

**Evaluation of Initial Flavor Fade in Roasted Peanuts using Sensory  
Analysis, Gas Chromatography-Olfactometry, Gas Chromatography-  
Flame Ionization Detection and Chemosensory Techniques**

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**Keywords:** Pyrazines, Roasted Peanuts, Gas Chromatography-Olfactometry, Solid  
Phase Microextraction, Flavor Fade, Oxidation, Pyrazine Decomposition

# **Evaluation of Initial Flavor Fade in Roasted Peanuts using Sensory Analysis, Gas Chromatography-Olfactometry, Gas Chromatography Flame Ionization Detection and Chemosensory Techniques**

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## **Abstract**

Preventing flavor fade requires an understanding of the relationship between carbonyl amine and lipid oxidation reactions. The polyunsaturated fatty acid content of lipids in peanuts makes them more susceptible to lipid oxidation. The major by-products of the oxidation reaction are nonanal, hexanal, octanal, and decanal. These chemicals are associated with cardboardy, painty, and oxidized flavors associated with flavor fade. The carbonyl-amine reaction yields a variety of pyrazines with positive flavor attributes.

Initial flavor notes were explored through sensory work, Gas Chromatography-Olfactometry, and chemical analysis. The fresh roasted volatiles produced from roasted peanuts and the aldehydes resulting from oxidation were also evaluated using GC-FID to quantify and identify the pyrazines and hexanal over a 21 day storage period. Electronic Nose was used to determine differences between storage periods.

Gas chromatography-Olfactometry identified potent pyrazines contributing to fresh roasted peanutty aroma in fresh peanuts. Using GC-FID a significant decrease ( $p < .05$ ) in 2-ethylpyrazine and 2,3-diethylpyrazine concentration was found over a 21-day period. No significant difference ( $p > 0.05$ ) was noted in the other pyrazines evaluated. A significant increase ( $p < 0.05$ ) was noted in the hexanal concentration over a 21-day period. The peroxide values and sensory analysis correlated directly with the GC-FID results with a significant increase ( $p < 0.05$ ) in peroxide value at Day 14 and Day 21, and a significant decrease ( $p < 0.05$ ) in fresh roasted peanutty flavor from days 0–21 and a significant increase ( $p < .05$ ) in painty, cardboardy and bitter from days 7-21. The electronic nose successfully separated Day 0 and Day 21 samples from Day 7 and 14, which were also separated, but with minimal overlap.

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## **Dedications**

I dedicate this work to my grandfather, the late, Mr. Clarence Powell, who has called me his little doctor from the age of 12. I am the first of many more. I would also like to dedicate this to my parents Mr. and Mrs. Adrian and Christine Powell who have given me an enormous amount of love, guidance, be it solicited or unsolicited, and financial support throughout my life. Without them I would be nowhere. I would also like to dedicate this to my brother Adrian Powell II, and my niece/god daughter Jaelyn Jodi Powell who keeps me inspired and feeling loved and missed when I am trapped in Blacksburg, VA for long spells. I will always remember “Call my name, and I’ll.....”. I would like to also thank my extended family, Aunts, Uncles, and cousins, who kept me in their prayers, and hearts through this trying time in my life. My friends, Ms. Jocelyn Smith, Ms. Alicia Dickerson, Mrs. Tameika Hollis, Ms. Cicely Washington, Ms. Lenese Colson, Ms. Naya McMillan, and my roommate, Mr. Nonye Onyewu who ensured that I would not keep my nose stuck in a book, and were all determined to drag me on vacations or to just hang out.

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# Chapter I

## Introduction

Peanuts are grown in the warm climates of Asia, Africa, Australia, and North and South America. Americans consume over 6 pounds of peanuts and peanut products per year, worth more than \$2 billion at retail level. The U.S., after China and India, is the third largest producer of peanuts, and produces over 29 million metric tons per year. The U.S. has about 3% of the world acreage of peanuts, but grows nearly 10% of the world's crop because of higher yields per acre. Although peanuts come in many varieties, there are four basic market types in the US: Virginia, Runner, Spanish, and Valencia (Woodruff 1982). The peanut is a very diverse crop with many uses such as roasted, peanut oil, peanut butter, peanut spread, peanut flour and meal, fermented peanut products, and peanut confections.

Lipids are the major component in peanuts. Lipids comprise ~52% of the dry weight of peanuts, with ~80% being unsaturated. Of the total fatty acid composition, ~48% is oleic and ~31% linoleic (Cobb and Johnson 1973). Lipid oxidation is the main cause of off flavor. The major by-products of the oxidation reaction are nonanal, hexanal, octanal, and decanal with hexanal being the most prominent (Nawar 1985). These chemicals are associated with cardboardy, painty, and oxidized flavors associated with flavor fade. The products of the carbonyl-amine reaction, or Maillard browning reaction, yields the positive flavor attributes of roasted peanuts. Some of these pyrazines include 2,6-dimethylpyrazine, 2-methylpyrazine, 2-ethyl-3-methylpyrazine, 6-methylpyrazine and 2,3,4-trimethylpyrazine.

A considerable amount of prior work has been done pertaining to the lipid oxidation of peanuts, but a lot of discrepancy exists in the literature relating to initial oxidation. Dimick (1994) noted a large increase in concentration of aldehydes and no significant increase or decrease in pyrazine concentrations. The data indicate that the off-flavor associated with roasted peanuts over storage is most likely due to masking of pyrazines by the larger concentrations of aldehydes produced during autoxidation. Vercellotti and others (1992), using a sniff port and GC retention times to evaluate the chemical compounds, determined that the volatile heterocyclic compounds, pyrazines, providing the positive attributes to the flavor of fresh roasted peanuts disappeared in rancid peanuts. They noted that this disappearance is due to degradation of heterocyclic compounds by lipid radicals and hydroperoxides or flavor entrapment between proteins and lipid hydroperoxides.

Food aromas and flavors produced by the Maillard browning reaction, most importantly heterocyclic compounds, are significant because of their contribution to the flavor of processed foods. These compounds are formed during roasting, baking, and cooking. Pyrazine development is thought to occur through the Maillard browning reaction, a non-enzymatic process.

Pyrazines constitute a very important class among flavor compounds. The alkyl-derivatives of pyrazines produce roasted nut-like, pleasant flavoring. The browning reaction is the pivotal reaction that yields pyrazines, but currently gaps exist in the literature with regards to the important pyrazines that contribute to the fresh-roasted peanutty aroma. A large variety of pyrazines are found in all foods, including coffee, roasted barley, casein, popcorn, rye crisp bread, soybeans, hydrolyzed soy protein,

chicken broth, nonfat dry milk, roasted pecans, dehydrated potato, potato chips, tomato, spray-dried whey, bell peppers, and roasted peanuts, to name a few (Collins and others 1971; Farretti and others 1970; Walradt and others 1970; Wilkens and Lin 1970; Wilson and Katz 1972; Wang and Odell 1972; and Liebach and others 1972). In order to understand flavor fade it is necessary to first determine which pyrazines are key to the fresh peanutty aroma, and find ways to maintain their stability over time.

Pyrazines are heterocyclic nitrogen compounds and their formation is a complex process. Newell and others (1967) evaluated the precursors of typical and atypical roasted peanut flavor and derived a relationship that related flavor precursor concentration to sensory flavor. They found that aspartic acid, glutamic acid, glutamine, asparagine, histidine, and phenylalanine were associated with the production of typical peanut flavor. From this information, Newell and others (1967) theorized a mechanism for the production of flavor components from amino acids and carbohydrates.

Amino acids, lipids, peptides and carbohydrates are precursors to roasted peanut flavors. Lipid oxidation in stored peanuts leads indirectly to the formation of aliphatic aldehydes, ketones, and alcohols. Free radicals and hydroperoxides formed during autoxidation also interact with other components of the food system such as amino acids, proteins, and other nitrogen containing compounds.

The interaction of peroxidizing lipids with other nitrogen containing food components has been heavily reviewed with regards to protein and amino acid degradation. Although, no research has focused solely on pyrazine declination due to these radicals, enough information is provided to infer that pyrazines may be degraded in the same way as proteins and amino acids (St. Angelo and Graves 1986; St. Angelo and

others 1979; Gardner 1983 and 1979; Hidalgo and Zamora 2002; and Funes and others 1982).

Current research has determined that pyrazines are the major volatile component in fresh, roasted peanut flavor but, of the numerous pyrazines in peanuts, key contributors need to be determined (Johnson and others 1971a and 1971b, Pattee and others 1982; Ho and others 1981; Baker and others 2002). Current research also shows inconsistency in the data with regards to the stability and contribution to flavor fade over time (Warner and others 1996; Vercellotti 1992). The explanation has been flavor degradation by oxidation materials along with flavor masking by aldehydes. But the question still remains, which pyrazines contribute to the overall roasted peanut flavor, and which pyrazines are interactions of products with lipid oxidation? This research seeks to answer these questions by exploring the following objectives.

- I) Analyze fresh roasted peanut aroma using SPME-GC-FID as a quantification and identification method, and identify key potent pyrazine components of fresh roasted peanuts using SPME-GC-O.
- II) Evaluate overall differences in fresh roasted peanuts over short-term storage using SPME-GC-FID.
- III) Use chemosensory techniques to evaluate overall differences found in roasted peanuts during a 21-day storage.

## Chapter II

### Literature Review

#### ***Lipid Oxidation of Roasted Peanuts***

Lipids are the major component in peanuts. Lipids comprise ~52% of the dry weight of peanuts, with ~80% being unsaturated. Of the total fatty acid composition, ~48% is oleic and ~31%, linoleic. Lipid oxidation is the main cause of flavor deterioration and off flavor formation in peanuts (Reed and others 2002). These off flavors and off odors are generally called oxidative rancidity, which renders the roasted peanuts less acceptable. Autoxidation, that is the reaction with molecular oxygen via a self-catalytic mechanism, is the main reaction involved in oxidative deterioration of lipids (Nawar 1996).

Autoxidation of fats can be divided into the following three parts: initiation, propagation, and termination (Figure I).

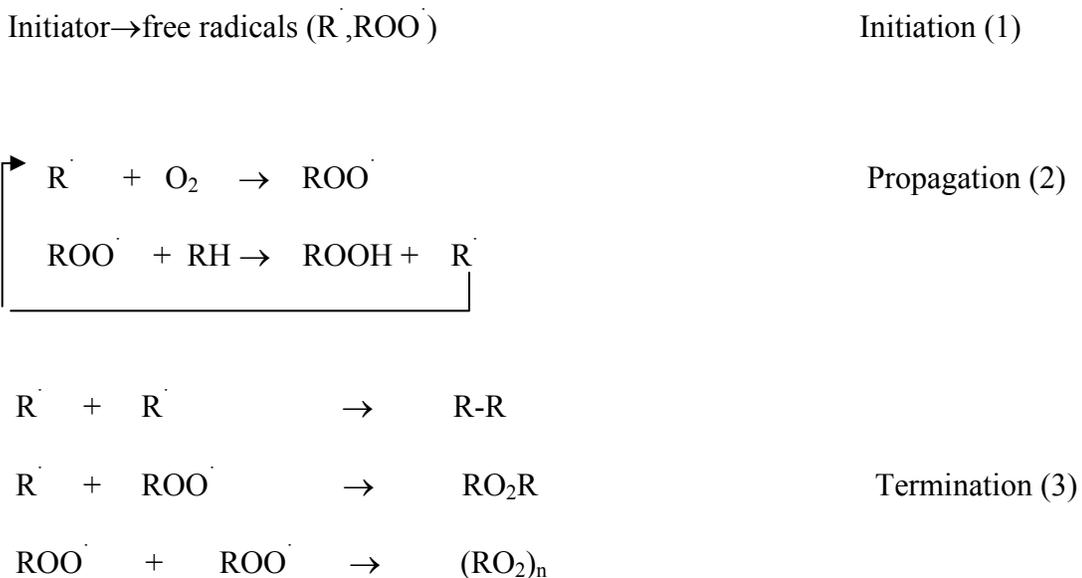


Figure I. Autoxidation of lipids, initiation, propagation, and termination step (Nawar 1996)

During the initiation step, hydrogen is removed from a fatty acid at the carbon atom next to the double bond (allylic) producing a free radical. Once this free radical is formed, it will combine with available oxygen to form a peroxy-free radical which can in turn remove hydrogen from another unsaturated molecule and give a peroxide and a new free radical, thus beginning the propagation step (deMann 1999). The propagation step is a chain reaction and will occur until there is no available O<sub>2</sub>. The propagation step is followed by the termination step, which occurs when the free radicals begin to react with themselves and yield non-reactive products.

The hydroperoxides formed in the propagation step of the reaction are the primary oxidation products. These oxidation products enter into numerous complex reactions involving substrate degradation and interactions. Oxidation products are generally unstable and decompose into secondary oxidation products, of various molecular weights and flavor thresholds.

Lipid oxidation can be accelerated by a variety of factors such as fatty acid composition, oxygen concentration, temperature, surface area, moisture, molecular orientation, radiant energy and antioxidants. When discussing peanut oils, the most obvious factor involved in oxidation would be the fatty acid composition. The number, position, and geometry of double bonds affect the rate of oxidation. Cis acids oxidize more readily than their trans isomers and conjugated double bonds are more reactive than non-conjugated (DeMann 1999). Since peanut oils are largely unsaturated with ~48% being oleic, and ~31% being linoleic, autoxidation under the right conditions is inevitable (Bett and Boylston 1992). For example, the autoxidation of oleic acid will proceed by the abstraction of hydrogen from carbons 8 or 11 resulting in two pairs of resonance

hybrids leading to the formation of four isomeric hydroperoxides. In linoleic acid, three isomeric hydroperoxides are formed. These hydroperoxides decompose resulting in the short chain volatile compounds, which contribute to the off flavors in oxidized peanuts (Nawar 1996).

Table I. An example of fatty acid decomposition and the specific aldehydes produced via autoxidation with oleic and linoleic acids. (Nawar 1996)

Fatty Acid	Methylene Group Involved	Aldehydes formed from decomposition of hydroperoxides
Oleic	11	Octanal, 2-decenal
	8	2-undecenal, nonanal
Linoleic	11	Hexanal, 2-octenal, 2,4-decadienal

Divino and others (1996) evaluated the effects of decreasing oil contents on the oxidation of roasted peanuts. They evaluated defatted peanuts as compared to regular peanuts based on peroxide value (PV) and gas chromatography (GC) to evaluate oxidation rates of peanuts with varying oil concentrations. The PV of the roasted peanuts decreased with decreasing oil concentrations. Peroxide value has a direct correlation with oxidation, meaning a decrease in PV would indicate a decrease in oxidation. The GC analysis did not reveal any difference between the oil content of the peanuts and the hexanal concentrations. Peroxide value is defined as the milliequivalents (meq) of peroxide (formed during oxidation) per kg of fat (Nielson 1994).

O'Keefe and others (1993) compared the oxidative stability between high oleic (HOP) and normal oleic peanut oils (NOP) by chemical accelerated oxidation techniques. The data indicated that the oxidative stability of HOP oil is much higher than that of NOP oil. The increased stability is due to the decrease in concentration of the linoleic acid (18:2 $\omega$ 6) in the high oleic peanut oil (Table 2). The higher the degree of saturation, the less oxidatively stable the oil (Nawar 1996).

Table II. Fatty acid composition of normal oleic peanut oils vs. high oleic peanut oils (% weight), and the impact of degree of saturation on oil stability.

Fatty Acid	wt % fatty acid	
	High Oleic	Normal Oleic
16:0	7.5	9.5
16:1 $\omega$ 7	.12	.002
18:0	3.3	2.6
18:1 $\omega$ 9	75.6	56.1
18:1 $\omega$ 7	.65	.50
18:2 $\omega$ 6	4.7	24.2
18:3 $\omega$ 3	.03	.03
20:0	1.6	1.3
20:1 $\omega$ 9	1.6	1.3
22:0	3.3	2.8
22:1 $\omega$ 11	.06	.05
24:0	1.5	1.5

Oxidation of saturated fatty acids is very slow, and only escalated at very high temperatures, so the more saturated fats, at lower heat, the less oxidation. Oxygen concentration is also a large factor affecting lipid oxidation. When oxygen is abundant, the rate of oxidation is independent of oxygen concentration, but at very low oxygen concentration the rate is proportional to oxygen concentration.

Roasting of peanuts initiates lipid oxidation and formation of carbonyl compounds (St. Angelo and Ory 1975). St. Angelo and Ory (1975) stored fresh roasted peanuts and raw peanuts for 1 year at 25°C, and found the raw peanuts, once roasted to be have all the characteristics of a fresh roasted peanut, while the roasted, then stored peanuts were rancid. This oxidation continues during storage at high temperature, and increases the content of aliphatic aldehydes, ketones, and alcohols. Hexanal, heptanal, 2-heptenal, octanal, 2-octenal, 2-decenal, 2,4-decadienal, 1-octene-3-ol, and 2-pentylfuran are products of the oxidation of linoleic acid (Nawar 1996). Heptanal, octanal, 2-decenal, nonanal, and decanal are products of the oxidation of oleic acid (Nawar 1996).

Reed and others (2002) determined that decreases in water activity from  $a_w=60$  to  $a_w=19$ , decreased peroxide values that signified a decrease in oxidation. They also found a decrease in aldehyde concentrations at the lower water activity ( $a_w=19$ ).

Warner and others (1996) observed Thiobarbituric Acid (TBA) values in peanuts and found a direct correlation between the increase in TBA values and an increase in aldehyde concentration, pentanal, hexanal, heptanal, octanal, and nonanal, as determined by GC/MS. The corresponding characteristic ion chromatograms demonstrated that pyrazine concentrations, methylpyrazine, dimethylpyrazine, trimethylpyrazine, and ethylmethylpyrazine, did not decrease during storage.

Preliminary studies show oxidation rates were important and necessary to understand the mechanism of flavor fade, the effects of degradation and polymerization reactions of heterocyclic nitrogen compounds. Flavor fade prevention requires the understanding of the relationship between carbonyl amines and lipid oxidation reactions. The polyunsaturated fatty acid content of lipids in peanuts makes them susceptible to lipid oxidation. Linoleate can be oxidized to form monohydroperoxides that are precursors for volatile decomposition products such as nonanal, octanal, decanal, and hexanal with hexanal being the most predominant (Nawar 1996).

### ***Pyrazines in Foods and their Development (Maillard browning)***

#### ***Pyrazine Development***

Maillard browning is a key reaction affecting the color and taste of foods. The major results of the Maillard browning reaction are the following: (1) formation of color and discoloration, (2) formation of flavors and off-flavors, (3) the production of

antioxidant compounds, (4) reduction of nutritional value of the product, and (5) the formation of potentially toxic compounds (Nursten, 1986).

Food aromas and flavors produced by the Maillard browning reaction, most importantly heterocyclic compounds, are significant because of their contribution to the flavor of processed foods. Pyrazines are formed during roasting, baking, and cooking. Pyrazine development is thought to occur through the Maillard browning processes. Under optimal conditions, reducing sugars will interact with an amino acid, a free amino group, or an amino acid that is part of a protein chain to produce nitrogen-containing insoluble compounds. These compounds will provide foods with flavors, aromas and colors, which may be either desirable or undesirable.

The Maillard browning reaction proceeds by a reducing sugar reacting reversibly with an amine to produce a glucosylamine. This glucosylamine undergoes an Amadori rearrangement to give a derivative of 1-amino-1-deoxy-D-fructose. At  $\text{pH} < 5$ , a hexose is formed, 5-hydroxymethyl-2-furaldehyde (HMF), and at  $\text{pH} > 5$  the HMF and other cyclic compounds polymerize to insoluble materials containing nitrogen (Bemiller and Whistler 1996).

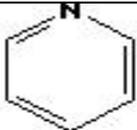
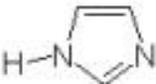
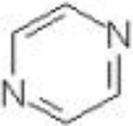
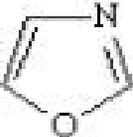
Pyrazines are heterocyclic nitrogen compounds and their formation is a complicated process. The alkyl pyrazines produce a roasted-nutty sensory impression, and the acetylpyrazines have a more popcorn impression (Fors, 1983). Newell and others (1967) evaluated the precursors of typical and atypical roasted peanut flavor and related flavor precursor concentration to sensory flavor. They found that aspartic acid, glutamic acid, glutamine, asparagine, histidine, and phenylalanine were associated with the production of typical peanut flavor. From this information, Newell and others (1967)

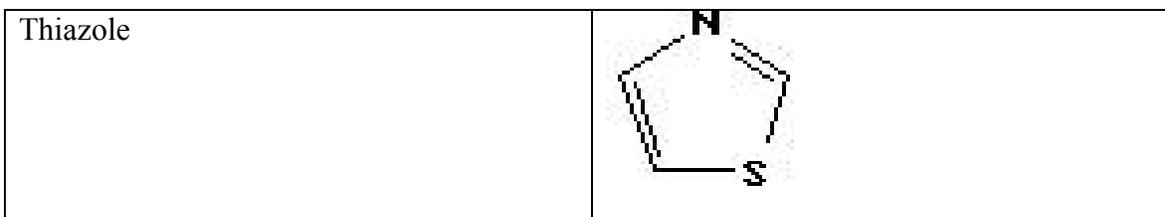
theorized a mechanism for the production of flavor components from amino acids and carbohydrates.

### *Pyrazines in Foods*

The Maillard reaction products can be classified into three groups, simple sugar dehydration/fragmentation products, simple amino acid degradation products, and volatiles produced by further interactions (Fors 1983). The group of greatest interest would be the volatiles produced by further interactions, which includes pyrroles, pyridines, imidazoles, pyrazines, oxazoles, and thiazoles (Table III.) (Fors 1983).

Table III. Volatiles produced by further interactions from the conversion of amino acids and carbohydrates.

Chemical	Structure
Pyrrole	
Pyridine	
Imidazoles	
Pyrazine	
Oxazole	



Pyrazines have been found in a wide variety of food products including but not limited to, barley, casein, coffee, peanuts, popcorn, rye crisp bread, soy beans, soy protein, beef, chicken broth, cocoa products, nonfat dry milk (stale), pecans, potatoes, potato chips, tomato, whey, green peas and bell peppers (Collins 1971; Ferretti and others 1970; Walradt and others 1971; Walradt and others 1971, von Sydow and Anjou 1969; Wilkens and Lin 1970; Manley and Fagerson 1970; Wang and others 1969; Watanabe and Sato 1971; Wilson and Katz 1972; Rizi 1967; Ferretti and Flanagan 1972; Mason and others 1966; Wang and Odell 1972; Sapers and others 1971; Buttery and others 1971; Ryder 1966; Murray and others 1970; Buttery and others 1969). Although most of these compounds are found in roasted or toasted foods, not all are. The methoxypyrazines known to have been found in green bell peppers give a green flavor or aroma, but methoxypyrazines are found in roasted peanuts also, although they apparently do not contribute this same aroma at the concentrations they are found in peanuts.

Mason and Johnson (1966) were the earliest researchers to identify pyrazines in peanuts. They found methylpyrazine, 2,5-dimethylpyrazine, 2-methyl-5-ethylpyrazine, trimethylpyrazine, and 2,5-dimethyl-3-ethylpyrazine. The importance of the pyrazines in the food industry can be determined by the fact that 1429 patents that have been issued in the area of pyrazine synthesis (U.S. Patent Office 2004). Some of the patents include chocolate flavor, coffee flavor, nut-like flavor, and baked flavor (Maga and Sizer 1973).

### ***Hydroperoxide Decomposition***

Amino acids, lipids, peptides and carbohydrates are precursors to roasted peanut flavors. Lipid oxidation in stored peanuts leads indirectly to the formation of aliphatic aldehydes, ketones, and alcohols. Free radicals and hydroperoxides formed during autoxidation also interact with other components of the food system such as amino acids, proteins, and other nitrogen containing compounds.

The interaction of peroxidizing lipids with other nitrogen containing food components have been well reviewed in regards to protein and amino acid degradation (Gardner 1983 and 1979; St. Angelo and others 1979; St. Angelo and Graves 1986; Funes and others 1982; Hidalgo and Zamora 2002; Bett and Boylston 1992). Although, no research has been specifically designed to evaluate pyrazine declination due to lipid radicals, enough information is provided to make an intelligent assumption that pyrazines can be degraded in the same way as proteins and amino acids.

Research has shown that proteins can be affected by lipid hydroperoxides in three ways: (a) formation of noncovalent complexes with lipid hydroperoxides, (b) free radical reactions, and (c) reactions with non-radicals from secondary oxidation reaction products (Gardener 1983). Radical reactions of proteins occur in three ways; (a) protein-protein or protein-lipid crosslinking, (b) protein scission, and (c) protein oxidation.

Decreases in flavor in roasted peanuts and flavor fade is caused by several factors including flavor entrapment and flavor masking (Bett and Boylston 1992). Gardener (1983) suggests that non-covalent bonding causes flavor entrapment. When proteins are exposed to peroxidized lipid, a large proportion of the lipid complexes with the protein through hydrogen bonds and hydrophobic interactions (Gardner 1979). The extraction

technique performed by several researchers to first extract polar lipids, noncovalently bound by hydrophobic interactions and last, the lipids bound by hydrogen bonds was performed by using a stepwise increase in solvent polarity (Pokorny 1963; Pokorny and others 1975; Kanazawa and others 1975). This technique only removes the noncovalently bound lipid peroxides while hydrolysis is used for removal of the covalently bound lipid peroxides.

Because the hydroperoxide-protein reaction is so complex, many of the protein/lipid oxidation reactions have been performed using model systems that typically include only lipid and protein. Schaich and Karel (1975) and Karel and others (1975) evaluated models using a lysozyme-peroxidized methyl linoleate mixture. They used electron paramagnetic resonance (EPR) to analyze protein radicals in protein-peroxidized lipid mixtures. The research found that the main signal, assigned to a carbon centered radical, increased in intensity in proportion to the peroxide value of the extracted lipid. They also found that the signal intensity increased after the lipid peroxides decomposed, thereby indicating the presence of protein radicals, and that the radicals are lipid peroxide induced.

Hidalgo and Zamora (2002) also evaluated lipid peroxidation in the methyl linoleate model system using bovine serum albumin (BSA) to evaluate the disappearance of methyl linoleate and the formation of primary and secondary products of lipid peroxidation as well as the loss of essential amino acids and the production of oxidized lipid/amino acid reaction products. Over increased incubation time, the amino acid residues recovered decreased upon exposure to methyl linoleate, and the oxidized lipid/amino acid reaction products concentration increased. Their results suggest the

oxidized lipid/amino acid reaction products formation is parallel to secondary products production and the lipid/amino acid products are formed directly from lipid hydroperoxides or very rapidly from secondary products.

Schaich and Karel (1976) suggested that radical transfers take place by complexes between lipid hydroperoxides and the nitrogen or sulfur residues of proteins (Figure II).

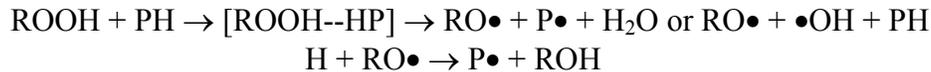


Figure II. Mechanism proposing the formation of free radical reaction between lipids (RH) and proteins (PH).

Funes and others (1982) determined the influence of concentration of lipids and protein on the depolymerization of lysozyme. Their results suggested that an increase in lipid-protein concentration does promote a higher degree of protein polymerization. This mechanism would be dependent upon the ability of the lipid to be oxidized, lipid hydroperoxides and lipid free radicals formed, and on the lipid and protein concentrations. Higher protein and lipid concentrations would increase the rate of free radical transfer and thereby increase protein polymerization (Funes and others 1982).

### ***Flavor Analysis of Roasted Peanuts***

Roasted peanuts are a desirable food product with a pleasant and unique flavor. The volatile components of roasted peanuts have been extensively studied and identified by several investigators. Amino acids, proteins, sugar, and carbohydrates are the major components in the roasted peanutty flavor. Many of these components are involved in the Maillard browning reactions. The browning reactions involved in model systems comprised of monosaccharides and amino acids, revealed the formation of pyrazines, pyrroles, furans, and other low molecular weight products (Pattee and others 1982).

Twenty-four compounds have been found and identified in the neutral fraction of peanut volatiles, and 22 found in the basic fraction (Johnson and others 1971 and Johnson and others 1971). Several volatile components isolated from roasted peanuts were described as contributors to nutty odor or a nut-like note, some of which are 2-crotalactone, 3-methyl-2-crotolactone, 5-hydroxy-4-nonenic acid, pyroxene, 2-isopropyl-4,5-dimethylthiazole, and 2-propyl-4,5-diethylthiazole (Pattee and others 1982).

Rodriguez and others (1989) observed that specific amino acids and monosaccharides are the precursors of roasted flavor. They also indicated that aspartic acid, glutamic acid, glutamine, asparagine, histidine and phenylalanine are associated with the production of typical roasted peanut flavor while threonine, tyrosine, and lysine are the precursors of atypical flavors. Browning reactions in the presence of monosaccharides and amino acids revealed the formation of pyrazine, pyrroles, furans and other low molecular weight products (deMann 1999). Baker and others (2002) identified five pyrazine compounds which affect fresh roasted peanut flavor: 2-methylpyrazine, 2,5-dimethylpyrazine, 2,3-dimethylpyrazine, 2-methoxy-3-methylpyrazine, and 2,3,5-trimethylpyrazine (Table IV).

Table IV. The effects of roasting times (5 min, 10 min, and 15 min) and temperatures (125,150,175, and 200°C) on pyrazines (2-methylpyrazine, 2,5-DMP – 2,5-dimethylpyrazine, 2,3-DMP – 2,3-dimethylpyrazine, 2-meo-3 – 2-methoxy-3-methylpyrazine, 2,3,5-TMP – 2,3,5-trimethylpyrazine) found in four different peanut genotypes (Florunner, Florida MDR 98, Georgia Green, and SunOleic 97R) (Baker and others 2002).

Florunner	2-methylpyr	2,5-DMP	2,3-DMP	2-meo-3	2,3,5-TMP
125C/15min	ND	ND	ND	.36	.23
150C/5min	ND	ND	ND	ND	ND
150C/10 min	ND	5.55	.61	ND	.89
150C/15min	2.75	9.80	ND	.57	1.15
175C/5min	5.24	15.73	ND	ND	1.76
175C/10min	27.06	47.6	1.00	1.79	4.77

175C/15min	38.88	49.66	1.64	.76	5.55
200C/5 min	13.66	33.28	.88	.92	4.23
Florida MDR 98					
125C/15min	ND	ND	ND	.31	.34
150C/5min	ND	ND	ND	.06	ND
150C/10 min	1.63	7.72	ND	.63	.77
150C/15min	2.64	24.32	.12	.061	2.47
175C/5min	1.39	9.67	ND	.07	1.17
175C/10min	13.03	26.23	1.09	.93	3.50
175C/15min	37.19	49.17	1.61	.73	5.70
200C/5 min	5.73	13.50	ND	1.13	1.96
Georgia Green					
125C/15min	ND	ND	ND	.88	ND
150C/5min	ND	ND	ND	ND	ND
150C/10 min	ND	5.16	ND	.87	.59
150C/15min	4.90	9.98	ND	ND	.84
175C/5min	4.91	10.1	ND	1.02	1.05
175C/10min	11.74	19.63	.63	.41	1.28
175C/15min	24.89	32.88	1.24	.70	3.06
200C/5 min	9.84	16.22	ND	1.69	1.70
SunOleic 97R					
125C/15min	ND	ND	ND	ND	ND
150C/5min	ND	ND	ND	ND	ND
150C/10 min	ND	2.72	ND	ND	1.43
150C/15min	2.43	6.02	ND	.35	.58
175C/5min	ND	3.54	ND	ND	.86
175C/10min	10.32	20.50	.86	.58	3.40
175C/15 min	27.76	32.82	1.01	.59	3.56
200C/5min	9.84	22.20	ND	ND	3.01

The prevention of flavor fade requires an understanding of the relationship between carbonyl-amine and lipid oxidation reactions. The polyunsaturated fatty acid content of lipids in peanuts, as discussed previously, makes the lipids more susceptible to lipid oxidation.

The major by-products of this reaction are nonanal, octanal, decanal, and hexanal, with hexanal being the most prominent. These chemicals contribute to the cardboardy, painty, and oxidized flavors associated with flavor fade. The products of the carbonyl-amine reaction, or browning reaction, yields the positive flavor attributes of roasted

peanuts. The major products of this reaction evaluated by Warner and others (1996) were 2,6-dimethylpyrazine, 2-methylpyrazine, 2-ethyl-3-methylpyrazine, 6-methylpyrazine, and 2,3,5-trimethylpyrazine. This research showed that during storage, the concentrations of hexanal, octanal, nonanal, and heptanal all increased along with the TBA values and decreasing flavor scores by a sensory panel. These results indicated that off flavor development in ground roasted peanuts during storage occurred in part as a result of a production of low molecular weight aldehydes produced from lipid oxidation. The pyrazines and furans however showed no distinct trends with this research, and the researchers found it difficult to determine any changes due to storage. This research indicates that flavor fade associated with roasted peanuts is due to the masking of pyrazines by large quantities of lower molecular weight aldehydes produced during lipid oxidation. However, Vercellotti and others (1992) determined that the positive volatile compounds that attributes to the flavor of fresh roasted peanuts disappeared when evaluating the rancid peanuts using a sniff port and GC retention times to evaluate the chemical compounds.

Ho and others (1981) reported a systematic chemical analysis of the volatile flavor components isolated from freshly roasted peanuts. They identified 131 compounds, which consisted of 12 aliphatic hydrocarbons, 7 aromatic hydrocarbons, 7 alcohols, 7 aldehydes and ketones, 5 acids, 9 esters, and lactones, 21 pyrazines, 7 pyrroles, 6 pyridines, 15 sulfides, 8 thiazoles, 3 thiophenes, 11 furanoids, 10 oxazoles and oxazolines, and 2 miscellaneous compounds. The identifications were based on mass spectral data and the infrared data were used to aid the identifications whenever samples were large enough to obtain the infrared spectra. The technology used by Ho and others

(1981) was much more current than the technology used by previous researchers, and therefore, they were able to acquire more information using this technology.

Ho and others (1981) separated these compounds into several groups: hydrocarbons, alcohols, aldehydes, ketones, and acids; esters and lactones; pyrazines; pyrroles and pyridines; thiazoles; oxazoles and oxazolines; and other heterocyclic compounds. Of the hydrocarbons, alcohols, aldehydes, ketones, and acids, only the isobutyric acid had a major impact on the fresh roasted peanut flavor with a strong butter-like aroma. The pyrazines had a major impact on flavor with a pleasant roasted nutlike note. The pyrroles and pyridines imparted a sweet woody aroma, and 2-acetylpyrrole has a rather unpleasant, plastic, antiseptic aroma. The thiazole compounds imparted a pleasant nutty aroma and could be important to the flavor of roasted peanuts. The oxazole compounds were characterized as having a green, nutty aroma.

### ***Effects of Storage on Quality of Peanuts***

Peanuts are a semiperishable crop and are subject to loss in quality during storage through microbial proliferation, insect and rodent infestation, biochemical changes (flavor change, rancidity, and viability loss), and physical changes (shrinkage and weight loss) (Pattee and others 1982). When held in suitable storage environments, clean peanuts can be stored for several years with minimal damage. Like most agricultural products, as moisture and temperature are decreased, the rate of deterioration in storage also decreases (Pattee and others 1982).

Dimick and others (1994) stored fresh roasted peanuts for 68 days and evaluated the flavor fade by measuring aldehyde concentration, pyrazine concentration, TBA values, and sensory analysis. With increasing storage, headspace hexanal, heptanal,

octanal, and nonanal concentrations increased concentrations along with the higher TBA value and 'rancid' flavor scores indicating off flavor development in roasted peanuts. Unlike the aldehydes, the headspace concentrations of the pyrazines showed no trends toward either increasing or decreasing as a result of storage.

Bett and Boylston (1992) studied the effect of elevated storage temperature on the flavor of roasted peanuts and on volatile compound concentrations. The samples were stored in a 37°C oven for 12 weeks, and samples were removed for GC analysis and sensory analysis periodically throughout storage. Bett and Boylston (1992) found that the content of alkylpyrazines decreased significantly with storage with the greatest decreases early in storage. This decrease in the content of heterocyclic compounds may be due to degradation by lipid radicals and peroxides or flavor entrapment by complexes between proteins and lipid hydroperoxides or hydroperoxide secondary products. Storage time also significantly affected roasted peanutty flavor. Roasted peanutty intensity decreased during storage. Over 12 weeks of storage at elevated temperatures, an increase in the concentration of hexanal, heptanal, 2-heptenal, octanal, 2-octenal, 2-decenal, 2,3-decadienal, 1-octen-3-ol, and 2-pentylfuran were observed. In contrast to the pyrazines, the greatest increases in the content of these compounds occurred at the end of the storage time. The painty and cardboardy flavors, noted by sensory testing, increased over elevated temperature with increasing storage (Bett and Boylston 1992). St. Angelo and Ory (1975) examined the effects of 12- month cold storage on the peroxidation of lipids in both roasted and green peanuts. They determined that storage of roasted peanuts at 4°C in jars containing air would not retard peroxidation.

Reed and others (2002) showed that off flavor components formed from oxidative reactions during storage at varying water activities ( $a_w$ ) can have a direct effect on sensory attributes of peanuts. At a low water activity ( $a_w=0.19$ ) the peanuts oxidized at higher rates than at higher water ( $a_w=0.60$ ) activities. The high oxidation rates resulted in an increase of unwanted flavor compounds. The GC-SPME analysis revealed that a decrease in pyrazines occurred during storage, while lipid oxidation products increased. The low  $a_w$  treatment showed higher levels of off flavor compounds and a greater decline in pyrazines as compared to the high  $a_w$  treatment. Baker and others (2002) stored peanuts under different water activities for fourteen weeks measuring peroxide values, percent moisture, and sensory attributes at 2-4 week intervals. Peroxide values increased over time for all treatments. The highest oxidation values were observed in the peanuts held at  $a_w = 0.67$ . Moisture percentages increased over time for all samples and correlated with the water activity of storage. Roasted peanut flavor decreased with time and the decrease was greater at higher  $a_w$ .

### ***Sensory Quality of Roasted Peanuts***

The sensory quality is the summation of all physical and chemical characteristics of the edible peanut seed that influences human senses and results in acceptability judgments by the consumer. The characteristic color of roasted peanuts is due primarily to Maillard browning reactions with the furfural browning products and Amadori rearrangements play a minor role in the coloration of the heated peanut seed (Pattee and others 1982). The brown color deepens as temperature and roasting time increases. The color of roasted peanuts can be assessed instrumentally by using colorimetry or reflectance spectrophotometry.

Texture also plays an important role in food acceptance. Roasted peanuts should be firm and crisp. Consumers, regardless of good coloring and flavor, will reject soft or mushy peanuts. Instrumental techniques that can be used to evaluate the texture are based on the force required to crush the peanut. Roasted peanut seeds are a desirable food product with a pleasant and unique flavor (Pattee and others 1982). Buckholz and others (1980) determined that roasting time has a significant influence on strength of odor and flavor. They evaluated two varieties of peanuts, Spanish and Runner, at light, medium, and dark roast. Using the Tukey test of significant difference to evaluate differences, a significant difference in the samples was observed.

Table V. Strength of odor and flavor based on peanut variety, runner and Spanish and peanut roast level, light, medium, and dark.

Roast Levels	Strength of Odor <sup>a</sup>	Strength of Flavor
Runner		
Light	4.98a	4.78a
Medium	6.07b	5.69b
Dark	6.84b	6.74c
Spanish		
Light	5.46a	5.26ab
Medium	6.01ab	5.80b
Dark	6.21b	6.02bc

<sup>a</sup> Letters within columns represent significant difference between samples

Consequently the dark roasted samples had the highest values for strength of odor and flavor (Table V).

In order to evaluate off flavors in peanut products, United States Department of Agriculture, Agricultural Research Service developed a quality/flavor research program at its Southern Regional Center to combat this issue. The first step in this program was to develop a common, standardized vocabulary to describe both desired and unacceptable flavors in peanuts and peanut products. Once this vocabulary was developed, trained taste panels could complement the work of peanut specialists in determining the biological,

physical, and chemical causes of unacceptable flavors that occasionally arise in the peanut industry (Johnsen 1986). Johnsen (1986) developed a lexicon of 19 terms to describe both desirable and undesirable flavors in peanuts. The terms are to be used with flavor intensity ratings ranging from 0-10 in evaluations of roasted peanut kernels and butters. The lexicon and intensity scale was developed by a 13 member panel of flavor and peanut specialists (Table VI) (Johnsen 1986).

Table VI. Lexicon of Peanut Flavor Descriptors as formulated by a 13 member panel of peanut and flavor specialists (Aromatics and Tastes) (Johnsen 1986)

Flavor Terminology	Description
Roasted Peanuttty	Aromatic associated with medium roast peanuts; having a fragrant character such as methylpyrazine
Raw bean/ peanut	Aromatic associated with light roast peanuts and having legume like character (specify beans or pea if possible)
Dark Roasted Peanut	Aromatic associated with dark roast peanuts, and having very browned character
Sweet Aromatic	Aromatic associated with sweet material such as caramel, vanilla, molasses, or fruit
Woody/hulls/skins	Aromatic associated with base peanut character and related to dry wood, peanut hulls, and skins
Cardboard	Aromatic associated with somewhat oxidized fats and oils and reminiscent of cardboard
Painty	Aromatic associated with linseed oil/ oil based paint
Burnt	Aromatic associated with very dark roast, burnt starches, and carbohydrates (burnt toast or espresso coffee)
Green	Aromatic associated with uncooked vegetables/ grass/ twigs, cis-3-hexanal
Earthy	Aromatic associated with wet dirt and mulch
Grainy	Aromatic associated with raw grain (bran, starch, corn, sorghum)
Fishy	Aromatic associated with trimethylamine, cod liver oil, or old fish
Chemical/plastic	Aromatic associated with plastic and burnt plastics
Skunky/mercaptan	Aromatic associated with sulfur compounds such as mercaptan, which exhibit skunk like or rubber like character
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
Astringent	The chemical feeling factor on the tongue described as puckering/dry and associated with tannins or alum.
Metallic	The chemical feeling on the tongue described as flat and metallic.

### ***Solid Phase Microextraction***

Flavor or fragrance components have been traditionally extracted by concentrating the analytes using liquid-liquid extraction, solid phase extraction, purge-and-trap, or headspace techniques. These procedures require a large amount of time, complicated equipment and solvents. The solid phase microextraction (SPME) technique eliminates the need for solvent or complicated apparatus by concentrating volatiles in headspace or nonvolatiles in liquids. Solid phase microextraction is a solvent free technique used for sample preparation that can integrate sampling, extraction, concentration, and sample introduction into a single step (Zhang and others 1994). SPME consists of two processes: partitioning of analytes between headspace and the liquid fiber coating and the desorption of concentrated analytes into an analytical instrument. In the first process, the coated fiber is exposed to the sample and the target analytes are extracted from the sample matrix into the coating. The fiber with the concentrated analytes is then placed into the instrument for desorption, separation, and quantification.

The fiber is a fused silica coated with a gas chromatographic stationary phase. The sample size of the fiber allows the fiber to be incorporated into a syringe-like device, which can then be operated like an ordinary syringe. Extraction techniques from the matrix are based on their sensitivity. Several factors that can influence the sensitivity of the fiber are the volume of fiber-coating, the fiber-coating characteristics, derivatization of target analytes, modification of matrices, heating the sample and cooling the coating (Zhang and others 1994). The proper coating is determined by the polarity of the compounds that are not to be extracted. Because both the sample matrix and the coating

compete for the target compound, the affinity of the coating for target analytes is crucial in SPME sampling. The nature of the matrix can be modified by adding salt or heating the sample. The addition of salt to aqueous samples increases the ionic strength thereby increasing the partitioning of polar organic compounds into the headspace. For thermally stable analytes, heating the sample is a convenient way to release analytes from the matrix into the headspace and improve sensitivity.

In order to obtain high precision and accuracy while using SPME, sampling time and other sampling parameters such as vial size, sample volume, and depth of immersion with liquid samples must be held constant. These factors are more important than full equilibration (Anonymous 1998). The number of extractions that can be performed with a single fiber is governed by the care in which the fiber is handled and the components in the sample being analyzed, but can range from 50-100 extractions, at which time, the accuracy and precision of the technique decreases (Anonymous 1998).

Coleman (1997) evaluated the qualitative and quantitative aspects of using both polar and nonpolar SPME fibers to analyze aqueous solutions of volatile and semivolatile Maillard reaction products including thiazoles, pyrazines, pyridines, and furfurals with two fibers, carbowax divinylbenzene and polydimethylsiloxane (PDMS) evaluated at varying concentrations from .50-50 ppm. Coleman (1997) also evaluated the matrix effects by measuring the standards alone and as mixtures. The research found that the carbowax divinylbenzene (CWDVB) fiber (slightly polar) was more selective for the more polar Maillard reaction products, when compared to the PDMS (nonpolar). They showed that SPME is an excellent technique for the qualitative analysis of Maillard reaction products, but for quantitative analysis matrix effect and competitive adsorption

of analytes needs to be taken into account when this method is employed. This matrix affect and analyte competition was further observed in research by Coleman (1997). Using the same techniques, Coleman (1996) showed the amount of compound absorbed from an aqueous sample increased with the degree of alkyl substitution and headspace volatility even though the concentration of all the compounds were the same.

### ***GC-Olfactometry***

Gas Chromatography-Olfactometry (GC-O) refers to the sniffing of GC effluents to determine which component(s) possesses odor. Many peaks detected by GC do not contribute to our perception of flavors or fragrances because the sample concentrations are below the detection threshold. Identification and quantification of such aroma or odor active compounds has many important advantages including: correlating sensory responses with volatile chemicals, selecting compounds useful for monitoring flavor changes during shelf life, resolving off flavor problems, understanding flavor release during eating, augmenting creative flavor compounds, targeting flavor compounds for thermal or biological generation, accessing olfactory activity of individuals, and elucidating the mechanisms of odor perception.

Soon after GC was invented, chemists interested in studying smell began to sniff the GC effluent to determine which components had odor. The first GC-O consisted of a non-destructive but insensitive thermal conductivity detector with the outlet sent to a sniff port located in a telephone booth to isolate the sniffer from other ambient smells. The first “true GC-O” design diminished the discomfort and inaccuracy associated with sniffing a stream of hot GC effluent (Mayol and others 2001). Further improvements

included the use of a venturi to retain the resolution of capillary columns and to deliver the effluent ergonomically to the “sniffer” (Figure III).

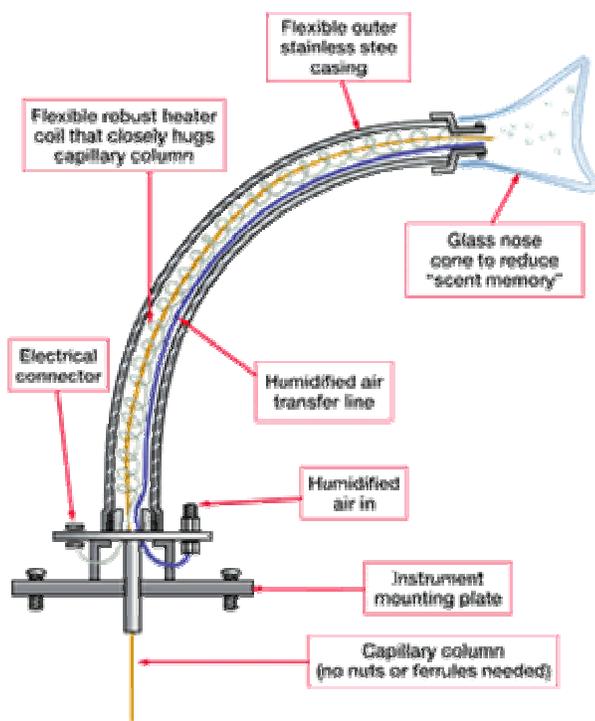


Figure III. Schematic of sniff port attached to gas chromatograph-olfactometer. ODO II, SGE International ([www.sge.com](http://www.sge.com))

Gas chromatography-olfactometry (GC-O) has become the most widely used technique for evaluation of complex food flavors because the GC-O directly provides important information about the presence of compounds with aromatic properties in foodstuffs (Ferreira and others 2002). The main purpose of the GC-O research is to list and order the aroma compounds present in the foodstuff according to their potential importance in the food flavor. These lists change according to the different GC-O techniques which can be classified into three categories: determination of threshold concentration (aroma extraction dilution analysis (AEDA) or CHARM, frequency of citations, intensity assessment (OSME), cross modality matching, or flavor impact values (Ferreira and others 2002).

Dilution factors (DF) and Charm values are two similar and effective means of estimating the odor active volatiles (OAV) of compounds in completely vaporized samples. A DF is a number of times, expressed as fold, that a sample can be diluted before an odor disappears from a GC-O aromagram. CHARM values are measures of the area under the GC-O peaks, which are derived from the dilution, and the duration of odor events.

An immediate problem is to identify those chemicals that contribute significantly to flavor. That is, a list of constituents needs to be arranged in order of decreasing flavor significance. A list requires the application of sensory techniques capable of associating flavor descriptors with chemical constituents (Acree and others 1984). CHARM is a procedure for the quantitative and qualitative analysis of gas chromatographic effluents. CHARM combines high resolution gas chromatography with use of n-paraffin standards, and uses computerized data collection and reduction, and a sensory procedure based on odor-detection thresholds rather than psychological estimations of stimulus intensity (Acree and others 1984). The analysis uses precise retention index, relative to normal paraffins, of the odor active constituents in a chromatographic effluent, and then applies intensive and/or nominal sensory descriptors to each of them. A solution of n-paraffin standards is then chromatographed under identical conditions and their retention times used to convert the times in the sensory response table to retention indices. A linear interpolation model is used for temperature-programmed chromatography and a logarithmic model is used for isothermal runs (Acree and others 1984).

Two experimental procedures are used each producing different types of sensory developed chromatograms, CHARM response chromatogram and the sensory response

chromatogram. The simplest technique is to repeat the chromatography several times with one subject, or different subjects, and add the resulting response tables to produce the coincident response chromatogram.

The objective of CHARM analysis is to determine the precise retention index relative to normal paraffins of the odor active constituents in a chromatographic effluent, and to apply intensive and/or nominal sensory descriptors of each of the odor active compounds. The CHARM response chromatogram is produced by considering the ratio of the total amount of odor active compounds eluting at a particular index to the threshold amount for that same mixture of compound (Acree and others 1984). This ratio can be estimated by performing the same analysis at decreasing dilutions, and when the odor is no longer detected, an upper bound for the ratio,  $c$ , is a function of the dilution factor,  $d$ , and the number of coincident responses,  $n$ :  $c = d^{n-1}$ . For a given retention index,  $c$ , is equal to the ratio  $a_1/a_n$ , where  $a_1$  is the amount of odor-active compound eluting from the most concentrated sample and  $a_n$  is the amount eluting from the most dilute sample. This gives,  $c = a_1/a_n = d^{n-1}$ , and for any value  $n$ ,  $d = a_1^{1/n} / a_n^{1/n}$ . The CHARM response chromatogram is made by plotting  $c$  against the retention index. The resulting peak areas are relative measures of the odor intensities of the substances eluting from the gas chromatograph in a particular region. The use of CHARM in the methodology of sensory analysis formalizes the process of sniffing gas chromatographic effluents. The concept of CHARM was systematically constructed from the idea of odor values.

The second method is the Aroma Extraction Dilution Analysis (AEDA). The aim of this technique in food flavor research is to determine the relative odor potency of compounds present in an extract. This method gives the priority order for chemical

identification and adds to the understanding of the chemical origins of olfactory differences (Grosch 1993). AEDA is the most frequently used method for the screening of flavor impact compounds because of its simplicity. The dilution factor (FD) value is the last dilution at which an odor active compound is detected at a certain retention index (RI) (Abbott and others 1993). According to this technique, the flavor extract is sequentially diluted (following a rate R where R is usually 2, 3, 5 or 10) and each dilution is analyzed by GC-O by a small number of judges. The FD of an odorant corresponds to the maximum dilution at which that odorant can be perceived by at least one of the judges. Numerically, if the last dilution at which the compound c was perceived was P (where P is usually is 0, 1,2, .....n), its FD is  $R^P$  (Ferreira and others 2002). The results are generally expressed as the logarithm of the factor of dilution (log FD) vs. the RI or by listing the factors of dilution.

Abbot and others (1993) evaluated the differences between the CHARM analysis method and the FD analysis method. They used a sniffing panel that consisted of six people. The sensory data was recorded by each panel member, by pressing the space bar on the computer keyboard when an odor was detected and immediately releasing when the odor was no longer detected. The yes/no response data (the start time and end time measured in seconds) were collected directly into a program. The panelists were also asked to put a descriptor onto the perceived odors. Serial dilutions were then produced and analyzed in the same way until the odor active regions were no longer detected. The researchers used the same data collection method, and analyzed the data differently using the CHARM analysis and the FD analysis to determine which gave more conclusive data. Abbott and others (1993) determined that the contribution of odor of a product by an

individual compound may be better determined by CHARM analysis than by FD as the surface area of the peak because the length of time the odor is perceived is taken into account rather than just the final dilution at which a compound was detected. The results also indicated difficulty for a panelist to detect the end of an odor active region than the beginning. Therefore, the period over which a compound is detected will vary and thereby influence the surface area of each odor active region.

GC-O method is preferred over GC-Dynamic Headspace Analysis. When using dynamic headspace analysis to evaluate peanut butter flavor, heating is required along with an inert gas sweep and vacuum, and the volatiles will contain not only the natural flavor volatiles of the peanut butter, but also compounds formed through precursors during the heating period used for trapping the headspace. The kinetics of Maillard reaction are such that when precursors are present, the products form slowly even at ambient temperature. Peanut butter that has been warmed for four hours at 90°C would result in a considerable amount of browning reaction products forming in peanut butter and should be taken into account as part of the assessment of a roasted peanut sample (Vercellotti and others 1992). Due to the previously mentioned problems with dynamic headspace, Vercellotti and others (1992) decided to perform a relatively short (15min) inert gas sweep of the sample through the cartridge. They chose to evaluate the correlation of identified important volatiles through high resolution capillary GC with sensory attributes from controlled human olfactory bioassay through the sniffer port that led to very valuable information about a product. The project used a good quality roasted Florunner peanut, a moderately rancid (PV=33 meq/kg), and a very rancid peanut (PV=111 meq/kg). The peanuts were compared for olfactory composition and GC

profiles. The positive attributes of the roasted peanuts disappeared in the rancid samples shown both by GC retention times of positive components, and olfactory identification with the sniffer port (Vercellotti and others 1992). They also determined that the two rancid peanut samples not only increased in other volatiles, hexanal, over those of the control, but also many of the key markers for acceptable flavor in the control were much more diminished or lacking in the rancid peanuts. Similarly, expected roasted peanut aroma attributes decreased in the rancid peanuts and negative odors were perceived as predominant olfactory characteristics. Vercellotti and others (1992) also evaluated the samples for good reproducibility of the method and determined that after fourteen runs good standard deviations at  $\pm 2\%$  for FID responses and olfactory perceptions in sequence were found. The standard deviations are an improvement over various trapping methods previously used, which had standard deviations of about  $\pm 18\%$ .

### ***Electronic Nose***

The human sense of smell is still the primary instrument used for evaluating the quality of a wide range of products through sensory analysis using both trained and untrained panels. Sensory analysis is an expensive process with large variations in the human sense of smell with age, health, and diet. Electronic nose (EN) sensory information is usually correlated with data acquired from analytical instruments such as GC and mass spectrometer in order to determine the odor or flavor of a product, but these techniques are very expensive and time consuming. A need exists in industry for an analytical instrument that is capable of mimicking the human sense of smell.

A typical EN consists of two key subsystems: an array of semi-conducting gas sensors and a data analysis technique. The gas sensors provide raw data by individually

changing electrical conductivity when exposed to different volatiles. The data analysis technique is then used to classify the sensor response data from individual receptors into an overall response pattern that represents the aroma. This pattern of individual sensor responses, rather than the response of any one specific sensor, is used to fingerprint the aroma (Que and others 2001). Each sensor in the array has the potential to respond individually to the many different volatiles that compose an aroma.

Semi conducting polymer sensors are used in many electronic noses and are generally made from polypyrrole that is modified through electrochemical or other chemical processes to respond to a variety of volatile compounds (Que and others 2001). The semi conducting polymer sensors operate at 30°C or less. This technology is based on unique sorption/desorption dynamics between volatile chemical compounds and an array of proprietary conducting polymers. Each polymer in the sensor array exhibits specific changes in electrical resistance upon exposure to different odor molecules. One constituent of the chemical mixture exposed to the array may interact with certain individual sensors, but not with others. This selective interaction produces a pattern or a “fingerprint”. When an odor is comprised of multiple chemicals, the fingerprint is the sum of their combined interactions with all sensors in the array. Odor concentrations or intensity can also generate different responses on the electronic nose (Hatfield and others 1994). Commercially manufactured electronic nose units typically include a built in data analysis package that is used to categorize samples based on aroma profile. These data analysis packages typically normalize all data and then utilize a wide range of statistical analysis techniques to classify and separate aroma profiles between treatments. These

statistical techniques may include principle component analysis, neural networks, k-means data clustering, and other pattern recognition methodologies.

Osborn and others (2001) wanted to determine if a commercially available electronic nose could statistically differentiate between off-flavored and non-off-flavored cured, ground peanuts and to develop sampling techniques to maximize the differentiation between the representative off-flavored and non-off-flavored peanuts using the available electronic nose. Through their research they determined an electronic nose can produce a statistically significant differentiation between off-flavored and non-off-flavored ground peanut kernels in the cured, unfrosted state. All the individual data points collected at a sampling time of 210 s for each sensor and, with the exception of a few sensors, all readings for off flavored ground kernels were greater than for kernels with questionable flavor. They also determined that the electronic nose effectively detected differences between treatments using only three replications with a sampling time of 60 s. The t-values for the electronic nose data were greater than both the GC and OVM.

Some researchers have made attempts to correlate the response of a sensor array in an electronic nose to that of a sensory panel in order to measure odor concentration. Hobbs and others (1995) attempted to overcome the difficulties of sensitivity and identification of currently used methods to determine odor concentration by using an electronic nose. Hobbs and others (1995) method would not only make the process less complicated, it would also be portable. Hobbs and others (1995) used the EN, photoionization detection (PID), and GC/MS to examine odors in pig and poultry slurries. Hobbs and others (1995) used these different instruments to determine which would be

optimal for poultry and pig slurries' odor concentration determination. PID responds to compounds with photoionization potential equal or less than that of the energy source, and is a general detector that does not directly detect water. The EN responds selectively to different types of compounds and the sensors that the device contains can be tailored to optimize discrimination between different compounds. The GC/MS was used to identify individual compounds to confirm the chemical differences between odors for the evaluation of the two detectors, PID and EN. Using these different techniques, they concluded that PID and EN had potential for measuring concentrations from pig and poultry slurries, but the sensitivity of both instruments were very low compared to olfactometry which was also used to compare with the sensitivity of the EN and PID.

Mallikarjunan and others (2002) used electronic nose as an alternative method for evaluating the rancidity of three different oils, fresh, marginally used and discarded. According to the results of extensive differences in signal responses for the sensors and the visual graphic plots using discriminate analysis, they determined that fresh, marginal, and discarded oil qualities can be effectively discriminated and identified from each other using the EN (Mallikarjunan and others 2002).

Aboukinane and others (2002) examined the use of a portable EN in determining different levels of volatility associated with peanut off flavors. They wanted to specifically determine if the EN could differentiate between samples stored at varying water activities and varying temperatures. The EN sensors showed a significant change in resistance upon being exposed to peanuts that were stored at varying humidity environments. Acetaldehyde and ethanol off-volatiles were produced when a set of the peanut pods were exposed to high temperatures, and the EN could also detect the

presence of these off volatiles. This off-volatile information gathered from the EN data was associated with the GC analysis, which also indicated high levels of ethanol and acetaldehyde.

Osborn and others (2001) determined the minimum level of destruction of cured, unroasted peanut samples required for the EN to significantly separate the means of high-temperature and room-temperature curing treatments. One lot of peanuts were dried in a convection oven 50°C for 65 hrs, and another lot of peanuts were dried at room temperature for 8 days. They determined that the EN was able to significantly separate high-temperature cured off-flavor peanuts from non-off-flavor peanuts for a completely non-destructive test using whole pods. Significant separation was also obtained using a sampling time of 60s with three replications. These results suggests that readings from an electronic nose can be directly correlated to flavor potential by analyzing a sample, roasting the same sample, and then evaluating the flavor using a sensory panel. The non-destructive test also has the potential to lead to the development of an “on-line” type sensor.

Miettinen and others (2002) compared sensory evaluation and two instrumental methods, EN, and headspace GC (HSGC) for their capability to detect aroma changes in oil-in-water emulsion models. The sensory panel easily detected increasing concentrations of the aromas in all matrices. The GC method determined the varying concentrations of linalool and diacetyl could be detected in all the matrices. The EN was less sensitive in detecting the differences in aromas in different matrices. The EN could only detect the highest concentrations of the linalool, and it was unable to distinguish between different concentrations in the diacetyl sample. Miettinen and others (2002)

determined that this variability in the sensitivity for different compounds may be a limiting factor for possible aroma applications, however, the inexpensiveness, speed, and possible applications for on-line detection make the EN an attractive screening tool for aroma measurements for the applications where the EN is sufficiently sensitive and selective.

Shaw and others (2000) evaluated commercial orange juice samples using EN and HSGC analysis with the goal of comparing the effectiveness of the new array detection systems with the more traditional gas chromatographic information. In this research, the use of quantitative data by HSGC for 11 volatile juice constituents gave better consistent separation of orange juice into three groups using canonical discriminate analysis based on a degree of processing than did the EN. The juices that were separated by the EN afforded a separation pattern similar to that found using data from HSGC, but the group most completely separated was different for the two techniques. The research suggests that additional volatile constituents not quantified by the HSGC influenced the electronic sensors (Shaw and others 2002).

Bleibaum and others (2002) evaluated varying brands of bottled apple juice in an attempt to correlate electronic instruments and human perception. They determined that electronic noses can be used to track “consumer defined quality” of apple juice by using results from correlation analysis and predictive equations between qualitative descriptive analysis (QDA) and consumer data. Electronic sensors can be used to predict acceptance in a more precise manner allowing greater tolerances on less important attributes while maintaining stricter control over key QDA sensory attributes.

Rye and others (2003) attempted to determine whether differences are detectable between samples of apple cider processed using high temperature short time pasteurization as monitored by EN technology. Heat treatments of 60, 70, and 80°C did not impose large differences in the aromatic profile of apple cider, measured using the EN when compared to untreated fresh apple cider after 24hr storage at 4°C. The heat treatment of 90°C imposed large differences in the aromatic profile as determined by the EN. The research determined that the EN was effective in identifying differences among processed apple cider samples, however, further examination into identification of GC compounds and sensory evaluation are required to apply the EN system to quality control and further organoleptic quality control in apple cider.

The EN can be used in different areas because of speed and ease of use as compared to gas chromatography. The EN is an important break through in the area of flavor chemistry and food stability, not only in product development, but also in quality assurance. For these reasons, an expansion of reliable techniques that can be used to detect product differences using the EN is critical to its expanded capabilities.

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**Identification and Quantification of Select Pyrazines in Fresh Roasted Peanuts using Solid Phase Microextraction-Gas Chromatography-Olfactometry and Gas Chromatography-Flame Ionization Detection**

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Identification of Pyrazines in Peanuts

## Abstract

Flavor fade prevention in peanuts requires an understanding of the primary contributors to the positive flavor components involved in roasted peanuty aroma, and the original concentrations in the product. This research used GC-FID and GC-O to identify and quantify the important flavor volatiles in fresh roasted peanuty aroma.

Ethylpyrazine, 2-methylpyrazine, 2-ethyl-3-methylpyrazine, 2,3-diethylpyrazine, and 2,3,5-trimethylpyrazine were found to be contributors to the roasted nutty aroma by the Gas Chromatography-Olfactometry trained panel at concentrations of .23, .70, 19.7, 1.5, and 6.5 ppm, respectively. 2-Methoxypyrazine and 2,3-dimethylpyrazine were also quantified at 3.1 and 4.8 ppm, respectively, and identified as samples not being traditionally correlated with fresh roasted peanut flavor, with aromas described as earthy and green. The GC-O intensities and the GC-FID signal were correlated to show a relationship between chemical concentration and perceived aroma. It was determined that pyrazines that were traditionally evaluated as having direct impact on fresh roasted peanuty aroma had low or no roasted peanuty aromas including 2,3-dimethylpyrazine and 2-methoxypyrazine.

**Keywords:** Pyrazines, Roasted Peanuts, Gas Chromatography-Olfactometry, Solid Phase Microextraction

## Introduction

Food aromas and flavors produced by the Maillard browning reaction, most importantly heterocyclic compounds including pyrazines, are significant because of their contribution to the flavor of processed foods. Pyrazines are formed during roasting, baking, and cooking. Pyrazine development is thought to occur through the Maillard or nonenzymatic browning process.

The browning or carbonyl-amine reactions involved in model systems comprised of monosaccharides and amino acids revealed the formation of pyrazines, pyrroles, furans, and other low molecular weight products (Pattee and others 1982). This reaction probably proceeds by the addition of a carbonyl group to an amino acid, protein or peptide yielding a glucosylamine. This glucosylamine will undergo Amadori rearrangements and become 1-amino-1-deoxy-ketose. These products are merely intermediate reactions, and the Maillard browning reaction will continue yielding nitrogen-containing compounds dependent upon the sample conditions, temperature, pH, sugars and amino groups.

A further reaction sequence, the Strecker degradation, begins from the  $\alpha$ -dicarbonyl compounds and other intermediate products. Strecker degradation occurs in foods with higher concentrations of amino acids and under higher temperatures and pressures. The aminoketone formed can yield pyrazine derivatives. These compounds will provide foods with flavors, aromas or colors which may be either desirable or undesirable.

Pyrazines constitute a very important class among flavor compounds. The alkyl-derivatives of pyrazines produce roasted nut-like, pleasant flavoring. The Maillard

browning reaction is the pivotal reaction that produces pyrazines, but currently gaps exist in the literature regarding the important pyrazines that contribute to the fresh roasted-peanutty aroma. A large variety of pyrazines are found in all foods, including, coffee, roasted barley, cooked casein, popcorn, rye crisp bread, soybeans, hydrolyzed soy protein, chicken broth, nonfat dry milk, roasted pecans, dehydrated potato, potato chips, tomato, spray dried whey, bell peppers, and roasted peanuts, to name a few (Collins 1971; Farretti and others 1970; Walardt and others 1971; Wilkens and Lin 1970, Wilson and Katz 1972; Wang and Odell 1972; Buttery and others 1969; and Liebach and others 1972). Current research has determined that pyrazines are the major volatile component in fresh roasted peanut flavor, but of the numerous pyrazines in peanuts, a need to determine the key flavor contributors exists (Johnson and others 1971a, 1971b; Pattee and others 1982; Ho and others 1981; Baker and others 2002). Current research also shows that, out of the considerable number of pyrazines that contribute to this overall fresh peanutty aroma, inconsistent data is available regarding stability and contribution to flavor fade over time, as determined by both sensory analysis and GC-FID (Warner and others 1996; Vercellotti and others 1992). The explanation has been flavor masking by aldehydes and other flavor degradation by oxidation materials (Bett and Boylston 1992; Dimick 1994). But the question still remains, which pyrazines contribute to the overall roasted peanutty flavor, and which pyrazines are affected by flavor entrapment and masking? This research seeks to answer these questions by evaluating fresh roasted peanut aroma using SPME-GC-FID standard addition as a quantification and identification method and SPME-GC-O in order to identify key potent pyrazine components of fresh roasted peanuts.

## **Materials and Methods**

### *Peanut Sample Preparation*

Smooth blanched Virginia Peanuts were obtained from Hampton Blanching (Severn, NC) (XL Virginia Peanuts/2002:0353HB1B—Lot 300) and stored in the cooler at 4-7°C until ready to be roasted. Twelve hundred grams of peanuts were dry roasted at 365°F (185-190°C). The pan was shaken every five minutes for 15 min. The roast degree uniformity was measured by sampling 50 gram groups of peanuts using a Minolta Chromameter (Tokyo, Japan) with Hunter Lab values at L=50-51 indicating medium roast.

### *SPME-GC-FID*

Pure standards of hexanal, 2-methylpyrazine, 2-methoxypyrazine, 2-ethylpyrazine, 2,5-dimethylpyrazine, 2,3-dimethylpyrazine, 2-ethyl-3-methylpyrazine, 2,3,5-trimethylpyrazine, and 2,3-diethylpyrazine were purchased from Sigma Aldrich Chemical Company (Milwaukee, WI). Standard solutions were prepared for each compound by adding the compound independently to the 10g peanut paste at a measured concentration between 2-3 ppm, independently. Fresh peanut paste was prepared by grinding 10g of roasted peanuts in a food processor (Black and Decker North American Power Tools and Accessories, Quick 'N Easy) for 30 seconds and adding 2.5 ml of a standard solution. The control sample contained the fresh peanut paste with distilled water in place of the standard solutions. Both the standard peanut slurry and the sample peanut slurry were placed into 40mL SPME vials with a septum-lined cap. A Carbowax divinylbenzene (CDVB) solid phase microextraction (SPME) fiber was then inserted through the pre-drilled septum into the headspace of the vial. The vial was then heated for 15 min in a hot water bath at 75°C to allow for the volatilization and adsorption of analytes onto the

fiber. The fiber was removed from the vial and inserted into the gas chromatograph (GC) injector port where it remained for 10 min to ensure complete desorption.

Samples were analyzed on a Hewlett Packard (HP) 5890 GC (Hewlett Packard, Palo Alto, CA) fitted with a sniff port (ODO II SGE International, Austin, TX). The GC was equipped with a DB-5 column (30m x.32 mm.i.d. x .25 $\mu$ m film thickness), flame ionization detector (FID), and an HP 3396 A integrator. The injector and detector temperatures were set at 250°C and 300°C, respectively. The initial column temperature was 50°C, and then increased at a rate of 10°C/min to a final temperature of 200°C. A GC splitter was used to split the column effluent between the FID and olfactometer in a 1:2 ratio, respectively. Helium was used as a carrier gas at 30cm/sec, linear flow velocity. Moist air was pumped through the sniffing port at 100mL/min. The GC-FID samples were all run in duplicate. Concentrations were calculated in parts per million (ppm) using the single point calibration method.

#### *GC-O*

Three trained panelists evaluated the fresh roasted peanut aroma, prepared using the method for the control vial as discussed above in triplicate. Panelists sat on a stool sniffing the GC-effluent as it exited the sniff port at 100mL/min. They recorded retention times, odor character and intensities of the odors on a 10- point scale. GC-O panelists were trained using a set of 25 compounds at varying concentrations and aromatic intensities that have been found to contribute to roasted peanutty flavor (Table I). The panelists underwent training sessions that focused on sample intensity, identification, and vocabulary. The training of panelists were concluded when panelists could consistently replicate several fresh roasted peanut samples using the GC-O, with proper vocabulary.

Table I. Flavor standards previously identified to have fresh roasted peanutty aroma

Peanut Flavor Standards	
1	Methyl-2-pyrrolyl ketone
2	2-Furyl methyl ketone
3	2-Acetylthiophene
4	2-Acetylthiazole
5	2-Acetylpyridine
6	2-Acetylpyrazine
7	Thiazole
8	4-Methylthiazole
9	4,5-Dimethylthiazole
10	2,4,5-Trimethylthiazole
11	2-Isobutylthiazole
12	2-Ethoxythiazole
13	2-Methylpyrazine
14	2,3-Dimethylpyrazine
15	2,5-Dimethylpyrazine
16	2,6-Dimethylpyrazine
17	2,3,5-Trimethylpyrazine
18	2,3,5,6-Tetramethylpyrazine
19	2-Ethylpyrazine
20	2,3-Deethylpyrazine
21	3-ethyl-2-methylpyrazine
22	2-Methoxypyrazine
23	2-Methoxy-3-methylpyrazine
24	2-Isobutyl-3-methoxypyrazine
25	2-Methoxy-4-vinylphenol

The compounds that were detected by individual panel members in two of three of their individual replications were then compared with aroma peaks identified by the two other panelists (Guen and others 2000). Aroma peaks detected by two of three panelists in two of three replications were included in the aromagram. Identification was completed by a comparison of retention times of GC-FID to retention times of GC-O recorded by the panelists. This data was compiled into a figure that displays average intensities of the GC-O signal corresponding with the GC-FID chromatogram.

## Results

Eight pyrazine compounds, and hexanal were quantified using GC-O. The GC-O panelists identified these compounds at varying intensities based upon their training and sense of acuity. The pyrazine compounds in fresh roasted peanuts were found to have varying intensities but the descriptors were very consistent in terms of roasted nutty and peanuty aroma (Figure I). The GC-O chromatogram displays seven standards and depicts the GC-FID intensities along with the trained panels perceived intensities which adds a direct sensory evaluation to an otherwise analytical technique. Figure I shows the large discrepancy between pyrazine concentration and pyrazine detection in aroma analysis.

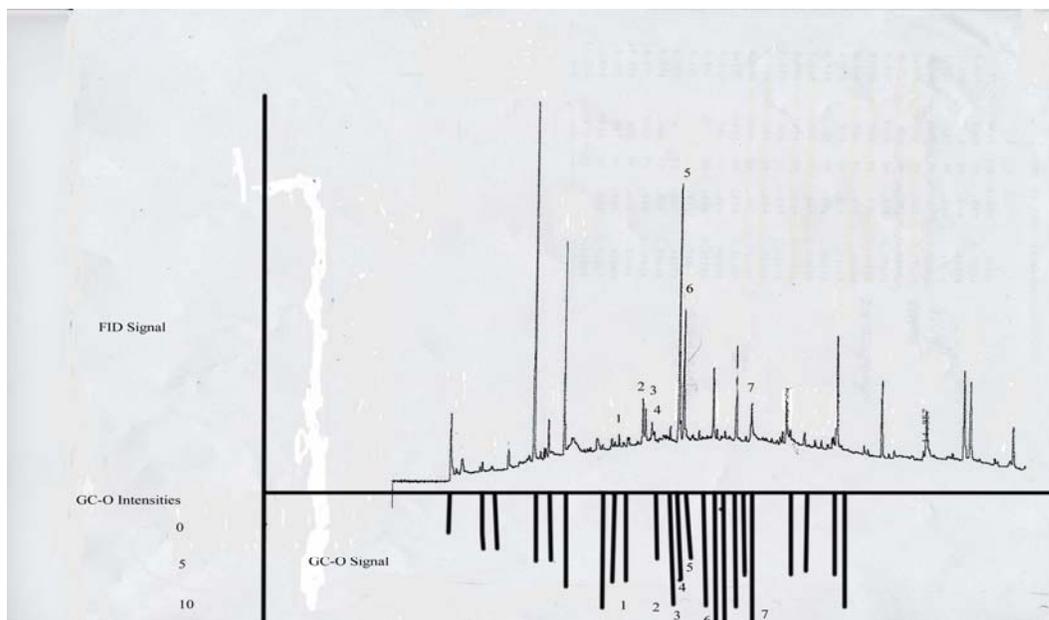


Figure I. GC-O intensities, as determined by a three member trained panel vs. GC-FID signals and intensities.

The odor intensities and the aromas associated with the samples are important because, although many pyrazines are associated with roasted peanuty flavor, the

pyrazines have significant intensities and odors that may not necessarily add to the peanuty aroma (Ho and others 1981; Walardt and others 1971; Johnson and others 1971a; Mason and Johnson 1966). Baker and others (2002) determined methoxypyrazine to be an important pyrazine contributing to fresh peanuty aroma, but Buttery and others (1969) determined methoxypyrazine to be a significant contributor to the odor of green bell peppers. According to the retention times, and identification of samples through standards using GC-FID, the GC-O panelists determined the methoxypyrazine compound to have an earthy aroma, and thereby not contributing a fresh, roasted peanuty aroma to the peanut flavor matrix (Table II).

Table II. Concentrations of identified pyrazines that contribute to fresh roasted peanut flavor in peanuts as determined by three trained panelists using GC-O paired with GC-FID.

Peak Number <sup>1</sup>	Retention Times	Aroma	Pyrazine	Concentration (ppm)
1	5.06	peanuts	2-methylpyrazine	0.70
2	6.10	Green	2-methoxypyrazine	3.08
3	6.40	Nutty	2-ethylpyrazine	0.23
4	6.61	Earthy	2,3-dimethylpyrazine	4.76
5	7.66	Roasted Grain	2-ethyl-3-methylpyrazine	19.70
6	7.95	Floral	2,3,5-trimethylpyrazine	6.48
7	9.39	Roasted peanuts	2,3-diethylpyrazine	1.49

<sup>1</sup> Peak number related to chromatogram in Figure 1.

Along with this earthy aroma, there were other aromas that would not traditionally be included in the roasted peanut matrix including floral, plastic, metallic, sulfur/skunky, perfume, and citrus (Table III). The aromas are caused by chemicals that are associated with the flavor volatiles of peanuts as they elute from the GC column. These aromas may be important in the overall aroma of the roasted peanut, but are not significant as individual compounds when evaluating fresh roasted peanut flavor. The important, or critical, aromas that were identified were roasted peanuty, nutty, roasted grain, popcorn, and caramel/sweet (Table II and III). These are all terms traditionally

used in the descriptors of peanuts through the lexicon of peanut descriptors (Johnsen and others 1986).

Compounds identified through GC-O as having potent aromas that contribute to fresh roasted peanuts were associated with the chemicals 2-ethylpyrazine, 2,3-diethylpyrazine, 2,5-dimethylpyrazine, 2-methylpyrazine, 2,3-dimethylpyrazine, 2-ethyl-3-methylpyrazine, and 2,3,5-trimethylpyrazine (Table II). From the GC-O chromatogram and Table II, it can be shown that the 2,3-diethylpyrazine and the 2-ethylpyrazine both contribute a significant amount of roasted nutty flavor to the flavor matrices of the roasted peanuty aroma found in fresh roasted peanuts. Both the 2-ethylpyrazine and the 2,3-diethylpyrazine have signal intensities as determined by a trained panel at greater than 7 on a scale from 1-10 with 1 being less intense and 10 being more intense (Table III). Identified pyrazines were also quantified using GC-FID (Table II).

Table III. Retention times, odors, and average intensities of fresh roasted peanuts as determined by three trained panelists using GC-O.

Retention Time	Odor	Intensity	Retention Time	Odor	Intensity
2.59	Peanut	3	8.48	Plastic	2
3.11	Peanut	2	8.71	Burned nutty	6
4.01	Green	3	8.74	Roasted nuts	2
4.55	Roasted grain/nutty	2	8.96	Burnt popcorn	10
4.76	Skunk	3	9.10	Nutty/popcorn	6
5.06	Peanuts	6	9.14	Roasted peanuts	6
5.33	Popcorn	6	9.37	Roasted peanuts	8
5.65	Roasted grain	6	9.43	Fruity	2
5.77	Roasted nuts	4	9.52	Nutty	8
5.93	Chemical solvent	2	9.66	Green peanuts	3
5.99	Skunk	6	9.67	Green	1
6.10	Green	5	9.93	Roasted	2
6.23	Earthy	5	9.97	Green	6
6.40	Nutty	7	10.2	Nutty	7
6.64	Earthy	5	10.39	Grapefruit	1
6.7	Roasted grain	3	10.43	Earthy	4
7.00	Caramel popcorn	2	10.63	Roasted peanut	1
7.23	Citrus	2	10.72	Skunky	5

7.40	Earthy	3	10.97	Sulfur	1
7.67	Roasted grain	3	11.08	Plastic	1
7.72	Earthy	6	11.67	Earthy	2
7.92	Caramel popcorn	7	11.96	Burnt	4
7.98	Floral	8	12.46	Nutty	1
8.11	Floral	4	12.52	Nutty	2
8.39	Floral	9	12.87	Floral	3
8.46	Plastic	5			

## Conclusion

GC-O in combination with GC-FID helps researchers to identify the important or key pyrazine compounds that contribute significantly to peanut aroma including 2-ethylpyrazine, 2,3-diethylpyrazine, 2,5-dimethylpyrazine, 2-methylpyrazine, 2,3-dimethylpyrazine, 2-ethyl-3-methylpyrazine, and 2,3,5-trimethylpyrazine. GC-O also adds a direct sensory component to an otherwise analytical procedure without having to use a sensory panel. This information is important when determining the mechanisms involved with flavor fade because all of the pyrazine compounds do not have the same response to storage. It has been found that some pyrazines decrease in concentration during storage at varying conditions, while others remain constant although there are decreases in roasted peanuty flavor and increases in painty, cardboardy, and bitter flavors as determined by sensory panels. Upon identification of the proper volatiles, researchers can then determine the trend of those compounds identified to contribute an intense peanut aroma, and perhaps better understand flavor fade and attempts to lower its impact on peanut quality loss during storage.

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## **Evaluation of Flavor Fade in Peanuts Over Short Term Storage**

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Flavor Fade in Peanuts

## Abstract

Initial flavor characteristics of roasted peanuts over short term storage were explored through sensory work and chemical analysis. The fresh-roasted volatiles produced from roasting peanuts and the aldehydes resulting from oxidation were also evaluated over short time using GC-FID, chemosensory techniques, and a sensory panel to quantify and identify the pyrazines and hexanal over the 21-day storage period. A significant decrease ( $p < .05$ ) was noted in 2,3-diethylpyrazine, 2-methoxypyrazine, 2,3-dimethylpyrazine, 2-ethyl-3-methylpyrazine, and 2,3,5-trimethylpyrazine concentrations over a 21-day period. No significant difference ( $p > 0.05$ ) was noted in the 2-methylpyrazine, and 2-ethylpyrazine concentrations. A significant increase ( $p < 0.05$ ) in the hexanal concentration over the 21-day period. The peroxide values and sensory analysis correlated directly with these results with a significant increase ( $p < 0.05$ ) in peroxide value at Day 14 and Day 21, and a significant decrease ( $p < 0.05$ ) in fresh roasted peanuty flavor from days 0–21 and a significant increase ( $p < .05$ ) in painty, cardboardy and bitter from days 7-21 with the sensory analysis. The electronic nose successfully separated Day 0 and Day 21 samples from Day 7 and 14, which were also separated, but with minimal overlap.

Keywords: Flavor Fade, Roasted Peanuts, Storage, Oxidation, Pyrazine Decomposition

## **Introduction**

Peanuts are grown in the warm climates of Asia, Africa, Australia, and North and South America. Americans consume over 6 pounds of peanuts and peanut products per year, worth more than \$2 billion at retail level. The U.S. produces over 29 million metric tons per year, and is the third largest producer of peanuts after China and India. The U.S. has about 3% of the world acreage of peanuts, but grows nearly 10% of the world's crop because of higher yields per acre. Although peanuts come in many varieties, four basic market types are in the US: Virginia, Runner, Spanish, and Valencia (Woodruff 1973). The peanut is a very diverse crop with many uses such as roasted, peanut oil, peanut butter, peanut spread, peanut flour and meal, fermented peanut products, and peanut confections.

The popularity of peanuts is due to both its crop diversity, stability, and natural flavorings of the edible peanut seeds. The flavor of the roasted peanut is attributed to the pyrazines that are formed upon roasting. Pyrazines are heterocyclic nitrogen compounds and their formation is a complicated process. The alkyl pyrazines produce a roasted-nutty sensory impression, and the acetylpyrazines have a more popcorn impression. Newell and others (1967) evaluated the precursors of typical and atypical roasted peanut flavor and derived a relationship that related flavor precursor concentration to sensory flavor. They found that aspartic acid, glutamic acid, glutamine, asparagine, histidine, and phenylalanine were associated with the production of typical peanut flavor. From this information, Newell and others (1967) theorized a mechanism for the production of flavor components from amino acids and carbohydrates.

Amino acids, lipids, peptides and carbohydrates are precursors to roasted peanut flavors (Buckholz 1981). Lipid oxidation in stored peanuts leads indirectly to the formation of aliphatic aldehydes, ketones, and alcohols. Free radicals and hydroperoxides formed during autoxidation also interact with the previously mentioned components of the food system and have been shown to decrease nitrogen containing compound concentrations by means of lipid-protein entrapment and hydroperoxide degradation (Gardner 1979 and 1983; St. Angelo and others 1979; St. Angelo and Graves 1986; Funes and others 1982; Bett and Boylston 1992).

The interaction of peroxidizing lipids with other nitrogen containing food components has been reviewed relating to protein and amino acid degradation (Gardner 1979 and 1983; St. Angelo and others 1979; St. Angelo and Graves 1986 and Funes and others 1982). Currently no research is available in regards to pyrazine declination due to free radicals, but enough information is available to infer that pyrazines may be degraded in the same way as proteins and amino acids (St. Angelo and Graves 1986; St. Angelo and others 1979; Gardner 1979 and 1983; Hidalgo and Zamora 2002; and Funes and others 1982).

Lipids are the major component in peanuts. They are ~52% of the dry weight of peanuts, with ~80% of the lipids being unsaturated. Of the total fatty acid composition, ~48% is oleic and ~31%, linoleic (Cobb and Johnson 1973). Lipid oxidation is the main cause of off-flavors. The major by-products of the oxidation reaction are nonanal, hexanal, octanal, and decanal, with hexanal being the most prominent (Nawar 1985). These chemicals are associated with cardboardy, painty, and oxidized flavors associated with flavor fade. The products of the carbonyl-amine reaction, or Maillard browning

reaction, yield the positive flavor attributes of roasted peanuts. Some of these pyrazines include 2,6-dimethylpyrazine, 2-methylpyrazine, 2-ethyl-3-methylpyrazine, 6-methylpyrazine and 2,3,4-trimethylpyrazine (Bett and Boylston 1992).

A considerable amount of prior work pertaining to the lipid oxidation of peanuts has been performed, but a lot of discrepancy in the literature exists relating to initial oxidation. Dimick (1994) noted a large increase in concentration of aldehydes and no significant change in pyrazine concentrations in samples stored at 65°C in an air convection oven for 1-68 days. Dimick's (1994) research indicates that the off-flavor associated with roasted peanuts is most likely due to aldehydes masking pyrazines by the larger concentrations of aldehydes. Vercellotti and others (1992) determined that the positive volatile compounds that contributes to the flavor of fresh roasted peanuts disappeared when evaluating the rancid peanuts using a sniff port and GC retention times to evaluate the chemical compounds. They noted that this disappearance is due to either degradation of heterocyclic compounds by lipid radicals and hydroperoxides or flavor entrapment between proteins and lipid hydroperoxides. Braddock and others (1995) evaluated volatile characteristics of high oleic and normal oleic acid peanuts stored at 25°C. The hexanal content was higher for the normal oleic peanut than the high oleic peanut. The pyrazines also were more stable in the high oleic peanuts than the normal oleic peanuts over storage. Baker and others (2002) evaluated the effects of water activity of peanuts on oxidation over a 14 week storage period, and found increased peroxide values over time for all treatments with the highest oxidation values observed at  $a_w = .67$ . From the current review of literature, it is unclear how pyrazines relate to flavor fade.

The purpose of this research is to better understand the mechanism and pinpoint the factors involved in flavor fade of peanuts over short term storage by quantifying and identifying the pyrazines that are directly involved with flavor fade of peanuts.

## **Materials and Methodologies**

### *Peanut Sample Preparation*

Smooth blanched Virginia Peanuts were obtained from Hampton Blanching (Severn, NC) (XL Virginia Peanuts/2002:0353HB1B—Lot 300) and stored in the cooler at 4-7°C until ready to be roasted. Twelve hundred grams of peanuts were dry roasted at 365°F (185-190°C). The pan was shaken every five minutes for 15 min. The roast degree uniformity was measured by sampling 50 gram groups of peanuts using a Minolta Chromameter (Tokyo, Japan) with Hunter Lab values at L=50-51 indicating medium roast.

### *Peanut Storage*

Treatment peanuts were roasted to a medium roast (L=50-51), stored under air in 1 pint Mason Jars with bands and dome lids, and sampled at days 0, 7, 14, and 21 for descriptive sensory analysis, chemosensory analysis, chemical analysis to evaluate oxidation, and volatiles by GC-FID. Control roasted peanut samples were placed in 1 pint Mason Jars (Ball, Ball®, and TM's Ball Corporation, Muncie, Indiana) with bands and dome lids, flushed with N<sub>2</sub>, and stored at -20°C until evaluation.

### *SPME-GC-FID*

Pure standards of hexanal, 2-methylpyrazine, 2-methoxypyrazine, 2-ethylpyrazine, 2,5-dimethylpyrazine, 2,3-dimethylpyrazine, 2-ethyl-3-methylpyrazine, 2,3,5-trimethylpyrazine, and 2,3-diethylpyrazine were purchased from Sigma Aldrich Chemical Company (Milwaukee, WI). Standard solutions were prepared for each compound by adding the compound independently to the 10g peanut paste at a measured concentration between 2-3 ppm, independently. Fresh peanut paste was prepared by

grinding 10g of roasted peanuts in a food processor (Black and Decker North American Power Tools and Accessories, Quick 'N Easy) for 30 seconds and adding 2.5 ml of a standard solution. The control sample contained the fresh peanut paste with distilled water in place of the standard solutions. Both the standard peanut slurry and the sample peanut slurry were placed into 40mL SPME vials with a septum-lined cap. A Carbowax divinylbenzene (CDVB) solid phase microextraction (SPME) fiber was inserted through the pre-drilled septum into the vial headspace. The vial was then heated for 15 min in a hot water bath at 75°C to allow for the volatilization and adsorption of analytes onto the fiber. The fiber was removed from the vial and inserted into the gas chromatograph (GC) injector port where the fiber remained for 10 min to ensure complete desorption.

Samples were analyzed on a Hewlett Packard (HP) 5890 GC (Hewlett Packard, Palo Alto, CA) fitted with a sniff port (ODO II, SGE International, Austin, TX). The GC was equipped with a DB-5 column (30m x.32 mm,i.d. .25µm film), Flame Ionization Detector (FID), and an HP 3396A integrator. The injector and detector temperatures were set at 250°C and 300°C, respectively. Helium was used as carrier gas at a linear flow velocity of 30cm/sec. The initial column temperature was 50°C, and then increased at a rate of 10°C/min to a final temperature of 200°C. A GC splitter was used to split the column effluent between the FID and olfactometer in a 1:2 ratio, respectively. Moist air was pumped through the sniffing port at 100mL/min. The GC-FID samples were all run in duplicate. Concentrations were calculated in parts per million (ppm) using the single point calibration method.

### *Chemosensory System*

Two grams of whole peanuts were placed in 40mL vials with aluminum crimp caps and septa were at Day 0, 7, 14, and 21 sampled using the HKR Sensory Systems Model QMB-6 (Munich, Germany) connected to a HS40-XL (Perkin-Elmer, Norwalk, CT) auto sampler. The QMB-6 model has six sensors with differing polarities. QMB-Soft NT software was used to provide Canonical Discrimination Analysis to reveal differences among samples.

### *Sensory Panel Training*

Seven panel members (5 females and 2 males) from the Virginia Tech community were trained using a lexicon of peanut flavor descriptors (Johnson and others 1987). Panelists were graduate students at Virginia Tech between the ages of 21 and 30. Panelists were trained to recognize roasted peanutty, cardboard, painty, and bitter flavors associated with both fresh roasted peanuts and “flavor-faded” peanuts through a series of ten, 45-60 minute training sessions. During training sessions, panelists were given a variety of peanut samples having undergone differing storage times and conditions in order to enhance or degrade various descriptors. Panelists were trained to scale cardboardy samples using a water soaked cardboard square and a peanut exhibiting slightly cardboardy characteristics for anchors. The painty descriptor was evaluated using a several year old peanut oil sample, and a 1-week peanut oil sample as anchors. The fresh roasted peanutty samples were anchored using a fresh roasted peanut and a 4-month peanut. Panelists were trained to discriminate fresh roasted peanuts from roasted samples stored from 0 days to 21 days using several sensory tests including triangle, and multiple comparisons. The panelists were trained to scale samples on all five descriptors using a

scale from 1-10 with 1 being less intense and 10 being most intense (Reed and others 2002). During the test evaluations, the panelists were given reference samples that were stored under N<sub>2</sub>, and at -20°C to prevent oxidation.

### *Sensory Analysis*

Sensory testing was performed at Virginia Tech in the Department of Food Science and Technology sensory labs with each of the seven panelists in an individual booth. The panelists were given three, three-digit coded, randomized samples and a reference and asked to rate the intensity of three samples on a scale of 1-10 with one being less intense and 10 being most intense based on five descriptors, roasted peanutty, bitter, sweet, cardboardy, and painty. The panelists were asked to rinse between samples and were given unsalted saltines to clean their palates between samples. Panelists were asked to take three-minute breaks, as timed by the facilitator, between samples.

### *Peroxide Value Determination*

Peanut oil was extracted from samples using the Christie modification of the Bligh and Dyer oil extraction method (1959). First, 10g of peanuts and 40g of water were blended (to provide ~80% sample moisture required in the Bligh and Dyer extraction) for four min in a blender with 50mL of chloroform and 100mL of methanol. The mixture was then filtered on a Buchner funnel using Whatman filter paper No. 4, which was rinsed with 50mL of chloroform. The filtrate was transferred to a 1L separatory funnel with 50 mL of 0.88% KCl solution added. The solution was mixed, allowed to settle, and the lower layer removed. Anhydrous sodium sulfate (Fischer, St. Louis, MO) was added to remove water. The solution was filtered (Whatman No. 1) into a round bottom flask and the solvent was removed using a rotary evaporator. The

oxidation levels in the peanut oils were measured using the American Oil Chemists' Society (AOCS) peroxide value method (1989) and reported in milliequivalents of peroxide per 1,000 grams of sample.

### **Statistical Analysis**

The statistical design was a nested completely randomized design with storage nested within replications. Three replications of all analyses were performed except GC-FID, which were performed in duplicate. The alpha level of all analyses was at 0.05. All statistical analyses used Analysis of Variance (ANOVA) to determine overall difference and Least Significant Difference (LSD) to determine differences between samples. All statistical analyses were performed using Statistical Analysis Package (SAS) (SAS Institute, Inc., 1982).

## **Results**

### **Peroxide Values**

There was no significant difference ( $p>0.05$ ) found between days 0 and 7, with peroxide values (PV) both at 0 meq/kg (Figure I), but over storage, a significant increase ( $p<0.05$ ) in peroxide value occurred at Day 14 and Day 21 with values at 5.0 and 6.0 meq/kg, respectively (Figure I). Peroxide value is directly correlated with early stages of oxidation. Roasting of peanuts initiates lipid oxidation, which continues during storage and increases the PV of roasted peanuts (St. Angelo and Ory 1975). This data is in agreement with research by Divino and others (1996) who evaluated the effects of decreasing oil contents on the oxidation of roasted peanuts. The PV of the roasted peanuts was decreased with decreasing oil concentrations which shows the effects of storage on lipids in roasted peanuts. Reed and others (2002) also noted a lower peroxide value of high oleic peanuts versus normal oleic peanuts. This indicates the higher the degree of unsaturation, the higher the peroxide values, and therefore, the higher the oxidation levels.

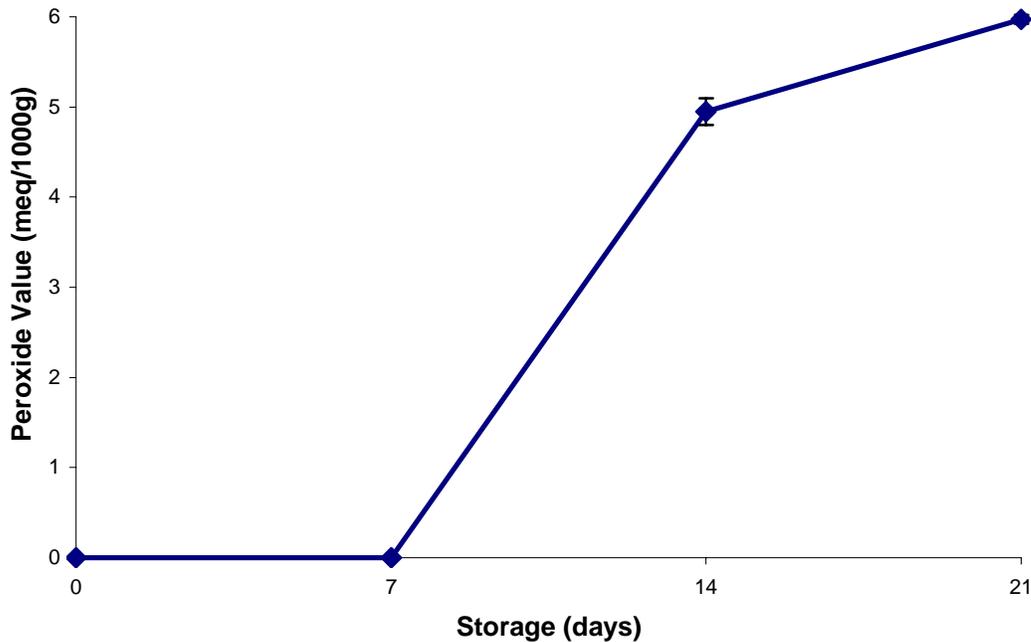


Figure I. Peroxide values of roasted peanuts over 21-day storage period stored at 25°C.

### **Descriptive Sensory Analysis**

Peanut flavors were evaluated using the terms roasted peanutty, sweet, bitter, cardboardy, and painty on 10-point scales, with 1 being least intense and 10 being most intense. Storage samples (25°C) were evaluated and a reference sample (-20°C) of a fresh roasted peanut with scores of roasted peanutty (7-8), sweet (5-6), cardboardy (1-2), painty (1-2), and bitter (1-2) was used. The roasted peanutty attribute decreased significantly ( $P < 0.05$ ) from Day 0 (7.33) to Day 21 (4.00) (Figure II), as expected. Researchers have found slight to significant decreases in roasted peanutty flavor beginning at Day 4 and extending through Week 7 (Dimick 1994; Warner and others 1996; Braddock and others 1995; Reed and others 2002). The sweet attribute significantly decreased ( $P < 0.05$ ) from Day 0 (5.29) to Day 7 (4.57) (Figure II). No

significant decrease ( $P>0.05$ ) was noted in the sweet attribute from Days 7-21 (Figure II). Bitterness increased throughout storage, but was only significant between Days 0 and 7 ( $P<0.05$ ) from Day 0 (1.90) to Day 7 (2.05) (Figure III). The painty attribute also increased significantly ( $P<0.05$ ) over time with Day 0 and 7 not being significantly different ( $P>0.05$ ) at 1.76 and 1.86, respectively (Figure III), but on Day 14 and Day 21 there was a significant increase ( $P<0.05$ ) to 2.86 and 3.19, respectively. In contrast, the cardboardy attribute increased significantly ( $P<0.05$ ) over time from Day 0 (1.67) to Day 21 (2.91) (Figure III). These data are in agreement with previous research pertaining to sensory flavor scores over time (Pattee and others 1982, Braddock and others 1995; Dimick 1994; Warner and others 1996; Reed and others 2002).

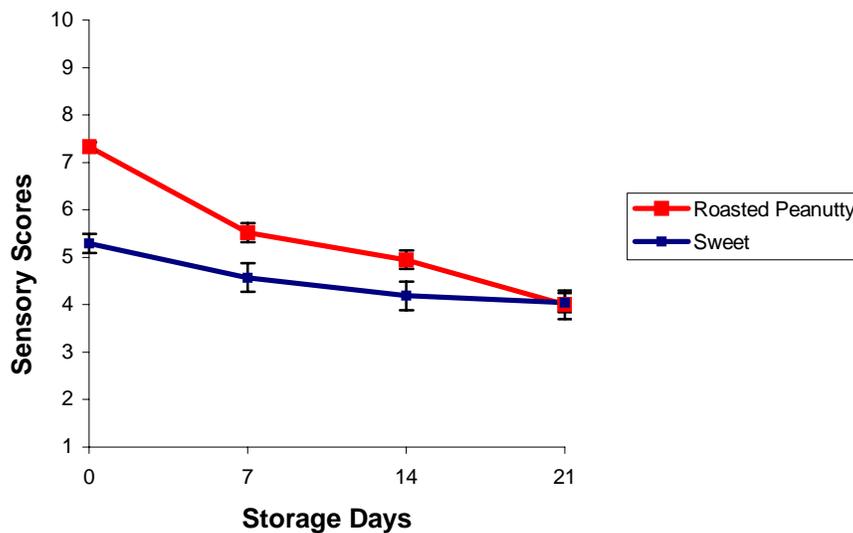


Figure II. Sensory scores for positive flavor descriptors roasted peanuttty and sweet, over 21 days storage at 25°C with 1 being less intense and 10 being most intense

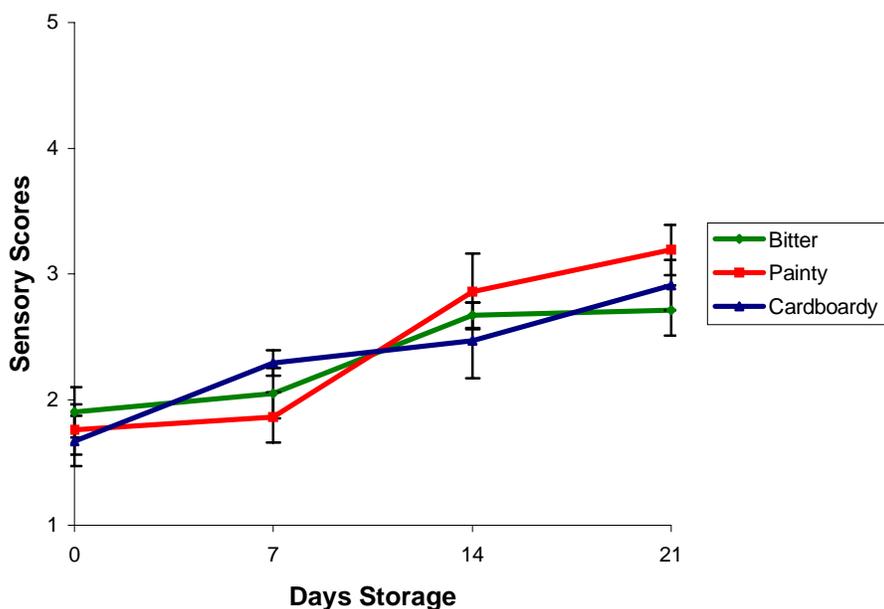


Figure III. Sensory scores for negative flavor attributes, bitter, painty, and cardboardy after 21 days storage at 25°C with 1 being least intense and 10 being most intense.

#### SPME-GC/FID

Single point calibration was used to calculate concentrations of pyrazines and aldehydes. The pyrazines evaluated and their retention times are listed in Table I, with a corresponding chromatogram illustrated in Figure IV.

Table I. Peak number and retention times of standards as they correspond to Figure IV

Peak number	Standards	Retention times
1	Hexanal	4.26
2	2-Methylpyrazine	5.01
3	2-Methoxypyrazine	6.23
4	2-Ethylpyrazine	6.40
5	2,5-Dimethylpyrazine	6.50
6	2,3-Dimethylpyrazine	6.61
7	2-Ethyl-3-Methylpyrazine	7.67
8	2,3,5-Trimethylpyrazine	7.96
9	2,3-Diethylpyrazine	9.39

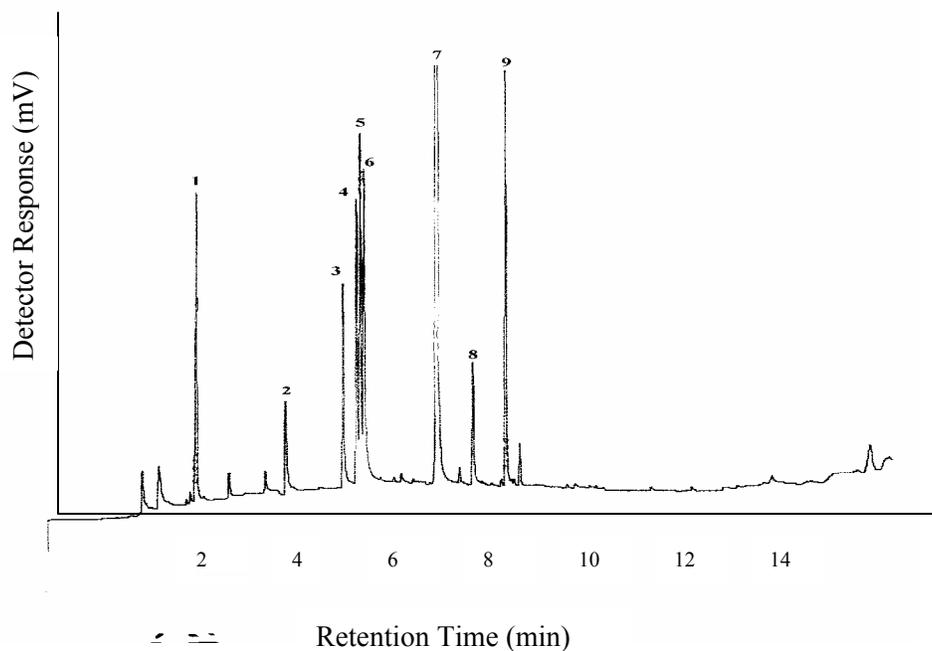


Figure IV. Gas chromatogram of pyrazine and aldehyde standards—Numbers refer to identifications shown in Table I.

A significant decrease ( $P < 0.05$ ) in concentration of 2-methoxypyrazine, 2,3-dimethylpyrazine, 2-ethyl-3-methylpyrazine, and 2,3,5-trimethylpyrazine occurred with storage time. There were no significant concentration ( $p > 0.05$ ) changes in 2-methylpyrazine, 2-ethylpyrazine, and 2,3-diethylpyrazine over a 21-day storage period (Figure V). There was a significant increase ( $P < 0.05$ ) in hexanal concentration with time.

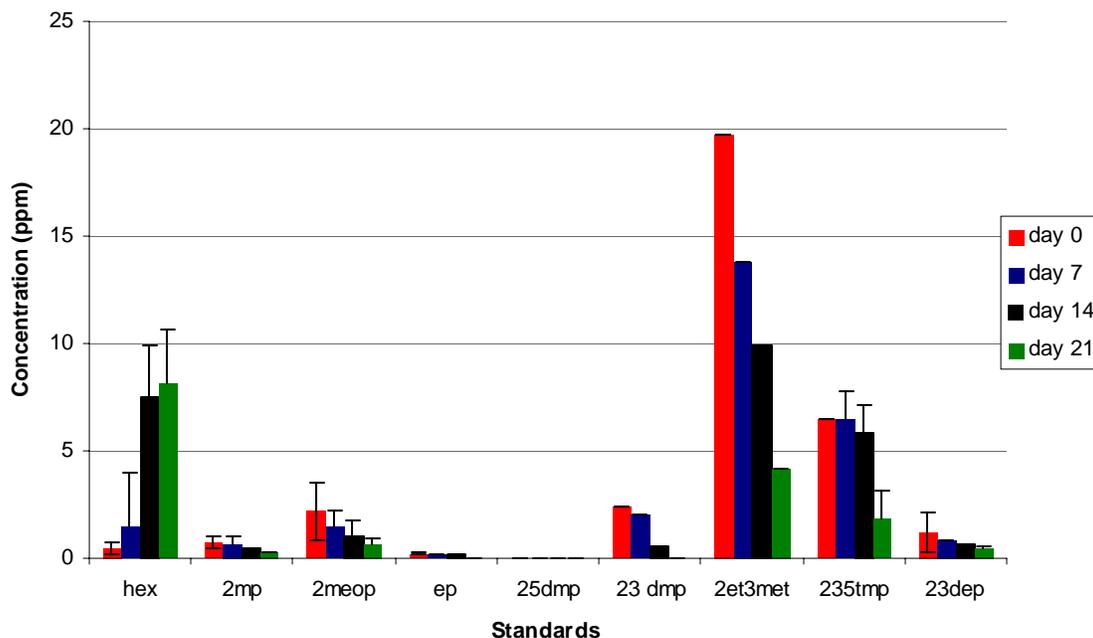


Figure V. Pyrazine and aldehyde concentrations (ppm) using GC-FID over 21-day storage period at 25°C analyzed on Days 0,7,14, and 21. Standards include (hex) hexanal, (2mp) 2-methylpyrazine, (2meop) 2-methoxypyrazine, (ep) ethylpyrazine, (25dmp) 2,5-dimethylpyrazine, (23dmp) 2,3-dimethylpyrazine, (2et3met) 2-ethyl-3-methylpyrazine, (235tmp) 2,3,5-trimethylpyrazine, (23dep) 2,3-diethylpyrazine.

These data agree with previous research reporting significant decreases in concentrations of pyrazines over storage, while other research differed. Dimick and others (1994) stored fresh roasted peanuts for 68 days at 25°C and evaluated the flavor fade by measuring aldehyde concentration, pyrazine concentration, TBA values, and sensory analysis. With increased storage time, an increasing trend in headspace hexanal, heptanal, octanal, and nonanal concentrations were found along with higher TBA values and ‘rancid’ flavor scores indicating off flavor development in roasted peanuts. Although, Dimick (1994) and Warner and others (1996) found increasing trends in aldehyde levels, neither researchers found significant ( $p>0.05$ ) decreasing trends in

pyrazine concentrations, but Warner and others (1996) reported fluctuations in pyrazine concentrations. Both Warner and others (1996) and Dimick (1994) found different trends depending on whether single ion monitoring or total ion analysis was used. Dimick (1994) also stored samples at a very high temperature (100°C).

Bett and Boylston (1992) found that the content of alkylpyrazines decreased significantly with storage with the greatest decreases early in storage. Reed and others (2002) also found that decreasing  $a_w$  significantly increased loss in pyrazine concentration, while lipid oxidation products increased. This decrease in the content of heterocyclic compounds, pyrazines, may be due to degradation by lipid radicals and peroxides or flavor entrapment by complexes between proteins and lipid hydroperoxides or their secondary products. Gardner (1983) suggested that non-covalent bonding causes flavor entrapment. When proteins are exposed to peroxidized lipid, a large proportion of the lipid complexes with the protein through hydrogen bonds and hydrophobic interactions (Gardner 1979). The current research also supports these theories of pyrazine degradation by lipid hydroperoxides, by showing significant decreases in pyrazine concentration over time.

A considerable amount of data describes the degradation of nitrogen containing compounds such as proteins, enzymes, and amino acids by lipid peroxide radicals (Schaich and Karel 1976; Funes and others 1982; Hidalgo and Zamora 2002). Although there was no research found in regards to the degradation of pyrazines in particular, one can infer that pyrazines are affected by the same mechanisms as the amino acids, proteins, and other nitrogen-containing compounds that have been previously reported. Zirlin and Karel (1967) found that by increasing water activity in a freeze-dried gelatin

exposed to peroxidizing methyl linoleate, scission was inhibited. This agrees with research performed by Reed and others (2002) which shows lower losses in the pyrazine content of peanuts with higher  $a_w$ . This current research assists in further confirming the mechanism of lipid peroxide decomposition leading to decreases in pyrazine content.

### *Chemosensory Analysis*

Electronic nose (EN) noted a clear separation of Day 0 and Day 21 from Days 7 and 14. The volatile characteristics of roasted peanuts on Days 14 and 21 are separated but with minimal overlap. From this graphic feature space display, an inference can be made that flavor fade set in at Day 7 and Day 14, as they are in the same graphic space only changing linearly, and at Day 21, oxidation or oxidative rancidity may set in and this causes the greater separation evidenced in the plot (Figure VI). These data can be related to the sensory analysis (Figure II and III). All the sensory descriptors between Day 0 and Day 21 are significantly different ( $P < 0.05$ ), and for Day 7 and 14 only the roasted peanutty and painty were significantly different ( $P < .05$ ) (Figure II).

Aboukinane and others (2002) evaluated peanut pods stored at varying water activities and temperatures and found the EN can decipher changes in aroma between odor of peanut pods with varying water activity levels and storage temperatures. This work suggests that there are key aromatic contributors to the separation caused between the samples at Day 0 and Day 21, but these aromatics are difficult to pinpoint using the EN. One can infer that the major cause of this separation can either be due to an increase or decrease in aroma active compounds found in the stored roasted peanuts. Osborn and others (2001) also found that an electronic nose can differentiate between odors of off-flavored and non-off-flavored cured, ground peanuts.

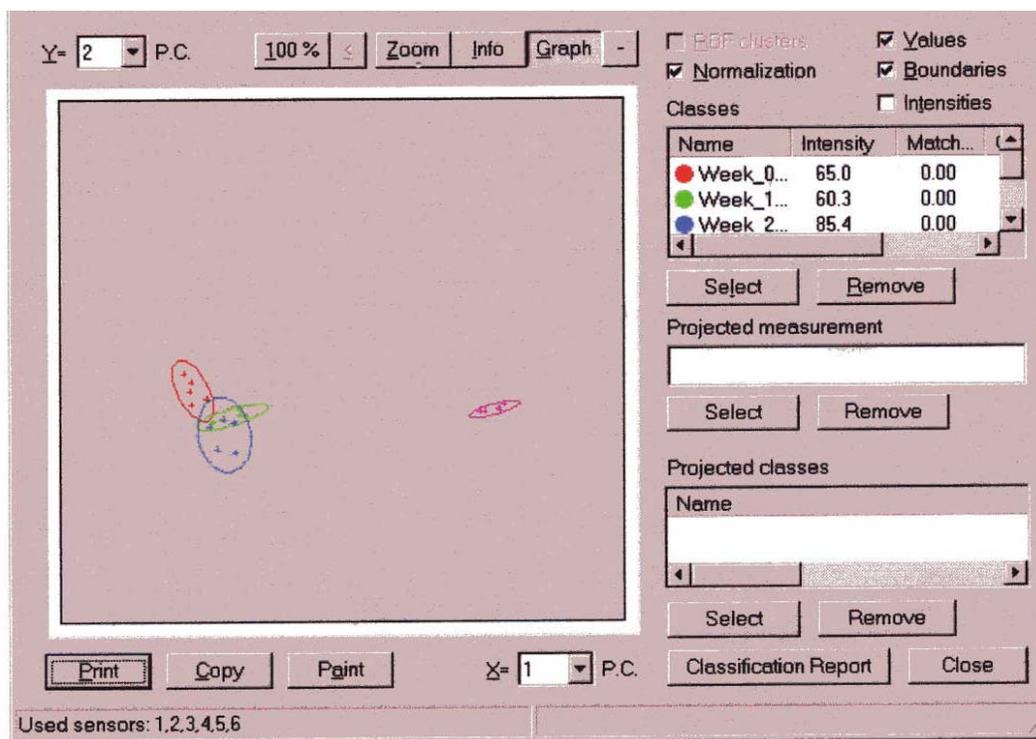


Figure VI. Principle Component Analysis plot of Day 0 (Week ), Day 7 (Week 1), Day 14 (Week 2), and Day 21 (Week 3) performed using QMBSOFTNT software on a QMB electronic nose.

## Conclusions

Previous research on flavor fade typically had storage periods anywhere from 68 to 360 days, compared to 21 days in our study. The shorter storage period would affect the changes in the pyrazines concentrations. The reasoning behind the shorter storage period was to examine the changes in volatile compounds over the early stages of peanut storage and ensure that initial flavor fade is being observed so that the mechanism for early flavor fade could be better understood.

The ability of the EN to differentiate between samples is not clearly understood in terms of the specific mechanism, or volatile chemicals that are triggering these differences. Although Aboukinane and others (2002) determined it to be the acetaldehyde and ethanol producing these results, there needs to be more work to determine its validity. The EN can therefore be a helpful tool in validating the overall shelf stability of samples over time, mainly because of the short preparation time. Forty samples were evaluated for this project, with a 25-minute preparation time as compared to the GC-FID sample preparation that was 15 minutes per sample for over 35 samples.

This research further describes the important pyrazines, 2-methylpyrazine, 2-methoxypyrazine, 2-ethylpyrazine, 2,5-dimethylpyrazine, 2,3-dimethylpyrazine, 2-ethyl-3-methylpyrazine, 2,3,5-trimethylpyrazine, and 2,3-diethylpyrazine, as the positive contributors to fresh roasted peanutty flavor. The decrease in pyrazine concentration may be due to decomposition by lipid radicals due to the peroxides. This research does dispel the idea that the main cause of flavor fade is masking of pyrazines alone, because there was a significant decrease in pyrazine concentration noted throughout this research, and if aldehyde masking was the only reason for decreased roasted peanutty flavor, pyrazine concentration would have very little effect on the results. Although aldehyde masking may have an effect over long term storage once the peanuts have become rancid and unpalatable, it is not likely for short term storage of peanuts.

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## **APPENDICES**

## Sensory Evaluation of Peanuts

Name \_\_\_\_\_ Date \_\_\_\_\_

First, taste the reference sample. The chart below gives an overview of the flavor intensity levels for each attribute of the reference sample. Then, rate the following samples from 1-10 for each flavor attribute (1 being less intense 10 being more intense).

Reference sample:

Roast peanuty: 7-8

Sweet: 5-6

Cardboardy: 1-2

Painty: 1-2

### Sample 231

Roast Peanuty

1 2 3 4 5 6 7 8 9 10

Sweet

1 2 3 4 5 6 7 8 9 10

Cardboardy

1 2 3 4 5 6 7 8 9 10

Painty

1 2 3 4 5 6 7 8 9 10

### Sample 502

Roast Peanuty

1 2 3 4 5 6 7 8 9 10

Sweet

1 2 3 4 5 6 7 8 9 10

Cardboardy

1 2 3 4 5 6 7 8 9 10

Painty

1 2 3 4 5 6 7 8 9 10

### Sample 998

Roast Peanuty

1 2 3 4 5 6 7 8 9 10

Sweet

1 2 3 4 5 6 7 8 9 10

Cardboardy

1 2 3 4 5 6 7 8 9 10

Painty

1 2 3 4 5 6 7 8 9 10

Prescreening questionnaire for a flavor panel

History:

Name: \_\_\_\_\_

e-mail: \_\_\_\_\_

Time:

Are Thursdays at 3:00-3:30 good for you?    YES            NO

Will you be in Blacksburg through June 1?    YES            NO

Health

Do you have any of the following?

Dentures

Diabetes

Oral or gum disease

Hypoglycemia

Food Allergies

Hypertension

Do you take any medications that may affect your senses, especially taste and smell?

Food Habits

Are you currently on a diet? If so, explain.

How often do you eat out in a month?

How often do you eat fast foods out in a month?

How often in a month do you eat a complete frozen meal?

What is (are) your favorite food(s)?

What foods can you not eat?

What foods do you not like to eat?

Is your ability to distinguish smell and tastes

	Smell	Taste
Better than average	_____	_____
Average	_____	_____
Worse than average	_____	_____

## Flavor Quiz

If a recipe calls for black pepper and there is none available, what would you substitute?

What are some foods that taste like yogurt?

How would you describe the difference between flavor and aroma?

How would you describe the difference between flavor and texture?

Describe some of the notable flavors in peanut butter?

Describe some of the notable flavors in black coffee?

Describe some of the notable flavors in Ritz crackers?

Peanut Panel Training: Session I.

Flavor Attributes

Roasted peanuty

The aromatic associated with medium roast peanuts-----and having fragrant character such as methylpyrazines

Cardboard

The aromatic associated with somewhat oxidized fats and oils and reminiscent of cardboard.

Painty

The aromatic associated with linseed oil and oil based paint

Bitter

The taste on the tongue associated with bitter agents such as caffeine or quinine

Triangle Test

Name: \_\_\_\_\_

Sample: Roasted Peanut

---

Instructions

Taste samples from left to right. Two are identical, determine which is the odd sample.

If no difference is apparent, you must guess.

Sets of three samples

Which is odd?

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

## **Jodi Powell**

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### **Objective**

To obtain a post doctoral position in the area of flavor chemistry

### **Education**

Ph.D. Candidate Food Science and Technology, Summer 2004  
Virginia Polytechnic Institute and State University  
Blacksburg, VA 24060

Dissertation: Evaluation of Initial Flavor Fade in Peanuts Using Sensory, Gas Chromatography/Olfactometry, and Chemosensory Techniques

M.S. Food Science and Technology, August 2001  
Virginia Polytechnic Institute and State University  
Blacksburg, VA 24060

Thesis: Sensory and Analytical Analysis of Sweet Buttermilk Formulations with Skim Milk, and their Stability to Light Oxidation and Pasteurization

B.S. Chemistry May 1998  
Delaware State University  
Dover, DE 19901

### **Employment**

**Program Assistant-GS-344-07-STEP Program**  
**United States Department of Agriculture / Cooperative State**  
**Research Education and Extension Service – Science and Education**  
**Resources Development**  
**Washington, DC**  
**Dr. George Cooper**  
**October 2003-Current**

- Create spreadsheets using Microsoft Excel and databases using Microsoft Access and CREEMS for several higher education grants programs depicting total funding for the programs, objectives, outcomes and impacts extrapolated from final reports submitted from Principle Investigators at the Universities
- Create professional PowerPoint presentations to be used at NASULGC conferences and various other national University meetings

- Represented CSREES at Future Farmers of America Conference in Kentucky
- Represented SERD at Agriculture in the Classroom National Conference
- Facilitated several meetings at the Agriculture in the Classroom National Conference in the place of a National Program Leader and a Program Specialist
- Summarizes funded projects and programs for the USDA-1890 Capacity Building Grants Program for preparation of award jackets
- Use Statistical Analysis Procedure that I independently designed to normalize panelists scores in order to rank proposals for funding using Microsoft Excel

**Food Analysis Teaching Assistant  
Virginia Polytechnic Institute and State University  
College of Agriculture and Life Sciences  
Department of Food Science and Technology  
Blacksburg, VA  
January 2002 - May 2004**

- Conducted 13 food analysis labs to prepare undergraduate students for jobs in the areas of quality assurance or quality control
- Lectured on a variety of chromatography and separation techniques, chemical titration techniques, protein and lipid assays and analyses
- Prepared laboratory reagents and standards prior to lab and clean and dispose of chemicals upon laboratory completion
- Implemented on the spot changes in laboratory manuals when necessary for successful completion of laboratory assignments
- Answer questions and assist students with analytical techniques, and make corrections when required
- Used Microsoft Excel spreadsheets to manage students laboratory grades and homework

**Dr. Betty Shabazz's Delta Academy  
Program Chair  
Delta Sigma Theta Sorority, Inc., Blacksburg Alumnae Chapter  
Blacksburg, VA  
Ms. Renia Edwards, Chapter President  
August 2002-August 2003**

- Independently assembled, coordinated, and lead a committee in planning and arranging all aspects of a youth development program

- Developed a curriculum which incorporated academic growth and development as well as promoting self esteem
- Coordinated seminars, field trips, and weekend travel excursions for over 11 young ladies between the ages of 10 and 14 years
- Coordinated funding for a completely non-profit, community funded program by independently soliciting donations and preparing proposals for local funding sources including a Virginia State Community grant for \$2500.
- Completed progress reports for the program indicating programmatic outcomes and impacts on both the community and the individual students involved in the program to be reported to the National Chapter of Delta Sigma Theta Sorority, Inc.
- Developed fliers and brochures using MSPublisher and MSPowerpoint
- Fliers and brochures were distributed electronically through various electronic listserves using MSOutlook

**Office Automation GS-344-05**

**United States Department of Agriculture / Cooperative State Research, Education and Extension Service /SERD-Higher Education Programs Office**

**Dr. Jeffrey Gilmore**

**May 1994 – October 2003**

- Review, summarize and organize research proposals and awards requested from Higher Education Institutions
- Assist with conducting and organizing professional peer panel review boards for several higher education grants programs
- Created statistical program used to facilitate the normalization of scores used in the peer panel review process
- Created Microsoft Access database to maintain data system for HEP - Multicultural Scholars Program and HEP- Fellowships Program
- Developed reports from the database which were used for program management and accountability

**VT STARS**

**Virginia Tech Summer Training Academy for Rising Stars  
Blacksburg, VA**

**Dr. Vernard Harrington/Mr. Ed McPherson**

**540-231-2436**

**July 2000-August 2002**

- Residential Counselor
- Assisted with curriculum development
- Instructed students on the uses of Microsoft Powerpoint to develop scientific poster presentations

- Assisted with locating qualified and well trained instructors for facilitating sessions
- Assisted with programmatic advertising and brochure development
- Aided director in developing annual summaries for the program's outcomes and impacts

**Minority Academic Opportunities Program  
Virginia Tech  
Graduate Assistant  
Dr. Randolph Grayson  
540-231-4209  
Summer 2000**

- Developed tri-fold, two-sided, color brochures for programmatic advertisement to be distributed to over 200 universities using Microsoft Publisher
- Assisted with coordination of a summer enrichment research program for undergraduate students to gain experience with independent research projects
- Maintained a university mailing list database in Microsoft Excel

**Food Chemistry Graduate Teaching Assistant  
Virginia Polytechnic Institute and State University  
College of Agriculture and Life Sciences  
Department of Food Science and Technology  
Blacksburg, VA  
August 1999 – December 2001**

- Independently conducted 12 food chemistry labs for 15 students
- Lectured students on pertinent information pertaining to the labs prior to execution
- Discussed professional technical writing style with the students as a part of their training
- Graded writing intensive laboratory reports according to Journal of Food Science Style Guide rules and regulations and assigned semester grades
- Set up and cleaned up chemicals, analytical instruments and glassware
- Accessible for tutoring and other guidance in the areas of Food Chemistry and General Food Science courses

**Chemistry Teaching Assistant  
Virginia Polytechnic Institute and State University  
College of Arts and Sciences  
Department of Chemistry  
Blacksburg, VA  
August 1998 – May 1999**

- Independently conducted 13 general chemistry wet laboratories per semester for 75 students
- Developed laboratory curriculum
- Lectured students prior to the two hour lab on pertinent information required for successful completion of the lab and the laboratory reports
- Graded laboratory reports for each lab and assigned overall semester grades to students
- Prepared and cleaned up lab areas upon completion of student lab assignments
- Tutored undergraduate chemistry and non-chemistry majors in environmental chemistry, general chemistry, and analytical chemistry

**Summer Intern in Environmental Chemistry  
University of California Center for Environmental Research  
and Technology  
Riverside, CA  
May 1997-August 1997**

- Conducted individual and original research on the kinetics of octanol isomers in the atmosphere
- Gained experience in Gas Chromatography/Flame Ionization Detection, MSEXcel, and ChemStation software
- Formal write up and presentation of data and knowledge gained from experimentation

**Honors and Activities**

- United States Department of Agriculture 1890 National Scholar
- Presidential Scholar – Delaware State University, 1994-1998
- American Chemical Society, Treasurer – Delaware State University National Chapter, 1994-1998
- Minority Academic Opportunities Program Fellowship – Virginia Tech, 1999-2004
- Minority Academic Opportunities Program Peer Mentor –1998-2003
- Food Science Club – Virginia Tech, 1999-2003
- American Dairy Science Association – National Chapter

- Minorities in Agriculture, Natural Resources, and Related Sciences –Virginia Tech/National Chapter
- Black Graduate Student Organization, Vice President – Virginia Tech
- Peer Review Panelist – United States Department of Ag/CSREES/SERD —Higher Education Programs Office
- National Dairy Products Evaluation Team Competition – Virginia Tech
- Christiansburg Institute High School Volunteer Tutor – Math, Chemistry
- Delta Sigma Theta Sorority, Inc.—Treasurer – 2002
- Delta Sigma Theta Sorority, Inc. – Chair Dr. Betty Shabazz’s Delta Academy – 2002 – 2003

## **Presentations**

- Minorities in Agriculture, Natural Resources and Related Sciences – “Evaluation of Initial Flavor Fade in Peanuts using Sensory, Gas Chromatography-Olfactometry, and Chemosensory Techniques” March 2004
- Food Science and Technology Research Symposium, “Evaluation of Initial Flavor Fade in Peanuts using Sensory, Gas Chromatography-Olfactometry, Electronic Nose” Virginia Tech, April 2003
- Human Nutrition, Foods and Exercise, Carbohydrates Symposium, Virginia Tech – April 2002
- American Dairy Science Association –“The Sensory and Analytical Analyses of Nonfat Milk Formulations: Stability to Light Oxidation and Pasteurization” – National Conference, July 2001
- Minorities in Agriculture, Natural Resources and Related Sciences –“The Sensory and Analytical Analyses of Nonfat Milk Formulations: Stability to Light Oxidation and Pasteurization” National Conference, March 2001
- Food and Animal Sciences Research Symposium – “Sugar Substitutes” – Alabama A&M University – November 2001
- Graduate Student Association Research Symposium, Virginia Tech “Sensory Analysis of Nonfat Milk Formulations” December 2000
- Summer Intern Research Presentation “Using Gas Chromatography and a Simulated Atmosphere Chamber to Determine the Kinetics of Octanol Isomers in the Atmosphere” – University of California, Riverside, August 1997

## **References**

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Dr. George Cooper – 202-720-4870  
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Dr. Susan E. Duncan – 540-231-8675