# FLAVOR PROFILES OF

# **OKLAHOMA-GROWN PEANUTS**

By

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# NOMENCLATURE

- ANOVA Analysis of variance
- GC Gas chromatography
- GMP Genetically modified peanuts
- HPLC High performance liquid chromatography
- MS Mass spectrometer
- RI Reflective index
- rpm Rotation per minute
- w/w Weight to weight

## Units

- % Percentage
- °C Degree Centigrade
- g Gram
- hr Hour
- kg Kilogram
- mL Milliliter
- min Minute
- μL Microliter
- μm Micrometer
- sec Second

# **CHAPTER 1**

## **INTRODUCTION**

### **1.1 PROBLEM STATEMENT**

Both conventional breeding and genetic engineering have been used to modify peanut varieties for improved agronomic performance and pest resistance. Flavor is an important attribute influencing consumer acceptance of peanuts. It is crucial that flavor quality is at least maintained or improved during modification of peanuts. Genotype, environment and their interaction have significant effects on peanut flavor. To our knowledge there is no comprehensive study on the flavor profile of Oklahoma grown peanut varieties. Furthermore, the effect of conventional breeding and genetic modifications on peanut flavor have not been examined.

# **1.2 HYPOTHESIS**

Peanut cultivars developed through conventional breeding and genetic modifications may exhibit differences in flavor and sensory characteristics.

# **1.3 OBJECTIVES**

The main objective of this research project is to examine effect of conventional breeding and genetic modification on the flavor profile of peanut varieties grown in Oklahoma. The specific objectives include:

- Characterization of the chemical composition of peanut varieties developed through conventional breeding and genetic engineering.
- 2) Evaluation of sensory characteristics of the peanut varieties grown in Oklahoma.
- 3) Examination of volatile components of peanuts.
- 4) Depiction of olfactory characteristics of peanut varieties.
- Comparison of chemical composition, sensory and olfactory characteristics and volatile components of peanut varieties.

# **CHAPTER 2**

## **REVIEW OF LITERATURE**

#### **2.1 OVERVIEW**

Peanut is an important oilseed crop grown in the United States (Ho and others 1981). Although production acreage is declining peanut remains an important crop for Oklahoma. Peanuts (Arachis hypogaea L.) are unique plant species that produce flowers above the ground, but develops fruits below ground (Ory and others 1992). Peanuts are characterized by high oil and protein, and low ash and carbohydrate contents (Grosso and others 2000). A large percentage of peanut seeds are used for oil production in many countries, whereas in the United States approximately 60% of production is consumed as foods (Didzbalis and others 2004), such as peanut butter, roasted snacks and candies (Ory and others 1992). The United States leads the world in direct consumption of peanuts. According to the USDA's Foreign Agricultural Service Trade Data, from October 2005 to May 2006, 7,946 metric tons of peanuts and peanut products were imported, with a combined value of \$8,467,000. America's favorite food, peanut butter, is also one of the major products imported into the United States, with 14,286 metric tons imported from October 2005 to May 2006 alone, totaling \$20,548,000. The United States is also a major exporter of peanuts and peanut products to Canada, United Kingdom, and Netherlands.

From August 2005 to May 2006, the exports of in-shell peanuts and peanut butter were 14,205 and 19,974 metric tons, respectively.

In the United States, four major market-type peanuts are grown: Virginia, Runner, Spanish, and Valencia (Pattee and others 2000; Pattee and others 2001). These peanut types are genetically different in parentage (Pattee and others 2001). *Arachis hypogaea* species includes Virginia and Runner types. Spanish and Valencia are developed from the same species, *A. hypogaea fastigiata*, but different botanical varieties, vulgaris and fastigiata, respectively. These four market-types have also different usage. The runner type is used in peanut butter, the large-seeded Virginia market-type for ballpark and grocery in-shell, the Spanish market-type for confections, and the Valencia market-type for grocery in-shell (Pattee and others 2001).

### **2.2. PEANUT BIOTECHNOLOGY**

The main objectives of the peanut breeding and genetic engineering programs at the Oklahoma State University and the Stillwater laboratories of United States Department of Agriculture, Agricultural Research Service (USDA-ARS) have been to develop high yielding, early maturing peanut cultivars with resistance to Sclerotinia blight and improved post harvest characteristics for Oklahoma (Anonymous 2002). Emphasis has been on the development of runner and Spanish market types. The peanut varieties examined in this study, NC 7, Florunner, Jupiter, Tamrun OL 01, Tamrun OL 02, Tamrun 96, Tamspan 90, OLin and Okrun, were developed by the major peanut breeding projects in the US and evaluated for yield, grade, seed weight and disease resistance as a part of the Uniform Peanut Performance Test (UPPT) run by Oklahoma State University and South West High Oleic Peanut Program (SWHOPP) conducted by the Texas Agricultural Experiment Station, Oklahoma Agricultural Experiment Station, and the USDA-ARS (Anonymous 2002). All the peanut varieties examined in this study came from Oklahoma State University field trial plots in Fort-Cobb, Oklahoma.

Genetic engineering of peanuts for disease resistance is a potential solution for reducing use of chemicals to manage crop production. Scientists at the USDA-ARS Plant Science Research Laboratory in Stillwater, OK, carry out research on development of transgenic peanut lines adaptable to the Southwest with value-added characteristics to reduce the impact of biotic and abiotic stress. This laboratory has developed transgenic peanut lines containing anti-fungal genes from rice (chitinase gene) and alfalfa ( $\beta$ -1-3-glucanase) (Chenault and others 2002). Modified peanut lines have been tested for Sclerotinia blight resistance (Chenault and others 2005). Three transgenic lines, 188, 540 and 654, did show a significant increase in disease resistance compared to the parent cultivar Okrun. This study also examines the flavor profile of transgenic peanut lines 188, 540 and 654 provided by the USDA-ARS laboratories.

### **2.3 CHEMICAL COMPOSITION**

The chemical composition of peanut seeds is affected by genotype, climate conditions, soil type, biotic stress and agronomic practices (Brown and others 1975; Sanders 1982). Peanut seeds contain 36-54% oil (Dwivedi and others 1990; Hashim and others 1993; Isleib and others 2004), 16-34% protein (Young and Hammons 1973; Pancholy and others 1978; Jambunathan and others 1985), 10-30% starch, 3-5% total soluble sugar, about 11% total fiber (Lintas and Cappelloni 1992; Cardozo and Li 1994),

and 2-3% ash (Grosso and Guzman 1995; Grosso and others 1997). Oil is an important component of food because it has the capability to carry flavors and aromas. Peanuts are high fat foods due to their high oil content (36-54%). However, peanuts containing 320-400 ppm oil have been reported (Grimm and others 1996; Jakkula and others 1997a; Jakkula and others 1997b; Barrientos-Priego and others 2002).

The oil content of peanut varieties grown in Oklahoma was examined by Jonnala and others (2005a). Tamrun OL 01 (also known as TX 977006) (Simpson and others 2003a), Tamrun OL 02 (also designated as TX 977053) (Simpson and others 2006), TX 977164 and TX 977239 were developed through conventional breeding with SunOleic 95R and Tamrun 96 as the parent lines. SunOleic 95R was developed by University of Florida. Tamrun OL 02 and TX 977164 had the lowest and highest oil content, 41.7% and 48.6%, respectively. Parent peanut lines had significantly different oil content (about 44%) than these two varieties. However, these results were within the range of oil content reported in the literature (Ory and others 1992; Baker and others 2002; Isleib and others 2004). The oil contents of genetically modified peanuts (GMP), 188, 540 and 654, were similar to that of the parent variety Okrun. The GMP seeds contained about 46% oil (Jonnala and others 2005b).

About 80% of the fatty acids in peanuts are unsaturated, with oleic and linoleic acids accounting for the majority of total unsaturated fatty acids (Baker and others 2002). The high levels of polyunsaturated fatty acids make peanuts highly susceptible to rancidity and off-flavor (Ory and others 1992; Braddock and others 1995; Mugendi and others 1998). The ratio of oleic to linoleic acid (O/L) had been used to predict the stability and shelf-life of oil (Casini and others 2003). According on Bolton and Sanders

(2002), conventional peanuts have O/L ratio of 1.5. A comprehensive study on the fatty acid content of new high-oleic peanut cultivars was reported by Jonnala and others (2005a). According to their study, the SunOleic 95R peanut variety had an O/L ratio of 4.5 whereas the same ratio for the new high-oleic varieties was about 35, which indicates tremendous improvement in peanut shelf-life. All the transgenic peanut lines and parent variety Okrun had similar O/L ratios, about 1 (Jonnala and others 2005b).

Similar protein content was reported for both cultivated and wild peanuts, in the 23-30% range (Pancholy and others 1978; Dwivedi and others 1990; Ory and others 1992; Grosso and others 2000). Jonnala and others (2005a) reported that new high-oleic peanuts developed through conventional breeding had 25-29% protein. This range was considerably lower than those reported by Basha and others (1992b). According to the latter study four cultivars of peanut grown in Oklahoma (Florunner, Florigiant, GA T-2524, and TP 107-11) had approximately 45-50% protein. The three GMP lines grown in Oklahoma had approximately 27% protein content (Jonnala and others 2005b). The parent cultivar Okrun had a similar amount of protein to the GMP lines. Some amino acids such as aspartic acid, asparagine, glutamic acid, phenylalanine, and histidine were shown to be the precursors of typical peanut flavors (Newell and others 1967).

The mineral or ash content in conventional peanut varieties is approximately 2% (Hung 1994). Jonnala and others (2005a, 2005b) reported similar ash content for higholeic and GMP peanut lines. Phosphorous, calcium and magnesium are the major minerals present in peanuts. Similar mineral compositions have been reported for various peanut varieties developed through conventional breeding and genetic modifications (Derise and others 1974; Wong and Johnston 1986; Jonnala and others 2005a; Jonnala and others 2005b).

### 2.4 SUGAR CONTENT AND COMPOSITION OF PEANUT VARIETIES

Peanut breeders are interested in selecting cultivars that produce the best flavors. The sweet attribute of peanuts is a heritable trait (Pattee and others 2000). Identification of the sugar content and composition in peanut varieties will help breeders in selecting the most promising cultivars for further improvements. Sugar is known to be one of the precursors for development of peanut flavor (Newell and others 1967). Higher sweetness sensory scores are associated with generally superior flavor profiles that are low in bitter and high in roasted peanut flavor (Pattee and others 2000). Significant differences in free sugar content were detected among peanut varieties and the same varieties grown at different locations (Oupadissakoon and others 1980).

It has been shown that sucrose (12-37 mg/g peanut) is the main sugar component in all peanut varieties examined up to date (Newel and others 1967; Mason and others 1969; Tharanathan and others 1975; Oudipassakoon and others 1980; Ross and Mixon 1989). The range for total sugars among 52 genotypes examined by Pattee and others (2000) was 2.5% for Virginias, 1.7% for runners and 1.2% for fastigiates. It was also reported that sugar content was higher in the Argentina-grown peanuts than that from USA- and China-grown peanuts (Bett and others 1994). It is believed that the sweetness found in peanuts in mainly due to the presence of large amount of free sucrose (Mason and others 1969). Occurrence of free glucose, fructose, mannose (0.2-0.3%) along with sucrose in alcoholic extracts of Spanish peanuts has been reported (Mason and others 1969). Basha (1992a) reported similar free sugar composition for 20 peanut varieties grown in Florida. Glucosamine (Basha 1992a), verbascose, and xylose (Tharanathan and others 1975) are other carbohydrates detected in peanut seeds.

Seed maturity affects the sugar content and composition in peanuts (Oudipassakoon and others 1980; Ross and Mixon 1989). According to Ross and Mixon (1989) the stachyose concentration increased while other sugars decreased as the peanut seeds developed. Basha and others (1976) examined six peanut cultivars and noted that the total carbohydrate concentration decreased with increasing seed maturity. A similar trend was reported for Spanish peanuts: sucrose concentrations declined with seed maturation (Mason and other 1969).

Seed size and storage time have significant effects on sugar content and composition of peanuts. Pattee and others (1981) stored peanut kernels of selected sizes at 4°C, 65% relative humidity and monitored the changes in carbohydrate content and composition. Seed size significantly affected the concentrations of all carbohydrates except ribose. In general the smallest seeds had the highest carbohydrate concentrations. Although the amount of total carbohydrate in peanuts did not change significantly during storage, individual sugar contents did change indicating potential effects on peanut quality.

Information on sugar content of Oklahoma-grown peanuts is limited. Basha (1992b) examined four peanut cultivars grown in the state of Oklahoma (Florunner, Florigiant, GA T-2524, and TP 107-11) for their sugar content and reported that approximately 48-50% of the total sugars were in soluble form in these varieties. However, individual sugars were not identified. One of the objectives of this study is to

fill in the information gap on sugar content of peanut varieties developed through conventional breeding and genetic modifications and grown in Oklahoma.

### **2.5 LEXICON FOR THE DESCRIPTION OF PEANUT FLAVOR**

A peanut lexicon "*is intended to provide definitive, common terminology for use in communicating differences in peanut flavor variables among all phases of peanut research and industry*" (Johnsen and others 1988). In 1984 Oupadissakoon and Young developed a lexicon which contained many important terms, but failed to take into account the effect of degree of roast from light to dark. Syarief and others (1985) developed a set of off-flavor terminologies which were limited to oxidized, mold, earthy and petroleum. Descriptors for the sweet/caramel character and various off-flavors were missing from the peanut lexicon.

In 1988, Johnsen and others developed a complete peanut flavor lexicon that provides a means to communicate quality issues related to flavor beyond the hedonic "good/bad" or "like/dislike" responses. A panel comprised of industry personnel and scientists from USDA-ARS evaluated eighteen peanut samples for this purpose. A pilot scale gas heated surface combustion dryer set at 325°F and a convection oven with horizontal air flow were used to roast peanut samples to be used for the sensory evaluations. The degree of roasting was determined by a colorimeter. All the roasted peanuts were screened to represent four roast levels, which were "very light", "light", "dark" and "very dark". The panel evaluated both blanched splits and peanut butter at room temperature. The first step in developing a peanut flavor lexicon was to define terms to characterize the aromatics, basic tastes, feeling factors, and off-flavors typically

present in peanuts. In order to achieve this, each panelist evaluated peanut samples which represented optimum roast, over and under roast, and very over and very under roast. This step produced terms that described desirable peanut flavors as well as terms for roast variations. The panelists also evaluated peanut samples that represented off-flavors. A 10-point scale was used to evaluate the intensity of these flavors. Then the terminology was validated by evaluating the peanut samples using both the lexicon and the established intensity scale. Panelists were presented with three samples, which included a reference, a rancid sample, and a sample that was stored in a warehouse involved in a fire. The data collected from the sensory analysis showed that the lexicon was able to describe the flavor variations present in the samples. A total of 20 terms are described in the lexicon. The flavors are as follows: roasted peanutty, raw bean/peanutty, dark roasted peanut, sweet aromatic, painty, woody/hulls/skins, cardboard, burnt, green, earthy, grainy, fishy, chemical/plastic, skunky/mercaptan, sweet, sour, salty, bitter, astringent, and metallic. The definitions of the terms are summarized below (Johnsen and others 1988):

**Roasted peanutty:** Flavors associated with medium-roast peanuts, and having fragrant characteristics of methyl pyrazine.

**Raw bean/peanutty:** Flavors associated with light-roast peanuts and has characteristics of legume (like beans or peas).

**Dark roasted peanut:** Flavors associated with dark-roast peanuts and having very brown and toasted characteristics.

**Sweet aromatic:** Flavors used to describe sweet taste like caramel, vanilla, molasses, and fruits.

**Woody/hulls/skins:** Flavors associated with base peanut character, and related to dry wood, peanut hulls, and skins.

**Cardboardy:** Flavors used to describe somewhat oxidized fats and oils and reminiscent of cardboard.

Painty: Flavors associated with linseed oil, oil based paint.

**Burnt:** Aromatic compounds used to describe very dark roast and burnt starches, toast or espresso coffee.

Green: Flavors associated with uncooked vegetables/grass wigs, cis-3-hexanal.

Earthy: Flavors associated with wet dirt and mulch.

Grainy: Aroma used to describe raw grain, like bran, starch, corn or sorghum.

Fishy: Aroma associated with trimethylamine, cod liver oil, or old fish.

**Chemical/plastic:** Aroma associated with plastic or burnt plastic.

Skunky/mercaptan: Aromatic compounds associated with smell of sulfur compounds,

such as mercaptan; which exhibit skunk-like character.

Sweet: Taste on the tongue associated with sugars.

**Sour:** Taste on the tongue associated with acids.

**Salty:** Taste on the tongue associated with sodium ions.

**Bitter:** Taste on tongue associated with caffeine or quinine.

Astringent: Chemical feeling factor on the tongue, and can be described as

puckering/dry, and associated with tannins or alum.

**Metallic:** Chemical feeling factor on the tongue, and can be described as flat, metallic, and associated with iron and copper salts.

This lexicon of peanut flavor provides a comprehensive, non-redundant list of terms (Johnsen and others 1988). In this thesis we will use this lexicon to compare instrumental data (GC volatile analysis, olfactory evaluation) with sensory evaluation results.

### 2.6 SENSORY ATTRIBUTES OF PEANUT VARIETIES

environmental conditions, Agronomic practices, seed maturity and handling/storage practices may cause variations in peanut flavor (McNeill and Sanders 1998). Pattee and others (1998) examined 1136 peanut samples obtained from Southeast, Southwest and Virginia-Carolina peanut production regions for their sweet, bitter and roasted flavor attributes. These samples represented 122 genotypes, including the most common peanut cultivars in the Runner and Virginia market types and 42 year-bylocation combinations. Genotypic variation was significant for all three attributes as was location-to-location variation within year and region. It was also noted that New Mexico Valencia C was the sweetest and least bitter cultivar. The Runner type peanuts had the highest roasted peanut score, followed by the Valencia, Spanish and Virginia types.

Flavor quality of peanuts grown in Argentina, China and USA for the crop year 1986, 1987 and 1988 were evaluated (Bett and others 1994). There were distinct differences among peanuts from various countries. Peanuts from US had a better quality described by high roasted peanutty and low fruity/fermented sensory scores than those of the peanut obtained from Argentina and China.

Sanders and others (1989) have shown that intensities of "off" flavors such as painty and fruity fermented were higher in immature peanuts. Sensory evaluation scores

for sour and bitter attributes were consistently high and scores for sweetness and high roast intensity were low for immature peanuts. Mugendi and others (1998) examined the flavor stability of high-oleic roasted peanuts. It was found that the two high-oleic peanuts were not significantly different from each other in flavor quality and stability but had better flavor characteristics than those of the normal oleic acid content peanuts. The latter peanut samples oxidized to a greater extent and produced painty "off" flavors. Braddock and others (1995) reported similar sensory evaluation data for high oleic peanuts. High oleic peanuts maintained a more desirable flavor quality during storage. Loss of roasted peanut flavor and development of painty off-flavor were slower for high-oleic peanuts as compared to traditional peanut varieties. According to Sanders and others (1990), differences in flavor scores between cold- and farmers stock-stored peanuts were not significant. However, peanut curing temperatures above 35-38°C have been often associated with off-flavors (Sanders and others 1990). It has been reported that peanuts cured at low temperature received high favorable flavor scores (Singleton and others 1971). Sanders and Bett (1995) reported that harvest date has a significant effect on sensory quality of peanuts. Their study showed that intensities of roasted peanutty and sweet aromatic attributes were lower and intensities of dark roast and bitter taste were higher for peanuts harvested earlier than optimum harvest date.

### **2.7 VOLATILE FLAVOR COMPOUNDS IN PEANUTS**

#### **2.7.1 Analytical Techniques**

Volatile compounds are responsible for the aroma and contribute to the flavor of peanuts. Several analytical methods have been used to examine volatile components in

peanuts. Gas chromatograph (GC) equipped with a headspace analyzer is commonly used to determine quality of peanut seeds and off-flavors. In this method ground peanut samples are sealed in a vial and the partial vapor pressure of volatile compounds in the headspace is allowed to reach equilibrium. Then a portion of the headspace gas is injected onto a GC column for separation of individual compounds. Pattee and others (1990) used this technique to develop correlations among volatile components, marketing grades, and flavor in Runner-type peanuts. This technique is simple, fast and can eliminate column degradation by non-volatile residues (Rouseff and Cadwallader 2001). Use of a GC/MS olfactory unit with a sniff port can simultaneously identify the structure of the individual flavor chemical and its sensory strength and character (Didzbalis and others 2004).

Volatile compounds can be extracted into a liquid medium instead of concentrating them in the headspace of a vial (Didzbalis and others 2004). The extraction of flavor volatiles into a liquid medium may help to identify the lower concentration analytes, which could be undetectable if stripped from solid samples using a headspace analyzer.

In 1987, Dickens and others devised a headspace volatile concentration (HSVC) test to determine the volatile compounds in peanuts. They measured the total concentration in the headspace by using an organic volatile meter (OVM), which is essentially a commercially available semiconductor sensor. A schematic diagram of the sensor circuit of the OVM is published by Dickens and others (1987). OVM is good for determining the total organic volatile concentration in peanut samples, but lacks the sophistication to identify individual volatile compounds. Later on, the HSVC test was

adopted as a part of the Federal-State Inspection Service (FSIS) peanut grading procedure (Pattee and others 1989).

The solid phase micro extraction (SPME) method has also been utilized to analyze flavor compounds in normal oleic and high oleic peanuts (Reed and others 2002). The SPME method is a solventless technique, where a fiber is used to concentrate volatile compounds released from a sample by heating, which are then placed in a GC injection port to allow desorption. A similar method was also used by Buckholz and others (1980) to trap volatiles from peanut samples. In this study, nitrogen gas was used to strip volatiles from peanuts contained in a jacketed glass column, and these volatiles were adsorbed onto polymers followed by GC analysis of the compounds desorped from the adsorbent in the GC injection port.

The application of an electronic nose in the detection of volatiles in peanuts is a rather new and promising technique. The electronic nose is simple and fast compared to GC. The electronic nose essentially consists of 32 individual sensors that could identify differences in aroma, making this technique attractive for analysis of various food products. The gas sensors work by changing conductivity when exposed to different volatiles. Each of the 32 gas sensors have the ability to respond individually to the different volatiles that make up an aroma, making it easier to "fingerprint" a specific aroma (Osborn and others 2001). Compared to the OVM, the electronic nose has the capability to identify individual volatile compounds that can be helpful in selecting the best peanut cultivars to breed or purchase. In 2001, Osborn and others (2001) employed the electronic nose to detect off-flavors in Florunner type peanuts. However, the reproducibility of data obtained by using an E-nose is questionable.

Leunissen and others (1996) utilized supercritical fluid extraction (SFE) to extract flavor compounds from roasted peanuts and then a gas chromatograph/mass spectrometer (GC/MS) for identification of the extracted compounds. Supercritical fluids have lower viscosities and higher diffusivities than liquids. Hence, mass transfer is improved during the SFE process. Peanut samples were extracted at 50°C and 96 bar using carbon dioxide (Leunissen and others 1996). Major flavor compounds extracted from roasted peanuts were 2,3- and 2,6-dimethylpyrazines. SFE is a rapid and solvent-free method for extracting flavor compounds from food samples.

### 2.7.2 Volatiles in Peanuts

Volatile compounds in peanuts have been studied extensively and hundreds of compounds which may be responsible for peanut flavor have been identified (Young and Hovis 1990; Bett and others 1994; Reed and others 2002). The majority of the research studies carried out in this field has focused on roasted peanut flavor which is very important for consumer acceptance. Studies using raw peanuts are rare.

Ho and others (1981) identified as many as 131 volatile flavor components in fresh roasted peanuts. Some of these compounds include hydrocarbons, alcohols, aldehydes, acids, ketones, esters, lactones, pyrazines, pyrroles, pyridines, sulfides, thiazoles, thiophenes, furanoids, oxazoles, and oxazolines. Pyrazines are the compounds responsible for the roasted peanut flavor and aroma (Ho and others 1981; Ho and others 1983; Baker and others 2003). 2-Methylpyrazine, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, ethylpyrazine, and 2,3,5-trimethylpyrazine are the main pyrazine derivatives found in peanuts (Warner and others 1996; Reed and others 2002). Acetic

acid, benzaldehyde, acetaldehyde, benzothiazole, 3-methylpyridine, hexanal, and nonanal were also found along with pyrazines in high oleic (501/1250 Sunrunner) and traditional (612/612 Florunner) peanut cultivars (Braddock and others 1995). The latter study was performed by trained sensory panelists using a GC sniff port (olfactory detection). The panelists characterized the chemical components of peanuts as follows: pyrazines-nutty/roasted, acetic acid-yeasty, benzeneacetaldehyde-sweet/floral, hexanal-intense green, grassy and nonanal-strong floral aroma.

Young and Hovis (1990) examined raw and roasted peanut volatiles by using a GC/MS method and pure chemicals as standards. A trained panel also evaluated the same samples for sensory characteristics. Sixteen volatiles identified in these samples were different that those found by Braddock and others (1995). The volatile compounds identified in the former study were methanediol, ethanol, acetone, methyl acetate, N-methylpyrrole, and 2-methylpropanal. The panelists described N-methylpyrrole as "musty", 2-methylpropanal as "fruity" and hexanal as "beany".

Low molecular-weight aldehydes such as hexanal and heptanal are generally associated with the off-flavors in peanuts (Warner and others 1996). These compounds are products of lipid oxidation reactions taking place during peanut storage. The presence of polyunsaturated fatty acids in peanuts makes them susceptible to lipid oxidation (Warner and others 1996). The presence of a large amount of aldehydes in peanuts may mask roasted peanut flavors generated by pyrazines (Warner and others 1996). Concentrations of aldehydes such as hexanal, heptanal, octanal and nonanal in peanuts increase as peanut storage time increases. Usually hexanal is the main aldehyde present in rancid peanuts (Warner and others 1996). Burroni and others (1997) investigated volatile components of raw, roasted and fried peanuts from Argentina. Hexanal, 1-methylpyrrole, 1-hexanol, acetic acid and trace amount of cyclobutanol were found in raw peanuts. 2,6-Dimethylpyrazine was present in both roasted and fried peanuts but not in raw samples. Hexanal was the main compound present in abundance in three types of peanuts.

Current research on genetic modification of peanuts generally focuses on increasing pod disease resistance. To the best of our knowledge no literature is currently available on the flavor and volatile components of genetically modified peanut varieties. In this thesis work, peanuts developed through both conventional breeding and genetic engineering techniques were analyzed for their flavor characteristics. This study reports both sensory characteristics and instrumental analysis of volatile compounds in raw and roasted peanuts grown in Oklahoma.

# **CHAPTER 3**

### **MATERIALS AND METHODS**

## **3.1 VARIETY SELECTION**

In this study, twelve cultivars of peanut, developed through conventional breeding or genetic modifications were analyzed for their chemical composition and flavor characteristics. Three of these were genetically-modified peanuts (GMP) developed by the United States Department of Agricultural-Agricultural Research Service (USDA-ARS) in Stillwater, Oklahoma, and includes lines 188, 540, and 654. Okrun was the parent cultivar and was analyzed as a comparison. The rest of the eight conventional breeding cultivars were NC 7, Jupiter, Florunner, Tamrun 96, Tamrun OL 01, Tamrun OL 02, Tamspan 90, and OLin. All the peanut samples were grown in Oklahoma. The characteristics of these peanut lines are summarized below:

### NC 7:

NC 7 is a Virginia variety developed by the North Carolina Agricultural Research Service and released in 1978 (Wynne and others 1979). It is resistant to early leaf spot disease and has a high yield potential. It produces a high percentage of extra large kernels and fancy pods, but is vulnerable to diseases such as cylindrocladium black rot and Sclerotinia blight (The Peanut Grower 2004).

## Jupiter:

Jupiter is a Virginia variety jointly released by the Oklahoma Agricultural Experiment Station and the USDA-ARS. It had shown to have improved performance capability. This variety produces greater yield, extra large kernels, and total sound mature kernels compared to NC 7. It also exhibit greater tolerance to Sclerotinia blight and pod rot than NC 7 (Anonymous 2000).

### Florunner:

Florunner is a runner variety and was released in 1969 by the Florida Agricultural Experiment Station. Florunner was derived from a cross of the varieties 'Early Runner' and 'Florispan'. This variety had shown to exhibit better flavor, quality, and yield than Early Runner (Norden and others 1969). This variety has been used as the industry standard for evaluation of sensory characteristics of peanuts (Pattee and others 2002).

### Tamrun 96:

Tamrun 96 belongs to the runner variety and was released by the Texas Agricultural Experiment Station in 1996. It is known for its high yield and disease resistance. In terms of yield, Tamrun 96 produces a higher yield compared to Florunner and the seed size is slightly larger than Florunner. Tamrun 96 exhibits better performance than Florunner with regards to disease resistance, such as tomato spotted wilt, southern blight, and Sclerotinia blight (Smith and others 1998).

#### Tamrun OL 01:

Tamrun OL 01 was released by Texas Agricultural Experiment Station in January 2002. This variety was the result of crosses between 'Tamrun 96' and 'SunOleic

95R'. It is a runner type peanut with pods much larger than Tamrun 96, and has moderate level of the same disease tolerance attributes as Tamrun 96. This line has a high O/L ratio (Simpson and others 2003a).

## Tamrun OL 02:

Tamrun OL 02 is a sister line to Tamrun OL 01, which is a high O/L ratio runner variety with excellent yield and grade potential. This variety had shown to have moderate level of disease tolerance attributes as Tamrun 96 and Tamrun OL 01. It has lower sugar content and smaller seed size than Tamrun OL 01 (Simpson and others 2006).

### Tamspan 90:

Tamspan 90 was developed and released by the Texas Agricultural Experiment Station and the USDA in April 1990. It is a Spanish-type variety with good resistance to pod rot and sclerotinia blight (Smith and others 1991).

### OLin:

OLin was released in January 2002 by Texas Agricultural Experiment Station. It is a Spanish variety that has a high O/L ratio. The yield is slightly lower than Tamspan 90. Pods of OLin are similar in size and shape as Tamspan 90. Occasionally, this variety produces three seeded pods (Simpson and others 2003b).

### Okrun:

This line was developed by the Oklahoma Agricultural Experimental Station and released in 1986. Okrun was the result of the crosses of Florunner and Spanhoma, and it is commercially classified as a runner variety. Okrun is susceptible to diseases such as Sclerotinia blight. However, tests in Oklahoma showed that it has a higher resistance to leaf spots and pot rods that the current runner varieties (Banks and others 1989).

188:

This is a transgenic peanut line developed from Okrun somatic embryos that contains a single copy of rice chitinase transgene (Chenault and others 2005).

### 540 and 654:

These are transgenic peanut lines developed from Okrun somatic embryos that contain both chitinase transgene from rice, and  $\beta$ -1-3-glucanase transgene from alfalfa (Chenault and others 2005).

### **3.2 SAMPLE COLLECTION**

Raw peanut (in shell) samples were obtained from Oklahoma State University Research Station field trial plots in Fort Cobb, Oklahoma. There were four replications for each variety. Approximately one pound of sound and mature pods was collected from each replicate after harvest. Four samples were mixed and shelled thoroughly to obtain a representation of each cultivar. The peanut seeds were stored in airtight containers in a freezer at -20°C until further analysis.

### **3.3 SAMPLE PREPARATION**

Approximately 200 g of peanut seeds was brought to room temperature before grinding. The seeds were ground for 1 min using a coffee grinder (Black & Decker CBG5, Miami, FL) at medium speed. The seeds were pooled and mixed well before storing in airtight plastic containers at -20°C until further analysis.

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## **3.4 ACCELERATED SOLVENT EXTRACTION (ASE)**

An accelerated solvent extraction unit (Dionex Co., Model ASE 300, Sunnyvale, CA) was utilized to remove oil from peanut samples. The extraction system was described in detail elsewhere (Dunford and Zhang 2003). Approximately 10 g of finely ground peanut sample was placed in a 34 mL stainless steel extraction cell. Extraction parameters were programmed on the unit as follows: temperature 80°C, 4 extraction cycle, 15 min extraction time/cycle, 50% flush volume and 90 sec purge time. The solvent used for oil extraction was 100% hexane (Pharmco-AAPER, Brookfield, CT). The extraction process can be summarized in the following steps: loading the cell into the oven, filling the cell with solvent, heating the cell, static extraction, flushing with fresh solvent, purging solvent from cell into collection bottle, release of pressure, and unloading the cell (Dunford and Zhang 2003). Extracted oil in hexane was collected into a 250 mL collection bottle. Nitrogen gas was used to purge the system until all remaining extract was transferred to the bottle. The defatted peanut sample remaining in the extraction cell was transferred into clean glass vials and stored in a freezer (-20°C) until utilized for sugar analysis.

#### **3.5 PEANUT CHEMICAL COMPOSITION**

### 3.5.1 Moisture Content

Moisture content of the samples was determined using AOAC method 950.46 (1995). Peanut samples were taken out of the freezer and brought to room temperature before use. Aluminum moisture dishes were pre-dried in a forced-air oven (VWR Scientific, Model 1370 FM, Bristol, CT) for an hour at 100°C prior to analysis.

Approximately 0.5 g of sample was weighed in the dried aluminum dish. Then the sample was dried in the oven for 5 hrs at 100°C until constant weight was reached. The loss in sample weight as percent of the initial sample weight was reported as the moisture content of the sample.

# 3.5.2 Ash Content

Ash content of the peanut samples was determined according to the AOAC method 923.03 (1995). Crucibles were pre-dried in the furnace (Fisher Scientific, Model 58 Isotemp® Muffle Furnace 600 Series, Fair Lawn, NJ) for 5 hrs at 525°C. About 2 g of fine ground peanut sample was weighed into the dried crucible and sample was ashed in the furnace for 5 hrs at 525°C. Percentage residual weight in the crucibles was reported as the ash content in the sample.

### 3.5.3 Oil Content

The oil content of the peanut samples was determined according to the AOAC method 960.39 (1995). Approximately 2 g of finely ground peanut sample was weighed into a cellulose thimble. The thimbles were then placed in the Soxtec extraction unit (Tecator, Model 1043 Extraction Unit, Sweden), and 40 mL of petroleum ether (Mallinckrodt, Paris, KE) was used to extract the oil from the sample. The aluminum cup with the extracted oil was placed into vacuum oven (Fisher Scientific, Isotemp® Oven, Fair Lawn, NJ) for 15 min to evaporate the excess moisture. The amount of extracted oil was determined gravimetrically.

## 3.5.4 Protein Content

The protein content of the peanut samples was analyzed according to the AOAC method 928.08 (1995). In summary approximately 0.5 g of finely ground sample was weighed on a nitrogen-free paper. Sample wrapped in the paper was digested with concentrated sulfuric acid (Pharmco-AAPER, Brookfield, CT), hydrogen peroxide (Fisher Scientific, Fair Lawn, NJ), and two Kjeldahl catalyst tablets (Fisher Scientific, FisherTab<sup>TM</sup> ST-35, Fair Lawn, NJ) using a Kjeltec block digester unit (Tecator, Model 2020 Digester, Sweden) for 40 min. Total nitrogen amount in the sample was determined by distillation and titration of the extracts using a Kjeltec instrument (Tecator, Model 2300 Kjeltec analyzer unit, Sweden). A conversion factor of 6.25 was used to convert the amount of nitrogen to amount of protein present in the samples.

#### **3.6 SUGAR ANALYSIS**

#### **3.6.1 Sample Preparation**

Defatted peanut flour was used to determine sugar content of the samples. Oil was stripped off the peanut samples (Refer to section 3.4 in this chapter) using 100% hexane (pharmco-AAPER, Brookfield, CT) and an accelerated solvent extraction unit (Dionex, Model ASE 300, Sunnyvale, CA).

### **3.6.2 Sugar Extraction and HPLC Analysis**

Approximately 0.5 g of defatted peanut flour was extracted with 4 mL of 80% methanol (pharmco-AAPER, Brookfield, CT) in a clean centrifuge tube. A reflux apparatus was constructed by protruding a 9" Pasteur pipette (Fisher Scientific,
Fisherbrand <sup>®</sup>, Fair Lawn, NJ) through an open-top cap and fitting it securely on the centrifuge tubes. The tubes fitted with reflux apparatus were placed on a dry block heater (Pierce Reacti-Therm<sup>™</sup>, Model 18970, Rockford, IL) at 80°C. The extraction time was 20 min. Then extract was centrifuged (Fisher Scientific, Model 225 Centrific™ Centrifuge, Fair Lawn, NJ) at 2000 rpm for 10 min. The supernatant was transferred into clean test tubes. The extraction was performed one more time (for a total of two extractions) and the combined supernatant was filtered using a 25 mm syringe filter with 0.45µm nylon membrane (VWR International, Bristol, CT). The filtered supernatant was evaporated under vacuum using a RapidVap evaporation system (Labconco, Model 79000-02, Kansas City, MO). The sugar residue in the tube was dissolved in 1 mL deionized water and subsequently utilized for sugar determination. A high-performance liquid chromatography (HPLC) (Waters, Model 2695 Separation Module, Milford, MA) equipped with a reflective index (RI) detector (Waters, Model 410, Milford, MA) was used for sugar analysis. The separation of sugar components was performed on a carbohydrate analysis column (3.9 x 300 mm) with a covalently bonded amino packing material (Waters, Milford, MA). The mobile phase flow rate and run time were 2.0 mL/min, and 20 min, respectively. Column temperature was maintained at  $30 \pm 5^{\circ}$ C. Mobile phase consisted of 80% HPLC grade acetonitrile (Pharmco-AAPER, Brookfield, CT) and 20% de-ionized water. Sugar standards such as glucose, sucrose, and fructose were obtained from Sigma-Aldrich, Inc. (St Louis, MO). Stock solutions of 125 mg/mL were prepared for all the sugar standards and dilutions were made from these stocks for preparation of calibration curves. Sugar amount in the samples was determined from calibration curves prepared for each compound.

# **3.7 SENSORY ANALYSIS**

The sensory tests were performed by a sensory analysis team at North Carolina State University supervised by Dr. Timothy Sanders. The details of the tests are described below:

# 3.7.1 Roasting

The peanut samples were dry-roasted on a conveyor belt in a gas-fired Aeroglide Roaster (Aeroglide Corp., Raleigh, NC) at 177°C. During the roasting, the samples were taken out periodically to measure the roast color using a HunterLab DP-9000ä (Hunter Associate Laboratory, Reston, VA). The target Hunter L value was  $49.0 \pm 1.0$ . The conveyor belt speed was adjusted accordingly to the target Hunter L value. After roasting the samples were cooled immediately and stored in freezer bags at -22°C until the sensory tests.

## **3.7.2 Sensory Testing**

Peanut paste was prepared using a food processor (Cuisinart Corp, East Windsor, NJ). A grind-cool procedure was followed to maintain paste temperature below 32°C (Sanders and others 1989). This procedure involved two 2-min grindings with 1 min cooling in between followed by several 1-min grindings with 30-sec cooling intervals. Grinding was continued until desired paste consistency was achieved. Paste samples were left at room temperature overnight prior to sensory testing.

The eight panelists were trained over a 5-month period in accordance to the Spectrum® Descriptive Analysis method by Meilgaard and others (1987). Paste samples

were presented to the panelists, who then rated the intensity of the various attributes on a 0 (zero) to 15 intensity scale (Johnsen and others 1988). A total of eighteen attributes were evaluated including roast peanutty, sweet aromatic, dark roast, raw beany, earthy, and painty. The attribute descriptors can be found in Johnsen and others (1988). All the samples were assigned a three digit code and were randomly presented to the panelists at each session along with a reference sample of known descriptor intensity rating. Panelists used water and salt-less crackers to cleanse their palette between testing.

#### **3.8 HEADSPACE ANALYSIS**

### **3.8.1 Extraction of Headspace Volatiles**

Raw and roasted ground peanut samples were utilized for headspace analysis. Approximately 2.0 g of samples was weighed into 10 mL headspace vials, along with 0.5 g of sodium sulfate. A headspace sampler (Hewlett Packard, Model 7694, Palo Alto, CA) was used to extract volatiles from the samples. Samples were equilibrated in the headspace sampler for 30 min at 150°C. The temperatures of the sample valve, and transfer line were 160 °C and 165°C, respectively. The rest of the headspace sampler parameters were as follows: vial pressurization 0.20 min, sample loop fill 0.05 min, loop equilibration 0.20 min, and sample injection 1.00 min. The vial "shaking" mode was set to "low".

#### 3.8.2 Gas chromatography/Mass spectrometry and Olfactory Detection

Volatile compounds from the peanuts were analyzed using a gas chromatograph from Hewlett Packard (Model 6890, Palo Alto, CA) equipped with a mass spectrometer (Agilent, Model 5973, Palo Alto, CA) and an olfactory detector. Volatiles were separated using an Equity<sup>TM</sup>-5 fused silica capillary column (30m x 0.25mm x 0.5µm) from Supelco (Bellefonte, PA). The split ratio was 6:1. The injector and MS temperatures were 250°C, and 230 °C, respectively. The initial oven temperature of 35°C was increased to 60°C at 5°C/min, and hold for 5 min. From 60°C, the temperature was raised to 230°C at 15°C/min and held at 230°C for 10 min. The total run time was 31.33 min. The carrier gas (helium) flow rate was 1.5 mL/min. The effluent from the capillary column was split into 2:1 using a fused silica y-connector between the olfactory sniff-port and the mass spectrometer. GC-MS operating temperatures were as follows: MS transfer line 280°C, ion source 230°C and MS quadruple 150°C. The ionization energy was 70 eV. The scan range and rate were 29-400 amu and 4 scans/sec, respectively. The data collection and analysis were managed using an HP Chemstation (Enhanced Chemstation G1701DA Version D.00.00.38, Agilent Technologies, Palo Alto, CA). The volatile compounds in the samples were identified by direct comparison of their chromatographic retention times and the mass spectra with those of the authentic compounds. Pure standards were obtained from Sigma-Aldrich, Inc (St Loius, MO), VWR (Suwanee, GA) and Fisher Scientific (Fair Lawn, NJ). These standards included 2,5-dimethylpyrazine, benzaldehyde, benzeneacetaldehyde, hexanal, acetic acid, pentanoic acid, propionic acid, hexanoic acid, cyclohexanol, and  $\gamma$ -butyrolactone. The peaks were also confirmed with NIST/EPA/NIH Mass Spectral Library (Version 2.0).

The odor of volatile compounds from the capillary column was evaluated via an olfactory detection port (ODP)/sniffing port (Gerstel GmbH, Mülheim an der Ruhr, Germany). The ODP allows the sensing of compounds by the human nose as they elute

from the gas chromatograph. The effluent is split as it leaves the column so that it arrives simultaneously at the nose and at the detector. This way additional information is gained on compounds that are responsible for specific odors. Perceived description and intensity of the compounds sensed at the port by the user is recorded using ODP-recorder software (Gerstel GmbH, Mülheim an der Ruhr, Germany) which is incorporated into the MS ChemStation<sup>™</sup>. When a user identifies an odor at the port/nose cone, he/she can record voice comments via a microphone and a voice recognition software, Dragon Naturally Speaking Preferred Version 8.10.000.285 (Marysville, CA), which works alongside the ODP-recorder. The ODP system comes with a pad that has four buttons representing four intensity levels: 1 for low, 2 for medium, 3 for high and 4 for very high. At the time the user detects an odor at the ODP port she/he presses one of the intensity buttons on the pad while recording voice comments using the microphone. The ODP-recorder software will record the intensity, the voice comments, and the time the olfactory pad was suppressed in special folders on the computer. The voice recognition software converts the recorded voice comments into text peak annotations, which can be overlaid on the MS chromatograms. Before the Dragon Naturally Speaking voice recognition software can be used, each user will have to undergo a voice training incorporated in the software itself. This training enables each user to create their personal pronunciation profile, as the user profile needs to be loaded onto the ODP-recorder software at the beginning of each run.

# **3.9 STATISTICAL ANALYSIS**

All analysis was conducted in duplicates, except sugar, which was conducted in triplicates. All samples were randomized, and mean values were reported. Analysis of variance (ANOVA) of the results was performed using General Linear Model procedure of SAS (Statistical Analysis System, Version 9.1, Cary, NC). Multiple comparison of the various means were carried out by LSD (Least Significant Difference) test at  $\alpha = 0.05$  except the sensory test results which were analyzed using Duncan's New Multiple range test.

# **CHAPTER 4**

# **RESULTS AND DISCUSSIONS**

### **4.1 PEANUT CHEMICAL COMPOSITION**

### 4.1.1 Oil Content

Oil content has an important effect on the sensory characteristic of foods because it contributes to mouth feel and carries flavors and aromas. Peanuts are high oil content foods. Oil content of peanut lines examined in this study varied between 45.7% and 50.1% (w/w) (Tables 1 and 2). These results are similar to the oil content of peanuts published in the literature (Jonnala and other 2005a). Florunner and Tamrun OL 02 had significantly lower oil content than the other peanut lines developed through conventional breeding.

Genetically modified peanut line 188 had similar oil content as the parent line, Okrun (Table 2). Although differences among Okrun and GMP lines 540 and 654 were statistically significant, variations were not large and within the values published for conventional peanuts in the literature (Dwivedi and others 1990; Hashim and others 1993; Isleib and others 2004). The results obtained in this thesis are also similar to that of the GMP lines reported by Jonnala and others (2005b).

# 4.1.2 Protein Content

Peanuts are an excellent source of protein. Tables 1 and 2 show protein contents of peanut lines developed through conventional breeding and genetic modifications, respectively. Tamspan 90 had significantly higher protein content, about 33%, (w/w) than other peanut lines (Table 1). The protein content of GMP line 188, 32.35% (w/w), was significantly higher than that of the parent and other GMP lines examined in this study (Table 2). However, similar to the results on oil content of these samples, the variations were not large and within the protein contents published for conventional peanuts (Pancholy and others 1978; Dwivedi and others 1990; Ory and others 1992; Grosso and others 2000). The experimental results reported in this thesis also confirm that protein content of GMP crops harvested in previous years (Jonnala and others 2005b) are similar to the protein content of the same varieties grown in consecutive years indicating stability of the chemical composition of GMP.

### 4.1.3 Moisture and Ash Content

Peanut samples examined in this thesis were stored at room temperature until received in our laboratory for testing after which samples were stored frozen in sealed containers. Tables 1 and 2 show that moisture contents of all the samples were rather low, about 4%. We also received samples from a farmer who stores his peanuts at a cool temperature (10°C). His samples had significantly higher moisture (about 7%) content than the samples reported in this thesis. It is important to note that cool storage of peanuts could help to reduce the formation of off flavors and maintain relatively higher moisture levels in the seeds which could have a positive effect on the mouth feel of these products.

Ash content of peanuts varied between 2.17% and 2.55% (Tables 1 and 2). GMP lines had similar ash content as parent Okrun and conventional peanut varieties reported in the literature (Derise and others 1974; Wong and Johnston 1986; Jonnala and others 2005a; Jonnala and others 2005b).

### **4.2 SUGAR CONTENT AND COMPOSITION**

Free sugars are key components in formation and development of peanut flavor. Tables 3 and 4 show the sugar content of peanut cultivars examined in this study. Sucrose was the major sugar present in peanuts. These results are in agreement with the literature (Newel and others 1967; Mason and others 1969; Tharanathan and others 1975; Oudipassakoon and others 1980; Ross and Mixon 1989). Other sugars such as glucose and fructose were under the detection limit of the analytical test used in this study (HPLC/IR detection). The presence of small amounts of glucose, fructose, raffinose, and stachyose in peanut seeds has also been reported in the literature (Basha 1992b). The sucrose content of the peanut cultivars developed using conventional breeding was between 56 to 73 mg/g. These results are significantly higher than those reported by Oupadissakoon and others (1980) because in this thesis sucrose content was expressed on an oil free basis rather than mg/g full fat peanut flour. Florunner and OLin had the highest and lowest amount of free sugars, respectively, among the peanut seed developed through conventional breeding. The free sugar content of parent line Okrun and GMP 188 and 654 were similar but GMP 540 had significantly lower sugar content. The variations among the free sugar content of GMP (56.7%-64.9%) and conventional breeding lines (56.1%-73.1%) were not extensive. To our knowledge this thesis is the first report on the sugar content of GMP.

### **4.3 FLAVOR OF PEANUTS**

#### **4.3.1 Sensory Characteristics**

Only the conventional breeding varieties were subjected to sensory evaluation as the GMP lines have not yet been approved for human consumption. Tables 5a and 5b show the average score for each attribute analyzed by the panelists. The means with no letters in Table 5a and 5b are not significantly different at  $\alpha = 0.05$  level. "Roast peanutty" (RP)" is the attribute that correlates directly to consumers' perception of "good peanut flavor". All the peanut lines received RP scores above 4. The average RP scores for the peanut samples examined in this study were within the RP range reported in the literature (Pattee and others 2002). Although Florunner had the highest RP score, 5.13, the difference between the RP scores for Florunner and that for NC 7, Jupiter and Tamrun OL 02 were not statistically significant. Means for the sensory attribute Sweet Aromatic (SA) varied between 2.33 and 2.93 and there was no significant difference among the samples. Dark Roast (DR) sensory attribute intensity scores varied between 2.33 and 3.08 and showed some significant differences among the samples. However, differences were not extensive. Florunner and Tamspan 90 had the lowest and highest Raw-Beany (RB) intensity scores, respectively. Earth, painty, fruity fermented, sour, tongue taste bitter and ashy sensory attribute intensity scores for all the peanut samples examined in this thesis were very low, <1. The panelists detected wood/hulls/skin flavor notes in all the peanut samples and intensity scores were relatively high, 3.04-3.16. Sweet (1.78-2.37) and bitter (2.67-3.47 intensity scores reported in this thesis is similar to those reported in literature (Pattee and others 2002). Florunner had the lowest total off-note intensity score (Table 5b).

In summary there were some statistically significant differences among the sensory attributes of peanut samples. However, the differences were not large enough to cause any concerns or benefits in terms of flavor quality of Oklahoma grown peanuts.

#### **4.3.2 Volatile Components of Raw and Roasted Peanuts**

Volatile compounds are responsible for the aroma and have a significant effect on peanut flavor. Typical headspace/GC chromatograms of raw and roasted peanuts examined in this study are shown in Figures 1-16. The identity of each peak was confirmed by direct comparison of their chromatographic retention times and the mass spectra with those of the authentic compounds and/or data in the NIST/EPA/NIH Mass Spectral Library (Version 2.0). The headspace tests were carried out using raw peanuts at a relatively high temperature, 150°C, for two reasons: 1) the low detection limit of the GC/MS/headspace system used for this study dictated the use of a high temperature to concentrate volatile compounds; 2) high temperature maximized the release of flavor compounds with relatively high boiling point. Furthermore, peanuts are subjected to 130-150°C during the roasting process which releases highly desirable flavor compounds (roasted and nutty peanut flavors). Hence heating raw peanut samples at 150°C simulates roasting conditions.

Chemical derivatives of acetic acid, aldehydes, alcohols, pyrazine and pyrrole were detected in all the samples examined in this study. The presence of these

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compounds in peanuts has been also reported by several other research groups (Vercellotti and others 1992; Burroni and others 1997). Oxo-methylester acetic acid was the main volatile compound in all the samples. This compound consisted of 46-63% of the total GC area count for volatile compounds. Young and Hovis (1990) and Vercellotti and others (1992) reported that free methanol was the major volatile compound present in the peanut samples they analyzed. In this study a NIST/EPA/NIH Mass Spectral Library search for the peak at 2 min gave the highest probability for methanol. However, a closer examination of the mass fragmentation pattern for methanol and oxo-methylester acetic acid standards confirms that target and qualifier ions (largest mass fragments) for oxo-methylester acetic acid and the peak at 2 min have a better match than methanol.

1-Methyl-1H-pyrrole was the second largest peak on the chromatograms. N-Methylpyrrole was described as having a sweet and woody odor (Ho and others 1981). Aldehydes such as 2-methylpropanal, 3-methylbutanal, pentanal, octanal, hexanal and nonanal were also present in the peanut samples. These compounds are usually associated with off-flavors formed during oil oxidation. As mentioned earlier in this thesis peanut samples examined in this study were stored at room temperature before they were received in our laboratory. These compounds might have formed during storage. Benzeneacetaldehyde, benzaldehyde, pentanol and 2,5-dimethylpyrazine were detected in most of the samples in relatively low quantities. Benzeneacetaldehyde and 2,5dimethylpyrazine are associated with floral, sweet, caramel and malty, chocolate flavors, respectively (Braddock and others 1995). Only one sample, Tamrun OL 02, had a significant amount of acetone, about 14% (of the GC area count for volatile compounds) (Figure 5, Table 10). The volatile components of four roasted peanut samples which were used for sensory analysis were also identified. The roasted peanut samples Florunner (Figure 13, Table 18), Jupiter (Figure 14, Table 19) and Tamspan 90 (Figure 15, Table 20) contained (Z)-2-heptanal, 2-pentyl-furan and (E)-2-octenal along with other volatile compounds identified in raw peanuts. Furanoid compounds are usually formed by degradation of carbohydrates (Ho and others 1981). It has been reported that 2-pentyl-furan is formed by autoxidation of linoleic acid and is associated with beany and grassy flavor (Smouse and Chang 1967).

GC/headspace chromatograms for GMP and parent line Okrun were very similar (Figure 9-12). The same volatile compounds found in conventional breeding lines were also detected in GMP (Table 14-17). These results indicate that genetic modifications did not cause any significant change in volatile components of peanuts.

# 4.3.3 Olfactory Characteristics of Peanut Varieties

The average human nose can detect nearly 10,000 distinct scents, a feat that requires about 1,000 olfactory genes, or roughly 3% of the human genome (Breer 2003). Certain volatiles are detected in concentrations as low as few parts per trillion and parts per thousands; moreover, even stereo-isomeric compounds can be distinguished (Breer 1997).

Flavor components of foods have been analyzed by using an olfactory detection port installed on a GC/MS. This technique allows sensing of compounds by the human nose as they elute from the column of a gas chromatograph. The analysis of flavors is very challenging, because of the wide range of odor thresholds of the individual

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compounds. Furthermore human beings are much more sensitive to some odors than others (Adahchour and others 2002). The aroma-active compounds, or key flavors, are usually present in ultra-trace amounts and are not usually the major volatile constituents of the food. When using GC with olfactory detection, the human nose can often detect a distinctive smell where the chromatogram produces a flat baseline. Even more challenging, a substance that does elute at the proper retention time is not necessarily responsible for that odor. The aroma compounds might well be hidden by artifacts present at higher concentrations (Adahchour and others 2002). However, GC/olfactory analysis is still a versatile technique to identify volatile compounds in foods and evaluate their flavor perceptions.

Olfactory analyses of the peanut samples studied in this thesis were carried out by untrained users to evaluate consumer response/perception of the products. Rancid, sour, raw peanut, roasted peanut, sweet-floral, burnt latex, beany, green and burnt butter were the terms used to describe compounds coming out of the GC column by "User 1". Sweet, musty, bitter, green grass, peanut butter, stale, floral, roasted peanut and peanut were the descriptors used by "User 2". Although retention times of the peaks on the chromatogram and the "User" responses to smell did not match exactly they were fairly close (Table 6-21). The reasons for differences in chromatographic peak retention and "user" response times are several fold; 1) GC data analysis software labels the retention time of the chromatographic peaks which is shaped as a bell curve at the tip of the curve whereas "user" respond is recorded at soon as the smell reaches to the nose, 2) "user" response times to a smell is likely to be slightly different, 3) the most important reason is that, as

mentioned earlier, when using GC with olfactory detection the human nose can often detect a distinctive smell where the chromatogram produces a flat baseline.

In general rancid, musty and sour type negative descriptor were used in the region where aldehydes eluted from the GC column. The "user" responses such as sweet and floral were in the region where 2,5-dimethylpyrazine and benzeneacetaldehyde eluted from the column. These olfactory "user" responses are very similar to the reports in the literature which associate aldehydes with off flavors and lipid oxidation products (Vercellotti and others 1992; Burroni and others 1997), 2,5-dimethylpyrazine with and malty, chocolate flavors and benzeneacetaldehyde with floral, sweet, caramel, respectively (Braddock and others 1995).

# CONCLUSIONS

Oklahoma grown peanuts contain about 50% (w/w) oil. Hence they are high oil content foods. Oil is an important component in a food system because of its role as a flavor carrier and contribution to "mouth-feel". Protein provides texture and structure, which are important characteristics influencing consumer's acceptance of a food product. Peanuts are good source of protein, about 30%, w/w protein. Sucrose is the major free sugar in all the peanut lines. Proximate composition of the conventional breeding and GMP lines examined in this study was similar to that reported in the literature. Sensory analysis carried out by trained panelists showed that there were minor differences among the samples. Florunner had high roasted peanut and low total off-flavors. It was rated as the best line in terms of flavor. All the sensory scores for 18 flavor attributes for Oklahoma grown peanuts were within the sensory scores published for good quality peanuts.

To the best of our knowledge this is the first study examining flavor profile of conventional and GMP lines using a dynamic headspace GC system equipped with an olfactory detector. Flavor/aroma attributes of all the conventional breeding and GMP lines analyzed in this study were described by similar terms by two olfactory "users". Hence there was no significant detectable flavor difference among the samples. Results of the olfactory evaluation of the peanut varieties support the sensory tests conducted on the same samples by trained panelists. The Oklahoma grown peanut varieties portrayed similar flavor characteristics as good quality peanuts reported in the literature.

# **FUTURE RESEARCH**

The focus of this study was the evaluation and comparison of proximate composition and flavor characteristics of peanut lines developed for Oklahoma region by using both conventional breeding and genetic engineering techniques. Due to the large number of peanut lines examined and analytical and sensory tests carried out, samples for only one year were included in this study. However, it is imperative that stability of the chemical composition and flavor properties of the modified peanut lines in different climates, under various agronomic practices and management systems and over time requires further research. Such an extensive study on stability of peanut chemical and flavor characteristics could lead to identification of cultivars with exceptionally good flavor profile and healthy chemical composition. This study is a first step toward generating a resource base for future peanut breeding and genetic engineering programs that focus on development of new peanut lines for the Oklahoma region.

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Cultivar	Oil	Protein	Moisture	Ash
NC 7	$48.64 \pm 0.01^{a}$	$29.63 \pm 0.25^{d,e}$	$4.25\pm0.07^{d,e}$	$2.53 \pm 0.003^{a}$
Florunner	$45.77 \pm 0.01^{e}$	$30.95\pm0.04^{c}$	$4.4\pm0.3^{b,c,d}$	$2.27 \pm 0.001^{\circ}$
Jupiter	$47.70 \pm 0.10^{c,d}$	$29.18 \pm 0.49^{e}$	$4.40\pm0.10^{b,c,d}$	$2.43\pm0.004^{b}$
Tamrun OL 01	$45.66 \pm 0.44^{e}$	$29.70 \pm 0.03^{d,e}$	$4.67\pm0.11^{a,b}$	$2.17\pm0.004^{d}$
Tamrun OL 02	$47.53 \pm 0.36^{d}$	$30.25\pm0.18^{c,d}$	$4.37\pm0.14^{c,d}$	$2.17\pm0.026^d$
Tamrun 96	$48.70 \pm 0.03^{a}$	$29.82 \pm 0.06^{d,e}$	$3.99 \pm 0.09^{e}$	$2.17\pm0.019^{d}$
Tamspan 90	$48.04 \pm 0.01^{b,c,d}$	$33.35\pm0.44^a$	$4.90\pm0.02^{a}$	$2.35\pm0.011^{b}$
OLin	$48.19 \pm 0.02^{a,b,c}$	$32.12 \pm 1.11^{b}$	$4.56 \pm 0.11^{b,c}$	$2.37 \pm 0.019^{b}$
Okrun	$48.48 \pm 0.35^{a,b}$	$30.59 \pm 0.16^{c,d}$	$4.13\pm0.08^{d,e}$	$2.24\pm0.108^{c,d}$

**Table 1:** Proximate composition of peanut seeds developed through conventional breeding (%, w/w, dry basis).

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 $^{a,b,c,d,e}$  Means in the same column with the same letters are not significantly different at  $\alpha$  = 0.05

**Table 2:** Proximate composition of peanut seeds developed through genetic modifications (%, w/w, dry basis).

Cultivar	Oil	Protein	Moisture	Ash
Okrun	$48.48 \pm 0.35^{b}$	$30.59 \pm 0.16^{b}$	$4.13\pm0.08^a$	$2.24 \pm 0.11^{\circ}$
188	$48.42 \pm 0.55^{b}$	$32.35 \pm 0.62^{a}$	$4.31\pm0.03^a$	$2.55\pm0.001^{a}$
540	$50.10\pm0.09^{a}$	$29.49\pm0.47^{b}$	$3.73\pm0.07^{b}$	$2.45\pm0.033^{a,b}$
654	$49.78 \pm 0.02^{a}$	$29.56\pm0.78^b$	$3.94\pm0.26^{a,b}$	$2.33 \pm 0.003^{b,c}$

 $^{a,b,c}$  Means in the same column with the same letters are not significantly different at  $\alpha = 0.05$ 

Cultivar	Sucrose
NC 7	$69.7 \pm 4.4^{b}$
Florunner	$73.1 \pm 3.8^{a}$
Jupiter	$62.3 \pm 1.9^{d,e}$
Tamrun OL 01	$66.6 \pm 3.8^{\circ}$
Tamrun OL 02	$65.7 \pm 0.4^{\circ}$
Tamrun 96	$60.3 \pm 0.4^{e}$
Tamspan 90	$60.4 \pm 0.4^{e}$
OLin	$56.1 \pm 0.5^{\rm f}$
Okrun	$64.9 \pm 1.9^{c,d}$

**Table 3:** Sugar composition of peanut seeds developed through conventional breeding (mg/g, oil free basis).

 $^{a,b,c,d,e,f\!}$  Means in the same column with the same letters are not significantly different at  $\alpha$  =0.05

Cultivar	Sucrose
Okrun	$64.9 \pm 1.9^{a}$
188	$62.7\pm4.0^a$
540	$56.7 \pm 2.1^{b}$
654	$62.4 \pm 2.0^{a}$

**Table 4:** Sugar composition of peanut seeds developed through genetic modifications (mg/g, oil free basis).

<sup>a,b</sup> Means in the same column with the same letters are not significantly different at  $\alpha = 0.05$ 

Cultivar	RP	SA	DR	RB	WHS	Card	Earth	Painty	PC
NC 7	4.83 <sup>a,b</sup>	2.81	2.81 <sup>a</sup>	2.37	3.14	0.88	0.00	0.08	0.08
Florunner	5.13 <sup>a</sup>	2.93	3.08 <sup>a</sup>	2.00	3.16	0.68	0.00	0.00	0.00
Jupiter	4.83 <sup>a,b</sup>	2.79	2.75 <sup>a,b</sup>	2.23	3.13	0.79	0.00	0.00	0.00
Tamrun OL 01	4.64 <sup>b</sup>	2.79	2.78 <sup>a,b</sup>	2.26	3.07	0.93	0.00	0.00	0.00
Tamrun OL 02	4.73 <sup>a,b</sup>	2.79	3.00 <sup>a</sup>	2.28	3.07	1.14	0.00	0.00	0.00
Tamrun 96	4.49 <sup>b</sup>	2.72	2.58 <sup>a,b,c</sup>	2.38	3.11	1.43	0.00	0.21	0.13
Tamspan 90	4.50 <sup>b</sup>	2.33	2.33 <sup>b,c</sup>	2.76	3.13	1.46	0.13	0.29	0.00
OLin	4.58 <sup>b</sup>	2.50	2.44 <sup>b,c</sup>	2.49	3.04	1.54	0.08	0.00	0.08

**Table 5a:** Sensory scores for peanut varieties developed through traditional breeding<sup>1</sup>.

<sup>1</sup>RP – roast peanutty, SA – sweet aromatic, DR – dark roast, RB – raw beany, WHS – wood/hulls/skins, Card – cardboardy, Earth – earthy, Painty – painty, PC – plastic chemical

<sup>a,b,c</sup> Means in the same column with the same letter are not significantly different at  $\alpha = 0.05$  level. Comparison of means was analyzed by Duncan's New Multiple Range Test.

Cultivar	Μ	FrF	SW	Sour	Bitter	Astr	ТТВ	Ashy	Total
NC 7	0.00	0.00	2.09 <sup>a,b,c</sup>	0.00	3.05 <sup>a,b,c</sup>	1.00	0.08	0.11	1.65 <sup>a,b</sup>
Florunner	0.00	0.00	2.37 <sup>a</sup>	0.00	2.67 <sup>c</sup>	1.00	0.00	0.23	0.88 <sup>c</sup>
Jupiter	0.00	0.00	2.13 <sup>a,b,c</sup>	0.00	2.90 <sup>b,c</sup>	1.00	0.29	0.11	1.38 <sup>b,c</sup>
Tamrun OL 01	0.13	0.00	2.21 <sup>a,b</sup>	0.00	3.47 <sup>a</sup>	1.00	0.08	0.33	1.83 <sup>a,b</sup>
Tamrun OL 02	0.00	0.00	2.35 <sup>a</sup>	0.00	3.38 <sup>a,b</sup>	1.08	0.21	0.39	1.88 <sup>a,b</sup>
Tamrun 96	0.00	0.00	1.92 <sup>b,c</sup>	0.00	2.98 <sup>a,b,c</sup>	1.00	0.08	0.23	2.00 <sup>a,b</sup>
Tamspan 90	0.00	0.00	1.99 <sup>a,b,c</sup>	0.00	2.82 <sup>b,c</sup>	1.00	0.17	0.41	2.39 <sup>a</sup>
OLlin	0.00	0.00	1.78 <sup>c</sup>	0.00	2.96 <sup>b,c</sup>	1.06	0.00	0.36	2.31 <sup>a</sup>

**Table 5b:** Sensory scores for peanut varieties developed through traditional breeding<sup>1</sup>.

 $^{1}M$  – metallic, FrF – fruity fermented, SW – sweet, Sour – sour, Bitter – bitter, Astr – astringent, TTB – tongue taste bitter, Ashy – ashy, Total – total "offnote".

<sup>a,b,c</sup> Means in the same column with the same letter are not significantly different at  $\alpha = 0.05$  level. Comparison of means was analyzed by Duncan's New Multiple Range Test.



Figure 1: A typical chromatogram showing headspace composition for NC 7(raw).
Peak	Retention	Chemical compound	GC	Olfactory
#	time (min)		% Area	response:
				User #1
-	1.06	-	-	Smoky (2)
-	2.00			Rancid (4)
1	2.01	Oxo-methylester acetic	50.7	-
		acid		
2	2.59	2-Methyl-propanal	3.6	-
-	2.61	-	-	Burnt butter (3)
3	3.46	3-Methyl-butanal	2.0	-
-	3.53	-	-	Sour (3)
4	3.58	2-Methyl-butanal	3.3	-
-	3.65	-	-	Sour (2)
5	4.05	Pentanal	3.0	-
6	4.85	1-Methyl-1H-pyrrole	10.5	-
7	6.17	Hexanal	2.8	-
-	6.23	-	-	Acidic (2)
-	6.73	-	-	Smoky (2)
-	8.75	-	-	Raw peanut (3)
-	8.89	-	-	Raw peanut (4)
-	10.67	-	-	Beany (3)
-	11.61	-	-	Sweet (2)
8	12.41	Benzaldehyde	0.7	-
9	14.16	Benzeneacetaldehyde	1.6	-
-	14.19	-	-	Sweet, floral (4)
-	14.83	-	-	Burnt latex (4)
-	14.98	-	-	Beany (3)
10	15.03	Nonanal	0.4	-
_	15.14	-	-	Raw peanut (2)
-	16.17	-	-	Raw peanut (3)
-	18.15	-	-	Green (3)
-	18.27	-	-	Green (3)
-	18.75	-	-	Sour (2)

**Table 6:** Chemical composition and olfactory description of peanut flavor components for NC 7 (raw).



Figure 2: A typical chromatogram showing headspace composition for Florunner (raw).

Peak	Retention	Chemical compound	GC	Olfactory	Olfactory
#	time (min)		%	response:	response:
			Area	User #1	User #2
-	1.64	-	-	Rancid (3)	-
1	1.71	Oxo-methylester	51.1	-	-
		acetic acid			
-	1.84	-	-	Rancid (4)	-
-	2.07	-	-	-	Sweet, musty (4)
2	2.32	2-Methyl-propanal	3.7	-	-
-	2.34	-	-	Beany (3)	-
-	2.79	-	-	-	Butter (3)
3	3.23	3-Methyl-butanal	3.0	-	-
-	3.29	-	-	Green (2)	-
4	3.36	2-Methyl-butanal	3.7	-	-
-	3.55	-	-	-	Sweet (3)
5	3.84	Pentanal	3.3	-	-
6	4.68	1-Methyl-1H-pyrrole	9.4	-	-
7	5.31	1-Pentanol	0.9	-	-
8	6.02	Hexanal	2.9	-	-
-	6.08	-	-	Sour (2)	-
-	6.23	-	-	-	Green grass (3)
-	8.61	-		Raw peanut (3)	-
-	8.78	-	-	Roast peanut (2)	-
-	8.83	-		-	Peanut butter (4)
-	10.53	-	-	Raw peanut (4)	-
-	10.66	-	-	-	Musty (3)
-	10.76	-	-	Butter (3)	-
9	10.78	2,5-Dimethylpyrazine	0.7	-	-
-	11.24	-	-	-	Stale (3)
-	11.39	-	-	Sweet (3)	-
-	11.48	-	-	Sweet (3)	-
-	11.95	-	-	-	Butter (3)
10	12.38	Benzaldehyde	0.7	-	-
-	13.24	-	-	-	Peanuts (3)
11	13.32	Octanal	0.5	-	-
12	14.13	Benzeneacetaldehyde	1.5	-	-
-	14.17	-	-	Sweet, floral (4)	-

**Table 7:** Chemical composition and olfactory description of peanut flavor components for Florunner (raw).

-	14.24	-	-	-	Floral (4)
-	14.85	-	-	Burnt latex (3)	-
-	14.97	-	-	Raw peanut (3)	-
13	15.03	Nonanal	0.3	-	-
-	15.16	-	-	-	Roasted
					peanuts (3)
-	16.00	-	-	-	Green (3)
-	16.17	-	-	Roast peanut	-
				(3)	
-	18.15	-	-	-	Stale peanuts
					(4)
-	18.18	-	-	Beany $(2)$	-



Figure 3: A typical chromatogram showing headspace composition for Jupiter (raw).

Peak	Retention	Chemical compound	GC	Olfactory
#	time (min)		% Area	response:
				User #1
-	0.69	-	-	Smoky (3)
1	2.15	Oxo-methylester acetic	50.4	-
		acid		
-	2.16	-	-	Rancid (4)
2	2.71	2-Methyl-propanal	4.1	-
-	2.74	-	-	Sour (3)
3	3.56	3-Methyl-butanal	2.3	-
4	3.68	2-Methyl-butanal	4.0	-
5	4.13	Pentanal	3.1	-
6	4.92	1-Methyl-1H-pyrrole	9.4	-
7	5.52	1-Pentanol	0.9	-
8	6.24	Hexanal	2.7	-
-	6.26	-	-	Sour (2)
-	8.87	-	-	Raw peanut (3)
-	9.00	-	-	Raw peanut (3)
-	9.27	-	-	Sweet (2)
-	10.72	-	-	Rancid (3)
9	10.91	2,5-Dimethylpyrazine	0.6	-
-	11.57	-	-	Sweet (3)
-	11.74	-	-	Sweet (2)
10	12.43	Benzaldehyde	0.9	-
11	13.35	Octanal	0.4	-
12	14.16	Benzeneacetaldehyde	1.9	-
-	14.21	-	-	Sweet, floral (4)
_	14.87	-	-	Burnt latex (4)
_	15.00	-	-	Raw peanut (3)
13	15.04	Nonanal	0.3	-
_	15.33	-	-	Roast peanut (3)
-	18.15	-	-	Green (3)
-	18.77	-	-	Acidic (2)

**Table 8:** Chemical composition and olfactory description of peanut flavor components for Jupiter (raw).



**Figure 4:** A typical chromatogram showing headspace composition for Tamrun OL 01 (raw).

Peak	Retention	Chemical compound	GC	Olfactory
#	time (min)		% Area	response:
				User #1
1	2.01	Oxo-methylester acetic	51.2	-
		acid		
-	1.98	-	-	Rancid (4)
2	2.59	2-Methyl-propanal	6.7	-
-	2.62	-	-	Smoky (3)
3	3.46	3-Methyl-butanal	4.4	-
-	3.48	-	-	Sour (2)
4	3.58	2-Methyl-butanal	5.9	-
-	3.63	-	-	Sour (2)
5	4.05	Pentanal	1.0	-
6	4.85	1-Methyl-1H-pyrrole	8.0	-
7	6.18	Hexanal	0.8	-
-	8.75	-	-	Raw peanut (3)
-	8.87	-	-	Raw peanut (4)
-	10.65	-	-	Beany (3)
8	10.96	2,5-Dimethylpyrazine	0.5	-
-	11.47	-	-	Sweet (2)
-	11.81	-		Sweet (3)
9	12.41	Benzaldehyde	0.9	-
10	14.16	Benzeneacetaldehyde	1.2	-
-	14.21	-	-	Sweet, floral (4)
-	14.86	-	-	Burnt latex (4)
-	14.98	-	-	Beany (3)
11	15.04	Nonanal	0.5	_
-	15.08	-	-	Raw peanut (2)
-	16.73	-	-	Green (2)
-	17.97	-	-	Green (3)
-	19.28	-	-	Burnt (3)

**Table 9:** Chemical composition and olfactory description of peanut flavor components for Tamrun OL 01 (raw).



**Figure 5:** A typical chromatogram showing headspace composition for Tamrun OL 02 (raw).

Peak	Retention	Chemical compound	GC	Olfactory
#	time (min)		% Area	response:
				User #1
-	1.93	-	-	Rancid (4)
1	1.95	Oxo-methylester acetic	56.3	
		acid		
2	2.20	Acetone	13.6	
-	2.52	-	-	Sour (3)
3	2.53	2-Methyl-propanal	6.1	
4	3.40	3-Methyl-butanal	4.4	
-	3.41	-	-	Sour (4)
5	3.52	2-Methyl-butanal	5.8	
6	4.80	1-Methyl-1H-pyrrole	8.4	
-	8.72	-	-	Raw peanut (2)
-	8.84	-	-	Raw peanut (3)
-	10.62	-	-	Sour (3)
-	11.38	-	-	Roast peanut (2)
7	12.40	Benzaldehyde	1.0	
8	14.14	Benzeneacetaldehyde	1.9	
-	14.18	-	-	Sweet, floral (4)
-	14.83	-	-	Burnt latex (3)
-	14.91	-	-	Burnt latex (4)
	14.97	-	-	Raw peanut (3)
9	15.03	Nonanal	0.3	
-	16.14	-	-	Burnt butter (3)

**Table 10:** Chemical composition and olfactory description of peanut flavor components for Tamrun OL 02 (raw).



Figure 6: A typical chromatogram showing headspace composition for Tamrun 96 (raw).

Peak	Retention	Chemical compound	GC	Olfactory
#	time (min)		% Area	response:
				User #1
1	1.71	Oxo-methylester acetic	50.8	-
		acid		
-	1.74	-	-	Rancid (4)
2	2.32	2-Methyl-propanal	4.5	-
-	2.34	-	-	Sour (3)
3	3.23	3-Methyl-butanal	2.7	-
-	3.26	-	-	Sour (2)
4	3.36	2-Methyl-butanal	4.2	-
5	3.85	Pentanal	3.3	-
6	4.68	1-Methyl-1H-pyrrole	7.5	-
7	5.31	1-Pentanol	1.0	-
8	6.03	Hexanal	3.2	-
-	8.68	-	-	Raw peanut (3)
-	8.78	-	-	Raw peanut (3)
-	9.15	-	-	Sweet (2)
-	10.54	-	-	Sour (3)
-	10.65	-	-	Sour (3)
9	10.81	2,5-Dimethylpyrazine	0.5	-
-	11.41	-	-	Raw peanut (3)
-	11.52	-	-	Raw peanut (3)
-	11.69	-	-	Roast peanut (2)
-	12.29	-	-	Sweet (2)
10	12.38	Benzaldehyde	0.9	-
11	13.32	Octanal	0.4	-
12	14.14	Benzeneacetaldehyde	1.9	-
-	14.17	-	-	Sweet, floral (4)
-	14.89	-	-	Burnt latex (3)
-	14.98	-	-	Beany (3)
13	15.03	Nonanal	0.3	-
-	15.94	-	-	Burnt latex (2)
	16.13	-	-	Raw peanut (3)
-	18.09	-	-	Green (2)
-	18.23	-	-	Green (2)

**Table 11:** Chemical composition and olfactory description of peanut flavor components for Tamrun 96 (raw).



**Figure 7:** A typical chromatogram showing headspace composition for Tamspan 90 (raw).

Peak	Retention	Chemical	GC	Olfactory	Olfactory
#	time	compound	%	response:	response:
	(min)		Area	User #1	User #2
-	0.52	-	-	Sour (4)	-
1	2.00	Oxo-methylester	53.7	-	-
		acetic acid			
-	2.05	-	-	Rancid (4)	-
-	2.07	-	-	-	Butter (4)
2	2.59	2-Methyl-propanal	3.9	-	-
-	2.68	-	-	Sour (3)	Sweet (3)
-	2.90	-	-	-	Butter (4)
3	3.47	3-Methyl-butanal	1.8	-	-
-	3.50	-	-	Burnt butter (3)	-
_	3.55	-	-	-	Sweet (3)
4	3.59	2-Methyl-butanal	2.8	-	-
5	4.05	Pentanal	3.8	-	-
6	4.85	1-Methyl-1H-pyrrole	5.7	-	-
7	5.46	1-Pentanol	1.3	-	-
8	6.18	Hexanal	4.0	-	-
-	6.21	-	-	Sour (2)	-
-	6.23	-	-	-	Grass (3)
-	6.80	-	-	-	Burned
					peanuts (3)
-	8.76	-	-	Raw peanut (3)	-
-	8.77	-	-	-	Peanuts (4)
-	8.87	-	-	Raw peanut (4)	-
-	10.72	-	-	Sour (3)	-
-	10.77	-	-	-	Sour (3)
9	10.87	2,5-	0.5	-	-
		Dimethylpyrazine			
-	11.21	-	-	-	Burning (4)
-	11.40	-	-	Sweet (3)	-
-	11.56	-	-	Sweet (3)	-
-	11.70	-	-	-	Peanuts (3)
10	12.42	Benzaldehyde	0.7	-	-
11	13.34	Octanal	0.4	-	-
_	13.77	-	-	-	Rubber (4)
12	14.15	Benzeneacetaldehyde	1.1	-	-
-	14.19	-	-	Sweet, floral (4)	-

**Table 12:** Chemical composition and olfactory description of peanut flavor components for Tamspan 90 (raw).

-	14.27	-	-	-	Floral (4)
-	14.97	-	-	Beany (3)	-
-	15.00	-	-	-	Green (4)
13	15.04	Nonanal	0.3	-	-
-	15.13	-	-	Roast peanut	-
				(2)	
-	15.93	-	-	Burnt latex (4)	-
_	18.12	-	-	Beany (3)	-
_	18.71	-	-	Sweet (2)	-



Figure 8: A typical chromatogram showing headspace composition for OLin (raw).

Peak	Retention	Chemical compound	GC	Olfactory
#	time (min)		% Area	response:
				User #1
-	0.79	-	-	Sour (2)
-	1.07	-	-	Sour (2)
1	2.12	Oxo-methylester acetic	57.5	-
		acid		
	2.14	-	-	Rancid (4)
2	2.69	2-Methyl-propanal	5.3	-
3	3.54	3-Methyl-butanal	3.3	-
-	3.60	-	-	Smoky (2)
4	3.66	2-Methyl-butanal	4.7	-
-	3.72	-	-	Sour (3)
5	4.12	Pentanal	1.2	-
6	4.91	1-Methyl-1H-pyrrole	9.1	-
7	6.22	Hexanal	0.8	-
-	8.85	-	-	Raw peanut (4)
-	9.00	-	-	Raw peanut (3)
-	10.67	-	-	Sour (4)
8	10.90	2,5-Dimethylpyrazine	0.6	-
-	11.44	-	-	Sweet (4)
-	11.57	-	-	Sweet (4)
9	12.43	Benzaldehyde	0.9	-
10	13.34	Octanal	0.4	-
11	14.16	Benzeneacetaldehyde	2.0	-
-	14.18	-	-	Sweet, floral (4)
-	14.89	-	-	Burnt latex (3)
-	14.98	-	-	Green (3)
12	15.04	Nonanal	0.3	-
-	15.27	-	-	Roast peanut (2)
	16.20	-	-	Raw peanut (3)
-	17.10	-	-	Smoky (2)
-	18.17	-	-	Beany (2)
-	18.69	-	-	Sour (3)

**Table 13:** Chemical composition and olfactory description of peanut flavor components for OLin (raw).



Figure 9: A typical chromatogram showing headspace composition for Okrun (raw).

Peak	Retention	Chemical	GC	Olfactory	Olfactory
#	time (min)	compound	%	response:	response:
			Area	User #1	User #2
-	0.77	-	-	Sour (3)	-
1	2.00	Oxo-methylester	47.8	-	-
		acetic acid			
-	2.03	-	-	Rancid (4)	-
-	2.30	-	-	-	Musty (3)
2	2.59	2-Methyl-propanal	5.6	-	-
-	2.61	-	-	Sour (3)	-
-	2.79	-	-	-	Sweet (3)
-	2.98	-	-	-	Butter (4)
3	3.45	3-Methyl-butanal	4.5	-	-
-	3.49	-	-	Burnt (3)	-
4	3.58	2-Methyl-butanal	5.2	-	-
-	3.65	-	-	-	Butter (3)
-	3.88	-	-	-	Musty (3)
5	4.04	Pentanal	2.2	-	-
6	4.85	1-Methyl-1H-pyrrole	13.7	-	-
7	5.46	1-Pentanol	0.8	-	-
8	6.17	Hexanal	2.0	-	-
-	8.79	-	-	Raw peanut (4)	-
-	8.95	-	-	-	Peanuts (4)
-	9.05	-	-	Beany (3)	-
-	10.66	-	-	Sour (4)	-
-	10.69	-	-	-	Rancid (4)
-	10.81	-	-	Sour (3)	-
9	10.86	2,5-	0.7	-	-
		Dimethylpyrazine			
-	11.43	-	-	Beany (3)	-
-	11.57	-	-	-	Butter (3)
-	11.58	-	-	Sweet (3)	-
-	11.79	-	-	Sweet (2)	-
10	12.41	Benzaldehyde	0.6	-	-
-	13.81	-	-	-	Bitter (3)
11	14.15	Benzeneacetaldehyde	1.2	-	-
-	14.18	-	-	Sweet, floral (4)	-
	14.24	-	-	-	Floral (4)
-	14.94	-	-	Burnt latex (4)	-
_	15.02	-	-	-	Stale (3)

**Table 14:** Chemical composition and olfactory description of peanut flavor components for Okrun (raw).

12	15.03	Nonanal	0.2	Raw peanut (3)	-
-	15.24	-	-	Raw peanut (3)	-
	15.96	-	-	-	Musty (3)
-	16.18	-	-	Burnt butter	-
				(3)	
-	18.12	-	-	Green (3)	-
-	18.39	-	-	Green (3)	-
-	18.40	-	-	-	Pungent (4)



Figure 10: A typical chromatogram showing headspace composition for 188 (raw).

Peak	Retention	Chemical compound	GC	Olfactory	Olfactory
#	time (min)		%	response:	response:
			Area	User #1	User #2
-	0.72	-	-	Sour (3)	-
1	2.03	Oxo-methylester	62.6	-	-
		acetic acid			
-	2.05	-	-	Rancid (4)	-
-	2.19	-	-	-	Musty
					peanuts (4)
2	2.60	2-Methyl-propanal	2.9	-	-
-	2.63	-	-	Sour (3)	-
-	2.93	-	-	-	Butter (2)
3	3.47	3-Methyl-butanal	1.9	-	-
-	3.52	-	-	Burnt butter	-
				(2)	
-	3.55	-	-	-	Butter (3)
4	3.59	2-Methyl-butanal	3.5	-	-
5	4.06	Pentanal	1.7	-	-
6	4.86	1-Methyl-1H-pyrrole	8.9	-	-
7	5.47	1-Pentanol	0.8	-	-
8	6.18	Hexanal	1.9	-	-
-	6.21	-	-	Sour (2)	-
-	6.27	-	-	-	Green (2)
-	6.97	-		-	Skunk (2)
-	8.80	-	-	Raw peanut (4)	-
-	8.88	-	-	-	Peanuts (3)
-	9.16	-	-	Raw peanut (3)	-
-	10.34	-	-	-	Peanuts (3)
-	10.65	-	-	Sour (3)	-
-	10.71	-	-	-	Bitter (3)
9	10.87	2,5-Dimethylpyrazine	0.7	-	-
-	10.92	-	-	Burnt butter	-
				(2)	
-	11.43	-	-	Sweet (2)	-
-	11.51	-	-	Sweet (3)	-
-	11.58	-	-	-	Stale peanuts
					(3)
-	11.74	-	-	Sweet (3)	-
10	12.42	Benzaldehyde	0.6	-	-
-	12.64	-	-	-	Rancid (4)
-	13.69	-	-	-	Butter (2)

**Table 15:** Chemical composition and olfactory description of peanut flavor components for 188 (raw).

11	14.15	Benzeneacetaldehyde	2.8	-	-
-	14.19	-	-	Sweet, floral	-
				(4)	
-	14.34	-	-	-	Floral (3)
-	14.83	-	-	Burnt latex (4)	-
-	14.97	-	-	Beany (3)	-
-	14.99	-	-	-	Bitter, green
					(3)
-	15.17	-	-	Raw peanut (3)	-
-	16.14	-	-	Green (3)	-
-	16.18	-	-	-	Peanuts (2)
-	18.12	-	-	Beany (3)	-
-	18.72	-	-	Sour (2)	-
-	20.67	-	-	-	Peanuts (2)



Figure 11: A typical chromatogram showing headspace composition for 540 (raw).

Peak	Retention	Chemical	GC	Olfactory	Olfactory
#	time (min)	compound	%	response:	response:
			Area	User #1	User #2
1	2.01	Oxo-methylester	46.7	-	-
		acetic acid			
-	2.05	-	-	Rancid (4)	-
-	2.21	-	-	-	Musty (3)
2	2.60	2-Methyl-propanal	7.9	-	-
-	2.61	-	-	Rancid (3)	-
-	2.73	-	I	-	Bitter (2)
-	2.92	-	-	-	Sweet (3)
3	3.46	3-Methyl-butanal	5.7	-	-
-	3.48	-	-	Sour (2)	-
4	3.58	2-Methyl-butanal	7.1	-	-
-	3.69	-	-	Raw peanut (3)	-
-	3.90	-	-	-	Sour (2)
5	4.05	Pentanal	2.1	-	-
6	4.85	1-Methyl-1H-pyrrole	11.4	-	-
7	5.46	1-Pentanol	1.0	-	-
8	6.17	Hexanal	2.4	-	-
-	8.83		-	-	Butter (3)
-	8.84	-	-	Raw peanut (3)	-
-	10.61	-	-	-	Sweet (2)
-	10.65	-	-	Sour (3)	-
-	10.71	-	-	-	Sour (3)
9	10.85	2,5-	0.6	-	-
		Dimethylpyrazine			
-	11.40	-	-	Sweet (2)	-
-	11.51	-	-	Sweet (3)	-
-	11.52	-	-	-	Peanuts (2)
10	12.41	Benzaldehyde	0.5	-	-
-	12.70	-	-	-	Rancid (4)
11	14.15	Benzeneacetaldehyde	0.9	-	-
-	14.20	-	-	Sweet, floral	-
				(4)	
-	14.23	-	-	-	Floral (3)
-	14.82	-	-	Burnt latex (4)	-
-	14.89	-	-	Burnt latex (3)	-
	14.95	-	-	-	Stale peanuts
					(3)
-	14.98	-	-	Raw peanut (3)	-

**Table 16:** Chemical composition and olfactory description of peanut flavor components for 540 (raw).

12	15.03	Nonanal	0.2	-	-
	15.15			Roast peanut	-
				(2)	
	15.21				Bitter (2)
-	15.95	-	-	Burnt (3)	-
-	16.15	-	-	-	Peanuts (2)
-	18.18	-	-	Beany (2)	-
-	18.36	-	-	-	Stale (2)



Figure 12: A typical chromatogram showing headspace composition for 654 (raw).

Peak	Retention	Chemical	GC	Olfactory	Olfactory
#	time (min)	compound	%	response:	response:
			Area	User #1	User #2
1	1.99	Oxo-methylester	45.5	-	-
		acetic acid			
-	2.02	-	-	Rancid (4)	-
-	2.14	-	-	-	Butter (3)
2	2.57	2-Methyl-propanal	7.1	-	-
-	2.60	-	-	Sour (3)	-
-	2.85	-	-	-	Butter (2)
3	3.44	3-Methyl-butanal	5.4	-	-
-	3.47	-	-	Sour (3)	-
4	3.57	2-Methyl-butanal	6.6	-	-
-	3.58	-	-	-	Sweet (2)
5	4.03	Pentanal	2.5	-	-
6	4.84	1-Methyl-1H-pyrrole	11.0	-	-
7	5.44	1-Pentanol	1.0	-	-
8	6.16	Hexanal	2.3	-	-
-	8.78	-	I	Sweet (3)	Peanuts (2)
-	8.94	-	I	Sweet (3)	-
-	10.65	-	-	Sour (3)	-
-	10.74	-	-	-	Bitter (3)
9	10.84	2,5-	0.8	-	-
		Dimethylpyrazine			
-	11.35	-	-	Sweet (4)	-
-	11.44	-	-	Sweet (4)	-
-	11.64	-	-	-	Sweet (2)
10	12.40	Benzaldehyde	0.7	-	-
-	12.58	-	-	-	Butter (2)
-	12.71	-	-	-	Rancid (4)
-	13.85	-	-	-	Burning (2)
11	14.14	Benzeneacetaldehyde	1.7	-	-
-	14.20	-	-	Sweet, floral	-
				(4)	
-	14.22	-	-	-	Floral (4)
-	14.86	-	-	Burnt latex	-
				(4)	
-	14.99	-	-	Raw peanut	-
				(3)	
-	15.00	-	-	-	Green (3)
12	15.03	Nonanal	0.2	-	-

**Table 17:** Chemical composition and olfactory description of peanut flavor components for 654 (raw).

-	15.19	-	-	Raw peanut	-
				(2)	
-	15.43	-	-	Roast peanut	-
				(2)	
-	15.92	-	-	Burnt latex	-
				(3)	
-	16.14	-	-	Burnt butter	-
				(3)	
-	16.15	-	-	-	Sweet (2)
-	18.06	-	-	Beany (2)	-



**Figure 13:** A typical chromatogram showing headspace composition for Florunner (roasted).

Peak	Retention	Chemical compound	GC	Olfactory
#	time (min)		% Area	response:
				User #1
1	2.07	Oxo-methylester acetic	39.9	-
		acid		
-	2.04	-	-	Rancid (4)
2	2.65	2-Methyl-propanal	3.9	-
-	2.67	-	-	Sour (3)
3	3.63	2-Methyl-butanal	2.4	-
4	4.08	Pentanal	4.3	-
5	4.88	1-Methyl-1H-pyrrole	10.6	-
6	5.46	1-Pentanol	3.1	-
7	6.20	Hexanal	7.5	Green (2)
-	10.67	-	-	Sour (3)
-	11.49	-	-	Sweet (3)
8	12.28	(Z)-2-Heptenal	0.8	-
-	12.88	-	-	Sour (2)
9	13.34	Octanal	0.6	-
-	14.22	-	-	Sweet, floral (4)
-	14.82	-	-	Burnt latex (4)
-	14.98	-	-	Raw peanut (3)
10	15.03	Nonanal	1.0	-
-	15.94	-	_	Pesticide (3)
-	18.18	-	-	Beany (3)

**Table 18:** Chemical composition and olfactory description of peanut flavor components for Florunner (roasted).



**Figure 14:** A typical chromatogram showing headspace composition for Jupiter (roasted).

Peak	Retention	Chemical compound	GC	Olfactory
#	time (min)		% Area	response:
				User #1
1	2.01	Oxo-methylester acetic	48.1	-
		acid		
-	2.05	-	-	Sour (2)
-	2.09	-	-	Rancid (4)
2	2.60	2-Methyl-propanal	3.0	-
-	3.48	-	-	Sour (2)
3	3.59	2-Methyl-butanal	2.4	-
4	4.05	Pentanal	3.6	-
5	4.86	1-Methyl-1H-pyrrole	8.7	-
6	5.43	1-Pentanol	2.8	-
7	6.17	Hexanal	8.4	-
-	6.20	-	-	Green (2)
-	10.64	-	-	Sour (2)
-	11.46	-	-	Raw peanut (2)
8	12.28	(Z)-2-Heptenal	1.1	-
-	12.87	-	-	Sour (2)
9	13.34	Octanal	0.6	-
-	14.22	-	-	Sweet, floral (4)
-	14.92	-	-	Burnt latex (4)
-	14.98	-	-	Beany (3)
10	15.03	Nonanal	1.2	-
-	15.96	-	-	Green (2)
-	18.10	-	-	Beany (2)

**Table 19:** Chemical composition and olfactory description of peanut flavor components for Jupiter (roasted).



**Figure 15:** A typical chromatogram showing headspace composition for Tamspan 90 (roasted).

Peak	Retention	Chemical compound	GC	Olfactory
#	time (min)		% Area	response:
				User #1
-	0.62	-	-	Sour (2)
1	2.02	Oxo-methylester acetic	18.6	-
		acid		
-	2.05	-	-	Rancid (4)
2	2.60	2-Methyl-propanal	2.2	-
-	2.65	-	-	Smoky (2)
3	2.84	Butanal	1.7	-
4	3.59	2-Methyl-butanal	1.5	-
5	4.04	Pentanal	8.6	-
-	4.06	-	-	Sour (2)
6	4.85	1-Methyl-1H-pyrrole	2.8	-
7	5.43	1-Pentanol	5.5	-
-	5.61	-	-	Acidic (3)
8	6.18	Hexanal	20.9	-
-	6.19	-	-	Green (3)
-	8.92	-	-	Burnt butter (2)
9	10.35	Heptanal	0.8	-
-	10.59	-	-	Sweet (2)
-	10.68	-	-	Raw peanut (3)
10	12.27	(Z)-2-Heptenal	3.7	-
11	12.87	1-Octen-3-ol	1.4	-
-	12.90	-	-	Sour (1)
12	13.10	2-Pentyl-furan	1.0	-
13	13.33	Octanal	1.0	-
-	14.23	-	-	Sweet, floral (4)
14	14.35	(E)-2-Octenal	0.9	-
-	14.88	-	-	Burnt latex (4)
-	14.97	-	-	Beany (3)
15	15.03	Nonanal	1.7	-
-	15.80	-	-	Chemical (3)
-	15.93	-	-	Green (2)
-	16.77	-	-	Beany (2)
-	18.26	-	-	Green (3)

**Table 20:** Chemical composition and olfactory description of peanut flavor components for Tamspan 90 (roasted).



Figure 16: A typical chromatogram showing headspace composition for OLin (roasted).
Peak	Retention	Chemical compound	GC	Olfactory
#	time (min)		% Area	response:
				User #1
-	0.53	-	-	Smoky (2)
1	1.96	Oxo-methylester acetic	60.7	-
		acid		
-	1.93	-	-	Rancid (4)
-	2.00	-	-	Sour (3)
2	2.56	2-Methyl-propanal	5.1	-
-	2.59	-	-	Burnt butter (2)
-	3.48	-	-	Sour (2)
3	3.56	2-Methyl-butanal	3.7	-
4	4.02	Pentanal	1.9	-
5	4.83	1-Methyl-1H-pyrrole	7.3	-
6	6.15	Hexanal	2.1	-
-	6.19	-	-	Green (3)
-	6.70	-	-	Sour (2)
-	10.67	-	-	Sour (3)
7	10.83	2,5-Dimethylpyrazine	0.9	-
-	10.87	-	-	Burnt popcorn
				(2)
-	11.38	-	-	Raw peanut (3)
_	11.49		-	Sweet (3)
-	14.20	-	-	Sweet, floral (4)
-	14.83	-	-	Burnt latex (3)
8	15.03	Nonanal	0.8	-
-	15.84	-	-	Beany (2)

Table 21: Chemical	composition and	olfactory	description	of peanut flav	vor components
for OLin (roasted).					

\* The number in parentheses indicates the level of intensity perceived: 1 - low, 2 - medium, 3 - high, 4 - very high.

## VITA

#### Ee Chin Ng

#### Candidate for the Degree of

# Master of Science

## Thesis: FLAVOR PROFILES OF OKLAHOMA-GROWN PEANUTS

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- Personal Data: Born in Perak, Malaysia, On February 14, 1981, the daughter of Mr. and Mrs. Chew Sooi Ng
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Pages in Study: 99

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Major Field: Food Science

Scope and Method of Study: The main objective of this study is to examine flavor characteristics of peanut varieties developed through both traditional breeding and genetic engineering. Conventionally bred peanut varieties, NC 7, Jupiter, Florunner, Tamrun 96, Tamrun OL 01, Tamrun OL 02, Tamspan 90, and OLin were grown at Fort Cobb, Oklahoma. The genetically modified peanut (GMP) varieties, 188, 540 and 654 were from the USDA-ARS in Stillwater, Oklahoma. The peanut variety Okrun was examined as a control for the genetically engineered peanut lines. The flavor analysis was performed using two techniques, sensory evaluation and a gas chromatograph/mass spectrometer (GC/MS) equipped with an olfactory sniff-port. The identification of volatile flavor compounds was performed using the library search on the MS and standard references. Traditional varieties were evaluated by a trained sensory panel for 18 attributes including roasty peanutty, sweet aromatic, raw beany and fruity fermented flavors. The peanut samples were also analyzed for their moisture, ash, protein, sugar, and oil composition.

Findings and Conclusions: Experimental results showed that the variations in nutritional composition of peanut varieties examined in this study were minimal. There were minor differences in flavor attributes among the samples. Florunner had lowest total off-notes and significantly higher roasted peanut flavor than the rest of the samples. The implications of this study are significant, as peanut breeders are able to breed peanut cultivars that have greater pest and fungal resistance, and yet, able to maintain the desirable flavor characteristics.