ABSTRACT

DA CONCEICAO NETA, EDITH RAMOS. Application of Sensory and Instrumental Analyses for the Characterization of Flavor in Roasted Peanuts and Farmstead Cheddar Cheese. (Under the direction of Dr. Timothy H. Sanders and Dr. MaryAnne Drake).

The driving force for the consumption of peanuts is the unique flavor that is developed during roasting. The peanut plant has an indeterminate flowering pattern, which means that peanuts of different maturity classes exist in lots of a seed-sized commercial grade. Due to compositional variations, the seed maturity distribution within a lot may affect flavor development during roasting. The main objectives of this study were to characterize the flavor of roasted peanuts from five maturity classes, and to reconstitute the aroma of roasted peanuts in model systems. A secondary objective was to correlate the chemical and sensory characteristics of roasted peanuts from five maturity classes. Different statistical techniques including Pearson's correlations, principal component analysis (PCA), and partial least square regression (PLSR) were used to establish relationships where appropriate. An additional study characterized the earthy/bell pepper (EBP) flavor in Farmstead Cheddar cheeses.

In the first study, HS-SPME (headspace solid-phase microextraction) sampling conditions were optimized for the analysis of roasted peanuts. Eight compounds were used to assess the extraction efficiency of various testing conditions. For gas chromatography-mass spectrometry (GC/MS) analysis, the use of DVB/CAR/PDMS fiber led to greater peak areas than the PDMS fiber. For a given extraction time and temperature, addition of salt increased extraction efficiency. Peak areas also increased

with increasing extraction time and temperature. Optimized conditions increased response of target compounds by 40% to 1760% when compared to undiluted paste extracted at 40ºC/30 min with a DVB/CAR/PDMS fiber. Optimized conditions also resulted in higher number of detected compounds by gas chromatography-olfactometry (GC/O) analysis.

In the second study, the flavor of roasted peanuts from five maturity classes was characterized, and the aroma of roasted peanuts was reconstituted in model systems. Twenty-five compounds were selected by aroma extract dilution analysis (AEDA) as being potential contributors to roasted peanut flavor. Five additional compounds detected by headspace solid-phase microextraction (HS-SPME) were selected based on their high intensity scores on the GC-O analysis. Similarity score against the aroma of roasted peanuts for the optimized model system was 8 a 10-point scale. Omission experiments indicated that methional, 1-octen-3-one, nonanal, hexanal, octanal, 2-acetyl-1-pyrroline, carbon disulfide, and phenylacetaldehyde were the main contributors to roasted peanut aroma.

In the third study, the compositional variations in peanuts of five maturity classes were determined, and correlations among their chemical and sensory characteristics were established. Roasted peanutty was positively correlated with sweet aromatic and sweet taste, and negatively correlated with bitter. Free amino acids decreased with increasing maturity ($P<0.05$), possibly due to protein synthesis during maturation. Free amino acids also decreased during roasting in all maturity classes, due to involvement of these compounds in Maillard reactions. Oleic/linoleic ratio significantly increased with seed

maturity, which suggests that more mature peanuts may be less susceptible to lipid oxidation.

In the final study, the earthy/bell pepper (EBP) flavor that has been previously recognized in some Farmstead Cheddar cheeses with natural bandage wrappings was characterized. EBP flavor was detected in four out of eight Farmstead Cheddar cheeses by a trained sensory panel. 2-secbutyl-3-methoxypyrazine and 2-isopropyl-3-methoxypyrazine were identified as the main sources of EBP flavor in these cheeses by GC/O and GC/MS analysis. In general, those pyrazines were prevalent towards the exterior of the cheeses, which suggests they are formed near the surface and migrate into the cheese during ripening. Sensory analysis of mild Cheddar cheese model systems confirmed that direct addition of those individual pyrazines resulted in EBP flavor.

Application of Sensory and Instrumental Analyses for the Characterization of Flavor in Roasted Peanuts and Farmstead Cheddar Cheese

by
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DEDICATION

Aos meus amados pais, Maria Aparecida e José Carlos, os quais sempre me incentivaram a alcançar meus objetivos e a buscar meus sonhos.

To my loving parents, Maria Aparecida and José Carlos, who have always encouraged me to reach my goals and follow my dreams.

BIOGRAPHY

Edith Ramos da Conceicao Neta was born on February 28, 1979 in Brazil. She grew up in Juiz de Fora with her parents, sister and brother. Edith obtained a Bachelor's degree in Food Engineering at Federal University of Viçosa, Brazil in December 2003. As an undergraduate, she completed internships at two fruit pulp processing centers and also gained experience in a dairy product manufacturing facility. In her senior year, Edith worked as an exchange student in the USDA ARS Food Science Research Unit at NC State University for six months. After completing her undergraduate degree, Edith pursued her Master's degree in Food Science at NC State under the direction of Dr. Roger McFeeters. Her thesis research focused on understanding the chemical basis for sour taste perception in fresh pack dill pickles. During this time, she participated in the PepsiCo summer internship program (Valhalla, NY) where her focus was on analysis of factors affecting rate of color loss in fruit juices. Upon graduating with her Master's degree, Edith joined Nestlé R&D (Marysville, OH) for a one-year co-op, working on formulation and sensory analysis of ice cream coatings. In January 2007, she returned to NC State to begin her Ph.D. program in the Department of Food, Bioprocessing and Nutrition Sciences, under the direction of Dr. Timothy Sanders and Dr. Maryanne Drake. During her doctoral degree, Edith was awarded several highly competitive scholarships including two from the Institute of Food Technology, and the Department's first annual Frito-Lay Product Development Graduate Fellowship. Edith was an active member of the NC State Food Science Club, chairing several committees and speaking at local K-12 schools, but her greatest contribution was to lead the Club's Product Development Team

to a National Championship at the Institute of Food Technologist's annual meeting in 2009 with the instant smoothie product, *Shiverrs*. From her Masters degree, Edith published four refereed papers. Her doctoral research has resulted in four papers, published or in revision, and one book chapter. In total, her research has been presented at seven national or international conferences. In the summer of 2009, Edith participated in a summer internship at The Clorox Company (Pleasanton, CA) in the Hidden Valley division where she worked on flavor optimization. Upon completion of her degree, Edith will join The Clorox Company as a Research Scientist in California, where she looks forward to enjoying their nearly perfect weather and laid-back lifestyle.

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'No one can whistle a symphony. It takes an orchestra to play it.' (HE Luccock)

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LITERATURE REVIEW

UNDERSTANDING PEANUT FLAVOR – A CURRENT REVIEW

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Introduction

Peanuts are a significant commodity in the United States, with average consumption of approximately one million tons per year (American Peanut Council 2007). In contrast with other countries where the end products are peanut oil, cake and meal, the major market for U.S. peanuts is in edible products. Peanut butter accounts for about 50% of the market, while the other half is divided equally between snack nuts and confectionary products. Only about 15% of the U.S. crop serves as base for peanut oil production (American Peanut Council 2007).

The driving force for the consumption of peanuts is the unique and pleasant flavor that is developed during roasting (Sanders et al. 1997). Peanut flavor is influenced by genetic, environmental, physiological, and biochemical processes occurring in the seed, as well as handling, processing, and storage conditions (Sanders et al. 1995; Sanders et al. 1995; Young et al. 1974). The numerous combinations of interactions of these factors affect the final chemical composition of peanuts, which in turn determine flavor quality. The flavor of roasted peanuts is determined by the composition and concentration of volatiles present, with over 200 aroma-active compounds identified to date (Schirack et al. 2006). Understanding the role of these aroma-active compounds may allow for better control of the flavor of peanut products, which would benefit both consumers and the peanut industry. Although significant efforts have been made to identify the potent odorants in peanuts, the specific compound(s) or groups of compounds responsible for the roasted peanut flavor remain elusive.

This review presents a current summary of the progress in peanut flavor research and advances in understanding the chemical basis of peanut flavor by means of sensory and instrumental analysis. Pyrazines have long been considered important components of roasted peanut flavor (Mason et al. 1966; Mason et al. 1967; Mason et al. 1969; Maga 1973; Shibamoto and Bernhard 1976), but there is limited conclusive information showing that they are the basis for the roasted peanut flavor. This review provides an overview of current flavor chemistry techniques and common mechanisms for pyrazine formation in several model systems. In addition, numerous compounds that have been associated with typical roasted peanut flavors are reviewed. Finally, the major reactions and chemical changes associated with the most common off-flavors developed in roasted peanuts during processing and storage are discussed.

Flavor Isolation Techniques

Development of reliable, efficient methods to extract analytes from food systems has been a challenge to researchers. A proficient technique for extraction of volatile compounds from foods should meet the following requirements: (1) extract the key compounds contributing to flavor; (2) not destroy or alter the structure of relevant aroma compounds; (3) not create new aroma compounds or artifacts; and (4) eliminate non-volatile compounds that might interfere with gas chromatographic separation (Engel et al. 1999).

Several different analytical methods have been developed to study the volatile composition of foods including steam distillation, solvent extraction, simultaneous

distillation/extraction (SDE), high vacuum distillation, solvent assisted evaporation method (SAFE), and headspace analysis (Curioni and Bosset 2002; Sides et al. 2000). Numerous studies have shown that the composition of aroma extracts is highly dependent on the isolation technique employed (Fischer et al. 1995; Vandeweghe and Reineccius 1990). There is no single method that allows simultaneous recovery of the full range of aroma compounds present in a food system, thus a combination of extraction techniques must be used to completely characterize the aroma active volatile compounds (Drake et al. 2006).

Steam distillation is one of the oldest techniques for extracting volatile compounds from foods. Although the method is rapid and simple, the high temperatures utilized may lead to formation of artifacts in the samples. In addition, the volatiles are highly diluted in water after collection in cold traps (Sides et al. 2000).

Direct solvent extraction is a valuable technique for isolating higher molecular weight volatile compounds from non-volatile food matrices (Wong and Park 1968). The extract is usually concentrated under nitrogen or in a rotatory evaporator. The shortcomings of this method are co-extraction of matrix components and extraction of relatively low amounts of high volatile compounds (Sides et al. 2000).

Currently, simultaneous distillation/extraction (SDE) is one of the most popular flavor isolation technique. This method allows simultaneous extraction of steam distillates by solvents, but has the limitation of forming thermally induced artifacts (Nickerson and Likens 1966). High vacuum transfer (HVT) is a technique that allows transferring of volatiles between two vessels based on an extreme temperature

differential, thereby reducing the possibility of artifact formation (Schieberle and Grosch 1985; Weurman et al. 1970). Solvent Assisted Flavor Evaporation (SAFE) is an improved and specialized version of the HVT technique (Engel et al. 1999). SAFE provides rapid isolation of volatiles from food suspensions, matrices with high fat content, and aqueous foods (Engel et al. 1999). SAFE has been widely employed in flavor chemistry research including analysis of volatile compounds in milk (Bendall and Olney 2001; Havemose et al. 2007), sweet cream butter (Lozano et al. 2007), coffee beans (Scheidig et al. 2007), peanuts (Didzbalis et al. 2004; Schirack et al. 2006) and cheese (Suriyaphan et al. 2001; Whetsine et al. 2005)

Headspace analysis includes dynamic and static methods. Dynamic headspace analysis (DHA) or purge-and-trap is an effective method for the extraction of aroma compounds from foods. In this technique, an inert gas is continuously passed through the sample and the volatile compounds are collected in a trap containing an adsorbent (Sides et al. 2000). This method has the advantages of being nondestructive, sensitive, and rapid. The main disadvantage is that water is collected with the volatile material. Static headspace is a technique based on establishment of equilibrium between the food sample and the gas phase above it. Solid-phase microextraction (SPME) is a variant of the static headspace method that has gained popularity in recent years. A fused silica fiber coated with an absorbent is immersed into the headspace above a liquid or solid sample and analytes are extracted by diffusion onto the coating (Sides et al. 2000). SPME has been extensively used for quantitative analysis of flavor compounds because analytes are isolated without interferences from the matrix components (Steffen and Pawliszyn 1996).

The main advantages of this method are simplicity, high speed, low cost, small sample volume, and high sensitivity (Kataoka et al. 2000). In general, extracts obtained by headspace analysis (dynamic and static) contain fewer compounds than those obtained by solvent extraction or distillation methods. In addition, headspace analysis is usually not suitable for isolating high molecular weight compounds, as well as tightly bound and encapsulated volatiles (Sides et al. 2000).

Instrumental Analysis

Separation of volatiles extracted from foods is generally performed using gas chromatography (GC). Following or during GC separation of compounds, identification may be accomplished using mass spectrometry and tentative identifications may be made using retention indices and aroma profiles from GC/O analysis. Gas chromatography combined with mass spectrometry (GC/MS) has been considered the ideal device for identification of volatile compounds. In general, a mass spectrometer consists of an ion source, a mass-selective analyzer, and an ion detector. Atoms or molecules are ionized and fragments of specific mass and charge are created. The mass-selective analyzer then separates the ions based on their mass-to-charge ratio (m/z). Molecules have distinctive fragmentation patterns, which can provide structural information, chemical formula, and molecular weight of the molecule, depending on the type of MS technique applied (Ravindranath 1989).

Although traditional GC analysis is a powerful tool for identification and quantification of volatile compounds in foods, it does not provide information regarding

the relative contribution of individual components to flavor. Usually, only a small percentage of the total compounds present are aroma-active and thus contribute significantly to a particular flavor. Also, the total amount of a given compound is not necessarily related to its flavor impact, as the concentration present may still fall below sensory threshold (McGorrin 2002).

Gas chromatography – olfactometry (GC/O) is a semi-quantitative technique that provides links between GC data and odor active compounds that potentially play a role in flavor (Fuller et al., 1964). In this technique, the GC has been modified with an olfactometer or sniffer port at the detector end of the column and a trained panelist (sniffer) describes the aroma of individual compounds as they elute from the GC column. Although GC/O is a valuable technique in identifying odor-active compounds, it may be difficult to determine the sensory relevance of these volatiles from a single chromatogram.

Dilution analysis such as Aroma Extract Dilution Analysis (AEDA) and CharmAnalysis^{TM} are commonly used to determine the potential relative contribution of a compound to the flavor of the food (Acree et al. 1984; Grosh 1993). In dilution analysis, an extract is serially diluted and sniffed until no odorants are detected at the sniffer port. The lowest detectable amount eluting from the column is converted to a flavor dilution value (FD) in AEDA, or to a Charm Value in CharmAnalysis^{TM} (Acree et al. 1984; Grosh 1993).

Correlating Sensory and Instrumental Analysis

Descriptive sensory analysis (DSA) is a powerful tool for flavor characterization of foods. DSA utilizes a lexicon of terms (descriptors) that define the various flavor characteristics of a food. A lexicon is developed in a moderated session of trained or experienced panelists who use words to describe the various flavor characteristics of a particular food. Upon completion, a lexicon is a defined and nonredundant list of flavor descriptors that describe the sensory characteristics found in a wide range of available samples of a particular food (Drake and Civille 2003). DSA makes use of a panel trained in the use of a lexicon that functions as an instrument to evaluate both quantitative (intensity) and qualitative (lexicon) components of a product (Meilgaard et al. 1991). Use of a highly trained panel results in reproducibility of results such that effective correlation of DSA with instrumental and/or consumer data can be achieved (Drake and Civille 2003). The most common DSA methods are: The Flavour Profile Method™, Quantitative Descriptive Analysis™, Quantitative Flavour Profiling™, Free-ChoiceProfiling™, and Spectrum™ Method (Murray et al. 2001).

In general, relationships between sensory and instrumental analysis of foods can be determined by following three steps: (1) selection of flavor(s) of interest using descriptive sensory analysis; (2) instrumental analysis of volatile extracts; (3) and confirmation of aroma-active compounds via quantitation, threshold testing and descriptive sensory analysis of model systems (Drake et al. 2006). Schirack et al. (2006) used this approach to show that phenylacetaldehyde, guaicol and 2,6-dimethylpyrazine were responsible for the stale/floral and ashy off-flavor in high temperature microwave-

blanched peanuts. Using a similar tactic, Didzbalis et al. (2004) showed that fruit-like esthers such as ethyl 2-methylpropanoate, ethyl 2-methylbutanoate, and ethyl 3-methylbutanoate along with short chain organic acids (butanoic, 3-methlbutanoic, and hexanoic) were responsible for the fruity/fermented off-flavor developed in immature peanuts cured at high temperatures.

Formation of Volatile Compounds

The predominant reactions involved in the formation of volatile compounds in roasted peanuts are the Maillard reaction, Strecker degradation, thermal degradation of sugars (caramelization), and lipid oxidation (Cammarn et al. 1990; Coleman et al. 1994). The Maillard reaction involves the interaction of reducing sugars and amino compounds such as amines, amino acids, peptides, and proteins. The main products from this reaction are heterocyclic nitrogen compounds, such as furans, thiazoles, thiophenes, oxazoles, pyrroles, imidazoles, pyridines, and pyrazines (Hodge 1953; Hwang et al. 1995). The Strecker degradation involves degradation of α-amino acids by reductones (α-dicarbonyls), which are usually derived from the Maillard reaction (Hodge 1967; Schonberg and Moubacher 1952). During the Strecker degradation, the α-dicarbonyls are converted into α-aminocarbonyls, which eventually condense to form alkylpyrazines (Fennema 1996) (Figure 1). Thermal degradation of sugars (caramelization) produces low molecular weight open-chain oxygen-containing compounds, as well as heterocyclic oxygen-containing species such as furan derivatives (Coleman et al. 1994). Lipid oxidation leads to the formation of oxygen containing compounds such as aliphatic

aldehydes, ketones, and alcohols (Coleman et al. 1994). In addition, free radicals, hydroperoxides, and secondary products formed during lipid oxidation may interact with nitrogen-containing compounds such as proteins and possibly pyrazines, modifying the precursors available for Maillard reactions as well as the flavor compounds already formed during roasting (Alzagtat and Alli 2002; Funes et al. 1982; Gardner 1979; St. Angelo and Graves 1986; Vercellotti et al. 1992; Williams et al. 2006). Lipids can be oxidized by both enzymatic and non-enzymatic mechanisms. In plant systems such as peanuts, lipoxygenase acts as a catalyst to oxidize polyunsaturated fatty acids to form hydroperoxides, which then break down to produce a number of secondary products (Fennema 1996). Although roasting of peanuts inactivates enzymes, lipid oxidation may still occur because lipoxygenase contains transition metals such as iron and copper that become catalysts in non-enzymatic mechanisms (Ory et al. 1992).

Peanuts Composition and Flavor Precursors

Peanut seeds consist of about 47% fat, 25% protein, 19% carbohydrates, and 7% water. The proximate composition of raw and roasted Virginia-type peanuts is shown in Table 1 (Derise et al. 1974). Unsaturated fatty acid concentrations are high in peanut oil, which makes peanut products very susceptible to lipid oxidation (Ory et al. 1992; St. Angelo 1996). Oleic acid and linoleic acids are the most abundant fatty acids in the peanut seed (Jonnala et al. 2005). The main proteins are albumins, and two globulins, arachin and conarachin. The carbohydrates include starch, pectin, cellulose, and sucrose (Hoffpauir 1953). Peanuts also contained a variety of minerals, as illustrated in Table 2

for the runner market-type (Jonnala et al. 2005). Talcott et al. (2005) found that peanuts are a good source of antioxidant polyphenolics such as p-coumaric acid. They reported that the amount of p-coumaric acid increased during roasting probably as a result of hydrolytic reactions from the native esterified or bound forms

A flavor precursor is usually a non-volatile compound which can be converted to a volatile compound under certain conditions such as thermal processing, fermentation, storage, etc. (Newell et al. 1967). The unique roasted peanut flavor developed during the roasting of peanuts is influenced by a number of factors, such as genetics, seed size, maturity, curing, production, handling, and storage conditions (Mason et al. 1969; Oupadissakoon et al. 1980; Pattee et al. 1981; Vercellotti et al. 1994).

Sugars, proteins, and free amino acids have been considered the main precursors of roasted peanut flavor (Chiou et al. 1991; Koehler et al. 1969; Mason et al. 1969). Mason and Waller (1964) used particulate fractionation to identify the specific location of flavor precursors in peanuts. Using a density gradient centrifugation procedure, three fractions (upper, middle and bottom layers) were obtained from re-suspension of particulates in oil. The upper layer, which was rich in aleurone grains and protein bodies, produced the typical aroma of roasted peanuts when heated in oil, and thereby was considered the main source of flavor precursors. The critical minimum temperature for flavor formation was 132ºC, which excluded enzymatically catalyzed reactions giving rise to flavor components.

Previous studies have reported the incidence of sucrose, fructose and glucose in peanuts, with sucrose being the major sugar (Mason et al. 1969; Newell et al. 1967;

Oupadissakoon and Young 1984). During roasting, sucrose is hydrolyzed into fructose and glucose by invertase, and these reducing sugars take part in browning reactions (Mason et al. 1969). Basha (1992) found that sucrose was the major soluble sugar constituent in peanuts, followed by glucosamine, stachyose, and raffinose, while the insoluble fraction contained glucosamide, arabinose, and trace levels of glucose and thamnose.

Amino acids have been considered as the primary source of nitrogen for pyrazine structures formed during roasting (Koehler et al. 1969). Newell et al. (1967) tracked the changes in the concentration of several amino acids during the roasting of mature and immature peanuts, and proposed that aspartic acid, glutamic acid, glutamine, asparagine, histidine, and phenylalanine were the main precursors of the typical peanut flavors, and threonine, tyrosine, and lysine the major precursors of the atypical or off-flavors. Arginine has also been considered the precursor of atypical bitter taste in roasted peanuts (Cobb and Johnson 1973; Woodroof 1983).

Flavor development is sensitive to peanut maturity (Sanders et al. 1982; Sanders et al. 1989). The amount of carbohydrates and α-amino nitrogen in peanuts decreases with increasing maturity, possibly due to the utilization of these compounds in the seed for syntheses of starch, lipid, and protein during maturation (Oupadissakoon et al. 1980; Rodriguez et al. 1989; Vercellotti et al. 1994). Mature peanuts contain more of the amino acid precursors of typical roasted peanuts than immature peanuts (Pattee and Young 1987).

Moisture content is important in flavor development reactions. Raw peanuts containing 10.5 % moisture had more soluble carbohydrates and glucose than peanuts containing 3.4% moisture (Chiou et al. 1991). This is in agreement with Pattee et al. (1982), who found that peanuts stored at higher moisture contents (8.7-9.2 %) contained more glucose, fructose, inositol, and raffinose than peanuts stored at lower moisture contents (6.2-6.3%). Higher moisture peanuts had lower concentrations of pyrazines and roasted peanut flavor, as well as higher sensory scores for undesirable flavors associated with lipid oxidation, such as painty and cardboardy (Abegaz et al. 2004).

Understanding the changes in flavor precursor content during roasting is also essential for peanut flavor optimization. Chiou et al. (1991) noticed that the amount of precursor amino acids in peanuts increased in the early stages of roasting, most likely due to hydrolysis. However, a decrease in the concentration of these compounds was observed as roasting proceeded, probably due to their involvement in further chemical reactions. Thus, the original content of flavor precursors in raw peanuts may not be a final indicator of flavor quality. Basha and Young (1985) observed a decrease in methionine-rich proteins during roasting, and suggested that they might be involved in the formation of pyrazines compounds.

Volatile Composition of Roasted Peanuts

During roasting, flavor precursors in peanuts undergo Maillard or nonenzymatic browning reactions, producing numerous heterocyclic compounds that give rise to roasted peanut flavor. Heat-treated aqueous extracts of roasted peanuts contained several

volatile compounds that were consistent with Maillard reactions such as pyrazines (Coleman et al. 1994).

Early studies into peanut flavor chemistry focused on isolating and identifying thermal products of roasting, but their importance and balance for the overall flavor were not discussed. In a study conducted by Walradt et al. (1971), steam volatiles from roasted spanish peanuts were fractionated using preparative gas chromatography, and the individual fractions were analyzed by GC/MS. A total of 187 compounds were identified, including phenols, carbonyls, alcohols, aromatic hydrocarbons, esters, terpenes, and pyrazines. Several alkyl and alkenyl pyrazines were reported for the first time in roasted peanuts. Johnson et al. (1971b) isolated several volatile compounds of roasted peanuts by vacuum degassing of the pressed oil, followed by fractionation of the condensate. A total of 24 new compounds were identified in the neutral fraction, including seven furans, six pyrroles, three 2-phenyl-2-alkenals, and two thiophenes. Johnson et al. (1971b)Ho et al. (1981) isolated flavor compounds from Florunner variety roasted peanuts by removal of volatiles from the headspace and subsequent condensation. The isolated compounds were fractionated using preparative gas chromatography, and the fractions were identified by a combination of infrared and mass spectrometry. A total of 131 compounds were identified (seventy for the first time) in roasted peanuts, including lactones, pyrazines, pyrroles, pyridines, sulfides, thiazoles, thiophenes, furanoids, oxazoles, oxazolines, and sulfides. Using a similar extraction method, Lee et al. (1981) identified several new volatile compounds in Florunner roasted peanuts, including eight thiazoles, seven oxazoles, and three oxazolines. Buckholdz et al. (1980b) identified the following

compounds using a polymer adsorption method followed by mass spectrometry: isobutyraldehyde, 2-methylbutanal, 1-methylpyrrole, 2-methylpyrazine, and 2-5-dimethylpyrazine.

Brown et al. (1972) found that roasted peanuts contained higher amounts of total carbonyl (compounds containing the functional group C=0) than raw peanuts, and concluded that this was probably due to acceleration of lipid oxidation at high temperatures, and to a lesser extent to Maillard reactions and Strecker degradation. In the same study, 2-methylpropanal, 3-methylbutanal, and 2-methylbutanal were present in roasted peanuts, whereas they were absent in raw peanuts. These aldehydes are produced by Strecker degradation of the corresponding amino acids valine, leucine, and isoleucine, and are thought to be associated with the harsh aroma of freshly roasted peanuts (Mason et al. 1967).

Although early studies characterized the volatile compounds in roasted peanuts, limited information was made available regarding odor quality and intensity. In addition, the flavor isolation and separation techniques used in these studies may have led to formation of artifacts and loss of volatiles.

Active Aroma Compounds and Sensory Perception

The human sensory perception of peanut flavor is determined by a combination of the gustatory, olfactory and trigeminal systems. The gustatory system detects basic tastes such as sweet, bitter, salty, sour, and umami; the trigeminal system relates to perception of chemical feeling factors such as astringency, pungency, and acridness, and the

olfactory system detects volatile compounds (Lawless and Heymann 1998). Volatile compounds are the key element in the flavor profile of roasted peanuts, and they can be perceived orthonasally or retronasally.

In the early 1980's, a descriptive lexicon containing several terms and definitions was developed for sensory evaluation of roasted peanuts (Oupadissakoon and Young 1984). Among the attributes were: astringent, bite, burnt, chemical, earthy, green, nutty, oil, rancid, roasted peanut, sour, stale, bitter, and sweet. A few years later, Johnsen et al. (1988) developed a more complete language, which included terms that described the degree of roast, such as raw bean and dark roast, as well as the flavors generated at different stages of oxidation, such as cardboardy and painty (Table 3). Later on, the terminology was modified and the new descriptor 'fruity', which is an off-flavor associated with high temperature curing, was added (Sanders et al. 1989).

The chemical basis for peanut flavor quality has been extensively investigated over the past several years. Bett et al. (1994) compared the flavor of roasted peanuts from different origins and declared that US-grown peanuts had more intense peanut flavor and less intense fruity/fermented flavor than peanuts from Argentina and China. Peanuts from Argentina had the highest levels of off-flavor related compounds, while Chinese grown peanuts were characterized as cardboardy. Similarly, Young et al. (2005) used descriptive sensory analysis and consumer testing to compare peanuts from different origins. Argentina peanuts were described as musty and sweet, Chinese peanuts exhibited woody/hull/skins flavors as well as bitter and sour tastes, and US-grown peanuts were characterized by sweet aromatic, roasted peanut and dark roast. Consumer testing showed

that US-grown had the highest overall liking scores, followed by China and Argentina grown peanuts, respectively.

Many studies have identified and described the odor characteristics of volatile compounds (Table 4), but their odor potency and actual contribution to flavor was not elucidated. Although GC/O is not a new technology (Fuller et al. 1964), application to peanut flavor volatiles is relatively new. GC/O was not applied in most, if any of the early studies dealing with aroma description of volatile compounds isolated from roasted peanuts. Ho et al. (1981) identified several compounds in the aroma of roasted peanuts and suggested that a few of them might contribute to the flavor profile of roasted peanuts. For example, 2-isopropyl-4,5-dimethylthiazole and 2-propyl-4,5-diethylthiazole had a pleasant nutty odor. 2-crotolactone and 3-methyl-2-crotolactone were also described as nutty. N-methylpyrrole was characterized as having a sweet, woody odor. Most of the oxazoles identified in their study were described as having a green nutty aroma. In general, the pyrazines were described as producing pleasant roasted nut-like notes.

Similarly, Lee et al. (1981) proposed that numerous compounds might play a role in the aroma of roasted peanuts. For example, they reported that 2-isopropyl-4,5-dimethylthiazole and 2-propyl-4,5-diethylpyrazole had a pleasant nutty flavor. 2-acetyloxazole was described as having nutty and popcorn-like character; while 2,4,5-trimethyloxazole and 2,4-diethyl-5-propyloxazole were described as nutty, sweet and green. The authors also suggested that 4,5-dimethyloxazole and 5-butyloxazole might impart a green, sweet, and vegetable note to the aroma of roasted peanuts. Finally, 2,4,5-trimethyl-3-oxazoline had woody and green odors; and 2-methyl-3-oxazoline and 2,4-

dimethyl-3-oxazoline were characterized as nutty and sweet. Brown et al. (1973) reported that hexanal and octanal and possibly nonanal and 2-nonenal were associated with beany flavor. Buckholz and Daun (1981) suggested that 2,4-dimethyl-3-thiazoline was associated with the nut skin attribute in the flavor of roasted peanuts.

Low molecular weight aldehydes have been associated with the harsh green notes from fresh roasted peanuts (Johnson et al. 1971a; Johnson et al. 1971b; Mason et al. 1966). Pentanal has been described as having a solvent-like note, and was negatively related to sensory preference of roasted peanuts (Buckholz et al. 1980). Brown et al. (1972) suggested that 2-heptanal, 2-octenal, 2-nonenal and 2,4-decadienal were associated with fatty and deep-fried notes of cold- pressed oil from freshly roasted peanuts. The sweet aroma from roasted peanuts has been attributed to phenylacetaldehyde (Mason et al. 1967).

More recent peanut flavor research has benefited from descriptive sensory analysis as well as GC/O analytical methods that facilitate identification of key odor-active compounds (Braddock et al. 1995; Didzbalis et al. 2004; Greene et al. 2008; Matsui et al. 1998; Schirack et al. 2006). Further, several techniques such as Aroma Extract Dilution Analysis (AEDA) and Charm Analysis have been used in flavor chemistry research to determine the relative strength of odor components and thus identify the most potent odorants contributing to a flavor (Braddock et al. 1995; Grosch 1993; Matsui et al. 1998; Schirack et al. 2006).

Braddock et al. (1995) used GC/O to evaluate the odor characteristics of several compounds isolated from roasted peanuts. 3-methylpyridine had an intense roasted

peanut odor, benzeneacetaldyde was described as flowery and sweet, and 2-3-dihydrobenzofuran provided a harsh, sulfur like aroma. Benzothiazole smelled burnt at the sniffer port, and hexanal provided a green, grassy off-note. Nonanal was described as floral, while heptan-2-ol and 1-pentanol had slightly pungent and green aromas. The most potent odorants in the volatiles extracted from headspace of freshly roasted peanuts were: 2,5-dimethylpyrazine, methylpyrazine, and 2-ethyl-3-methylpyrazine, as determined by Aroma Extract Dilution Analysis (AEDA). These compounds were described as malty/chocolate, grilled chicken/savory, and roasted, respectively.

Schirack et al. (2006) used GC/O to characterize the aroma properties of solvent extracts from roasted peanuts, and subsequently used aroma extract dilution (AEDA) to determine those with the highest impact to the overall flavor. They identified over 200 aroma-active compounds, and thirty-eight were reported as the main flavor active volatiles of roasted peanuts. Among the pyrazines were: 2,6-dimethylpyrazine (FD_3= 6), 2-ethyl-5-methylpyrazine (FD_3= 4); 2,5-dimethyl-3-ethylpyrazine (FD_3= 7); 2-ethyl-3,5-dimethylpyrazine (FD_3= 8); 2,3-diethyl-2-methylpyrazine (FD_3= 6) and 3-methoxy-2,5-dimethylpyrazine (FD_3= 4) (Table 5). Several other classes of compounds were reported to contribute to the overall flavor of roasted peanuts, as shown in Table 4.

Basha and Young (1996) separated peanut seed proteins into 10 fractions by gel filtration, and found that one of these fractions produced several volatile compounds associated with off-flavors in roasted peanuts. In a subsequent study, they concluded that the protein fraction that was capable of producing off-flavor volatiles was lipoprotein in nature and rich in oleic acid (Basha et al. 1998). Some of the compounds produced by

this protein fraction were N-methylpyrrole pentane, acetone, dimethyl sulfide, 2-methylpropanol, pentanal, and hexanal. These compounds have been associated with a variety of off-flavors such as musty, fruity, tongue burn, and beany (Young and Hovis 1990). It was suggested that in addition to lipid oxidation, thermal degradation of proteins might be a source of the off-flavor compounds in roasted peanuts.

Ethyl acetate was associated with fruity off-flavors developed at high curing temperatures, and was considered an indicator of deterioration of aroma and flavor (Singleton et al. 1971). Similarly, Pattee et al. (1965) reported that ethyl acetate and acetaldehyde contributed to off-flavors developed in high-temperature cured peanuts. Sanders et al. (1989) reported that immature peanuts cured at higher temperatures had lower intensities of sweet aromatic and roasted peanut and higher intensities of fruity/fermented, painty, sour, and bitter flavor attributes. Some reductones such as hydroxyfuranones and furaneols have been associated with the fruity/fermented off-flavors found in improperly dried or freeze damaged peanuts (Vercellotti et al. 1994).

In a recent study, Didzbalis et al. (2004) used gas chromatography-olfactometry (GC/O) and solvent assisted flavor evaporation (SAFE) to identify the compounds responsible for fruity/fermented off-flavor developed in immature peanuts artificially subjected to constant high temperature (40ºC) curing. Fruity esters such as ethyl 2-methylpropanoate, ethyl 2-methylbutanoate, and ethyl 3-methylbutanoate, and high levels of short chain organic acids such as butanoic, 3-methylbutanoic, and hexanoic were associated with the fruity fermented off-note. Model system studies showed that the short chain organic acids were responsible for the cheesy/fermented aroma, while the esters

contributed to the fruity, apple-like aromas. Greene (2007) and Green and othes (2008) used instrumental, and descriptive sensory analysis to compare roasted peanuts containing naturally occurring and artificially created fruity fermented off-flavors. The esters identified by Didzbalis et al. (2004) were not identified in samples having naturally occurring fruity fermented off-flavor. In addition, methylpropanoate was absent in both natural and artificially created fruity fermented samples. Immature peanuts cured at high temperature (40^{o}C) (artificially created off-flavors) contained ethyl-2-methylbutanoate and ethyl 3-methylbutanoate, which was in agreement with Didzbalis et al. (2004). Sensory differences between natural and artificially created fruity fermented were also reported. The former was usually described as having an overripe fruit note, whereas the latter was described as rotten garbage/soured off-flavor. Greene (2007) suggested that differences between natural and artificially created samples were responsible for the observed differences.

Pyrazines and Roasted Peanut Flavor

Investigation of roasted peanut flavor has been an ongoing effort for over 40 years (Brown et al. 1968; Mason and Waller 1964; Mason et al. 1966; Newell et al. 1967; Waller et al. 1969). Pyrazines, which are heterocyclic compounds containing two nitrogen atoms, have been traditionally suggested to be the key element for the typical aroma of roasted peanuts (Mason et al. 1966; Mason et al. 1967; Mason et al. 1969; Maga 1973; Shibamoto and Bernhard 1976). Table 5 shows the major pyrazine compounds that

have been identified in roasted peanuts, along with the aroma descriptors associated with them.

Mason et al. (1966) reported that the following pyrazines produced typical nutty flavors: methylpyrazine; 2,5-dimethylpyrazine; trimethylpyrazine, methylethylpyrazine, and dimethylethylpyrazine. Johnson et al. (1971a) analyzed the basic fraction volatiles of roasted peanuts, which were isolated by vacuum degassing of the pressed oil. Several alkylpyrazines were reported for the first time, and as a group they contributed to the 'nut-like' character of typical roasted peanuts. Buckholz et al. (1980) correlated sensory evaluation with instrumental analysis of peanuts roasted for various lengths of time and found that a decrease in carbonyls and subsequent increase in pyrazines was an indication of good quality peanut flavor. For instance, a positive correlation was noticed between the concentration of 2-ethyl-6-methyl pyrazine, which has roasted, nutty notes, and sensory preference. Crippen et al. (1992) reported that pyrazines, methylbutanal, methylpropanal, and sulfur compounds (eg. methanethiol, carbon disulfide and dimethyl-sulfide) were associated with dark roasted flavors. In a study conducted by Leunissen et al. (1996), methylpyrazine was directly related to perception of roasted flavor at low concentrations.

Tsantili-Kakoulidou and Kier (1992) developed a mathematical model to predict odor strength of pyrazines as a function of their topology, shape and electronic features. They found that as the alkyl substituents became longer and more branched in the molecule, odor strength of pyrazines increased (lower threshold values). Ho et al. (1983) reported that, in general, alkylpyrazines have nutty, green, and vegetable-like aromas.

Mono-substituted pyrazines and substituted methylpyrazines have been described as nutty and roasted peanutty (Masuda and Mihara 1988).

Using gas chromatograph olfactory (GC/O), Braddock et al. (1995) identified several aroma-active compounds from freshly roasted peanuts. 2,5-dimethylpyrazine, methylpyrazine, and 2-ethyl-3-methylpyrazine were described as malty/chocolate, grilled chicken/savory, and roasted, respectively. In addition, 2,5-dimethyl-pyrazine was characterized as chocolaty/malty, and ethylpyrazine as dark/roasted. Baker et al. (2003) examined 4 peanut genotypes and found that among the pyrazines tested, 2,5-dimethylpyrazine correlated the most with roasted peanut flavor and aroma obtained from sensory data (Flavor: $R^2 > 0.83$; Aroma: $R^2 > 0.87$). They concluded that this compound could be used as a sensory predictor of roasting in peanut products. Roasted peanuts from the Fall crop in Taiwan were found to contain higher levels of total pyrazines than peanuts from the Spring crop, which was consistent with the general consumer concept that the former is richer in peanut flavor (Ku et al. 1998).

In an early study by Koehler et al. (1971), the threshold concentrations of several pyrazines in water and oil were determined. Although none of the compounds alone had the typical aroma of roasted peanuts, several alkylpyrazines, especially 2-ethylpyrazine, 2-methylpyrazine, and methylethylpyrazine, produced ‘roasted’ and ‘nutty’ responses. However the concentration of 2-methylpyrazine in roasted peanuts was below the odor threshold level in both water and oil.

Theories for Pyrazine Formation

Roasting conditions directly influence the formation of pyrazines and perception of roasted peanut flavor. In a study by Leunissen et al. (1996), the amount of pyrazines increased as roasting conditions changed from mild (low temperature/ short time) to severe (high temperature/ long time). The authors also reported that methyl and other pyrazines were desirable at low concentrations, but they became increasingly bitter at high concentrations as determined by descriptive sensory analysis. In another study, the optimum roasting condition tested for 4 peanut genotypes was 175^{o}C/ 15 minutes, where the highest level of pyrazines and sensory scores for roasted peanut flavor were achieved (Baker et al. 2003).

The effect of reaction temperature on the formation of pyrazines has also been investigated. Hwang et al. (1994) found that the total amount of pyrazines increased with reaction temperature in a model system containing glucose and lysine. In addition, long chain alkyl-pyrazines had higher activation energy, thus they required higher temperatures for formation. Koehler and Odell (1970) studied the effect of temperature for pyrazine synthesis in a sugar-amino acid model system and found that few pyrazines were formed below temperatures of 100^{o}C. They also reported that the yield of pyrazines increased as the temperature increased up to 150^{o}C. Coleman and Steichen (2006) noticed that reaction temperature not only influenced the total yield but also affected the distribution of pyrazines on microwave heat-treated systems containing rhamnose, ammonium hydroxide, leucine, and valine. In a model system where glucose and asparagine were heated at 120^{o}C for 24 hours, the major compound formed in early

stages of the reaction was methylpyrazine. However, as the reaction proceeded the amount of dimethylpyrazine increased until its ratio to methylpyrazine became constant at about 3 (Koehler and Odell 1970). Shibamoto and Bernhard (1976) found that reactant ratio, as well as reaction time and temperature, influenced total yield but not the distribution of pyrazines formed in ammonia-glucose model systems.

Several mechanisms for pyrazine formation have been proposed. In general, sugars and sugar degradation products are considered as the primary carbon sources for pyrazine carbon structures formed during roasting, while amino acids are considered to be the nitrogen supply (Dawes and Edwards 1966; Koehler et al. 1969). The most currently accepted pathway for pyrazine formation involves the reaction of amino acids with α- dicarbonyls compounds (intermediate products in the Maillard reaction) through Strecker degradation, producing α-amino carbonyls, which then condense to give rise to alkylpyrazines (Figure 1) (Fennema 1996)

Shibamoto and Bernhard (1977a,b) proposed ten α-amino carbonyl fragments (Figure 2) that can produce the following upon condensation: pyrazine, 2-methylpyrazine, 2,5- and 2,6-dimethlypyrazine, trimethylpyrazine, 2-ethylpyrazine, 2-ethyl-5-methyl- and 2-ethyl-6-methylpyrazine, and 2-ethyl-3,5-dimethyl- and 2-ethyl-3,6-dimethylpyrazine. For example, Figure 3 illustrates the synthesis of methylpyrazine from condensation of the fragments I and V.

Another well-accepted pathway assumes that ammonia is released from amino acids during pyrolysis and serves as the intermediate for pyrazine formation. A proposed mechanism of deamination from α- and β-amino acids is shown in Figure 4 (Shu 1998).

It is well known that glutamine releases ammonia easier than other amino acids through the process of deamidation and deamination during thermal degradation (Sohn and Ho 1995). Thus, if ammonia served as the intermediate during pyrazine formation, higher yield of pyrazine compounds would be expected in reaction systems involving glutamine as compared to other amino acids. In fact, Chen and Ho (1999) investigated the formation of pyrazines in ribose/glucose/fructose–glutamine/serine/threonine model systems, and found that the glutamine-containing systems generated more total pyrazines than serine and threonine-containing systems. Furthermore, if ammonia functions as an intermediate in the formation of pyrazines, a similar distribution pattern of these compounds would be expected regardless of the precursor amino acid. Van Praag et al. (1968) reported that a similar series of pyrazines was formed when fructose reacted with different amino acids such as glycine, serine, leucine, isoleucine, valine, and alanine. Similarly, Newell et al. (1967) indicated that essentially the same pyrazines were formed in sugar amino acid systems, regardless of the nitrogen source employed. Shu (1998) obtained tetramethylpyrazine from the reaction between acyloins (α-Hydroxy ketones) and amino acids. They concluded that ammonia was released from the amino acids and served as the intermediate because acyloins are not involved with Strecker degradation, and are known to produce pyrazines when reacted with ammonia.

Conversely, Koehler et al. (1969) reported that the distribution of pyrazines formed in glucose-amino acid and glucose-ammonium chloride systems was dependent on the nitrogen source. Ammonium chloride yielded mostly pyrazine and only traces of alkylated pyrazines, while amino acids gave mostly alkylated pyrazines with very small

amounts of pyrazine. They concluded that nitrogen was still bound to the amino acid upon condensation with the sugar. Similarly, Wong and Bernhard (1988) reported that the types and amounts of pyrazines were dependent upon the nitrogen source in model systems containing glucose mixed with ammonium hydroxide, ammonium formate, ammonium acetate, glycine, and monosodium glutamate. Hwang et al. (1995) compared the total yield of pyrazines for several amino acid –glucose model systems and reported that lysine and glycine were among the most and least reactive amino acids in the synthesis of pyrazines, respectively. Arnoldi et al. (1988) reacted fructose with eight different amino acids in deodorized cocoa-butter-water model systems and concluded that the synthesis of some pyrazines was aspecific, i.e., it occurred with every amino acid, but that others were specific, i.e., it occurred only with certain amino acids. Pyrazines formed from specific amino acids were generally high molecular weigh compounds, having long, branched, or oxygenated substituents, which probably originated from the precursor amino acid.

Koehler et al. (1969) proposed that condensation of nitrogen with two or three-carbon fragments from hexoses led to formation of alkalized pyrazines. For example, methylpyrazine would be formed from condensation of nitrogen with one two-carbon fragment and one three-carbon fragment; dimethylpyrazine from two two-carbon fragments and so on. These sugar fragments could be formed by retro-aldol condensation, which is catalyzed by amines (Shibamoto and Russel 1977). Amino groups of lysine have been shown to catalyze the fragmentation of sugar molecules during Maillard reaction (Hwang et al. 1994), which is consistent with the observation of Whistler and BeMiller

(1958) that the higher the pH, the faster the rate of sugar fragmentation. Shibamoto and Bernhard (1976) also reported that adding sodium hydroxide or ammonia to glucose-ammonia model systems increased total pyrazine yield in a similar way. They concluded that besides serving as a reactant for the synthesis of pyrazines, ammonia functions as a basic catalyst for sugar fragmentation. The carbon source has been shown to affect the yield of pyrazines, probably due to differences in fragmentation. Fructose produced the highest yields of alkylpyrazines as well as dimethylpyrazine to methylpyrazine ratio among glucose, sucrose, and arabinose (Koehler and Odell 1970). In another model system study, rhamnose was shown to yield more alkyl substituted pyrazines such as ethyldimethylpyrazines than glucose when reacting with ammonia (Shibamoto and Russel 1977; Shibamoto and Bernhard 1978).

An alternate pathway for pyrazine formation involves pyrolysis of amino acids and does not require the presence of sugar. Several pyrazines were obtained from heating individual nitrogenous organic compounds under air flow (Wang and Odell 1973). Alkylpyrazines were also obtained without a carbohydrate source from the pyrolysis of α-hydroxy amino acids (Kato et al. 1970). Similarly, Shu (1999) obtained several pyrazines by heating serine or threonine as well as a combination of these at different temperatures. They suggested that conversion of α- hydroxycarbonyls into α-aminocarbonyls by decarbonylation followed by dehydration gave rise to alkylpyrazines.

Lipid oxidation and off-flavors

Peanuts contain approximately 50% fat, of which about 80% is unsaturated. Because of the high level of unsaturated fatty acids, peanuts are susceptible to lipid oxidation, which produces monohydroperoxides that may become precursors for volatile aldehydes such as nonanal, octanal, decanal, and hexanal (Min 1988; Min et al. 1989; Nawar 1985; Warner et al. 1996). Several studies have shown that off-notes such as cardboardy, painty and oxidized are associated with these compounds (Braddock et al. 1995; Reed et al. 2002). Figure 5 shows the mechanism of lipid oxidation and formation of primary and secondary degradation products (Shahidi 2000).

Oleic acid and linoleic acids represent about 50% and 30% of the total fatty acid composition of normal peanuts, respectively (Cobb and Johnson 1973; Mercer et al. 1990). The University of Florida developed a high-oleic peanut line containing > 80% oleic acid and < 3% linoleic acid (Gorbet and Knauft 1997; Knauft et al. 2000). Because oxidation of linoleic acid occurs about ten times faster than oxidation of oleic acid ((Nawar 1985), high oleic acid peanut varieties have higher stability against lipid oxidation and thereby longer shelf life than normal oleic lines (Braddock et al. 1995; Mugendi et al. 1998; Nepote et al. 2006a; O'Keefe et al. 1993; Reed et al. 2002).

Isleib et al. (2006) compared the sensory profile and chemical composition of normal and high oleic peanuts from two databases. From the first one, they found no differences in off-flavors, but small differences in roasted peanut, astringency, over-roasted, and nutty attributes between these two varieties. From the second database, they observed that the high oleic line had slightly lower intensities of cardboardy and painty

notes. However, they concluded that the sensory differences between freshly roasted normal and high oleic peanuts were too small in magnitude for an average consumer to detect. Indeed, Nepote et al. (2006a) found no difference between normal and high oleic freshly roasted peanuts in a consumer acceptance test. Talcott et al. (2005) reported that high-oleic roasted peanuts had higher burnt peanut flavor and aroma than normal oleic lines, but declared that no differences existed for roasted peanut flavor and aroma, sweetness, and bitterness.

Peanut Storage and Flavor Fade

Flavor fade is defined as the loss of positive attributes associated with fresh-roasted peanuts such as 'roasted peanut flavor' accompanied by development of off-flavors during storage (Abegaz et al. 2004). Understanding the sources of flavor fade in peanuts is beneficial for both processors and consumers. It requires knowledge of lipid oxidation and carbonyl-amine reactions, flavor entrapment between proteins and lipid hydroperoxides, as well degradation of heterocyclic nitrogen compounds by lipid radicals and hydroperoxides (Alzagtat and Alli 2002; Warner et al. 1996; Williams et al. 2006).

Lipid oxidation leads to the formation of numerous undesirable compounds such as hexanal, heptanal, octanal, and nonanal, which are associated with painty, cardboardy, and oxidized flavors. In addition, subproducts of lipid oxidation may interact with roasted peanut flavor compounds such as pyrazines, ultimately leading to flavor fade (Warner et al. 1996; Williams et al. 2006). Flavor fade may also be associated with the masking of

roasted peanut flavor compounds, such as pyrazines, by large quantities of low molecular weight aldehydes formed during lipid oxidation (Dimick 1994).

Braddock et al. (1995) compared the flavor stability of normal and high oleic roasted peanuts stored at 25°C for 74 days, and concluded that pyrazines and peanut flavor were more stable in the high oleic variety than in normal peanuts. Normal oleic variety peanuts had significantly lower pyrazine FD values than high oleic peanuts. Hexanal content as well as painty and cardboardy attributes were lower for the high oleic variety. After 74 days of storage, the FD-value for hexanal in the normal oleic variety was twice as much as the high-oleic variety. Similar results were reported by Nepote et al. (2006a), who found that the high oleic variety developed less cardboardy flavor and had a slower rate of peanut flavor loss than normal oleic peanuts stored at 23 and 40°C. They predicted the shelf life of high oleic roasted peanuts to be 25 and 10 times longer than normal roasted peanuts when stored at 23 and 40°C, respectively.

Williams et al. (2006) conducted short-term storage studies with fresh roasted peanuts to evaluate changes in the concentrations of hexanal and pyrazines over time. An increase in the concentration of hexanal with parallel increases in painty and cardboardy flavors was reported. In addition, decreases in the concentrations of several pyrazines such as 2,3-diethylpyrazine, 2-3-dimethylpyrazine, 2-ethyl-3-methylpyrazine, and 2,3,5-trimethylpyrazine were accompanied by a decrease in roasted peanut flavor.

Similarly, Bett and Boylston (1992) found a decrease in the concentration of alkylpyrazines in roasted peanuts stored at 37°C for 12 weeks, especially during the initial weeks. Reed et al. (2002) also showed that pyrazines and peanut flavor decreased

during storage of high and normal oleic peanuts, and that the concentration of pyrazines for the high oleic were higher than the normal oleic during storage. This is in agreement with Vercellotti et al. (1992), who reported that compounds responsible for fresh roasted peanut flavor disappeared in rancid peanuts. The decrease in the content of pyrazines in these studies might be explained by flavor entrapment or degradation of pyrazines by free radicals or hydroperoxides from lipid oxidation (Funes et al. 1982; Gardner 1979; St. Angelo and Vercellotti et al. 1992; Williams et al. 2006).

In contrast, Warner et al. (1996) found no change trends in the concentration of several pyrazines such as 2,6-dimethylpyrazine, 2-methylpyrazine, 2-ethyl-5-methylpyrazine, and 6-methylpyrazine, but a decrease in the roasted peanut flavor over 65-days storage at 65^{o}C. They did notice an increase in the concentration of hexanal, heptanal, octanal, and nonanal during storage, which was accompanied by an increase of oxidative rancid flavor. They suggested that peanut roasted flavor was masked by the low-molecular weight aldehydes produced during lipid oxidation during storage. The authors suggested that the high storage temperature at which their study was conducted (65^{o}C) might have influenced the differences observed between their results and previous reports dealing with pyrazine changes over time. However, several studies have shown that pyrazines are not usually formed below 70^{o}C (Koehler and Odell 1970; Maga 1982; Shibamoto and Bernhard 1976).

Pattee et al. (1999) conducted low-temperature-long term stability studies in roasted peanut paste, and found that lipid oxidation occurred even at temperature as low as -23 ^{o}C. At this condition, the intensity of stale flavor, as well as fruity/fermented flavor

increased over time up to 13 months. Peanut flavor, however, seemed to be stable. This is in agreement with the observation of Pattee et al. (2002) who reported that roasted peanut flavor was stable in normal and high oleic roasted peanuts stored at -20°C for two months.

Pattee et al. (1971) stored unshelled peanuts under simulated warehouse conditions and shelled peanuts under controlled environmental conditions, and found that total volatiles increased with storage time. Pentane, acetaldehyde, and methanol represented the majority of these compounds, which were probably formed due to enzyme activity. In a more recent study, Nepote et al. (2006b) tested the effect of adding a honey coating to roasted peanuts to prevent lipid oxidation during storage. They found that the intensity of cardboardy and oxidized notes increased, while peanut flavor decreased during storage at 23 and 40°C for both the coated and control roasted peanuts. However, the degree of change for the honey coated samples was less pronounced, and thus they were more resistant to lipid oxidation.

The role of moisture in flavor fade of peanut products is not clearly understood. Reed et al. (2002) studied the effect of water activity (a_w) in the flavor stability of roasted peanuts and found that lowering a_w augmented formation of lipid oxidation products as well as loss of pyrazine compounds. Mate et al. (1996) also found that low relative humidity (~20%) increased the rate of lipid oxidation of peanuts compared to high relative humidity (~60%). In contrast, Felland and Koehler (1997) found that adding 2.5 and 5% moisture to peanut butter decreased perceived roasted aroma and flavor and increased development of off-flavors during 29 days of storage at 25ºC. Abegaz et al.

(2004) also reported that peanut butter containing 2 and 5% added moisture had lower roasted peanut flavor intensity and lower concentrations of pyrazines than samples without added moisture during storage at 21ºC for 52 weeks. Baker et al. (2002) found that intermediate water activities (0.33 and 0.44) were the best to control lipid oxidation in high-oleic peanuts, as measured by peroxide value.

Conclusions and Areas for Future Research

The unique flavor developed during the roasting of peanuts is one of the major factors influencing consumer choice and acceptance. Investigation of peanut flavor quality has been an ongoing challenge and although pyrazines have been considered the source of roasted peanut flavor for over 40 years, there is limited information that unquestionably proves it to be true. The fact that many pyrazines have been identified in the volatile composition of roasted peanuts does not necessarily mean they play a major role in peanut flavor. Compounds may be present in a food while having little contribution to flavor if their concentrations are below sensory threshold (McGorrin 2002). In addition, the fact that many studies have reported that pyrazines produce nutty/roasted notes does not specifically imply they are the basis for roasted peanut flavor. The aroma of individual compounds may not be directly related to their actual role in flavor due to interactions with the matrix and other chemical compounds present in the food (Drake and Civille 2003).

Most of the studies that have investigated the flavor chemistry of peanuts were conducted several decades ago. The isolation and separation techniques of flavor

components used in these studies may have led to formation of several artifacts and loss of volatiles. Descriptive sensory analysis using references and a defined language was not conducted nor were model system studies carried out to specifically determine the role of pyrazine compounds in the flavor profile of roasted peanuts. Odor activity value (OAV), which is the ratio of concentration to the sensory odor threshold, may indicate the importance of a given compound to flavor (Drake 2007). To the best of our knowledge, OAV values of the compounds isolated in early studies were not reported. The relative importance and balance of these compounds for the overall flavor of roasted peanuts had not been elucidated by any other means.

More recent peanut flavor research has benefited from analytical methods that facilitate identification of key odor-active compounds such as Gas chromatography – olfactometry (GC/O) and Aroma Extract Dilution Analysis AEDA. Schirack et al. (2006) found that thirty-eight compounds were the main contributors for the aroma of roasted peanuts. Only seven of these compounds were pyrazines. It is plausible that several other compounds other than pyrazines might be associated with the roasted peanut flavor.

Although GC/O is a valuable technique to screen compounds that fall into threshold ranges, it does not necessarily indicate that they are essential for the aroma of a food. Accurate links cannot be established due to possible interactions of these individual compounds with the matrix and other compounds present in the food (Drake and Civille 2003). Consequently, confirmation of the key aroma contributing compounds by means of model systems that mimic the food matrix is needed. Further research into peanut

flavor quality demands the use of model systems to undoubtedly pinpoint the compounds contributing to roasted peanut flavor.

Table 1: Proximate composition for Virginia-type raw and roasted peanuts.

Constituent	Raw (%)	Roasted (%)
Moisture	6.7	2.5
Fiber	4.9	5.4
Fat	47	48.8
Nitrogen	4.53	4.76
Protein	24.7	26
Ash	2.54	3.39

Adapted from Derise et al. (1974).

Table 2: Mineral composition (mg/100g) for runner market-type peanuts.

P	Ca	K	Mg	Cu	Fe	Zn	Na
355.9	76.4	577.3	190.8	1.0	2.2	2.9	27.1

Adapted from Jonnala et al. (2005)

Table 3: Lexicon of Peanut flavors developed by Johnsen et al. (1988).

	Descriptor	Definition
Aromatics	Roasted Peanutty	The aromatic associated with medium-roast peanuts (about 3-4 USDA color chips) and having fragrant character such as methylpyrazine
	Raw Beany	The aromatic associated with light-roast peanuts (about 1-2 on USDA color chips) and having legume-like character (specify beans or pea if possible)
	Dark Roasted Peanut	The aromatic associated with dark roasted peanuts (4+ on USDA color chips) and having very browned or toasted character
	Sweet Aromatic	The aromatics associated with sweet material such as caramel, vanilla, molasses, fruit (specify type)
	Woody/Hulls/Skins	The aromatics associated with base peanut character (absence of fragrant top notes) and related to dry wood, peanut hulls, and skins
	Cardboardy	The aromatic associated with somewhat oxidized fats and oils and reminiscent of cardboard
	Painty	The aromatic associated with linseed oil, or oil based paint
	Burnt	The aromatic associated with very dark roast, burnt starches, and carbohydrates (burnt toast or espresso coffee)
	Green	The aromatic associated with uncooked vegetables/grasstwigs, *cis*-3-hexanal
	Earthy	The aromatic associated with wet dirt and mulch
	Grainy	The aromatic associated with raw grain (bran, starch, corn, sorghum)
	Fishy	The aromatic associated with trimethylamine, cod liver oil, or old fish
	Chemical/Plastic	The aromatic associated with plastic and burnt plastics
	Skunky/Mercaptan	The aromatic associated with sulfur compounds, such as mercaptan, which exhibit skunk-like character
Tastes	Sweet	The taste on the tongue associated with sugars
	Sour	The taste on the tongue associated with acids
	Salty	The taste on the tongue associated with sodium ions
	Bitter	The taste on the tongue associated with bitter agents such as caffeine or quinine
Chemical Feelings	Astringent	The chemical feeling factor on the tongue, described as puckering/dry and associated with tannins or alum
	Metallic	The chemical feeling factor on the tongue described as flat, metallic and associated with iron and copper

Table 4: Chemical compounds isolated from roasted peanuts

Compounds	Isolation* Method (and fraction)	Odor detection Method	Odor Quality	Reference
2-isopropyl-4,5-dimethylthiazole	Purge and trap	Sniffing of isolated compounds obtained by preparative GC	Nutty	Ho et al. (1981)
2-propyl-4,5-diethylthiazole	Purge and trap	Sniffing of isolated compounds obtained by preparative GC	Nutty	Ho et al. (1981)
2-crotolactone	Purge and trap	Sniffing of isolated compounds obtained by preparative GC	Nutty	Ho et al. (1981)
3-methyl-2-crotolactone	Purge and trap	Sniffing of isolated compounds obtained by preparative GC	Nutty	Ho et al. (1981)
N-methylpyrrole	Purge and trap	Sniffing of isolated compounds obtained by preparative GC	Sweet/ woody	Ho et al. 1981
2-isopropyl-4,5-dimethylthiazole	Purge and trap	Sniffing of isolated compounds obtained by preparative GC	Nutty	Lee et al. (1981)
2-propyl-4,5-diethylpyrazole	Purge and trap	Sniffing of isolated compounds obtained by preparative GC	Nutty	Lee et al. (1981)
2-acetyloxazole	Purge and trap	Sniffing of isolated compounds obtained by preparative GC	Nutty/popcorn	Lee et al. (1981)
2,4,5-trimethyloxazole	Purge and trap	Sniffing of isolated compounds obtained by preparative GC	Nutty/ sweet/ green	Lee et al. (1981)

Table 4. Continued

2,4-diethyl-5-propyloxazole	Purge and trap	Sniffing of isolated compounds obtained by preparative GC	Nutty/ sweet / green	Lee et al. (1981)
4,5-dimethyloxazole	Purge and trap	Sniffing of isolated compounds obtained by preparative GC	Green/ sweet/ vegetable	Lee et al. (1981)
5-butyloxazole	Purge and trap	Sniffing of isolated compounds obtained by preparative GC	Green/ sweet/ vegetable	Lee et al. (1981)
2,4,5-trimethyl-3-oxazoline	Purge and trap	Sniffing of isolated compounds obtained by preparative GC	Woody/green	Lee et al. (1981)
2-methyl-3-oxazoline	Purge and trap	Sniffing of isolated compounds obtained by preparative GC	Nutty /sweet	Lee et al. (1981)
2,4-dimethyl-3-oxazoline	Purge and trap	Sniffing of isolated compounds obtained by preparative GC	Nutty /sweet	Lee et al. (1981)
Pentanal	Purge and trap	N/A	Solventy /green	Buckholz et al. (1980).
3-methylpyridine	Dynamic headspace	GC/O	Roasted peanut butter	Braddock et al. (1995)
Benzeneacetaldehyde	Dynamic headspace	GC/O	Floral/ sweet/caramel	Braddock et al. (1995)

Table 4. Continued

2-3-dihydrobenzofuran	Dynamic headspace	GC/O	Rubbery/harsh	Braddock et al. (1995)
Benzothiazole	Dynamic headspace	GC/O	Harsh/burnt/ rubbery	Braddock et al. (1995)
Hexanal	Dynamic headspace	GC/O	Green, grassy	Braddock et al. (1995)
Nonanal	Dynamic headspace	GC/O	Floral	Braddock et al. (1995)
heptan-2-ol	Dynamic headspace	GC/O	Pungent/ green	Braddock et al. (1995)
Acetic acid	Dynamic headspace	GC/O	Bread dough/yeasty	Braddock et al. (1995)
Phenylacetaldehyde	SAFE (NB fraction)	AEDA	Rosy/green	Schirack et al. (2006)
Guaiacol	SAFE (NB fraction)	AEDA	Burnt	Schirack et al. (2006)
2-methylbutanal	SAFE (NB fraction)	AEDA	Chocolate/malt	Schirack et al. (2006)

Table 4. Continued

Toluene	SAFE (NB fraction)	AEDA	Sweet/chemical	Schirack et al. (2006)
2,3-butanediol	SAFE (NB fraction)	AEDA	Fruity	Schirack et al. (2006)
Furfural	SAFE (A fraction)	AEDA	Sweet	Schirack et al. (2006)
(E)-2-hexenal	SAFE (A fraction)	AEDA	Fruity	Schirack et al. (2006)
Ethyl valerate	SAFE (A fraction)	AEDA	Fruity	Schirack et al. (2006)
Heptanal	SAFE (NB fraction)	AEDA	Fatty	Schirack et al. (2006)
(E,Z)-2,4-heptadienal	SAFE (NB fraction)	AEDA	Fatty	Schirack et al. (2006)
Methyl hexanoate	SAFE (A fraction)	AEDA	Sweet	Schirack et al. (2006)
Furaneol™ (2,5-dimethyl-4-hydroxy-3(2H)-furanone)	SAFE (A fraction)	AEDA	Burnt sugar	Schirack et al. (2006)

Table 4. Continued

Acetophenone	SAFE (NB fraction)	AEDA	Fruity/sweet	Schirack et al. (2006)
Maltol (3-hydroxyl-2-methyl-4H-pyran-4-one)	SAFE (A fraction)	AEDA	Cotton candy	Schirack et al. (2006)
Nonanal	SAFE (NB fraction)	AEDA	Green/floral	Schirack et al. (2006)
4-ethylbenzaldehyde	SAFE (A fraction)	AEDA	Burnt sugar	Schirack et al. (2006)
3-ethylphenol	SAFE (NB fraction)	AEDA	Old books/musty	Schirack et al. (2006)
Decanal	SAFE (NB fraction)	AEDA	Fried	Schirack et al. (2006)
(E,E)-2,4-decadienal	SAFE (NB fraction)	AEDA	Fried/oxidized	Schirack et al. (2006)
Decanoic acid	SAFE (NB fraction)	AEDA	Oxidized	Schirack et al. (2006)
Delta-elemene	SAFE (NB fraction)	AEDA	Wood	Schirack et al. (2006)
4-acetoxy-2,5-dimethyl-3(2H)-furanone	SAFE (A fraction)	AEDA	Burnt sugar	Schirack et al. (2006)

Table 4. Continued

Delta-decalactone	SAFE (A fraction)	AEDA	Sweet/fruity	Schirack et al. (2006)
Geranyl butyrate	SAFE (NB fraction)	AEDA	Rosy	Schirack et al. (2006)
Tetradecanal	SAFE (NB fraction)	AEDA	Honey/hay	Schirack et al. (2006)
(E)-2-hexenoic acid	SAFE (NB fraction)	AEDA	Fatty	Schirack et al. (2006)
Pantolactone	SAFE (A fraction)	AEDA	Burnt sugar	Schirack et al. (2006)
Benzaldehyde	SAFE (A fraction)	AEDA	Sweet/malty	Schirack et al. (2006)
Methyl cinnamate	SAFE (A fraction)	AEDA	Strawberry	Schirack et al. (2006)
Ethyl 2-methylpropanoate	SAFE	GC/O	Fruity-fermented	Didzbalis et al. (2004)
2,3-butanedione	SAFE	GC/O	Buttery	Didzbalis et al. (2004)

Table 4. Continued

2,3-pentanedione	SAFE	GC/O	Buttery	Didzbalis et al. (2004)
Ethyl 2-methylbutanoate	SAFE	GC/O	Green/fruity	Didzbalis et al. (2004)
Ethyl 3-methylbutanoate	SAFE	GC/O	Fruity/apple-like	Didzbalis et al. (2004)
Hexanal	SAFE	GC/O	Grassy	Didzbalis et al. (2004)
Octanal	SAFE	GC/O	Citrus-like	Didzbalis et al. (2004)
2-furanmethanethiol	SAFE	GC/O	Coffee-like	Didzbalis et al. (2004)
Methional	SAFE	GC/O	Potato-like	Didzbalis et al. (2004)
Butanoic acid	SAFE	GC/O	Sharp/sour	Didzbalis et al. (2004)
Phenylacetaldehyde	SAFE	GC/O	Honey-like	Didzbalis et al. (2004)

Table 4. Continued

3-methylbutanoic acid	SAFE	GC/O	Rancid/cheese	Didzbalis et al. (2004)
Hexanoic acid	SAFE	GC/O	Cheesy/fatty	Didzbalis et al. (2004)
2-methoxyphenol	SAFE	GC/O	Sweet/smoky	Didzbalis et al. (2004)
4-hydroxy-2,5-dimethyl-3(2H)-furanone	SAFE	GC/O	Strawberry like	Didzbalis et al. (2004)
Acetoin / 2,3-butadione	SAFE (NB fraction)	AEDA	buttery/butterscotch	Greene (2007)
3-methylbutanal	SAFE (NB fraction)	AEDA	malty/chocolate	Greene (2007)
Hexanal	(SAFE (NB fraction)	AEDA	green/grassy	Greene (2007)
Methional	SAFE (NB fraction)	AEDA	potato	Greene (2007)
2-acetyl-1-pyrroline	SAFE (NB fraction)	AEDA	popcorn	Greene (2007)

Table 4. Continued

1-octen-3-one	SAFE (NB fraction)	AEDA	metallic/mushroom	Greene (2007)
2-ethyl-6-methylpyrazine	SAFE (NB fraction)	AEDA	sweet	Greene (2007)
Trimethylpyrazine	SAFE (NB fraction)	AEDA	earthy/soil/dirt	Greene (2007)
Phenylacetaldehyde	SAFE (NB fraction)	AEDA	rosy/floral	Greene (2007)
2-ethyl-3,5-dimethylpyrazine	SAFE (NB fraction)	AEDA	earthy/soil/dirt	Greene (2007)
2,3-diethyl-5-methylpyrazine	SAFE (NB fraction)	AEDA	earthy/soil/dirt	Greene (2007)
2-methoxy-4-vinylphenol	SAFE (NB fraction)	AEDA	licorice/sweet	Greene (2007)
2-ethyl-3,5-dimethylpyrazine	SAFE (A fraction)	AEDA	earthy/soil/dirt	Greene (2007)
Acetic acid	SAFE (A fraction)	AEDA	vinegar/acetic acid	Greene (2007)

Table 4. Continued

Methional	SAFE (A fraction)	AEDA	potato	Greene (2007)
Butanoic acid	SAFE (A fraction)	AEDA	sweaty/musty/cheesy	Greene (2007)
Ethyl-2-methylbutanoate	Simultaneous distillation/extraction (N fraction)	AEDA	Fruity	Matsui et al. (1998)
(Z)-2-nonenal	Simultaneous distillation/extraction (N fraction)	AEDA	Fatty/Green	Matsui et al. (1998)
(E,E)-2,4-decadienal	Simultaneous distillation/extraction (N fraction)	AEDA	Fatty, deep fried	Matsui et al. (1998)
(E)-β-damascenone	Simultaneous distillation/extraction (N fraction)	AEDA	Boiled apple like	Matsui et al. (1998)
Ethyl isobutyrate	Simultaneous distillation/extraction (N fraction)	AEDA	fruity	Matsui et al. (1998)

Table 4. Continued

3-mercapto-2-butanone	Simultaneous distillation/extraction (N fraction)	AEDA	Cooked meat-like	Matsui et al. (1998)
2-acetyl-1-pyrroline	Simultaneous distillation/extraction (B fraction)	AEDA	Roasty/sweet	Matsui et al. (1998)
Dimethyltrisulfide	Simultaneous distillation/extraction (N fraction)	AEDA	Sulfurous	Matsui et al. (1998)
2-propionyl-1-pyrroline	Simultaneous distillation/extraction (B fraction)	AEDA	Roasty	Matsui et al. (1998)
2-furfurylthiol	Simultaneous distillation/extraction (N fraction)	AEDA	Sweet/ Smoky	Matsui et al. (1998)
(E,Z)-2,4- nonadienal	Simultaneous distillation/extraction (N fraction)	AEDA	Green	Matsui et al. (1998)
(E,Z)-2,4-decadienal	Simultaneous distillation/extraction (N fraction)	AEDA	Fatty/Green	Matsui et al. (1998)

Table 4. Continued

2-methoxyphenol (guaiacol)	Simultaneous distillation/extraction (N fraction)	AEDA	Burnt	Matsui et al. (1998)
2-phenyl-2-butenal	Simultaneous distillation/extraction (N fraction)	AEDA	Green/phenolic	Matsui et al. (1998)
3-methoxy-4-hydroxybenzaldehyde (vanillin)	Simultaneous distillation/extraction (A fraction)	AEDA	Vanilla-like	Matsui et al. (1998)
3-methylbutanal	Simultaneous distillation/extraction (N fraction)	AEDA	Malty	Matsui et al. (1998)
d-limonene	Simultaneous distillation/extraction (N fraction)	AEDA	Lemon-like	Matsui et al. (1998)
Octanal	Simultaneous distillation/extraction (N fraction)	AEDA	Fatty	Matsui et al. (1998)
(E)-2-nonenal	Simultaneous distillation/extraction (N fraction)	AEDA	Fatty/Green	Matsui et al. (1998)

Table 4. Continued

Phenylacetaldehyde	Simultaneous distillation/extraction (N fraction)	AEDA	Sweet/honey-like	Matsui et al. (1998)
(E,E)-2,4- nonadienal	Simultaneous distillation/extraction (N fraction)	AEDA	Fatty, deep fried	Matsui et al. (1998)
(E)-2-undecenal	Simultaneous distillation/extraction (N fraction)	AEDA	Fatty	Matsui et al. (1998)
Hexanoic acid	Simultaneous distillation/extraction (A fraction)	AEDA	Sweaty	Matsui et al. (1998)
2-methoxy-4-vinylphenol (4-vinylguaicol)	Simultaneous distillation/extraction (N fraction)	AEDA	Spicy/phenolic	Matsui et al. (1998)
δ-dodecalactone	Simultaneous distillation/extraction (N fraction)	AEDA	Sweet	Matsui et al. (1998)

* Fraction in which compound appeared. A: Acidic; B: Basic; and N: Neutral

Table 5: Pyrazine compounds isolated from roasted peanuts.

Compound	Isolation Method (and fraction*)	Odor detection Method	Odor quality	Reference
2,5-dimethyl-3-ethylpyrazine	SAFE (AC fraction)	GC/O	Brothy	Schirack et al. (2006)
2,6-dimethyl-3-ethylpyrazine	SAFE (NB fraction)	GC/O	Nutty/ earthy	Schirack et al. (2006)
2,6-dimethylpyrazine	SAFE (NB fraction)	GC/O	Nutty/ earthy	Schirack et al. (2006)
2,3-diethyl-5-methylpyrazine	SAFE (NB fraction)	GC/O	Roasted	Schirack et al. (2006)
3,5 diethyl-2-methylpyrazine	SAFE (NB fraction)	GC/O	Roasted	Schirack et al. (2006)
2-ethyl-5-methylpyrazine	SAFE (AC fraction)	GC/O	Fruity/ sweet	Schirack et al. (2006)
2-ethyl-5-methylpyrazine	Dynamic headspace	GC/O	Nutty/roasted	Braddock et al. (1995)
Methylpyrazine	Dynamic headspace	GC/O	Grilled chicken, savory	Braddock et al. (1995)

Table 5. Continued

2,5-dimethyl-pyrazine	Dynamic headspace	GC/O	Chocolaty/malty	Braddock et al. (1995)
ethylpyrazine	Dynamic headspace	GC/O	Dark roasted/ toasted	Braddock et al. (1995)
2,3- dimethylpyrazine	Dynamic headspace	GC/O	Roasted	Braddock et al. (1995)
2-ethyl-3-methylpyrazine	Dynamic headspace	GC/O	Roasted	Braddock et al. (1995)
3-ethyl-2,5-dimethylpyrazine	Dynamic headspace	GC/O	Roasted/ slightly sweet	Braddock et al. (1995)
2-ethylpyrazine	Solvent extraction	Sniffing of purified compound (preparative gas-liquid chromatography)	Roasted/nutty	Koehler et al. (1971)
2-methylpyrazine	Solvent extraction	Sniffing of purified compound (preparative gas-liquid chromatography)	Roasted/nutty	Koehler et al. (1971)
Methylethylpyrazine	Solvent extraction	Sniffing of purified compound (preparative gas-liquid chromatography)	Roasted/nutty	Koehler et al. (1971)

Table 5. Continued

2-ethyl-3,5-dimethylpyrazine	Simultaneous distillation/extraction (B fraction)	GC/O	Roast	Matsui et al. (1998)
2,3-diethyl-5-methylpyrazine	Simultaneous distillation/extraction (B fraction)	GC/O	Roasty	Matsui et al. (1998)
3-ethyl-2,5-dimethylpyrazine	Simultaneous distillation/extraction (B fraction)	GC/O	Roasty	Matsui et al. (1998)
2-ethenyl-3,5-dimethylpyrazine	Simultaneous distillation/extraction (B fraction)	GC/O	Roasty	Matsui et al. (1998)
2,5-(or 2,6)-diethylpyrazine	Simultaneous distillation/extraction (B fraction)	GC/O	Sweet	Matsui et al. (1998)
2-ethyl-5-methylpyrazine	Simultaneous distillation/extraction (B fraction)	GC/O	Sweet	Matsui et al. (1998)
2-ethyl-3-methylpyrazine	Simultaneous distillation/extraction (B fraction)	GC/O	Sweet	Matsui et al. (1998)

Table 5. Continued

2-methoxy-5-((E)-1-propenyl) pyrazine	Simultaneous distillation/extraction (B fraction)	GC/O	Sweet/earthy	Matsui et al. (1998)
2,6-dimethylpyrazine	SAFE	GC/O	Nutty	Didzbalis et al. (2004)
2-ethylpyrazine	SAFE	GC/O	Nutty	Didzbalis et al. (2004)
2,3-dimethylpyrazine	SAFE	GC/O	Nutty	Didzbalis et al. (2004)
2-ethyl-5-methylpyrazine	SAFE	GC/O	Sweet/nutty	Didzbalis et al. (2004)
Trimethylpyrazine	SAFE	GC/O	Nutty	Didzbalis et al. (2004)
2,ethyl-3,6-dimethylpyrazine	SAFE	GC/O	Roasty	Didzbalis et al. (2004)
2-furanmethanethiol	SAFE	GC/O	Coffee-like	Didzbalis et al. (2004)
2-ethyl-3,5-dimethylpyrazine	SAFE	GC/O	Roasty	Didzbalis et al. (2004)

* Fraction in which compound appeared. A: Acidic; B: Basic; and N: Neutral

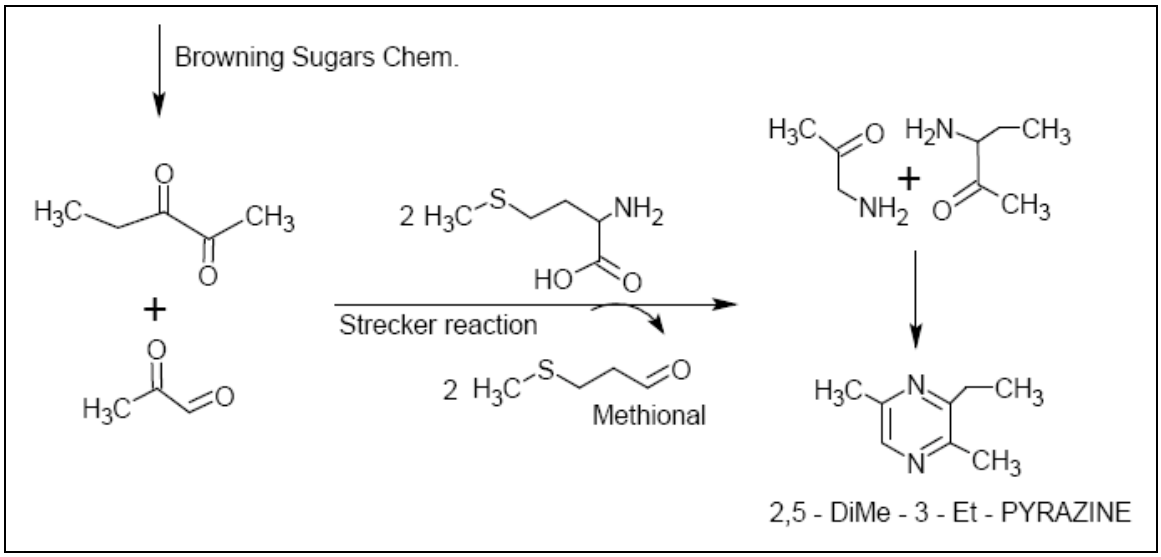

Figure 1: Formation of an alkyl pyrazine through the Strecker degradation reaction

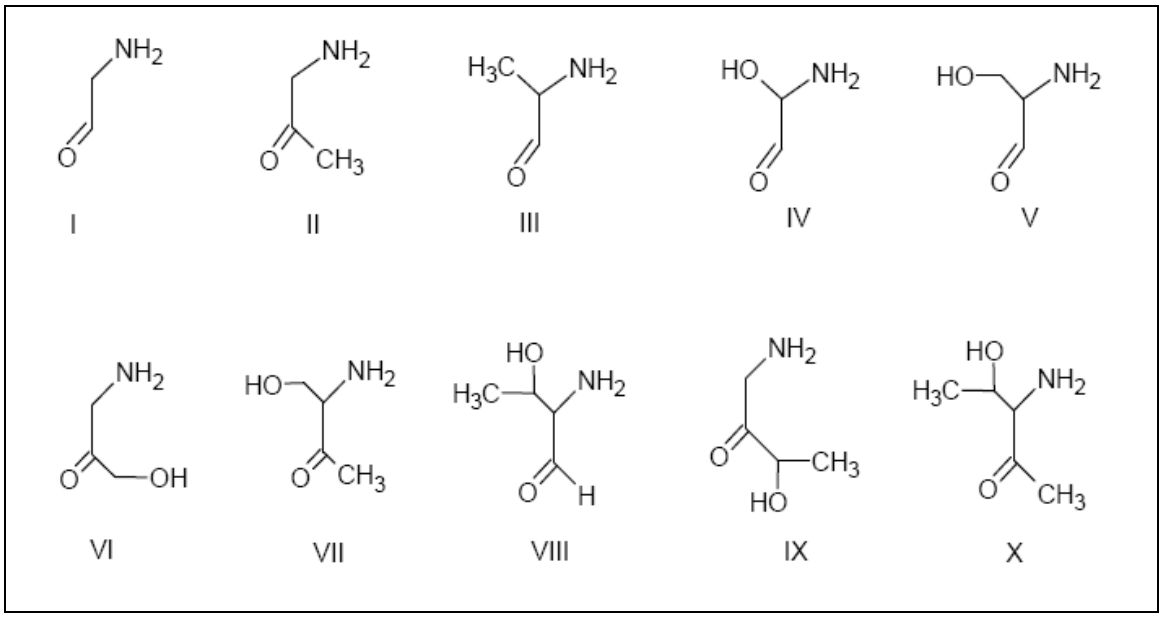

Figure 2: α-amino carbonyl intermediates proposed by Shibamoto and Bernhard (1977a, b)

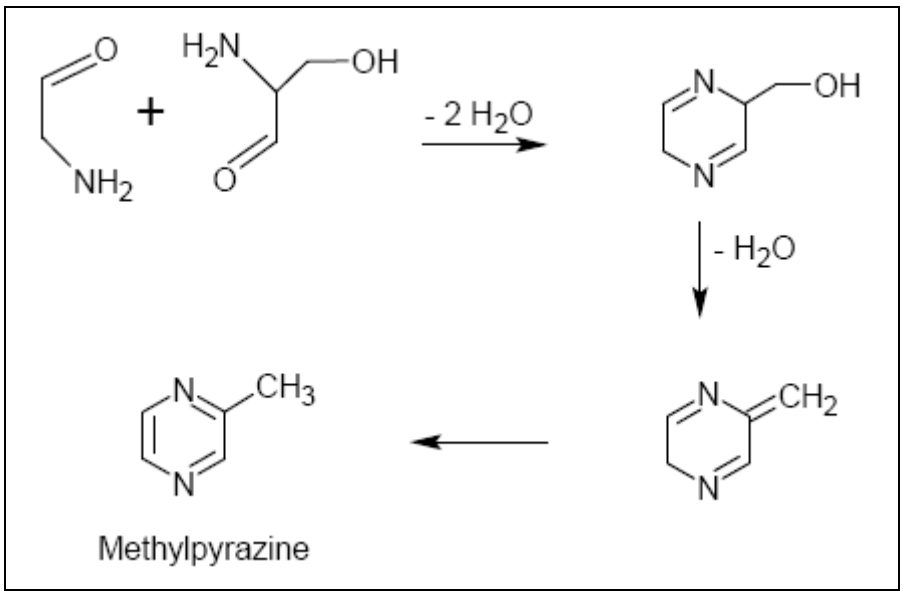

Figure 3: Mechanism for the formation of methylpyrazine from fragments proposed by Shibamoto and Bernhard (1977a, b)

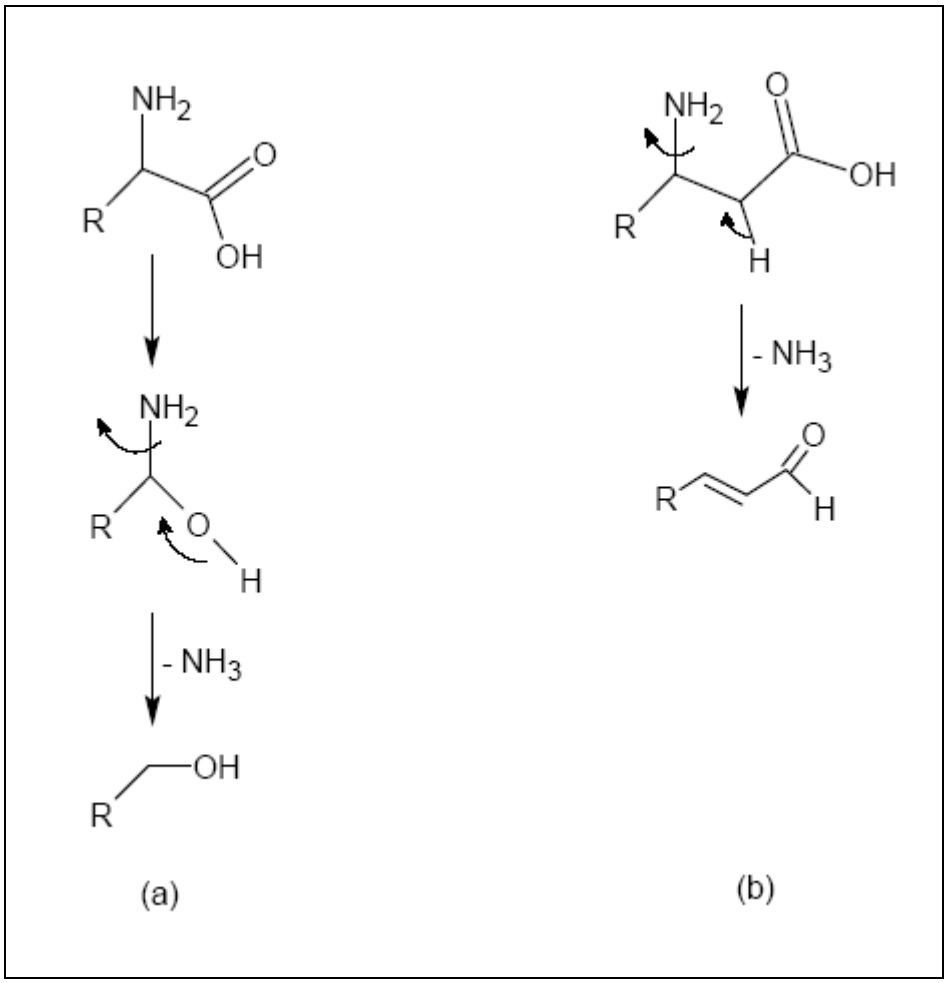

Figure 4: Mechanism of deamination from α- amino acid (a) and β-amino acid (b) proposed by Shu (1998).

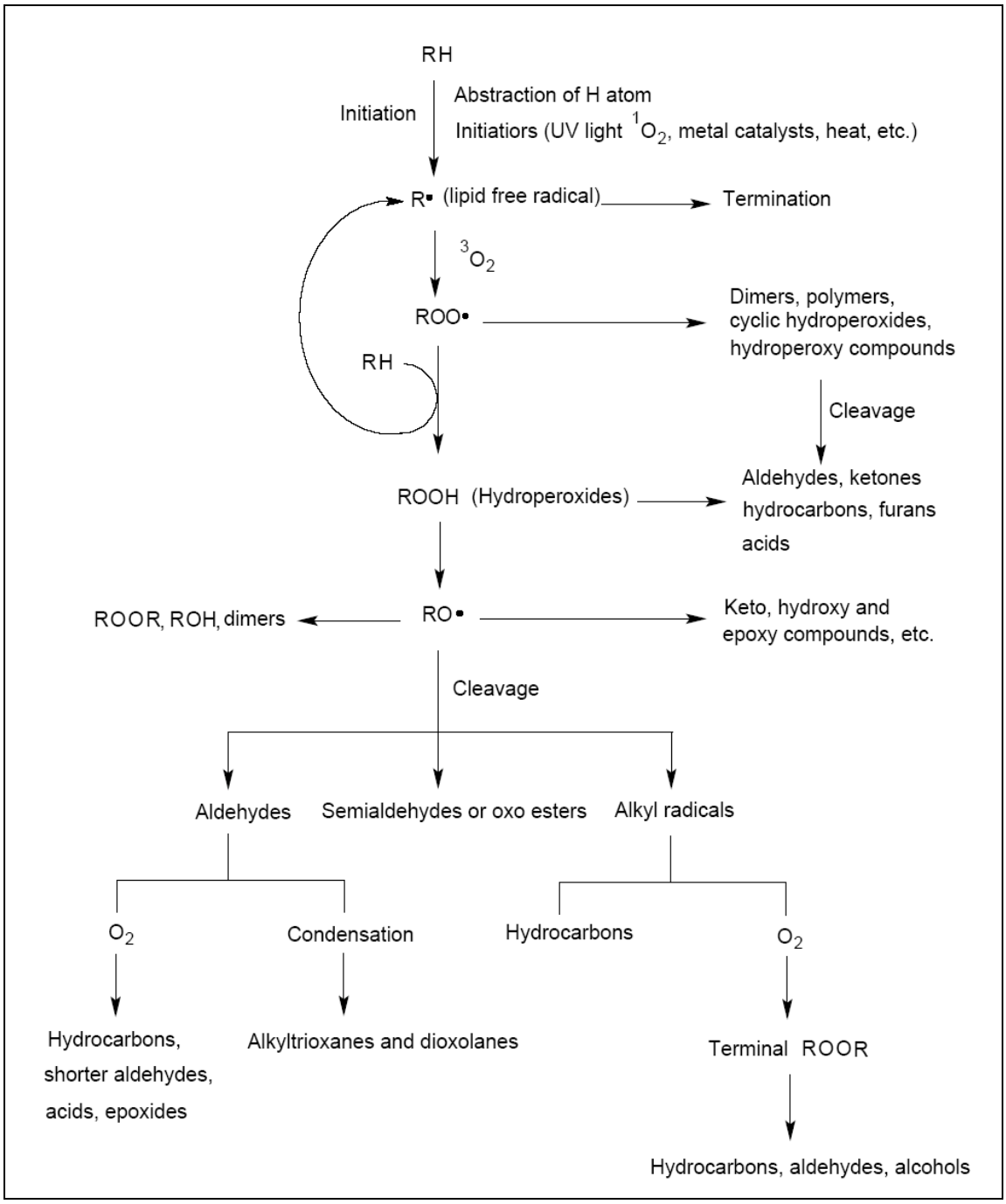

Figure 5: Mechanism of lipid oxidation adapted from Shahid (2000).

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CHAPTER 2:

OPTIMIZATION OF HEADSPACE SOLID-PHASE MICROEXTRACTION (HS-SPME) SAMPLING CONDITIONS FOR THE ANALYSIS OF VOLATILE COMPOUNDS IN ROASTED PEANUT PASTE

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Abstract

Headspace solid-phase microextraction (HS-SPME) is a valuable technique for the extraction of volatile compounds in foods. The objective of this study was to optimize headspace sampling conditions of roasted peanut paste for HS-SPME analysis. The effects of fiber type, salt, extraction time, and extraction temperature were evaluated. Volatiles were analyzed using gas chromatography-mass spectrometry (GC/MS) and gas chromatography-olfactometry (GC/O). Eight compounds in the headspace were used to assess the extraction efficiency of various testing conditions. Use of DVB/CAR/PDMS fiber led to greater peak area responses for all compounds tested as compared to PDMS fiber. For a given extraction time and temperature, addition of 6 mL saturated NaCl solution increased extraction efficiency of target compounds. In general, peak area responses increased with increasing extraction time and temperature. Optimized conditions increased response of the target compounds by 40% to 1760% when compared to undiluted paste extracted at 40ºC/30 min with a DVB/CAR/PDMS fiber. Compared to the later method, optimized conditions resulted in higher number of detected compounds by GC/O analysis. This study provides optimal headspace sampling conditions for efficient extraction of components from roasted peanut paste.

KEYWORDS: Solid-phase microextraction, volatile, peanuts, method optimization

Introduction

The basis for the consumption of peanuts in U.S. is the unique and pleasant flavor that is developed during roasting (Sanders et al. 1997). The flavor of roasted peanuts is determined by the composition of volatiles present, with over 200 aroma-active compounds identified to date (Schirack et al. 2006). The main sources for formation of volatile compounds in roasted peanuts are the Maillard reaction, thermal degradation of sugars (caramelization), and lipid oxidation (Cammarn et al. 1990; Coleman et al. 1994). The Maillard reaction involves the interaction of reducing sugars and amino compounds and the main products from this reaction are heterocyclic nitrogen compounds, such as furans, thiazoles, thiophenes, oxazoles, pyrroles, imidazoles, pyridines, and pyrazines (Hodge 1953; Hwang et al. 1995). Thermal degradation of sugars (caramelization) produces low molecular weight open-chain oxygen-containing compounds, as well as heterocyclic oxygen-containing species such as furan derivatives (Coleman et al. 1994). Lipid oxidation leads to the formation of oxygen containing compounds such as aliphatic aldehydes, ketones, and alcohols (Coleman et al. 1994).

Several different analytical methods have been developed to study the volatile composition of foods including steam distillation, solvent extraction, simultaneous distillation/extraction (SDE), high vacuum distillation, and solvent assisted evaporation (SAFE) (Curioni and Bosset 2002; Sides et al. 2000). However, these methods have the disadvantages of relatively low reproducibility, low selectivity, long preparation time, high cost, and potential solvent contamination. Headspace solid-phase microextraction (HS-SPME) is a variant of the static headspace method that has been widely practiced in

recent years. A fused silica fiber coated with an absorbent is immersed into the headspace above a liquid or solid sample and analytes are extracted by diffusion onto the coating based on the equilibrium among three phases: Sample ⇔ Headspace ⇔ SPME fiber (Sides et al. 2000, Kalua and Boss 2008). Analyzing HS-SPME volatile extracts by gas chromatography-mass spectrometry (GC/MS) is an effective way to detect, identify and quantify chemical compounds in foods. This technique involves thermal desorption of HS-SPME fiber-extracted analytes in the GC injection port, which are then separated on the GC column and detected by the MS analyzer. HS-SPME has been extensively used for the analysis of flavor compounds in foods because analytes are isolated without interference from the matrix (Steffen and Pawliszyn 1996). The main advantages of this method are simplicity, high speed, low cost, small sample volume, and high sensitivity (Kataoka et al. 2000). However, HS-SPME is very susceptible to experimental conditions such as fiber type, extraction time, extraction temperature, headspace volume, sample pH, and salt content. Therefore, these parameters should be kept constant to obtain consistent results (Sides et al. 2000; Quanch et al. 1999).

Optimization of extraction parameters for HS-SPME headspace sampling has been examined in various food matrixes. Carrillo et al. (2007) optimized several parameters for extracting phthalates in wine including fiber type, extraction temperature, sample volume, and NaCl concentration. Ho et al. (2006) determined optimum sampling temperature, sampling time, and type of fiber for the analysis of palm sugar volatile compounds. Similarly, Zhang et al. (2009) optimized the HS-SPME fiber type, and then used a response surface experimental design to examine the effects of extraction

temperature, equilibrium time, and extraction time for the analysis of volatile compounds in longan.

Although HS-SPME has been widely used for flavor characterization of roasted peanuts (Greene at al. 2008; Williams et al. 2006; Abegaz et al. 2004; Baker et al. 2003), optimization of experimental conditions for volatile extraction has not yet been determined. The objective of this study was to optimize HS-SPME extraction conditions including fiber type, salt addition, extraction temperature, and extraction time for the analysis of the volatile compounds in roasted peanut paste.

Materials and methods

Peanut Sample Preparation

Runner-type peanut pods were water blasted to remove the exocarp and visually sorted into increasing maturity levels based on mesocarp color (yellow, orange A, orange B, brown and black). Peanut pods were dried with ambient air, shelled, and sized by diameter over slotted hole screens, as previously described (Sanders, 1989). Brown maturity class, medium grade size peanut seeds (<8.3 >6.3 mm) were roasted in a convection oven at 169ºC/25 min to a Hunter L-value of 50 ± 1. Peanut pastes were prepared using a Beixer 3 food processor (Robot Coupe Inc., Ridgeland, MS), packaged in 8 oz glass jars, and stored at -20ºC until analysis.

Chemicals

Analyte standards of 2-methyl-3-heptanone and 2-methylvaleric acid were purchased from Sigma-Aldrich (St. Louis, MO). Methanol served as solvent and was also obtained from Sigma-Aldrich (St. Louis, MO). Stock solutions were prepared from the pure compounds diluted in absolute methanol and were stored at -20ºC until use for a maximum of 1 month.

HS-SPME Procedure

Peanut volatiles were isolated by headspace solid-phase microextraction (HS-SPME) using a CTC Analytics combiPAL auto sampler (Zwingen, Switzerland). Five grams of peanut paste were placed into 20 mL clear screw cap vials (Microliter Analytical Supplies Inc.; Suwanee, GA). SPME fibers were inserted through the vial septa at 31 mm depth, and exposed to the headspace above the sample, which was agitated at 250 rpm. 2 ppm 2-methyl-3-heptanone and 6 ppm 2-methylvaleric acid were added to each vial as internal standards.

A full factorial design was used to optimize experimental conditions including fiber type, salt addition, extraction time, and extraction temperature for GC/MS analysis. Two fibers having different stationary phases and thickness were tested: A three-phase 1 cm divinylbenzene/carbonex/polydimethylsiloxane (DVB/CAR/PDMS) with a 50/30 μm coating thickness, and a single-phase 1 cm polydimethylsiloxane (PDMS) with a 100 μm coating thickness (Supelco, Bellefonte, PA). Fibers were conditioned in the GC injector port before analysis, according to manufacture' recommendations. Samples were as

follows: undiluted peanut paste; peanut paste with addition of 3 or 6 mL saturated salt solution (2.2 and 3.2 M NaCl final concentration in the sample, respectively); and peanut paste with addition of 3 or 6 mL water to account for the effects of sample mixing and dilution. Samples were equilibrated for 30 min before extraction for 30 or 40 min, at temperatures of 40 or 50ºC. Eight compounds previously identified in roasted peanuts were selected to evaluate the extraction efficiency of different headspace sampling conditions (Greene et al. 2008; Schirack et al. 2006; Didzbalis et al. 2004; Buckholz et al. 1980; Crippen et al. 1992).

Gas chromatography/mass spectrometry (GC/MS)

Extracted volatiles were analyzed on a 6890N GC/ HP5973 mass selective detector (Agilent Technologies., Palo Alto, CA). The GC was equipped with a nonpolar capillary column DB-5MS, 30 m length x 0.25 mm i.d. x 0.25 μm d_f (J&W Scientific, Folsom, CA). Helium gas was used as carrier gas at a constant flow rate of 1 mL/ min. Oven temperature was programmed to increase from 40 to 250ºC at a rate of 8ºC/min with initial and final hold times of 5 and 30 min, respectively. Mass selective detector conditions were as follows: capillary direct interface temperature, 250ºC; ionization energy, 70 eV; mass range, 35-350 m/z; EM Voltage (Atune +200 V); scan rate, 2.94 scans/s. All samples were run in duplicate using the splitless mode.

Gas chromatography-olfactometry (GC/O)

After optimizing HS-SPME conditions for the analysis of volatile compounds in peanut paste using GC/MS, two sampling methods were examined using GC/O analysis: (1) a previously published HS-SPME method (Greene et al. 2008), and (2) the optimized HS-SPME method from this study.

In the first method, 10 g of peanut paste were placed into a 40 mL amber screw cap vial (Supelco, Bellefonte, PA), while in the second method 10 g of peanut paste plus 12 mL saturated salt solution (3.2 M NaCl final concentration in the sample) were used. Samples were allowed to equilibrate at 40ºC for 30 min and the fiber was manually inserted into the sample vial using a SPME holder (Supelco, Bellefonte, PA) at 2 cm depth. Volatile compounds were extracted at 40ºC/30 min for the first method and 40ºC/40 min for the second method, followed by desorption in the GC/O injector for 5 min at 250ºC. Prior to each extraction, the fiber was conditioned in a GC injector for 5 min at 250ºC to reduce possible contamination.

Samples were analyzed on a HP5890 series II gas chromatograph (Hewlett-Packard Co.; Palo Alto, CA) fitted with a flame ionization detector (FID), splitless injector, and a sniff port. The GC was equipped with a nonpolar column (ZB-5ms, 30 m length x 0.25 μm film thickness d_f (J&W Scientific; Folsom, CA). The oven temperature was programmed to increase from 40ºC to 200ºC at a rate of 10ºC/min with an initial hold of 3 min and a final hold of 20 min. Column effluent was split 1:1 between the FID and sniffing port using deactivated fused silica capillaries (1 m length x 0.25 mm i.d.). The FID and sniffing port were kept at 250ºC. Humidified air was supplied at the end of

the sniffing port at a rate of 30 mL/min to prevent dehydration of the nasal membranes of the panelists. Two experienced panelists sniffed each sample. Panelists described the odors and scored aroma intensities using a 5-point numerical intensity scale (Van Ruth 2001).

Results and discussion

Gas Chromatography Mass Spectrometry (GC/MS)

Selection of appropriate SPME fiber is usually performed based on the volatility and polarity of target analytes. In this study, DVB/CAR/PDMS fiber had a strong extraction capacity, and was the most effective fiber coating for all sampling conditions tested. For example, DVB/CAR/PDMS fiber resulted in 8-12 fold greater adsorption capacity than PDMS fiber with the following sampling conditions: 6 mL saturated salt solution added; 40ºC extraction temperature; 40 min extraction time (Figure 1). Thus, DVB/CAR/PDMS was selected as the best option for extracting target volatiles in peanuts. Although PDMS was less effective for the extraction of target compounds in the present study, this fiber coating has been used for the analysis of volatile compounds in various food matrixes including onion, apple, soft drinks, coffee, orange juice, wine, cheese, and whey protein (Kataoka et al. 2000). However, several optimization studies have reported superior extraction efficiency for DVB/CAR/PDMS compared to PDMS, which is in agreement with our results. For instance, Ho et al. 2006 selected DVB/CAR/PDMS for the analysis of palm sugar after comparison with PDMS, PA and PDMS/DVB fibers. The authors suggested that DVB/CAR/PDMS had a better

performance because it combined the characteristics of multiple phases (Ho et al. 2006). Similarly, Zhang et al. (2009) found that DVB/CAR/PDMS fiber produced the best results for the volatile analysis of longan after comparison with PDMS, PA, and CAR/PDMS fibers.

The presence of salt may decrease the solubility of hydrophobic compounds in the food matrix and facilitate their diffusion to the headspace by the phenomenon described as 'salting out' (Yang and Peppard 1994; Liu and Yang 2002). The effect of salt concentration on the adsorption capacity of target compounds was evaluated based on the sum of their peak areas (Zhang et al. 2009; Liu and Yang 2002). Addition of salt solution resulted in increased fiber adsorption capacity of the target compounds for all temperature/time extraction conditions tested ($P<0.05$) (Figure 2). Compared to undiluted peanut paste, addition of 6 mL saturated salt solution (3.2 M NaCl concentration in the sample) resulted in greater extraction efficiency, with 125-264% increase in the sum of peak areas of target compounds for all extraction time/temperature conditions tested (Figure 2). Steffen and Pawliszyn (1996) evaluated the effect salt on the efficiency of HS-SPME extractions in orange juice. They reported that addition of 6.2 M resulted in the highest enrichment of volatile compound onto the fiber coating when compared to 0, 2.6, and 7.4 M NaCl (Steffen and Pawliszyn 1996). Liu and Yang (2002) also examined the effect of salt on the extraction efficiency of eight volatile compounds in banana juice. Depending on the type of compound tested, different responses were observed with salt addition. Overall, the authors suggested that 4.2 M NaCl was the optimum level for extraction of volatiles in banana juice. Yang and Peppard (1994) reported that for most

compounds, increasing concentrations of NaCl from 0 to 4.2 M resulted in increased adsorption capacity; however for a few compounds adsorption increased with salt concentration then leveled off or decreased; and for a few other compounds, the response decreased with increasing salt concentration.

Although peak area responses were higher for samples containing salt, efficiency of extractions also increased with water addition when compared to undiluted peanut paste ($P< 0.05$) (Figure 2). Increasing the amount of water added from 3 to 6 mL resulted in greater peak areas for samples extracted at 40ºC; however, a decrease or no change in response was observed for samples extracted at 50ºC (Figure 2). Addition of water may change the inter-molecular interactions in the matrix and partitioning of volatile compounds between sample and headspace (Kalua and Boss 2008). Dilution may also augment sample agitation, which results in greater diffusion of flavor compounds from the matrix to the headspace (Kataoka et al. 2000). However, the rate-limiting step in HS-SPME is generally considered to be the diffusion of analytes from the aqueous phase to the headspace; thus increasing water content may also negatively affect extraction efficiency (Steffen and Pawliszyn 1996). In fact, Liu and Yang (2002) found that an intermediate water dilution resulted in greatest adsorption capacity of eight target compounds extracted from banana headspace.

The efficiency of HS-SPME is influenced by the vapor pressure of analytes in the testing vial, which is primarily affected by extraction time and temperature (Liu and Yang 2002). The amount of an analyte adsorbed onto the SPME fiber is generally accepted to be proportional to initial concentration in the sample; thus, full equilibration

is not required for quantification (Kataoka et al. 2000). However, maximum sensitivity occurs at the partition equilibrium among the three phases: Sample ⇔ Headspace ⇔ SPME fiber (Kalua and Boss 2008). Therefore, optimizing extraction time is crucial for increased adsorption capacity of target compounds. In general, prolonging extraction time from 30 to 40 min resulted in higher enrichment of compounds onto the fiber coating for a given extraction temperature (Figure 3). Longer extraction time resulted in 11-23% and 15-33% greater peak areas for the majority of compounds extracted at 40 and 50°C, respectively. However, peak areas of hexanal did not change considerably with increasing extraction time for both extraction temperatures tested. Peak areas of 2-methylbutanal decreased by 16% from 30 to 40 min when extracted at 40°C but remained unchanged at 50°C with increasing extraction time. This might be due to the capacity volume of the fiber as well as the volatile profile of the sample being tested (Ho et al. 2006). Longer extraction times are usually required for less volatile compounds; thus increasing the extraction time might have caused displacement of the more volatile compounds such as 2-methylbutanal from the fiber (Camara et al. 2007).

Temperature is crucial for greater efficiency of HS-SPME extractions as it affects the diffusion rate and partial pressure of analytes into the headspace. Usually, high temperatures increase the mass transfer process, and facilitate dispersion of analytes from the food matrix to the headspace. However, high temperatures may also negatively affect the adsorption of analytes onto SPME fiber due to a decrease of partition coefficients between the headspace and fiber (Zhang and Pawliszyn 1993). In this study, an increase in adsorption capacity was observed with increasing extraction temperature for most

compounds. With the exception of 2-methylbutanal and hexanal, increasing extraction temperature from 40 to 50°C led to a 20-46% increase in peak areas with the following conditions: 6mL saturated salt solution; 40 min extraction time; DVB-CAR-PDMS fiber (Figure 3). The higher responses observed at 50°C could be due to higher mobility of analytes from the matrix into the headspace, which may lead to better recoveries of compounds (Camara et al. 2007). However, peak areas for hexanal and 2-methylbutanal decreased by 27 and 37%, respectively with increasing extraction temperature. A possible explanation is that the higher extraction temperature affected not only the vaporization of these analytes from the matrix to the headspace but also their desorption from the fiber to the headspace (Ho et al. 2006). Quanch et al. (1999) optimized sampling conditions for HS-SPME analysis of whey protein concentrate solutions, and found that extracting compounds at 40ºC for 30 min produced the highest recovery from the headspace. They also reported that increasing the extraction temperature to 50°C did not substantially change the volatile profile of whey protein concentrate solutions. The influence of extraction temperature (25, 40, 50 and 60°C) was also investigated for the analysis of volatile compounds in whisky at constant extraction time of 60 min (Camara et al. 2007). Optimized extraction temperature for whisky samples was selected as 40°C, with additional increase in temperature resulting in decreased adsorption capacity for a few classes of compounds (Camara et al. 2007). Although 50°C resulted in considerably higher extraction efficiency in the present study, 40°C was selected as the optimum temperature for the HS-SPME analysis of peanut paste. A lower extraction temperature was favored because high temperatures may introduce artifacts by thermally generating

compounds not originally present in the sample. For instance, decomposition of hydroperoxides at high extraction temperature and long time may interfere with quantitation of volatile compounds from lipid oxidation (Jelen 2006).

After evaluation of the main parameters affecting the efficiency of HS-SPME extraction in peanut paste, the following sampling conditions were selected as optimum within the range tested: 50/30 μm DVB/CAR/PDMS fiber; 6 mL saturated salt solution added (3.2 M NaCl final concentration); 40ºC extraction temperature; 40 min extraction time. When comparing the optimized method with a published method (50/30 μm DVB/CAR/PDMS fiber, no salt added, 40ºC extraction temperature, 30 min extraction time) (Greene et al. 2008), a considerable increase in response was observed for all compounds tested (Table 1). Peak areas for 2-methylbutanal and hexanal increased by 39 and 67%, respectively; pyrazine compounds were enriched by 119-220%; and efficiency of phenylacetaldehyde extraction improved by 1763%.

Gas Chromatography Olfactometry (GC/O)

Gas chromatography–olfactometry (GC/O) is a valuable technique for identifying the aroma active compounds that may potentially affect the flavor of foods (Fuller et al., 1964). In this method, the GC has been modified with a sniffer port and a trained panelist describes the aroma of individual compounds as they elute from the column. GC/O analysis was carried out to compare the aroma profile of peanut pastes extracted with two HS-SPME methods: (A) a published method: 50/30 μm DVB/CAR/PDMS fiber; undiluted peanut paste; 40ºC extraction temperature; 30 min extraction time (Greene et

al. 2008), and (B) the optimized method from the present study: 50/30 μm DVB/CAR/PDMS fiber; added saturated salt solution (3.2 M final NaCl concentration); 40ºC extraction temperature; 40 min extraction time. Similar gas chromatographic patterns were obtained from samples extracted using these two HS-SPME methods (Figure 4). However, when comparing the aroma profile eluting from the GC column, qualitative and quantitative differences were observed (Table 2). For example, 38 odorants were detected with the optimized method, but only 25 odorants were perceived with the published method. In addition, 17 out 38 odorants were only detected by the optimized method, not with the alternate method. Four odorants that were detected using the alternate method were not detected with the optimized method. Odor descriptors for these compounds included sweet/roasted, chroline, cheese/sweaty, and solvent-like. Overall, the optimized method resulted in increased number of aroma-active compounds eluting from the GC/O column.

Conclusions

Optimized sampling conditions for HS-SPME analysis of roasted peanuts are crucial for efficient extraction of aroma compounds. In this study, the effects of fiber type, salt addition, extraction time, and extraction temperature were evaluated, and a method for HS-SPME extraction of volatile compounds in peanut paste was developed. DVB/CAR/PDMS fiber was more effective than PDMS for extraction of target compounds. Addition of 6 mL saturated salt solution (3.2 M NaCl final concentration) resulted in increased peak area responses for all temperature/time extraction conditions

tested. For a given temperature, higher extraction time resulted in higher responses for target compounds. Although extractions performed at 50°C resulted in higher efficiency when compared to 40°C, the latter was selected as the optimum extraction temperature to prevent formation of artifacts in the samples. Optimized method resulted in higher number of aroma-active compounds eluting from the GC/O column. Results from this study indicate that the efficiency of HS-SPME extractions can be greatly improved by optimizing headspace sampling conditions. This study provides a new method for HS-SPME extraction that can be used to better characterize the flavor of peanut paste.

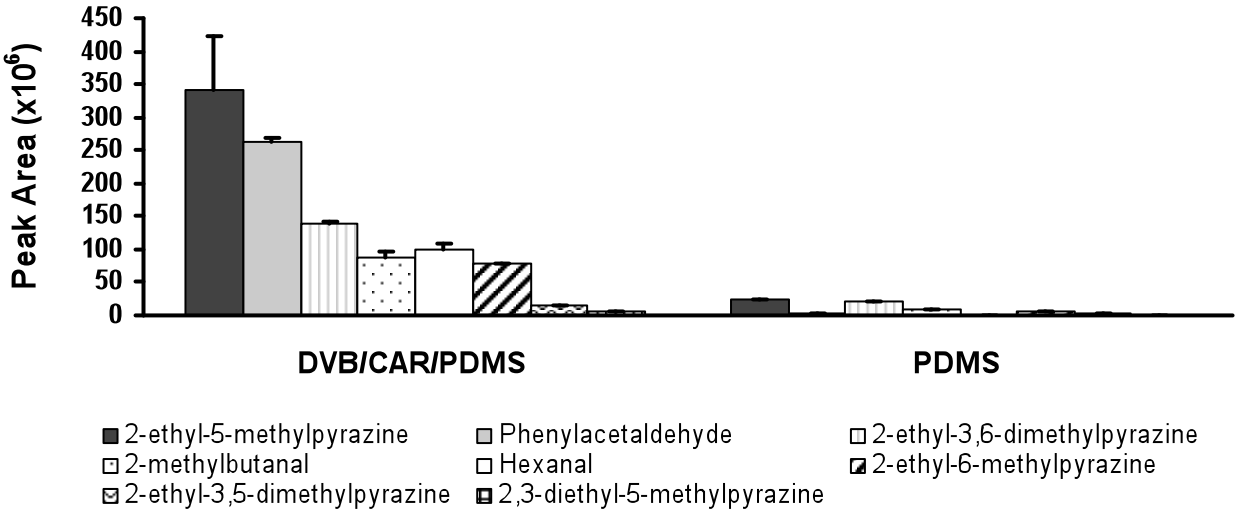

Figure 1: Effect of fiber type on adsorption capacity of target compounds. Extractions performed using undiluted peanut paste, with no salt added; 40ºC extraction temperature; 30 min extraction time (Greene et al. 2008).

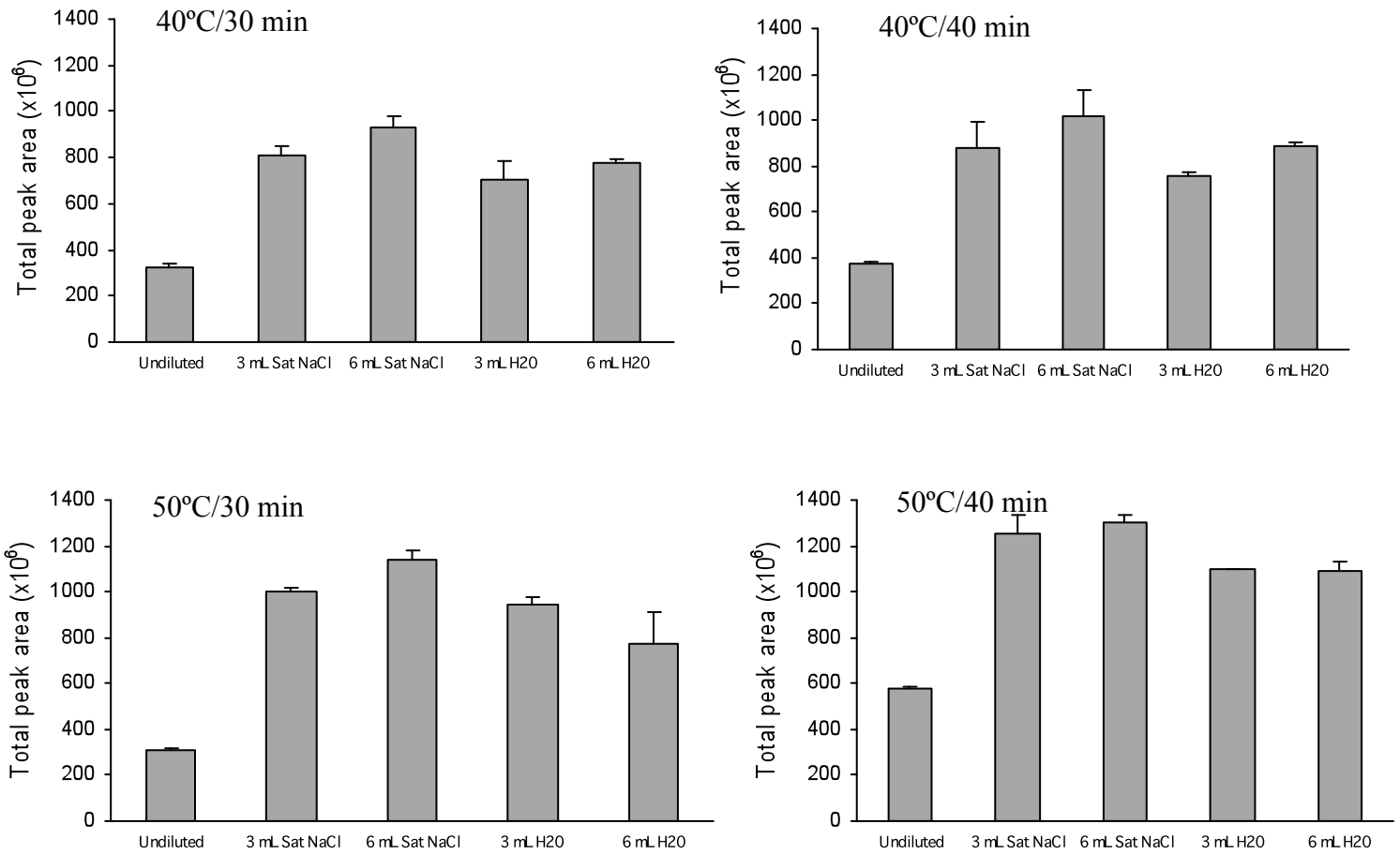

Figure 2: Effect of salt addition on adsorption capacity of target compounds expressed as the sum of peak areas. Samples extracted at different temperature/time using 50/30 μm DVB/CAR/PDMS fiber.

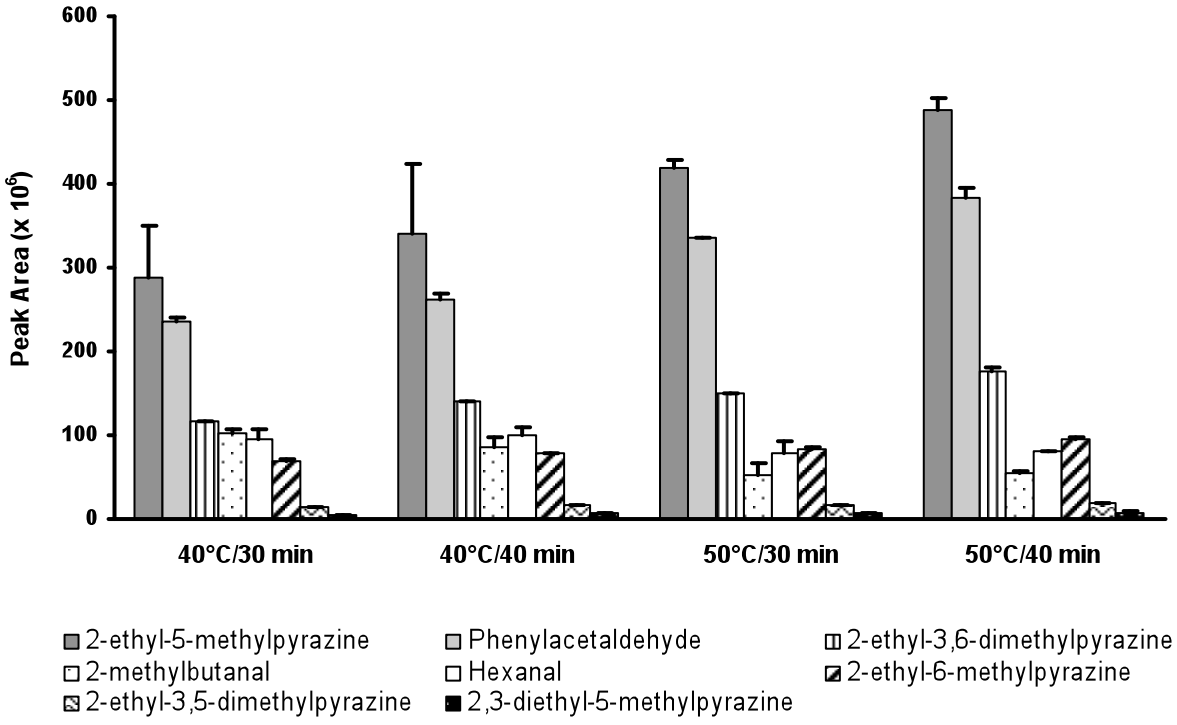

Figure 3: Effect of extraction time and temperature on peak area of target compounds from peanut pastes containing 6 mL saturated salt solution (3.2 M NaCl final concentration) and extracted using 50/30 μm DVB/CAR/PDMS fiber.

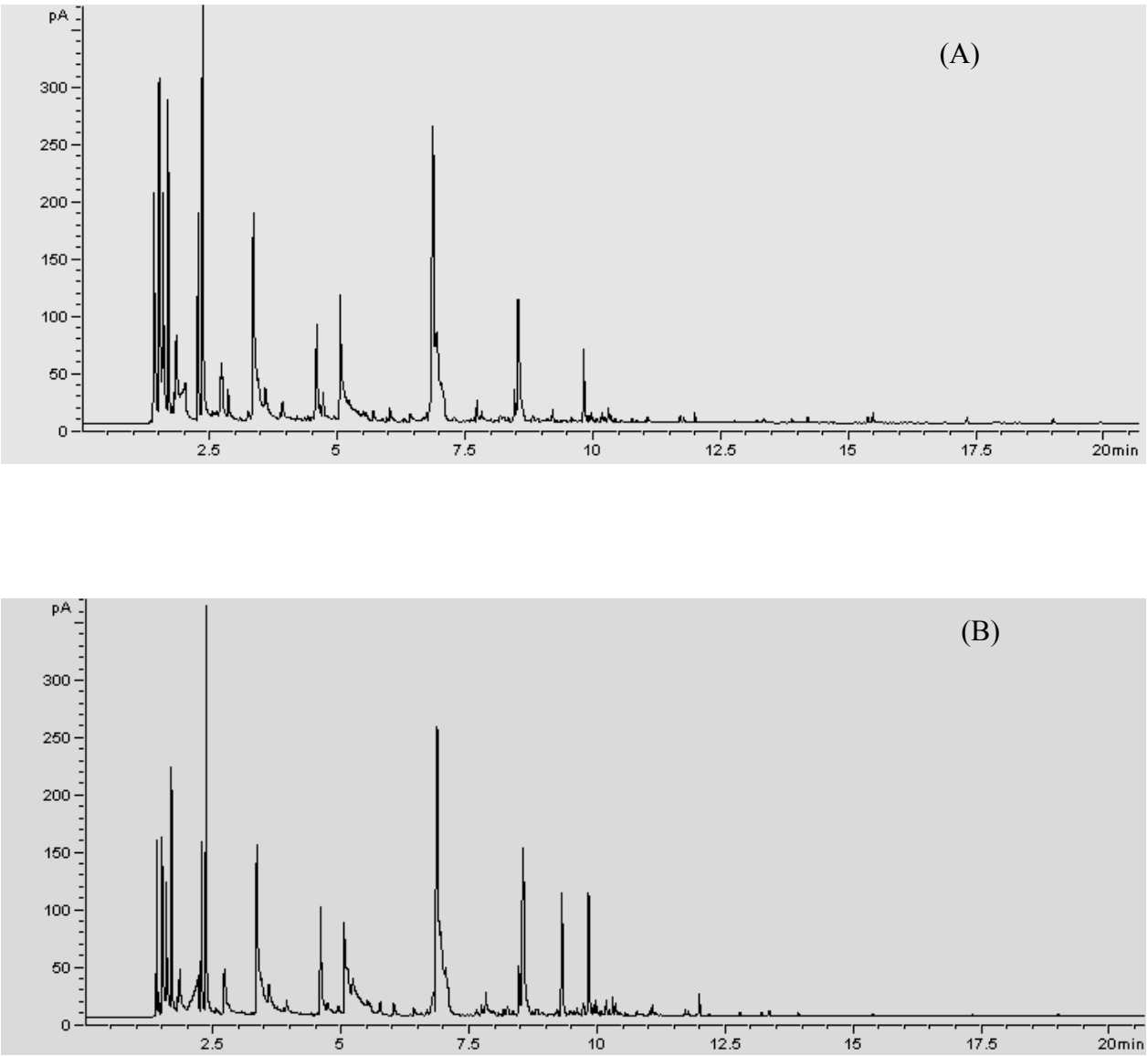

Figure 4: HS-SPME-GC/O chromatograms of peanut paste obtained using: (A) published method: undiluted peanut paste; 40ºC extraction temperature; 30 min extraction time; 50/30 μm DVB/CAR/PDMS fiber (Greene et al. 2008). (B): Optimized method: saturated salt solution added (3.2 M final NaCl concentration); 40ºC extraction temperature; 40 min extraction time; 50/30 μm DVB/CAR/PDMS fiber.

Table 1: Comparison of extraction efficiency between published and optimized HS-SPME methods in peanut paste. (A) Published method: undiluted peanut paste; 40ºC extraction temperature; 30 min extraction time; 50/30 μm DVB/CAR/PDMS fiber (Greene et al. 2008). (B) Optimized method: saturated salt solution added (3.2 M final NaCl concentration); 40ºC extraction temperature; 40 min extraction time; 50/30 μm DVB/CAR/PDMS fiber.

Compound	**Peak area (x 10^6)**		**% Increase**
	Published method (A)	**Optimized method (B)**	
Phenylacetaldehyde	14	262	1763
2-ethyl-5-methylpyrazine	107	341	220
2-ethyl-3,6-dimethylpyrazine	46	140	202
2,3-diethyl-5-methylpyrazine	2	7	197
2-ethyl-3,5-dimethylpyrazine	6	16	157
2-ethyl-6-methylpyrazine	35	78	119
Hexanal	52	87	67
2-methylbutanal	62	87	39

Table 2: Comparison of aroma profile from GC/O analysis between published and optimized HS-SPME methods in peanut paste. (A) Published method: undiluted peanut paste; 40ºC extraction temperature; 30 min extraction time; 50/30 μm DVB/CAR/PDMS fiber (Greene et al. 2008). (B) Optimized method: saturated salt solution added (3.2 M final NaCl concentration); 40ºC extraction temperature; 40 min extraction time; 50/30 μm DVB/CAR/PDMS fiber.

RT	Aroma description	Aroma intensity	
		Published method (A)	Optimized method (B)
1.4	sweet/roasted	2	nd*
1.5	garbage	nd	2
1.7	sweet	nd	1.5
1.8	buttery	3	2.5
2.3	malty	2.5	3
2.6	chlorine	3	nd
2.7	buttery	nd	2.5
3.0	rubbery	nd	3
3.2	burnt plastic	nd	1.5
3.6	stinky/fatty	nd	2
4.1	cheesy/sweaty	2	nd
4.5	grassy/floral	2	2
4.7	plastic	nd	1.5
4.9	brothy	nd	1.5
5.5	solvent-like	2	3
5.8	plastic	nd	1.5
5.9	rubbery/burnt plastic	3.5	3
6.6	rotten/sour	nd	1.5
6.7	potato	2.5	2.5
7.0	cheesy/sweaty	2.5	2.5
7.4	sweat/garbage	2	2
7.6	cheesy	nd	2
7.9	garlic/onion	2.5	2
8.1	mushroom	1.5	2.5
8.4	fruity	2	1.5
8.5	solvent-like	2.5	2.5
8.7	burnt plastic	2	2.5
8.9	popcorn/cheesy	3	2.5
9.1	rubbery	nd	2
9.2	rosy	nd	3
9.4	cotton candy	3	3
9.8	solvent-like	2	nd
9.9	earthy/bell pepper	2.5	2.5
10.2	maple/sweet	2	2

Table 2. Continuted

10.6	plastic/stinky	nd	1.5
11.1	earthy	nd	1.5
11.3	medicinal/rubbery	3	2.5
12.0	medicinal/cough syrup	2.5	3
12.7	roasted/nutty	2	1.5
13.1	perfume/fruity	nd	1
13.3	earthy/roasted	1.5	2
14.4	solvent-like	nd	1.5

*nd: not detected

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CHAPTER 3:

CHARACTERIZATION OF ROASTED PEANUT FLAVOR: DESCRIPTIVE SENSORY ANALYSIS, QUANTITATION OF KEY AROMA-ACTIVE COMPOUNDS AND RECONSTITUTION STUDIES IN AROMA MODELS

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Abstract

The present study characterized the flavor of roasted peanuts from five maturity classes using sensory and instrumental analyses. The exocarp was removed from peanut pods to accommodate visual sorting into five maturity classes based on mesocarp color. Pods were cured, shelled, and sized to obtain medium grade size seeds, which were roasted and processed into paste. The key aroma-active compounds from peanut extracts were screened by aroma extract dilution analysis (AEDA). Twenty-five compounds were selected as being potential contributors to roasted peanut flavor. Five additional compounds detected by headspace solid-phase microextraction (HS-SPME) were selected based on their high intensity scores on the GC-O analysis. Based on instrumental data, five model systems were developed by combining different concentrations of selected odorants. Optimized model system received a similarity score against the aroma of roasted peanuts of 8 on a 10-point scale. Omission experiments indicated that methional, 1-octen-3-one, nonanal, hexanal, octanal, 2-acetyl-1-pyrroline, carbon disulfide, and phenylacetaldehyde were the main contributors to roasted peanut aroma. Pyrazines were not found to have a major effect on the aroma of model mixtures. This study characterized the aroma of roasted peanuts using reconstituted model mixtures and contributes to a better understanding of the roasted, nutty flavor that is developed during peanut roasting.

KEYWORDS: roasted peanut flavor, model system, reconstitution study, instrumental analysis, GCxGC- TOFMS, GC-O, AEDA.

Introduction

Peanuts are well-liked among consumers because of the pleasant flavor that is developed during roasting (Sanders et al. 1997). During peanut roasting, flavor precursors undergo Maillard or nonenzymatic browning reactions, producing numerous heterocyclic nitrogen compounds that give rise to roasted peanut flavor (Hodge 1953; Hwang et al. 1995). Other reactions involved in the formation of volatile compounds in roasted peanuts include Strecker degradation, thermal degradation of sugars (caramelization), and lipid oxidation (Cammarn et al. 1990; Coleman et al. 1994). The flavor of roasted peanuts is associated with their volatile composition and over 300 compounds have been identified to date (Chetschik et al. 2008; Neta et al. 2010).

Investigation of roasted peanut flavor has been an ongoing effort for over 40 years. Early studies focused on the isolation and identification of compounds formed during roasting. Mason et al. (1966) reported several pyrazines as part of the volatile profile of roasted peanuts including methylpyrazine, 2,5-dimethylpyrazine, trimethylpyrazine, methylethylpyrazine, and dimethylethylpyrazine. Walradt et al. (1971) identified a total of 187 compounds in roasted peanuts and several alkyl and alkenyl pyrazines were reported for the first time in their study. Johnson et al. (1971) identified a total of 24 new compounds in roasted peanuts, including seven furans, six pyrroles, three 2-phenyl-2-alkenals, and two thiophenes. Ho et al. (1981) identified 131 compounds (seventy for the first time) in roasted peanuts, including lactones, pyrazines, pyrroles, pyridines, sulfides, thiazoles, thiophenes, furanoids, oxazoles, oxazolines, and sulfides.

Despite the extensive list of compounds identified in these early studies, limited information was made available regarding the odor properties of the compounds and the overall contribution to roasted peanut flavor. More recent research has applied descriptive sensory and gas-chromatography olfactometry (GC-O) analyses to identify key aroma-active compounds having high potential of contributing to roasted peanut flavor. GC-O is not a new technology (Fuller et al. 1964), its application to peanut flavor volatiles is relatively new. Braddock et al. (1995) used GC-O to evaluate the odor characteristics of several compounds isolated from roasted peanuts. 3-methylpyridine had an intense roasted peanut odor, phenylacetaldehyde was described as flowery and sweet, and 2-3-dihydrobenzofuran provided a sulfur-like aroma. Benzothiazone smelled burnt, and hexanal had a green, grassy note. Nonanal was described as floral, while heptan-2-ol and 1-pentanol had slightly pungent and green aromas. The most potent odorants in the volatiles extracted from headspace of freshly roasted peanuts were: 2,5-dimethylpyrazine, methylpyrazine, and 2-ethyl-3-methylpyrazine, as determined by aroma extract dilution analysis (AEDA). Similarly, Schirack et al. (2006) used GC-O to characterize the aroma properties of solvent extracts from roasted peanuts, and subsequently used AEDA to determine those with the highest aroma activity. Thirty-eight compounds were reported as the main flavor active volatiles of roasted peanuts including pyrazines, aldehydes, alcohols, esters, ketones, furanones, organic acids, and phenolic compounds. Recently, Chetschik et al. (2008) applied AEDA to compare the aroma-active compounds isolated from raw and roasted peanuts. 2-isopropyl-3-methoxypyrazine, 2-isobutyl-3-methoxypyrazine and trans-4,5-epoxy-(E)-2-decenal were among the compounds with

highest FD (flavor dilution) factors in raw peanuts. 2-acetyl-1-pyrroline and 4-hydroxy-2,5-dimethyl-3-(2H)-furanone contributed the most to roasted peanut aroma, and had the most pronounced increase in FD factors after roasting.

While the use of GC-O coupled with AEDA is a powerful tool for screening potent odorants in foods, these techniques have some weaknesses. For instance, they only consider the effect of isolated aroma-active compounds, neglecting the potential interactions with other chemical compounds present in the food (Drake and Civille 2003). In addition, GC-O data provide information of odor thresholds in air, which may not reflect the threshold of compounds in the food due to matrix interactions (Stephan et al. 2000). Other limitations include losses of odorants during the isolation process leading to underestimation of FD factors, and the assumption of linearity between sample dilution and odor intensity of volatiles (Stephan et al. 2000). Therefore, sensory studies of GC-O data using reconstitution aroma models are critical to validate the importance of selected aroma-active compounds to flavor. Reconstitution model studies involve the addition of key compounds to an odorless matrix in the same concentration of that found in the food. The aroma and/or flavor of the mixture models are then compared to the original food using a descriptive sensory panel (Grosch 2001). Omission experiments may be performed to further identify the character impact odorants by excluding a single or multiple odorants in the mixture and comparing it to the complete model. Aroma reconstitution studies have been successfully applied to several food products including wines (Guth 1998; Ferreira et al. 2002; Lorrain et al. 2006), fruit juices (Schieberle and Hofmann 1997; Buettner and Schieberle 2001), coffee (Semmelroch and Grosch 1996;

Mayer et al. 2000), french fries (Wagner and Grosch 1998), and dairy products (Preininger et al. 1996; Karagul-Yuceer et al. 2004). In general, close similarities between the sensory profiles of the model mixture and the original food were reported.

Although the flavor of roasted peanuts has been the subject of research for several years, quantification of key aroma compounds and reconstitution studies using model systems have not yet been reported. In addition, while the effect of seed maturity on sensory quality of peanuts has been previously reported (McNeill and Sanders 1998; Sanders et al. 1989), links between instrumental and sensory data have not yet been determined. Therefore, the objectives of this study were (1) to characterize roasted peanut flavor in peanuts of five mesocarp-color based maturity classes using descriptive sensory analysis; (2) to identify and quantify the key aroma-active compounds in roasted peanuts of 5 maturity classes using instrumental analyses; (3) to correlate descriptive sensory analysis with instrumental analysis; (4) to recreate roasted peanut aroma in model systems; and (5) to validate the importance of selected aroma-active compounds by conducting sensory studies of reconstituted aroma models.

Materials and methods

Peanut samples

Runner-type peanut pods (5,000 kg; crop 2007) were water blasted to remove the exocarp and visually sorted into increasing maturity classes based on mesocarp color (yellow, orange A, orange B, brown and black) as previously described elsewhere (Drexler and Williams 1979; Sanders et al. 1989). Peanut pods were dried using ambient

air, shelled, and sized by diameter over slotted hole screens (Sanders et al. 1989). Medium grade size peanut seeds from each maturity class (<8.3 and >6.3 mm) were roasted in the center of a convection oven at 169 ºC for 25 min (Please see appendix 3 for seed size distribution). Roast time was optimized on seeds from brown pods, and Hunter L-value ranged from 48 to 52 among maturity classes (Appendix 2). Peanut pastes were prepared using a Beixer 3 food processor (Robot Coupe Inc.; Ridgeland, MS, USA), packaged in 8 oz glass jars, and stored at -20 ºC until analysis. Peanuts were freshly roasted and pasted before sensory and instrumental analyses were conducted.

Sensory analysis of roasted peanuts

An estabilished descriptive sensory panel (n=10) with over 700 hundred hours experience on peanut flavor evaluated the flavor of roasted peanut pastes. Panelists were students and staff from the USDA Agricultural Research Service, Market Quality and Handling Research Unit, and the Department of Food, Bioprocessing and Nutrition Sciences at North Carolina State University, Raleigh, NC.

At each sensory session, samples were tempered to room temperature and randomly presented to panelists along with a reference peanut paste as a warm-up. Samples were evaluated in 4 replicates using a 15-point intensity scale, according to the Spectrum^{TM} method (Meilgaard et al. 1999). Water and non-salted crackers were provided as palate cleansers between samples. Sensory evaluation was performed using the peanut lexicon previously developed by Johnsen et al. (1988) and Sanders et al. (1989 (Figure 1).

Chemicals

Ethyl ether (anhydrous, 99.8%), sodium chloride (99%), sodium sulfate (99%), and the internal standards 2-methyl-3-heptanone and 2-methyl pentanoic acid were obtained from Aldrich Chemical Co. (St. Louis, MO, USA). Sodium bicarbonate (99.7%) and hydrochloric acid (36.5%) were purchased from Fisher Scientific (Pittsburgh, PA, USA). With the exception of the following, all reference standards (Table 1) were obtained from Sigma-Aldrich (Sigma-Aldrich, St. Louis, MO, USA). 2-acetyl-1-pyrroline and 2-propyl-1-pyrroline were acquired from Chemstep (Carbon Blanc, France). 3,5-diethyl-2-methylpyrazine was sourced from Penta (Livingston, NJ, USA), and trans-4,5-epoxy-(E)-2-decenal was obtained from Cayman Chemical Company (Ann Arbor, MI, USA). Stock solutions for instrumental analysis were prepared from the pure compounds diluted in absolute methanol (Sigma-Aldrich; St. Louis, MO, USA), and were stored at -20 ºC until use for a maximum of 1 month.

Sample preparation for instrumental analysis

Direct solvent extraction and solvent assisted flavor evaporation (SAFE)

Each sample replicate consisted of 100 g of peanut paste, which was equally divided into two teflon centrifuge bottles. An internal standard mixture of 2-methyl-3-heptanone (neutral/basic fraction) and 2-methyl pentanoic acid (acidic fraction) suspended in ether was added to each bottle for a final concentration of 2.5 ppm each. Subsequently, 100 mL of NaCl and 100 mL ethyl ether were added to each sample, and shaken for 30 min at a speed of 8 on a Roto mix (Barnstead/Thermolyne Type 50800; Dubuque, IO, USA). The

bottles were centrifuged for 15 min at 3000 rpm in a Sorvall RC- 5B refrigerated (3 ºC) superspeed centrifuge (DuPont Instruments; Parkersburg, WV, USA) to separate the solvent phase from the peanut paste, which was then transferred to a closed glass jar. This procedure was repeated three times, each with addition of 100 mL of ethyl ether. The combined solvent extracts were stored at -20ºC until further analysis. Roasted peanut volatiles from each maturity class were extracted in duplicate. Volatile compounds from peanut paste solvent extracts were collected using SAFE, with an apparatus similar to that described by Engel et al. (1999). Solvent extracts were loaded into the top of the SAFE apparatus, and slowly released into the vacuum flask. The non-volatiles were collected in the round-bottom flask and the volatile compounds were collected in the first trap of the SAFE apparatus, which was surrounded by liquid nitrogen. Distillation was carried out for 2 h under vacuum (ca. 10^{-4} Torr). The distillate was then concentrated to 20 mL under a gentle stream of nitrogen gas at ambient temperature. The concentrated distillate was washed twice with 3 mL of 0.5 M sodium bicarbonate and shaken using a vortex. After each wash, the water phase (bottom layer) was set aside in a screw cap test tube. The concentrate was washed three times with 2 mL saturated sodium chloride solution, and the water phase (bottom layer) was transferred to the previous screw cap tube. The remaining ether (top layer) was designated as the neutral/basic fraction. The aqueous phase was acidified with hydrochloric acid (18% w/v) to pH 2 and then washed three times with 5 mL ethyl ether. After each addition, the ether phase (top layer) was transferred to another tube and the combined extract was designed as the acidic fraction. Each fraction was filtered through anhydrous sodium sulfate twice to remove water

residue, concentrated to 0.5 mL under a gentle stream of nitrogen gas, and stored at -80 ºC. Immediately before analysis, 100 uL of each fraction was transferred to graduated 2 mL screw cap vials with a glass insert (Supelco, Bellefonte, PA, USA), and further concentrated to 50 uL under a gentle stream of nitrogen gas at ambient temperature.

Headspace solid-phase microextraction (HS-SPME) for gas chromatography-olfactometry *(GC-O) analysis*

Ten grams of peanut paste plus 12 mL saturated NaCl solution were placed into a 40 mL amber screw cap vial (Supelco, Bellefonte, PA, USA). Samples were allowed to equilibrate at 40 ºC for 30 min and the fiber was manually inserted into the sample vial using a SPME holder (Supelco; Bellefonte, PA, USA) at 2.5 cm depth. Volatile compounds were extracted at 40 ºC/40 min, followed by desorption in the GC-O injector for 5 min at 250 ºC. Prior to each extraction, the fiber was conditioned in a GC injector for 5 min at 250 ºC to reduce possible contamination.

HS-SPME for comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry (GCxGC-TOFMS) analysis

Peanut volatiles were isolated using headspace solid-phase microextraction (HS-SPME) using a CTC Analytics combiPAL auto sampler (Zwingen, Switzerland). For the quantification of less abundant compounds such as 1-octen-3-one, pentanoic acid, methional, and 2,3-diethyl-5-methylpyrazine, 2 grams peanut paste were placed into 10 mL clear screw cap vials (Microliter Analytical Supplies Inc.; Suwanee, GA) containing

2.5 mL saturated NaCl solution and 100 uL methanol. For the quantification of all remaining compounds (Table 2), 1 g peanut paste, 1.25 mL saturated NaCl solution (6.2 M), and 250 uL methanol were used. A higher sample size was used for the less abundant odorants to enhance sensitivity. After addition of 100 ppb 2-methyl-3-heptanone as the internal standard, the vials were shaken using a vortex for 1 min. Volatiles were extracted using a three-phase 1 cm divinylbenzene/carbonex/polydimethylsiloxane (DVB/CAR/PDMS) with a 50/30 μm coating thickness (Supelco; Bellefonte, PA, USA). SPME fibers were inserted through the vial septa at 12 mm depth, and exposed to the headspace above the sample. Samples were equilibrated for 15 min at 40 ºC with agitation of 500 rpm. Samples were then extracted for 40 min at 40 ºC with 100 rpm agitation, and desorbed into the GCxGC-TOFMS instrument for 15 min.

Gas chromatography-olfactometry (GC/O)

Neutral/basic and acidic fractions of solvent extracts (1 uL) as well as volatiles from HS-SPME were analyzed on a HP5890 series II gas chromatograph (Hewlett-Packard Co.; Palo Alto, CA, USA) fitted with a flame ionization detector (FID), splitless injector, and a sniff port. The GC was equipped with either a polar capillary column (DB-WAX, 30 m length × 0.25 mm i.d. × 0.25 μm film thickness (Agilent J&W Scientific; Santa Clara CA, USA), or a nonpolar column (DB-5 ms, 30 m length × 0.25 μm film thickness) (Agilent J&W Scientific; Santa Clara CA, USA). The oven temperature was programmed to increase from 40ºC to 200ºC at a rate of 8ºC/min with an initial hold of 3 min and a final hold of 20 min. Column effluent was split 1:1 between the FID and

sniffing port using deactivated fused silica capillaries (1 m length x 0.25 mm i.d.). The FID and sniffing port were kept at 250 ºC. Humidified air was supplied at the end of the sniffing port at a rate of 30 mL/min to prevent dehydration of the nasal membranes of the panelists. Two experienced panelists sniffed each sample. Panelists described the odors and scored aroma intensities using a 5-point numerical intensity scale (Van Ruth 2001).

Aroma extract dilution analysis (AEDA) was applied to peanut solvent extracts to determine the most potent aroma-active compounds from each maturity level. Extracts were diluted at a ratio of 1:3 and injected into the GC-O (1 uL) after each dilution until no odor could be detected. The flavor dilution (FD) factor for each compound was determined as the last dilution at which the odorant was detected (Grosch 1993). Compounds having higher FD values were considered to play a larger role in overall roasted peanut flavor than those with lower FD values.

Comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry (GCxGC-TOFMS)

Peanut volatiles were quantified on a LECO Pegasus III two-dimensional gas chromatrograph (GCxGC) coupled with time of flight mass spectrometer (TOFMS) (Model # 614-100-700; Leco Corporation; St. Josep, MI, USA). The instrument was connected to an Agilent GC (Model# 6890N; Agilent Technologies; Santa Clara, CA, USA) fitted with a secondary oven. The system was equipped with a thermal modulator cooled with liquid nitrogen with a modulation time of 1.75 sec and hot jet pulse time of 0.35 sec. Cool time between stages was set as 0.53 sec. Analyses were performed using a

SolGel-Wax™ 30 m x 0.25 mm ID x 0.25 μm d_f (SGE, Austin, TX) (polyethylene glycol) as the first dimension column and a RTX 17-01 1 m x 0.1 mm ID x 0.1 μm d_f (Restek, Bellefonte, PA) (14% cyanopropylphenyl – 86% dimethyl polysiloxane) as the second dimension column. Helium was used as the carrier gas at a constant flow rate of 1.3 mL/ min. The transfer line was set as 250°C and operated in pulsed splitless mode with a pulse pressure of 37 psi for 1 min, and the split vent was opened for 2 min. The primary oven temperature was programmed to increase from 40ºC to 140ºC at a rate of 5 ºC/min, then 10 ºC/min to 250 ºC with an initial hold of 2 min and a final hold of 3 min. Secondary oven temperature increased from 55ºC to 155ºC at 5 ºC/min then 10 ºC/min to 250ºC with an initial hold of 2 min and a final hold of 4 min. Time of flight mass spectrometer detector conditions were as follows: ionization energy, -70 eV; ion source temperature, 200ºC; mass range, 25-500 m/z; detector voltage 1500 V; scan rate, 200 spectra/s. Data analyses were performed using the ChromaTOF® software (Leco Corporation, St. Joseph, MI, USA). The unique mass was used to quantify peaks as determined by the deconvolution algorithm.

For each aroma compound, a five-point internal standard curve was constructed by spiking different concentrations of the compound in a model system consisting of 25% egg white powder as the protein source (Michaels Foods; Minnetonka, MN, USA), 51% hydrogenated oil (Crisco; Food Lion, Raleigh, NC, USA), 4% sugar (Food Lion; Raleigh, NC, USA) 18% starch and (H-50; National Starch; Bridgewater, NJ, USA) and 2% water. The model system base was created to have the proximate chemical composition of roasted peanuts (Kim and Hung 1991). 1-octen-3-one, pentanoic acid, methional, and 2,3-

diethyl-5-methylpyrazine, 2 grams peanut paste were added to 1 g base and 1.25 mL saturated NaCl solution (6.2 M) in 10 mL clear screw cap vials (Microliter Analytical Supplies Inc.; Suwanee, GA). Stock solutions of these odorants were prepared such that the total amount of methanol added was 250 uL. All remaining compounds (Table 2), which were present in lower concentrations in peanut pastes, were added to 2 g base containing 2.5 mL saturated NaCl solution. In this case, stock solutions in methanol were prepared to add up to 100 uL total volume. 2-methyl-3-heptanone was added as the internal standard in all samples (100 ppb in the base). Standard curves were constructed by plotting the peak area ratio of the analyte to 2-methyl-3-heptanone versus the concentration ratio of analyte to 2-methyl-3-heptanone. Resultant peak areas were fitted using linear regressions, with the concentrations averaged across three replications.

Identification of odorants

Positive identification of aroma-active compounds in peanuts was performed by comparison of the volatile compound's mass spectra, retention index, and aroma quality with those of standard compounds analyzed under the same conditions. For the calculation of retention indices (RI), an n-alkane series was used (Van den Dool and Kratz 1963).

Reconstitution studies on aroma models

Aroma models

The initial aroma model (Model #1) was prepared on the basis of volatile data. Odorants quantified by GCxGC-TOFMS were spiked into the matrix in concentrations equal to those determined in roasted peanuts of the black maturity level (Table 2). Studies have shown that 'on' flavors such as 'roasted peanutty' and 'sweet aromatic' are higher for the more mature peanuts (Sanders et al. 1989; McNeill and Sanders 1998). Based on the solubility of compounds, stock solutions were prepared in either oil or water (Table 2), and added to the matrix (50g) described above. The model system was stirred and allowed to equilibrate for 24 hours at 4ºC. Based on sensory evaluation of the initial model, four additional model systems were developed to improve its overall aroma profile (Table 2).

Similarity tests and aroma profiling

Model systems were evaluated orthonasally using the sensory panel (n=10) described above. Panelists received an additional ~15 h training to identify and quantify different aroma attributes of peanut paste on a 15-point intensity scale using the Spectrum^{TM} Method (Meilgaard et al. 1999). Panelists were also trained to describe the aroma of about 25 odorant references, most of which were present in the model systems. Samples (3 grams each) were presented in 1 oz aluminum foil wrapped glass jars (Fisher Scientific, Hampton, NH, USA) with screw caps, labeled with a 3-digit code. Samples were equilibrated for 2 hours at room temperature prior to sensory analysis.

Panelists evaluated Model #1, which was developed based on volatile data, and were asked to provide descriptions for improvement of the aroma profile. This step was repeated until an aroma similar to that of roasted peanuts was achieved. The composition of each aroma model is shown in Table 4.

Similarity tests were conducted using a 10-point intensity scale to compare the aroma profile of five aroma models to roasted peanuts. In each session, a reference roasted peanut paste (black) and five aroma models were presented in random order. Samples were evaluated in triplicate on three consecutive days.

The model system with the highest similarity score (Model #5) as well as two roasted peanut paste samples (black and yellow) were profiled using a 15-point scale, according to the Spectrum™ method (Meilgaard et al. 1999). Results from three replications were averaged and plotted in a spider web diagram.

Omission experiments

Thirteen reduced compound models were prepared to assess changes in the aroma of the optimized model (Model #5) by omitting one or more odorants (Table 5). The reduced models were individually compared to the original aroma model via triangle tests. Each triangle test was presented to 10 panelists and repeated in the same day.

Statistical analysis

Statistical analysis was performed using the SAS® System for Windows™ version 8.2 (SAS Institute Inc.; Cary, NC). Principle component analysis (PCA) was

carried out separately for the sensory and instrumental data obtained from roasted peanuts of five different maturity classes. The correlation between these two data sets was carried out using PCA and partial least squares regression (PLSR).

Data obtained from the reconstitution experiments were analyzed using one-way analysis of variance (ANOVA). Means separation was carried out using the Tukey test. Significant differences for the triangle tests were determined using the method described by Roessler et al. (1978), with n=20 being the maximum number of corrected responses.

Results and discussion

Sensory analysis of roasted peanut pastes

The peanut plant has an indeterminate flowering pattern which results in peanuts of different maturity at harvest. The physiological differences across maturity classes may affect the flavor profile that is developed during the roasting of a sized peanut lot (Sanders et al. 1982; Sanders et al. 1989). The sensory attributes of roasted peanut pastes from five mesocarp-color based maturity classes were evaluated by a descriptive panel using a previously developed lexicon (Figure 1). The intensity of roasted peanutty, which is the desirable sensory attribute associated with freshly roasted peanuts, increased with seed maturity ($P=0.0009$), with intensities ranging from 4.4 for 'yellow' to 5.1 for 'black'. Similarly, sweet aromatic and sweet taste increased with maturity level ($P=0.003$). Bitter taste was lower for the more mature classes (black, brown) than for the immature classes (orange B, orange A, yellow) ($P<0.05$) (Table 3). These results are in agreement with Sanders et al. (1989), who reported that 'on' flavors such as roasted

peanutty flavor and sweet aromatic were higher in more mature peanuts, while 'off' flavors such as painty and fruity-fermented were higher in immature peanuts. McNeill and Sanders (1998) also found that the intensity of roasted peanutty flavor was significantly higher for the more mature classes. Pattee and Young (1987) suggested that mature peanuts contain more of the amino acid precursors of typical roasted peanuts than immature peanuts, which may explain the sensory differences observed in this study.

Principal component analysis (PCA) of the data obtained from the sensory profiling of peanuts from different maturity classes allowed the reduction of 8 variables to 2 independent components accounting for 88% of the total variation. High variable loads usually translate into greater contribution to what the PC explains of the data structure (Gardiner, 1997). PC1 was highly loaded with raw beany, woody/hull/skins and bitter in the positive direction, and with sweet taste, roasted peanutty and sweet aromatic in the negative direction. For PC2, astringency was high in the positive direction and dark roast and bitter were high in the negative direction. Maturity classes were well separated into the two dimensional space and were highly differentiated on PC1, with the more mature classes such as black and brown being loaded on the negative direction of PC1, and the least mature classes such as orange A and yellow being loaded on the positive PC1 dimension. Black and Brown, the most mature classes, were located close to roasted peanutty, sweet aromatic and sweet taste, while yellow, the least mature sample, was placed close to bitter and dark roast. Because maturity and seed size are not absolutely correlated, peanuts of different maturity classes are found in all grade sizes. Results from

this study suggest that the maturity distribution in a grade size may influence flavor development when peanuts are roasted to the same degree (Sanders 1989).

Identification and quantification of selected aroma-active compounds in peanuts

Volatile analysis of compounds from roasted peanuts typically results in complex chromatograms and several hundreds of compounds have been identified to date (Neta et al. 2010; Schirack et al. 2006, Chetschik et al. 2008; Braddock et al. 1995; Didzbalis et al. 2004; Greene et al. 2008; Matsui et al. 1998). However, research has shown that only about 5% of the volatile compounds identified in foods impact flavor (Grosh 2000). Approximately 200 aroma-active compounds were detected in peanut extracts using GC-O, which is consistent with previous reports (Schirack et al. 2006). About 50 compounds were detected with HS-SPME, but a number of overlapping compounds were found between these two extraction techniques (Appendix 1). In order to determine the impact odorants in roasted peanuts, AEDA, which is a dilution to odor technique, was applied on the neutral/basic and acidic fractions of peanut extracts from five different maturity classes. A total of 20 compounds (17 in the neutral/basic fraction and 3 in the acidic fraction) were selected from peanut extracts as the key odorants in roasted peanuts. Flavor dilution (FD) factors for the neutral/basic and acidic fractions were ≥ 243 ($\log_3$ FD ≥ 5), and ≥ 27 ($\log_3$ FD ≥ 3), respectively, for at least one maturity level. Five additional compounds detected by HS-SPME were selected based on their high intensity scores on the GC-O analysis (Table 1). Compounds were positively identified using retention index (RI) on two different stationary GC phases, odor quality, mass spectra, and comparison to

authentic standards subjected to the same analytical conditions. Tentative identifications were based on matching the RI values and odor properties of unknown compounds against those of authentic standards. Twenty compounds were positively identified, four were tentatively identified and one remains unknown (Table 1).

Methional, dimethyl trisulfide, phenylacetaldehyde, 1-octen-3-one, and 2-acetyl-1-pyrroline were among the compounds with highest FD factors within a maturity class. Methional, which has a potato/brothy odor, has been recently reported in roasted peanuts (Greene et al. 2008; Chetschik et al. 2008), and may be formed through Strecker degradation of methionine (Ballance 1961). The amino acid methionine may also be involved in the formation of dimethyl trisulfide either by direct oxidation or through reactions with hydrogen sulfide (Yu and Ho 1995). Dimethyl trisulfide has a garlic/onion aroma, and has been previously reported as a key component in the aroma of boiled meat (Golovnja and Rothie 1980), vegetables such as cabbage, broccoli and cauliflower (Buttery et al. 1976), wine (Guth 1997), and roasted peanuts (Chetschik et al. 2008). Phenylacetaldehyde has a rosy/floral aroma profile and is formed by the reaction of phenylalanine and α-dicarbonyl compounds via Strecker degradation (Hofmann and Schieberle 2000). Phenylacetaldehyde has been shown to contribute to the overall aroma of several foods including chocolate (Welty et al. 2000), ham (Jurado et al. 2009), honey (Radovic et al., 2001), wine (Campo et al. 2006), Cheddar cheese (Whetstine et al. 2005), and roasted peanuts (Schirack et al. 2006; Greene et al. 2008; Chetschick et al. 2008). 1-octen-3-one, which has a mushroom-like odor, may be formed from the auto-oxidation of arachidonic acid (Blank et al. 2001) and has been reported in apple cider (Xu et al. 2007),

mushroom (Cho et al. 2006), Cheddar cheese (Christensen and Reineccius 2006) and roasted peanuts (Greene et al. 2008; Chetschik et al. 2008). 2-acetyl-1-pyrroline is formed from the reaction between the amino acid proline and carbohydrates such as 2-oxopropanol and fructose (Schieberle 1995). This compound has been identified as an important constituent of the aroma of popcorn (Schieberle 1991), cooked rice (Buttery et al. 1982), whey protein (Whetstine et al. 2005), bread crust (Schieberle and Grosch 1985), and has been recently reported in roasted peanuts (Chetschik et al. 2008).

Peanuts from the Black maturity class exhibited the highest FD factors for 2-methoxy-4-vinylphenol, 2-acetyl-3-methylpyrazine, and phenylacetaldehyde. 2-methoxy-4-vinylphenol may be produced by thermal decarboxylation of the polyphenol ferulic acid (Fiddler et al. 1967), which has been reported in peanuts at levels of about 9 mg/ 100g (Mattila and Hellstrom 2007; Zhao et al. 2008). Pyrazine compounds have been traditionally associated with the aroma of roasted peanuts (Mason et al. 1966; Shibamoto and Bernhard 1976; Buckholz et al. 1980; Baker et al. 2003). Based on GC/O analysis, five pyrazines were found among the potential odorants contributing to roasted peanut flavor, including 2-ethyl-3,5-dimethylpyrazine (nutty/brothy), 2-isopropyl-3-methylpyrazine (earthy/bell pepper), 2-ethyl-3-methylpyrazine (rosted/nutty), 2-acetyl-3-methylpyrazine (roasted/nutty), 2-5-dimethylpyrazine (earthy) and 3,5-diethyl-2-methylpyrazine (earthy/soil). However, latter reconstitution studies in model systems revealed that pyrazines were not among the key aroma compounds in roasted peanuts. The most currently accepted pathway for pyrazine formation involves the reaction of amino acids with α-dicarbonyl compounds through Strecker degradation, producing α-

amino carbonyls, which then condense to give rise to alkylpyrazines (Fennema 1996). An alternate pathway involves pyrolysis of amino acids and does not require the presence of sugar. Pyrazines reported in this study have been associated with the flavor of several thermally processed foods such as coffee (Cordero et al. 2008), cocoa (Brunetto 2009), french fries (Wagner and Grosch 1998), hazelnuts (Cordero et al. 2008), almonds (Vazquez-Araujo et al. 2009), and peanuts (Schirack et al. 2006; Chetschik et al. 2008).

Lipid oxidation products such as hexanal (green/ grassy), octanal (fruity/fatty) and nonanal (fruity/fatty) were also among the key odorants in roasted peanuts. Brown et al. (1973) suggested that hexanal, octanal and possibly nonanal were associated with the beany aroma of peanuts. These compounds have been previously found in heated butter (Lee et al. 1991), grapefruit (Buettner and Schieberle 2001), coffee and hazelnut (Cordero et al. 2008), as well as in roasted peanuts (Chetschik et al. 2008).

Other aroma-active compounds that are also likely to contribute to the aroma of roasted peanuts include 2,3-pentanedione (buttery), 2-methylbutanal (malty/chocolate), carbon disulfide (garbage/rotten), maltol (cotton candy), pentanoic acid (cheesy/sweaty), and acidic acid (vinegar). Trans-4,5-epoxy-(E)-2-decenal (medicinal), indole (mothball/hay), and estragole (licorice) were tentatively identified by RI values and odor properties compared with those of the respective reference standard; however, satisfactory MS signals were not achieved for these compounds. Trans-4,5-epoxy-(E)-2-decenal has been recently identified as a constituent of roasted peanuts (Chetschik et al. 2008), whereas indole and estragole have not been yet been reported in peanuts.

Compared to the most recent studies on flavor characterization of peanuts (Schirack et al. 2006; Chetschik et al. 2008), GC/O analysis results are in agreement with the potential role of phenylacetaldehyde, 2-ethyl-3,5-dimethylpyrazine, 2-methylbutanal, and nonanal to the aroma of roasted peanuts. Schirack et al. (2006) used AEDA to determine the compounds with the highest impact on roasted peanut flavor. Thirty-eight compounds were reported in their study; however several of them were only tentatively identified. Among the compounds identified by Schirack et al. (2006), only six were also detected in our study. Chetschik et al. (2008) conducted an extensive study to characterize the flavor of raw and roasted peanuts. Odorants identified in our study were in agreement with the majority of compounds with the highest FD factors from their study. However, a few of the most aroma-active compounds reported by Chetschik et al. (2008), such as (E,E)-2,4-decadienal (FD=1024) and 4-hydroxy-2,5-dimethyl-3-(2H)-furanone (FD=2048), were not detected in our study. The differences observed among these studies may be attributed to the different handling and peanut varieties used. Compared to these two most recent studies, we have identified the following compounds as further odorants in roasted peanuts: 2-ethyl-3-methylpyrazine, 2,5-dimethylpyrazine, carbon disulfide, and pentanoic acid.

Quantitative data for positively identified compounds were determined in peanuts from five maturity classes using GCxGC-TOFMS. GCxGC is a powerful analytical tool that has been commonly used in flavor chemistry in recent years. In this technique, two independent columns are used to separate individual constituents of a food sample. Narrow fractions of the first GC column effluent are continuously focused via a

cryogenic modulator, which traps and reinjects the slices into the second column (Wu et al. 2004; Adahchour et al. 2006). This process results in improved separation of analytes, with increased peak capacity and higher sensitivity when compared to one-dimensional gas chromatography (Adahchour et al. 2003; Pierce et al. 2008). The column combination used in this study facilitated the identification and quantification of 19 compounds selected by GC-O analysis (Figure 3). Compounds were quantified using multiple point internal standard curves by spiking different levels of each compound to model systems, which were then extracted by HS-SPME and analyzed using GCxGC-TOFMS. Table 2 shows the regression equation, correlation coefficient, and the unique mass used for quantification of each compound.

Principal component analysis (PCA) was carried out to visually examine the relationships between volatile composition and maturity classes (Figure 4). The first two principle components (PC1 and PC2) explained 48% and 27% of the total variance, respectively. PC1 was highly loaded with phenylacetaldehyde, 2,5-dimethylpyrazine, nonanal, octanal, and 2,3-pentanedione in the positive direction, and with 1-octen-3-one and 2-methylbutanal in the negative direction. PC2 was high in acetic acid, 2,3-diethyl-5-methylpyrazine and carbon disulfide in the positive direction and with 2-methoxy-4-vinylphenol, maltol, pentanoic acid and 2-ethyl-3,5-dimethylpyrazine in the negative direction. The more mature classes such as black and brown were located on the positive side of PC1, while the least mature classes such as orange B orange A and yellow were located on the left dimension. Black was mostly associated with nonanal, octanal, phenylacetaldehyde and 2,5-dimethylpyrazine. Brown and yellow were located in

opposite sides of the bi-plot. Yellow was associated with pentanoic acid, 2-methoxy-4-vinylphenol, maltol and 2-ethyl-3,5-dimethylpyrazine, while the brown was negatively associated with those compounds and positively related to acetic acid. Orange B was mostly associated with 1-octen-3-one and 2-methyl butanal.

Partial least square regression (PLSR) and PCA were applied to determine the relationships between sensory and instrumental data. PLSR is a multivariate technique for constructing predictive models from many collinear variables (Tobias 1996). The two first latent factors extracted from the PLSR of instrumental on sensory data explained 82% of total variance of the sensory data. This suggests that the sensory attributes could be well predicted from the volatile composition of the samples examined in this study. Figure 5 shows the results of PCA obtained from sensory and instrumental data. The first two PC explained 77% of the total variance. Maturity classes were well discriminated by PC1, which accounted for 51% of the total variance. The interrelations between variables were also examined in the biplot of PC1 vs. PC2. Roasted peanutty, sweet aromatic and sweet taste were most associated with phenylacetaldehyde, and 2,3-pentanedione, whereas woody/hull/skins was mostly associated with 2-methylbutanal. Dark roast was associated with 2-ethyl-3,5-dimethylpyrazine and 2-methoxy-4-vinylphenol. This study provided information on the relationships between sensory and instrumental data in peanuts of different maturity classes and may contribute to a better understanding of the flavor that is developed during peanut roasting.

Aroma models and omissions experiments

A well accepted approach for the characterization of complex flavors, such as that of roasted peanuts, involves the use of GC-O and AEDA to screen potentially key odorants, identification and quantification of aroma-active compounds, and reconstitution studies on aroma models. In reconstitution studies, model systems are prepared by mixing odorants in the same proportion of that found in the food. Omission experiments are then conducted to validate the importance of odorants to flavor by eliminating selected compounds from the mixture and comparing it to the full model. An initial aroma model for roasted peanuts was developed by adding 19 compounds to a neutral matrix in the same concentrations found in the black peanuts (Table 4). The model was evaluated by a descriptive sensory panel, and had an overall aroma resembling that of roasted peanuts, but with low intensities of roasted peanutty and sweet aromatic. Quantification studies using conventional techniques such as the one used in this study usually provides a good estimation of concentration of odorants in foods. However, precise quantifications can not be achieved due to complexity of the sample matrix, differences in concentration, volatility and reactivity of compounds (Grosch 2001; Schieberle and Grosch 1987). A highly trained panel was used to evaluate the model systems and direct the formulation of additional models in order to attain the typical aroma of roasted peanuts. A total of four additional model systems were developed by changing the concentration of selected compounds according to descriptions provided by the panel (Table 4).

Once all models were developed, a similarly test was conducted using a 10-point scale, with ‘0’ representing no similarity and ‘10’ representing high similarity to roasted

peanut aroma. The initial model system (Model #1) received a relatively low similarity score of 4.7; however modification of the concentrations of some volatiles in the model led to significant improvement of the aroma profile (Figure 6). The optimized model system (Model #5) was very similar to the aroma of roasted peanuts, with a similarity score of 8. A comparison of the aroma profile of the optimized model system with peanuts showed that the intensity of roasted peanutty was about 1-point lower than roasted peanutty in the yellow class and about 1.5-points lower than the roasted peanutty intensity in the black class. The optimized model system was also slightly lower in sweet aromatic, dark roast and woody/hulls/skins, but had a higher intensity of raw beany than both the black and yellow class peanuts (Figure 7). Overall, panelists agreed that the optimized model system imparted the typical aroma of roasted peanuts.

Omission experiments were carried out to examine the relative importance of odorants to the aroma profile of model mixtures. Thirteen model systems were prepared by omitting one or more compounds and comparing it to the complete model via triangle tests (Table 5). Several incomplete models were significant different from the full model, suggesting that missing compounds played a major role in the aroma of roasted peanuts. As an example, the aroma model lacking methional, 1-octen-3-one and dimethyl trisulfide was significantly different ($P<0.001$) from the complete model, with 18 out of 20 panelists detecting an odor difference. When these compounds were individually omitted from the model, panelists could detect a difference for methional ($P<0.03$) and 1-octen-3-one ($P< 0.05$), but not for dimethyl trisulfide ($P>0.05$). The absence of aldehydes in the model was also detectable. A total of 15, 14 and 13 panelists detected an odor

difference on incomplete models missing nonanal ($P<0.001$), hexanal ($P< 0.001$), and octanal ($P<0.005$), respectively. The panel was also able to discriminate the aroma of the model missing 2-acetyl-1-pyrroline ($P<0.03$). This compound has been recently reported to play a major role on the aroma of roasted peanuts (Chetschik et al. 2008). In addition, the lack of phenylacetaldehyde resulted in a significant change in the model, suggesting that this compound is an important component of the aroma of roasted peanuts. The panel also detected a change when carbon disulfide was missing from the model ($P< 0.03$). Many pyrazine compounds have been associated with the aroma of roasted peanuts (Neta et al. 2010). Although results from the GC-O and AEDA analysis indicated that five pyrazines were among the compounds contributing the most to roasted peanut aroma, omission experiments revealed that pyrazine compounds as a group could be removed from the model system without any noticeable change ($P>0.05$). Other compounds that did not impact the aroma of the model mixtures included 2-methylbutanal, 2,3-pentanedione, and maltol (Table 5).

Conclusions

This study characterized the roasted peanut flavor of five mesocarp-color maturity classes using sensory and instrumental analyses. Maturity had a marked effect on the flavor profile of peanuts, with more mature peanuts exhibiting higher intensities of positive attributes such as roasted peanutty, sweet aromatic and sweet taste. Over 200 aroma-active compounds were found to contribute to the overall flavor of roasted peanuts. A total of 19 compounds selected by GC-O and AEDA, were identified and

quantified in peanuts using GCxGC-TOFMS. While the use of instrumental analysis is an effective way to characterize the flavor of foods, sensory studies using reconstitution aroma models are critical to validate the importance of selected aroma-active compounds. Results from the model mixtures and omission experiments revealed the following odorants as being the major contributors to roasted peanut flavor: methional, 1-octen-3-one, nonanal, hexanal, octanal, 2-acetyl-1-pyrroline, carbon disulfide, and phenylacetaldehyde. Although five pyrazine compounds were identified among the potential major contributors to roasted peanut flavor by GC-O analysis, they were not found to have a major effect on the aroma of model mixtures. This study is the first to characterize the aroma of roasted peanuts using reconstituted model mixtures and contributes to a better understanding of the roasted, nutty flavor that is developed during peanut roasting.

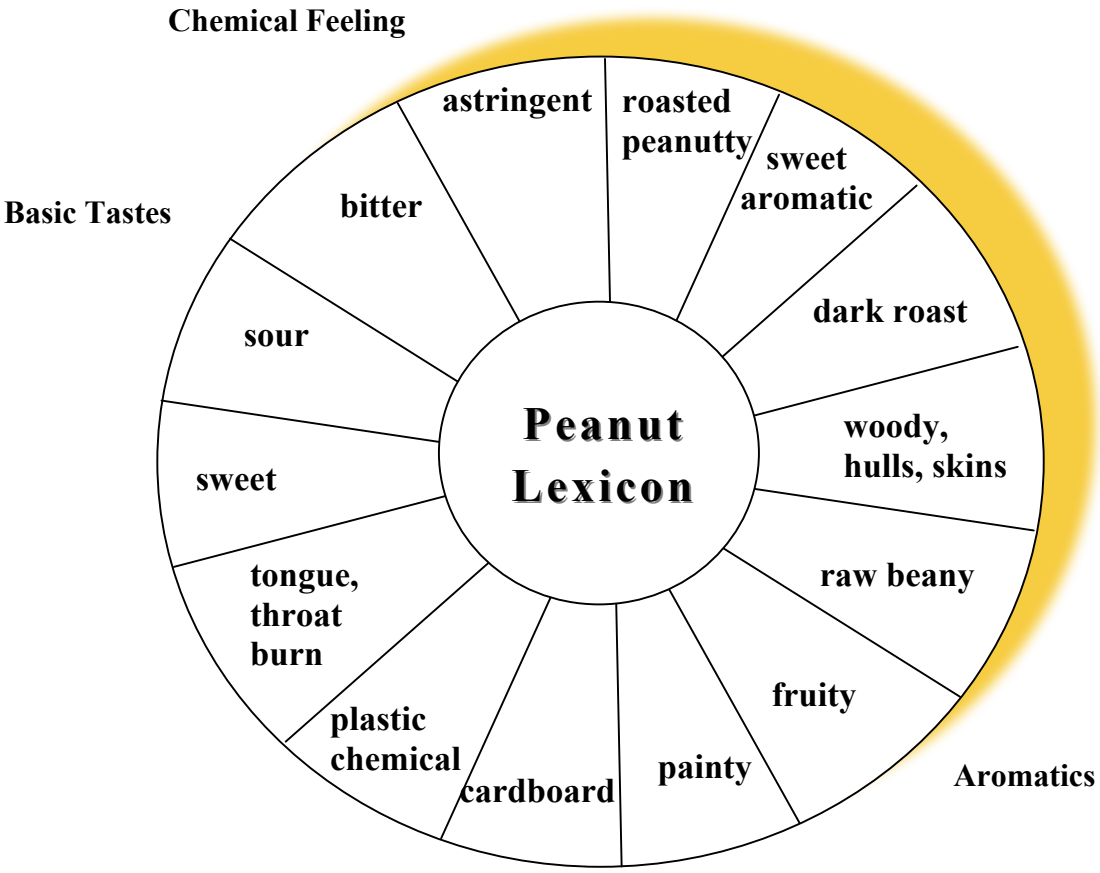

Figure 1: Peanut lexicon used for the flavor evaluation of roasted peanut pastes. Lexicon was previously developed by Johnsen et al. (1988) and Sanders et al. (1989).

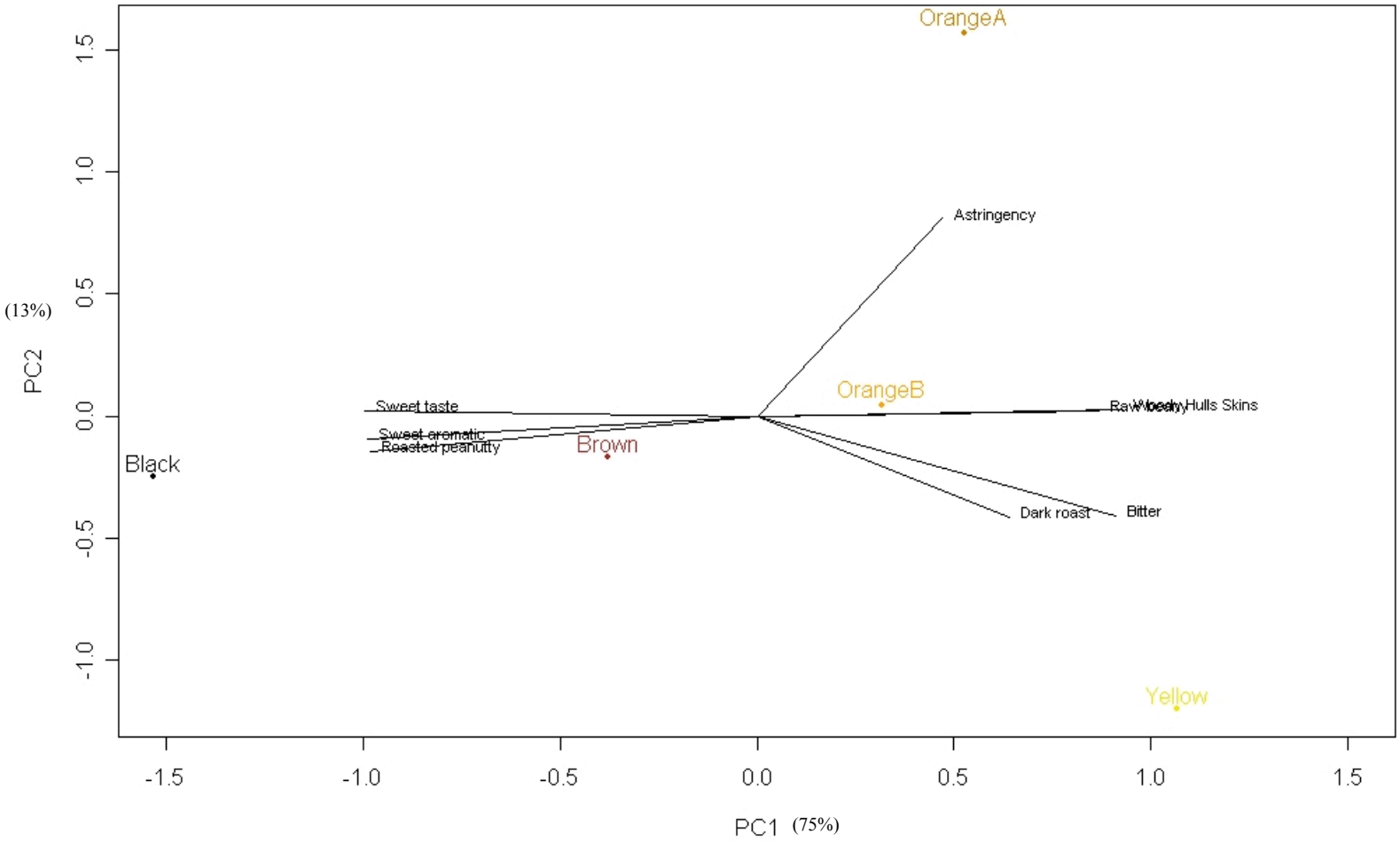

Figure 2: Principal component analysis (PC1 vs. PC2) of descriptive sensory attributes of peanuts from five mesocarp-color based maturity classes.

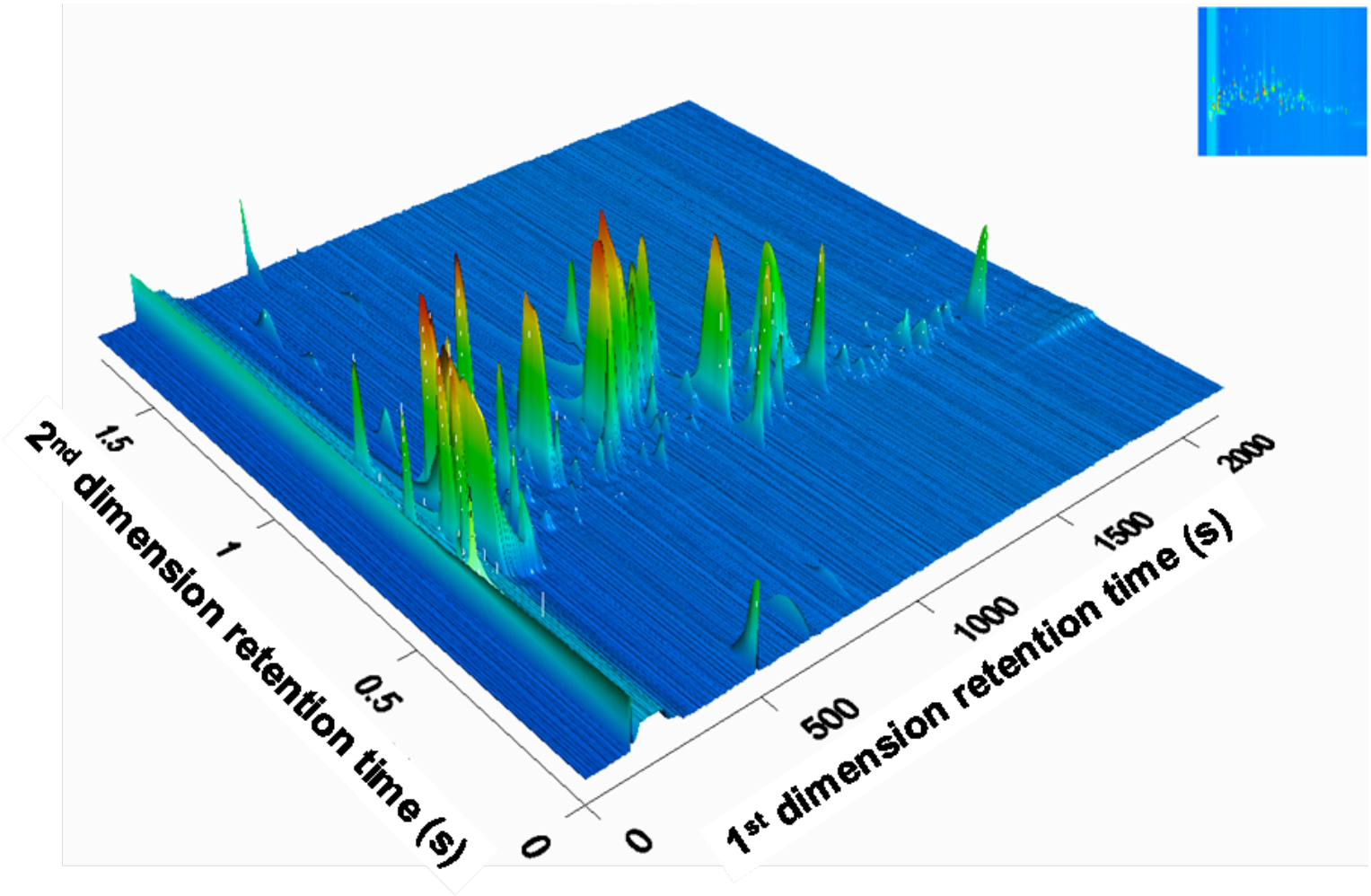

Figure 3: GCxGC-TOFMS total ion (TIC) chromatogram of peanut paste (black maturity level) extracted using HS-SPME.

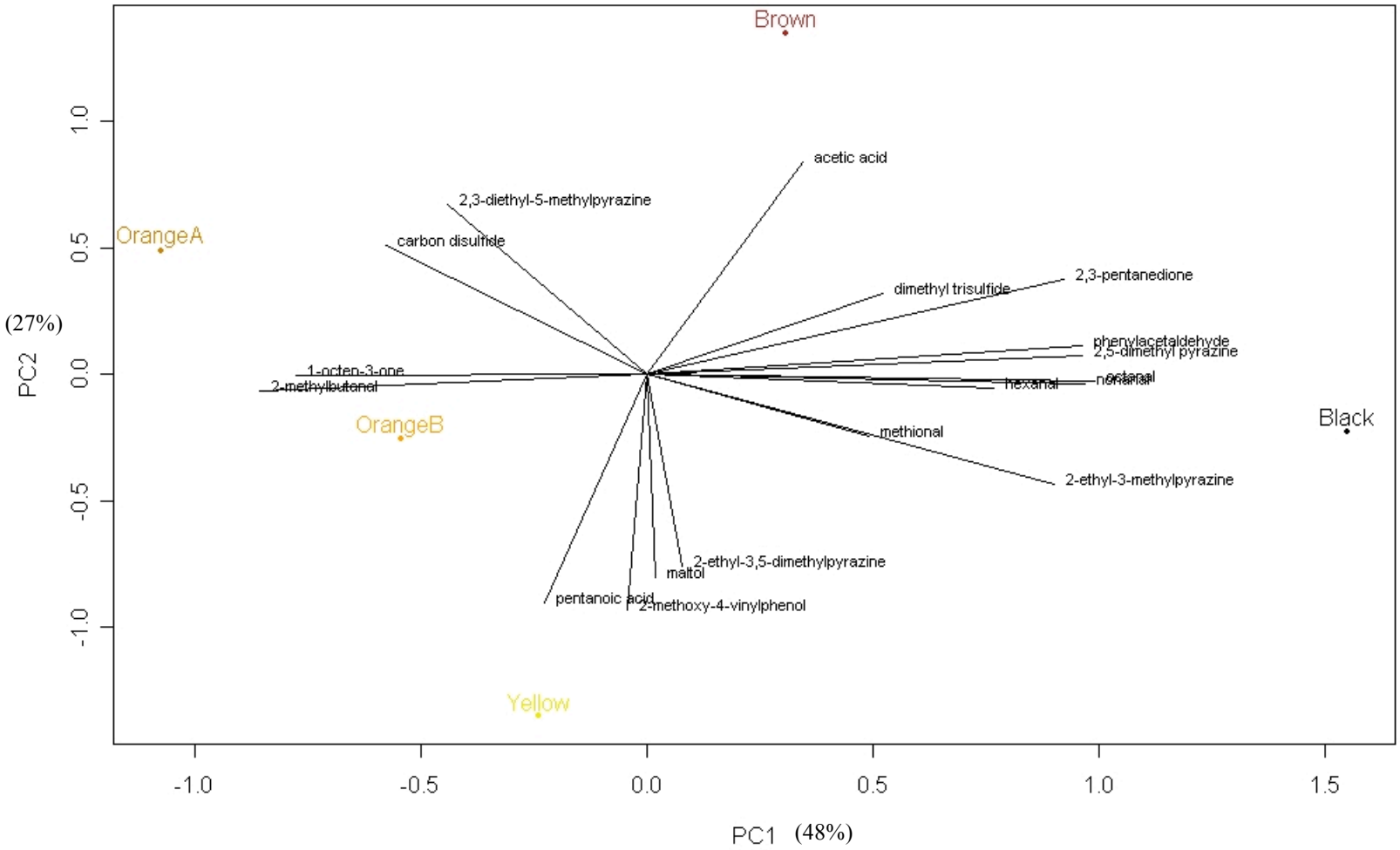

Figure 4: Principal component analysis (PC1 vs. PC2) of volatile profile of peanuts from five mesocarp-color based maturity classes.

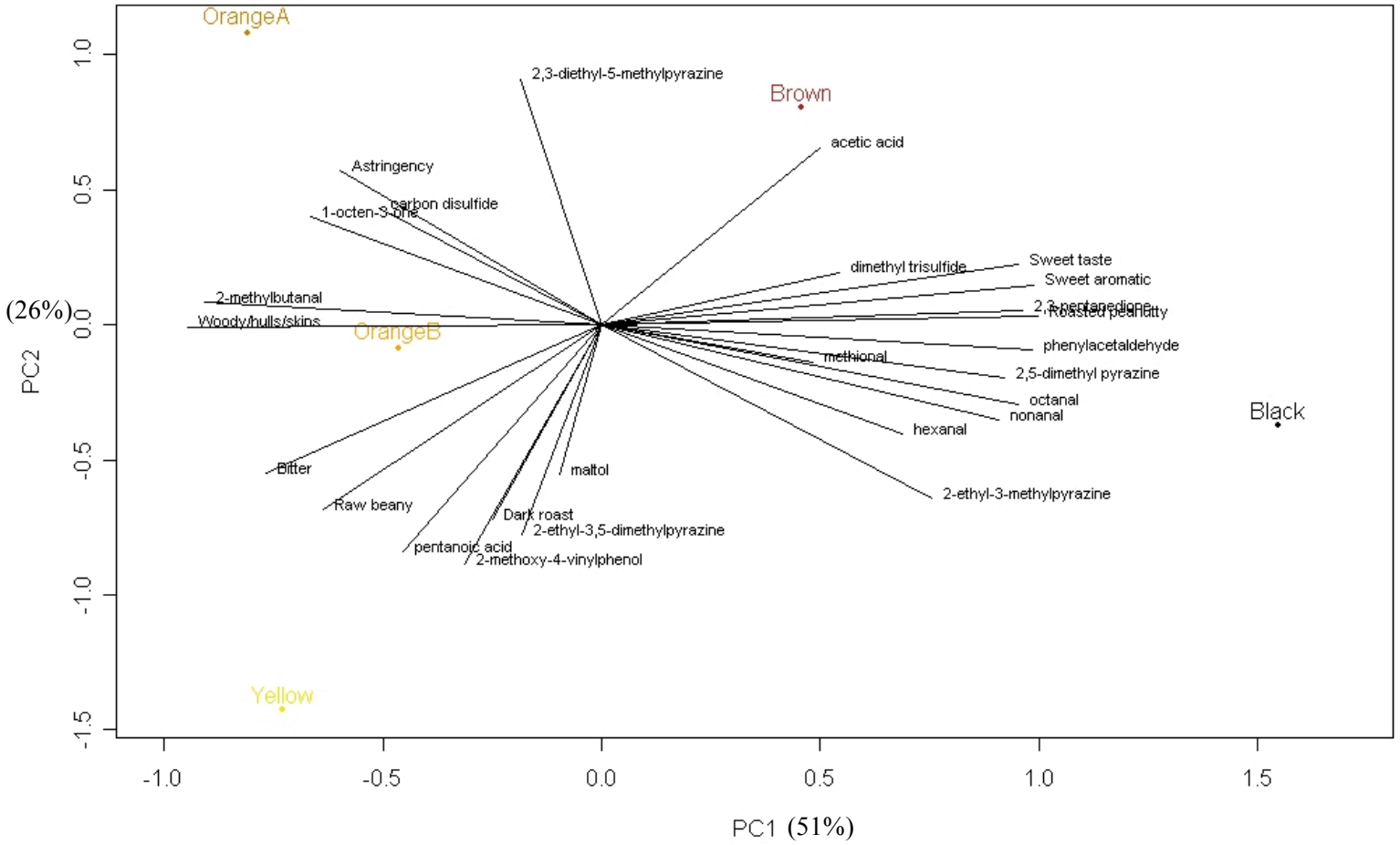

Figure 5: Principal component analysis (PC1 vs. PC2) of descriptive sensory analysis and volatile profile of peanuts from five mesocarp-color based maturity classes.

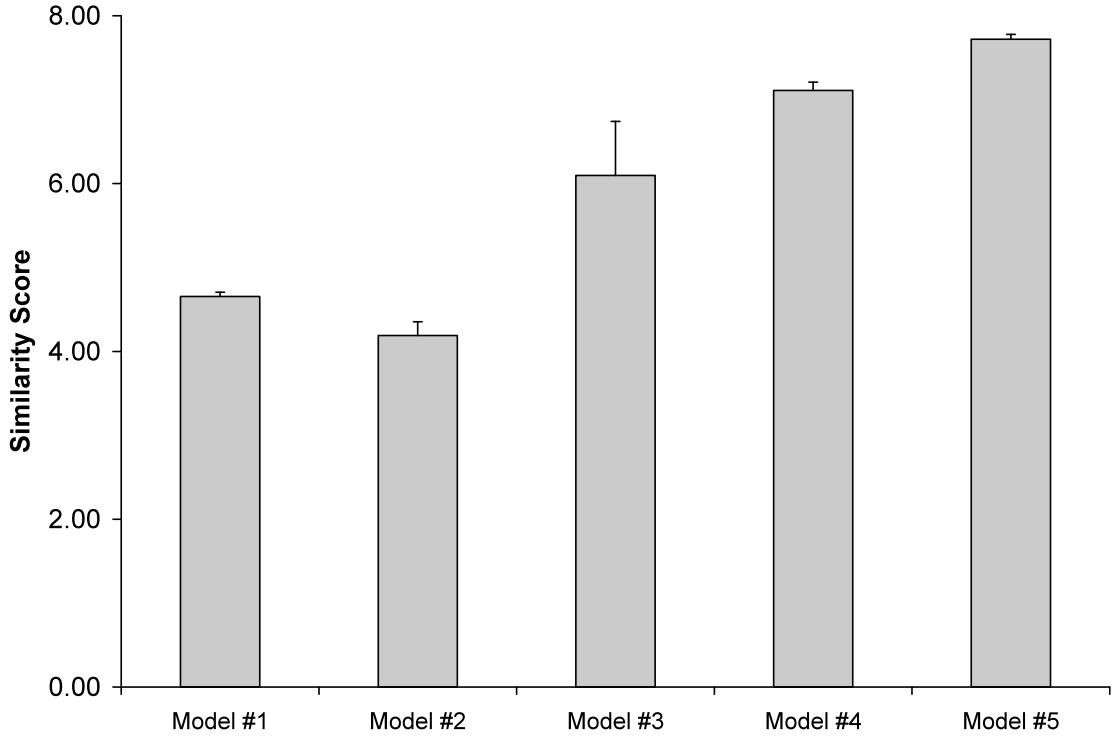

Figure 6: Similarity of the aroma of model systems with that of a reference roasted peanut paste using a 10-point scale.

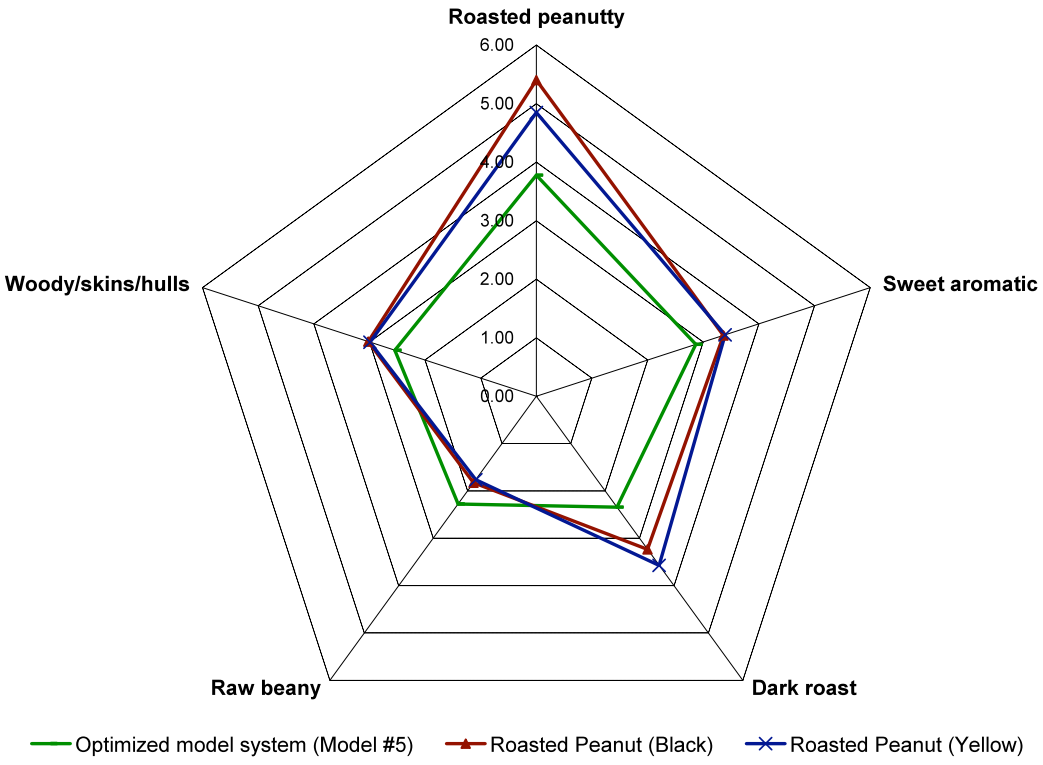

Figure 7: Comparative aroma profile of roasted peanut paste (black and yellow maturity classes) and optimized aroma model (Model #5)

Table 1: Most potent odorants identified in roasted peanuts.

Nr	Compound	Aroma description	Extract Source[a]	RI (SAFE)[b] DB-5MS	RI (SAFE)[b] *DB-WAX*	RI (HS-SPME)[c] DB-5MS	RI (HS-SPME)[c] *DB-WAX*	Log_3FD factors[d] BLA	BRO	ORB	ORA	YEL	Method of Identification
1	2-methylbutanal	malty/chocolate	NB	656	*910*	648	*906*	6	4	6	7	5	RI, odor, MS[e]
2	2,3-pentanedione	buttery	NB	705	*1054*	701	*1052*	4	5	4	7	5	RI, odor, MS
3	hexanal	green/grassy	NB	794	*1070*	803	*1071*	5	4	6	6	5	RI, odor, MS
4	2-methyl-3-furanthiol	vitamin/nutty	NB	867	*1290*	871	*1295*	10	6	5	5	2	RI, odor[f]
5	methional	potato	NB	913	*1434*	913	*1454*	9	9	8	9	9	RI, odor, MS
6	2-acetyl-1-pyrroline	popcorn	NB	925	*1327*	925	*1329*	8	9	10	9	9	RI, odor, MS
7	dimethyl trisulfide	garlic/onion	NB	976	*1364*	978	*1369*	7	6	6	8	6	RI, odor, MS
8	1-octen-3-one	mushroom	NB	983	*1288*	984	*ND*	7	9	7	9	7	RI, odor, MS
9	octanal	fruity	NB	1005	*1300*	1006	*ND*	8	5	1	0	3	RI, odor, MS
10	2-propionyl-1-pyrroline	popcorn/roast	NB	1034	*1436*	1033	*1432*	8	7	7	8	6	RI, odor
11	phenylacetaldehyde	rosy/floral	NB	1061	*ND*[g]	1059	*ND*	9	8	6	7	5	RI, odor, MS
12	2-ethyl-3,5-dimethylpyrazine	nutty/brothy	NB	1079	*1452*	1075	*ND*	6	5	0	8	2	RI, odor, MS
13	nonanal	fruity	NB	1091	*1395*	1098	*1380*	7	6	4	1	3	RI, odor, MS
14	unknown	mothball/hay	NB	1165	*ND*	ND	*ND*	5	6	6	7	3	RI, odor
15	2-methoxy-4-vinylphenol	medicinal	NB	1320	*ND*	ND	*ND*	7	4	3	2	4	RI, odor, MS
16	estragole	licorice	NB	1208	*ND*	ND	*ND*	6	3	5	7	2	RI, odor
17	trans-4,5-epoxy-(E)-2-decenal	sickening/metallic	NB	1378	*ND*	ND	*ND*	6	6	4	2	3	RI, odor
18	acetic acid	vinegar	AC	663	*1419*	619	*ND*	3	5	6	3	5	RI, odor, MS
19	2-ethyl-3-methylpyrazine	roasted/nutty	AC	1016	*1436*	1009	*1424*	5	4	5	7	7	RI, odor, MS
20	2-acetyl-3-methylpyrazine	roasted/nutty	AC	1105	*1630*	1118	*1600*	7	5	3	6	1	RI, odor
21	carbon disulfide	garbage/sulfur	HS-SPME	=	=	<600	*640*	=	=	=	=	=	RI, odor, MS
22	2,5-dimethyl pyrazine	earthy	HS-SPME	=	=	922	*1239*	=	=	=	=	=	RI, odor, MS

Table 1. Continued

23	Maltol	cotton candy	HS-SPME	=	=	1113	*ND*	=	=	=	=	=	RI, odor, MS
24	2,3-diethyl-5-methylpyrazine	earthy/soil	HS-SPME	=	=	1173	1483	=	=	=	=	=	RI, odor, MS
25	pentanoic acid	sweaty/fatty	HS-SPME	=	=	906	ND	=	=	=	=	=	RI, odor, MS

[a]NB: neutral/basic fraction; AC: acidic fraction; HS-SPME: compounds extracted using HS-SPME. [b]Retention indexes calculated from GC-O data using roasted peanut solvent extracts [c]Retention indexes calculated from GC-O data using HS-SPME. [d]Log_3FD factors: Flavor dilution factors (FD) were determined on a DB-5MS for the neutral/basic fraction and on a DB-WAX for the acidic fraction; BLA: black; BRO: brown; ORB: orange B; ORA: orange A; YEL: yellow. [e]Compounds positively identified based on the comparison of mass spectra, retention index, and aroma quality with those of standard compounds. [f] Compounds tentatively identified based on the comparison of retention index, and aroma quality with those of standard compounds. [g]ND: not detected

Table 2: Concentration of most potent odorants identified in roasted peanuts.

Compound	QuantMass[a]	Equation[b]	R^{2c}	Concentration (ppb)[d]				
				Black	Brown	Orange A	Orange B	Yellow
2,3-pentanedione	43	y = 0.0037x + 0.1106	0.94	465 ± 55a	442 ± 115a	324 ± 130a	353 ± 140a	342 ± 126a
2-acetyl-1-pyrroline	43	y = 0.0005x + 0.1706	0.91	4 ± 0	54 ± 0	14 ± 0	ND	ND
2-methoxy-4-vinylphenol	135	y = 0.0017x - 0.7673	0.67	797 ± 48a	749 ± 48a	781 ± 47a	784 ± 81a	873 ± 122a
phenylacetaldehyde	91	y = 0.0028x - 6.8241	0.95	25719 ± 3941a	14195 ± 2105b	7194 ± 893bc	9107 ± 863c	8163 ± 1550c
2-methylbutanal	41	y = 0.0029x + 0.2666	0.96	6436 ± 2278a	8596 ± 749a	9591 ± 2000a	8240 ± 995a	9630 ± 4499a
carbon disulfide	76	y = 0.0059x - 0.4273	0.91	124 ± 8b	229 ± 62a	186 ± 14ab	196 ± 23ab	180 ± 22ab
dimethyl trisulfide	126	y = 0.0039x - 0.2541	0.97	162 ± 18a	160 ± 19a	159 ± 24a	147 ± 23a	156 ± 33a
hexanal	44	y = 0.0059x + 1.6891	0.98	773 ± 55a	757 ± 196a	541 ± 226a	730 ± 184a	697 ± 264a
maltol	126	y = 0.0001x - 0.963	0.84	13199 ± 244a	10507 ± 1519a	12674 ± 913a	12654 ± 1103a	13014 ± 1168a
nonanal	57	y = 0.0014x + 0.0256	0.95	894 ± 165a	724 ± 130ab	416 ± 65b	621 ± 149ab	625 ± 207ab
octanal	43	y = 0.0079x - 3.0938	0.97	518 ± 8a	492 ± 13ab	463 ± 15b	481 ± 9b	481 ± 17b
2,5-dimethylpyrazine	42	y = 0.0144x - 12.554	0.97	6242 ± 1429a	5376 ± 531a	4330 ± 140a	4182 ± 264a	4896 ± 1242a
2-ethyl-3-methylpyrazine	121	y = 0.0091x - 0.164	0.94	220 ± 91a	152 ± 75a	121 ± 22a	149 ± 42a	178 ± 99a
2-ethyl-3,5-dimethylpyrazine	135	y = 0.0016x + 0.166	0.97	2868 ± 724a	2447 ± 379a	2677 ± 472a	2330 ± 308a	3703 ± 922a
acetic acid	45	y = 6E-05x - 6.3932	0.91	250204 ± 27733a	273471 ± 59886a	250122 ± 14125a	218743 ± 25494a	224527 ± 17001a
1-octen-3-one	55	y = 0.0058x - 0.125	0.97	103 ± 5	97 ± 8	150 ± 4	124 ± 8	111 ± 7
pentanoic acid	60	y = 0.0015x - 0.0577	0.87	60 ± 19a	52 ± 23a	56 ± 31a	76 ± 4a	83 ± 14a
methional	48	y = 0.002x - 0.0297	0.98	92 ± 5a	54 ± 4c	76 ± 9ab	55 ± 6c	67 ± 7bc
2,3-diethyl-5-methylpyrazine	135	y = 0.0015x - 0.0242	0.98	58 ± 21b	70 ± 21ab	109 ± 21a	64 ± 11ab	34 ± 16b

[a]Selected ion (m/z) used for compound quantification. [b]Linear regression equation obtained from a five-point internal standard curve. [c]Coefficient of determination from the linear regression model. [d]Means followed by different letters are significantly different ($P<0.05$) among maturity classes.

Table 3: Flavor profile of peanuts from five mesocarp-color based maturity classes.

	Sensory intensity[a]				
	Black	Brown	Orange B	Orange A	Yellow
roasted peanutty	5.1 ± 0.1a	4.7 ± 0.1ab	4.5 ± 0.2b	4.5 ± 0.2b	4.4 ± 0.3b
sweet aromatic	3.1 ± 0.1a	2.9 ± 0.2ab	2.7 ± 0.1b	2.7 ± 0.2b	2.6 ± 0.2b
dark roast	3.1 ± 0.1a	3.2 ± 0.2a	3.1 ± 0.1a	3.2 ± 0.1a	3.4 ± 0.1a
raw beany	2.1 ± 0.1a	2.1 ± 0.1a	2.1 ± 0.1a	2.1 ± 0.2a	2.1 ± 0.2a
woody/hulls/skins	3.1 ± 0.1a	3.2 ± 0.0a	3.3 ± 0.0a	3.3 ± 0.1a	3.3 ± 0.1a
sweet taste	2.6 ± 0.0a	2.4 ± 0.0ab	2.3 ± 0.1bc	2.2 ± 0.1bc	2.1 ± 0.2c
bitter	2.3 ± 0.1b	2.6 ± 0.2ab	2.7 ± 0.2ab	2.6 ± 0.0ab	3.0 ± 0.3a
astringency	1.0 ± 0.0b	1.0 ± 0.0ab	1.0 ± 0.0ab	1.1 ± 0.1a	1.0 ± 0.0ab

[a]Means followed by different letters are significantly different ($P<0.05$) among maturity classes.

Table 4: Model systems prepared for sensory evaluation.

	Concentration (ppb)[a]				
	Model #1[a]	Model #2	Model #3	Model #4	Model #5
2,3-pentanedione	460	120	200	200	200
2-acetyl-1-pyrroline	10	20,000	4,000	3,000	3,000
2-methoxy-4-vinylphenol	800	0	0	0	0
phenylacetaldehyde	25,700	200,000	25,000	25,000	25,000
2-methylbutanal	6,500	100	800	800	800
carbon disulfide	125	1,500	100	100	100
dimethyl trisulfide	160	100	100	100	100
hexanal	780	890	1,000	1,200	1200
maltol	13,320	80,000	24,500	24,500	24,500
nonanal	915	20,000	1,000	1,000	1,000
octanal	520	15,000	1,000	1,000	1,000
2,5-dimethylpyrazine	6,250	2,000	6,000	6,000	6,000
2-ethyl-3-methylpyrazine,	222	100	100	100	100
2-ethyl-3,5-dimethylpyrazine	2,900	120	1,000	1,000	1,000
acetic acid	251,000	251,000	0	0	0
1-octen-3-one	100	100	100	80	100
pentanoic acid	60	70	70	0	0
methional	90	100	100	80	100
2,3-diethyl-5-methylpyrazine	60	100	100	100	100

[a]Amount of compound spiked in a model system consisting of 25% egg white powder, 51% hydrogenated oil, 4% sugar, 18% starch, and 2% water. [b]Initial model system was in concentrations equal to those determined in roasted peanuts of the black maturity level

Table 5: Aroma of optimized model mixture affected by the omission of one or more odorants.

Odorant (s) omitted in the model	# of correct responses (out of 20 panelists)
methional, 1-octen-3-one, dimethyl trisulfide	18[a]
nonanal	15[a]
hexanal	14[a]
octanal	13[b]
2-acetyl pyrroline	12[c]
methional	12[c]
carbon disulfide	12[c]
1-octen-3-one	11[d]
phenylacetaldehyde	11[d]
2-methylbutanal, 2,3-pentanedione	10[e]
maltol	9[e]
dimethyl trisulfide	9[e]
pyrazine compounds[f]	8[e]

[a]$p < 0.001$; [b]$p < 0.005$; [c]$p < 0.03$; [d]$p < 0.05$; [e]Not significantly different at 95% probability level. [f]Pyrazine compounds missing from the model included 2-ethyl-3,5-dimethylpyrazine, 2-ethyl-3-methylpyrazine, 2,5-dimethylpyrazine, and 2,3-diethyl-5-methylpyrazine.

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CHAPTER 4:

CHEMICAL COMPOSITION AND SENSORY CHARACTERISTICS OF RUNNER-TYPE PEANUTS FROM FIVE MATURITY CLASSES

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Abstract

The compositional variations in runner-type peanuts of five maturity classes were determined and correlated with peanut chemical and sensory characteristics. Peanut pods were water blasted to remove the exocarp and visually sorted into increasing maturity classes based on mesocarp color (yellow, orange A, orange B, brown and black). Pods were dried and shelled to obtain the medium grade size seeds from each maturity class which were roasted in a convection oven. Sucrose was the primary sugar in the raw and roasted peanuts, and was significantly higher for the black maturity class. Stachyose, raffinose and inositol concentrations decreased with increasing seed maturity. Sucrose content was positively associated with roasted peanutty, sweet aromatic and sweet taste, and negatively associated with woody/hull/skins. Roasted peanutty was positively correlated with sweet aromatic and sweet taste, and negatively correlated with bitter taste. Taste. Free amino acids decreased with increasing maturity ($P<0.05$) and due to roasting in all maturity classes. Oleic/linoleic acid ratio ranged from 1.27 to 1.55 for the yellow and black classes, respectively. The sum of saturated fatty acids gradually decreased with seed maturity, ranging from 19.4 to 18.8% from yellow to black, respectively. Oleic acid content was inversely related to linoleic, arachidic, eicosenoic, behenic, lignoceric, et al. fatty acids. This study provides information on compositional differences in peanuts of five maturity classes and may allow for a better understanding of flavor development during roasting.

KEYWORDS: peanuts, chemical composition, maturity, roasting

Introduction

Peanuts are a valuable commodity in the United States and are typically consumed as a snack or used as an ingredient in the production of numerous foods such as peanut butter and confectionary products (American Peanut Council 2010). Four market-type peanuts are commercially grown in the U.S. including runner, virginia, spanish, and valencia. The runner market-type accounts for about 80% of the total crop in the U.S. and is mostly used in the manufacture of peanut butter (American Peanut Council 2010).

The flavor quality of peanuts is influenced by a number of factors including genetic, environmental, physiological, and biochemical processes occurring in the seed, as well as handling, processing, and storage conditions (Sanders et al. 1995; Young et al. 1974b). Sugars and free amino acids are considered to be the main precursors of roasted peanut flavor (Koehler et al. 1969; Mason et al. 1969). Previous studies have reported that sucrose is the major soluble sugar in peanuts (Basha 1992; Oupadissakoon et al. 1980a,b). During peanut roasting, sucrose is inverted into fructose and glucose, which are involved in Maillard reactions (Mason et al. 1969). Newell et al. (1967) reported that aspartic acid, glutamic acid, glutamine, asparagine, histidine and phenylalanine were the main precursors of the typical peanut flavors, while threonine, tyrosine and lysine were the major precursors of off-flavors.

The peanut plant has an indeterminate flowering pattern, which means that peanuts of different maturity classes exist in all seed-sized commercial grades (Sanders et al. 1982; Sanders 1989). Several methods have been developed to determine optimum

time of harvest including arginine maturity index (AMI), oil color, internal hull color (Shellout Method), kernel density, seed/hull ratio (SHMI) (Pattee et al. 1980) and the hull-scrape method (Drexler and Williams 1979). Harvest timing based on the hull-scrape method (Drexler and Williams 1979) is the most accepted and widely used method in the peanut industry. In this method, the exocarp is removed from the pods, which are then visually classified into different categories according to mesocarp color. The hull-scrape method has been used in numerous investigations to examine the effects of seed maturity on chemical composition and flavor quality of peanuts (Sanders 1989; Sanders et al. 1990; Sanders et al. 1989; McNeill and Sanders 1998; Rodriguez et al. 1989; Sanders and Bett 1995).

Seed maturity has been shown to influence the fatty acid composition in peanuts (Sanders et al. 1982; Sanders 1982). Oleic acid and linoleic acids are the most abundant fatty acids in the peanut seed, accounting for about 50% and 30% of the total fatty acid composition, respectively (Jonnala et al. 2005; Cobb and Johnson 1973; Mercer et al. 1990). Oxidation of linoleic acid occurs about ten times faster than oleic acid (Nawar 1985); therefore, peanuts with a higher oleic/linoleic (O/L) acid ratio tend to have better stability against lipid oxidation and thereby longer shelf life than those with lower O/L rations (Braddock et al. 1995; Mugendi et al. 1998; Nepote et al. 2006; O'Keefe et al. 1993; Reed et al. 2002). In general, previous studies on the effects of maturity on fatty acid profile of peanuts have reported that O/L ratio increases with seed maturity (Sanders et al. 1982; Worthington et al. 1972; Kim and Hung 1991). However, Hashim et al.

(1993) reported a decrease in oleic acid and increase in linoleic acid with maturity, and Knauf et al. (1986) observed no change in fatty acid composition due to maturity.

The effect of seed maturity has also been investigated in relation to amino acid and sugar composition. Oupadissakoon et al. (1980b) reported a decreasing pattern for free amino acid and sugar content during the early harvest period, followed a plateau near optimum harvest date, and increase during the late harvests. Basha et al. (1980) found that alanine, phenylalanine, isoleucine and threonine decreased as the seeds matured, and suggested that synthesis of proteins from free amino acids in peanuts occurred during the early stages of maturation. Arginine has been used as an indication of peanut immaturity, and it has also been correlated with off-flavors developed during roasting (Young and Mason 1972; Cobb and Johnson 1973; Woodroof 1983; Young et al. 1974a; Young et al. 1974b).

Seed maturity has been show to affect flavor development during roasting, with more mature peanuts having greater potential for full flavor than immature peanuts (McNeill and Sanders 1998). Sanders (1989) reported that 'on' flavors such as roasted peanutty and sweet aromatic were higher in more mature peanuts, while 'off' flavors such as painty and fruity-fermented were higher in immature peanuts. McNeill and Sanders (1998) also found that the intensity of roasted peanutty flavor was significantly higher for the more mature classes. Although previous studies have shown that seed maturity affects peanut quality, further research is needed to understand the interrelationship between sensory attributes and chemical composition of peanuts from different maturity classes. The objectives of this study were to determine the chemical composition of peanuts from

five mesocarp-color based maturity classes and to establish correlations between chemical parameters and descriptive sensory analysis.

Materials and methods

Peanuts

Runner-type peanut pods (var. Georgia Green, 5,000 kg; crop 2007) obtained from a single field at the USDA. ARS, National Peanut Research Laboratory, Dawson, GA were water blasted to remove the exocarp and visually sorted into increasing maturity classes based on mesocarp color (yellow, orange A, orange B, brown and black), according to the hull-scrape method (Drexler and Williams 1979). Peanut pods were dried using ambient air, shelled, and sized by diameter over slotted-hole screens (Sanders 1989). A portion of the medium grade size (<8.3 and >6.3 mm) peanut seeds from each maturity class was roasted in the center of a convection oven at 169ºC for 25 min and another portion was used as raw seed. Roast time was optimized on seeds from brown pods, and Hunter L-value ranged from 48 to 52 among maturity classes. Peanut pastes were prepared using a Beixer 3 food processor (Robot Coupe Inc.; Ridgeland, MS, USA), packaged in 8 oz glass jars, and stored at -20ºC until analysis. Data reported are the mean of three replications.

Sample handling

Raw peanuts. Peanut oil was extracted from whole raw seeds using a hydraulic press (Carver, model 2822-1; Wabash, IN USA). The oil was use for fatty acid analysis.

The residual peanut oil from the cake was extracted using a Soxhlet apparatus. The samples were extracted for 6 h with hexane. Defatted peanut meal was used for carbohydrate and free amino acid analyses. Oil and moisture were determined using the Minispec Mq-one Seed Analyzer (Bruker Optics; Billerica, MA, USA), and were utilized in conversion of the concentrations of free amino acid and carbohydrate on the defatted meal samples back to a fresh seed weight basis.

Roasted peanut paste. Peanut oil was extracted from roasted peanut paste using solvent extraction. Samples of roasted peanut paste (2g) and 30 mL of hexane were placed in 50 mL plastic culture tubes with a screw cap (Sarstedt Inc., Newton, NC, USA). The tube contents were mixed using a vortex for 10 sec and shaken on an orbital shaker (Reliable Scientific Inc., Nesbit, MS, USA) for 30 min at a speed setting of 80. The tube was then centrifuged for 10 min at a speed of 1075 rpm (Damon/IEC Division, model K; Needham Heights, MA, USA), and the supernatant was transferred to a test tube. This procedure was repeated two times, each with addition of 30 mL of hexane. Following extraction, the collected hexane was evaporated under a gentle stream of nitrogen gas at ambient temperature and the residual oil was used for fatty acid analysis. Hexane left on the pellet was evaporated under a gentle stream of nitrogen gas at ambient temperature and the defatted peanut meal was submitted to carbohydrate and free amino acid analyses.

Carbohydrate analysis

Triplicate 100 mg meal samples from each maturity class were extracted

according to the method described by Oupadissakoon et al. (1980b). After evaporation of the solvent, 800 ppm lactose and 400 ppm cellobiose were added to the residue as internal standards. Samples (50 μL) were diluted with 2 mL distilled water then filtered through Dionex OnGuard-H cartridges into autosampler vials (Supelco; Bellefonte, PA, USA). Carbohydrates were analyzed using high-performance liquid chromatography (HPLC) (Dionex BioLC; Dionex Corporation, Sunnyvale, CA, USA) with a Dionex PA-1 column (250 mm length and 4 mm i.d.) at a controlled temperature of 30 °C. A 10 μL sample was injected. The eluent was 200 mM NaOH at an isocratic flow rate of 1.0 mL/min (column pressure of 1645 psi). A Pulsed Amperometric Detector (PAD) was used with a change in sensitivity from 100 to 500 nC between 4 and 7 min to allow sucrose to be quantified with the other sugars. With the exception of sucrose, each carbohydrate was quantified by calculating the ratio of the unknown's peak height to the cellobiose peak height. The lactose internal standard was used for quantifying sucrose. All the reference standards were purchased from Sigma Chemical Corp. (St. Louis, MO, USA) with the exception of cellobiose (Fluka Chemie; Steinheim, Germany).

Fatty acid analysis

Fatty acids were methylated with 14% boron trifluoride in methanol, according to the method described by Bannon et al (1982). Fatty acid methyl esters were extracted into hexane and analysed using a Perkin Elmer Autosampler XL gas chromatograph (Norwalk, CN) with a flame ionization detector (FID) fitted with a capillary column of 70% cyanopropyl polysilphenylene-siloxane as the stationary phase (30 m length 0.25

mm i.d., 0.25 μm film thickness) (BPX-70, SGE Analytical Science, Austin, TX, USA). The oven temperature was programmed to increase from 60ºC to 180ºC at a rate of 10ºC/min, then 4ºC/min to 235 ºC, with an initial hold time of 2 min. Helium was used as the carrier gas at 1.85 mL/min. Fatty acids were identified by comparison of retention times with those of authentic standards (Matreya, Inc., Pleasant Gap, PA, USA).

Free amino acid analysis

Free amino acid analysis was performed following a procedure adapted from Grimm et al. (1996). A solution (30 mL) containing 60% methanol, 25% chloroform and 15% H_2O was added to defatted peanut meal (2 g), then mixed by a vortex for 30 s and centrifuged for 10 min at a speed of 1075 rpm (Damon/IEC Division, model K; Needham Heights, MA, USA). The supernatant was transferred to a small beaker and dried for 12 hours. The dried samples were resuspended in 5mL of extraction buffer, which contained 0.71 g Na_2HPO_4 in 1 L of water and 5% acetonitrile (ACN) with pH adjusted to 7.4. Samples were derivatizated using the Waters AccQ•Tag system (Waters Corp., Milford, MA, USA). Borate buffer (60 μL) was added to the sample (20 μL), and mixed for 30 s using a vortex. Freshly prepared derivatization reagent (20 μL) was added to 2 mL sample tubes, which were mixed by a vortex and left at room temperature for 1 min. Contents of the tubes were then transferred to 2 mL screw cap vials with a glass insert (Supelco, Bellefonte, PA, USA), immediately capped and heated for 10 min at 55°C. Aliquots of external standards of amino acids were derivatized as described for samples to construct standard curves.

Derivatives (20 μL) were analyzed using a Summit Model HPLC (Dionex Corp., Sunnyvale, CA) with a Waters AccQ•Tag column (C18, 4μ, 150 mm x 3.9 mm) (Waters Corp., Milford, MA, USA) and a fluorescence detector with excitation/emission wavelengths of 250/395 nm. The mobile phase consisted of two eluents: (A) AccQ·Tag$_{ultra}$ eluent A concentrate diluted with water (10%, v/v) (Waters Corp., Milford, MA, USA), and (B) 60% acetonitrile in water. The following gradient elution was used: eluent B increased from 0% to 2.5% in 0.5 min, to 7% in 15 min, to 10% in 19 min, to 33% in 32 min and held for 1 min. This was followed by a column wash with 100% eluent B for 3 min before equilibration with eluent A. Total run time was 50 min at 37 ºC with a flow rate of 1.0 mL per min.

Descriptive sensory analysis

Descriptive sensory data from roasted peanut pastes of the same five mesocarp-color based maturity class samples were obtained from Neta et al. (2010).

Statistical analysis

Statistical analysis was performed using the SAS® System for Windows™ version 8.2 (SAS Institute Inc.; Cary, NC). The maturity and processing (raw vs. roasted) effects and their interactions were evaluated by two-way analysis of variance (ANOVA). Data from carbohydrates, free amino acids and fatty acids were also analyzed using one-way ANOVA. Means separation was carried out using the Tukey test and significance of results was established at the $P<0.05$ level. The relationship between carbohydrates and

descriptive sensory analysis was carried out using principal component analysis (PCA), partial least squares regression (PLSR), and Pearson correlations. In addition, the relationships between Hunter L-values of roasted peanuts and carbohydrates in the raw seeds as well as relationships among fatty acids in the raw seeds were established using Pearson correlations.

Results and discussion

Carbohydrates

Carbohydrate composition was determined in raw and roasted peanuts from five mesocarp-color based maturity classes (Table 1). Free carbohydrates in raw and roasted peanuts consisted mainly of sucrose, stachyose, raffinose, inositol, glucose and fructose, which is consistent with previous studies (Oupadissakoon et al. 1980b; Pattee et al. 2000).

Glucose, fructose and sucrose have been consistently reported in peanuts, with the latter being the major sugar (Holey and Hammons 1968; Cobb and Swaisgood 1971; Mason et al. 1969; Newell et al. 1967; Basha 1992). Sucrose was the most abundant sugar in both raw and roasted peanuts, and was significantly higher in the black maturity class ($P<0.01$) (Table 1). Contents of stachyose, raffinose and inositol in both raw and roasted peanuts decreased with seed maturity. Two-way ANOVA showed significant interactions between maturity and processing (raw vs. roasted) effects for glucose ($P<0.0001$) and fructose ($P=0.0002$). In the raw seeds, glucose and fructose contents were higher in more immature seeds such as orange A and yellow. However, in roasted

peanuts these sugars tended to be higher for the more mature seeds. This may be explained by the different involvement of these sugars in Maillard reactions during the roasting process. Fructose and glucose are known as the key reducing sugars involved in the non-enzymatic browning in peanuts (Mason et al. 1969; Newell et al. 1967). Besides being naturally found in the raw seed, fructose and glucose are formed during peanut roasting from the hydrolysis of sucrose (Mason et al. 1969). Hunter L-value of roasted peanut pastes was negatively correlated with fructose (r=-0.68; P=0.005), glucose (r=-0.62; P=0.013), and sucrose (r-0.56; P=0.03) in the raw seeds, which indicates that high contents of these sugars in the raw seeds resulted in darker roasted peanut pastes.

Different statistical techniques were applied to examine the relationships between carbohydrate composition in the raw seeds and sensory attributes of roasted peanuts. Application of principal component analysis (PCA) allowed the reduction of 13 variables into two principle components (PC1 and PC2) explaining 67% and 16% of the total variance, respectively (Figure 1). PC1 was highly loaded with bitter, woody/hulls/skins, raffinose and stachyose in the positive direction, and with sweet taste, sweet aromatic, roasted peanutty and sucrose in the negative direction. Peanut maturity classes were well separated into the two dimensional space and were highly differentiated on PC1, with the more mature classes such as black and brown being loaded on the negative direction of PC1, and the least mature classes such as orange A and yellow being loaded on the positive PC1 dimension. In summary, woody/hull/skins and bitter attributes in roasted peanut paste were mostly associated with raffinose and stachyose contents in the raw

seed. Sweet taste, sweet aromatic and roasted peanutty were associated with sucrose content in the raw seeds.

The sensory-carbohydrate relationships were also examined using partial least square regression (PLSR), which is a multivariate statistic technique that allows correlations of a block of dependent variables with many collinear independent variables (Tobias 1996). The two first latent factors extracted from the PLSR of carbohydrate content in raw seeds on sensory attributes of roasted peanuts explained 91% of total variance of the sensory data. A visual examination of the biplot from the first two latent factors extracted by the PLSR model reveals that roasted peanutty, sweet aromatic, sweet taste and sucrose contents clustered together, which indicates they are related to each other (Figure 2). Similarly, bitter and woody/hulls/skins in roasted peanuts were associated with the contents of raffinose and inositol in the raw seeds. Dark roast was mostly associated with glucose, fructose and stachyose (Figure 2).

The sensory-carbohydrate and sensory-sensory relationships were also examined using Pearson correlation coefficients, which is a measure of the strength of linear dependence between any two variables. Generally speaking, correlation coefficients between the sensory variables concurred with the results from multivariate statistical analyses (Table 2). Roasted peanutty was positively correlated with sweet aromatic ($r=0.859$) and sweet taste ($r=0.803$), and negatively correlated with bitter taste ($r=-0.725$). In addition, sweet aromatic and sweet taste were positively correlated with each other ($r=0.774$). Previous studies have also reported significant positive and negative correlations of roasted peanutty attribute to sweet and bitter tastes, respectively (Pattee et

al. 1998; Pattee et al. 2000). Woody/hull/skins was positively correlated with dark roast (r=0.553) and bitter taste (r=0.741), and negatively correlated with roast peanutty (r=-0.604) and sweet taste (r=-0.727). Bitter was negatively associated with sweet taste (r=-0.799) and sweet aromatic (r=-0.725). In addition, bitter was positively associated with dark roast (r=0.588).

Correlation coefficients between carbohydrates and sensory variables were also consistent with the results from multivariate statistics (Table 3). Sucrose content in raw seeds was positively associated with roasted peanutty (r=0.834), sweet aromatic (r=0.728) and sweet taste (r=0.810), and negatively associated with woody/hull/skins (r=-0.678). These results are in agreement with Pattee et al. (2000), but contrast the findings of Oupadissakoon and Young (1984), who reported a negative correlation between sucrose and roasted peanutty flavor. Glucose was positively associated with dark roast (r=0.508) and astringency (r=0.546). No significant correlations were found between fructose and sensory attributes, which is in agreement with Pattee et al. (2000). Stachyose content was inversely related with sweet taste (r=-0.530). Raffinose and inositol were negatively correlated to roasted peanutty (r=-0.728; r=-0.692), sweet aromatic (r=-0.730; r=-0.691), and sweet taste (r=-0.861; r-0.850), respectively. In addition, inositol was positively associated with bitter taste (r=0.741). Pattee et al. (2000) examined the carbohydrate-sensory relationships in three market-type peanuts, and found no significant correlations between inositol and sensory characteristics of roasted peanuts. However, in their study a positive correlation between raffinose and roasted peanutty was observed for one market-type, which is in agreement with our results.

Free amino acids

The concentration of the following free amino acids in the raw seed significantly decreased with increasing maturity: aspartic acid, serine, glutamic acid, glycine, histidine, $(NH_4)_2SO_4$, arginine, alanine, proline, tyrosine, valine, isoleucine and leucine (Figure 3). This is likely due to utilization of free amino acids for synthesis of proteins during seed maturation (Rodriguez et al. 1989; Basha et al. 1980). Results from this study are in agreement with the findings of Rodriguez et al. (1989), who reported that immature classes had considerably lower amounts of α-amino nitrogen content, which is a measure of total amino-acids. In addition, the fact that arginine was higher for the most immature classes (orange A and yellow) is consistent with results from Young and Mason (1972), who found a substantial decrease in arginine content as the peanut fruit matured. Oupadissakoon et al. (1980b) observed that contents of free amino acids reached a minimum near the optimum harvest date. Similarly, Basha et al. (1980) reported that the rate of free amino acid decrease in the peanut seed was more pronounced during the early stages of maturation. In their study, the amount of free amino acid in the raw seed was higher for the more immature classes than for the intermediate and more mature classes. Methionine, lysine and threonine contents were not significantly different across maturity classes (Figures 3-4). Seed from black pods had the highest content of phenylalanine among all maturity classes. Phenylalanine is involved in the synthesis of phenylacetaldehyde by the reaction with α-dicarbonyl compounds via Strecker degradation (Hofmann and Schieberle 2000), and has been previously shown to be

associated with the aroma of roasted peanuts (Schirack et al. 2006; Greene et al. 2008; Chetschik et al. 2008; Braddock et al. 1995).

Free amino acids have been shown to be one of the major flavor precursors in roasted peanuts (Newell et al. 1967). It has been suggested that free aspartic acid, glutamic acid, glutamine, asparagine, histidine and phenylalanine are precursors of typical peanut flavors, while arginine, threonine, tyrosine and lysine are the major precursors of off-flavors (Newell et al. 1967). Free amino acids in the raw seed significantly decreased during roasting for all maturity classes, possibly due to involvement of these compounds in Maillard reactions (Oupadissakoon and Young 1984) (Figure 3-4). In contrast with the findings of Rodriguez et al. (1989), the differences between the contents of free amino acids in the raw and roasted peanuts were comparable across maturity classes (Table 4). This divergence might be explained by the different roasting procedure applied in these studies. In the present investigation, peanuts from all maturity classes were roasted using a defined, optimized time/temperature combination, while in the study conducted by Rodriguez et al. (1989), they were roasted for various lengths of time to achieve a similar Hunter L-value.

Fatty acids

The peanut seed consists of about 44-56% oil, most of which is composed of unsaturated fatty acids (Cobb and Johnson 1973). Consistent with previous reports, oleic and linoleic acids were the predominant fatty acids in peanuts, accounting for about 80% of the total profile (Table 5) (Jonnala et al. 2005; Cobb and Johnson 1973; Mercer et al.

1990; Young et al. 1972). Oleic/linoleic acid (O/L) ratio ranged from 1.27 to 1.55 for the yellow and black, respectively. Kim and Hung (1991) reported similar ranges, 1.33 to 1.68, in peanuts from five mesocarp-color based maturity classes. The O/L ratio significantly increased with increasing maturity ($P<0.0001$), which suggests that more mature peanuts may be less susceptible to lipid oxidation (Table 5). Generally speaking, previous studies have found that O/L ratio increases with increasing maturity (Sanders et al. 1982; Worthington et al. 1972; Kim and Hung 1991; Sanders 1980a,b). However, a few studies have reported a different pattern. For instance, Hashim et al. (1993) found a decrease in oleic acid and increase in linoleic acid with seed maturity, while Knauft et al. (1986) observed no change in fatty acid composition due to maturity.

Saturated fatty acids accounted for the additional ~20% of the total profile in the raw seeds. Palmitic represented about 11% of the total, while behenic and stearic represented about 3% and 2%, respectively. Other saturated fatty acids such arachidic and lignoceric represented between 1 and 2% of the total profile. The sum of saturated fatty acids gradually decreased with seed maturity, ranging from 19.4 to 18.8% from yellow to black, respectively (Table 5). Nevertheless, more mature peanuts contained relatively higher amounts of arachidic, which is in agreement with the findings of Young et al. (1972). Among all maturity classes, orange B had the highest content of palmitic, the major saturated fatty acid in peanuts. However, orange B generally contained the lowest contents of the remaining saturated fatty acids. Stearic content decreased progressively from yellow to brown; however black contained the highest amount of this fatty acid among all maturity classes. In a study conducted by Sanders (1980b), the amount of fatty

acids decreased as peanuts matured with the exception of oleic acid. Similarly, Sanders et al. (1982) reported that palmitic, arachidic, eicosenoic, behenic and lignoceric decreased with seed maturity. However, in their study the highest contents of stearic were found in the extremes of the maturity classes, black and yellow. In contrast, Kim and Hung (1991) reported that stearic content increased with seed maturity while palmitic remained unchanged. They also found that contents of linoleic, arachidic, behenic, and lignoceric decreased as peanuts matured. The discrepancies among these studies could be attributed to the different peanut varieties, handling, processing, and storage conditions used (Sanders et al. 1995; Young et al. 1974b).

Correlations between fatty acids were also examined in this study (Table 6). Oleic acid had a strong negative association with linoleic acid (r=-0.997). The proportions of these fatty acids during different stages of maturation are regulated by the Δ12 desaturase activity, which converts oleic to linoleic (Groff et al. 1996). Oleic was also inversely related to arachidic (r=-0.865), eicosenoic (r=-0.790), behenic (r=-0.597), lignoceric (r=-0.873), and 'others' (r=-0.572), while linoleic was positively associated with these fatty acids (r=0.884, 0.753, 0.548, 0.842, and 0.561, respectively). Andersen and Gorbet (2002) examined the effect of year and planting date on fatty acid composition of high oleic and normal oleic peanuts, and found that palmitic was negatively correlated with oleic acid. They suggested this was a result of an augmented rate of elongation of palmitic to stearic acid, followed by desaturation to oleic acid via Δ9 desaturase. In the present study, a similar justification may be given for the negative correlation found between stearic and eicosenoic. This would translate into an increase rate of elongation of

stearic to arachidic acid, followed by desaturation to eicosenoic acid via Δ12 desaturase. Stearic was negatively correlated with behenic (r=-0.762) and was the only fatty acid associated with palmitic acid (r=0.523). The former may be synthesized by acylation of palmitic, which is catalyzed by fatty acid elongases (Groff et al. 1996). This may also account for the positive correlation observed between behenic and lignoceric acids (r=0.548). In this study, a positive association was also found between behenic and eicosenoic (r=0.955); arachinidic and lignoceric (r=0.603); and eicosenoic and lignoceric (r=0.980) acids.

Conclusions

The peanut plant has an indeterminate flowering pattern, which results in peanuts of different maturity classes in all seed-sized commercial grade. This study determined the compositional variations in medium grade size peanuts of five mesocarp-color maturity classes and established correlations among their chemical and sensory characteristics. Sucrose content in the raw seed was positively associated with roasted peanutty, sweet aromatic and sweet taste, and negatively associated with woody/hull/skins in roasted peanuts. Free amino acids decreased during roasting, as a result of their involvement in Maillard reactions. Oleic/linoleic acid ratio increased with seed maturity, indicating that more mature peanuts may be less susceptible to lipid oxidation. This study underscores the effects of seed maturity on sensory and chemical composition of peanuts, and may allow for a better understanding of flavor development in roasted peanuts.

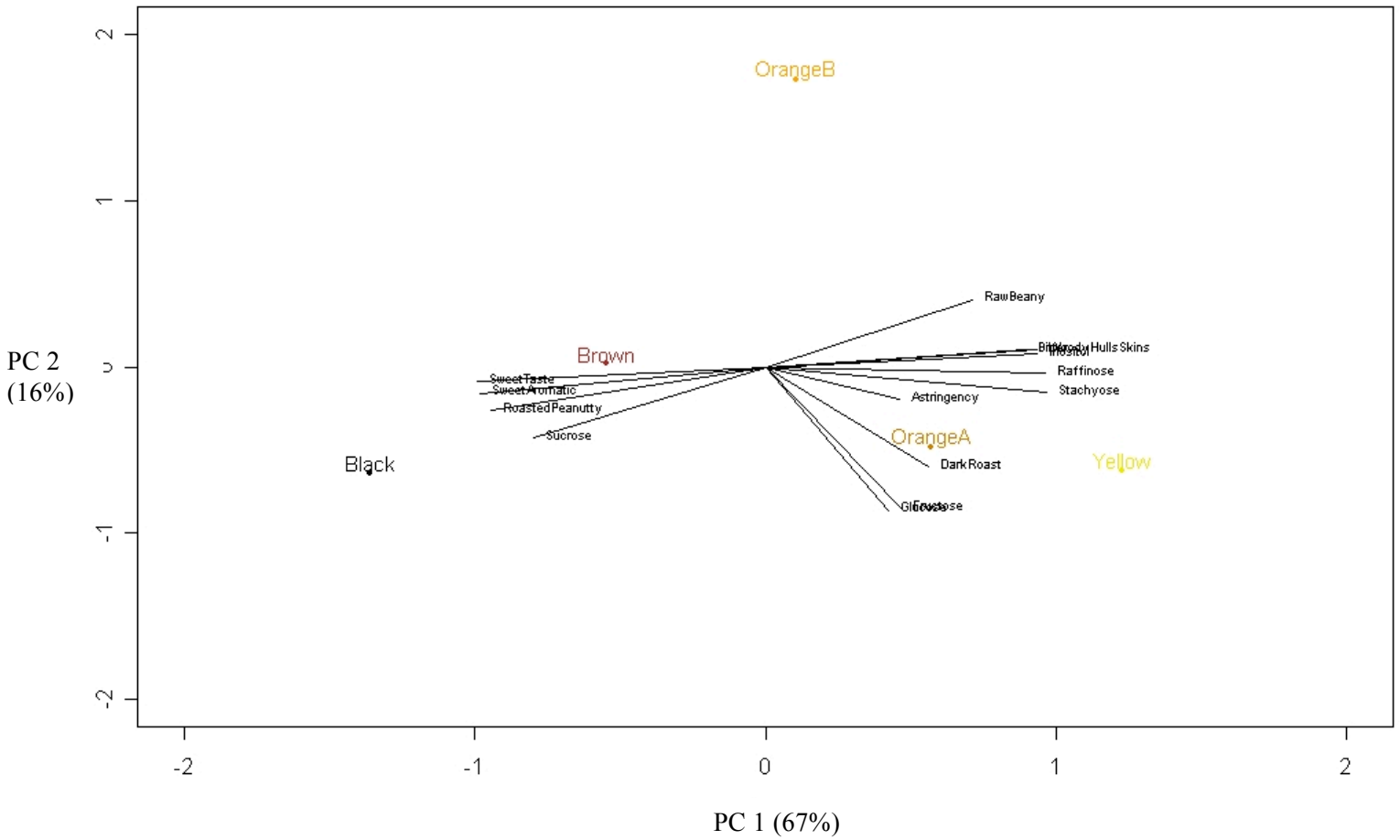

Figure 1. Principal component analysis biplot (PC1 vs. PC2) of carbohydrates and descriptive sensory attributes of peanuts from five mesocarp-color based maturity classes.

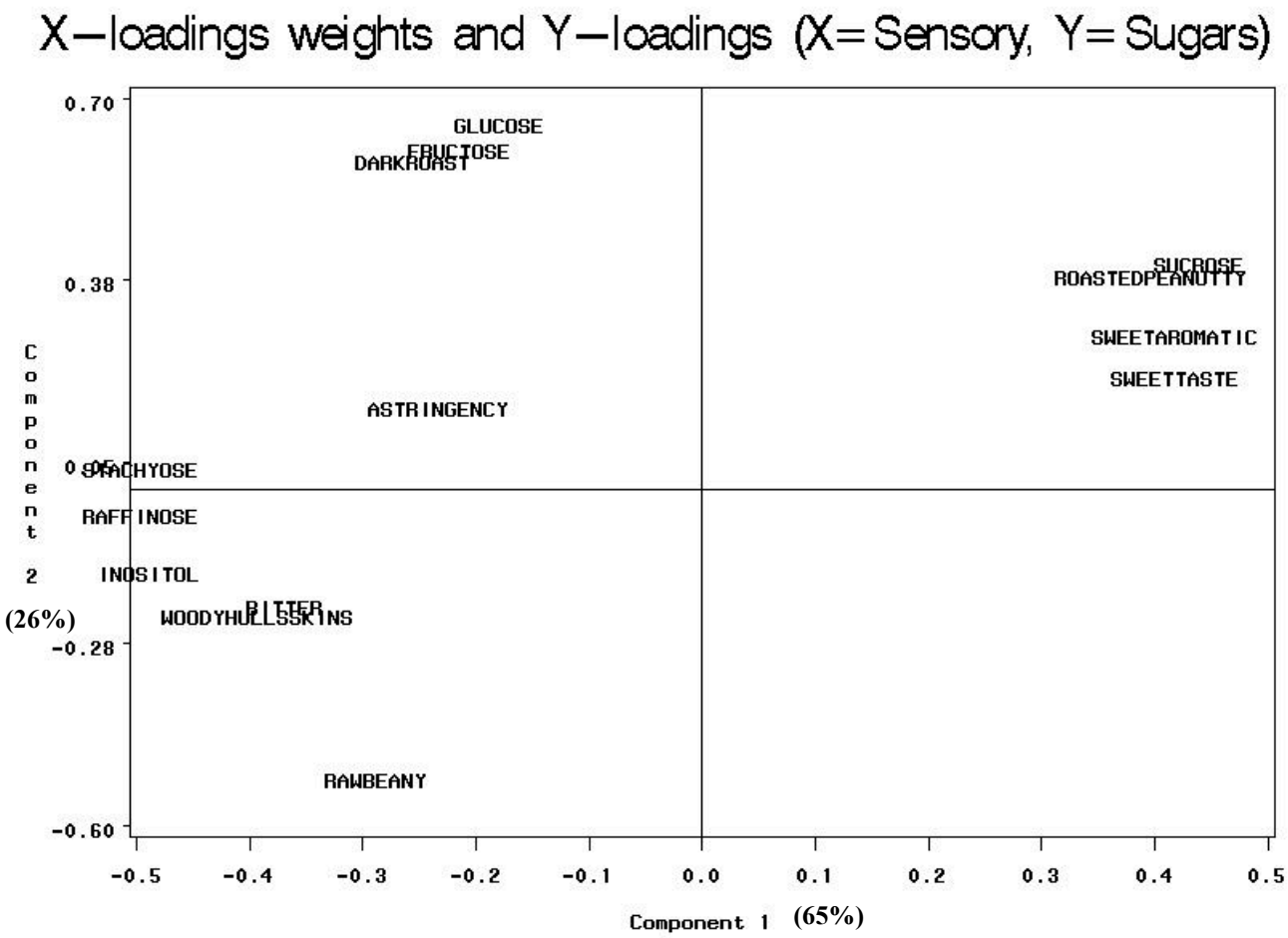

Figure 2. Biplot of the first two latent factors extracted from the partial least square regression (PLSR) of carbohydrates on descriptive sensory attributes.

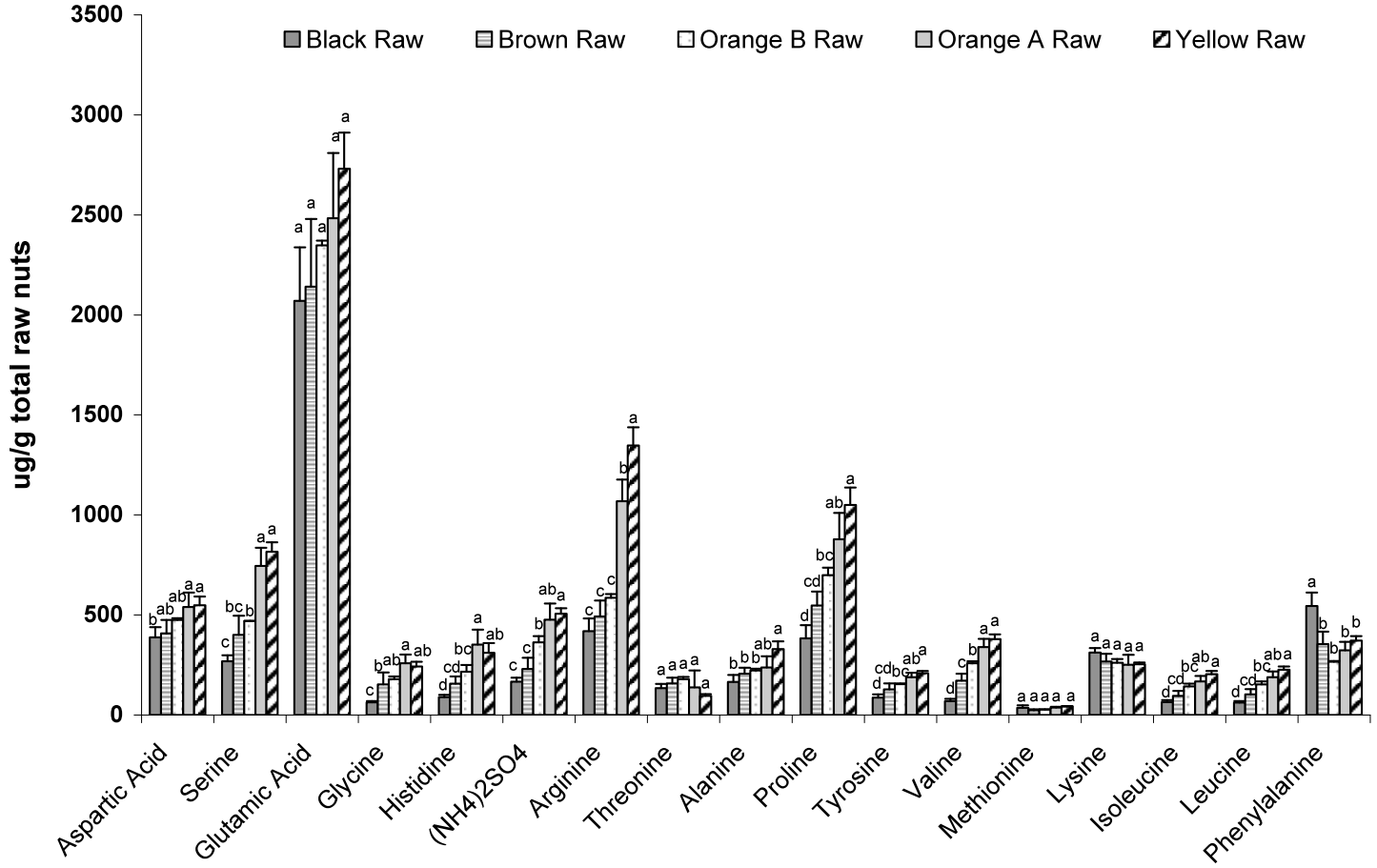

Figure 3. Free amino acid composition in raw seeds from five mesocarp-color based maturity classes. Means followed by different letters are significantly different ($P<0.05$) among maturity classes.

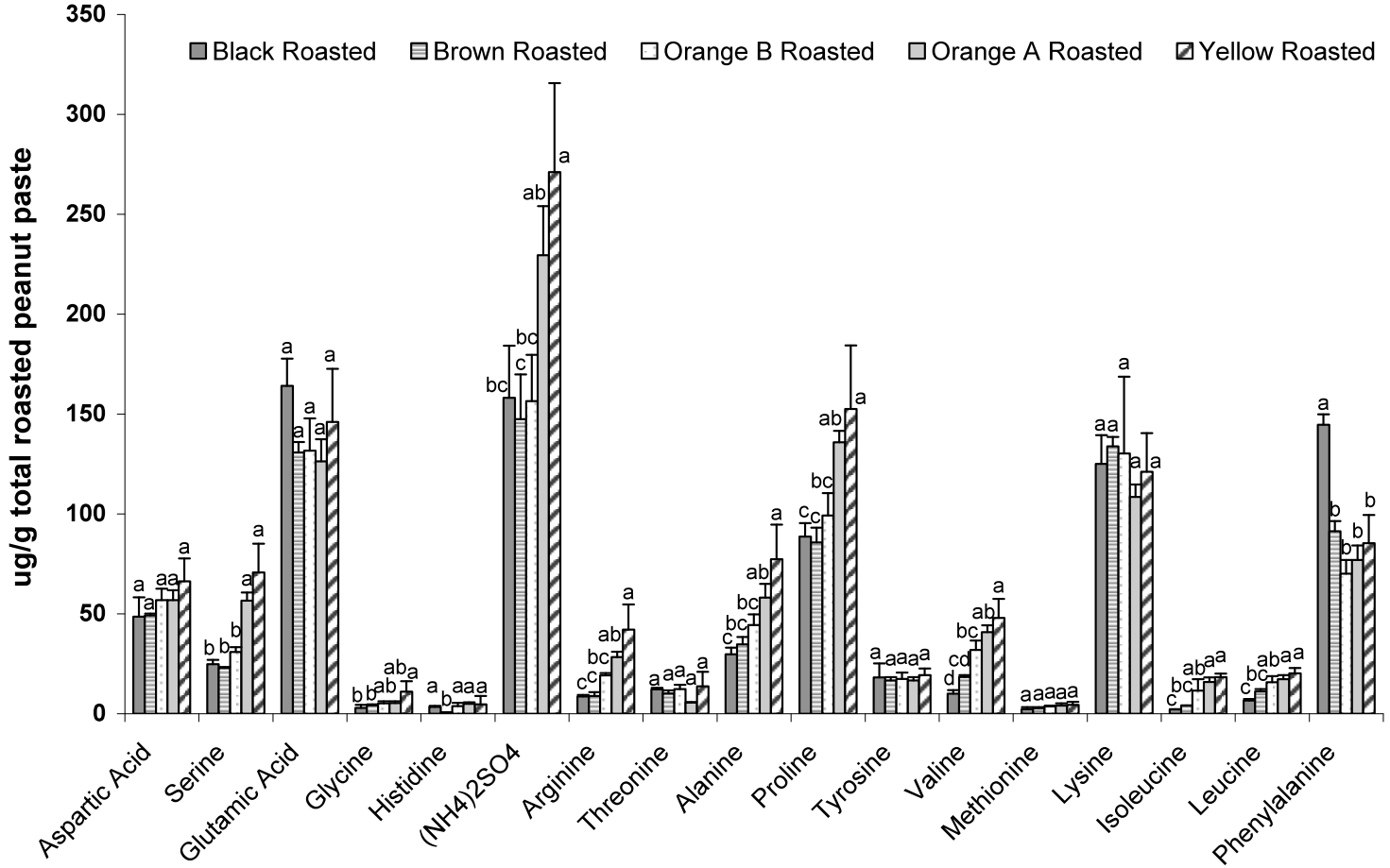

Figure 4. Free amino acid composition in roasted peanuts from five mesocarp-color based maturity classes. Means followed by different letters are significantly different ($P<0.05$) among maturity classes.

Table 1: Carbohydrate composition in raw and roasted peanuts from five mesocarp-color based maturity classes.

		Concentration (μg/g total sample)*				
Processing	Carbohydrate	Black	Brown	Orange B	Orange A	Yellow
Raw Peanuts	Sucrose	28,138±1,737a	21,182±866b	18,998±917b	19,038±934b	20,120±330b
	Stachyose	2,750±267d	2,884±75cd	3430±79bc	3,913±265ab	4,433±252a
	Raffinose	480±48c	555±23c	744±34b	910±56a	925±54a
	Inositol	187±14c	189±2c	238±5b	231±5b	275±9a
	Glucose	59±5ab	56±8ab	49±6c	67±1a	65±2a
	Fructose	15±3bc	17±2ab	8 ±2c	19±5ab	23±1a
	Total sugars	31,628±2,031a	24,883±957b	22,421±1,553b	24,178±1,243b	25,842±1,640b
Roasted Peanuts	Sucrose	27,994±1016a	23,223±724b	20,498±649b	21,026±2042b	21,281±1,285b
	Stachyose	2,592±19c	3,087±182c	3,775±213b	4,314±407ab	4,634±165a
	Raffinose	452±11c	562±26bc	681±18b	900±87a	910±51a
	Inositol	194±6c	212± 7c	253±3b	257±18b	298±12a
	Glucose	96±3a	87±6a	73±3b	72 ± 5b	73±1b
	Fructose	29±2a	27±2ab	22±1b	25±2ab	23±2b
	Total sugars	31,357±1035a	27,199±916b	25,302±714b	26,593±2,551b	27,220±457b

*Means followed by different letters are significantly different ($P<0.05$) among maturity classes.

Table 2. Pearson correlation coefficients among sensory attributes in roasted peanuts.

	Roasted peanutty	sweet aromatic	dark roast	raw beany	woody/hull/ skins	sweet taste	bitter	astringency
roasted peanutty		**0.859*** **	-0.191	-0.225	**-0.604***	**0.803****	**-0.725***	-0.359
sweet aromatic	**0.859*** **		-0.324	-0.035	-0.451	**0.774****	**-0.725***	-0.291
dark roast	-0.191	-0.324		-0.359	**0.553***	-0.372	**0.588***	-0.210
raw beany	-0.225	-0.035	-0.359		-0.017	-0.269	0.055	0.235
woody/hull/skins	**-0.604***	-0.451	**0.553***	-0.017		**-0.727***	**0.741****	0.223
sweet taste	**0.803****	**0.774****	-0.372	-0.269	**-0.727***		**-0.799****	-0.372
bitter	**-0.725***	**-0.725***	**0.588***	0.055	**0.741****	**-0.799****		0.006
astringency	-0.359	-0.291	-0.210	0.235	0.223	-0.372	0.006	

Significant correlations are established as follows: *P<0.05; **P<0.001; ***P<0.0001

Table 3. Pearson correlation coefficients between carbohydrates in raw seeds and sensory attributes of roasted peanuts.

	Roasted peanutty	sweet aromatic	dark roast	raw beany	woody/hull/ skins	sweet taste	bitter	astringency
inositol	**-0.692***	**-0.691***	0.362	0.293	0.446	**-0.850*****	**0.741****	0.217
glucose	-0.269	-0.285	**0.508***	-0.124	0.368	-0.311	0.309	**0.546***
fructose	-0.202	-0.302	0.446	-0.107	0.194	-0.352	-0.352	0.215
sucrose	**0.834****	**0.728****	-0.125	-0.093	**-0.678***	**0.810****	**-0.630****	-0.423
raffinose	**-0.728****	**-0.730****	0.2125	0.222	0.466	**-0.861*****	**0.587***	**0.564***
stachyose	-0.348	-0.284	0.192	0.303	0.139	**-0.530***	0.210	0.422

Significant correlations are established as follows: *P<0.05; **P<0.001; ***P<0.0001

Table 4. Percentage decrease of free amino acid contents during peanut roasting.

	% reduction of amino acids during roasting*				
	Black	Brown	Orange B	Orange A	Yellow
aspartic acid	87.5±1.9a	87.7±2.0a	88.0±0.7a	89.2±0.9a	87.8±2.6a
serine	90.7±0.7a	94.0±1.6a	93.4±0.3a	92.3±0.6a	91.3±1.5a
glutamic acid	92.0±0.6a	93.8±1.2a	94.4±0.5a	94.8±0.4a	94.6±1.0a
glycine	95.2±1.2a	97.1±0.8a	97.1±0.3a	97.9±0.3a	95.5±0.9a
histidine	96.1±0.4a	99.8±0.3b	98.2±0.5b	98.5±0.2b	98.5±0.9b
$(NH_4)_2SO_4$	4.9±9.3a	31.4±27.6ab	57.1±1.7b	50.4±4.7b	46.2±8.7ab
arginine	97.9±0.2a	98.2±0.2a	96.7±0.1a	97.3±0.3a	96.8±0.7a
threonine	90.8±0.6a	93.4±1.3a	93.1±0.7a	95.0±1.6a	85.3±4.6a
alanine	81.6±0.9a	82.7±4.3a	80.0±1.1a	74.8±2.2a	75.9±5.7a
proline	76.1±2.3a	84.0±3.1a	85.8±1.2a	84.2±1.2a	85.2±3.5a
tyrosine	78.9±7.1a	86.4±4.4ab	88.6±1.4b	91.0±0.7b	90.7±1.6b
valine	85.6±1.4a	88.8±2.8a	87.6±1.8a	87.8±0.9a	87.3±2.8a
methionine	93.1±2.8a	87.9±3.8a	86.2±0.5a	88.8±3.0a	89.6±2.0a
lysine	59.7±6.4a	49.2±5.8a	49.5±16.1a	56.0±4.9a	52.5±3.6a
isoleucine	96.7±0.2b	95.6±0.9b	91.6±3.6b	90.5±0.3a	91.0±0.8ab
leucine	89.2±0.9a	88.4±4.3a	89.7±1.5a	90.8±0.5a	91.0±0.7a
phenylalanine	73.2±1.1a	73.5±5.9a	73.6±1.9a	75.8±1.9a	76.9±4.0a

*Means followed by different letters are significantly different ($P<0.05$) among maturity classes.

Table 5. Fatty acid composition in peanut oil extracted from raw seeds.

	Percentage of Total Fatty Acid (%)				
Fatty acid	Black	Brown	Orange B	Orange A	Yellow
palmitic (C16:0)	10.681±0.070b	10.730±0.022b	10.869±0.015a	10.748±0.042b	10.783±0.029ab
stearic (C18:0)	2.226±0.004d	2.380±0.005a	2.389±0.002a	2.320±0.004b	2.264±0.007c
oleic (C18:1)	48.138±0.021a	47.761±0.080b	46.239±0.072c	44.677±0.033d	43.837±0.051e
linoleic (C18:2)	31.035±0.024e	31.408±0.053d	32.784±0.035c	33.968±0.021b	34.509±0.019a
arachidic (C20:0)	1.215±0.003d	1.249±0.005c	1.263±0.004bc	1.278±0.009a	1.276±0.003ab
eicosenoic (C20:1)	1.325±0.002c	1.271±0.006d	1.259±0.004d	1.392±0.022b	1.474±0.003a
behenic (C22:0)	3.015±0.017b	2.889±0.011c	2.812±0.005d	3.020±0.013b	3.209±0.011a
lignoceric (C24:0)	1.697±0.005c	1.673±0.003d	1.668±0.005d	1.809±0.012b	1.915±0.006a
Others	0.667±0.048a	0.640±0.038a	0.718±0.116a	0.788±0.056a	0.732±0.070a
O/L ratio	*1.551±0.002*	*1.521±0.005*	*1.410±0.001*	*1.315±0.000*	*1.270±0.001*
% saturated fat	*18.834±0.053*	*18.920±0.024*	*19.000±0.012*	*19.175±0.013*	*19.447±0.007*

*Means followed by different letters are significantly different ($P<0.05$) among maturity classes.

Table 6. Person correlations coefficients among fatty acids in raw seeds.

	palmitic C16:0	stearic C18:0	oleic C18:1	linoleic C18:2	arachidic C20:0	eicosenoic C20:1	behenic C22:0	lignoceric C24:0	others
palmitic C16:0		**0.523***	-0.391	0.429	0.484	-0.124	-0.310	-0.001	0.104
stearic C18:0	**0.523***		0.084	-0.036	0.374	**-0.613***	**-0.762****	-0.469	-0.049
oleic C18:1	-0.391	0.084		**-0.997*****	**-0.865****	**-0.790****	-0.597	**-0.873*****	**-0.572***
linoleic C18:2	0.429	-0.036	**-0.997*****		**0.884*****	**0.753****	**0.548***	**0.842*****	**0.561***
arachidic C20:0	0.484	0.374	**-0.865*****	**0.884***		0.47	0.228	**0.603***	0.462
eicosenoic C20:1	-0.124	**-0.613***	**-0.790****	**0.753***	0.475		**0.955*****	**0.980*****	0.380
behenic C22:0	-0.310	**-0.762****	**-0.597***	**0.548***	0.228	**0.955*****		**0.907*****	0.222
lignoceric C24:0	-0.0008	-0.469	**-0.873*****	**0.842*****	**0.603***	**0.980*****	**0.907*****		0.418
others	0.104	-0.04	**-0.572***	**0.561***	0.462	0.380	0.222	0.418	

Significant correlations are established as follows: *P<0.05; **P<0.001; ***P<0.0001

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CHAPTER 5:

CHARACTERIZATION OF PYRAZINE COMPOUNDS CONTRIBUTING TO EARTHY/BELL PEPPER FLAVOR IN FARMSTEAD CHEDDAR CHEESE

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Abstract

Farmstead Cheddar cheeses with natural bandage wrappings have a distinctive flavor profile that is appealing to many consumers. An earthy/bell pepper (EBP) flavor has been previously recognized in some of these cheeses. This study characterized the pyrazine compounds causing EBP flavor in Farmstead Cheddar cheeses. Eight cheeses were divided into inner, outer, rind and wrapper sections, and tested for descriptive sensory and instrumental analyses. To assess reproducibility of EBP flavor, cheeses from the same facilities were purchased and tested after 6 and 12 months. EBP flavor was detected in four out of eight Farmstead Cheddar cheeses by a trained sensory panel. 2-secbutyl-3-methoxypyrazine and 2-isopropyl-3-methoxypyrazine were identified as the main sources of EBP flavor in these cheeses by GC/O and GC/MS. In general, those pyrazines were prevalent in the wrapper (106-730 ppb) and rind (39-444 ppb) sections of the cheeses. They were either not detected in inner and outer sections of the cheeses or were present at low concentrations. These results suggest that 2-secbutyl-3-methoxypyrazine and 2-isopropyl-3-methoxypyrazine are formed near the surface of the cheeses and migrate into the cheese during ripening. Threshold values in water and whole milk were 1 ppt and 16 ppt for 2-secbutyl-3-methoxypyrazine, and 0.4 ppt and 2.3 ppt for 2-isopropyl-3-methoxypyarazine, respectively. Sensory analysis of mild Cheddar cheese model systems confirmed that direct addition of those individual pyrazines (0.4-20 ppb) resulted in EBP flavor.

KEYWORDS: earthy/bell pepper flavor, cheese flavor, alkylpyrazines

Introduction

Farmstead Cheddar cheeses with natural bandage wrappings have a distinct flavor profile that is appealing to many consumers (Lieberman 2007). A Farmstead cheese is defined as any type of cheese that is manufactured at the same location as the cows are milked (Lieberman 2007). Farmstead Cheddar cheeses are typically manufactured from unpasteurized milk and aged with a natural cloth or cheesecloth bandage for more than 1 year prior to marketing (Quickes Traditional; Devon, UK; personal communication). Studies have demonstrated that certain Farmstead Cheddar cheeses exhibit a characteristic earthy/bell pepper (EBP) flavor (Suriyaphan et al. 2001). Drake et al. (2001) developed a descriptive lexicon to characterize Cheddar cheese flavors, and reported that EBP flavor was one of several flavors that were not frequently observed in U.S. Cheddar cheeses. Among more than 250 Cheddar cheeses screened in their study, only one exhibited the EBP flavor: a British Farmstead Cheddar that was manufactured from raw milk and aged for 24 months. Suriyaphan et al. (2001) used gas chromatography coupled with olfactometry (GC/O) and mass spectrometry (GC/MS) to characterize the aroma properties of solvent extracts from a British Farmstead Cheddar cheese. They reported that the EBP flavor commonly found in this cheese was associated with alkylpyrazines such as 2-isopropyl-3-methoxypyrazine and 2-isobutyl-3-methoxypyrazine. They also hypothesized that these pyrazines were formed on the surface of the cheeses and subsequently migrated into the cheese. However, instrumental detection limits and their sampling technique were not able to confirm this hypothesis.

Pyrazines are six membered heterocyclic compounds that contain two nitrogen atoms in the ring (Barlin 1982). Alkylpyrazines are found naturally in a variety of vegetables including bell peppers (Buttery et al. 1969; Luning et al. 1994), chili peppers (Pino et al. 2006), potatoes (Buttery et al. 1973) and green peas (Murray et al. 1970). The herbaceous/bell pepper character of certain wines produced from Cabernet Sauvignon, Merlot, and Sauvignon Blanc grape varieties have been attributed to 2-isobutyl-3-methoxypyrazine, 2-secbutyl-3-methoxypyrazine, and 2-isopropyl-3-methoxypyrazine (Allen et al. 1994; Allen et al. 1995; Sala et al. 2002). Chitwood et al. (1983) reported that the 'green' aroma of three bell pepper cultivars (*Capsicum)* was associated with 2-isobutyl-3-methoxypyrazine and 2-secbutyl-3-methoxypyrazine.

Alkylpyrazines are generally formed by the Maillard reaction, Strecker degradation, or pyrolysis of amino acids under high temperature conditions. They contribute significantly to the flavor of heat-treated foods such as bread, roasted coffee, and roasted peanuts (Maga 1992). Moreover, alkylpyrazines can be formed as byproducts of metabolic processes in some plants and microorganisms (Cheng et al. 1991). For instance, alkylpyrazine mixtures similar to those produced in Maillard reactions have been identified in fermented cocoa (Barel et al. 1985; Gill et al. 1984), fermented soy (Liardon and Ledermann 1980) and cheese (Liardon et al. 1982). 2-isopropyl-3-methoxypyrazine, which has been considered the source of musty-earthy off-flavor in eggs, milk and fish, has been identified as a metabolite of *Pseudomonas perolans* and *Pseudomonas taetrolens* (Gallois et al. 1988; Cheng and Reineccius 1991; Cheng et al. 1991; McIver and Reineccius 1986; Morgan 1976). Brie and Camembert are cheeses that

undergo surface-ripening by *Pencillium sp.* and exhibit a characteristic musty-earthy flavor (Kosikowski 1982). Karahadian et al. (1985a) identified several volatile compounds with earthy-musty notes that were produced by pure cultures of *Penicillium caseicolum* and *Penicillium camembert,* including a group of eight-carbon alcohols and corresponding ketones, 2-methylisoborneol, and 2-isopropyl-3-methoxypyrazine. All of these compounds were considered as potential contributors to the earthy-musty off-flavor of Camembert and Brie cheeses.

The use of instrumental analysis in conjunction with sensory techniques is an effective way to characterize flavor of foods. In general, links between sensory and instrumental analysis may be accomplished by three steps: (1) selection of flavor(s) using descriptive sensory analysis; (2) analysis of isolated volatile compounds using gas chromatography-olfactometry (GC/O) and gas chromatography-mass spectrometry (GC/MS); and (3) confirmation of aroma-active compounds via instrumental quantitation, threshold testing and sensory analysis of model systems (Drake et al. 2006). The objectives of this study were to use this approach to characterize the volatile compounds contributing to EBP flavor in Farmstead Cheddar cheeses and to determine the concentrations and flavor impact of those compounds in cross-sections of the cheeses.

Materials and methods

Cheeses

Eight Farmstead Cheddar cheeses were used in this study (Table 1) and were purchased locally (Whole Foods; Raleigh, NC, USA) or ordered online and shipped by overnight delivery on ice packs (www.idealcheese.com). A 7 kg cross-sectional wheel of Farmstead Cheddar cheese #1 was freshly cut from a 28 kg drum. The wheel was immediately divided into 4 wedges so that each had the same proportion of inner, outer, rind, and wrapper sections (Figure 1). The rind was defined as the dark-yellow side section of about 0.5 cm thick from the outer border of the cheese wedge; the inner was defined as the intersection of the cheese wedge 1 cm away from the top and bottom rind; the outer was defined as the section located 1 cm away from the side, top and bottom rind (Figure 1). All four sections within a wedge, including the bandage wrapper, were evaluated by instrumental analysis. Inner and outer sections of the cheeses were evaluated using descriptive sensory analysis. To confirm the reproducibility of EBP flavor, additional wedges (2 kg) of Cheddar cheese #1 were subsequently purchased and analyzed after 6 and 12 months (Whole Foods; Raleigh, NC, USA).

Farmstead Cheddar cheese #2 was purchased as a small 0.5 kg truckle. Due to the small size of this cheese, samples (inner, outer, rind, and wrapper) were obtained from the entire truckle rather than from different wedges. An additional 2 kg wedge of this cheese, which was freshly cut from a 28 kg drum, was purchased and analyzed after one year to access reproducibility of EBP flavor. Six other Farmstead Cheddar cheeses (Table 1; cheeses #3 –8) were obtained as 2 kg wedges from 28 kg drums. An additional

sample of Farmstead Cheddar cheese #8 was purchased as a 2 kg truckle. Inner, outer, and rind sections were sampled for sensory and instrumental analyses as previously described.

Descriptive sensory analysis of cheeses

A trained sensory panel (n=8) with over 500 h experience in the descriptive sensory analysis of cheese evaluated the samples. Panelists were students and staff (ages 26 – 48 y) from the Department of Food, Bioprocessing and Nutrition Sciences at North Carolina State University, who were initially selected based on availability and ability to distinguish the basic tastes. Solutions of 250 ppt 2-secbutyl-3-methoxypyrazine and 100 ppt 2- isopropyl-3-methoxypyrazine in water were provided to panelists during training along with cheeses with and without EBP to familiarize panelists with this flavor. Analysis of data collected from preliminary sessions confirmed that panelists could consistently identify and scale Cheddar cheese flavors including EBP.

Inner and outer sections of cheeses were evaluated for flavor and aroma using a previously established cheese sensory lexicon (Drake et al. 2001). Cheeses were dispensed into lidded 58 mL soufflé cups with randomized 3-digit codes. Cheeses were tempered to 15ºC for sensory analysis. Panelists evaluated each sample in duplicate using a 15-point intensity scale, according to the Spectrum ^{TM} method (Meilgaard et al. 1999). Panelists were provided with a mild Cheddar cheese along with a previously identified profile as a warm-up sample. Water and crackers were provided as palate cleansers

between samples. Responses were collected with paper ballots or Compusense Five v4.6 (Compusense, Guelph, Ontario, Canada).

Chemicals

Analyte standards of 2-secbutyl-3-methoxypyrazine, 2-isopropyl-3-methoxyprazine, and 2-ethylpyrazine (internal standard) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Methanol and ethanol served as solvents and were also obtained from Sigma-Aldrich (St. Louis, MO, USA). Stock solutions were prepared from the pure compounds diluted in absolute ethanol for sensory analysis or absolute methanol for instrumental analysis. Stock solutions were stored at -20ºC until use for a maximum of 1 mo.

Gas chromatography/mass spectrometry (GC/MS)

Cheese volatiles were isolated by solid phase microextraction (SPME) using a CTC Analytics combiPAL auto sampler (Zwingen, Switzerland). Five grams of grated cheese (or 16 pieces of 2.5 cm^2 wrapper) were placed into 20 mL clear screw cap vials (Microliter Analytical Supplies Inc.; Suwanee, GA, USA), and 9.7 ppb of 2-ethylpyrazine was added as the internal standard. A three-phase 1 cm Stableflex 50/30μm divinylbenzene/carbonex/PDMS (DVB/CAR/PDMS) fiber (Supelco, Bellefonte, PA, USA) was exposed into the headspace above the sample at 40ºC with agitation at 250 rpm for 25 min. The fiber was inserted through the vial septa at 22 mm depth and remained in the headspace for 30 min before injection.

Extracted volatile components were analyzed on a 6890N GC/ HP5973 mass selective detector (Agilent Technologies., Palo Alto, CA, USA). The GC was equipped with a nonpolar capillary column (DB-5MS, 30 m length x 0.25 mm i.d. x 0.25 μm d_f; (J&W Scientific, Folsom, CA, USA). Helium gas was used as carrier gas at a constant flow rate of 1 mL/ min. Oven temperature was programmed to increase from 40ºC to 250ºC at a rate of 8ºC/min with initial and final hold times of 5 and 30 min, respectively. Mass selective detector conditions were as follows: capillary direct interface temperature, 250 ºC; ionization energy, 70 eV; mass range, 35-350 m/z; EM Voltage (Atune +200 V); scan rate, 2.94 scans/s. All samples were run in triplicate using the splitless mode.

Gas chromatography-olfactometry (GC/O)

Ten grams of grated cheese (or 16 pieces of 2.5 cm^2 wrapper) were placed into a 40 mL amber screw cap vial (Supelco, Bellefonte, PA, USA) to equilibrate for 30 min at 40ºC. The fiber was manually inserted into the sample vial at 2 cm depth for 30 min, followed immediately by desorption in the GC/O injector for 5 min at 250ºC. Prior to extraction, the fiber was conditioned in a GC injector for 5 min at 250ºC to prevent contamination.

Samples were analyzed on a HP5890 series II gas chromatograph (Hewlett-Packard Co.; Palo Alto, CA, USA) fitted with a flame ionization detector (FID), splitless injector, and a sniff port. The GC was equipped with either a polar capillary column (BD-WAX, 30m length x 0.25 mm i.d. x 0.25 μm film thickness d_f, (J&W Scientific; Folsom, CA, USA), or a nonpolar column (DB-5ms, 30 m length x 0.25 μm film thickness d_f,

(J&W Scientific; Folsom, CA, USA). The oven temperature was programmed to increase from 40ºC to 200ºC at a rate of 10ºC/ min with an initial hold of 3 min and a final hold of 20 min. Column effluent was split 1:1 between the FID and sniffing port using deactivated fused silica capillaries (1 m length x 0.25 mm i.d.). The FID and sniffing port were kept at 250ºC. Humidified air was supplied at the end of sniffing port at a rate of 30 mL/min to prevent dehydration of the nasal membranes of the panelists. Two experienced panelists (each with > 100 h experience with GC-O of dairy products) sniffed each sample in duplicate on the 2 different columns. Panelists described the odors and scored aroma intensities using a 5-point numerical intensity scale.

Identification and quantification of pyrazines

Positive identification of 2-secbutyl-3-methoxypyrazine and 2-isopropyl-3-methoxypyrazine in cheese was performed by comparison of volatile compounds mass spectra, retention index, and aroma quality with those of standard compounds analyzed under the same conditions. For the calculation of retention indices (RI), an n-alkane series was used (Van den Dool and Kratz 1963).

Standard curves were generated using cheese model systems, as described by Whetstine et al. (2005). Mild Cheddar cheese served as the matrix because it neither contained EBP flavor nor the pyrazines of interest, as determined by preliminary sensory and GC/MS analysis. Mild Cheddar cheese was shredded and portioned into several 25 g samples. Pyrazine compounds were first dissolved in methanol as stock solutions and then added to individual cheese shred aliquots to achieve concentration ranges between

10 and 150 ppb. In preliminary studies, these levels provided similar peak areas for 2-secbutyl-3-methoxypyrazine and 2-isopropyl-3-methoxypyrazine in Farmstead Cheddar cheese containing EBP flavor. Cheese samples were kneaded by hand for 5 min, shaped into spheres and equilibrated for 24 h at 4ºC. A six-point internal standard curve was constructed for each pyrazine by plotting the peak area ratio of the analyte to 2-ethylpyrazine (internal standard) versus the concentration ratio of analyte to 2-ethylpyrazine. Resultant peaks area were fitted using linear regressions, with the concentrations averaged across three replications. GC/MS sensitivity was defined as the minimum concentration of an analyte that allowed accurate and reproducible determination of the peak height or area. The limit of detection for each pyrazine was determined in a model system as the concentration which produced a 'peak height-to-baseline noise' of at least 3 (signal-to-noise ratio ≥ 3).

Threshold testing

Orthonasal detection thresholds (i.e. the lowest concentration at which an odor can be detected) were determined for 2-secbutyl-3-methoxypyrazine and 2-isopropyl-3-methoxypyrazine using a 7-series forced choice ascending concentration method of limits (ASTM 1992). Threshold values were determined in both deodorized water and whole milk. Deodorized water was obtained by boiling 4 liters of distilled water to one-third of its volume. Whole milk was purchased from a local grocery store. From one row to the next, the stimulus concentration was increased by a factor of 3. Pyrazine compounds were diluted in methanol and appropriate amounts of the stock solutions were added to

samples. For each row, blank samples were adjusted with the same concentration of methanol to eliminate the effect of solvent. Samples were equilibrated at room temperature for 1 h before being presented for sensory evaluation in 58 mL lidded plastic soufflé cups labeled with a 3-digit code. Sample cups within each row were presented in random order.

Subjects (n = 40) were instructed to sniff samples from left to right and choose the odd sample among the three. Subjects were also asked to indicate judgment certainty (sure/not sure) within each row. The individual best estimate threshold (BET) was taken as the geometric mean of the last concentration with an incorrect response and the first concentration with a correct response with no further incorrect responses. If the subject indicated 'not sure' for the correct choice, that concentration was adjusted by a factor of 1.41 to account for guessing (Lawless et al. 2000). The group threshold was calculated as the geometric mean of the individual best estimate thresholds.

Sensory evaluation of cheese models

Sensory analysis of model systems was conducted to confirm that 2-secbutyl-3-methoxypyrazine and 2-isopropyl-3-methoxypazine contributed to EBP flavor found in Farmstead Cheddar cheeses. Samples were evaluated using the same protocol applied for descriptive sensory analysis of cheeses.

Cheese model samples were prepared using a modified procedure described by Whetstine et al. (2005). Pyrazine compounds were first dissolved in ethanol as stock solutions. Mild Cheddar cheese was shredded and portioned into 5 samples of 250 g.

Stock solutions were added to four of these samples to attain 2 and 20 ppb for 2-secbutyl-3-methoxypyrazine and 0.4 and 4 ppb for 2-isopropyl-3-methoxypyrazine. These concentrations were all above the orthonasal thresholds determined in water and whole milk. The control sample contained ethanol in place of standard solutions. Each of the five samples was kneaded by hand for 5 min and shaped into a sphere. Samples were equilibrated for 24 h at 4ºC, and re-shaped into small spheres (8 g) prior to sensory evaluation. Samples were equilibrated for 1 h at room temperature prior to sensory analysis.

Statistical analysis

Data from descriptive sensory and instrumental analyses were evaluated by analysis of variance using the general linear models procedure of SAS (version 9.1; Cary, NC, USA). Means separation was performed using the Fisher's least significant difference (LSD) test.

Results and discussion

Descriptive sensory analysis

The presence of EBP flavor was confirmed by trained panelists in four out of eight Farmstead Cheddar cheeses (cheeses #1, 2, 7, and 8) (data not shown). Intensities of EBP flavor in the inner and outer sections ranged from approximately 1 to 3.5 on a 15-point scale. Previous work has demonstrated that most cheese flavors fall between 0 and

6 on this scale (Drake et al. 2005, 2008). The remaining four Farmstead Cheddar cheeses did not exhibit EBP flavor in any of the sections.

Characteristic descriptive profiles of inner and outer sections of Farmstead Cheddar cheese #1, purchased on two different occasions approximately one year apart were similar (Table 2). Cheeses were characterized by typical aged/developed flavors such as sulfur, brothy, and nutty. This is in agreement with the flavor profiles previously reported by Drake et al. (2001) and Suriyaphan et al. (2001) for British Farmstead Cheddar cheeses. EBP flavor was detected in both of these cheeses, with trends towards higher intensities for the outer sections as compared to the inner sections. Cheeses purchased and tested after one year showed larger differences in EBP flavor across sections, with sensory scores being 0.8 and 3.4 for the inner and outer sections, respectively (Table 2).

Identification and quantification of pyrazines

Volatile compounds responsible for EBP flavor in Farmstead Cheddar cheeses were identified by SPME GC/MS and GC/O as 2-isopropyl-3-methoxypyrazine and 2-secbutyl-3-methoxypyrazine (Table 3). Positive identification was achieved by comparison of volatile compound mass spectra, retention index, and aroma quality with those of standard compounds analyzed under the same conditions. Among twenty aroma active-compounds that were consistently identified in the cheeses by SPME GC/O, those pyrazines were the only two compounds described as EBP at the sniffing port (data not shown).

Suriyaphan et al. (2001) identified 2-isopropyl-3-methoxypyrazine and 2-isobutyl-3-methoxypyrazine as the sources of EBP flavor in a single British Farmstead Cheddar cheese. In the present study, the former compound was also identified as one of the contributors to EBP flavor. However, 2-secbutyl-3-methoxypyrazine, rather than 2-isobutyl-3-methoxypyrazine, was identified as the second source of EBP flavor in Farmstead Cheddar cheeses. In addition to similar chemical structures, 2-secbutyl-3-methoxypyrazine and 2-isobutyl-3-methoxypyrazine share 2 out of the 3 largest ion peaks in their mass spectra (m/z=124 and 151) (Figure 2). These two compounds also have similar aroma profiles and retention indices (Table 4). Therefore, they could be misidentified as one another if sufficient precautions were not taken. In the present study, 2-secbutyl-3-methoxypyrazine was positively identified in place of 2-isobutyl-3-methoxypyrazine by extracting the largest ion peak (m/z= 138) and comparing the mass spectra of eluting compounds to that of a 2-secbutyl-3-methoxypyrazine standard (Figures 2 and 3). The procedure was repeated for 2-isobutyl-3-methoxypyrazine (m/z = 124), however none of the eluting compounds had a similar mass spectra as the respective standard. The more sensitive mass selective detector used in this study allowed a more accurate distinction between these two pyrazine compounds compared to the earlier study conducted by Suriyaphan et al. (2001).

Quantification of 2-secbutyl-3-methoxypyrazine and 2-isopropyl-3-methoxypyrazine in the inner, outer, rind, and wrapper sections was performed using internal standard curves generated with model systems. Linear correlation coefficients (R^2) for calibration curves were 0.99 and 0.97 for 2-secbutyl-3-methoxypyrazine and 2-

isopropyl-3-methoxypyrazine, respectively. The instrumental limit of detection was determined as 1 ppb for both pyrazine compounds, with signal to noise ratios > 3. Quantification of these aroma components was achieved by solid-phase microextraction (SPME) and gas chromatography/mass spectrometry (GC/MS). Previous studies have shown that SPME is a powerful technique for extraction of 2-alklyl-3-methoxypyrazines in several complex matrices such as wine, fresh chili, and bell pepper (Hartmann et al. 2002, Sala et al. 2002; Mazida et al. 2005).

Both 2-secbutyl-3-methoxypyrazine and 2-isopropyl-3-methoxypyrazine were prevalent towards the exterior sections of Farmstead Cheddar cheese #1. The concentrations of these compounds in the rind and wrapper sections were reproducible across 3 different samples of this cheese purchased and tested in a one year interval (Table 5). In general, concentrations of 2-secbutyl-3-methoxypyrazine (150-444 ppb) were higher than 2-isopropyl-3-methoxypyrazine (39-115 ppb) in the wrapper ($P<0.05$). The opposite trend was observed for the rind sections, where concentrations of 2-isopropyl-3-methoxypyrazine (557-730 ppb) were higher than those of 2-sec-butyl-3-methoxypyrazine (106-187 ppb) ($P<0.05$). These pyrazines were not detected by GC/MS in the inner and outer sections of any of the three samples of Farmstead Cheddar cheese #1.

Pyrazines of interest were also present in the two samples of Farmstead Cheddar cheese #2 purchased and evaluated within a one year interval (0.5 kg cheese truckle and 2 kg cheese wedge from a 28 kg drum) (Table 6). Similar to results for cheese #1, 2-secbutyl-3-methoxypyrazine was prevalent in the wrapper (162 ppb), and 2-isopropyl-3-

methoxypyrazine was mostly found in the rind section (155 ppb) of the small truckle. Small concentrations of those pyrazines were found in the inner and outer sections (Table 6). Compared to the small truckle, the cheese wedge, obtained from a large drum, had significantly higher concentrations of 2-secbutyl-3-methoxypyrazine and 2-isopropyl-3-methoxypyrazine in the rind section (303 ppb and 1200 ppb, respectively) ($P<0.05$). Those pyrazines were not detected in the inner and outer sections of this cheese (Table 6). The higher concentrations of those pyrazines toward the exterior regions of the cheese wedge compared to the small truckle may be explained by differences in their size/total volume. During cheese ripening, pyrazine compounds migrate faster into the small truckle than into the large cheese drum because the former has a higher surface area to volume ratio. This may explain why those pyrazines were detected in the interior sections of the small truckle but not in the interior sections of the cheese wedge.

Detectable levels of 2-isopropyl-3-methoxypyrazine were found in the rind section of the remaining Farmstead Cheddar cheeses as follows: cheese #7 wedge (32 ± 29 ppb), cheese #8 truckle (111 ± 120 ppb) and cheese #8 wedge (70 ± 28 ppb). 2-isopropyl-3-methoxypyrazine was not found in inner and outer sections, and 2-sec-butyl-3-methoxypyrazine was not detected in any section of these cheeses. Instrumental volatile analysis of the remaining Farmstead Cheddar cheeses (cheeses # 3, 4 5, and 6) did not detect 2-secbutyl-3-methoxypyrazine and 2-isopropyl-3-methoxypyrazine in any section of the cheeses. This finding was consistent with descriptive sensory analysis results that these cheeses did not exhibit EBP flavor.

In an earlier study (Suriyaphan et al. 2001), the concentration of 2-isopropyl-3-methoxypyrazine was reported to be higher in the outer sections than at the center of a British Farmstead Cheddar cheese, which is in agreement with our results. The distribution pattern of pyrazine compounds in these cheeses suggests that the compounds are formed on or near the surface of the cheese and migrate towards the center during ripening. In the present study, Farmstead Cheddar cheeses had typical visible mold growth on the rind section, which suggests that EBP flavor related pyrazines might be formed as microbial metabolites from mold naturally occurring on the cheese exterior. Several studies have shown that alkylpyrazines can be formed via the primary and secondary metabolism of microorganisms (Cheng and Reineccius 1991; Beck et al. 2003). For example, 2-isopropyl-3-methoxyprazine has been identified as a metabolite of *Pseudomonas perolans* and *Pseudomonas taetrolens* (Gallois et al. 1988; Cheng and Reineccius 1991; McIver and Reineccius 1986; Morgan 1976). Beck et al. (2003) identified several pyrazines, including 2-isopropyl-3-methoxyprazine and 2-secbuty-3-methoxypyrazine, as metabolites of *Paenibacillus polymyxa*. Brie and Camembert are cheeses that are surface-ripened by *Pencillium sp.*, and their characteristic musty-earthy flavor has been linked to 2-isopropyl-3-methoxypyrazine produced by these fungi (Kosikowski 1982; Karahadian et al. 1985b).

Threshold testing

Threshold values in water and whole milk were 1 ± 0.8 ppt and 16 ± 1 ppt for 2-secbutyl-3-methoxypyrazine, and 0.4 ± 0.6 ppt and 2.3 ± 1.1 ppt for 2-isopropyl-3-

methoxypyarazine, respectively. Lower threshold values obtained for 2-isopropyl-3-methoxypyrazine in both matrices suggest that this compound is a more potent odorant as compared to 2-secbutyl-3-methoxypyrazine. Previous studies have reported threshold values for 2-isopropyl-3-methoxypyrazine in water ranging between 0.2 ppt (Young et al. 1996) and 1.0 ppt (Murray et al. 1970). A threshold concentration of 1 ppt in water has been reported for 2-secbutyl-3-methoxypyrazine (Fors 1988; Murray et al. 1970). Differences in threshold values may occur depending upon the testing method and number of panelists (Meilgaard et al. 1999). Although previously reported threshold values were not obtained using the ASTM method, they fell in the same range as experimental threshold values found in our study. Wagner et al. (1999) determined the odor threshold for 83 alkylpyrazines in air and classified 2-alkyl-3-methoxypyrazines as a special class of odorants due to their relative low threshold values. They suggested that odor perception was a function of both steric and electrostatic interactions with receptors. Threshold concentrations for 2-isopropyl-3-methoxypyrazine and 2-secbutyl-3-methoxypyrazine in air were 2 ppt and 3 ppt, respectively.

The exceptionally low threshold values of these pyrazine compounds indicate that they contribute significantly to flavor despite their low concentration found in some of the cheeses. In the case of Farmstead Cheddar cheese #1, pyrazines were not detected towards the interior of the cheese by instrumental analysis. However, the instrumental limit of detection was 1 ppb for both compounds, which is approximately 1000 times higher than experimental sensory threshold values. Descriptive sensory analysis showed that inner and outer sections of this cheese exhibited the EBP flavor (Table 2). This

suggests that human perception of these compounds was more sensitive than instrumental analysis.

Model systems

The odor characteristics of 2-secbutyl-3-methoxypyrazine and 2-isopropyl-3-methoxypyrazine were described as earthy/bell pepper/potato as they eluted from the GC/O column (Table 3). However, the individual aroma of a given compound may not reflect the actual role in flavor due to matrix interactions (Drake and Civille 2003; Singh et al. 2003). Thus, assessing the effect of volatile components in a matrix similar to the food that is being studied is important. The contribution of 2-secbutyl 3-methoxypyrazine and 2-isopropyl-3-methoxypyrazine to EBP flavor of cheeses was confirmed by sensory evaluation of model systems consisting of a mild domestic Cheddar cheese spiked with the pyrazine compounds. The addition of each pyrazine compound resulted in EBP flavor in cheese model systems (Figures 4 and 5). Panelists agreed that the EBP flavor resulting from addition of pyrazine compounds to cheeses were similar to that naturally found in Farmstead Cheddar cheeses. They also found no difference in the quality of EBP flavor resulting from addition of either pyrazine compound. However, 2-secbutyl-3-methoxypyrazine required a concentration five-fold higher than 2-isopropyl-3-methoxypyrazine to impart a similar sensory response, which was consistent with our threshold results that the latter is a more potent odorant.

Conclusions

This study revealed the importance of 2-secbutyl-3-methoxypyrazine and 2-isopropyl-3-methoxypyrazine as the sources of EBP flavor in bandage wrapped Farmstead Cheddar cheeses. In general, pyrazines were most prevalent in the cloth wrapper and the rind section of the cheeses. The two pyrazines were either not detected in inner and outer sections of the cheeses or they were present at low concentrations. Despite the low concentrations of 2-secbutyl-3-methoxypyrazine and 2-isopropyl-3-methoxypyrazine present in these sections, their contribution to flavor was significant because of their extremely low threshold values. This study suggests that pyrazines causing EBP flavor are formed as metabolites of fungi present on or near the surface of the cheese and that the compounds migrate into the cheese during ripening. These results imply that formation of EBP flavor in Cheddar cheese requires a natural rind exposed to the air during ripening/aging rather than vacuum-sealing in polyethylene bags as is done for most commercial Cheddar cheeses. This may explain why EBP flavor is only found in certain Farmstead Cheddar cheeses with natural air-exposed rinds.

Table 1. Farmstead Cheddar cheeses selected for descriptive and instrumental analyses. Cheese wedges and cross-sections were obtained from large 28 kg drums.

Cheese #	Country of Origin	January/ 07	July/ 07	January/ 08
1	England	7 kg cross-section	2 kg wedge	2 kg wedge
2	England	0.5 kg truckle	-	2 kg wedge
3	USA	-	2 kg wedge	2 kg wedge
4	Scotland	-	2 kg wedge	2 kg wedge
5	Australia	-	2 kg wedge	-
6	USA	-	2 kg wedge	-
7	England	-	-	2 kg wedge
8	England	-	-	2kg truckle and 2kg wedge

Table 2. Flavor profiles of inner and outer sections of Farmstead Cheddar cheese #1 purchased on two different occasions approximately one year apart.

	Jan/07[a]		Jan/08[a]	
	Inner	Outer	Inner	Outer
Earthy/Bell Pepper (EBP)	1.3a	1.7b	0.8a	3.4b
Cooked/milky	2.5a	3.0b	2.2a	2.2a
Milkfat	2.8a	3.3a	3.0a	2.5b
Sulfur	2.0a	2.0a	2.0a	2.5b
Brothy	3.4a	3.0b	3.0a	2.8b
Nutty	1.3a	1.5b	2.5a	1.3b
Sweet	2.3a	2.0a	2.5a	2.5a
Salty	3.5a	4.0b	3.5a	3.6a
Bitter	1.5a	1.0b	1.3a	1.0a
Sour	3.3a	3.3a	3.0a	3.0a
Phenolic	1.8a	1.5a	2.3a	1.0b
Umami	3.0a	3.3a	2.0a	2.0a
Prickle	-	-	1.8a	1.0a

[a] For each sample (Jan/07 and Jan/08), means in a row followed by different letters are different ($P<0.05$).
[b]ND: not detected

Table 3. Pyrazine compounds identified as the source of EBP flavor in Farmstead Cheddar cheeses.

Compound	CAS[a]	Formula	RI[b]		Odor Character[c]	Identification Method
			DB-5MS	C20M		
2-isopropyl-3-methoxypyrazine	25773-40-4	$C_8H_{12}N_2O$	1180	1410	Earthy, bell pepper, potato	RI, odor, MS
2-secbutyl-3-methoxypyrazine	24168-70-5	$C_9H_{14}N_2O$	1094	1567	Green, potato, earthy	RI, odor, MS

[a]CAS: Chemical Abstracts Service registry number
[b] Retention indices (RI) were calculated from GC-O analysis
[c]Odor character determined by GC/O analysis

Table 4. Published values of Kovats retention indices (RI) and odor characteristics of 2-secbutyl-3-methoxypyrazine and 2-isobutyl-3-methoxypyrazine (available at http://www.flavornet.org).

Compound	RI		Odor Character
	DB-5MS	C20M	
2-secbutyl-3-methoxypyrazine	1176	1500	Carrot, earthy
2-isobutyl-3-methoxypyrazine	1186	1510	Earth, spice, green pepper

Table 5. Concentrations of pyrazine compounds in Farmstead Cheddar cheese #1 purchased on three different occasions within one year.

		Concentration (ppb) [a]			
		Inner	Outer	Rind	Wrapper
2-secbutyl-3-methoxypyrazine	Jan/07	ND[c]	ND	108 ± 44a[b]	444 ± 208b
	July/07	ND	ND	106 ± 98a	150 ± 73a
	Jan/08	ND	ND	187 ± 52	NA[d]
2-isopropyl-3-methoxypyrazine	Jan/07	ND	ND	705 ± 118a	39 ± 5b
	July/07	ND	ND	730 ± 345a	115 ± 110b
	Jan/08	ND	ND	557 ± 116	NA

[a] Means for Jan/07 samples were obtained from 4 cheese wedges (7 kg). Means for July/07 and Jan/08 samples were obtained from 1 cheese wedge (2 kg)
[b]Means followed by different letters are significantly different across cheese sections
[c]ND: not detected
[d]NA: Wrapper not available at purchase

Table 6. Concentrations of pyrazine compounds in Farmstead Cheddar cheese #2 purchased on two different occasions approximately one year apart.

		Concentration (ppb) [a]			
		Inner	Outer	Rind	Wrapper
2-secbutyl-3-methoxypyrazine	Jan/07	16 ± 15a[b]	8 ± 11a	16 ± 15a	162 ± 64b
	Jan/08	ND[c]	ND	303 ± 93	NA[d]
2-isopropyl-3-methoxypyrazine	Jan/07	5 ± 4a	29 ± 12a	155 ± 72b	33 ± 14a
	Jan/08	ND	ND	1200 ± 480	NA

[a] Means for Jan/07 samples were obtained from small truckle (0.5 kg). Means for Jan/08 samples were obtained from a cheese wedge (2 kg)
[b]Means followed by different letters are significantly different across cheese sections
[c]ND: not detected
[d]NA: Wrapper not available at purchase

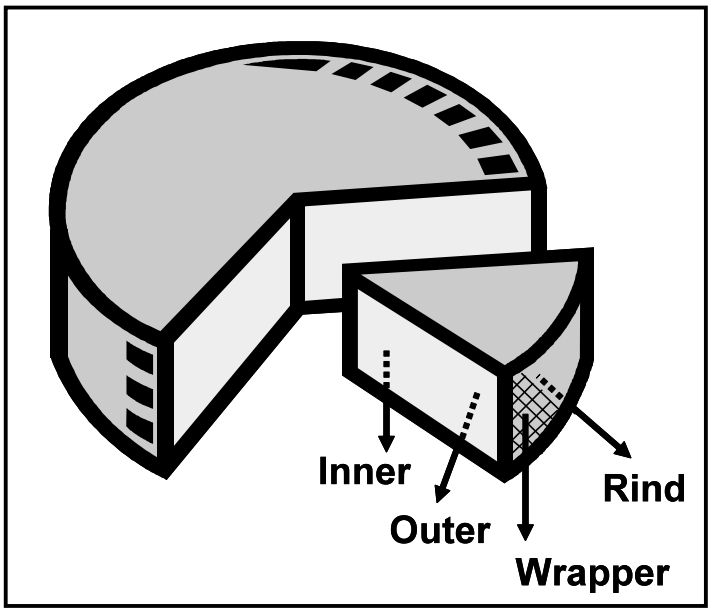

Figure 1. Schematic of Farmstead Cheddar cheese sampling used for descriptive sensory and instrumental analyses. Rind was the dark-yellow side section of about 0.5 cm thick from the outer border of the cheese wedge; inner was the intersection of the cheese wedge 1 cm away from the top and bottom rind; outer was the section located 1 cm away from the side, top and bottom rind; wrapper was the bandage cloth used during cheese ripening.

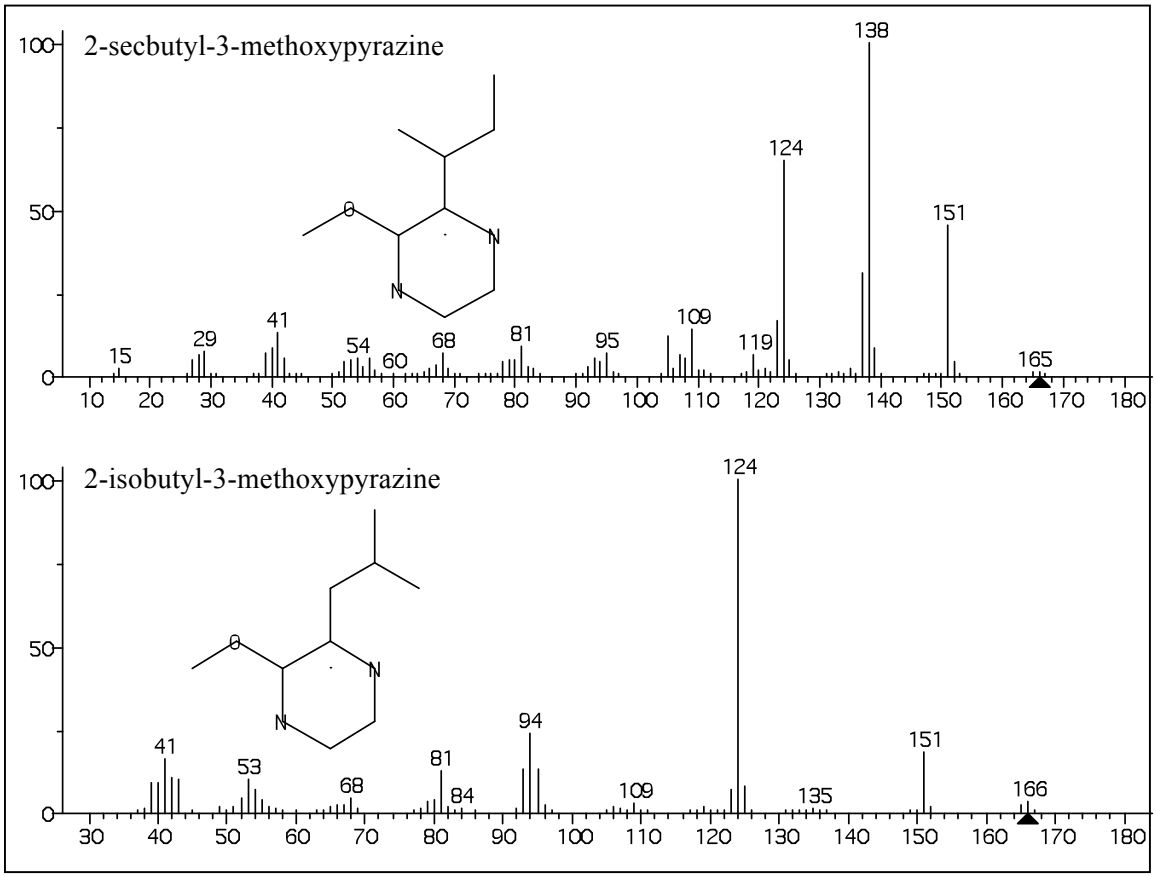

Figure 2. Chemical structure and mass spectra of 2-secbutyl-3-methoxypyrazine and 2-isobutyl-3-methoxypyrazine.

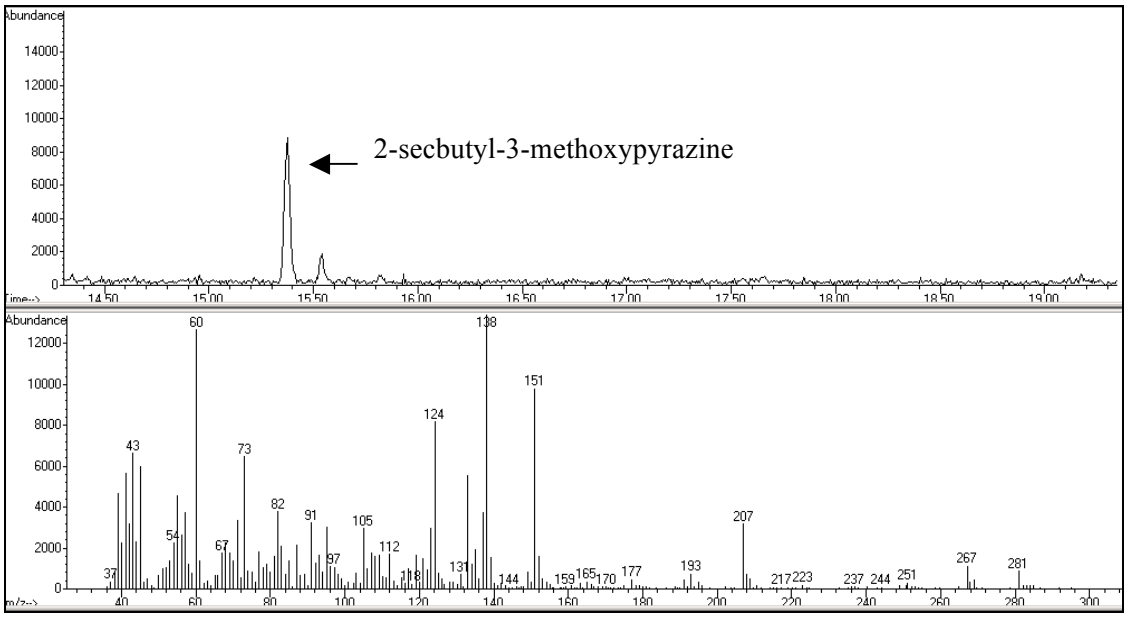

Figure 3. Chromatogram and mass spectra of eluting compounds obtained by extracting the largest ion peak (m/z=138) of 2-secbutyl-3-methoxypyrazine.

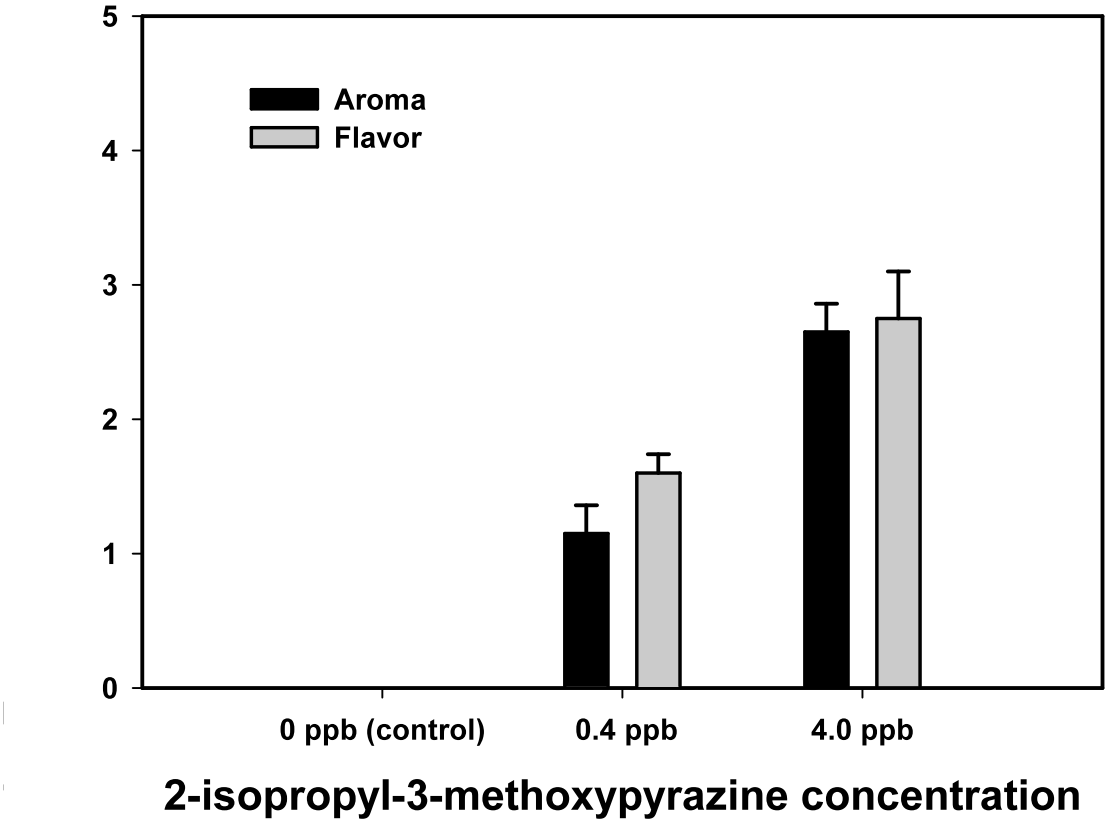

Figure 4. Impact of the addition of 2-isopropyl-3-methoxypyrazine on flavor and aroma of model cheeses. Error bars represent the standard deviation of panel means from duplicate analyses.

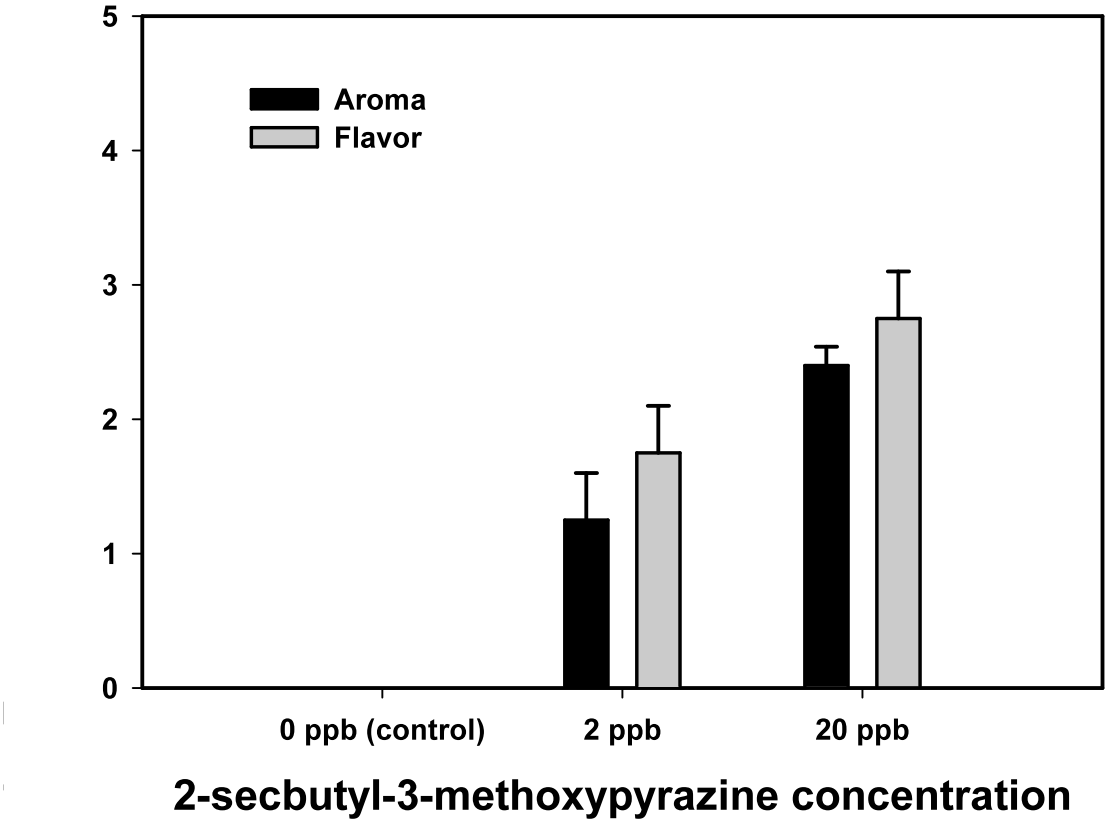

Figure 5. Impact of the addition of 2-secbutyl-3-methoxypyrazine on flavor and aroma of model cheeses. Error bars represent the standard deviation of panel means from duplicate analyses.

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APPENDICES

APPENDIX 1

Tables 1-20 comprise data from GC/O analysis of roasted peanuts from five maturity classes. Roasted peanuts were extracted using solvent-assisted flavor evaporation (SAFE), followed by phase separation into neutral/basic and acidic fractions. Each fraction was evaluated on two columns, DB-5MS and DB-WAX, by two experienced sniffers. Aroma description, retention time and intensity score for each odorant represent the average among 4 replications (2 sniffers x 2 extractions).

Table 1. GC/O data obtained from the neutral/basic fraction of black roasted peanuts on a DB-5MS column

Retention Index	Retention time (s)	Intensity Score	Aroma description
<600	1.86	1.25	malty/fishy
<600	2.03	1.50	malty
603	2.26	2.13	buttery/malty
613	2.38	1.83	onion/garlic/fishy
651	2.82	1.50	fruity
656	2.88	2.38	malty/sweet/chocolate
669	3.04	1.50	vinegar
676	3.12	1.50	fishy
691	3.29	1.33	sweet/malty/cotton candy/buttery
705	3.53	1.88	buttery/burnt
732	4.18	1.75	rubbery/vomit/sickening/fishy/green
751	4.64	1.00	sulfur
763	4.92	1.50	plastic/cooked
771	5.12	1.75	geranium/plastic/rubbery
784	5.44	2.00	garlic/onion/brothy
794	5.66	2.25	floral/green/grassy/immature nuts
803	5.88	2.17	onion/garlic/potato
807	5.98	2.00	potato/plastic/geranium
818	6.21	1.50	fishy/garlic
822	6.30	1.00	brothy/onion
836	6.63	1.25	chocolaty/malty/sweet
850	6.92	1.00	fruity
864	7.24	2.00	gasoline/rubbery
864	7.24	2.00	rubbery
867	7.31	3.00	vitamin/metallic/cooked/nutty/gasoline
868	7.33	1.50	roasted nuts
877	7.54	2.00	gasoline
890	7.82	1.50	rubbery
896	7.96	1.50	brothy
903	8.10	2.25	fatty

Table 1. Continued

905	8.16	3.00	sweaty/sweet/popcorn
913	8.33	2.63	potato/burnt/coffee//brothy
925	8.58	2.75	popcorn/toasted/fatty/cooked
941	8.93	1.83	green/mint
946	9.06	1.50	popcorn
952	9.17	2.17	cooked/green/nutty/peanut butter/sweaty
956	9.27	1.75	earthy/onion/garlic/fruity
965	9.46	1.75	nutty/brothy/licorice/cheesy
975	9.69	2.00	brothy
976	9.72	2.38	garlic/sulfur/onion
983	9.86	2.50	mushroom
1002	10.27	1.00	hay
1005	10.34	2.75	fruity/fatty/stale/popcorn/corn nuts
1010	10.44	2.50	fruity
1016	10.56	2.50	stale/corn nuts/roasted/candy/popcorn
1018	10.60	1.50	mushroom
1019	10.63	2.75	geranium/plastic/earthy/stale/mushroom
1034	10.93	2.17	cooked/popcorn/fishy/fatty/corn nuts/roasted
1039	11.04	2.00	cooked/brothy/geranium/mint
1047	11.19	3.00	geranium/cooked/fruity/rubbery
1050	11.26	1.50	brothy
1056	11.39	2.17	nutty/roasted/spicy/cooked
1061	11.49	2.88	rosy/floral
1072	11.71	2.40	spicy/cooked/rosy/floral/herbal/licorice
1079	11.85	2.00	nutty/popcorn/doughy/brothy
1085	11.98	1.50	popcorn
1091	12.09	2.00	fruity/popcorn/brothy
1096	12.21	2.83	solvent/nutty/earthy/bell pepper
1100	12.29	2.75	peanuts/fresh peanuts/peanut butter
1102	12.32	1.00	cooked/brothy/burnt
1108	12.44	2.25	fruity/popcorn/sweet
1110	12.47	2.67	roasted/peanuts cooked/sweet
1113	12.54	2.50	fruity/earthy/sweet/hay
1113	12.54	2.00	solvent/pungent/alcohol
1117	12.61	1.00	brothy/roasted/sweet
1118	12.63	2.75	fatty/cucumber/cotton candy/sweet/maple syrup
1128	12.82	2.00	cooked/nutty/roasted/fruity
1143	13.10	2.88	phenol/bandaid/barny/animal/public restroom
1160	13.43	2.38	fruity/cukes/fatty
1164	13.50	2.00	earthy/dusty
1165	13.52	2.00	mothball/hay
1172	13.66	1.75	earthy/cucumber/dough
1176	13.74	2.33	fruity/stale/spicy/peppers/earthy/dusty/hay

Table 1. Continued

1187	13.94	2.50	medicinal/licorice
1191	14.02	1.50	bell pepper
1204	14.28	2.00	cukes/fruity/fatty/sweet/citrus
1210	14.38	2.67	licorice/hay/mint/bandaid/barny
1217	14.49	3.00	licorice
1238	14.87	2.25	licorice/hay/lipstick/stale/spice
1245	14.99	1.50	mothball
1262	15.30	1.50	stale
1280	15.62	1.25	coconut/stale/earthy/dusty
1286	15.73	2.13	coconut/stale/fecal/mothball
1297	15.92	1.50	licorice
1301	16.00	2.50	sickening/stinky/toasted/solvent/burning leaves
1313	16.19	2.00	solvent
1320	16.31	2.00	rosy/honey/medicinal
1333	16.53	2.38	phenol/spicy/l/sickening/burning leaves
1344	16.72	2.17	rosy/medicinal/flowers/licorice
1366	17.09	2.33	sickening/vitamin
1378	17.29	2.00	coconut/toasted
1379	17.30	2.00	mothball/sickening/stale/burning leaves
1397	17.60	1.50	corn nuts roasted
1399	17.64	3.00	honey
1408	17.78	2.00	licorice
1415	17.89	2.75	fatty/cooked/stale/metallic/sickening
1445	18.37	1.50	cooked
1472	18.79	2.00	fecal/stinky/sickening animal (same)/garlic/hay
1513	19.44	1.25	nutty/cooked/sweet/coconut/canned corn

Table 2. GC/O data obtained from the acidic fraction of black roasted peanuts on a DB-5MS column

Retention Index	Retention time (s)	Intensity Score	Aroma description
602	2.25	1.50	buttery
616	2.42	1.50	brothy/fishy
652	2.84	2.00	malty/chocolaty/sweaty/plastic/cooked
663	2.97	2.25	vinegar/sour
689	3.27	2.50	spicy vinegar
698	3.38	1.00	buttery
710	3.65	1.25	buttery
717	3.82	1.00	plastic
733	4.20	1.67	sulfur/green/vomit/sickening
753	4.68	1.50	stinky/fruity/rubbery/fishy/green
762	4.91	1.00	sulfur/onion
772	5.13	1.50	plastic/nutty
783	5.41	1.00	fruity
795	5.69	1.63	green/grassy/plastic/raw/green nuts
816	6.17	1.00	plastic/raw/green
839	6.68	2.17	vomit/cheesy
854	7.02	2.00	sweaty
865	7.26	2.38	cooked/nutty/vitamin/rubbery/plastic/solvent
871	7.40	2.33	rubbery/sickening/latex/tasted/burnt/sweaty
895	7.94	2.38	cheesy/sweaty/stinky feet/brothy/fatty/sour
904	8.13	2.50	sweaty/cheesy/vomit/musty/brothy
909	8.25	1.83	rubbery/plastic/burnt
914	8.35	2.38	burnt/potato/brothy
926	8.62	2.25	popcorn/burnt
944	9.01	2.00	sweaty shirt
968	9.53	1.00	vomit
979	9.76	1.50	fishy
973	9.65	1.00	earthy/mushroom
977	9.72	2.00	mushroom/earthy
982	9.85	2.25	mushroom/earthy
992	10.06	2.00	burnt toast
999	10.21	1.00	fruity
1008	10.39	1.75	fruity
1010	10.44	2.00	earthy/fruity/sweet rosy
1016	10.57	2.00	peanut butter/roasted/nutty/fruity
1037	10.99	1.83	cooked/brothy/veggie/burnt sugar/popcorn
1047	11.20	2.25	plastic/fruity/rubbery/sweet/burnt
1059	11.44	2.88	rosy
1068	11.62	1.50	sweet
1088	12.04	2.00	cooked/sweet

Table 2. Continued

1098	12.24	2.50	sugar/cotton candy/
1092	12.13	2.67	earthy/bell pepper/burnt/cucumber
1100	12.29	2.38	peanuts/roasted
1111	12.50	2.25	burnt/alcohol/rotten/wet dog
1133	12.91	2.00	sweaty/sweet/burnt
1148	13.20	2.17	dirty/barny/animal/dirty restroom/hay
1149	13.22	1.00	plastic
1161	13.46	2.00	fruity/nutty/fatty
1173	13.68	2.00	lipstick/waxy
1187	13.95	2.50	solvent/pepper
1198	14.16	1.50	smoky
1208	14.34	1.83	fruity/sweet/floral/licorice/fatty/cucumber
1217	14.49	2.17	hay/mothball/licorice/fatty
1239	14.89	1.25	coconut/stale coconut
1251	15.11	2.00	sweet
1259	15.24	1.00	plastic
1269	15.43	1.00	burnt/roasted
1285	15.71	1.50	maple/curry
1300	15.98	1.50	burnt/plastic
1297	15.92	2.00	mothball/hay
1312	16.19	2.50	medicinal
1324	16.39	2.17	dirty restroom/sickening/hay/ mothball
1312	16.19	2.50	medicinal
1377	17.27	1.50	cooked/nutty/burnt/roasted peanuts
1431	18.14	1.50	stale coconut
1459	18.58	2.00	animal/hay
1532	19.71	1.50	cooked
1549	19.96	2.17	coconut/cooked

Table 3. GC/O data obtained from the neutral/basic fraction of brown roasted peanuts on a DB-5MS column

Retention Index	Retention time (s)	Intensity Score	Aroma description
<600	1.78	1.25	spice/garlic/brothy
<600	2.04	1.50	malty/onion
602	2.25	1.88	buttery
615	2.41	1.83	onion/garlic/fishy/sulfur
623	2.49	1.50	buttery/green
653	2.85	2.63	malty/chocolaty/sweaty
666	3.00	1.88	garlic/onion/brothy/fish/ranch dressing/herb
678	3.14	1.50	buttery/sweet
696	3.36	1.25	stale/nutty/fresh
704	3.49	2.33	buttery/sweet/mayo/fatty/herbs
712	3.70	1.75	buttery/fresh nutty
729	4.11	1.50	sweet/rubbery/burnt plastic
734	4.23	2.50	immature/putrid/vomit/sickening
763	4.93	1.50	plastic/toasted/green/fruity/onion/grassy/cooked
771	5.11	2.00	burnt plasctic/rubbery/plastic/nutty/fruity
773	5.17	1.50	cooked
792	5.64	2.00	green/grassy/fruity/apple
796	5.71	2.00	immature nuts/rubbery
803	5.89	1.75	garlic/brothy/onion/sweaty
810	6.05	2.00	immature nuts/rubbery
817	6.19	2.25	brothy/onion/veggies
837	6.65	1.50	fruity/sweet/green
866	7.28	2.88	sour/medicinal/vitamin/cooked nutty/pungent
892	7.86	1.50	rubbery/sweet/buttery
897	7.97	1.75	onion/garlic/brothy/sulfur
903	8.10	2.00	fatty lingers
904	8.14	2.63	sweaty/nutty/grassy
912	8.31	3.00	potato/spicy/brothy/burnt coffee/expresso beans
924	8.57	2.75	popcorn/toasted/fatty
942	8.97	1.50	minty
947	9.08	4.00	rubber/harsh
952	9.19	2.00	green
949	9.12	1.50	garlic/onion/brothy/barny
954	9.23	2.25	brothy/onion/veggies/garlic/sulfur/spicy/sweet
959	9.34	2.00	garlic/sulfur/spicy
972	9.62	1.50	cooked
976	9.71	2.75	rubbery/garlic/onion/brothy/sulfur
983	9.85	2.38	brothy/garlic/mushroom/fruity
991	10.04	1.83	mushroom/earthy/plastic ?
997	10.16	2.00	cooked/brothy/meaty/body odor/barny

Table 3. Continued

1005	10.33	2.50	fruity/corn nuts/fatty/citrus/sweet
1016	10.56	1.50	corn nuts//burnt/stale nutty/cooked corn nuts
1021	10.67	2.17	rubbery/plastic/fresh granola nutty/fatty/harsg
1032	10.88	2.00	popcorncheesy/meaty/brothy roasted meat
1040	11.05	1.50	brothy/cooked
1047	11.19	1.75	rubbery?/ granola nutty
1057	11.40	2.25	nutty/fruity/cooked/spicy green
1061	11.49	3.13	rosy/floral
1073	11.73	2.00	nutty/doughy/peanut butter/cooked nuts
1076	11.80	2.00	cooked/roasted/garlic
1086	12.00	2.38	fruity/corn nuts/sweet
1093	12.15	1.50	detergent
1095	12.18	2.63	earthy/bell pepper/peanut butter/nutty
1102	12.33	2.00	fruity/toasted/burnt/cooked peanuts
1106	12.41	2.00	cukes/fatty/citrus
1111	12.50	2.88	maple syrup/sweet/burnt/soy sauce/fatty/citrus
1127	12.81	1.50	cooked/nutty
1141	13.08	2.88	phenol/barny/public restroom/bandaid/burnt
1155	13.35	2.00	cucumber/wax/burnt
1160	13.44	2.00	fruity/stale
1176	13.73	2.17	fruity/stale/lipstick/earthy/dusty
1177	13.76	2.00	potato brothy/ sulfur (boiled potatos)
1185	13.91	3.00	roasted/spicy/licorice/hay/medicinal
1205	14.29	2.50	doughy
1209	14.36	2.25	fatty
1210	14.37	2.33	minty/hay/animal/mouthball
1229	14.71	1.50	fruity
1231	14.74	1.50	hay/animal/stale
1237	14.85	2.00	licorice
1251	15.11	1.00	hay
1265	15.36	1.50	mouthball/hay/phenol/stale/nutty
1276	15.55	1.00	garlic
1281	15.64	2.25	green tea/coconut lingers/earthy/dusty/licorice
1289	15.78	1.00	doughy
1290	15.79	2.75	barny/mouthball
1300	15.97	2.50	sickening/stinky/oatmeal/mettalic
1310	16.14	2.17	stinky/medicinal
1315	16.23	2.17	sickening/phenol
1323	16.36	1.75	stale fatty/spicy
1332	16.51	2.00	medicinal/spicy curry
1340	16.66	1.75	mouthbal/licorice/doughy/rubbery
1350	16.81	2.00	floral/stale/mouthball
1360	16.98	2.17	medicinal/licorice/cinnamon/spices/stale/animal

Table 3. Continued

1378	17.29	2.00	licorice/sickening/nutty/cooked
1388	17.46	1.50	fatty
1398	17.63	1.00	sweet
1405	17.74	2.50	sickening/stale/mettalic
1412	17.84	1.00	fatty
1469	18.74	3.00	cooked cereal/ animal
1481	18.94	3.00	canned corn

Table 4. GC/O data obtained from the acidic fraction of brown roasted peanuts on a DB-5MS column

Retention Index	Retention time (s)	Intensity Score	Aroma description
<600	2.02	1.00	buttery
604	2.27	1.67	buttery/fishy
616	2.41	1.63	brothy/garlic/onion/buttery
654	2.86	2.25	malty/sweet/chocolaty
658	2.91	1.75	vinegar
668	3.02	2.00	sour/onion/cooked/garlic
682	3.19	1.50	sour/vinegar
705	3.52	1.33	buttery/cooked butter/sweet
712	3.68	1.50	cheesy/buttery/burnt/sweet
734	4.22	2.25	sulfur/nutty/vomit/sickening
752	4.66	1.83	onion/sulfur/smoky/vomit/brothy
758	4.80	2.00	raw/immature
772	5.15	1.00	sulfur/green
772	5.13	2.00	plastic/sharp
791	5.59	1.00	fruity
794	5.68	2.25	brothy/cooked/skunk/grassy/raw/green nuts
812	6.08	1.50	brothy/cooked
822	6.30	1.50	fruity/sweet
838	6.67	2.10	vomit/cheesy
865	7.28	2.38	toasted/burnt/cooked/nutty/solvent/rotten fruit
872	7.41	1.75	cashews/green nutty/burnt green nuts
892	7.86	1.83	cooked/skunk/rubber/brothy/sweet/earthy
900	8.03	2.63	cheesy/sweet/vomit/sweaty
913	8.34	2.70	potato/coffee/burnt beans/spicy potato
927	8.63	1.75	popcorn
947	9.08	1.50	pungent/sour
960	9.35	1.00	dusty/earthy
977	9.72	2.67	mushroom/garlic/onion
983	9.86	2.63	earthy/mushroom
994	10.09	1.50	earthy/cooked
1000	10.24	1.50	fruity
1004	10.31	1.50	sweaty
1006	10.36	2.50	fruity/sweet popcorn/corn nuts
1014	10.52	1.75	fruity/nutty/earthy
1023	10.70	1.50	rubbery
1022	10.68	2.00	rosy
1028	10.80	2.50	rosy
1037	10.99	2.00	cooked/brothy/popcorn/rosy
1047	11.20	2.50	plastic/rubber/geranium
1054	11.35	2.00	sour

Table 4. Continued

1060	11.46	3.00	herbal/rosy/floral
1072	11.72	3.00	stink public restroom
1090	12.08	2.13	fruity/corn nuts
1095	12.18	2.63	earthy/bell pepper/green/stale
1105	12.39	2.67	maple/sweet/burnt/
1110	12.48	3.50	smoke/soy sauce/sulfur/burnt/match
1144	13.13	2.00	mothball/animal/sweaty
1164	13.52	2.88	spicy/sweet/burnt/green/herbal/public restroom
1176	13.73	2.00	carpet/fatty
1188	13.98	2.13	solvent/stale/spicy curry/sour/bell pepper
1216	14.49	1.63	mothball /hay/mint/herbal/roasted nuts
1234	14.81	1.75	medicinal/fruity/sweet
1285	15.71	1.50	mothball/hay
1298	15.94	2.17	sickening/stale/burnt/nutty
1306	16.08	2.75	medicinal
1315	16.24	2.50	mothball/licorice
1352	16.85	2.88	stale/stinky/metallic/stale/hay/sickening
1351	16.84	2.25	medicinal/rubber
1369	17.14	2.25	curry/burnt smoky roasted meat/floral/green
1429	18.12	1.50	floral
1447	18.41	1.75	grape/tortilla/floral/stale coconut
1469	18.75	2.50	hay/mothball
1480	18.92	2.50	sickening/stale
1545	19.90	1.50	metallic
1510	19.39	1.00	sweet
1564	20.20	2.25	coconut/stale coconut

Table 5. GC/O data obtained from the neutral/basic fraction of orange B roasted peanuts on a DB-5MS column

Retention Index	Retention time (s)	Intensity Score	Aroma description
<600	1.77	1.50	sulfur/garlic/malty
<600	2.02	1.50	malty
<600	2.10	1.00	brothy
603	2.26	2.17	buttery
612	2.37	2.00	onion/garlic
653	2.85	2.38	malty/chocolaty/fatty/sweet/bready
669	3.03	1.50	garlic/sour/cabbage
678	3.14	1.50	malty/chocolate
683	3.20	1.50	malty/sweet
691	3.29	2.00	brothy/veggies/garlic/onion
700	3.41	1.50	vitamin
705	3.53	1.75	buttery
706	3.55	1.00	green beans
708	3.59	1.50	rubbery
712	3.68	1.50	garlic
722	3.93	1.33	cooked/smoky/garlic/sulfur/cabbage/immature
734	4.22	2.00	sour/stinky/skunky/vomit/rubbery/green/sulfur
739	4.35	0.50	green bean/rubbery
745	4.45	1.00	salty rubbery
750	4.62	1.83	brothy/cabbage/sulfur/vomit//garlic/green
751	4.64	0.50	green/grassy/herbal
752	4.66	0.50	onion
760	4.86	1.50	bandaid
764	4.95	2.13	rubbery/sour/fishy/onion/plastic/chemical fridge
770	5.10	2.00	rubbery/plastic/fishy/fatty
781	5.35	1.00	cheesy/refrigerator/stale
783	5.40	1.00	fish/sulfur
786	5.49	0.50	green/onion
787	5.51	0.50	onion
788	5.52	1.50	rubbery
793	5.64	2.00	green/grassy/raw/fruity apple
797	5.74	1.50	stale
801	5.83	2.00	onion/garlic/sweaty
814	6.13	2.25	onion/garlic/brothy/skunk/fishy/rubbery/stale
827	6.42	0.50	sweaty/sulfur
844	6.81	1.75	fruity/popcorn/burnt/sweet/sweaty
850	6.93	1.50	poop/stinky
866	7.30	3.00	vitamin/sour/rotten fruity /skunky/cinnamon
868	7.34	1.00	burnt/cooked nutty

Table 5. Continued

873	7.44	2.50	body odor
876	7.51	1.00	gasoline
891	7.85	2.75	rubbery/plastic/sharp/pungent
893	7.89	2.00	onion/garlic/fishy/sweaty
893	7.89	0.50	coconut
903	8.10	1.83	sweet/fatty/fruity/grassy
905	8.16	3.25	sweaty
908	8.21	3.00	roasted nut
909	8.24	2.50	brothy
913	8.32	2.88	potato/sweaty/burnt/ coffee beans/smoky
923	8.55	2.75	popcorn/cheesy
942	8.97	1.83	corn/fruity/mint/sweaty
949	9.12	1.83	sulfur/onion/garlic/popcorn/body odor/vitamin
956	9.26	0.50	cucumber
962	9.40	2.13	potato/brothy/bell pepper/green/corn/oil/rancid
966	9.49	1.50	fruity
975	9.69	2.75	onion/garlic/burnt /mushroom
982	9.84	1.25	fruity/popcorn
984	9.89	2.00	fruity
985	9.91	2.50	mushroom/metallic
989	9.99	1.67	rubbery/plastic/sweet/tart/citrus
995	10.11	2.00	brothy/veggies/burnt/citrus/sweaty
1002	10.27	2.00	rubber
1006	10.35	3.00	fruity /popcorn/corn nuts/citrus/fatty
1011	10.45	1.00	plastic/fruity
1015	10.53	2.00	burnt
1022	10.68	2.50	plastic/lipstick
1024	10.72	2.00	toasted/roasted
1028	10.81	1.50	roasted
1032	10.89	2.13	popcorn/cooked popcorn/toasted/cooked brothy
1033	10.91	2.50	peanut/nutty
1038	11.02	1.50	fruity
1041	11.08	1.50	sulfur
1049	11.23	2.00	nutty/roasted/cabbage/rubbery/earthy/brothy
1054	11.34	2.00	curry/fruity
1061	11.48	3.00	rosy/fatty
1072	11.70	2.00	nutty/doughy/fruity
1079	11.86	1.50	nutty/brothy
1082	11.91	2.50	doughy/burnt
1088	12.04	2.25	fruity/fatty
1095	12.18	2.75	earthy/bell pepper/nutty/fruity/cukes
1095	12.19	2.00	sweet/molasses
1103	12.35	1.75	nutty/roasted/brothy

Table 5. Continued

1104	12.36	2.25	fruity
1109	12.47	3.00	toasted peanuts/peanut/nutty/peanut butter
1111	12.49	2.38	maple syrup/sweet/nuts/soy sauce/fatty/toasted
1116	12.59	2.00	fruity
1122	12.71	1.50	peanut butter/popcorn/creamy
1128	12.82	2.00	pepper
1133	12.92	2.00	smoky/plastic/alcohol/earthy
1134	12.94	1.50	cooked
1136	12.98	2.00	sweet/maple
1141	13.07	1.50	fatty
1143	13.10	3.13	bell pepper/fatty/mothball/hay/sweaty/bad odor
1148	13.21	1.00	medicinal
1149	13.23	2.00	lipstick/stale
1157	13.37	2.00	roasted
1159	13.42	2.00	green cucumber/floral/fatty/fruity
1161	13.46	1.00	earthy
1167	13.57	1.50	chocolate
1171	13.65	1.00	waxy/fatty/nutty
1174	13.70	2.00	cucumber/doughy/earthy /carpet fatty/nut
1181	13.84	2.00	mothball/garlic/spicy/licorice/barny
1186	13.93	2.67	licorice/mothball/peppers/banana jalapeño
1197	14.14	2.00	malty
1201	14.21	2.00	rubbery/plastic
1208	14.34	2.67	licorice/hay/mothball/mint/tortilla/ animal/nutty
1208	14.35	2.50	cucumber/doughy/tomato/fatty/ brothy/nutty
1218	14.52	2.50	cardboard/earthy/jalapeno pepper/rosy
1223	14.61	2.50	curry
1232	14.78	1.75	doughy/burnt/sweet/hay/tortilla
1237	14.85	2.17	licorice/grape/tortilla
1245	14.99	1.00	metallic/beany
1251	15.11	2.00	nutty
1264	15.33	2.00	mothball/floral
1273	15.50	1.67	earthy/green
1283	15.67	2.00	brothy/spice/doughy/fatty/fruity/oatmeal/nutty
1288	15.77	2.50	barny/animal/mothball/hay/
1301	15.99	2.00	licorice/medicinal
1309	16.13	2.25	sickening/stale/spicy/fatty/phenol
1324	16.38	2.67	medicinal/public restroom/animal/tortilla/soil/
1338	16.61	2.25	licorice
1346	16.75	2.00	sweet tortilla hay/rosy/fish
1367	17.11	2.17	fatty tortilla floral cloves/sickening /doughy
1377	17.27	1.50	fatty
1387	17.43	1.00	animal

Table 5. Continued

1394	17.55	1.00	plastic
1398	17.63	1.50	mint
1403	17.70	2.17	sickening/licorice/metallic/mint/animal/beefy
1409	17.80	1.00	mothball/mint
1416	17.91	2.25	sickening/metallic
1431	18.15	2.00	medicinal
1457	18.56	1.00	tortilla
1467	18.71	1.50	roasted/doughy
1469	18.74	1.50	cooked mothball
1510	19.39	1.00	grapey/tortilla
1514	19.45	1.00	burnt/popcorn
1522	19.57	1.50	corn/stale/cooked corn/tortilla
1530	19.68	1.50	licorice
1633	21.20	1.00	nutty
1648	21.40	1.50	sulfur

Table 6. GC/O data obtained from the acidic fraction of orange B roasted peanuts on a DB-5MS column

Retention Index	Retention time (s)	Intensity Score	Aroma description
<600	2.03	1.25	malty/chocolaty
604	2.28	2.00	buttery
612	2.37	1.67	brothy/veggies/sulfur
653	2.85	1.88	malty/sour/sweaty
660	2.93	2.17	vinegar/sour
684	3.21	1.67	cheesy/brothy/ranch dressing/mayo
700	3.41	1.50	brothy/toasted/nutty
705	3.53	2.00	buttery
729	4.09	1.00	garlic faint
734	4.23	1.75	sulfur/brothy/vomit
752	4.66	2.38	onion/garlic/fishy/sour/rotten fruity
756	4.75	2.50	sulfur/cabbage
778	5.29	1.00	roasted
793	5.65	2.00	green/grassy
797	5.74	1.50	fruity
796	5.72	1.50	onion/brothy
804	5.91	1.50	green beans/garlic/onion
816	6.17	2.00	spicy brothy
836	6.62	2.25	vomit/cheesy/sweaty
848	6.88	2.20	cheesy/FFA/garlic/sweaty
860	7.16	3.00	solvent/rubbery/sour/toasted
867	7.31	2.50	roasted/cooked/nutty/potato brothy
872	7.42	2.50	burnt/roasted meat/garlic
892	7.86	1.50	plastic
892	7.87	2.33	cheesy/vomit/sweaty
896	7.96	2.75	spicy brothy/sweaty/wet dog
907	8.20	2.33	vomit/earthy/sweaty/rubber
913	8.34	2.75	potato/spicy/coffee
927	8.64	2.00	corn chip
939	8.89	2.50	dirty/moldy
951	9.15	1.50	vomit/cheesy/sour
970	9.57	1.00	fruity
978	9.76	2.00	brothy/veggies/garlic/onion
984	9.87	2.38	mushroom
1000	10.24	1.50	nutty/plastic
1008	10.39	2.25	fruity/corn nuts
1015	10.55	2.00	nutty/roasted/toasted/brothy nutty/popcorn
1023	10.70	2.00	rosy
1033	10.91	1.50	spicy brothy/cheesy/popcorn
1039	11.02	2.33	popcorn

Table 6. Continued

1048	11.21	1.75	rubber
1056	11.38	1.50	rubbery
1060	11.47	2.88	rosy/floral
1066	11.58	2.00	green rose/sweet/fruity
1068	11.63	2.00	public restroom
1083	11.94	1.50	cooked/burnt
1089	12.06	2.13	fruity/corn nuts
1095	12.19	2.13	earthy/bell pepper/green/cukes
1100	12.29	2.50	roasted nuts
1101	12.31	1.75	fruity/burnt/popcorn
1107	12.43	2.63	sweet/maple syrup/burnt/soy sauce
1126	12.78	2.00	sweet/burnt
1139	13.03	3.50	public restroom
1149	13.22	2.17	mothball/hay/sweaty/burnt/cooked meat
1159	13.41	2.25	rubbery/toasted/stinky/sweet
1169	13.61	1.50	toasted
1187	13.95	1.83	toasted/spicy/curry/medicinal
1200	14.20	2.00	hay/mothball
1216	14.49	1.50	brothy
1238	14.87	1.63	lipstick/waxy/sweet/medicinal/licorice
1250	15.09	1.75	brothy/plastic/nutty
1261	15.29	1.00	medicinal/fruity
1277	15.57	1.50	brothy/nutty/roasted
1286	15.73	2.00	coconut
1312	16.18	2.67	licorice/mothball/medicinal
1331	16.50	2.50	stinky/sickening/stale
1342	16.68	2.33	medicinal
1352	16.86	2.67	sickening/stale/rosy
1363	17.04	2.50	medicinal/mothball/licorice/animal/brothy
1431	18.14	2.88	sickening/stale/hay/metallic
1495	19.16	2.00	mothball/fatty/dusty floral
1559	20.12	1.50	sweet/caramel
1591	20.59	1.00	coconut/sweet

Table 7. GC/O data obtained from the neutral/basic fraction of orange A roasted peanuts on a DB-5MS column

Retention Index	Retention time (s)	Intensity Score	Aroma description
<600	1.99	1.50	malty
600	2.23	1.00	mint
603	2.26	2.50	buttery/garlic
611	2.36	1.67	garlic/sulfur/brothy/vinegar
622	2.48	1.00	mint/malty
627	2.54	2.00	buttery
653	2.84	2.83	sweaty/malty/stinky feet
667	3.02	2.00	onion/fresh onions/garlic/ranch dressing
697	3.36	1.50	plastic
701	3.43	1.25	Rubbery/plastic/fresh nuts
701	3.43	2.00	buttery
705	3.53	2.33	buttery
719	3.87	1.67	rubbery/nutty/cabbage/bready/toasted/rubber
723	3.97	2.25	skunky/garlic/vomit/rye
731	4.16	1.75	sour/stale/chest nuts/immature/green
736	4.28	2.00	vomit
741	4.40	0.80	rotten fruit
752	4.65	2.33	cabbage/sulfur/garlic/garbage/sour
763	4.92	2.00	rubbery
765	4.97	2.17	brothy/onion/garlic/pungent/garlic/plastic/nutty
774	5.20	1.00	brothy
792	5.62	2.75	green/grassy
796	5.73	2.50	vinegar/sweaty
802	5.87	2.00	brothy/onion/garlic/herbs/sweaty/grassy
815	6.15	1.50	garlic/sour/rubbery/rotten fruit/green herbs
838	6.67	1.00	citrus
842	6.76	2.00	fruity/sweet/fishy/citrus/fatty
848	6.88	2.00	earthy/popcorn/cheesy/fatty/sweet
863	7.23	1.75	earthy
867	7.32	3.00	sour/vitamin/rotten fruit/cooked nutty
890	7.82	2.50	plastic/rubber/cooked/burnt/rubbery
896	7.95	2.67	onion/garlic/burnt cukes/sweaty/fatty/sour cloth
904	8.13	1.75	fruity/earthy
906	8.17	2.50	sweaty/nutty/garlic/brothy
909	8.25	1.50	fruity
913	8.33	2.83	potato/burnt coffee beans/beans/nutty/roasted
914	8.35	3.00	spicy potato
925	8.59	2.33	popcorn/corn nuts
938	8.89	1.50	medicinal/mint
948	9.10	1.50	green/nutty

Table 7. Continued

949	9.12	2.50	brothy/popcorn/cheesy/vomit
953	9.20	2.00	garlic/sulfur/onion/popcorn/roasted/nutty
957	9.30	1.75	earthy/bell pepper/paprika
961	9.38	2.00	stale
964	9.45	2.50	potato/brothy/earthy
974	9.66	2.75	garlic/brothy/popcorn/onion
978	9.74	3.25	onion/garlic /mushroom/stale
980	9.80	1.00	brothy
984	9.88	3.00	mushroom
993	10.07	1.50	rubbery
996	10.14	2.25	rubber/balloons/cooked mushroom/burnt
1006	10.34	2.50	fruity/sweet/solvent/floral
1009	10.41	2.00	sulfur/body odor
1013	10.50	2.50	fruity/sweet/floral/corn nuts
1021	10.67	3.00	malty/curry
1027	10.78	1.50	malty chocolate
1032	10.90	2.17	popcorn/popcorn/cooked/brothy/earthy/corn
1042	11.10	2.00	fruity/cooked/cheese nutty/plastic/cooked/fatty
1047	11.21	2.25	rubbery/ball pen ink/solvent/plastic
1056	11.38	2.50	jalapeño pepper/medicinal
1061	11.48	3.00	rosy
1065	11.56	1.50	solvent/plastic
1070	11.67	2.00	fruity/licorice
1071	11.70	2.00	brothy
1073	11.74	2.00	floral/honey
1084	11.95	1.75	nutty/roasted/popcorn/canned corn/cooked
1086	12.00	2.50	fruity
1094	12.15	2.33	earthy/bell pepper/green/cukes/cooked beans
1100	12.28	2.00	fruity/green/nutty
1106	12.41	1.50	rosy peanuts roasted
1107	12.42	3.00	maple syrup
1113	12.54	3.00	peanuts/roasted/peanut butter nutty cooked
1116	12.59	2.00	solvent/green/nutty/carpet
1118	12.63	2.00	fruity
1128	12.83	1.00	bean sprout
1132	12.91	3.00	peanut butter/sweet/cooked/nutty
1141	13.08	2.00	nutty/cooked/bell pepper
1143	13.10	2.50	grape/musty
1150	13.24	2.75	mothball/hay/musty/phenol/barny
1158	13.39	2.00	peanut butter/nutty/cooked/sweet
1173	13.68	1.75	earthy
1178	13.78	2.25	lipstick/licorice
1178	13.79	2.50	hay/mothball/rosy/tortilla/animal

Table 7. Continued

1179	13.80	2.00	canned corn
1184	13.89	2.88	licorice/jalapeño pepper/fermented/soy sauce/
1206	14.31	2.00	apple
1210	14.38	2.67	mothball /hay/musty/sweet/nutty/cardboard
1219	14.53	2.00	tortilla/mayo/honey
1238	14.87	2.50	licorice/fruity/fatty/stale
1251	15.11	2.00	oxidized peanut oil
1269	15.42	2.00	bell pepper/jalapeño peppers
1273	15.50	2.00	earthy garlic
1277	15.56	1.50	green bell pepper
1282	15.66	2.00	cinnamon oatmeal
1289	15.78	2.25	cooked/toasted licorice/sweet/mothball/animal
1303	16.03	2.50	medicinal/perfume
1317	16.26	2.25	sickening/stale
1317	16.27	1.50	fruity
1317	16.27	2.00	licorice
1325	16.39	2.50	medicinal
1329	16.47	2.00	mothball/hay/stale/animal
1345	16.74	2.63	licorice/fresh rubber/sweet
1356	16.93	3.25	fruity/mothball/rubber/animal/medicinal/licorice
1395	17.57	3.00	sickening/metallic//licorice/stale
1531	19.70	2.50	canned corn
1554	20.04	1.00	garlic

Table 8. GC/O data obtained from the acidic fraction of orange A roasted peanuts on a DB-5MS column

Retention Index	Retention time (s)	Intensity Score	Aroma description
<600	2.02	1.25	malty
602	2.25	1.50	buttery/garlic
615	2.40	1.75	garlic/brothy/buttery
655	2.88	2.25	malty/chocolaty/sweaty/cheesy
666	3.00	2.13	vinegar/sweaty
701	3.42	1.75	rubbery/toasted/nutty/cooked nuts//sweaty
707	3.57	1.67	brothy/buttery/vomit/cooked/sweaty
734	4.23	1.75	vomit/sickening
753	4.68	2.13	onion/garlic/cabbage/sulfur
773	5.17	1.33	rubbery/plastic
794	5.67	1.67	cooked egg/brothy/earthy
806	5.94	2.00	onion/garlic
817	6.21	2.00	rubbery/green/spicy
823	6.32	2.00	brothy/onion/garlic
839	6.69	2.13	vomit/cheesy
853	6.99	2.00	vomit/cheesy/fruity/stinky/popcorn/vitamin
860	7.15	2.00	glue
867	7.30	1.75	rubbery/cooked nutty
869	7.36	2.75	solvent/sour/rotten fruity/vitamin/rubbery/
892	7.86	2.50	plastic/burnt rubber/rubber
898	8.00	2.13	vomit/cheesy/sweaty/malty/bready fatty
908	8.22	2.25	bad body odor/sweat/vomit/cheesy/b.o.
914	8.36	2.75	potato/roasted/coffee beans/sweat
935	8.80	2.17	vomit/sweaty/popcorn/cheesy/fatty/bready/
936	8.82	2.00	barny/animal/mothball
953	9.20	1.50	cheesy/vomit
957	9.29	1.00	garlic
968	9.54	1.75	stale/sour/rubbery
979	9.77	1.88	brothy/garlic/onion
983	9.87	2.63	mushroom
996	10.15	2.00	licorice/medicinal
1001	10.26	1.67	burnt/brothy/earthy brothy
1007	10.37	1.67	sweet/apples/fruity
1014	10.52	2.00	solvent fruit
1016	10.57	2.25	earthy/cooked/fatty/nutty/peanut butter
1026	10.77	2.00	plastic/sharp
1037	10.99	1.67	nutty/roasted/cooked/peanut butter
1039	11.04	2.17	old socks/sweaty/popcorn/brothy
1048	11.22	2.13	rubbery/plastic/rubber
1061	11.48	3.13	floral/rosy

Table 8. Continued

1080	11.87	1.75	animal/floral/mothball
1076	11.80	2.25	curry/meaty//beefy
1091	12.10	2.13	green/fruity
1095	12.18	2.25	earthy/cukes/green/stale/burnt//bell pepper
1100	12.29	2.00	fruity
1104	12.37	2.00	peanut butter/sweet
1108	12.43	3.00	maple syrup/sweet/curry/maple/soy sauce
1115	12.58	2.50	cotton candy/burnt/soy sauce
1116	12.60	1.50	medicinal
1147	13.18	2.25	sweaty/burnt/sweet
1159	13.42	1.75	rubbery/sweet/sweaty/cukes
1167	13.57	2.00	insect/rubbery/grilled meat/charcoal/roasted
1189	13.99	1.88	jalapeño pepper/medicinal/spicy
1238	14.88	1.50	mint/hay
1252	15.12	2.00	fatty/lipstick
1319	16.30	1.88	licorice/mothball/burnt curry
1338	16.62	2.00	stinky/sickening/mothball/sweet/licorice
1338	16.61	2.50	stinky/sickening
1341	16.67	3.00	medicinal
1384	17.39	2.63	sickening/stale/hay/metallic
1355	16.90	2.50	licorice
1364	17.05	3.00	sweet/mothball/animal
1366	17.08	3.00	medicinal
1412	17.84	2.00	sickening/metallic/mothball
1434	18.19	2.25	spicy/sulfur/egg
1473	18.80	1.50	hay/mothball/floral/animal
1557	20.08	1.50	sickening/stale
1585	20.50	1.50	sweat/wax

Table 9. GC/O data obtained from the neutral/basic fraction of yellow roasted peanuts on a DB-5MS column

Retention Index	Retention time (s)	Intensity Score	Aroma description
<600	1.73	1.50	garlic
<600	2.06	1.25	malty/garlic
603	2.26	1.88	buttery/malty
615	2.40	1.00	garlic
641	2.71	1.00	buttery
653	2.85	2.75	malty/chocolaty/cheesy/sweaty/fatty/buttery
671	3.06	2.00	garlic/onion/ranch dressing
686	3.24	1.00	plastic/nutty/glue/garlic
702	3.44	2.00	cooked/nutty/buttery
707	3.57	2.00	sour butter/malty/butter/bready
717	3.81	1.50	rubbery/creamy/earthy
723	3.95	2.00	rubbery/meaty/brothy/garlic/beefy/fruity
734	4.21	1.75	sulfur/sickening/vomit/turkey/puke
752	4.66	2.13	garbage/sour/cheesy/vomit/sour/rotten/garlic
763	4.92	2.00	spicy/earthy/rubber/plastic/pungent/solvent
765	4.98	2.00	plastic/pungent/solvent/nutty
770	5.10	2.00	plastic/fruity/harsh
784	5.43	1.17	brothy/earthy/garlic/mushroom/sulfur
787	5.50	0.50	brothy
794	5.67	2.50	grassy/green/fruity
801	5.83	2.33	brothy/sweat/egg/onion/sulfur/sweaty/sweet
805	5.93	2.00	garlic/onion
815	6.15	2.50	brothy/garlic/skunk
816	6.17	1.50	popcorn/fatty
839	6.68	1.50	plastic/nutty
843	6.77	2.17	fruity
847	6.86	1.75	earthy
848	6.90	2.00	fruity/brothy
855	7.04	1.00	cheesy
859	7.14	2.00	canned corn/cooked
868	7.33	2.63	rubbery/sour/vitamin/cooked brothy nutty
879	7.59	2.00	green/sharp/nutty/fermented nutty
891	7.85	2.38	nutty/rubbery/brothy/plastic/sweet/fresh tires
901	8.07	1.00	vinegar
903	8.11	2.30	earthy/fruity/fatty/popcorn/malty/sweaty
912	8.31	2.75	onion/potato/cooked nutty/coffee roasted meat
917	8.42	3.00	cooked/burnt/burnt bread/fatty
925	8.59	2.38	popcorn/cheesy/toasted/peanut butter
930	8.71	2.00	metallic
934	8.78	2.00	popcorn/corn chip

Table 9. Continued

937	8.85	2.00	cooked
940	8.92	1.75	mint/hay
946	9.04	1.83	brothy/cooked/nutty/sweaty/meaty/sulfur/potato
953	9.19	2.50	popcorn/cheesy /earthy/garlic/brothy/onion
957	9.28	2.00	pickles/vinegar
958	9.31	1.50	potato/brothy
959	9.34	2.13	cooked/stale/burnt cukes/mushroom/brothy
963	9.43	0.50	garlic/sulfur
972	9.61	2.00	brothy
976	9.71	2.00	mushroom/oxidized mayo
979	9.76	3.00	onion/garlic
983	9.86	2.67	mushroom
990	10.02	2.50	earthy/plastic/sour earthy mushroom
998	10.18	2.00	latex balloon/body odor/beefy/beefy
1003	10.30	0.50	fatty
1006	10.36	2.25	fruity/green/earthy
1014	10.52	1.83	earthy/fruity/fatty/stale/cheesy
1019	10.63	2.50	fruity/earthy/dusty
1022	10.69	1.50	earthy/plastic/glue
1031	10.86	2.00	popcorn/cooked//vomit/cheesy
1036	10.98	0.50	Sweet
1039	11.04	2.00	peanut butter
1041	11.07	1.50	medicine
1047	11.21	2.00	rubbery/grapey/plastic
1048	11.21	0.80	cheesy/vomit
1053	11.32	2.00	vanilla cookie/bready/cookie
1056	11.39	2.25	medicinal/jalapeño peppers/licorice
1061	11.48	3.13	rosy/hay
1064	11.55	1.00	plastic
1068	11.62	0.50	cooked/sweet
1072	11.72	2.00	licorice
1075	11.78	1.00	cheesy
1082	11.92	2.00	solvent/animal/hay
1083	11.94	1.63	popcorn/cooked/toasted
1088	12.04	2.50	fruity/sweet/cukes
1089	12.07	1.00	plastic
1093	12.15	2.75	sweet/cukes/earthy/bell pepper
1103	12.34	2.00	fruity/nutty/burnt/fatty/smoky/cukes
1109	12.45	2.88	maple syrup/soy sauce/sweet/solvent/curry
1109	12.46	3.00	peanut butter/nutty/ earthy/roasted/fresh peanuts
1117	12.62	2.00	hay/phenol/fermented soy sauce
1122	12.70	0.50	toasted/sweet/popcorn
1124	12.74	2.00	cooked corn

Table 9. Continued

1132	12.90	2.00	nutty/roasted/ peanut butter
1134	12.94	1.50	earthy
1140	13.06	0.50	popcorn/cooked
1144	13.13	2.38	mothball/hay/barny/bandaid/animal
1149	13.23	2.25	garlic/rubber/plastic
1154	13.32	2.50	curry/maple/burnt/soy sauce
1160	13.44	2.00	fruity/fatty/stale bandaid
1164	13.52	2.00	earthy/cukes/stale/tortilla/fatty
1171	13.65	1.50	molasses/fatty
1180	13.82	1.50	hay/mothball/solvent/soy/animal/vitamin
1181	13.84	1.00	peanut butter/cooked
1186	13.94	2.38	licorice /jalapeño peppers/medicine
1196	14.12	3.00	lipstick/fruity
1207	14.33	1.50	green/fruity
1212	14.41	2.38	mothball/hay/mint/barny
1222	14.59	2.00	medicine/licorice/fatty/vitamin/animal
1236	14.84	2.50	licorice/medicinal
1274	15.51	1.67	potato/cooked/toasted/fruity/cabbage
1285	15.71	1.25	fruity/sweet
1291	15.81	2.17	coconut/mothball/metallic/hay/mint/tortilla
1307	16.10	2.17	medicinal
1319	16.29	2.00	hay/mothball/phenol /potato/malty
1330	16.48	2.00	peppers/licorice
1343	16.70	2.17	licorice/jalapeño peppers
1358	16.95	2.67	tortilla/metallic/mothball/mint/licorice
1389	17.47	2.17	mothball/licorice/hay/grapey metallic stale
1402	17.70	2.38	sickening/burning leaves/grapey metallic stale
1423	18.02	2.83	sickening/burning leaves
1450	18.45	2.00	hay/mothball
1572	20.31	1.50	malty/sweaty/canned corn
1632	21.18	1.50	mothball tortilla
1646	21.38	1.50	floral tortilla mothball

Table 10. GC/O data obtained from the acidic fraction of yellow roasted peanuts on a DB-5MS column

Retention Index	Retention time (s)	Intensity Score	Aroma description
<600	2.01	1.33	malty/solvent/note ether
605	2.29	1.75	buttery/cooked/sweet
622	2.48	1.00	buttery
636	2.65	1.00	sweet/buttery
655	2.87	2.17	malty/cheesy/sweaty
668	3.02	1.50	cooked
669	3.04	1.38	vinegar/acidic
697	3.36	1.25	vinegar
704	3.50	1.50	buttery
724	3.98	1.00	fruity
734	4.23	1.17	rubbery
737	4.29	2.00	sulfur/egg/vomit
749	4.59	1.17	fruity/burnt
753	4.69	2.50	garlic/egg/cabbage/sulfur/garbage/sour/rotten
774	5.20	2.00	plastic
795	5.70	1.63	green/grassy/fruit
798	5.77	1.50	fruity
805	5.93	1.50	brothy/garlic
815	6.14	1.00	brothy
820	6.26	1.67	sweet/rubbery/meet/onion/garlic
834	6.58	1.50	garlic
838	6.66	1.00	fruity
842	6.75	2.13	sweaty/popcorn/cheesy
851	6.95	1.67	popcorn/brothy/cheesy/green
866	7.29	2.67	rubbery/plastic/sharp
871	7.39	3.13	sour/nutty/cooked/vinegar/rotten fruit/rubbery
879	7.57	1.50	onion
892	7.87	2.00	rubbery
895	7.94	2.17	cheesy/popcorn/skunk/sweaty/bready
905	8.16	2.50	sweaty/cheesy/vomit/sweaty/onion/sulfur
910	8.27	3.00	phenolic/dirty
915	8.37	3.13	potato/roasted sweet meat/dirty/garbage/socks
929	8.69	1.67	brothy/rubber/malty/cheesy
953	9.21	3.00	catty
965	9.46	1.00	cooked/brothy
972	9.61	1.50	rubbery
979	9.76	2.00	brothy/garlic/malty
983	9.85	2.00	garlic
984	9.88	2.50	mushroom/metallic
1002	10.26	1.67	fruity/catty

Table 10. Continued

1009	10.41	2.00	fruity
1016	10.56	2.00	sweet/cooked/fatty/popcorn/earthy
1027	10.79	2.00	plastic/cooked
1034	10.93	1.75	cooked/popcorn
1039	11.03	1.00	fruity/sweet
1043	11.11	1.50	brothy/cooked
1049	11.24	2.25	rubbery/plastic/almonds
1059	11.45	3.00	rosy/sweaty/nutty
1072	11.72	1.50	fruity
1088	12.05	1.63	fruity/nutty/meat/pot roast
1096	12.20	2.50	earthy/bell pepper/cukes
1099	12.27	2.67	maple syrup/floral/sweet
1106	12.40	1.50	potato/meat
1127	12.80	3.00	curry/sweet
1146	13.16	2.00	sweaty
1150	13.24	1.00	spice
1159	13.42	2.50	mothball/hay
1168	13.58	1.88	sweet/earthy/dusty/roasted/burnt/earthy/meaty
1179	13.80	3.00	sweet/beef jerky
1188	13.97	2.00	licorice/jalapeño pepper
1202	14.23	1.50	sweet/maple syrup
1303	16.03	1.00	cooked
1323	16.36	2.17	medicinal/floral
1341	16.66	2.50	sickening/stale
1345	16.74	2.50	licorice/tortilla/animal/mint
1374	17.22	2.83	sickening/stale/metallic
1361	17.01	2.17	licorice
1397	17.60	2.00	sickening/stale
1378	17.29	1.50	mothball/meaty
1500	19.24	2.00	sickening/stale
1553	20.03	1.67	canned corn l
1577	20.38	1.00	sweet/coconut
1581	20.44	2.25	sickening/stale
1641	21.31	1.00	burnt

Table 11. GC/O data obtained from the neutral/basic fraction of black roasted peanuts on a DB-WAX column

Retention Index	Retention time (s)	Intensity Score	Aroma description
716	1.50	1.00	meaty
732	1.66	2.00	buttery/sweet/malty
794	2.26	1.00	greasy noodles
814	2.42	2.10	malty/chocolaty
916	3.24	3.00	buttery
938	3.54	1.00	dirty feet
973	4.04	1.67	rubbery/plastic/ashy
998	4.38	1.50	green/grassy
1025	4.88	2.13	buttery
1050	5.36	2.14	green/grassy/melon/fruity
1068	5.70	2.00	rubbery/rubber/resin/plastic
1077	5.87	2.50	grassy
1097	6.26	1.00	sweet/fruity
1105	6.42	1.50	plastic/burnt/cooked/earthy
1120	6.73	1.50	cooked/brothy/toasted/cooked/roasted
1145	7.24	1.00	musty/fruity
1152	7.38	1.50	fishy
1157	7.48	1.00	spicy/sweet/fruity
1169	7.71	1.33	sweet/buttery/cooked
1184	8.03	1.17	stale /roasted/peanutty/fruity/brothy
1198	8.31	2.25	brothy/cooked/herb/green
1209	8.53	1.50	stinky/sickening
1221	8.76	1.50	fruity/earthy
1228	8.91	1.80	grains/sweet/grassy/herbal/green/grassy
1236	9.07	1.50	mushroom/earthy
1242	9.19	2.00	solvent/sour/rotten fruit/vitamin/rubbery
1251	9.36	1.50	fruity
1257	9.48	1.88	sweet/caramelized/cooked/brothy/sharp
1288	10.09	2.50	mushroom/earthy
1300	10.32	1.50	fruity
1307	10.47	1.75	roasted/nutty
1315	10.61	2.25	brothy
1319	10.69	2.00	popcorn
1327	10.83	2.50	popcorn/sweet
1340	11.07	2.00	brothy/sweet/popcorn
1354	11.32	2.00	brothy
1362	11.48	2.00	roasted
1365	11.53	3.00	garlic/veggies/brothy potato
1374	11.71	2.00	sulfur/veggie/skunky/musty

Table 11. Continued

1383	11.88	2.50	brothy/veggies/potato
1402	12.22	1.50	solvent/sweet
1417	12.49	2.50	roasted/nutty/burnt coffee/cooked/burnt
1436	12.80	1.93	roasted/nutty/spicy
1448	13.02	3.00	potato/ brothy
1490	13.74	1.75	earthy/dusty
1508	14.05	1.50	roasted
1515	14.17	1.00	metallic
1541	14.59	1.50	fruity/sweet (nutty)
1576	15.16	2.00	roasted/nutty
1740	17.69	3.00	nutty/sweet/chocolate/cooked

Table 12. GC/O data obtained from the acidic fraction of black roasted peanuts on a DB-WAX column

Retention Index	Retention time (s)	Intensity Score	Aroma description
745	1.78	1.17	malty
791	2.23	1.00	sweet
810	2.39	1.88	malty/chocolate
830	2.53	2.00	chocolate/roasted lingers
849	2.66	1.00	brothy
920	3.30	2.13	buttery/roasted peanuty/cooked/sweet/nutty
980	4.12	1.00	earthy
1025	4.88	2.00	buttery/sweet
1049	5.34	1.50	green/grassy
1068	5.71	1.00	brothy/dirty
1077	5.87	1.00	plastic/ashy
1121	6.75	1.25	nutty/plastic
1133	6.99	1.70	garlic/onion/plastic/skunk/garbage/brothy
1173	7.81	1.50	sulfur/garlic/onion/brothy
1183	8.01	1.00	sweet ?/fruity
1196	8.27	1.17	brothy/plastic/sweet/cooked
1239	9.11	1.50	mushroom/earthy/green/roasted nuts
1253	9.40	2.38	pungent/solvent/pungent/stinky/rotten fruit
1274	9.81	1.00	sweet
1278	9.88	2.00	stinky/ solventy/rubbery/resinous/green
1287	10.07	1.00	sweet/veggie
1289	10.10	2.67	rubbery/chicken/meaty brothy/onion/garlic
1298	10.29	2.00	rubbery/solventy
1313	10.56	1.50	cooked
1328	10.85	1.83	roasted/nutty
1341	11.09	1.50	rubbery
1346	11.18	2.00	rubbery/sweet floral/plastic/floral/summertime
1365	11.53	1.83	earthy/brothy/mushroom
1375	11.73	2.25	rubbery/solventy ?/brothy/sweet/cooked
1419	12.52	2.25	vinegar
1420	12.53	2.50	roasted coffee
1436	12.80	2.00	cooked/sweet/brothy/veggies
1436	12.80	1.75	vinegar /sour/acidic
1440	12.88	1.50	earthy
1449	13.03	2.17	earthy/bell pepper
1456	13.15	2.17	potato
1476	13.50	1.00	fatty/fried lingers
1567	15.02	1.50	plastic
1475	13.48	1.00	roasted/peanutty lingers
1630	16.01	2.33	roasted nutty

Table 12. Continued

1693	17.00	1.00	almost rosy undertone lingers
1768	18.11	2.00	sweet/cooked
1983	21.07	2.25	roasted/nutty

Table 13. GC/O data obtained from the neutral/basic fraction of brown roasted peanuts on a DB-WAX column

Retention Index	Retention time (s)	Intensity Score	Aroma description
730	1.63	1.38	malty/sweet
735	1.68	2.00	fresh nutty
792	2.24	2.00	garlic/brothy/garlic/lettuce/herbs
815	2.43	2.25	malty/chocolate/sweaty cheesy malty
916	3.24	2.63	buttery/sour, cheese
943	3.61	1.00	mayo/herbs ranch dressing
970	3.99	1.63	sour/stale/fruity/nutty/burnt sugar
974	4.05	1.00	rubbery
998	4.38	1.17	green/grassy/fruity
1024	4.86	2.13	buttery/sweet/sour, cheese
1040	5.17	1.00	pineapple
1049	5.34	2.13	green/grassy/apple /fruity
1068	5.70	2.33	plastic/burnt/rubbery/plastic/plastic/nutty
1079	5.91	1.50	brothy
1104	6.41	1.67	mushroom/earthy/brothy/garlic/malty
1121	6.74	1.00	cooked
1129	6.91	1.25	strawberry (fresh)/earthy
1132	6.97	2.50	sweaty /puke
1156	7.46	1.00	herbal
1161	7.56	1.50	mint
1169	7.72	1.25	buttery/sweet
1181	7.96	1.67	plastic/burnt/rubbery/sweet/nutty/
1195	8.25	1.00	cooked/brothy
1199	8.33	1.50	vomit/puke/rubbery
1215	8.64	1.00	rubbery
1224	8.83	1.50	paint oil
1228	8.90	2.25	green/grassy/sweaty/fatty/damp cloth
1240	9.15	1.83	mushroom/brothy/veggies/oxidized
1255	9.45	2.00	sour/rotten fruity/rubbery
1263	9.60	1.50	cooked/brothy
1277	9.87	1.00	fruity
1286	10.05	2.00	burnt rubber/floral
1290	10.12	2.38	mushroom/brothy
1300	10.34	1.75	fruity
1320	10.71	2.70	popcorn/nutty/cooked/roasted
1336	11.00	2.13	popcorn/fruity
1349	11.23	1.75	brothy/veggies
1358	11.41	1.50	spicy/curry/buttery/popcorn
1364	11.51	2.50	onion garlic /autoclave
1375	11.71	2.17	onion garlic/earthy/brothy/nutty

Table 13. Continued

1395	12.09	1.50	brothy
1398	12.15	1.63	fruity/sweet candy
1412	12.39	2.00	mushroom/brothy/garlic
1424	12.59	2.25	roasted/nutty
1434	12.78	2.63	potato/cooked potato/roasted/coffee
1452	13.09	3.00	roasted peanuts
1470	13.40	1.50	fruity/earthy/bell pepper/nutty
1490	13.75	1.00	plastic
1499	13.91	1.00	fruity
1506	14.03	1.75	fruity/earthy
1517	14.20	1.50	cooked/mushroom/brothy
1535	14.50	1.25	fruity
1564	14.97	1.33	roasted nutty
1741	17.71	2.00	floral

Table 14. GC/O data obtained from the acidic fraction of brown roasted peanuts on a DB-WAX column

Retention Index	Retention time (s)	Intensity Score	Aroma description
735	1.68	1.38	malty
818	2.45	2.38	malty/chocolaty
923	3.34	1.13	buttery/sweet/buttery
970	3.99	1.00	rubbery
997	4.36	1.50	sweet
1027	4.92	1.70	buttery/cheesy
1050	5.36	1.50	green/grassy/fresh
1125	6.84	1.50	sulfur/floral
1133	6.99	1.88	onion/garlic/burnt
1168	7.71	1.00	cooked
1153	7.39	1.00	cooked/brothy
1184	8.02	1.33	Burnt/garlic/onion
1200	8.35	1.43	stale milk powder/sweet/burnt plastic
1233	9.00	2.00	damp laundry
1254	9.41	2.50	rotten/metallic/sour medicine/rotten fruit
1290	10.12	2.67	rubbery/medicine
1295	10.23	2.00	onion/garlic
1319	10.67	1.83	mushroom/earthy
1314	10.59	1.00	rubbery/plastic
1322	10.74	1.50	fruity
1338	11.04	1.50	cooked/brothy/veggies
1346	11.18	2.00	rubbery/stale
1362	11.48	1.50	ink (ball pen)
1369	11.61	2.00	mushroom/garlic
1393	12.05	1.50	cooked
1417	12.48	2.00	earthy/brothy
1411	12.37	2.13	vinegar
1420	12.53	2.17	coffee/brothy mushroom/chocolate
1457	13.17	2.13	earthy/bell pepper/potato/ cooked
1470	13.40	2.17	potato
1501	13.94	1.75	acetone painty
1553	14.79	2.25	soil/earthy
1557	14.85	1.50	mint
1575	15.15	1.00	cooked/brothy
1591	15.41	1.50	soil/chocolate
1650	16.33	1.00	rubber
1653	16.37	1.38	nutty/roasted/peanuts
1674	16.70	1.50	seaweed
1786	18.37	1.50	sour cheese

Table 14. Continued

1830	18.95	1.50	autoclave
1895	19.75	2.00	rotten beans (uncooked ground beans)
1856	19.27	2.00	sulfur
1944	20.47	1.00	autoclave

Table 15. GC/O data obtained from the neutral/basic fraction of orange B roasted peanuts on a DB-WAX column

Retention Index	Retention time (s)	Intensity Score	Aroma description
732	1.65	1.25	malty
785	2.17	1.67	fish/garlic/onion
809	2.39	2.50	malty/chocolaty
906	3.10	2.00	malty
913	3.20	2.75	buttery
959	3.84	2.00	onion/brothy/garlic
968	3.96	2.00	rubbery/onion
982	4.16	1.00	garlic
995	4.33	1.25	green/grassy/garlic/onion
1007	4.54	1.00	green/fruity
1023	4.85	2.63	buttery/sweet
1038	5.13	1.50	floral
1047	5.30	2.25	green/grassy
1053	5.41	1.00	mint/hay
1070	5.75	2.13	rubbery/plastic/latex/fishy/garlic
1102	6.37	1.67	garlic/onion
1113	6.59	1.33	brothy/roasted/ball pen ink
1121	6.75	1.00	sweet
1129	6.92	1.67	skunky/green/brothy/veggies/grassy
1136	7.06	1.50	brothy/veggies/painty
1141	7.15	1.25	buttery
1152	7.37	1.50	fruity/green/sweet
1170	7.74	1.50	buttery/malty/chocolate/grassy/rubbery
1180	7.95	2.00	brothy
1187	8.08	2.00	garlic/mushroom
1192	8.18	1.50	fruity/cooked
1197	8.30	2.17	earthy/cooked/skunky/green/rubbery
1209	8.52	1.50	herbal/basil
1223	8.80	2.75	brothy/sweaty
1227	8.88	1.50	green/grassy
1225	8.84	3.00	musty/sweaty
1240	9.15	1.50	earthy/mushroom/garlic
1253	9.41	2.50	sour/vitamin/musty/sweaty/plastic
1277	9.88	1.00	sweet
1265	9.64	1.50	paint
1290	10.13	2.50	mushroom
1292	10.16	2.33	malty/nutty/fruity
1306	10.44	2.50	roasted/bell pepper/rubbery/nutty
1319	10.68	2.50	popcorn/toasted/burnt corn nuts/nutty
1326	10.81	3.00	spicy/brothy/corn nuts/sweet

Table 15. Continued

1343	11.13	2.25	brothy
1349	11.24	2.67	brothy/veggies/potato/sweaty/earthy
1364	11.52	2.38	onion/garlic/fishy/veggies/rancid
1377	11.76	2.33	musty/rancid nutty
1390	12.00	2.50	burnt
1412	12.40	1.50	sweet
1417	12.48	2.63	roasted/burnt/brothy/potato/onion/garlic
1436	12.82	2.00	roasted/nutty/corn chips
1443	12.93	3.13	potato/spicy potato/roasted potato
1453	13.10	1.50	earthy/bell pepper
1466	13.33	2.75	toasted nuts/nutty
1470	13.40	1.00	fruity
1479	13.56	2.00	roasted potato
1484	13.64	1.50	fruity/nutty
1497	13.86	1.25	fruity/toasted
1505	14.01	1.00	fruity
1519	14.23	2.38	roasted/nutty/toasted nuts
1640	16.18	2.00	fatty

Table 16. GC/O data obtained from the acidic fraction of orange B roasted peanuts on a DB-WAX column

Retention Index	Retention time (s)	Intensity Score	Aroma description
730	1.63	1.00	malty
758	1.91	1.50	sweet/malty
814	2.42	2.25	malty/chocolate
921	3.31	2.00	buttery/cheesy
970	3.99	1.00	rubbery
1027	4.92	1.50	buttery/sweet/fruity
1048	5.33	1.00	green/grassy/fruity
1055	5.46	1.50	brothy/grassy
1079	5.92	1.00	barny/animal
1121	6.74	1.50	strawberry
1131	6.94	2.25	garbage/sour/stinky/burnt
1186	8.06	1.50	floral/herbal
1197	8.28	2.00	musty
1227	8.89	1.50	fruity
1242	9.19	1.50	brothy/onion/garlic
1253	9.39	3.00	sour/rotten fruit/sweaty
1278	9.89	1.50	sulfur/rubbery
1288	10.08	2.00	rubbery/solvent/nutty
1299	10.30	1.50	mushroom
1320	10.71	1.50	brothy/garlic
1347	11.20	2.25	brothy/potato/veggies/garbage
1370	11.62	2.00	onion/garlic
1412	12.40	2.83	vinegar
1418	12.50	2.00	brothy
1436	12.80	2.50	brothy/mushroom
1458	13.19	2.50	potato
1548	14.71	1.00	cheesy
1636	16.12	1.50	roasted/nutty

Table 17. GC/O data obtained from the neutral/basic fraction of orange A roasted peanuts on a DB-WAX column

Retention Index	Retention time (s)	Intensity Score	Aroma description
724	1.58	1.50	malty/chocolaty
753	1.86	1.50	garlic
783	2.15	1.83	garlic/onion/brothy
797	2.29	2.25	garlic/fishy
811	2.40	2.38	malty/chocolate
897	3.00	2.00	malty
913	3.20	2.63	buttery
953	3.76	1.50	brothy/garlic
961	3.87	2.00	garlic
966	3.94	2.17	body odor/sour/toasted ?/brothy
994	4.33	1.50	roasted/fruity
1006	4.52	1.00	green/grassy
1018	4.75	1.50	fruity
1022	4.83	2.25	buttery
1038	5.14	1.67	fruity/spicy/grape
1045	5.28	2.25	green/grassy
1057	5.50	2.00	brothy/herbal/skunky/veggies
1066	5.66	2.33	rubbery/burnt plastic/burnt plastic
1073	5.81	1.50	brothy/veggies
1088	6.09	2.00	garlic/onion
1103	6.38	1.88	earthy/mushroom/brothy/garlic/rubbery/nutty
1110	6.53	1.75	brothy/insects/earthy
1119	6.70	1.50	brothy
1125	6.83	1.63	grassy/sweaty/green
1130	6.94	2.25	sour/stale/immature nuts/skunky/brothy/fishy
1151	7.35	1.33	fruity/fatty/hay/?cooked
1179	7.93	1.63	garlic/brothy/green/herbal/cabbage
1184	8.02	2.00	burnt
1193	8.22	1.88	mushroom/brothy/veggies/skunk
1218	8.71	1.67	grassy/herbal/basil/green/grassy
1227	8.88	2.43	sweaty/earthy/insects/musty
1238	9.11	1.83	fruity/spicy potato/brothy
1252	9.39	2.67	sour/rotten fruit/rubbery/stinky/body odor
1266	9.65	1.00	rubbery
1280	9.92	2.17	stale/sour/immature/rubbery
1287	10.07	2.83	garlic/onion mushroom/nutty
1294	10.21	2.17	mushroom/earthy
1301	10.35	1.50	fruity
1309	10.49	3.50	nutty/toasted
1315	10.60	1.50	fruity

Table 17. Continued

1321	10.72	2.88	popcorn/corn nuts/cooked/nutty
1328	10.86	1.75	buttery/fruity
1345	11.16	2.38	brothy/body odor/mushroom/musty
1355	11.36	2.75	brothy/veggies
1363	11.50	3.00	garlic/onion
1374	11.71	2.50	brothy/veggies/fruity/fermented/earthy
1395	12.09	2.25	fermented/fruity/ popcorn
1405	12.28	2.17	fermented grape/earthy
1411	12.37	2.00	fruity
1415	12.45	2.88	potato/smoked ham/cooked/nutty/brothy
1424	12.60	2.25	earthy/nutty/roasted
1436	12.82	2.17	popcorn/cooked/nutty
1440	12.87	3.00	potato
1454	13.13	3.50	roasted/nut
1453	13.11	1.50	earthy/bell pepper
1470	13.40	2.50	potato
1502	13.95	1.50	earthy/bell pepper
1513	14.14	2.00	nutty/roasted/corn nuts

Table 18. GC/O data obtained from the acidic fraction of orange A roasted peanuts on a DB-WAX column

Retention Index	Retention time (s)	Intensity Score	Aroma description
729	1.63	1.00	malty
788	2.20	1.00	garlic/onion
812	2.40	1.83	malty
915	3.23	1.67	buttery
965	3.92	1.25	roasted/burnt/burnt/garlic/onion
987	4.23	1.50	green/roasted
1024	4.86	1.27	buttery
1045	5.26	1.00	green
1129	6.90	2.33	sour/garbage/skunky/stale
1181	7.97	1.50	rubbery/stale/burnt
1194	8.23	2.00	brothy/skunky
1213	8.60	1.00	sulfur/brothy
1228	8.90	1.50	sweaty
1239	9.13	1.50	green/immature/fruity
1250	9.33	2.33	solvent/sour/rotten fruit /burnt
1267	9.67	1.00	rubbery
1275	9.84	2.00	green/vomit/cheesy
1286	10.06	2.50	roasted nuts/rubbery
1289	10.12	2.00	mushroom/earthy
1296	10.25	2.50	mushroom/brothy
1319	10.68	1.00	brothy/veggies
1330	10.88	1.50	rubbery/popcorn
1342	11.10	2.25	brothy/veggies
1364	11.52	2.50	brothy/onion/garlic
1406	12.29	2.67	vinegar
1416	12.46	2.67	brothy/veggies/burnt coffee
1427	12.65	2.50	fermented sausage
1440	12.88	2.33	brothy/veggies/roasted
1448	13.02	2.83	potato/spicy potato
1472	13.43	3.00	toasted nuts
1482	13.61	1.50	bell pepper
1505	14.00	1.50	cheesy
1523	14.30	2.50	toasted nuts
1562	14.93	1.50	cheesy
1599	15.54	1.50	fruity
1629	16.01	1.83	nutty/roasted/toasted nuts

Table 19. GC/O data obtained from the neutral/basic fraction of yellow roasted peanuts on a DB-WAX column

Retention Index	Retention time (s)	Intensity Score	Aroma description
704	1.38	2.00	solvent
727	1.60	1.83	malty/chocolate
786	2.19	2.00	brothy/garlic/cabbage
806	2.36	2.63	malty/chocolate
911	3.18	2.50	buttery
926	3.38	2.00	rotten fruit
942	3.60	1.00	malty
953	3.75	2.38	brothy/garlic/fishy
959	3.84	3.00	fermented fruit
967	3.95	1.83	rubbery/toasted/green immature
982	4.15	1.50	garbage
1003	4.47	1.50	herbal/green
1018	4.76	2.25	fruity
1022	4.82	2.38	buttery
1035	5.08	2.00	fruity/sweet/grape
1046	5.28	2.38	green/grassy
1057	5.49	2.00	cooked/brothy
1067	5.68	1.83	rubbery/burnt plastic/green
1074	5.83	2.25	brothy/veggies/onion/garlic/green/fishy
1103	6.38	1.75	garlic/brothy
1113	6.59	1.00	mushroom/brothy
1120	6.72	1.50	brothy
1126	6.86	1.75	grassy/sweaty
1130	6.92	2.38	stale/rubbery/garbage/skunky/sour/vitamin
1149	7.32	1.25	earthy/herbal
1153	7.40	1.00	popcorn
1169	7.72	1.00	mint
1180	7.95	1.50	brothy/burnt/green/stinky/rubbery
1197	8.29	1.63	brothy/sweet/skunky/green/mint/immature
1219	8.74	1.25	herbal
1225	8.85	1.88	grassy/fatty/sweaty
1229	8.92	2.00	earthy
1234	9.03	2.00	grassy/green/herbal/sweaty/musty
1240	9.14	1.00	fruity
1248	9.30	2.00	rubbery
1251	9.36	1.50	mothball/stale hay
1254	9.41	2.25	sour/rotten/garbage/rotten fruit
1264	9.62	1.50	rubbery
1284	10.02	1.50	nutty/roasted/earthy
1288	10.09	2.63	mushroom

Table 19. Continued

1294	10.20	2.00	metallic
1298	10.28	2.00	nutty
1303	10.39	1.00	earthy
1318	10.67	3.00	nutty
1320	10.70	2.50	popcorn/cheesy/nutty
1336	11.00	1.75	roasted nut/fruity
1345	11.16	2.00	brothy/veggies/skunk/fermented fruit
1362	11.49	2.50	garlic/brothy/cabbage/mushroom
1375	11.72	2.67	burnt/brothy/spicy
1391	12.01	2.00	Spicy/curry
1396	12.11	2.00	corn nuts
1408	12.33	1.83	earthy/fruity/rotten fruit
1407	12.30	2.00	rotten fruit/gasoline/fruity
1418	12.49	2.63	popcorn/brothy/roasted/burnt coffee
1432	12.74	1.67	fruity/sweet
1439	12.87	2.75	potato
1448	13.02	1.50	earthy/bell pepper
1454	13.12	2.50	nutty
1463	13.28	1.50	potato
1500	13.92	2.00	toasted/nutty/roasted

Table 20. GC/O data obtained from the acidic fraction of yellow roasted peanuts on a DB-WAX column

Retention Index	Retention time (s)	Intensity Score	Aroma description
638	1.26	1.50	sweet
712	1.46	1.50	spicy/curry/sweet
747	1.80	1.00	malty/sweet/grainy
752	1.85	1.00	buttery
806	2.37	2.25	malty/chocolate/buttery
817	2.44	2.00	malty
831	2.54	2.50	malty/chocolate
861	2.75	1.00	stinky/rubber
915	3.23	2.00	buttery
966	3.94	1.25	meaty/brothy/rubbery/plastic
989	4.25	1.00	plastic
1002	4.44	1.50	garbage/cabbage
1014	4.68	2.00	nutty
1023	4.85	1.17	buttery/cooked/milky/sweet
1046	5.29	1.33	green/grassy/sweet
1056	5.48	1.50	musty/sweaty
1097	6.26	1.50	toasted/yeasty/brothy
1128	6.90	2.75	garbage/stale/sulfur/egg/skunky
1178	7.90	1.50	burnt plastic/rubbery
1195	8.24	2.00	green/immature
1226	8.87	3.00	sweaty
1239	9.12	1.25	brothy/rubber
1241	9.17	2.00	green/immature
1250	9.35	2.63	rotten fruit/stale /sweet/fruit/very green
1279	9.91	1.33	cheesy/vomit/rubbery/green/immature
1287	10.08	2.13	rubbery/cooked/brothy
1294	10.21	1.88	mushroom
1303	10.39	1.00	fruity
1309	10.49	1.25	brothy
1316	10.62	2.00	cooked
1330	10.89	1.50	cheesy/popcorn
1344	11.14	2.25	Popcorn/brothy/sour/body odor/skins
1353	11.31	1.50	spicy/earthy/brothy
1370	11.62	1.83	garlic/onion
1405	12.27	2.17	vinegar
1416	12.47	2.63	brothy/veggies/potato/toasted/burnt
1423	12.58	1.67	vinegar/sour
1442	12.91	2.13	cooked/potato/brothy/veggies/spicy potato
1457	13.17	1.75	earthy/sweet/fatty
1463	13.27	3.00	nutty/sweet

Table 20. Continued

1466	13.33	2.25	potato
1484	13.64	2.50	cooked/nutty
1516	14.18	2.00	brothy potato
1575	15.15	1.50	toasted/nutty/roast/corn

APPENDIX 2

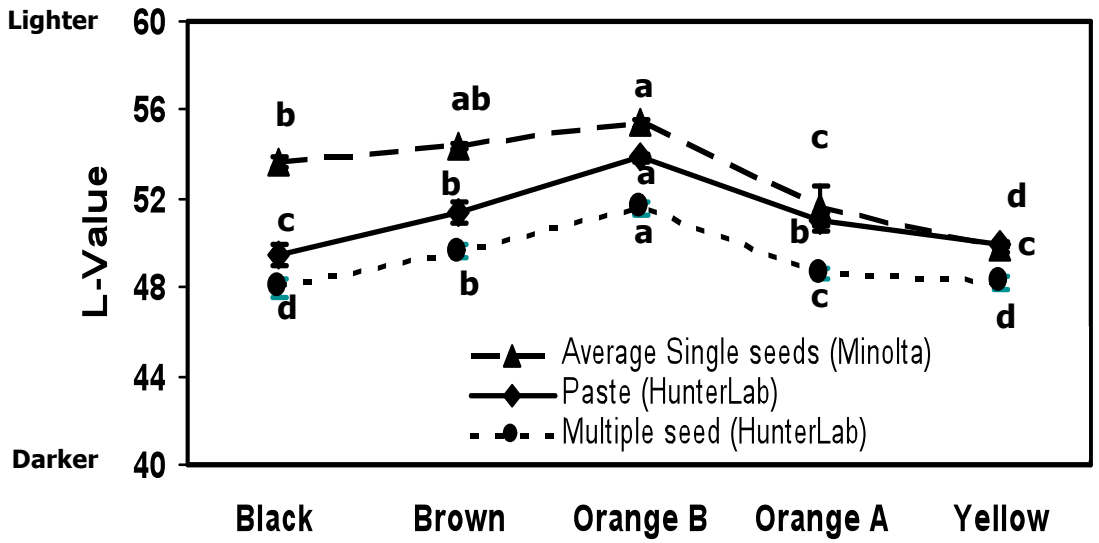

Figure 1. L-value distribution among roasted peanuts from five mesocarp-color maturity classes. 'Average single seeds' represents the mean of 100 individually measured seeds using a Konica Minolta colorimeter (Ramsey, NJ, USA). Peanut paste and multiple seed measurements were performed using a HunterLab colorimeter (Reston, VI, USA). For each line, means having different letters are significantly different ($P<0.05$) among maturity classes.

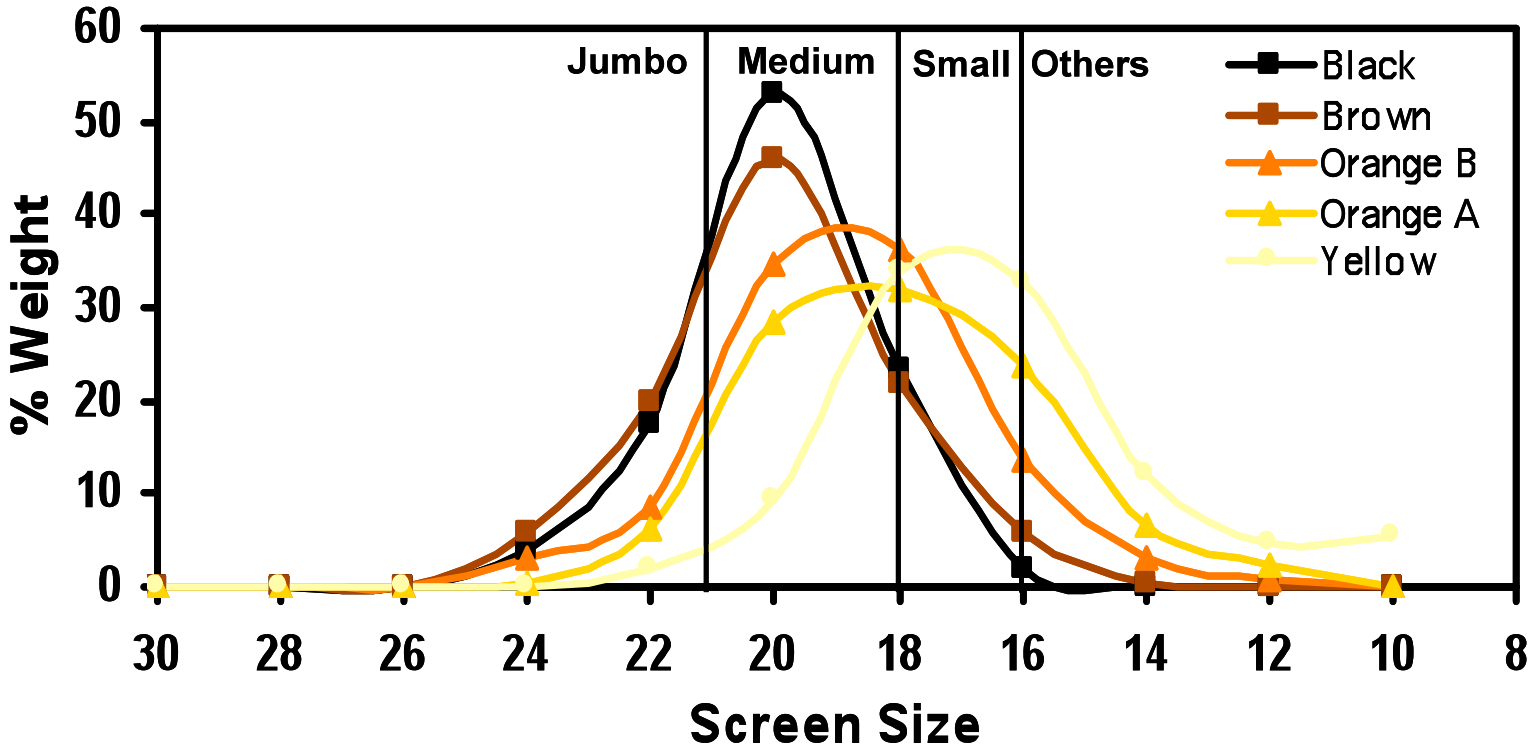

Figure 1. Seed size distribution of five mesocarp-color based maturity classes.