nonadecane	

Table 4

Sensor number	Maturity stages ^a		
	Green	Yellow	Red
1	2.69	3.37	5.05
2	2.56	3.15	4.62
3	2.64	3.16	4.76
4	2.62	3.16	4.73
5	2.44	3.03	4.48
6	2.39	2.98	4.39
7	2.65	3.23	4.79
8	2.67	3.28	4.86
9	2.34	2.91	4.28
10	2.65	3.24	4.90
11	2.45	3.02	4.45
12	2.43	3.02	4.50
13	2.42	2.00	4.41
14	2.56	3.13	4.78
15	2.16	2.69	4.03
16	2.20	2.71	3.99
17	2.19	2.68	3.98
18	2.05	2.54	3.80
19	2.60	3.27	4.89
20	2.42	3.00	4.44
21	2.57	3.17	4.66
22	2.17	2.71	4.05
23	2.18	2.75	4.12
24	2.19	2.75	4.15
25	2.38	2.94	4.39
26	2.47	3.03	4.50
27	2.64	3.16	4.81
28	2.53	3.10	4.60
29	2.65	3.36	5.00
30	2.68	3.27	4.84
31	2.69	3.36	4.99
32	2.74	3.41	4.94

^a Means of 20 replicates. Green, yellow, and red stages were significantly different in every sensor ($p < 0.05$).

FIGURE CAPTIONS

- Figure 1.** Headspace-gas chromatograms of serrano peppers at three different ripening stages.
- Figure 2.** Electronic nose mapping of headspace from serrano peppers at three different ripening stages: green, yellow, and red.
- Figure 3.** Electronic nose mapping of headspace of average values of three ripening stages of serrano peppers and different types of commercial chiles.

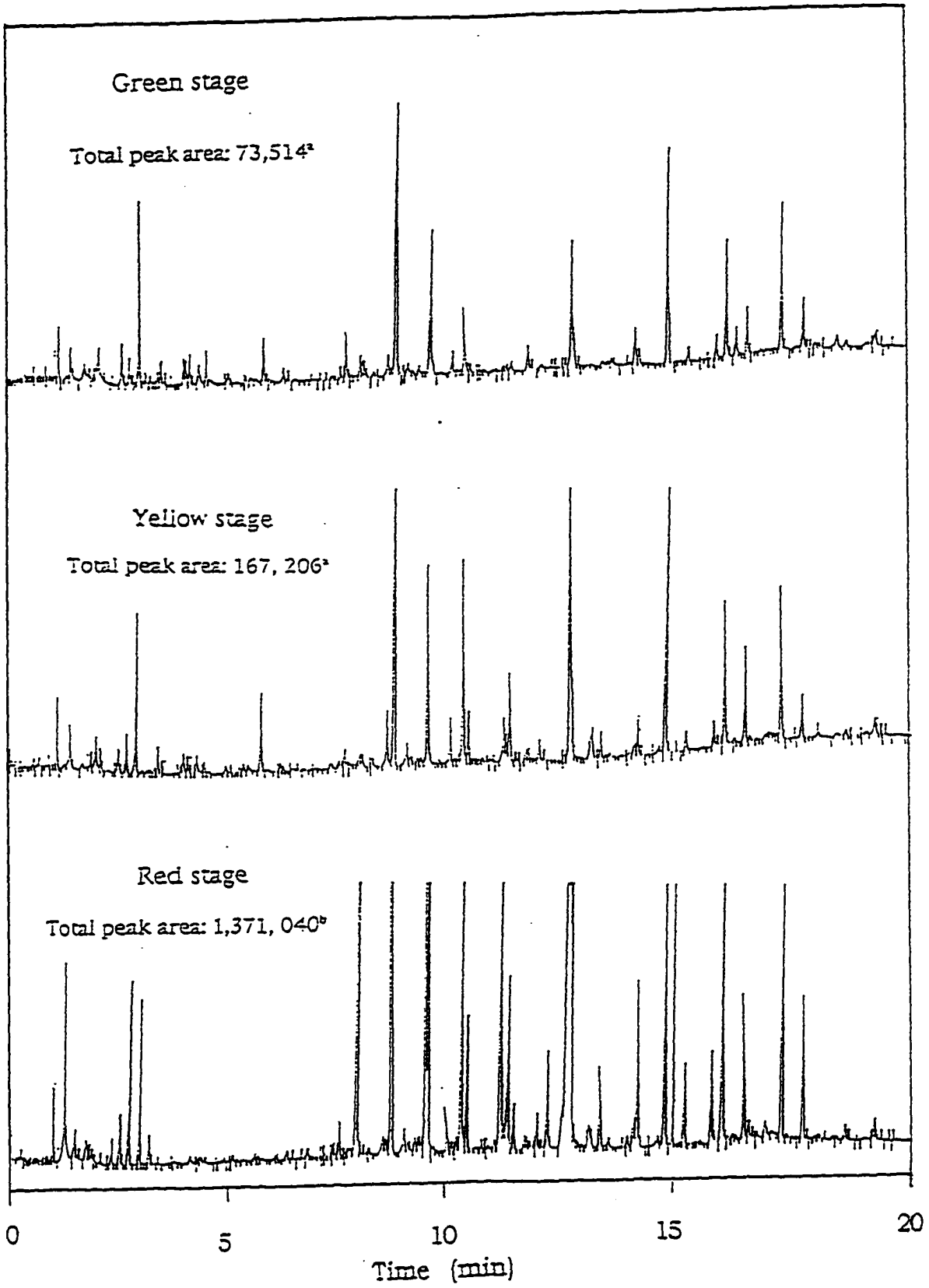


Figure 1

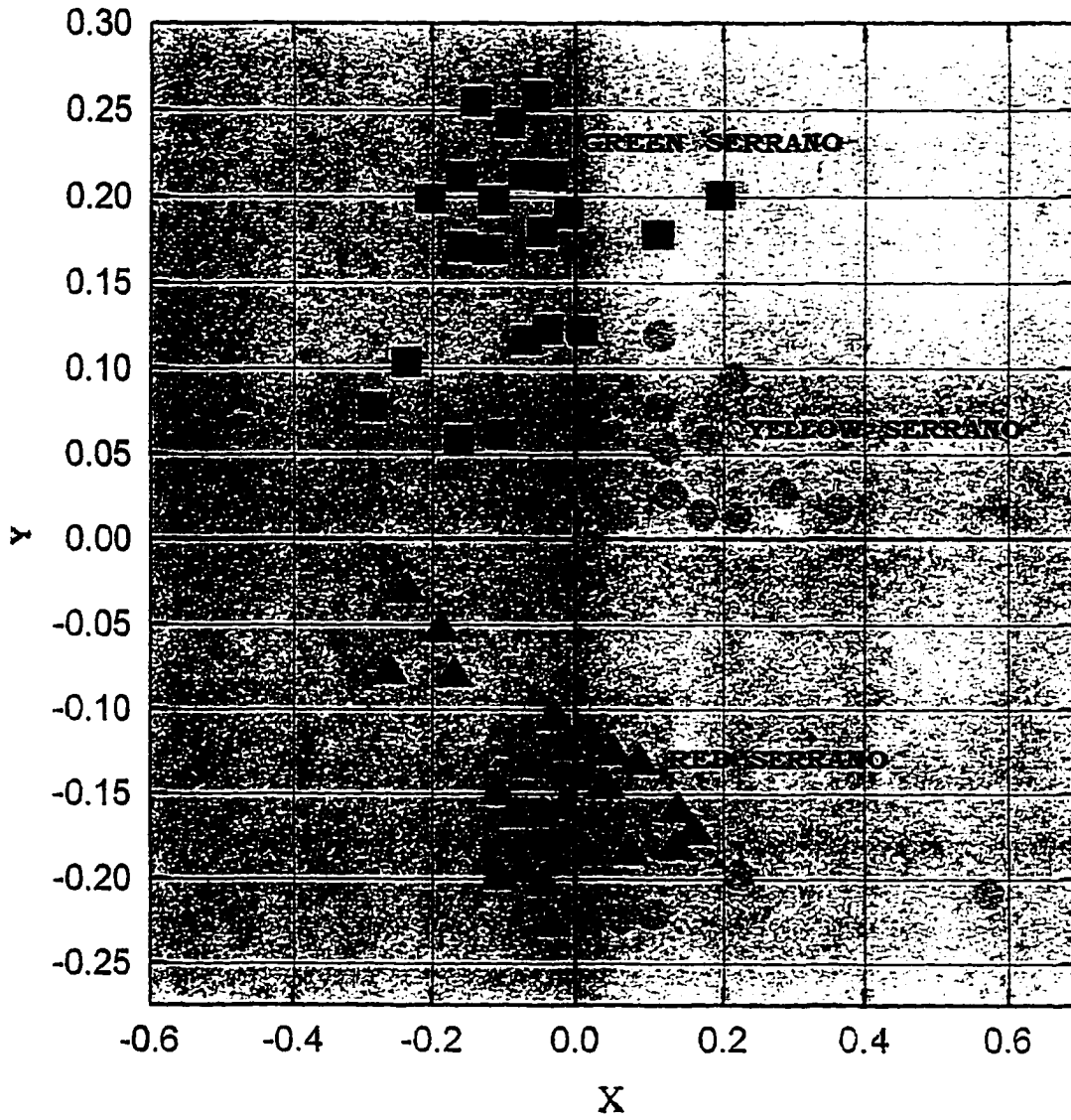


Figure 2

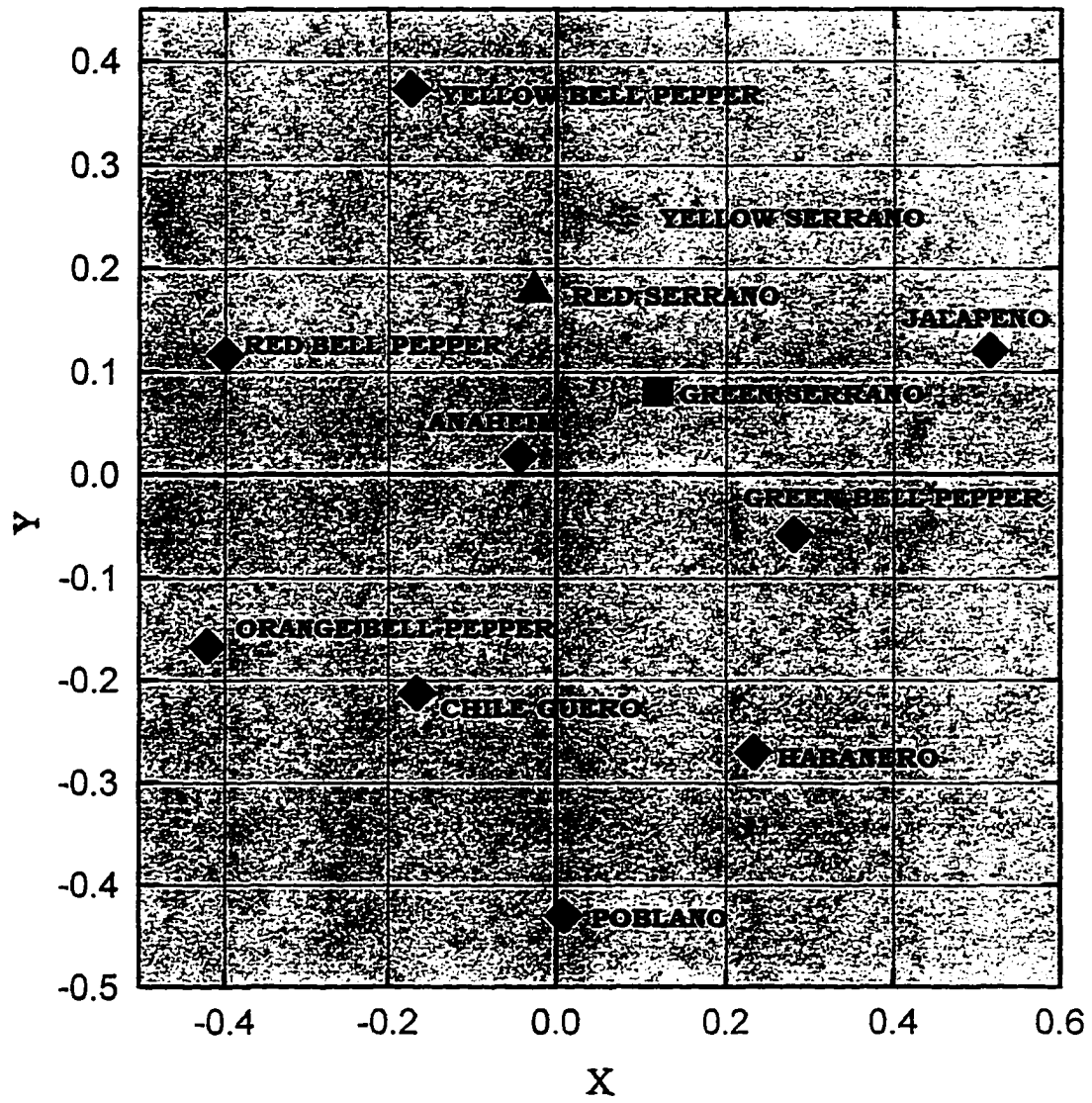


Figure 3

**THERMAL DEGRADATION OF CAPSAICIN
IN DIFFERENT MATRIXES UNDER CONVENTIONAL AND
MICROWAVE HEATING**

A paper to be submitted for publication to the Journal of Food Science

Alfonso R. Rocha-Herrera and Lester A. Wilson

ABSTRACT

Thermal degradation of capsaicin was evaluated in different matrixes (pure capsaicin, palmitic acid, soybean oil, a mixture of palmitic acid-soybean oil, and Cayenne pepper in soybean oil), heated in conventional and microwave ovens. Degradation of pure capsaicin and capsaicin in palmitic acid during conventional heating followed a pseudo first-order reaction rate. Activation energy was calculated as 9.3 Kcal/mol. No degradation was observed during the conventional heating of capsaicin and Cayenne pepper, in a soybean oil matrix. In the mixture palmitic acid-soybean oil, capsaicin degradation was observed at high concentrations of palmitic acid. No degradation was detected in any matrix during microwave heating.

INTRODUCTION

The trend for consumption of hot and spicy foods has increased in the last few years (Sloan, 1996). This effect has promoted the creation of new processed foods and the using of new ingredients that

were not common in traditional diets. Spices increase the flavor perception of foods and in some instances, like chiles, pungency is the basic quality characteristic which the consumers desire. Very little information is available about the effect of cooking on the characteristics of the compounds responsible for pungency. These compounds are a group of structurally related substances called capsaicinoids. Three of them constitute about the 95% of this group: capsaicin, dehydrocapsaicin, and nordihydrocapsaicin (Krajewska and Powers, 1988). Huffman et al. (1978) found that thermally processed Jalapeño peppers (*Capsicum annum*) heated at 100°C for 50 min contained higher capsaicinoid concentration than fresh samples. They indicated that the treatment freed capsaicin, making it more available for analysis. Harrison and Harris (1985) using the same treatment as Huffman et al. (1978), observed a reduction of approximately one-half of the capsaicin compared with the raw product. However, they found an increase in capsaicin concentration in Jalapeño pepper boiled at 100°C for 10 min. They suggested that during the thermal treatment, capsaicin leached in the cooling water after blanching or in the brine during storage. During boiling, these authors suggested that the process could destroy enzymes that could decompose the pepper. They indicated that capsaicin could complexed with other compounds liberated during cooking. However, they did not explain how these

effects could increase the concentration of this compound.

In another study, Wijeratne (1985) reported that degradation of capsaicin followed a first-order kinetic in a model system. He indicated that low water activity (0.5) and low pH (2.2) increased the degradation rate. Addition of linoleic acid at low water activity increased this degradation as well. Srinivasan et al. (1992) evaluated the loss of capsaicin during heat treatment (boiling temperature) at different pH and cooking times. They found a higher reduction at pH 5.1 compared with pH 6.1. Heating time also increased this degradation (30 min compared with 15 min-boiling).

Henderson and Henderson (1992) evaluated the degradation of capsaicin alone and in the presence of oleic acid. Decomposition products of capsaicin led to the formation of vanillin, methylnonenoic acid, and methylnonenamide. The mixture with oleic acid produced 9-octadecenamide and secondary amides.

Evaluating the effect of heat treatment on the flavor perception, several studies have reported the disadvantage of microwave cooking in producing foods with desirable flavors (Hassel, 1989; Whorton and Reineccius, 1990; Katz, 1994; Schiffmann, 1994; Stanford and McGorin, 1994). Because of the characteristics of these treatment, flavors are volatilized, degraded, or not permitted to migrate and distribute throughout the product. However, no references were found

about the effect of microwave treatment on the characteristics of capsaicinoids in chiles.

There exists the implication that conventional heat treatment may be critically important in the processing of chiles and can play a significant role on the perception of pungency. Therefore, our objective in this study was to evaluate the degradation of capsaicin mixed in different systems: palmitic acid, soybean oil, a variable mixture of palmitic acid-vegetable oil, and pure capsaicin, all of them cooked under two treatments, conventional and microwave heating.

MATERIALS AND METHODS

Materials

Capsaicin (8-methyl-N-vanillyl-6-nonenamide; approximately 60%) and palmitic acid (hexadecanoic acid) were purchased from Sigma Chemical Co. (St. Louis, MO). A standard solution containing the capsaicinoids was prepared with methanol. This solution was used as an external standard in the high-performance liquid chromatography (HPLC) analysis to determine the retention time of capsaicinoids. HPLC-grade methanol, 95% ethanol, HPLC-grade acetonitrile, HPLC-grade acetone, HPLC-grade acetic acid, and HPLC-grade chloroform were purchased from Fisher Scientific (Fair Lawn, NJ). Ground Cayenne pepper was donated by Burns Philp Food Inc., (Ankeny, IA). Vegetable

oil (Crisco[®] soybean oil, Procter & Gamble, Cincinnati, OH) was purchased from a grocery store in Ames, IA.

Sample preparation and heating condition

Pure capsaicinoids

Capsaicin was weighted (0.3308 g) and dissolved in 100 mL of HPLC-grade chloroform. A 2.5-mL sample was transferred to 5-mL scintillation vials and left uncapped overnight to allow to volatilize the chloroform. The solid residue was heated in a conventional oven at 177°C from 1 to 8 h. Samples were taken every hour and allowed to cool at room temperature. Methanol (2 mL) was added to each vial, capped with a Teflon-lined screw-cap and stirred manually. Samples were stored at 4.4°C until injection into the HPLC.

An exact amount of capsaicinoids (4.0 mg) was weighted in 10-mL scintillation vials. Uncapped samples were heated in a microwave oven (General Electric, Mod. J E2810A 002, 1.4Kw) for 10, 20, 30, and 40 min at 50 and 100% power. Samples were allowed to cool at room temperature and 8 mL of methanol was added to each vial. The vial was capped with a Teflon-lined screw-cap, stirred manually, and stored at 4.4°C until injection into the HPLC.

Capsaicin-palmitic acid mixture

Capsaicin (0.6144 g) was added to 246.6 g of melted palmitic acid

(at 65-67°C) and the mixture was stirred with a magnetic stirrer for 1 h keeping the temperature at 70°C to maintain the acid in a liquid state. The concentration of capsaicin in the final mixture was 2.5 mg per gram of palmitic acid. Exactly 4.0 g of this mixture was weighted in 15-mL scintillation vials and heated uncapped in a conventional oven at 93, 121, 149, 177, and 188°C for 1, 2, 3, and 4 h. Palmitic acid was heated separately under the same conditions as control. After the heat treatment, samples were cooled, capped, and stored at room temperature until extraction.

For the microwave heating, 4.0 g of the mixture was weighted in scintillation vials and treated under the same conditions than those for capsaicin. The samples were stored at room temperature until extraction. Pure palmitic acid was processed in similar way as control.

Capsaicin-vegetable oil mixture

Capsaicin (0.5177 g) was dissolved in 2 L of soybean oil at room temperature by stirring with a magnetic stirrer. A sample (5 g) was transferred to 10-mL scintillation vials and heated under the same conditions as those used for the mixture capsaicin-palmitic acid. After cooling, the sample was capped and stored at room temperature until extraction. The same process was carried out with pure soybean oil as control.

For the microwave heating, 4.0 g of the sample was heated under the same conditions as those for the mixture palmitic acid capsaicin.

Capsaicin-soybean oil-palmitic acid mixture

Capsaicin (0.1303 g) was mixed with 339.57 g of melted palmitic acid. Different concentrations of this mixture were prepared (from 10 to 90% w/w, in 10% increments) in soybean oil. Exactly 4.0 g of this mixture was transferred to scintillation vials and heated in a conventional oven at 177°C for 1, 2, 3, and 4 h. After the treatment, samples were allowed to cool at room temperature, capped, and stored at the same temperature for further extraction.

Cayenne pepper-soybean oil mixture

Ground Cayenne pepper (50 g) was thoroughly mixed with 1 L of soybean oil by magnetic stirring for 1 h at 45-50°C. The mixture was allowed to settle and the clear oil separated. A sample (10 mL) was transferred to 15-mL scintillation vials and heated under the same conditions than those for the capsaicin-palmitic acid mixture with one exception: samples were taken every 30 min.

For the microwave heating, 10 mL of the clarified oil was heated under the same conditions than those for the palmitic acid-capsaicin mixture.

Sample extraction

For the mixture capsaicin-palmitic acid, 1 g was weighted in a beaker and 15 mL of HPLC-methanol:water (80:20) was added. The mixture was stirred for 5 min in a magnetic stirrer at room temperature and then refrigerated at 4.4°C for 5 min to solidify part of the palmitic acid. After that, the sample was filtered through a No. 1 Whatman paper. The filtrate was stored for 15 min at -18°C and filtered again under the same conditions. A 5-mL sample was mixed with 10 mL of the mobile phase used in the HPLC analysis. This solution was stored for 15 min at -18°C and filtered with disposable syringes using 0.45- μ m polytetrafluoroethylene disposable filters (13 mm diameter, Alltech Assoc., Deerfield, IL). The filtrate was stored at 4.4°C until injection in the HPLC. The same procedure was used for the extraction of pure palmitic acid, the mixture capsaicin-palmitic acid heated in the microwave oven, and for the mixture capsaicin-soybean oil with different concentrations of palmitic acid.

For the mixture capsaicin-soybean oil, five solvents were tested (95% ethanol, methanol, acetonitrile, acetone, and chloroform). The mixture (1 mL) was added to 10 mL of the solvent (95% ethanol, methanol, or acetonitrile) and stirred for 10 min in a magnetic stirrer. The sample was allowed to separate in a separation funnel. The solvent was recollected, filtered with a 0.45 μ m disposable filter, and

injected into the HPLC. Since acetone and chloroform dissolved completely in the oil, they were only filtered and injected into the HPLC system.

Extraction of the mixture Cayenne pepper-soybean oil followed the same procedure as capsaicin-soybean oil using 95% ethanol.

HPLC analysis

A Beckman System Gold (Beckman Instruments, San Ramon, CA) system equipped with a Model 110 pump and a Beckman UV detector set at 280 nm was used. The reverse-phase separations were carried out on a Supelco LC-18 column (Supelco, Bellefonte, PA) with a 25 x 0.46 cm (i.d.) and a 5- μ m particle size. The mobile phase was acetonitrile:water:acetic acid (45:55:1), and an isocratic flow rate of 1.5 mL/min was maintained.

The major peaks corresponding to capsaicinoids were identified by their retention time as well as comparison with reported data in the literature. Quantification of these compounds was carried out by comparing their peak areas with those from the standard solution (Chiang, 1986).

Model for degradation kinetics

For the analysis of degradation kinetic data for capsaicin we used the general equation for a first order model:

$$\ln C/C_0 = -kt$$

where C_0 is the initial concentration of capsaicin, k is the temperature dependent rate constant (min^{-1}), and C is the capsaicin concentration at time, t . For a reaction following a first order kinetic model, the plot of $\ln (C_0/C)$ vs time would be a straight line and the slope would equal to k at a constant temperature (Steet and Tong, 1996).

Temperature dependence

The relationship of reaction rate to temperature was determined using the Arrhenius equation:

$$k = A_0 \exp (-E_a/RT)$$

where E_a is the activation energy of the reaction, R is the gas constant, T is the absolute temperature, and A_0 is a pre-exponential constant. This model was used to evaluate the degradation rate of capsaicin in a palmitic acid matrix at different temperatures.

Statistical evaluation

All data were analyzed with the use of a SAS statistical analysis program (SAS Institute, Inc., 1985). They were analyzed by one-factor completely randomized analysis of variance using the General Linear Model (GLM). Multiple comparison testing (Least Significant Difference (LSD) test) was used to identify which samples were different from

others for each treatment. Linear equations and determination coefficients were determined by the Regression Procedure (Proc Reg) program. Significance level was established at $p < 0.05$. Three replicates were used per treatment in every determination.

RESULTS AND DISCUSSION

Thermal degradation of pure capsaicin in conventional and microwave heating

Normalized concentration curves for the degradation of pure capsaicin heated at 177°C were developed (Figure 1). The initial concentration, C_0 , was calculated from the weight of capsaicinoids in the vial and considering, from preliminary HPLC analysis, that capsaicin corresponds to 63% (w/w) of the total capsaicinoids present in the standard. The degradation of pure capsaicin in conventional heating at 177°C followed a pseudo first-order reaction. The reaction rate value (k) was $2.1 \times 10^{-3} \text{ min}^{-1}$ and the coefficient of determination (r^2) from linear regression was 0.99. The calculated half-time ($t_{1/2}$) for this degradation was 330 min (5.5 h). Wijeratne (1985) reported that the degradation of capsaicin in a microcrystalline cellulose model followed a first order kinetic model. He indicated that low water activity (A_w) values (0.5) and low pH conditions (2.2) were favorable for a rapid degradation of capsaicin ($k = 3.3 \times 10^{-5} \text{ min}^{-1}$ at $A_w = 0.5$ and 37°C , and

$k=2.3 \times 10^{-5}$ at pH 2.2 at 37°C).

Conventional heating of capsaicinoids produced a brown coloration which became darker as the heating time increased from 1 to 8 h. HPLC chromatogram of capsaicinoids heated at 188°C for 8 h (Figure 2), showed the presence of many new peaks compared with the chromatogram of the standard solution (not shown). We observed that these new peaks eluted before the peaks corresponding to the capsaicinoids. Accordingly, we assumed that during heating of capsaicinoids at the conditions evaluated in this study, a possible fragmentation of this compounds generated a number of products with lower molecular weight. As the heating time increased, the number of new peaks increased as well, but the total area of the detected peaks decreased (Table 1). These results suggested that some low-molecular weight compounds could be lost by volatilization or that these new compounds could not be detected by the UV detector.

Henderson and Henderson (1992), evaluating the effect of heating at 200°C on capsaicin, found that the major decomposition product was 8-methyl-6-nonenamide and at a less significant level, vanillin and 8-methyl-6 nonenoic acid. Other identified products were substituted phenols and some amides. They suggested that the primary route for the formation of products is by cleavage of the bond between the amide group and the vanillin moiety. Identified

degradation products with lower molecular weight than capsaicin, ranged from heptene (MW 98) to 8-methyl-6-nonenoic acid (MW 170). This report supports our hypothesis that some volatile products formed during the heat treatment, could be lost by volatilization. Unfortunately, Harrison and Harris did not report kinetic data or mass losses that could have helped in the interpretative discussion.

Microwave heating of pure capsaicin resulted in no detectable degradation after 10, 20, 30, and 40 min at 50 or 100% power. A few new peaks appeared after the 10-min treatment (at 100% power), but they represented only 4% of the total area in the HPLC chromatograms. Although a browning coloration was observed after the treatments, no important peaks were detected that could suggest a significant degradation.

Degradation of capsaicin in a palmitic acid matrix

Thermal degradation of capsaicin in a palmitic acid matrix heated in a conventional oven at 93, 121, 149, 177, and 188°C followed a pseudo first-order rate (Figure 3). Heating at 188°C resulted in degradation of capsaicin only during the 1-h treatment. After this time, no detection of this compound was perceived. At 177°C, detection of capsaicin was observed only during 1, 2, and 3-h heating.

A brown coloration appeared as the heat treatment increased, though this coloration was not as deep as the color shown by the pure

capsaicin. The reaction rate constants (k) and r^2 values are presented in Table 2. Significant difference ($p < 0.05$) was found among every rate constant, indicating that the degradation of capsaicin occurs at different rates in the range of temperatures evaluated in this study. The marked influence of the temperature on degradation is apparent from the half life period of 641 min (at 93°C) compared with 40 min (at 188°C).

The Arrhenius plot for degradation of capsaicin is showed in Figure 4. The linearity of the data ($r^2 = 0.98$) indicated that the Arrhenius relationship was followed. Linear regression was performed on this plot to determine the pre-exponential factor, A_0 , and the activation energy, E_a . The calculated value for the E_a was 9.3 Kcal/mol. This value felt below the expected value for typical chemical reactions (15-50 Kcal/mol) as indicated by Johnson et al. (1995). However, Labuza (1980) reported several activation energy values for degradation of nutrients in foods, some of them starting at 8.0 Kcal/mol. Wijeratne (1985) calculated an activation energy for capsaicin in a microcrystalline cellulose system of 4.4 Kcal/mol at a water activity value of 0.50. At a water activity of 0.90, the Arrhenius energy calculated was 17 Kcal/mol. Because of the low water activity value (0.1) in our capsaicin-palmitic acid matrix, our calculated value of activation energy could be in close agreement with the value obtained

by Wijeratne. The pre-exponential value was calculated as 2.549 min^{-1} . Although the Arrhenius equation is frequently used as a theoretical basis for development of a mathematical model which describes the temperature sensitivity of a food product, Cohen and Saguy (1985) indicated that the activation energy generally depends on composition factors, such as water activity, moisture content, solid concentration, pH and others.

In our study, the kinetic parameters for loss of capsaicin showed that this degradation occurred 1.7, 4.6, 7.3, and 16 times faster at 121, 149, 177, and 188°C respectively, compared with the degradation rate at 93°C. The degradation rate of pure capsaicin at 177°C was 3.8 times slower than the degradation of capsaicin in the mixture with palmitic acid, at the same temperature. We assumed that capsaicin with palmitic acid received a higher integral heat treatment due to the high heat capacity value of the oil. Because of the high dilution used for the extraction of capsaicin, no peaks were detected corresponding to possible degradation products. Microwave heating of palmitic acid-capsaicin, did not show degradation compounds at 50 or 100% power.

Thermal treatment of a mixture capsaicin-vegetable oil

Capsaicinoids were dissolved in soybean oil and heated in a conventional oven under the same conditions as the mixture with palmitic acid. Preliminary extraction tests using 95% ethanol,

methanol, acetonitrile, acetone, and chloroform indicated that the highest yield of capsaicin recovery (97.4%) was obtained with 95% ethanol. No degradation of capsaicin was detected at any treatment conditions. However, as the heating process increased in time and temperature, the peak total area also increased. It was observed that as the treatment progressed, new peaks derived from the oil appeared in the HPLC chromatograms. This was corroborated by heating soybean oil alone as a control. The area of these peaks was larger as the treatment increased. No browning was observed at any condition. The lack of degradation of capsaicin in soybean oil, may be due to some protective action from the vegetable oil. The presence of vitamin E in the oil, because of its antioxidant properties, could avoid this deterioration. Wijeratne (1985) found that the degradation of capsaicin is accelerated by the addition of linoleic acid (1.5%) only if the system is at low water activity value (0.5). Comparing the HPLC chromatograms of heated palmitic acid and soybean oil, it was clear that after the same heating conditions, more peaks were produced from the vegetable oil. These results agreed with the statement indicated by Nawar (1996), where the unsaturated fatty acids are more susceptible to oxidation than the saturated analogs (soybean oil present a high content of linoleic acid), producing more degradation products. Henderson and Henderson (1992) reported that after heating a mixture of capsaicin

and oleic acid, the unique products formed as a result of this interaction were amides. They also mentioned that the oxidation of oleic acid appeared to be inhibited by capsaicin.

Heating capsaicin-soybean oil in microwave oven produced no detectable degradation forms of capsaicin. New peaks appeared through the treatment but, as happened with the conventional heating, these peaks resulted from changes in the oil.

Thermal treatment of ground Cayenne pepper in vegetable oil

The thermal degradation of Cayenne pepper mixed with soybean oil did not result in the detection of decomposition products derived from the chile. However, as in earlier findings, new peaks were developed coming from the oil degradation. The original mixture had a deep orange color. As the heating progressed, color changes occurred, from the orange to a yellow color. The more intense the heat treatment, the more pale-yellow coloration. Govindarajan (1986) indicated that heat treatment induces loss of color from oxidative degradation in carotenoids present in chiles, resulting in lightening of color. He reported that kinetic studies of loss of the major carotenoids in Cayenne pepper during the drying process, followed a first-order reaction. van Elbe and Schwartz (1996) indicated that, in addition to oxidation, changes in carotenoids during heat treatment can occur due to isomerization and fragmentation.

No thermal degradation of capsaicin was detected when the same mixture was heated in microwave oven. No changes in coloration was observed under the treatment at 50 or 100% power.

Degradation of capsaicin in a matrix palmitic acid-soybean oil

Different concentrations (10% w/w each) from 10 to 90% of palmitic acid were added to a soybean oil-capsaicin solution. Heating of this mixture in conventional oven at 177°C did not result in detectable degradation of capsaicin. However, heating in 80 and 90% palmitic acid resulted in a small degradation, mainly at the last two hours of the treatments. There was also a slight browning coloration observed during heating. This confirms our previous results where it seems that the soybean oil has a protective action against capsaicin degradation, even with some added palmitic acid. At high levels of this acid (80 and 90%), a small degradation was detected, although not enough to evaluate its reaction rate under the conditions of this study.

According to these results, we can assume that free fatty acids play an important role in capsaicin degradation during the thermal treatment. This fact can be relevant for the food industry. During frying process, there is an increase in the content of free fatty acids (Nawar, 1996), which might modify the capsaicinoids content of hot foods, reducing consequently the pungency sensation.

CONCLUSIONS

Degradation of pure capsaicin heated in conventional oven followed a pseudo first-order reaction. Degradation products from this treatment showed lower molecular weight than those from the capsaicinoids. Thermal degradation of capsaicin in a mixture with palmitic acid, followed a pseudo first-order model as well. The calculated activation energy (E_a) value was 9.3 Kcal/mol. Capsaicin degradation in this model was faster than the degradation of pure capsaicin at the same temperature. No decomposition was observed in the soybean oil matrix during conventional or microwave heating. This last process did not degrade capsaicin in a palmitic acid matrix or Cayenne pepper in soybean oil. A small level of degradation was observed in a mixture with soybean oil when the concentration of palmitic acid was 80 and 90% of the total.

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TABLE CAPTIONS

Table 1. Total area and total area/capsaicin ratio from HPLC analysis of capsaicinoids heated in conventional oven at 177°C.

Table 2. Kinetic parameters of capsaicin degradation in a palmitic acid matrix heated in conventional oven.

Table 1

Time (min)	Total area	Total area/capsaicin ratio
60	604 ^a (14.1)	1.74 ^a (0.01)
120	567 ^a (10.6)	1.85 ^b (>0.01)
180	540 ^a (14.1)	1.87 ^b (0.03)
240	440 ^b (10.6)	1.96 ^c (>0.01)
300	435 ^b (5.7)	1.99 ^c (0.01)
360	380 ^{b^c} (8.5)	2.10 ^d (>0.01)
420	349 ^{cd} (14.8)	2.15 ^c (>0.01)
480	288 ^d (43.4)	2.22 ^f (0.05)

^{a-f} Means (n=3) with different letters in the same column are significantly different (p<0.05). Standard deviations are showed in brackets.

Table 2

Temperature (°C)	$k^f \times 10^{-3}$ (min ⁻¹)	r^2g	$t_{1/2}^h$ (min)
93	1.08 ^a	-0.903	641.7
121	1.84 ^b	-0.933	376.6
149	4.96 ^c	-0.983	139.7
177	7.88 ^d	-0.971	87.9
188	17.2 ^e	-0.988	40.3

^{a-e} Means (n=3) with different letters are significantly different (p<0.05).

^f First-order rate constant

^g Coefficient of determination

^h Half-life period

FIGURE CAPTIONS

- Figure 1.** Degradation of pure capsaicin heated in conventional oven at 177°C. (C_0 : initial concentration of capsaicin at time $t=0$; C: final concentration of capsaicin at time t).
- Figure 2.** HPLC chromatogram of pure capsaicinoids after 8-h heating in conventional oven at 177°C. (NDC: nordihydrocapsaicin; C: capsaicin; DHC: dehydrocapsaicin).
- Figure 3.** Thermal degradation of capsaicin in a palmitic acid matrix heated in conventional oven. (C_0 : initial concentration of capsaicin at time $t=0$; C: final concentration of capsaicin at time t).
- Figure 4.** Arrhenius plot for the degradation of capsaicin in a palmitic acid matrix heated in conventional oven. (k : pseudo first-order rate constant; T: temperature, Kelvin).

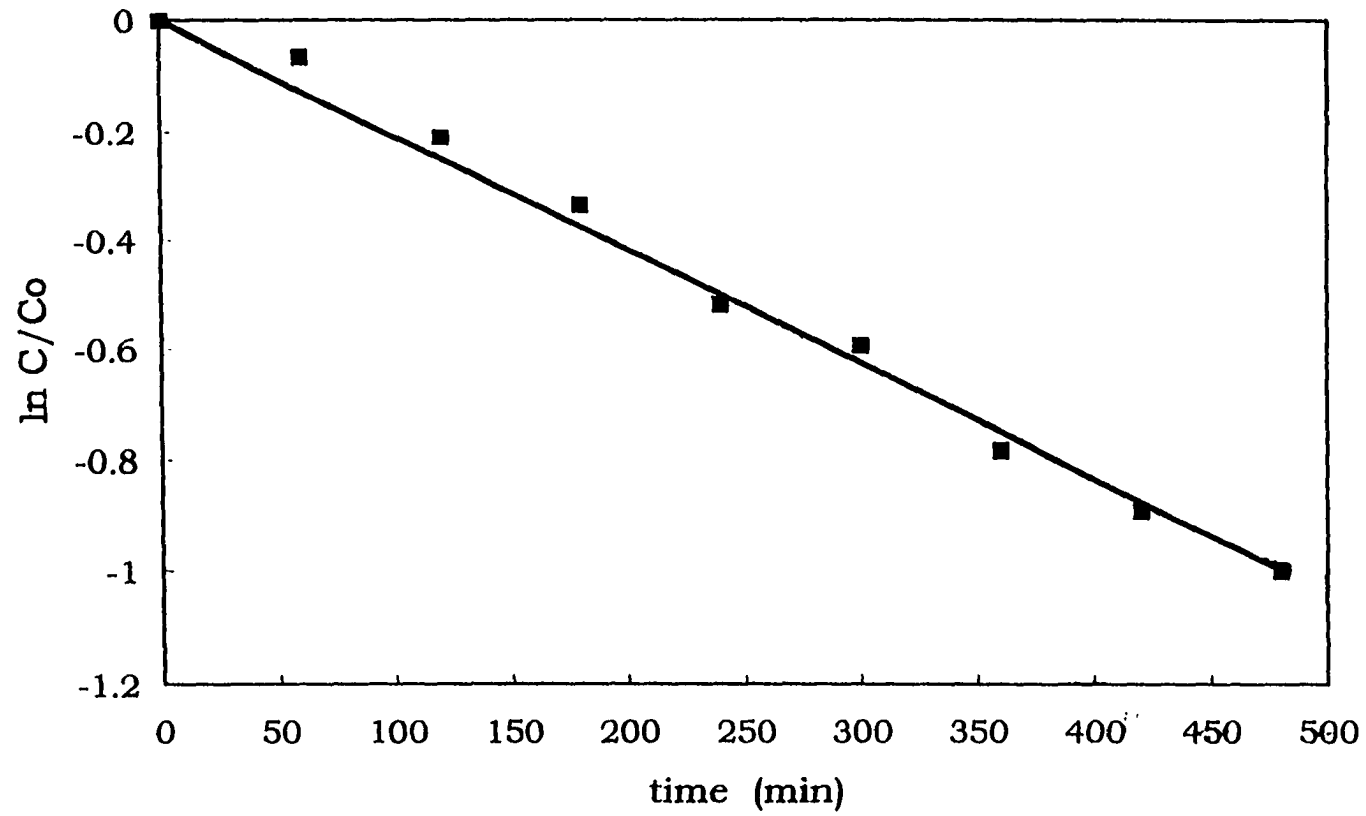


Figure 1

Detector Response

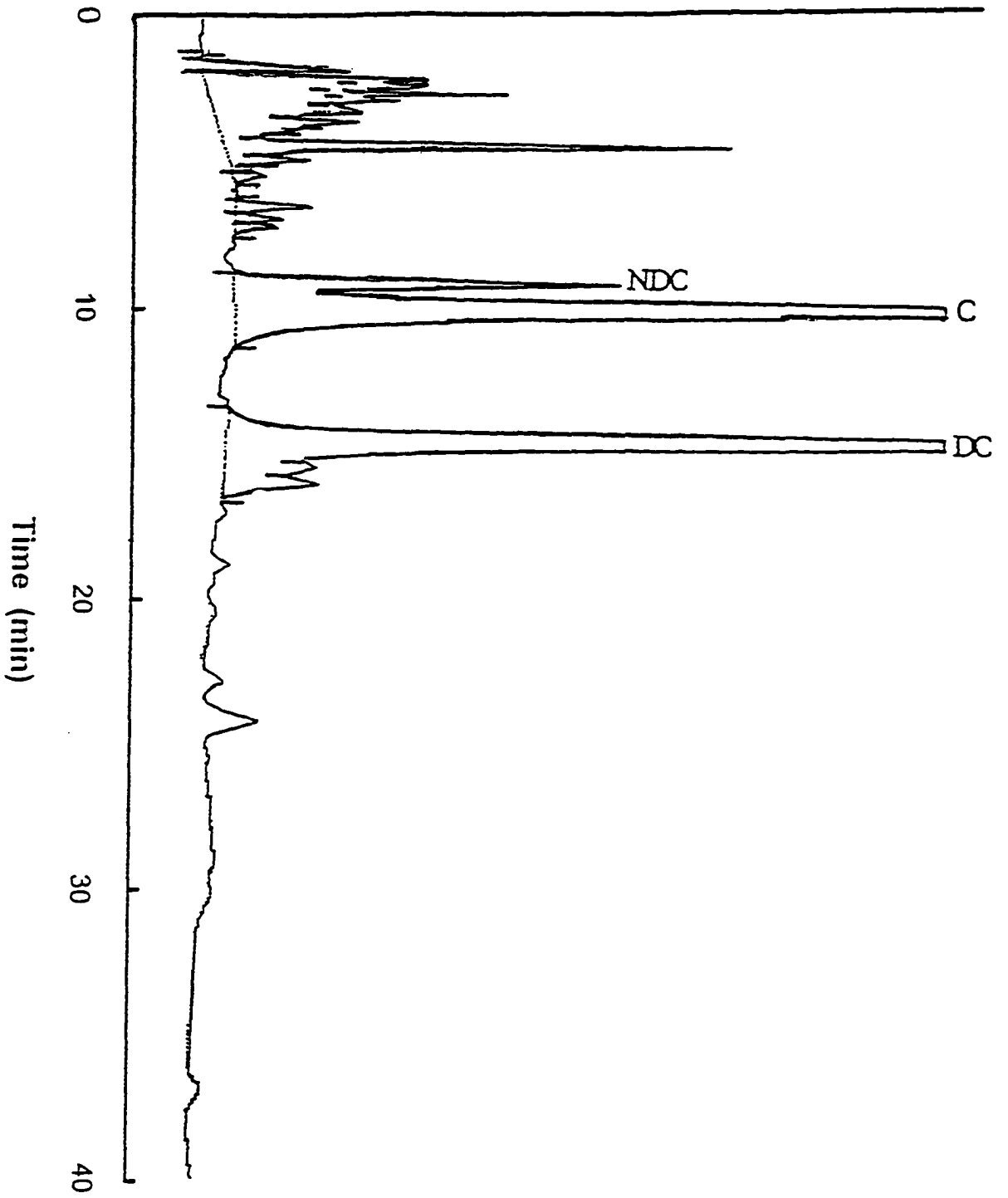


Figure 2

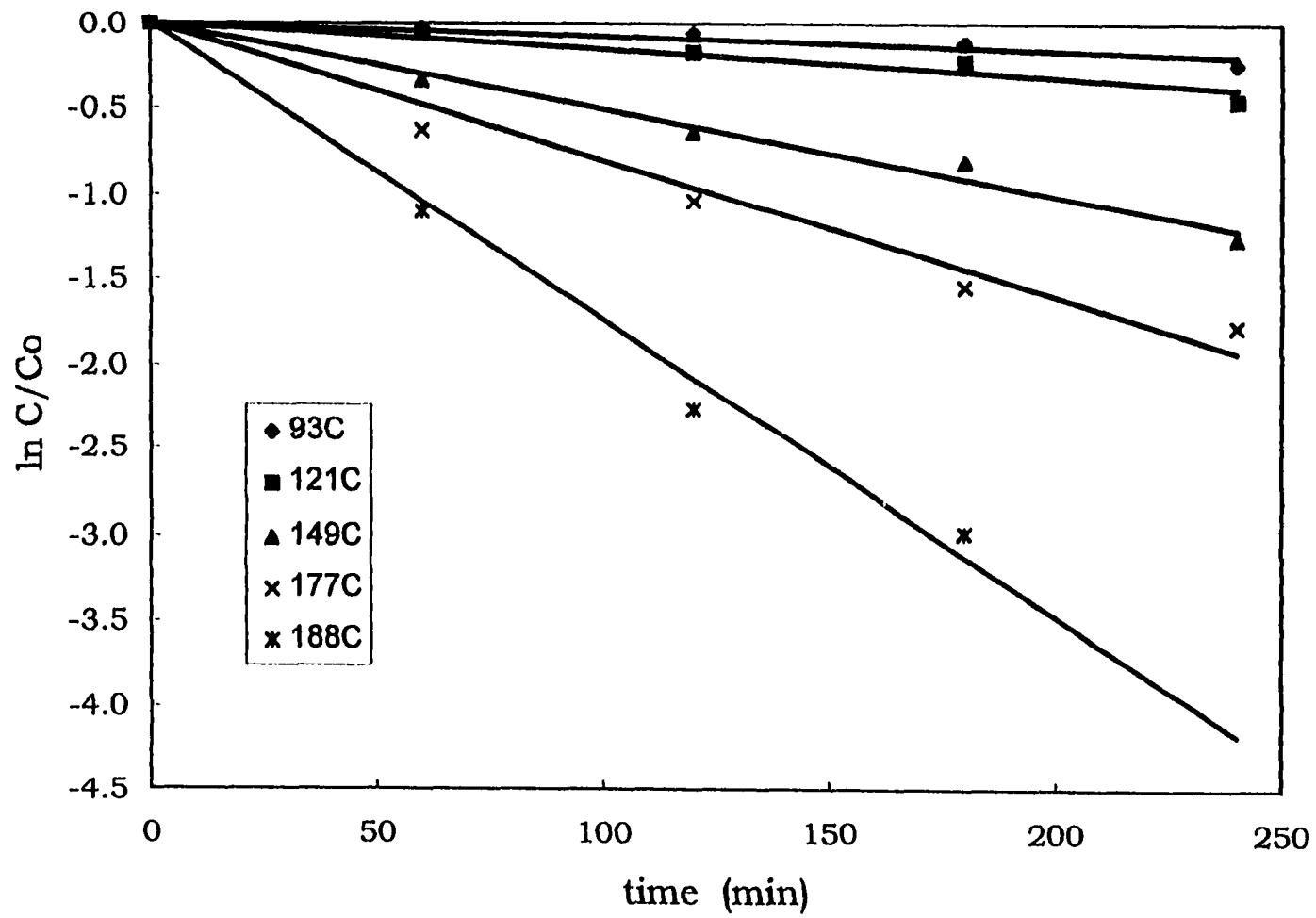


Figure 3

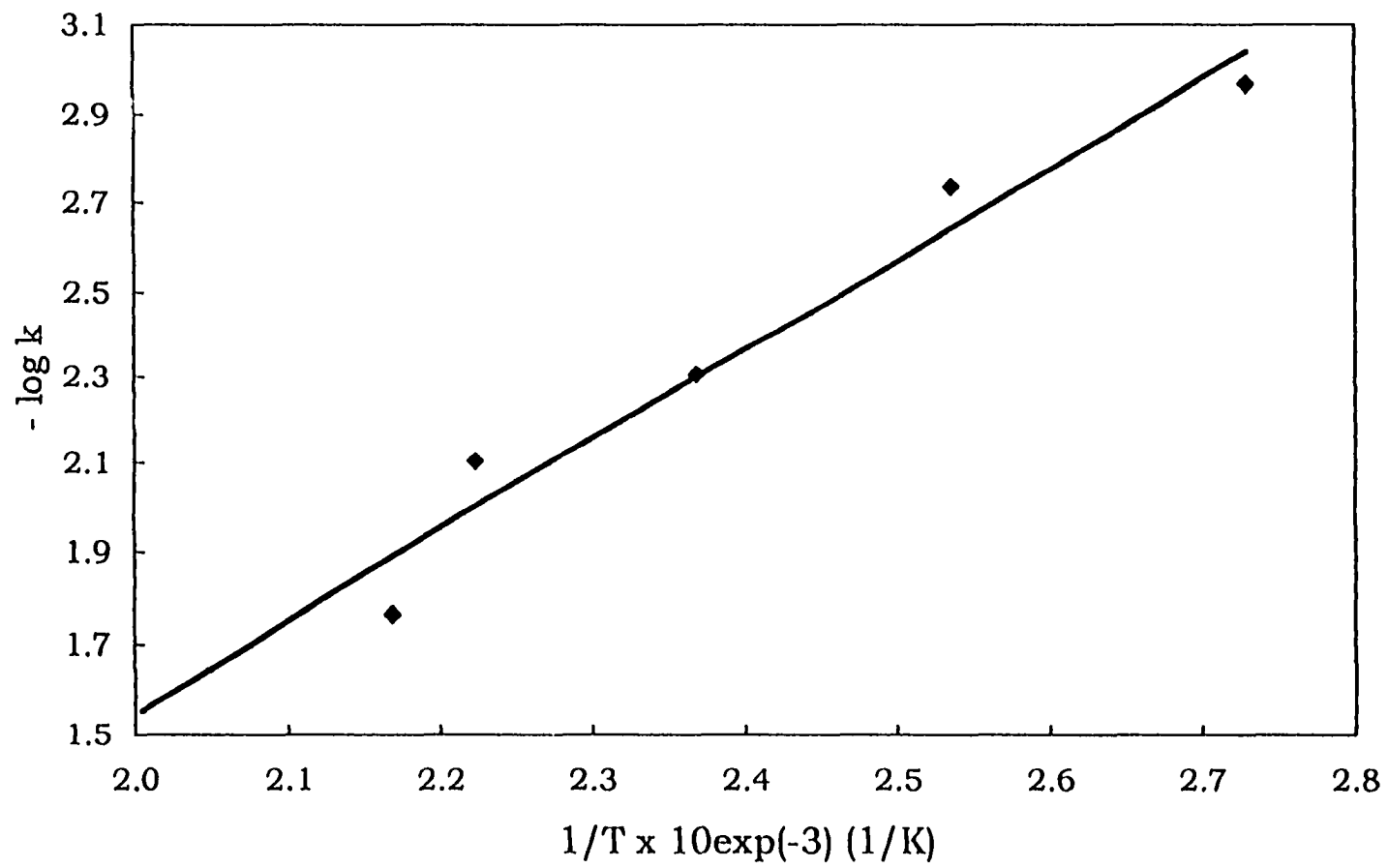


Figure 4

GENERAL CONCLUSIONS

Conclusions

In serrano peppers, dry matter increased with the ripening stage and a reduction in the average fresh weight of the chile was also observed. These results were related with the moisture loss during storage. The content of soluble solids and % of acidity was similar in the three stages. Between the green and yellow stage, an increase in the sugar/acid ratio was observed; however, this was not the case between the yellow and red stages.

Instrumental detection (HPLC analysis) did not show significant difference among the capsaicin content in the three maturity stages. However, a broad range of values was found in the results. The Scoville Heat Units calculated for the serrano peppers, were higher than the values reported in the literature.

The concentration of volatiles, as detected by gas chromatography, was higher in the red stage than in the other stages. Some identified volatiles matched with some aromatic compounds found in other chile varieties.

The green, yellow, and red stages resulted in different sensor-response from the electronic nose analysis. This showed that different aromatic characteristics were perceived by the apparatus. Different

capsicum types also produced different characteristic responses in comparison with those from the serrano peppers.

Panelists were unable to detect differences in the three stages of serrano peppers by aroma tests, and they could not differentiate among their intensities.

In relation with the extraction of salsas with different solvents, it was found that magnetic stirring of a dried chile, extracted the 95% of the pungent compounds, compared with more laborious and time-consuming methods. Ethanol proved to be an efficient solvent for extraction of capsaicinoids from dry peppers, compared with other solvents.

Extraction of salsas with ethanol demonstrated that in some instances, the level of pungency established in the label does not correlate with the level quantified instrumentally. Some salsas labeled as "mild" have a higher content of capsaicinoids than some salsas labeled as "hot".

The type of salsa preparation (blended and blended and dried) affected the efficiency of the capsaicinoids extraction. Salsas with a high content of moisture (about 95%) showed a significant difference when extracted blended compared with the blended and dried samples. Then, for high moisture salsas, we recommed that these products be blended and dried before extraction. Some factors affecting the

extraction efficiency were: the procedure of sample preparation, the capsaicinoids concentration, viscosity of the salsa, water content, and type of ingredients present.

Thermal degradation of pure capsaicin during conventional heating at 177°C, followed a pseudo first-order kinetic with a reaction rate value (k) of $2.1 \times 10^{-3} \text{ min}^{-1}$. The half-life for this reaction was 5.5 h. A brown coloration appeared after the heat treatment. The possible causes for this degradation was oxidation and molecular fragmentation with consequent volatilization of low-molecular weight products.

In a palmitic acid-capsaicin matrix, degradation of capsaicin also followed a pseudo first-order model. At 188°C, no detection of capsaicin was observed after the 2-h treatment. The activation energy calculated for this degradation was 9.3 Kcal/mol. Degradation of capsaicin in a palmitic acid matrix was 3.8 times faster than the degradation of pure capsaicin under similar conditions.

Thermal treatment of capsaicin in a soybean oil matrix, did not show any degradation of pungency. The presence of vitamin E could inhibit the degradative process. Similar results were obtained during the thermal process of Cayenne pepper in vegetable oil.

Using variable concentrations of palmitic acid in soybean oil, the

degradation of capsaicin was only observed when the palmitic acid concentration increased to 80 and 90%. Although the degree of decomposition was less compared with the effect on palmitic acid alone. These results suggest that during frying process, we might expect changes in the pungent content of chiles or their products. This process seems to be accelerated by the formation of free fatty acids during heating.

Microwave heating did not produce any degree of capsaicin degradation in the tested matrixes. Then it is possible that, under similar conditions, the pungency level might be maintained during microwave cooking.

Recommendations

Because of the different number of peaks found in serrano pepper, analysis and identification of the major volatiles at different ripening stages should be studied. This should be correlated with the sensory perception of flavor and pungency perceived by the consumer.

The lack of information about correlation between sensory and instrumental analysis in chile flavor, should motivate the analysis of this characteristics by using gas chromatography-mass spectrometry, electronic nose and sensory evaluation in different chile varieties. In the same way, studies should be made about different ripening stages

and their evaluation of flavor and pungency perception in different varieties.

Additional sensory evaluation is needed to determine, under controlled conditions, the effect of the characteristics of chiles at different ripening stages on the perception of aroma and pungency. Interesting evaluation would be to perform descriptive analysis comparing different varieties of chiles and determine the "note" they produce as the maturity stages change.

In this study it was demonstrated that the water content affects the efficiency of capsaicin extraction. However, it is important to consider other type of factors like type of salsa, ingredients, and particle size on the extraction with different solvents. This could help the salsa processors to evaluate more precisely the capsaicinoids content in their products.

Because of the variability in pungency levels found in different types of salsas, it is necessary to correlate the "real" capsaicinoid content in the product (evaluated instrumentally), the level indicated in the label, and the sensory response given by the consumer.

Because degradation of capsaicin might occur during conventional cooking of foods (depending of the matrix, presence of free fatty acids, pH, etc.) it is important to evaluate this effect on the pungency perception by sensorial methods. In the same way, studies should be

directed toward the determination of the mechanism of reaction for the degradation of capsaicin as well as identification of products from this reaction.

During cooking, important changes in flavor occur in a food system. Therefore, it would be desirable to evaluate whether these changes affect the perception of pungency. It would be important to relate this study by evaluating different types of capsicums under different food systems using different cooking methods.

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