

ABSTRACT

GREENE, JEFFREY L. Characterization of Naturally Occurring Fruity Fermented Off-flavor in Peanuts Using Descriptive Sensory, Consumer, and Instrumental Analyses. (Under the direction of Dr. Timothy H. Sanders and Dr. MaryAnne Drake.)

Peanuts are a valuable agricultural commodity and roasted peanut flavor is the driving force for consumer purchase and consumption. The development of off-flavors is a major concern to the peanut industry and physiological differences (i.e. oil and sugar content) among the different maturity classes can influence the presence and/or absence of specific flavors. Fruity fermented (FF) is a common off-flavor found in peanuts and is developed when peanuts are cured at excessive temperatures ($>35^{\circ}\text{C}$). Previous literature has characterized FF off-flavor using descriptive sensory analysis; however, there is little information on FF off-flavor using consumer evaluation. The peanut plant has an indeterminate flowering pattern meaning a range of maturities are present at harvest and the immature and mature peanuts differ in roasting and flavor quality. Immature peanuts tend to have more FF off-flavor than mature peanuts which results in a FF distribution within large peanut lots. The flowering pattern and heterogeneous distribution of immature and mature seed make it challenging to obtain an accurate determination of FF off-flavor in a bulk lot. Establishing links between flavor and volatile flavor compounds can be obtained by using sensory and instrumental analyses. Currently, there is little research published on the volatile components that contribute to naturally occurring FF off-flavor. The objectives of this research were to: i) characterize consumer's perception of FF off-flavor, ii) measure the variability and determine the FF distribution in bulk lots, iii) and identify the volatile compounds responsible for naturally occurring FF off-flavor using sensory and instrumental analyses. Descriptive sensory analysis was conducted to determine the no FF and FF

samples used for the consumer study. Two-hundred and eight consumers evaluated a control (no FF off-flavor), low (1.0 FF) intensity, and a high (3.0 FF) intensity using two different scaling techniques: category and line scales. Results indicated FF off-flavor negatively impacts consumer acceptance of peanuts and the line scales were more sensitive and showed more differences among the samples compared to the category scale. The second study investigated the distribution of FF off-flavor in peanut lots and the results indicated that FF intensity varied from lot to lot and within a single bulk lot. Solvent assisted flavor evaporation (SAFE), solidphase microextraction (SPME), gas chromatography-olfactometry (GC-O), gas chromatography-mass spectrometry (GC-MS), and model systems were conducted to identify the compounds responsible for naturally occurring FF off-flavor. Volatile analysis indicated that ethanol and the esters previously reported as causing FF off-flavor were not detected in natural FF samples by solvent extractions; however, they were present in natural and artificially created Georgia Green and Flavor Runner 458 samples by headspace extractions. These findings emphasizes that the use of laboratory created samples should not be used to identify off-flavor sources in peanuts. Additionally, the use of analytical techniques to identify FF off-flavor in bulk lots cannot be achieved using ethanol or esters as indicators.

Characterization of Naturally Occurring Fruity Fermented Off-flavor in Peanuts
using Descriptive Sensory, Consumer, and Instrumental Analyses

by

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DEDICATION

I dedicate this work to my mommy, Ms. Jeffrey Sutton Greene. Thank you for all the sacrifices you made throughout my childhood and graduate career so that I could continue to better myself through education.

BIOGRAPHY

Jeffrey Lynnette Greene was born on January 22, 1978, in San Antonio, Texas to Ms. Jeffrey S. Greene and Mr. Stonell B. Greene. Jeffrey has two older sisters, Ms. Jerilan D. Greene and Ms. Janiece B. Greene. She has two younger half-brothers, Silas S. Greene and Richmond H. Greene.

Jeffrey graduated from John Marshall High School in May 1997 and began her Bachelor of Science degree at Xavier University of Louisiana in New Orleans, LA in August 1997. As a Biology major Jeffrey worked in the Commodity Utilization unit at USDA-Agricultural Research Service-Southern Regional Research Center (ARS-SRRC) (August 1998-2001) investigating the genes involved in the biogenesis of specific conjugated fatty acid in Tung nuts. While there, she was able to explore all the various opportunities in the science field and she gained valuable experience in solving problems at the lab bench.

Jeffrey completed her B.S. in August 2001 and moved to Alabama to pursue her Masters degree at Tuskegee University in Tuskegee, AL as a NASA Scholar. After her first year in the NASA program, Jeffrey was selected to intern at NASA-Johnson Space Center in Houston, Texas. She worked in the Space Food Systems Laboratory where she worked on developing value-added food products for long term space missions and conducted analytical tests. Jeffrey loved conducting research and continued to develop her career by presenting her thesis research at various conferences both nationally and internationally. In March 2003, she was awarded 2nd place in the 30th Sigma Xi Research Society Graduate Oral competition. Jeffrey completed her Masters in May 2003 and returned to USDA-ARS-SRRC to intern in the Food Processing and Sensory Quality unit where she studied the development of off-flavors in fresh-cut cantaloupes using sensory and instrumental analyses.

Jeffrey began her doctoral program in January 2004 at North Carolina State University, Raleigh, NC under the direction of Dr. Timothy H. Sanders and Dr. MaryAnne Drake. Her research focused on relating sensory and instrumental analyses to identify the volatile compounds responsible for fruity fermented off-flavor in peanuts. Jeffrey worked diligently in the lab to be successful in her research studies. At the 2005 Annual Institute of Food Technologists (IFT) meeting, Jeffrey competed in the IFT Sensory Evaluation Division Rose Marie Pangborn Memorial Competition (2nd place award winner) and the IFT/Phi Tau Sigma/Procter & Gamble Graduate Research Paper Competition (4th place award winner). She placed in both competitions for her research entitled: *Effectiveness of Category and Line Scales to Characterize Consumer Perception of Fruity Fermented flavor in Peanuts*. Also, Jeffrey has presented in the Joe Sugg Graduate Research Competition at the American Peanut Research and Education Society (APRES) in Savannah, GA where she received an “honorable mention” for her research entitled: *Fruity fermented Off-flavor Distribution in Samples from Large Peanut Lots*. Jeffrey completed her graduate career by receiving the 1st place award in the Division I-Graduate Oral Research Paper Competition at the 2007 Minorities in Agriculture, Natural Resources, and Related Sciences (MANRRS) conference. The title of her oral presentation was: *Characterization of Aroma-active Compounds in Fruity Fermented Peanuts Using Aroma Extract Dilution Analysis*.

After graduating with her PhD in Food Science, Jeffrey will begin working at Kraft Foods in the Global Technology & Quality unit in Glenview, IL.

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“Trust in the Lord with all thy heart and lean not unto your own understanding. In all your ways, acknowledge Him and He will direct your path.”

Proverbs 3: 5-6.

“The race is not given to the swiftest nor to the strong, but to the one who endures it all.”

Ecclesiastes 9:11

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CHAPTER 1:
INTRODUCTION

Peanuts are a valuable agricultural commodity. India, China, and U.S. are the major peanut producing countries. In 2005, 4,821,250 million pounds of peanuts were produced in the U.S. (National Agriculture Statistics Service and USDA, 2006). Approximately 60% of peanuts produced are for human consumption. Roasted peanut flavor is the driving force for consumer purchase and consumption; therefore off-flavor development is of major concern to the peanut industry (Sanders et al., 1997).

Handling, processing, and storage of peanuts are the major factors that influence flavor quality (Sanders et al., 1995). Harvested peanuts have an average moisture content of approximately 50-60%. To facilitate good storage and high grade, the moisture levels in peanuts must be reduced to 8-10%. Moisture reduction is achieved after field drying in windrows. Curing is generally accomplished using forced heated air.

One of the most common off-flavors found in peanuts is known as fruity fermented (FF) (Sanders et al., 1989b). Studies have reported that FF flavor is developed when peanuts are cured at excessive temperatures ($>35^{\circ}\text{C}$) (Whitaker and Dickens, 1964). One of the mechanisms possibly involved in off-flavor development is the conversion of aerobic respiration to anaerobic respiration which can cause off-flavors (Sanders et al., 1989b; Pattee et al., 1965; Whitaker and Dickens, 1964). In general, off-flavors are developed during handling. Johnsen et al. (1988) reported that painty, cardboardy, and oxidized flavors account for the off-flavors found in processed peanuts and peanut butter products.

The peanut plant is unique because it flowers indeterminately, hence a range of peanuts of different maturity are present at harvest. Sanders et al. (1989b) reported that “small peanuts” are usually associated with immaturity but this relationship is not absolute. Williams et al. (1987) discussed that the size-maturity relationship is difficult to define

because of the indeterminate flowering which depends on the peanut variety and environmental conditions. The most common way to determine the maturity of peanuts is by using the Pod Maturity Profile. In this method, the peanut exocarp is removed to expose the mesocarp color. Peanuts are visually sorted into different color peanut maturity classes (black, brown, orange, yellow, and white). Black and brown mesocarps are the mature pods, orange colored pods are intermediate, and yellow and white pods are immature. Mature peanuts tend to have more roasted peanut flavor, slower flavor fade, less FF off-flavor, and lighter roast color. Immature peanuts usually exhibit less roasted peanut flavor, darker roast, faster flavor fade, more FF off-flavor, and darker roast color. The physiological differences in mature and immature peanuts affect many factors related to flavor and overall acceptability.

Sensory analysis is a powerful tool used to evoke, measure, and interpret the various characteristics of food products using the human senses (Lawless and Heymann, 1999). Aroma, appearance, flavor, texture, aftertaste, and sound properties of the product are associated with the qualitative aspects of the food. The quantitative aspect involves the intensity of attributes using discriminative, descriptive, and affective tests. Descriptive sensory analysis is a technique often used to detect and describe the qualitative and quantitative sensory components in food using trained panels (Meilgaard, Civille, and Carr, 1999). This procedure has been used to evaluate the descriptive profiles of peanuts (Sanders et al., 1989b; Young et al., 2005; Schirack, 2006; Katz, 2002). Affective tests have been used to characterize peanuts. Young et al. (2005) conducted consumer tests of peanuts from different origins and demonstrated that U.S. peanuts had the highest roasted peanut flavor compared to China and Argentina. Greene et al. (2005) evaluated consumer perception of FF

off-flavor using category and line scales. Results indicated that consumers can detect differences in FF and non-FF peanuts which affects overall liking.

FF off-flavor development in peanuts is a major concern for the peanut industry. Numerous manufacturers of peanut products rejected many lots of the 2003 crop because of FF flavor. This problem caused an economic loss to the industry and could have been avoided if FF off-flavor had been accurately detected. Determining the presence and intensity of FF off-flavor in a bulk lot can be difficult because of the relationship of FF to maturity and the mixing of lots in warehouse storage and shelling. Research has indicated that the use of sampling plans is an excellent way to get an accurate determination of a contaminant in a lot. Vandeven et al. (2002) stated that food manufacturers use acceptance sampling plans to obtain the best estimate of a wide range of factors in numerous commodities. Buyers and sellers can use sampling plans to detect heterogeneous contaminants in raw materials (Whitaker, 2003). There are numerous research studies on the development of sampling of lots to determine the presence and threshold level of aflatoxin in peanuts and corn (Whitaker and Johansson, 2005; Whitaker, 2003; Vandeven et al., 2002; Whitaker et al., 2004; Fonseca, 2002; Knutti and Schlatter, 1982), however there are no sampling plans for off-flavors in peanuts.

Understanding flavor is an important aspect to effective and strategic research and marketing (Drake et al., 2006). Establishing links between sensory and instrumental analyses of volatile compounds is a powerful method that can be used to further characterize flavor and enhance production technology. Descriptive sensory and volatile analyses are the two basic requirements needed to relate sensory profiles with instrumental measurements. In general, the approach to relating the two analyses include: i) descriptive sensory analysis,

ii) extraction/isolation, iii) gas chromatographic analysis, iv) threshold analysis, and v) model systems/addition studies.

Descriptive sensory analysis is the most important step because the identification of differences is the basis for further analysis. One extraction method alone may not extract all important volatiles present in the food matrix. To insure the greatest opportunity of obtaining all the important volatiles in a mixture, a combination of techniques should be used (Drake et al., 2006). There are various studies that have used solvent and headspace extractions in peanuts to establish links between instrumental and sensory (Didzbalis et al., 2004; Pattee et al., 1965; Pattee et al., 1990; Crippen et al., 1992; Vercellotti et al., 1992). Gas chromatography/mass spectrometry is used to identify extracted compound(s); however this identification does not indicate whether or not a compound is aroma-active. Aroma-active compounds are identified by Gas chromatography-Olfactometry. This methodology helps to identify the aroma-active compounds and compounds that are potentially involved with the flavor(s) of interest in a study.

There are several procedures used in gas chromatography-olfactometry and they are: postpeak intensity, nasal impact frequency, time-intensity, and dilution methods. The dilution techniques (CharmAnalysis™ and Aroma Extract Dilution Analysis) are the most common because odor activity values, flavor dilution factors, and the duration of an aroma can be calculated. These type data further strengthen the analysis by focusing on the odorants with higher values because the higher the value the more likely they are responsible for the flavor in the food. Threshold analysis is done to determine the concentration level at which people can detect specific compounds. Model systems are developed to confirm whether a compound is involved in the off-flavor.

The presence of FF off-flavor in peanuts is notable in peanuts and can potentially affect peanut products developed by manufacturers. The specific objectives of this research were to: i) characterize FF peanut lots using descriptive sensory analysis, ii) determine consumer's perception of FF peanuts using two different sensory methodologies, iii) measure the variability and characterize FF distribution among samples from large lots, and iv) identify the volatile compounds contributing to FF off-flavor in peanuts using sensory and instrumental analyses. The first objective will determine if FF off-flavor in peanuts impacts overall acceptability. The second objective will provide the framework for developing a sampling plan to accurately identify FF off-flavor in peanut lots. Lastly, identification of the compounds in FF peanuts will provide understanding of how specific volatile compounds impact flavor perception of FF peanuts. Further, knowledge of the compounds responsible for FF off-flavor will assist in designing analytical methods that can be used to more accurately identify FF peanut lots.

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CHAPTER 2:
LITERATURE REVIEW

HISTORICAL BACKGROUND OF PEANUTS

The peanut [*Arachis hypogaea*] is an important crop throughout numerous parts of the world. It is an annual soil-enriching, nitrogen-fixing legume, which is a native of South America and belongs to the Leguminosae family (Hammons, 1982). Although peanuts originated in South America, they are grown in other tropical and subtropical countries, including the United States, India, China, Argentina, Brazil, and West Africa (Ory et al., 1992). Overall, India, China, and the United States are the major peanut producing countries.

In 2003, the American Peanut Council reported that United States peanuts represented 10% of the world peanut production. The Worldwide production is almost 64 trillion pounds annually (Foreign Agriculture Service and USDA, 1998). In the United States, approximately 3.26 billion pounds of peanuts were produced in 2000, grossing close to \$1.0 billion (National Agriculture Statistics Service and USDA, 2001). Approximately 99% of all peanuts grown in the United States are grown in seven different states: Georgia (41%), Texas (24%), Alabama (10%), North Carolina (9%), Florida (6%), Virginia (5%), and Oklahoma (5%) (American Peanut Council, 2006).

There are four basic types of peanuts: runner, virginia, spanish, and valencia. These types are differentiated by plant shape, seed, and physiological differences. Runners are the most prevalent type and the majority of runner type peanuts are used to produce peanut butter (Virginia-North Carolina Peanuts Promotions, 2003). The Virginia type peanuts are characterized by large seed and are used in the roasted and processed in-the-shell market. These large sized peanuts are also marketed as cocktail peanuts or in mixed nuts for snacks (Virginia-North Carolina Peanuts Promotions, 2003). The small sized seed with reddish-brown skin are the Spanish type peanuts. These peanuts are used predominately in peanut

candies, with significant quantities used for snacks and peanut butter. Spanish peanuts have a high oil content which is an advantage when crushing for oil. Valencia types have three or more bright-red seed in a pod (Virginia-North Carolina Peanuts Promotions, 2003). Usually, they are roasted in-the-shell and are excellent for fresh use as boiled peanuts. Runner, virginia, spanish, and valencia type peanuts account for the total U.S. production at 80, 15, 4, and 1%, respectively (American Peanut Council, 2006).

UTILIZATION OF PEANUTS

Peanuts are a valuable agricultural commodity. The U.S. peanut crop is used for domestic edible products such as, peanuts, peanut butter, and peanut candy. The American Peanut Council reported that American consumers eat more than six pounds (2.7 kilograms) of peanut products every year. The principal food uses of peanuts include peanut butter, peanut confections, shelled, roasted, salted peanuts, and roasted in the shell. Additionally, peanuts can be processed for use in a variety of foods, such as breads, cookies, cakes/pies, non-milk beverages, soups, frozen desserts, and breakfast cereals (American Peanut Council, 2006). Of the peanuts used for domestic food and export in 1999-2000, 46% were processed into peanut butter, over 23% went into snack peanut products, 21% were used in peanut candy, and 9% were marketed as cleaned in-shell such as those sold in ballparks (Jurenas, 2001).

Peanuts are an excellent source of protein, providing over 10% of the U.S. recommended daily intake (RDI) per 1oz. serving of peanuts or 2 tablespoons of peanut butter. They are a good source of essential vitamins A, D, E, and C and calcium, phosphorus, magnesium, sodium, and potassium, fiber, and folic acid. In addition to food uses, peanuts can be processed for non-food uses. They have a variety of industrial

applications as paint, lubricating oil, insecticides, furniture polish, and leather dressings. The shells and skins may be used in fireplace logs, fiber roughage for livestock, kitty litter, and paper making.

GROWING, HARVESTING, AND CURING OF PEANUTS

Quality control of peanuts begins on the farm and continues successively through harvesting, curing, shelling, storing, and manufacturing. Since the 1940s, each step in handling peanuts from the field has been improved by the use of mechanical handling. These methods are beneficial because they reduce weathering, molding, and insect damage resulting in brighter, cleaner, and more uniform peanuts (Woodroof, 1966).

The peanut plant is an unusual crop because it produces flowers above the ground, but fruit develop below the soil surface (Figure 1).

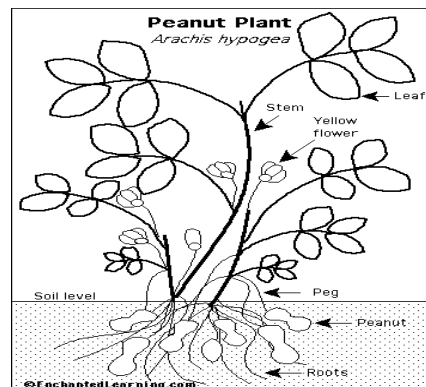


Figure 1. Peanut Plant Anatomy (Col, Jeananda. Enchanted Learning. <http://www.EnchantedLearning.com> 2001)

The fertilized ovary of the self-pollinating flowers elongates into peg which is positively geotropic and enters the soil. The embryos in the tip of the peg develop into a peanut pod.

The environmental conditions in which peanuts are planted may affect their growth.

Temperature has an important role in most aspects of peanut growth (Ketring et al., 1982).

Numerous research studies have investigated the effect of temperature on peanut yield. The

optimum day/night temperatures reported for efficient growth vary among studies. The temperatures reported include 35/25, 30/26, 35/20 and 30/35, and 25/25°C (Ono et al, 1974; Cox, 1979; Fortanier, 1957; Wood, 1968). Results indicated that plants are less sensitive to temperatures as they mature.

When peanuts are harvested at the optimum harvest date, approximately the highest yields and grades are obtained. At any harvest time there are always some immature pods; however, the peanut industry focuses on harvesting at the optimum time to obtain a high percentage of mature pods (Sanders et al., 1995). Harvesting of peanuts occurs in two stages: digging and combining. Digging consists of cutting the tap roots to loosen the plants from the soil. A shaker, which is located behind the blade, lifts the peanut plants from the soil, shakes off dirt from the peanuts, rotates the plants and drops it upside down into windrows where the peanuts are exposed to air drying for approximately 2-5 days. The second phase of harvesting involves combining. In this process, the combine lifts the plants, picks the pods from the vine into a hopper on the machine, and blows fragmented plants back into the field. The vines remain in the field and are used to improve soil fertility and organic matter.

Moisture content is probably the most important factor affecting the stability of peanuts during harvesting, drying, storage, and marketing (Shewfelt and Young, 1977). At the time of harvest, peanuts have a moisture content of 50-60%. In order to reduce lipid oxidation, fungal growth, and maintain overall quality, the moisture content must be reduced to 10%. Curing (drying) is a process during which the moisture content is reduced to a safe level for maintenance of quality (Young et al., 1982).

In the 1950's and 1960's, peanut equipment manufacturers developed various techniques to cure peanuts in windrows without the pods coming into contact with the soil.

The different types of curing methods include stack-pole, inverting, tenting, and sandwich windrows. Stack-pole curing is one of the oldest drying methods. In this technique the peanut plants are stacked around a pole with minimum exposure of most peanuts to the sun. The advantage of this method is that it provides good ventilation and allows slow curing which allows for continued maturation of pods (Sanders et al., 1997). Inverting involves rotating the plant upside down to expose the pods to the sun while the vines are touching the soil. Davidson et al. (1991) investigated the effects of soil temperature on the production of Florunner peanuts. Results indicated that soil temperatures within the range of 20-35°C will provide maximum field emergence. Inverting allows peanuts to dry more quickly and affords ease of harvest; however, since the pods are exposed directly to the sun they are more susceptible to developing off-flavors at high temperatures.

Tenting was developed to reduce the temperature of pod drying. In this method, the peanuts hang down in the vine mass which provides shade to the pods. This method is advantageous because the peanuts are cured at lower temperatures reducing the risk of off-flavor development. Sandwich windrows are also used to reduce drying temperatures. Sandwich windrows are constructed with an inverted plant on the bottom and a non-inverted plant on top resulting in peanuts sandwiched between the plants. The pods are shaded from high temperatures and sandwich windrows also protect peanuts from freeze injury because they are not directly exposed to the air (Williams, 2006). There is no single method that provides a better quality peanut; however, sandwich windrows help manage the risks faced when curing peanuts in hot climates. Sanders (2005) investigated the effectiveness of sandwiched, tent, and inverted windrows sprayed with Surround, a white kaolin clay which helps reduce temperature. Results indicated that sandwich windrows provided lower

temperatures. In the first study year differences were evident due to high temperature exposure; however, more moderate harvest temperatures resulted in no flavor differences.

Bulk curing of peanuts with heated air is an accepted practice in all peanut growing areas of the U.S. (Whitaker and Dickens, 1964). In typical curing processes, peanuts remain in windrows for 2-5 days until they reach 20-25% moisture content. Once this moisture content is reached the plants have dried to a brittle state and are easily combined. After peanut pods are combined they are transferred to drying wagons, in which they are dried with heated air until they reach a moisture content of ca.10%. Dried peanuts are stored in large warehouses until they are shelled. Peanut growing, harvesting, and curing are extremely important to overall quality of peanuts.

PEANUT MATURITY

Maturity affects yield, grade, size, milling quality, seed composition, flavor, and shelf life. Peanuts have an indeterminate flowering pattern which dictates that at any harvest date, fruit of a wide range of maturities will be harvested (McNeill and Sanders, 1996). This makes it difficult to determine when optimum maturity occurs because the fruiting pattern can vary considerably from year to year due to environmental conditions.

In-shell and shelled peanuts are sized without regard to maturity. In general, maturity and size are related; however, the maturity-size relationship of peanuts is not absolute (Sanders et al. 1989b); therefore, peanuts of different maturities are sized together (McNeill and Sanders, 1996). As peanut pods mature, physiological changes occur. In order to study these maturity related changes, there must be some separation of defined maturity levels. Each maturity stage necessarily represents a small and slightly overlapping range of

physiological characteristics; therefore, investigation of these differences requires a consistently accurate basis of classification (Sanders et al., 1982a).

There are several methods to classify the physiological maturity stages of development and they are all based on internal or external physical and morphological characteristics of the hull, seed coat, and seed (Sanders et al., 1982a). The methods for determination of maturity have been developed and used by the peanut industry (Sanders et al., 1982a). Internal hull color is one of the methods used and this procedure involves cracking the pods open to expose the color of the inside the hull. The percentages of pods with tan to brown internal hull are determined. Evaluating the oil color is a seldom used maturity determination method. Research has shown that oil pressed from mature peanuts is lighter in color than that from immature peanuts (Sharon, 1963; Holley and Young, 1963). Additionally, Holley and Young (1963) reported differences between oil color from slowly cured peanuts compared to oil from rapidly cured peanuts. Pattee and Purcell (1967) identified carotenoid compounds in peanut oil. Beta-carotene and lutein were present at higher levels in immature peanuts than mature peanuts. Pattee et al. (1969) investigated the color of peanut oil from various maturity stages finding a decrease in color as peanuts matured which is a result of the dilution of pigments by an increase in oil production. Methanolic extraction is another maturity method based on color. In this method, peanuts are extracted with methanol, which is cooled, filtered, and the % light transmittance is measured at 450nm. Light transmitted provides some indication of the maturity and whether pods are ready to be harvested. If the % light transmittance is 60 or lower, the crop should be dug; however, if the % light transmittance is in the low 60s, the harvest date should be recalculated. Sanders and Williams (1978) studied the use of methanolic extract to determine

when to dig peanuts. The results showed that under optimum conditions the methanolic extract provided an adequate prediction of optimum harvest date but generally did not reinforce the prediction on a sample taken on the initially predicted date. However, harvest date predictions made with this method are unreliable in areas with drought stress conditions.

The most used method for determining maturity is based on the color of the pod mesocarp. Williams and Drexler (1981) developed a harvest date prediction method which involves scarping off the hull exocarp to expose the color of the mesocarp. This method is called the Pod Maturity Profile or the Hull-Scrape Method, which classifies peanuts into seven maturity classes based on mesocarp color (Figure 2). In comparison to the methods previously mentioned this is a nondestructive method because the pod remains intact and is not cracked or cut open. This method provides useful information on the pod maturity distribution at the various stages of growth. Furthermore, the Pod Maturity Profile results in a determination of the number of pods within each maturity class for a single lot and indicate the number of pods with a potential to mature before harvest (Sanders et al., 1982b). As peanuts mature, the color of the mesocarp of the peanut shell changes from yellow to orange to brown and finally to black. Sanders et al. (1982b) conducted a study concluding that the Pod Maturity Profile classification method is reproducible throughout the growing season.



Figure 2. Florrynner mesocarps (exocarp removed) of 7 maturity classes subdivided into increment stages (Williams and Drexler, 1981)

Since peanuts are indeterminate in flowering, harvest at any given time always includes a certain number of immature and mature pods. Research has shown that there is a flavor-maturity relationship in peanuts. Sanders et al. (1982b) evaluated the flavor of medium-sized peanuts from each hull scrape maturity class and demonstrated that medium grade size peanuts from mature pods have greater potential for full flavor than peanuts from immature pods. Additionally, Sanders et al. (1989b) reported that peanuts from immature classes developed more fruity fermented off-flavor and less roasted peanutty flavor than mature peanuts of the same size when all samples were cured in-shell at 16.8°C above ambient temperature. McNeill and Sanders (1996) investigated the variability in pod maturity distributions within commercial sized in-shell lots for two different years. Results indicated that there was little difference in pod size distributions in the two different years. However, it was shown that maturation continues in peanuts without an increase in pod size.

OFF-FLAVOR DEVELOPMENT IN PEANUTS

The unique flavor of roasted peanuts is the underlying basis for consumer purchase and consumption (Sanders et al., 1997). Peanut flavor fade accompanied by off-flavor development in peanuts and peanut products is of major concern to peanut manufacturers. Peanuts and peanut butter products can develop off-flavors such as painty, cardboard, and oxidized flavors (Johnsen et al., 1988). Undesirable peanut flavors are a function of variety, production environment, handling, curing temperature, moisture content, and maturity stage of the peanut seed (Pattee et al., 1965). Attaining and maintaining consistent roasted flavor and roast color are important factors in peanut manufacturing operations (Sanders et al., 1989a). Ultimately, an increase in off-flavor usually decreases roasted peanut flavor. Therefore, it is pertinent to develop ways to decrease and/or eliminate off-flavors in peanuts.

Curing temperature is one of the most important factors that contribute to off-flavor development. Dickens (1957a, 1957b) reported that for a given curing temperature, immature peanuts have more off-flavor than mature, and peanuts cured in the absence of oxygen have more off-flavor than peanuts cured in the presence of oxygen. In addition, Dickens hypothesized that anaerobic respiration occurred in peanuts cured at about 35°C and that anaerobic by-products produced off-flavor(s). In 1964, Whitaker and Dickens reported that at high rates of respiration, which occurs with high curing temperatures, oxygen does not diffuse into the peanut seed at a sufficient rate to support complete aerobic respiration and anaerobic respiration may even predominate. Butt and Kummer (1951) reported that peanuts cured at 130°F had an undesirable flavor. Additionally, Schenk (1959) reported that the rate of gas exchange in curing peanuts is inversely proportional to the curing temperature. In order to decrease off-flavor development, research studies have determined that slow drying

rates and curing temperatures below 100°F (38°C) result in acceptable peanut quality and flavor.

One of the most prominent off-flavors today is commonly known as fruity fermented (FF) (Sanders et al., 1989b). The descriptive term was added to the published peanut lexicon (Johnsen et al., 1988). Whitaker and Dickens (1964) explained fruity fermented flavor as a natural physiological phenomenon that occurs when the diffusion of oxygen across the hull and skin of the peanut into the seed is less than the oxygen requirement of the respiration rate of the seed. Sanders et al. (1989b) investigated the effect of curing temperatures on flavor profiles of peanuts in different maturity classes. Results indicated an increase in sour and bitter attributes in immature peanuts. In addition, the immature peanuts had higher fruity fermented intensities compared to mature peanuts.

ROASTING AND FLAVOR PRODUCTION

The study of peanut flavor has been an ongoing challenge since the early 1950s (Baker et al., 2003). Among the most important volatile flavor compounds found in roasted peanuts are alkylpyrazines (Magaletta and Ho, 1996). Progress has been made in the identification of many of the important compounds present in the aroma of roasted peanuts. Volatile analysis studies have produced long lists of heterocyclic and other volatile compounds in roasted peanuts (Bett and Boylston, 1992). These compounds are formed through thermal reactions such as the Maillard reaction.

Lipid oxidation contributes to undesirable flavors in peanuts in the formation of aliphatic aldehydes, ketones, and alcohols. These compounds contribute to cardboardy and painty off-flavors. Pattee et al. (1965) used gas chromatography to separate the volatile components from high-temperature-cured off-flavor peanuts resulting in eleven of 21

compounds being identified. These compounds include formaldehyde, acetaldehyde, ethanol, acetone, isobutyraldehyde, ethyl acetate, butyraldehyde, isovaleraldehyde, 2-methyl valeraldehyde, methyl butyl ketone, and hexaldehyde. Mason et al. (1969) and Johnson et al. (1971 a, b) first reported that pyrazine and carbonyl compounds were responsible for roasted peanut flavor. The roasted nutty character of peanuts was caused by the reaction of reducing sugars liberated from sucrose with free amino acids (Mason et al., 1969). This study also reported that pyrazines found in roasted peanuts can arise from the glucose, fructose, and free amino acids found in raw peanuts. Magaletta and Ho (1996) reported that (Polyhydroxyalkyl) pyrazine compounds are formed during roasting of peanuts and may be isolated from the peanut matrix using a procedure of defatting with hexane, methanol extraction, and solid-phase extraction with anion and cation exchange cartridges. In 1967, Newell et al. conducted a study determining that monosaccharides, which participate in Maillard browning reactions, are extremely important in the formation of pyrazine compounds in roasted peanuts. Furthermore, the amino acids present in peanuts are aspartic acid, glutamine, asparagines, and phenylalanine. These compounds were found to be associated with typical or desired peanut flavor, while threonine, tyrosine, lysine, and an unknown amino acid were considered precursors of atypical or undesirable peanut flavor.

The majority of the published work on flavor analysis on roasted peanuts suggests pyrazines as the components responsible for roasted peanut flavor; however, not all researchers had similar results. The environmental conditions at which peanuts are processed is important to the volatile profile obtained. Young (1973) investigated the volatiles released during roasting of peanuts cured at 110, 135, and 160°F. The results showed that mercaptans, carbon dioxide, and carbonyls increased as the temperature increased in Argentine and Early

Runner varieties. El-Banna et al. (1983) identified six oxazoles, seven thiazoles, and two 2-aminoethylpiperidine as the compounds responsible for roasted peanut flavor in Runners.

SAMPLING PLANS

There are various chemical agents that can occur in foods developed for human consumption and the detection of these factors is needed (Whitaker and Johansson, 2005). One of the major concerns of the peanut industry, and other food industries, is the presence of off-flavors as consumers are generally not accepting of differences or variations in the flavor of products (Greene et al., 2007). There is a heterogeneous distribution that exists among seeds which makes it difficult to identify a lot that has FF off-flavor.

Food manufacturers have little to no control over the variation in raw materials and obtaining an accurate measure of the agent present in a single lot is extremely important so that good lots are not rejected and bad lots are not accepted (Vandeven et al., 2002). Research studies have suggested that sampling plans can be used to determine the level of contamination in a bulk lot. Vandeven et al. (2002) reported that acceptance sampling plans are used by food manufacturers to screen out lots with unacceptable levels of contamination. Designing a sampling plan is determined by several steps: i) sampling/sample sizes, ii) sample preparation, iii) analytical test methods, and iv) accept/reject criteria (Vandeven et al., 2002; Whitaker and Johansson, 2005). In the first step, the size of the sample and how the sample is removed from the lot is determined. Sample preparation involves minimal processing of the food product (i.e. riffing, milling, grinding) prior to collecting a subsample from the bulk lot. Third, analytical procedures are used to quantify the chemical agent of interest. Lastly, the criterion are set to determine the detection threshold limit at which the lot will be accepted or rejected (contaminant concentration < accept/reject limit = accepted

and processed into food for consumption; contaminant concentration > accept/limit = rejected and is not used for consumption). In general, no plan is 100% accurate in accepting good lots and rejecting bad lots because of the variability of the contaminant in a bulk lot (Vandeven et al., 2002).

Peanuts are one of the major crops that must be screened for contamination. The USDA Marketing Agreement specifies that peanuts be inspected for aflatoxin when farmers sell to the sheller at the buying point and after the peanuts have been shelled (Whitaker et al., 2002). There are numerous research studies on the presence of aflatoxin in peanut lots using sampling plans. Whitaker et al. (2004) conducted a study to determine the variability associated with aflatoxin contamination of peanuts from plants grown in specified row lengths. Data showed that the length of the row affected the variability of aflatoxin and the authors developed a regression equation to predict the various affects. In 2002, Vandeven et al. investigated the risk of incoming acceptance plans for raw materials that may be contaminated with mycotoxins. They were able to quantify the risks for a sampling plan designed for aflatoxin in raw shelled peanuts. Knutti and Schlatter (1982) proposed several different statistical distribution models concluding that a negative binomial distribution is a good statistical model for sampling lots for aflatoxin.

The development of sampling plans to test for aflatoxin has been widely studied; however, there is little information on sampling plans for off-flavors. Due to the range of peanut maturities at optimum harvest, FF intensity can vary among the individual peanut seed. This is an indication that a FF distribution exists within a lot and among lots. The development of a sampling plan for FF off-flavor is beneficial, in that it will aid in determining the presence and level of off-flavor in the lot prior to being purchased by the

manufacturer. Operating characteristic curves (OCC) can be developed to determine the performance of the sampling plan. Fonseca (2002) updated previously published work on a sampling plan for the analysis of mycotoxins in grains and developed an OCC for in-shell and shelled peanuts and corn.

SENSORY ANALYSIS

Sensory science is a scientific discipline used to evoke, measure, and interpret the characteristics of foods and materials as perceived by the human senses (Lawless and Heymann, 1999). Sensory is used to evaluate product consistency, product/process modifications, consumer acceptability, and development of new products.

There are various sensory tools used to evaluate and characterize food products. Qualitative aspects involve aroma, appearance, flavor, texture, aftertaste, and sound properties of a product. Quantitative methods can be further subdivided into discriminative, descriptive, and affective or consumer testing (Table 1).

Table 1. Classification of Different Sensory Tests

<u>Category</u>	<u>Test Types</u>
A. Qualitative	Focus groups, interviews
B. Quantitative	
• Discriminative	Difference: paired-comparison, duo-trio, triangle, multiple sample, dual and multiple standard tests.
• Descriptive	Descriptive analysis: flavor and texture profile, quantitative, descriptive analysis.
• Affective or Consumer	Acceptance preference: 9-point hedonic scale.

Adapted from Pal et al. (1996)

Qualitative tests are used to probe consumer responses on new products, identify consumer's perspectives, and generate ideas about new products (Lawless and Heymann, 1999). Focus groups are the most common form of qualitative research which involves 10 or more consumers discussing a product concept/idea with a moderator. Quantitative analysis involves discrimination, descriptive, and affective (consumer) tests.

Discriminative. Discrimination tests are used to determine whether differences exist between two samples and they include paired comparison, duo-trio, and triangle tests.

Descriptive. Descriptive sensory analysis (DSA) involves the detection (discrimination) and description of both qualitative and quantitative sensory components of a consumer product by trained panels of judges (Meilgaard et al., 1991). This type of analysis has enabled companies to make informed decisions and create greater awareness of sensory evaluation as an integral part of product development, ingredient solution, and consumer acceptability.

There are several different methods of descriptive analysis, such as Flavor Profile®, Quantitative Descriptive Analysis (QDA), and Spectrum™. The Flavor Profile® method was developed in the 1950s by Arthur D. Little Corporation and is described as the earliest form of descriptive analysis. This method is used primarily to perceive the aroma and flavor profiles of a product, the product intensities, product aftertaste, and the order of perception (Meilgaard et al., 1999; Lawless and Heymann, 1999). The terminology used to evaluate the samples is determined by the panel. The panel of judges is composed of four to six people and they evaluate samples using a consensus technique. Therefore, one consensus ballot is generated from the panelists. The Flavor Profile method uses a 7-point numerical type category scale anchored with words to interpret the attribute intensities (Meilgaard et al., 1999). After evaluation of the attributes, the panel leader conducts a general discussion

among panelists to derive a consensus profile based on the panel responses (Stone and Sidel, 1993). The advantages of this method are the size of the panel, consistency of results, and no extensive panel training. The disadvantage of the Flavor Profile method is that panelists use a series of numbers and symbols (1+) to indicate quantitative differences; therefore, the data cannot be used for statistical interpretation.

QDA was developed by Herbert Stone and Joel Sidel at Tragon Corporation in the 1980's. In the QDA method, the panel consists of 10-12 panelists and a panel leader. The panel leader guides the panel, but does not directly participate. With the assistance of the panel leader, the panel develops a sensory language to describe the product attributes. This is done by evaluating a variety of samples and generating attributes in the order perceived. The attributes are grouped by modality (appearance, flavor, texture, etc.), and the panel discusses the terms generated, eliminating any orthogonal terms. Definitions are developed for each attribute category. Additionally, references are assigned to assist panelist in perceiving specific attributes.

In QDA, products are evaluated using a line scale and panelists are trained to be product and attribute specific. A standardized evaluation procedure is developed to evaluate the products (Chambers and Wolf, 1996; Stone and Sidel, 1993). Furthermore, group discussions, statistical analysis, and visual assessments of panelist's scores are frequently conducted to maintain the accuracy and consistency of the panel. This enables the panel leader to determine whether the panel is using the scale properly to evaluate samples and adequately differentiating between samples. The amount of panel training depends on the research, product, panelists, and the research attributes being evaluated. The data generated is quantitative and qualitative and results are displayed in a web plot. The advantages of the

QDA are the short training period (6-10 hours) and the freedom of panel language development without panel leaders influence (Lawless and Heymann, 1999). The disadvantages are that panelists are trained to be product and attribute specific, therefore if there is a new product to be evaluated the panel needs to be retrained.

The Spectrum™ method was developed by Gail V. Civille from Sensory Spectrum™ in the 1970s. This procedure consists of complete, detailed, and descriptive characterization of the sensory attributes of a product (Meilgaard et al., 1999; Munoz and Civille, 1992). The panel consists of 10-12 panelists and an actively involved panel leader. The training procedure for Spectrum™ method is similar to QDA; however, the panelists use a universal scale to evaluate products allowing the panel to rate a variety of products using the same scale; therefore, they are not trained to be product and attribute specific.

A Spectrum™ panel is trained in a variety of attribute modalities and the panel is thus capable of evaluating an array of product categories that include foods, beverages, home care, personal care, paper, skinfeel/cosmetics, and other products (Munoz and Civille, 1992; Chambers and Wolf, 1996). The panelists use a universal, numerical 15-point intensity scale in which all attributes of any product are scored on the same scale. Definitions and references are used to train a panel to use the scale identically for evaluation of sensory attributes.

The panel data is reported in histograms and a wide variety of statistical methods may be used to differentiate among products. The advantages of the Spectrum™ method are that it gives specific emphasis to both qualitative and quantitative aspects of descriptive measurement, one panel can be used to evaluate and compare a wide range of products since the language and scale are not particular to one product (Munoz and Civille, 1992). The

disadvantage is that there is a more extensive training procedure, which can be relatively expensive.

The overall goal of descriptive analysis is to train a group of people to work as an instrument. Descriptive analysis is based on language so it is extremely important that panelists are properly trained and provided with the necessary materials (definitions/references) to be a reliable source of information. Flavor lexicons are widely used to document and describe sensory perception of selected foods (Drake and Civille, 2003). One key characteristic of a good flavor lexicon is that it be discriminating and descriptive (Drake and Civille 2003). Johnsen et al. (1988) developed the peanut flavor lexicon to describe desirable and undesirable flavors in peanuts (Table 2). Sanders et al. (1989b) added FF to the lexicon due to detection of the aromatic association with overripe fruit. The flavor of peanuts is extremely important to consumer acceptability (Bett el al., 1994).

Table 2. Lexicon of Peanut Flavor Descriptors (Johnsen et al., 1988)

AROMATICS	DEFINITIONS
Roasted Peanuttty	The aromatic associated with medium-roast peanuts (about 3-4 on USDA color chips) and having fragrant character such as methylpyrazine.
Raw Bean/Peanuttty	The aromatic associated with light-roast peanuts (about 1-2 on USDA color chips) and having legume-like character (specify beans or pea if possible).
Dark Roasted Peanut	The aromatic associated with dark roasted peanuts (4 + on USDA color chips) and having very browned or toasted character.
Sweet Aromatic	The aromatics associated with sweet material such as caramel, vanilla, molasses, fruit (specify type).
Woody/Hulls/Skins	The aromatics associated with base peanut character (absence of fragrant top notes) and related to dry wood, peanut hulls, and skins.
Cardboard	The aromatic associated with somewhat oxidized fats and oils and reminiscent of cardboard.
Painty	The aromatic associated with linseed oil, oil based paint.

Table 2 continued.

Burnt	The aromatic associated with very dark roast, burnt starches, and carbohydrates, (burnt toast or espresso coffee).
Green	The aromatic associated with uncooked vegetables/grasstwigs, cis-3-hexanal.
Earthy	The aromatic associated with wet dirt and mulch.
Grainy	The aromatic associated with raw grain (bran, cod liver oil, old fish).
Fishy	The aromatic associated with trimethylamine, cod liver oil, or old fish.
Chemical/Plastic	The aromatic associated with plastic and burnt plastics.
Skunky/Mercaptan	The aromatic associated with sulfur compounds, such as mercaptan, which exhibit skunk-like character.
Fruity Fermented	The aromatic associated with overripe fruit.
TASTES	
Sweet	The taste on the tongue associated with sugars.
Sour	The taste on the tongue associated with acids.
Salty	The taste on the tongue associated with sodium ions.
Bitter	The taste on the tongue associated with bitter agents such as caffeine or quinine.
CHEMICAL FEELING FACTORS	
Astringent	The chemical feeling factor on the tongue, described as puckering/dry and associated with tannins and aluminum.
Metallic	The chemical feeling factor on the tongue described as flat, metallic, and associated with iron and copper.

DSA has been used to understand many of the sensory properties of peanut flavor. Ory et al. (1992) discussed chemical changes, instrumental, and sensory analysis of peanut off-flavors. In 1994, descriptive sensory properties were reported on Argentina, China, and USA grown peanuts (Bett et al., 1994). Results indicated that an increase in fruity fermented off-flavor caused a decrease in roasted peanutty flavor. Bett and Boylston (1992) and Pattee et al. (1999) reported that during storage, there is an increase of off-flavor development and a decrease of roasted peanutty flavor. Sanders et al. (1989b) evaluated the effects of curing temperature on descriptive flavor of peanuts of different maturity. Additionally, previous research has established that immature peanuts have lower potential for high roast intensity and high off-flavor descriptors (Sanders et al., 1989a).

Affective (consumer). Consumer tests are used to determine consumer's acceptability of a newly developed product or a product that has been modified. The most widely used scale to test consumer liking/disliking is the 9-point hedonic scale. This scale has served as the food industry's standard scale since the 1940's and is relatively easy to use. There are several consumer studies on peanut flavors. McNeill et al. (2000) developed a quantitative consumer questionnaire for peanut butter to increase the understanding of consumer language. In 2002, McNeill et al. conducted a study to report the descriptive analysis of commercially available creamy style peanut butters. The sensory characteristics displayed in each of the products and in the category as a whole increased the overall understanding of the commercial creamy peanut category. Young et al. (2005) conducted descriptive sensory analysis and consumer acceptability of peanuts from different origins. The three peanut origins were characterized by different attributes: U.S.- sweet aromatic, roasted peanut and dark roast; China- woody/hull/skins, bitter, and sour; and Argentina- musty and sweet. The results indicated that U.S. peanuts received the highest scores for overall flavor liking. Greene et al. (2006) investigated consumer perception of fruity fermented off-flavor in peanuts using two different sensory methodologies. Results indicated that fruity fermented off-flavor negatively impacted consumer acceptance of peanuts.

INSTRUMENTAL ANALYSIS

Flavor research has been an evolutionary process driven by advances in instrumentation (Reineccius, 2000). In the late 1950s and early 1960s, gas chromatography became the main tool for determining the volatile compounds contributing to flavor in various food products. Gas chromatography (GC) involves the separation of chemical compounds based on partitioning between a mobile phase and a gas phase. The use of GC

has improved the separation, isolation, and quantification of volatile compounds in food products (Reineccius, 2000). Currently, there is ongoing research to improve and maintain current extraction methods, and the development of various mass spectrometers has helped to identify volatile compounds.

Instrumental analysis can be used to determine the numerous volatile compounds present in a food. There are various techniques that can be used to isolate volatile compounds from the food matrix. Solvent extraction and headspace sampling techniques are the two main types of extraction methods used in flavor analysis. Solvent techniques are based on differences in polarity between volatile compounds and the food matrix such as, solvent assisted flavor evaporation (SAFE), simultaneous distillation extraction (SDE), and supercritical fluid extraction. These techniques are destructive and highly sensitive to less volatile compounds (high molecular weight compounds).

SAFE is a relatively new and versatile method developed by Engel et al. (1999) to extract volatile compounds from a variety of complex food matrices. This is a reliable technique because it recovers the majority of all the volatile components. SAFE provides fast and accurate isolation of volatiles from either solvent extracts of foods, oils samples, or even fruit pulps (Engel et al., 1999). In SAFE, the sample is subjected to a high vacuum and the volatile compounds are separated from other organic substances that are non-volatile (i.e. sugars, carbohydrates, proteins). This technique has been used to analyze the volatile compounds of cheese and other dairy products; however, it has not been used often with peanuts. Didzbalis et al. (2004) used SAFE and gas chromatography-olfactometry to identify the volatile compounds contributing to fruity fermented off-flavor in 27 and 40°C cured immature peanuts. Results indicated that ethyl-2-methylpropanoate, ethyl-2-

methylbutanoate, ethyl-3-methylbutanoate, hexanoic acid, butanoic acid, and 3-methylbutanoic acid are responsible for fruity fermented off-flavor in high temperature cured samples. All three esters have been found in other products, such as white and red wine, melons, grapefruit, apples, and pineapples (Bauchot et al. 1998; Buettner and Schieberle 2001; Flath et al. 1969; Rettinger et al. 1991; Guth 1997, and Kotseridis and Baumes 2000). Schirack et al. (2006) used SAFE to extract the volatiles from microwave blanched peanuts.

SDE is another technique used in flavor analysis that was developed by Nickerson and Likens (1966). The SDE apparatus involves simultaneous condensation of the steam distillate and an immiscible organic solvent. Steam distillable-solvent soluble compounds are transferred from the aqueous phase to the solvent as both liquids are continuously recycled throughout the system (Parliament, 2002). Aroma extracts are obtained quickly by this method, but the elevated temperatures may cause artifacts to form (Engel et al., 1999).

For many years supercritical fluid extraction has been used widely for dealcoholization; decaffeination of coffee and tea; processing of tobacco, hops, spices, and fats and oils from vegetable and animal sources (Werkhoff et al., 2002). Additionally, this process can be used to extract specific compounds or active ingredients from the food. The principle of high-pressure extraction with supercritical CO₂ is to separate the flavor and fragrance chemicals from complex matrices without the use of organic solvents. Leunissen et al. (1996) investigated the volatile components present in roasted peanuts using supercritical fluid extraction and gas chromatography/mass spectrometry (GC/MS). The results indicated that roasting at high temperatures produces a wide range of chemicals in peanuts due to Maillard reactions and methylpyrazine at low concentrations is associated with good roasted flavor.

Headspace techniques are based on differences in volatility between volatile compounds and the food matrix. Solid phase microextraction (SPME), dynamic headspace, and static headspace are the principle headspace techniques used in flavor analysis. These procedures are nondestructive and sensitive to highly volatile compounds (low molecular weight compounds). Pattee et al. (1990) investigated the volatiles present in market grades of runner-type peanuts subjected to high temperatures using headspace volatile concentration (HSVC) test. The results indicated that the headspace volatile concentrations were higher in the large-seed marketing grades (jumbo) compared to the small-seed grades (other edibles). This suggests that larger seeds are generally more resistant to high temperature damage. Johnson et al. (1971 a,b) used a headspace vacuum degassing system to evaluate the volatile compounds found in the neutral and basic fraction in roasted peanuts. Nineteen alkylpyrazines, seven furans, six pyrroles, three 2-phenyl-2-alkenals, two thiophenes, and some miscellaneous compounds were identified.

SPME is a rapid, solventless extraction of volatile and semi-volatile compounds. The sample is placed in a vial sealed with a septum-type cap. A needle is inserted into the headspace of a liquid or solid sample and the fiber is lowered into the headspace. For a certain amount of time, the volatiles are absorbed onto the fiber. After adsorption and equilibrium is reached between the sample and headspace, the volatiles are desorbed from the fiber into the GC for analysis. SPME has been used to analyze dairy and peanut products. Williams et al. (2006) used SPME to study the volatile changes in roasted peanuts during short-term storage. They reported significant decreases in concentrations of 2-methoxypyrazine, 2,3-dimethylpyrazine, 2-ethyl-3-methylpyrazine, and 2,3,5-trimethylpyrazine occurred with storage time. There was a significant increase in hexanal concentration over

time indicating lipid oxidation. Baker et al. (2003) determined the pyrazine and flavor variations in peanut genotypes during roasting. Descriptive sensory results indicated that 2,5-dimethylpyrazine was most highly correlated to roasted peanut flavor and aroma. SPME techniques were used by Powell et al. (2005) to identify the volatile compounds responsible for fresh roasted peanut flavor. Ethylpyrazine, 2-methylpyrazine, 2-ethyl-3-methylpyrazine, 2,3-diethylpyrazine, and 2,3,5-trimethylpyrazine were found to be the key compounds associated with roasted peanut flavor. Coleman and Dube (2005) investigated the volatile compounds in chocolate, pears, peaches, caramel, ginger, and cinnamon using SPME.

Static and dynamic (purge and trap) are headspace techniques that have the same fundamental principle. In static headspace techniques, the sample is placed in a non-agitated vial. The headspace is sampled and injected on a GC. Dynamic headspace involves purging the sample with a gas (nitrogen) to move and concentrate volatile compounds in a trap. After equilibrium is reached, a sample from a trap is injected on the GC. Both of these methods are useful for various food products.

Bett et al. (1994) compared the flavor quality, volatiles, and proximate composition of peanuts from USA, China, and Argentina using sensory analysis and dynamic headspace analysis. Results indicated that the U.S. had better roasted peanut flavor and were sweeter than China and Argentina. Chinese peanuts had more cardboardy off-flavors while Argentina peanuts had fruity fermented off-flavor in conjunction with more volatiles associated with off-flavors such as hexanal and pentanal. Among these peanuts there were differences in flavor qualities of peanuts from various countries each year. In general, China and Argentina had higher total volatiles compared to the U.S. Vercellotti et al. (1992) used purge and trap at 60 and 127°C temperatures to study the volatiles present in commercial

sized peanuts. Results indicated that different sized peanuts had different volatile profiles. Braddock et al. (1995) compared the flavor and oxidative stability of normal and high oleic peanuts using dynamic headspace and sensory analysis. Results indicated that the most potent compounds were hexanal, methypyrazine, 2,5-dimethylpyrazine, and nonanal. Park (1993) investigated the loss and structural alterations of unsaturated aldehydes from lipid oxidation using purge and trap. Wellnitz-Ruen (1982) used headspace a purge and trap technique to analyze the presence of ethyl butyrate and ethyl hexanoate which cause fruity off-flavor in milk. Krist et al. (2004) evaluated the volatile compounds in Italian chestnuts using SPME analysis. Pentane, hexane, heptane, and butane were some of the aroma impact compounds identified.

RELATING SENSORY AND INSTRUMENTAL ANALYSES

The use of flavor related instrumental analysis alone has produced long lists of volatile compounds (Blank, 2002). However, the aroma/flavor of a food consists of many volatile compounds and only a few are sensorially relevant (Blank, 2002). Establishing links between the sensory perceptions of flavor and instrumental measurements provides an enhanced understanding of flavor. There are several reasons why flavor characterization is important. For instance, the ability to identify volatile compounds contributing to a specific off-flavor can aid in manipulation of processing parameters that prevent formation of those compound(s). The use of sensory and instrumental analyses can be used to develop artificial flavors that can be used in various food products. Additionally, product stability or variability of a food product can be evaluated using sensory and instrumental techniques to characterize flavor and chemical formation over time.

There are two basic requirements for establishing links between the chemical nature and structure of the principal flavor constituents: i) descriptive sensory analysis with a defined and anchored lexicon and ii) instrumental volatile analysis. GC in combination with olfactometry techniques is valuable for selecting those aroma-active compounds that potentially play a role in flavor. Research studies have shown that some compounds with very low thresholds may not be detected by GC, but gas chromatography-olfactometry (GC-O) may result in odor detection because of the sensitivity of the human nose. GC-O is a semi-quantitative technique that helps provide links between GC data and the compounds that may contribute to flavor. GC-O involves an extract or distilled sample from the food matrix which is injected onto a GC that has been modified with an olfactometer at the detector (Figure 3). A trained panelist (sniffer or human detector) sniffs compounds eluting from the GC and provides qualitative and quantitative data on what they smell.

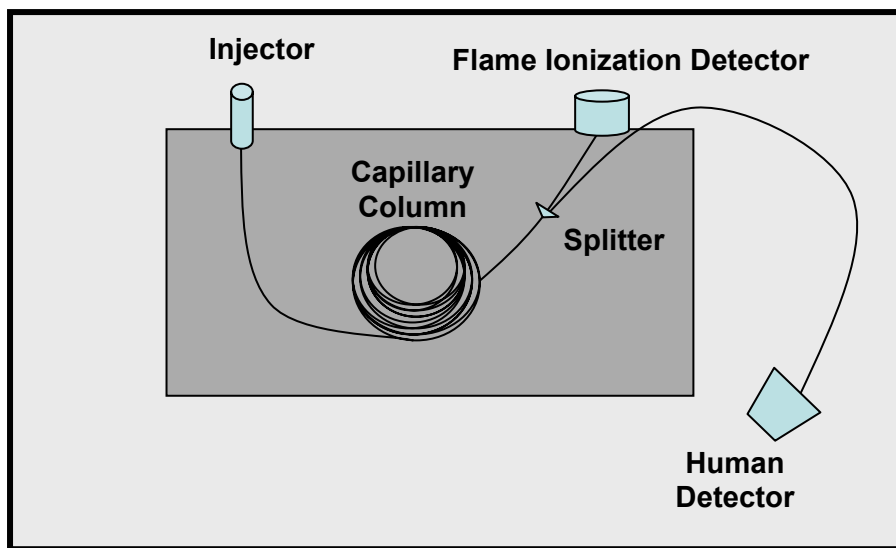


Figure 3. Gas Chromatography-Olfactometry

The qualitative data is produced when the sniffer describes the nature of their perception with a word or group of words. It is difficult to determine the sensory relevance of volatiles from a single GC-O chromatogram; therefore, several techniques have been developed to detect the

most potent odorants (Blank, 2002). These techniques include: Postpeak intensity, Nasal Impact Frequency (NIF), Time-intensity, and Dilution techniques which include CharmAnalysis™ and Aroma Extract Dilution Analysis (AEDA). Postpeak intensity is used as a screening technique for aroma-active compounds. This method involves two or more experienced sniffers that sniff the extracts as the compounds elute from the column. Sniffers record the retention time, odor characteristic, and intensity of compounds as they elute. NIF is also used to determine the most potent aroma-active compounds. Six to ten untrained sniffers verbally describe the aroma as the extracts elute from the GC. The data is recorded as the number of people that detected a specific odor. The principle of this method is that the more people that detect an odor, the more potential that a compound(s) has in flavor. Time-intensity techniques involve two or more experienced sniffers who verbally describe the intensity of an odor using a lever (slide bar). The higher the intensity of the odor, the more likely it plays a role in flavor.

Dilution techniques are powerful tools to determine the key compounds contributing to a flavor. CharmAnalysis™ was developed by Acree et al. (1993). In this method, serial dilutions of a sample are made and presented in a randomized order. The sniffer detects the beginning and end of the odor perceived (duration of smell). The dilution value is measured over the entire time of the eluting peak (Blank, 2002). A Charm value is calculated (sum peak areas of dilution factor / duration of odor response) and presented in a Charm chromatogram. Grosch (1993) developed Aroma Extract Dilution Analysis (AEDA). In this technique, the extract is diluted and injecting on the GC to determine the aroma-active compounds present. The sample is serially diluted and sniffed until nothing is smelled. Flavor dilution (FD) factors, the highest dilution at which a compound is smelled, are

determined for each compound. The compounds with the highest FD factors are presumed to be the compounds that are most important to the flavor. The major difference between CharmAnalysis™ and AEDA is that Charm data represents the dilution value over a period of time whereas AEDA determines the highest dilution at which value an odor is detected. Both analyses are comparable because they express the relative number of dilutions until an odor is no longer detectable by the sniffer (Friedrich and Acree, 1998a). It is important to remember that GC-O is not quantitative and does not prove that the compound(s) is present above sensory threshold; however, it is a extremely powerful when used in conjunction with other techniques.

Peanut flavor is very complex and is comprised of numerous volatile and non-volatile compounds (Young et al., 2005). The majority of volatile analysis on peanuts has been done using headspace techniques. There is limited information available using solvent extraction to isolate volatiles from peanuts. In 1994, Bett et al. compared the volatiles and sensory quality of USA, China, and Argentina peanuts using headspace techniques. Results indicated that peanuts from the USA had better roasted peanut flavor quality compared to the other origins. Alasalvar et al. (2004) compared the volatile composition of five Turkish hazelnut varieties using dynamic headspace, electronic-nose, and descriptive sensory analysis. A total of 46 volatile headspace compounds were identified: 11 ketones, 8 aldehydes, 6 alcohols, 6 aromatic hydrocarbons, 4 furans, 1 pyrazine, and 1 miscellaneous. Among these compounds, there were large differences from each variety. The relationships between pyrazines and aldehydes in peanuts throughout storage were investigated by Warner et al. (1996) using instrumental analyses. Results indicated that headspace concentrations increased during storage for hexanal, heptanal, octanal, and nonanal while pyrazines remained constant. Four

pyrazines were quantified and identified as contributing to fresh peanut flavor and aroma. These results suggest that the flavor-fade association with roasted peanuts was due to masking of pyrazines and other roasted peanut flavor compounds by large quantities of low-molecular weight aldehydes produced during lipid oxidation. The sensory results indicated differences in roasted peanut, green, and rancid flavors. Matsui et al. (1998) investigated the potent odorants of peanuts, hazelnuts, and pumpkin seeds using AEDA and GC-O in headspace samples. There were a total of 38 compounds in peanuts, 28 compounds in hazelnuts, and 27 compounds in pumpkin seeds using dilution analyses. Results indicated that 2-ethyl-3,5-dimethylpyrazine, 2,3-diethyl-5-methylpyrazine, 2-methylpropanal, 3-methylbutanal, (E,Z)- or (E,E)-2,4-decadienal were the potent odors in the three different types of seeds. Sensory analysis was not used to establish links between compounds and sensory perception.

Bett and Boylston (1992) reported that alkylpyrazines were primarily responsible for the roasted peanut attribute and Warner et al. (1996) reported that pyrazines influence peanut flavor. In both studies, the result suggested that the acceptability of the overall product is influenced by the presence of pyrazines. Crippen et al. (1992) used GC in an attempt to determine which compounds are responsible for roasted peanut flavor. Results indicated that pyrazines and some other compounds, such as methylbutanal and methylpropanal correlated highly with dark roasted peanuts. Ory et al. (1992) used GC data in a discussion of the chemical changes, instrumental, and sensory analysis of off-flavors. They identified acetic acid sulfur-containing methanethiol, dimethylsulfide, and dimethyldisulfide as compounds occasionally found in off-flavored peanuts.

Young and Hovis (1990) evaluated the change in volatile profiles in large numbers of peanut samples using GC-MS. These results indicated higher concentrations of n-methyl pyrrole in peanut samples, and a descriptive panel described n-methyl pyrrole as having a musty flavor. Singleton et al. (1971) cured peanuts at 22, 32, 45, and 50°C and evaluated the volatile compounds present. Peak ratios were used in conjunction with sensory evaluation to correlate instrumental data to curing temperature of peanuts. Acetaldehyde, ethanol, and ethyl acetate concentrations increased as curing temperature increased. Ethyl acetate was not present in 22, 32, and 45°C treatments, so the results suggest that ethyl acetate can serve as an indicator of high temperature curing. Sensory results indicated a higher intensity of off-flavor in higher curing temperatures. Burrioni et al. (1997) characterized volatile compounds of raw, roasted, and fried peanuts. The volatile components tentatively identified were hexanal, 1-methylpyrrole, cyclobutanol, 4-ethyl-2,5- dimethylisoxazolidine, 1-hexanol, 2,6-dimethylpyrazine, and acetic acid. Hexanal and 1-methylpyrrole were present in higher concentrations followed by acetic acid in the three types of peanuts. Bett et al. (1994) investigated the quality of several peanut origins using GC-MS and sensory analysis. The results indicated that desirable flavor compounds (pyrazines) do not increase much after roasting where undesirable flavor compounds (carbonyls) increase with storage.

Over the years, GC-O has become a popular tool in determining the specific compounds related to flavors in a wide variety of products. Of the numerous research studies that have used GC-O to identify compounds responsible for a flavor or off-flavor; few describe research studies using GC-O to identify the compounds responsible for flavors in peanuts. Didzbalis et al. (2004) used SAFE, GC-O, and descriptive analysis to investigate the compounds responsible for fruity fermented off-flavor. Results indicated that ethyl-2-

methylpropanoate, ethyl-2-methylbutanoate, and ethyl-3-methylbutanoate as the esters responsible for fruity fermented off-flavor in artificially created peanuts. Schirack et al. (2006) investigated the off-flavor developed in high temperature microwave blanched peanuts. Their results indicated that increased concentrations of phenylacetaldehyde, guaiacol, and 2,6-dimethylpyrazine were responsible for the stale/floral and ashy notes.

MODEL SYSTEM STUDIES

The subsequent steps in relating instrumental data to sensory perception in a food involve sensory analysis, threshold analysis, and the use of model systems or the addition of a suspected flavor compound directly to the food (Drake and Civille, 2003). Sensory threshold analysis is conducted on the key aroma active compounds that have been identified and quantified using instrumental analysis (Drake and Civille, 2003). Prior to model system studies, the odor activity values are determined and evaluated for significance. The ratio of the concentration of a compound to its odor threshold is expressed as the odor active value (OAV) (Drake and Civille, 2003). The OAV is considered an accurate determination of a compounds contribution to perceived flavor (Preininger and Grosch, 1994; Friedrich and Acree, 1998a).

Model system studies involve the addition of volatile compound(s) to a matrix of the particular food. The model is presented to a descriptive panel to confirm whether the compounds identified play a key role in the perceived flavor. There are numerous studies that have identified the volatile compounds responsible for specific flavor in dairy products using instrumental and sensory model systems; however, the use of model systems to evaluate aroma active compounds in off-flavored peanuts is limited. Caranchia-Whestine et al. (2003) identified >80 aroma compounds in fresh Chevre-style goat cheese including

ketones, alcohols, aldehydes, fatty acids, and furans. Sensory and model systems confirmed 4-methyl and 4-ethyloctanoic acids were responsible for waxy/animal flavor. Karagul-Yuceer et al. (2002) evaluated the chemical and sensory profiles of stored nonfat dry milk. AEDA results identified 3-(methylthio)propanal, o-aminoacetophenone, 2,5-dimethyl-4-hydroxy-3(2H)-furanone and 2-methyl-3-hydroxy-4H-pyran-4-one, butanoic acid, pentanoic acid, and hexanoic acid as having a significant role in nonfat dry milk flavor throughout storage. Sensory results indicated that the presence of these compounds in milk powder caused higher intensities of astringency. In 2003, Karagul-Yuceer et al. reported information on the predominant odorants responsible for the typical odor of rennet casein powder. AEDA results indicated o-aminoacetophenone to be the most potent odor; however, model system studies identified hexanoic acid, indole, guaiacol, and p-cresol as the key compounds responsible for stale flavor in rennet casein powder. Avsar et al. (2004) found that Strecker aldehydes in aged Cheddar cheese are responsible for nutty flavor in Cheddar cheese. Cheese models with 2/3-methylbutanal and 2-methylpropanal confirmed that these compounds were responsible for nutty flavor in aged Cheddar cheese. Carunchia-Whestine et al. (2005) identified phenylacetaldehyde and phenylacetic acid as the compounds responsible for rosy/floral flavor in cheddar cheese using model systems.

There is minimal research using model systems to confirm the volatile compounds responsible for off-flavors in peanuts. Didzbalis et al. (2004) used model systems to confirm if ethyl-2-methylpropanoate, ethyl-2-methylbutanoate, and ethyl-3-methylbutanoate and three acids were contributing to FF off-flavor. Schirack et al. (2006) conducted model system studies to confirm the compounds responsible for microwave blanched peanuts. Sensory results indicated that the combination of phenylacetaldehyde, guaiacol, and 2,6-

dimethylpyrazine closely mimicked the off-flavor in microwave blanched peanuts. The use of model systems is a powerful technique to confirm the role of specific compounds on flavor.

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CHAPTER 3

CHARACTERIZATION OF FRUITY FERMENTED PEANUT LOTS USING DESCRIPTIVE SENSORY ANALYSIS

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ABSTRACT

Fruity fermented (FF) off-flavor has been described in peanuts cured at excessively high ($>35^{\circ}\text{C}$) temperatures. Roasted peanut flavor (RPF) is the main factor that drives consumer purchase and consumption of peanuts and/or peanut products. Previous studies have indicated that RPF and FF off-flavor are inversely proportional which causes a negative impact on overall flavor and consumer acceptability. Descriptive sensory analysis is a powerful tool used in sensory science to identify and quantify peanut flavors. The objective of this study was to characterize the peanut flavor attributes in various FF lots that range in FF off-flavor intensities. Twenty, one ton bags of medium grade-size runner type peanuts were obtained from a commercial sheller. Twenty, 680 g (1.5 lb) subsamples were obtained by riffle-dividing each lot. The 400 samples were roasted at 350°C for 12 min. to obtain a Hunter L value of 50 ± 1 and further processed into paste using a standard protocol. The peanut pastes were randomly evaluated by a highly trained sensory panel. Sensory results indicated that there is a negative correlation between RPF and FF off-flavor. The peanut lots with high RPF had low FF intensities and high sweetness. Principal component analysis (PCA) was conducted to characterize the peanut flavor attributes within each lot. There were many differences in the intensities of flavors from lot to lot and the results confirmed that there is a relationship between RPF and FF off-flavor.

INTRODUCTION

The driving force for consumer purchase and consumption for peanuts is roasted peanut flavor and improper handling, processing, and storage of peanuts can affect this desirable flavor. One of the most common flavor issues in peanuts is the development of fruity fermented (FF) off-flavor. FF is a descriptive term used in sensory science to describe this off-flavor. It is defined as an aromatic associated with overripe fruit. The development of this off-flavor is acquired during high ($>35^{\circ}\text{C}$) temperature curing in the drying windrows or wagons.

Previous research has indicated that the presence of FF off-flavor negatively impacts consumer acceptance (Greene *et al.*, 2006). The indeterminate flowering pattern of the peanut plant produces a range of maturities on any harvest date. Immature peanuts are more susceptible to FF off-flavor and thus can affect the occurrence of FF off-flavor within a lot. Immature peanuts tend to roast darker, have more FF off-flavor, and a faster flavor fade throughout storage (Sanders *et al.*, 1989). Mature peanuts tend to roast lighter, have less FF off-flavor, and slower flavor fade (Sanders *et al.*, 1989). Due to the range of maturities and variation of FF off-flavor from seed to seed, a distribution of seed with FF exists in a single lot making it difficult to obtain an accurate determination of FF intensity in a lot (Greene *et al.*, 2007; Whitaker *et al.*, 2007). Roasted peanut flavor and FF off-flavor have been shown to be indirectly proportional and the presence and/or intensity of other peanut flavor attributes maybe influenced by FF off-flavor (Pattee *et al.*, 1990; Pattee and Giesbrecht, 1994; Pattee *et al.*, 1999).

Sensory analysis is used to evaluate the various attributes of food products. More specifically, descriptive sensory analysis is used to identify and quantify flavor attributes.

Flavor lexicons are used to document and describe the sensory perception of a certain food product. Johnsen *et al.* (1988) developed the peanut lexicon to describe the various flavor attributes in peanuts. In 1989, Sanders *et al.* added FF to the peanut lexicon. Bett and Boylston (1992) conducted a storage study on roasted peanuts and reported a decrease in roasted peanut flavor as off-flavors such as cardboardy, painty, and fruity fermented increased. Bett *et al.* (1994) investigated the sensory perception of peanuts from different origins: Argentina, China, and USA. Results indicated that Argentina peanuts had more FF off-flavor and had greater levels of off-flavor related compounds. Peanuts from China were characterized as cardboardy while the USA grown peanuts were significantly higher in roasted peanut flavor and sweet taste. Additionally, Young *et al.* (2005) characterized the Argentina, China, and USA peanuts origins using descriptive sensory analysis and consumer evaluation. The results indicated that Argentina peanuts were musty and sweet, Chinese peanuts exhibited woody/hull/skins, bitter, and sour flavor, and peanuts from the USA had the highest roasted peanut flavor intensity.

There are many flavor descriptors in peanuts and the presence and intensity of FF off-flavor may affect other attributes. To date, there are no studies that have characterized the flavor differences among the attributes in FF lots. The objective of this study was to examine the relationship of FF and other flavor descriptors over a range of FF off-flavor intensities.

MATERIALS AND METHODS

Peanut Samples. A total of twenty, one ton bags of medium grade-size runner type peanuts were obtained from a commercial sheller. The twenty peanut lots were identified by a single commercial analysis as having a range of fruity fermented off-flavor from 0 to 4 intensities.

Each peanut lot was riffle divided to obtain 20, 1.5 lb (680g) subsamples. The 400 samples (20 lots x 20 subsamples) were roasted at 350°C for ca. 12 min using an Aeroglide bench-top roaster to obtain a roast color Hunter L value of 50±1. Roasted peanuts were cooled using forced ambient air and seed coats were manually removed. After blanching, the roasted peanuts were ground into a paste using a Cuisinart Little Pro Plus Food Processor (Cuisinart, East Windsor, NJ) following the procedures of Sanders et al. (1989). This procedure consists of two 2-min grinds separated by 30s cooling intervals. The peanut pastes were stored at -40°C until evaluated by a descriptive sensory panel.

Descriptive Sensory Panel. Prior to sensory analysis, the peanut samples were tempered to room temperature (22°C). A descriptive sensory panel of twelve panelists with over 1000 h of training on peanut flavor evaluated the 400 peanut pastes. Samples were evaluated using a completely randomized design (CRD). Seven pastes were evaluated at each panel session. Panelists rinsed their mouths with water and cleansed their palates with unsalted crackers between each sample. Panelists evaluated the samples in duplicate.

Statistical Analysis. Analysis of Variance with Fisher's LSD was used to determine differences among means in peanuts lots. Pearson's correlations were conducted to determine correlations between flavor attributes. Principal Component Analysis (PCA) was used to characterize differences of peanut attributes within different peanut lots.

RESULTS AND DISCUSSION

Sensory differences occurred among the peanut lots (Table 1). Roast peanutty flavor (RPF) intensity ranged from 4.2 - 4.8 for the 20 different lots. Lot 1075 had the highest RPF intensity at 4.8 and was significantly ($P<0.05$) different from most of the other 20 peanut lots. The lots with the lowest FF intensities were Lot 1075, 2816, and 2821 at 0.3, 0.2, and

0.2, respectively and these lots had the highest RPF intensities among the 20 lots. On the contrary, Lot 1040 had the lowest RPF at 4.2 and the highest FF off-flavor intensity at 2.2 and was significantly ($P < 0.05$) different than other samples except 1039. This trend is in agreement with previously published data that reports that as FF intensity increases RPF decreases throughout storage (Pattee et al., 1990; Pattee and Giesbrecht, 1994; Pattee et al., 1999). The inverse relationship between RPF and FF among the 20 peanut lots was determined using the Pearson's correlation coefficient, and the results indicated a significant ($P < 0.05$) reduction in RPF as FF off-flavor increased ($R^2 = -0.23$) among all twenty lots and subsamples. Three individual peanuts lots were significantly different and there was an inverse relationship between RPF and FF for Lot 1035 ($R^2 = -0.46$), Lot 1065 ($R^2 = -0.52$), and Lot 1067 ($R^2 = -0.54$).

Previous research has indicated that heterocyclic nitrogen compounds (pyrazines) are responsible for roasted peanut flavor and these compounds decrease in concentration throughout storage (Mason et al., 1966; Newell et al., 1967; Singleton et al., 1971; Young et al., 1973; Warner et al., 1996; Williams et al., 2006). The inverse relationship between RPF and FF off-flavor may be due to a decrease in pyrazine concentrations, an imbalance of the key compounds contributing to roasted peanut flavor, or aldehyde masking of compounds responsible for roasted peanut flavor (Dimick, 1994; Braddock et al., 1995; Williams et al., 2006). Although the samples in this study were not subjected to long storage conditions, the results indicated a similar relationship between RPF and FF off-flavor as reported in previous studies.

Due to the fact that immature peanuts have higher sugar content and are more susceptible to developing off-flavors, there may be a relationship between the sweet

aromatic, sweet taste, and FF off-flavor. The sweet aromatic (SA) intensity ranged from 3.0-3.2 for the 20 peanut lots. Sweet taste (ST) of the 20 lots ranged from 2.4 - 2.7 with Lot 1020, 1064, 1065, and 1075 with the highest sweet taste intensities. Overall, the lots with the highest sweetness scores had low FF off-flavor intensities; therefore this suggests that there is no relationship between sugar content and FF off-flavor.

Principal component biplots of Lot 2816, 1040, and 1087 are shown in Figures 1-3. The biplots of the remaining 17 lots are located in the appendix 1-17. Figure 1 is the cluster analysis of the 20 subsamples from Lot 2816 (FF intensity = 0). PC1 makes up 39% variability and is composed of SA, ST, woody/hull/skins (WHS), and FF. PC2 (22% variability) was composed of RPF, raw beany (RB), and DR. In Lot 2816, subsamples 10, 11, 13, and 19 were characterized by SA and ST. Subsamples 1 and 17 were characterized by RPF and subsamples 6 and 14 were characterized by FF off-flavor. RPF and FF off-flavor were negatively correlated to each other in Lot 2816 which further validates the relationship between the two flavor attributes. Lot 1040 had the highest FF intensity of 2.2 and there was variation among the 20 subsamples. PC1 (33% variability) was composed of ST, SA, FF, and RPF. PC2 (25% variability) was characterized by RB, WHS, and DR. Although the majority of the subsamples had FF intensity of 2 and above, they were characterized by other attributes such as RPF, WHS, and DR. The FF intensity of Lot 1087 was 1.1 with a RPF intensity of 4.5. The total explained variability was 64% with FF, ST, and SA represented on PC1 (45%) and the PC2 (19%) was composed of RPF and RB. There is variability that exists from lot to lot and within a lot. PCA illustrated that the subsamples from different lots are characterized by various attributes even though the majority of the lots are FF.

Table 1. Sensory scores for peanut flavor attributes in fruity fermented lots

Lot	Roasted peanutty	Sweet aromatic	Dark roast	Raw beany	Woody/Hull/ Skins	Sweet Taste	Bitter	Astringency	Fruity fermented
1020	4.7 ^{ab}	3.1 ^{bcd}	3.6 ^{defg}	1.3 ^{bc}	3.0 ^{ghi}	2.7 ^{abcd}	3.1 ^{defg}	1.1 ^{ab}	1.1 ^{defg}
1022	4.5 ^{defg}	3.0 ^{cd}	3.6 ^{defgh}	1.2 ^{bc}	3.2 ^{bcd}	2.4 ^g	3.3 ^{ab}	1.0 ^b	0.9 ^{fgh}
1034	4.4 ^{fgh}	3.0 ^{cd}	3.9 ^{ab}	0.8 ^{de}	3.2 ^{bcd}	2.5 ^{abcde}	3.4 ^{ab}	1.0 ^b	1.1 ^{defg}
1035	4.5 ^{defgh}	3.0 ^{cd}	3.5 ^{defgh}	1.3 ^{bc}	3.2 ^{bcd}	2.5 ^{cdef}	3.2 ^{abcd}	1.0 ^b	1.0 ^{efg}
1036	4.5 ^{cd}	3.0 ^{ab}	3.6 ^{cd}	1.2 ^{ab}	3.2 ^a	2.5 ^{ab}	3.1 ^{abc}	1.0 ^b	1.1 ^{cde}
1039	4.3 ^{hi}	3.1 ^{bcd}	3.6 ^{cde}	1.1 ^{cd}	3.2 ^{bcd}	2.6 ^{abcd}	3.2 ^{bcde}	1.0 ^b	2.1 ^a
1040	4.2 ⁱ	3.0 ^{cd}	3.7 ^{cde}	1.1 ^{cd}	3.2 ^{ab}	2.6 ^{abcde}	3.2 ^{cdef}	1.0 ^b	2.2 ^a
1041	4.5 ^{de}	3.1 ^{abcd}	3.2 ^j	1.6 ^a	3.1 ^{efghi}	2.6 ^{abcde}	3.0 ^{fgh}	1.0 ^b	1.2 ^{cdef}
1063	4.4 ^{gh}	3.0 ^d	3.9 ^{ab}	0.9 ^{de}	3.2 ^{abc}	2.5 ^{defg}	3.2 ^{cdefg}	1.0 ^b	1.4 ^{bcd}
1064	4.5 ^{de}	3.0 ^{cd}	3.6 ^{cdef}	1.2 ^{bc}	3.0 ⁱ	2.7 ^{abc}	3.1 ^{efgh}	1.1 ^{ab}	1.7 ^b
1065	4.5 ^{de}	3.1 ^{abc}	3.4 ^{ghi}	1.4 ^{ab}	3.1 ^{cdefgh}	2.7 ^{ab}	3.1 ^{efgh}	1.0 ^b	1.3 ^{cde}
1066	4.4 ^{gh}	3.1 ^{abcd}	3.7 ^{bc}	0.9 ^{de}	3.2 ^{bcde}	2.5 ^{bcd}	3.2 ^{abcd}	1.0 ^b	1.1 ^{cdefg}
1067	4.6 ^{bc}	3.1 ^{bcd}	3.4 ^{hij}	1.5 ^{ab}	3.0 ^{hi}	2.6 ^{abcde}	2.9 ^h	1.1 ^b	1.6 ^{bc}
1075	4.8 ^a	3.2 ^{ab}	3.3 ^{ij}	1.6 ^a	3.1 ^{defghi}	2.7 ^a	3.0 ^{fgh}	1.0 ^b	0.3 ^{ij}
1086	4.6 ^{bc}	3.1 ^{abcd}	3.5 ^{efgh}	1.4 ^{ab}	3.2 ^{bcd}	2.6 ^{abcde}	3.2 ^{cdefg}	1.0 ^b	0.6 ^{hi}
1087	4.5 ^{cde}	3.1 ^{abcd}	3.5 ^{defgh}	1.3 ^b	3.1 ^{bcd}	2.5 ^{efg}	3.3 ^{abcd}	1.0 ^b	1.1 ^{defg}
1093	4.4 ^{efg}	3.0 ^{cd}	3.9 ^a	0.8 ^e	3.2 ^{abc}	2.4 ^{f^g}	3.4 ^a	1.0 ^b	0.8 ^{gh}
2816	4.7 ^{ab}	3.2 ^a	3.4 ^{fghi}	1.4 ^{ab}	3.1 ^{bcd}	2.6 ^{abcde}	3.1 ^{defg}	1.0 ^b	0.2 ^j
2821	4.7 ^{efg}	3.2 ^{bcd}	3.4 ^{defgh}	1.4 ^{bc}	3.1 ^{bcd}	2.6 ^{efg}	3.1 ^{abcd}	1.0 ^b	0.2 ^{defg}
6363	4.5 ^{def}	3.0 ^{cd}	3.5 ^{defgh}	1.3 ^{bc}	3.1 ^{fghi}	2.5 ^{defg}	3.0 ^{gh}	1.0 ^b	1.0 ^{efg}

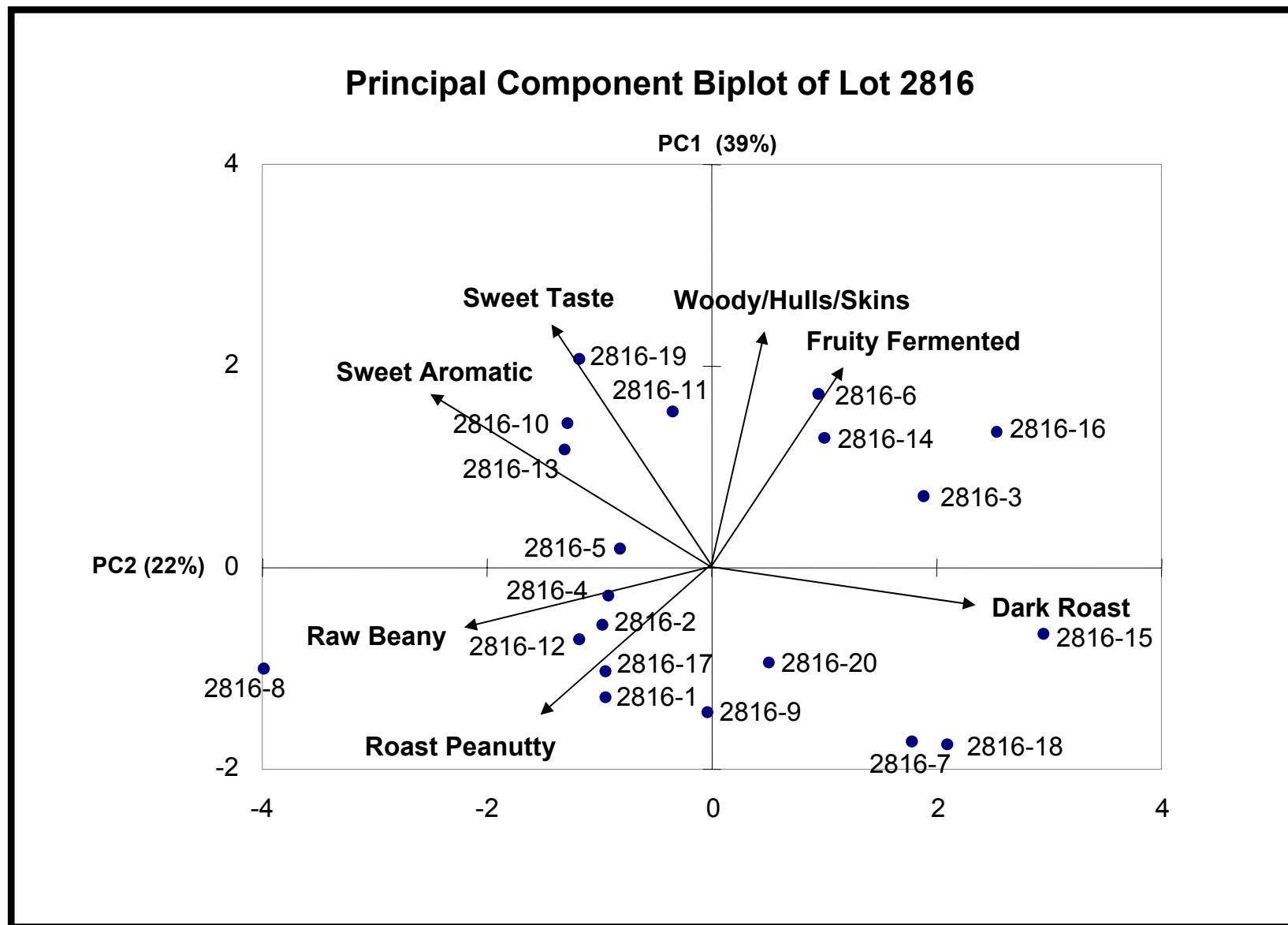


Figure 1. Principal Component Biplot of Lot 2816

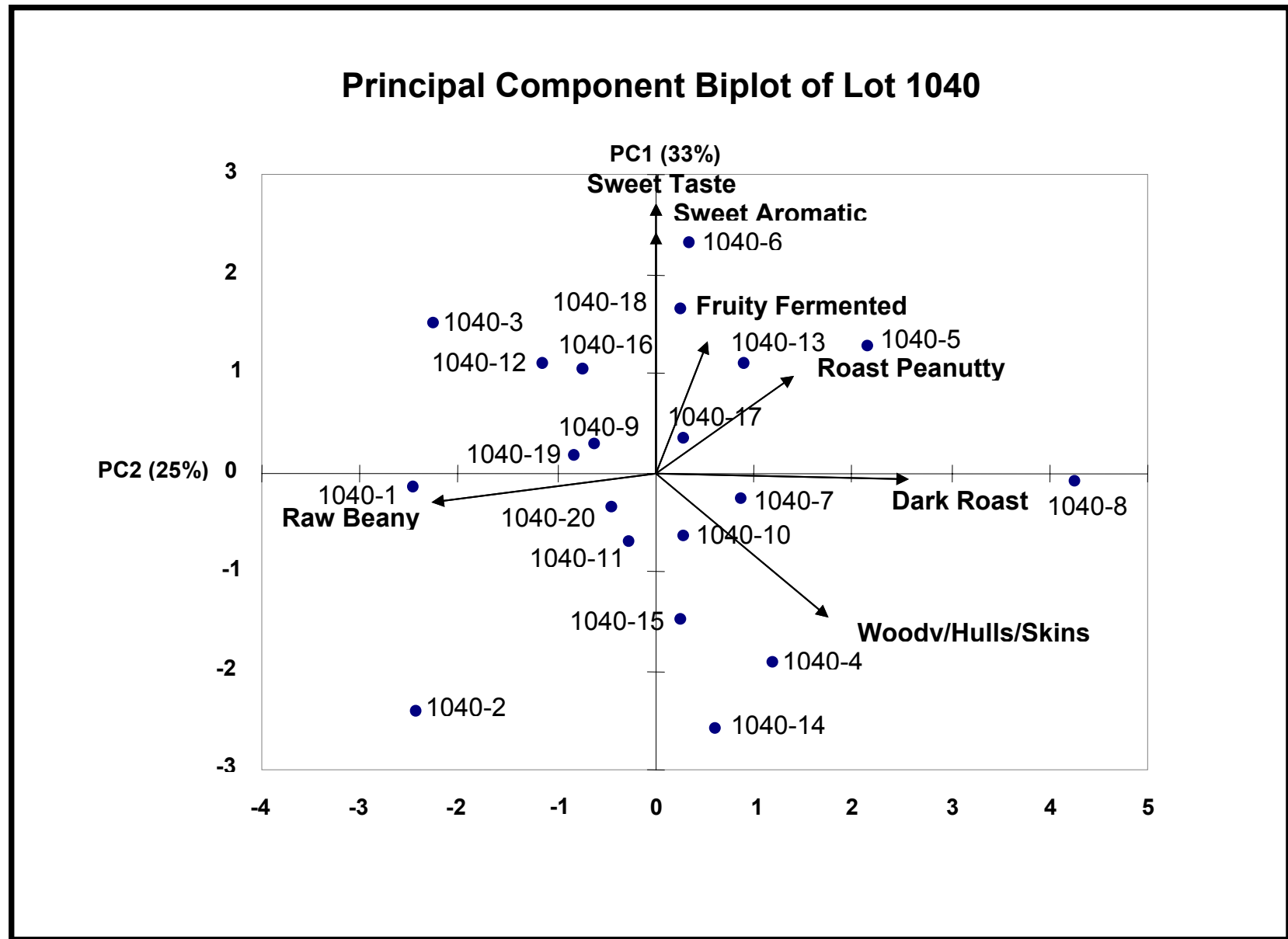


Figure 1. Principal Component Biplot of Lot 1040

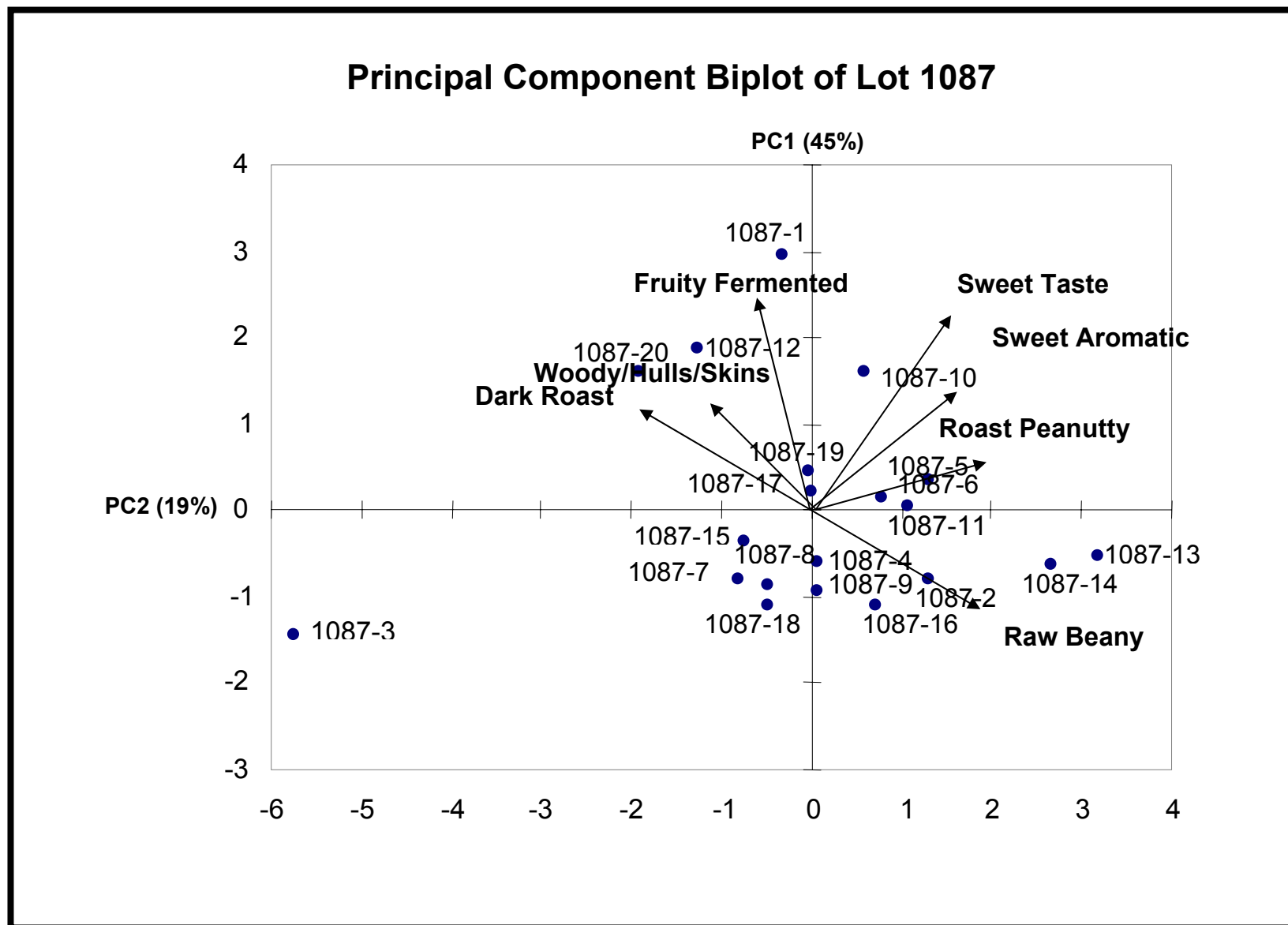


Figure 1. Principal Component Biplot of Lot 1087

CONCLUSIONS

There were differences seen among the various fruity fermented lots. Roast peanutty flavor and fruity fermented off-flavor were shown to have an inverse relationship, in that the lots with high roast peanutty flavor had lower fruity fermented intensities. Principal component analysis indicated that there is a lot of variability among the subsamples within a single lot making it difficult to determine the correct fruity fermented off-flavor intensity of the bulk lot.

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CHAPTER 4

EFFECTIVENESS OF CATEGORY AND LINE SCALES TO CHARACTERIZE CONSUMER PERCEPTION OF FRUITY FERMENTED FLAVOR IN PEANUTS

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EFFECTIVENESS OF CATEGORY AND LINE SCALES TO CHARACTERIZE CONSUMER PERCEPTION OF FRUITY FERMENTED FLAVOR IN PEANUTS

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ABSTRACT

Fruity fermented (FF) flavor is a common off-flavor in peanuts resulting from high-temperature curing. The 9-point hedonic scale is the most widely used scale to determine consumer acceptance; however, research has indicated that line scales may provide equal reliability and greater sensitivity. The objectives of this study were to characterize consumer perception of FF flavor in peanuts and to compare the effectiveness of the two scale types. Consumers (n = 208) evaluated control (no FF), low-intensity (1.0) FF and high-intensity (3.0) FF peanut pastes for the strength/intensity of roasted peanut flavor (RPF), sweet taste (ST), fresh peanut flavor (FPF) and overall liking (OV) using randomly assigned ballots. Sensitivity in defining consumer perception of off-flavor in peanuts was greater with use of line scales than with the hedonic scale. The line scale indicated that FF flavor in peanuts, even at low intensity, negatively impacted OV and further identified significantly lower RPF and FPF perception by consumers. The hedonic scale identified only a difference in FPF and was not sensitive enough to show a difference in OV.

INTRODUCTION

There are a variety of sensory tools that can be used in product evaluation to assess perceived intensity and acceptability of food products. The

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9-point hedonic scale is the most widely used scale to determine consumer acceptance. This scale was developed in the 1940s and has served as the industry standard scale for almost 60 years. However, because this scale utilizes unequal intervals resulting in central tendency, differentiating among liked or disliked foods is difficult (Schutz and Cardello 2001). The 9-point hedonic scale can be modified. For instance, the 9-point category scale has been reduced to 6- and 7-point scales and the labels have been changed into “children’s” language (Moskowitz *et al.* 1976; Kroll 1990; Epler *et al.* 1998). In 2003, Yao *et al.* (2003) used the 9-point scale with labels only, with numbers only and with both. One of the main findings of the study was that removing the labels from the 9-point hedonic scale elicited a wider range of scores on the scale. For the purpose of this study, we used the hedonic scale with both numbers and labels.

Studies suggest that line scales can be used in consumer testing. The labeled magnitude scale (LMS) is a vertical, semantic scale of perceptual intensity characterized by a quasi-logarithmic spacing of verbal labels (Green *et al.* 1996). Green *et al.* (1996) demonstrated that LMS was as easy to use as the 9-point hedonic scale and significantly less difficult to use than magnitude estimation (ME) in studies where consumers evaluated the taste intensity produced by sucrose or NaCl, or the odor intensity of acetic acid or phenyl ethyl alcohol. Their results indicated that LMS can be used to rate sensations of taste and smell when broadly defined; however, it should be modified for use in scaling specific taste (and probably odor) qualities.

The labeled affective magnitude (LAM) scale is a specialized type of LMS modified to evaluate liking/disliking of samples. Schutz and Cardello (2001) modified the LMS to liking/disliking and compared it with the 9-point hedonic scale and ME in several food preference and acceptability tests. The results indicated that the LAM scale may be used to effectively assess consumer like/dislike of food products. Additionally, the LAM scale was found to be equal in reliability and have greater sensitivity than the 9-point hedonic scale. The LAM scale was judged by consumers to be as easy to use as the 9-point hedonic scale and significantly less difficult than ME. Jeon *et al.* (2004) compared category and line scales within given protocols to study the variation among the mean errors when a given scale type was used. The results demonstrated that neither scale had an advantage in discriminating among samples. Use of proper methodology to evaluate consumer acceptance is an essential element in product marketing. It is extremely important that sensory methodologies be applicable to practical issues to determine the potential acceptance of the product.

Peanuts are a valuable agricultural commodity and approximately 1.7 million tons of peanuts are produced in the United States each year. Approximately 60% of the peanuts produced are consumed as food (Didzbalis *et al.*

2004) and the peanut product market is about \$7 billion. Research has indicated that the unique flavor of roasted peanuts is the underlying basis for consumer purchase and consumption of peanuts (Sanders *et al.* 1997). Production, handling and processing practices are key components in the development and maintenance of the desirable roasted peanut flavor (RPF). In postharvest curing of peanuts, heated air is normally utilized to reduce the moisture content to an average of 10% to facilitate safe storage (Sanders *et al.* 1989b).

Early studies reported that high-temperature curing off-flavor (as fruity fermented [FF] flavor was originally named) occurred at a curing temperature of $>35^{\circ}\text{C}$ and was more pronounced in immature peanuts (Butt and Kummer 1951; Bailey *et al.* 1954; Beasley and Dickens 1963). FF flavor is one of the most common off-flavors in peanuts (Sanders *et al.* 1989b). Sanders *et al.* (1989b) added the FF descriptor to the peanut lexicon previously published by Johnsen *et al.* (1988). Several research studies have investigated the development of FF flavor in peanuts and confirmed that high curing temperature, increased respiration and immaturity are key factors (Sanders 1989).

Several off-flavors have consistently been associated with “small, immature” peanuts. Immaturity is generally related to small size, although the relationship is not absolute (Sanders *et al.* 1989b), and peanuts of all grade sizes are found in various peanut-maturity classes. Sanders *et al.* (1989a) reported that medium-grade size peanuts from immature classes developed high intensities of FF flavor at high curing temperatures, while intensities were significantly lower or not present in mature peanuts of the same size.

Flavors documented by trained descriptive sensory panelists may not be detected by consumers or may not negatively influence consumer acceptance. There are few studies that have compared scaling techniques (9-point hedonic and LMS/LAM scale) among a large and diverse number of consumers and none comparing an off-flavor in peanuts. Therefore, a study to examine consumer perception of FF flavor was needed, and the objectives of this study were to characterize consumer perception of FF flavor in peanuts and to compare the effectiveness of the 9-point hedonic and line scales in determining consumer perception.

MATERIALS AND METHODS

Materials

Medium-grade size, runner-type peanuts (94.8 kg, 2003 crop) identified as having FF off-flavor (3.0 intensity), were obtained from a commercial sheller. The control sample was a uniform non-FF lot. A lab-scale roaster (Aeroglide Corporation, Raleigh, NC) was used to roast peanuts at 177°C for

12 min to a target roast color of Hunter $L = 50 \pm 1$. Roasted peanuts were uniformly cooled with forced ambient air and the seed coats were removed manually.

The roasted peanuts were processed into paste using a Cuisinart Little Pro Plus Food Processor (Cuisinart, East Windsor, NJ) as previously described by Sanders *et al.* (1989a). This procedure consisted of two 2-min grinds separated by 30-s cooling intervals until the desired paste consistency was acquired. A highly trained descriptive sensory panel (seven panelists with over 1000 h of experience each with descriptive analysis of products and more than 100 h of experience each with descriptive analysis of peanut flavor) confirmed the intensity of FF in the control, 1.0 (prepared by blending 0 FF and 3.0 FF) and 3.0 samples. The pastes were stored at -20°C and tempered to room temperature (20°C) the day before consumer panels were conducted.

Consumer Evaluation

A diverse population of 208 students, staff and faculty from North Carolina State University participated in evaluating the three peanut samples. Participants were recruited from four widespread locations on the university campus through personal communication, paper flyer advertisements and e-mail announcements. Prior to participation, panelists read and signed a written informed consent form, in which they were screened for peanut allergies and peanut product consumption. In addition, consumers completed a questionnaire to determine general demographic information and consumer purchase decisions for peanuts and/or peanut products. Consumers were randomly assigned ballots with either a 9-point hedonic or line scales (LMS/LAM) and received personal instructions on use of the scales. Green *et al.* (1996) found that the successful application of LMS was dependent on the instructions the subjects received.

Peanut paste samples (10 g) were placed into three-digit coded 2-oz soufflé cups with lids. All peanut samples were evaluated under red lights to mask any slight differences in color among samples. Consumers were provided with bottled water for rinsing between samples. The peanut pastes were presented using a balanced and randomized order. Consumers evaluated control (non-FF), low intensity (1.0) FF and high intensity (3.0) FF peanut samples for the strength/intensity of RPF, fresh peanut flavor (FPF), sweet taste (ST) and overall liking (OV). The FPF term was used to represent off-flavor without specifically describing the term FF. Previous consumer studies successfully used this approach to identify off-flavors in peanuts (Young *et al.* 2005). Consumers received a cash incentive (\$5) for participation. Approval for the study was obtained from the North Carolina State University Institutional Review Board.

TABLE 1.
CONSUMER ATTRIBUTE STRENGTH/INTENSITY MEANS
FOR FF PEANUTS: 9-POINT HEDONIC SCALE VERSUS LMS

Treatments	Attributes		
	RPF	ST	FPF
9-point hedonic			
Control	5.75 a	3.31 a	5.90 a
1.0 FF	5.82 a	3.62 a	5.19 b
3.0 FF	6.19 a	3.50 a	5.34 b
LMS			
Control	4.94 b	2.12 a	5.51 a
1.0 FF	5.01 b	2.30 a	4.80 a
3.0 FF	5.98 a	2.08 a	5.12 a

Means in a column followed by the same letter are not different ($P > 0.05$).

LMS, labeled magnitude scale; RPF, roasted peanut flavor; ST, sweet taste; FPF, fresh peanut flavor; FF, fruity fermented.

Scaling

Line scale data points were expressed in millimeters. Verbal labels on the line scales were positioned at specific geometric mean value locations (Schutz and Cardello 2004). For the LMS, the data points were measured from the lowest verbal label (barely detectable) to the perceived intensity of the specific descriptor. The LAM scale is a bidirectional scale and data points were determined by measuring from 0 mm (neither like nor dislike) to the point marked by the consumer. Thus, the 146-mm scale values could range from 0 to 73 and 0 to -73 (Schutz and Cardello 2004). Analysis of variance (ANOVA) with means separation (Fisher's LSD) was conducted to characterize differences among samples (SAS version 8.2, SAS Institute, Cary, NC). Quantile-quantile plots (Q-Q plots) were generated to compare the distributions of responses for each treatment and attribute using the different scales (SAS version 8.2).

RESULTS AND DISCUSSION

There was a similar balance of males to females (50 and 50%, respectively). About 49, 33 and 18% of the consumers were between the ages of 19 and 25, 26 and 45 and 46 and 65, respectively. The majority of the consumers (93.7%) purchased and consumed peanuts/peanut products at least once per month. Differences were observed among the three peanut pastes and between the two scaling techniques ($P < 0.05$) (Tables 1 and 2). Hedonic scale results

TABLE 2.
CONSUMER ATTRIBUTE LIKING/DISLIKING MEANS
FOR FF PEANUTS: 9-POINT HEDONIC SCALE VERSUS
LAM

Treatments	Attributes			
	RPF	ST	FPF	OV
9-point hedonic				
Control	5.21 a	4.47 a	5.37 a	5.10 a
1.0 FF	5.04 a	4.12 a	4.82 b	4.69 a
3.0 FF	4.97 a	4.11 a	4.87 ab	4.66 a
LAM				
Control	13.21 a	5.89 a	15.57 a	12.62 a
1.0 FF	4.33 b	2.18 a	7.94 b	6.91 ab
3.0 FF	7.68 ab	0.20 a	8.69 b	4.18 b

Means in a column followed by the same letter are not different ($P > 0.05$).

LAM, labeled affective magnitude; RPF, roasted peanut flavor; ST, sweet taste; FPF, fresh peanut flavor; OV, overall liking; FF, fruity fermented.

for strength/intensity were not significantly different ($P > 0.05$) for RPF and ST. The 0 FF (control) sample was significantly ($P < 0.05$) higher in FPF compared with the low (1.0) and high FF (3.0) samples. The LMS results indicated that ST and FPF were not significantly different among the samples; however, RPF was significantly higher for the high (3.0) FF intensity indicating that consumers perceived the high FF sample as more RPF (Table 1).

Liking/disliking differences for RPF, ST, FPF and OV varied between the hedonic and line scales (Table 2). Hedonic scale results indicated no significant ($P < 0.05$) differences among the peanut samples for RPF, ST and OV. The results for FPF hedonic scale scores were inconclusive in that the high (3.0) FF sample was not significantly different from either the control or the low (1.0) FF sample. These results suggest that consumers may not perceive FF as an off-flavor in unsalted peanut pastes. Hedonic scores for the control peanut paste are consistent with previous consumer studies of this product (Young *et al.* 2005). Products that score within the 7–8 range on the hedonic scale are confectionery products and/or dinner entrees (Young *et al.* 2005).

Differences among the samples were more clearly defined with the LAM scale (Table 2); although RPF in the high (3.0) intensity FF sample was not significantly different from the other samples. The control sample was significantly higher for FPF and the low (1.0) and high (3.0) FF samples were not significantly different from each other. For OV, the LAM scale results indicated that the control and low FF samples were not significantly different and

that the low and high FF samples were not significantly different. Using this scale, consumers were able to detect differences in liking (Table 2), but strength/intensity data (Table 1) indicated that they were unable to consistently characterize strength/intensity of any particular attribute. This may suggest that consumers knew that they liked one sample over another, although they were unable to determine why. The LAM scale has a greater sensitivity, compared with the hedonic scale, to detect differences among well-liked foods (Schutz and Cardello 2001). This is because of the LAM scale endpoints ("greatest imaginable liking/disliking") which enable more extreme ratings than "like/dislike extremely" (Schutz and Cardello 2001). Consumers scored all three samples in the same category range (5 = neither like nor dislike) with both scales and this may have reduced discrimination (Schutz and Cardello 2001). Q-Q plots for each treatment and attribute were linear (correlation coefficients [r^2] ≥ 0.80 ; $P < 0.01$), indicative of similarly shaped distributions for the hedonic and line scales (data not shown). Schutz and Cardello (2001) also noted similarly shaped distributions for responses to the 9-point hedonic and the LAM scales for evaluation of a variety of foods.

Although each scale was used with the same number of consumers, there were more differences among the peanut samples when the LAM scale was used. Consumer perception of FF off-flavor with the 9-point hedonic scale was difficult to determine. The LAM scale was more effective in providing data that demonstrated differences in consumer perception. The range and distribution of the semantic labels differ somewhat between the line and category scales and greater success might be expected with line scales (LMS/LAM) (Green *et al.* 1996).

CONCLUSIONS

The 9-point hedonic scale produced inconclusive differences among the three peanut samples. However, the LAM scale resulted in more differences among the samples and indicated that consumers were able to distinguish between the FF and non-FF peanuts. The differences in the scaling methods suggest that the 9-point hedonic scale is a more conservative sensory tool in determining differences among samples, while the line scales were more sensitive.

Consumers are able to detect FF flavor; however, the results indicate that they do not consider low levels of this flavor unacceptable in peanuts. Additional research is needed where FF peanuts are incorporated in peanut products to determine the potential consumer impact of FF peanuts. This study supports published concerns for consumer acceptance based on hedonic testing and is a

strong indication of the value of sensory methods testing using product ingredients. The use of proper methodology to evaluate consumer acceptance is essential to product marketing.

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CHAPTER 5
**FRUITY FERMENTED OFF-FLAVOR DISTRIBUTION IN SAMPLES FROM
LARGE PEANUT LOTS**

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ABSTRACT

Fruity fermented (FF) off-flavor develops when immature peanuts are cured at excessive temperatures ($>35^{\circ}\text{C}$). The objective of this study was to characterize FF distributions and determine the variability among samples from large peanut lots. Twenty peanut lots identified as having a range of FF off-flavor were sampled. Twenty samples from each lot were roasted and processed into paste for descriptive sensory analysis. Differences in FF intensity were noted within and among lots. The FF intensity mean of the lots was either greater or less than the median value for the samples, indicating that the distributions were skewed. The skewed distributions and the variation among samples from a single lot demonstrated the need to develop a sampling plan for FF off-flavor.

Key Words: fruity fermented, variability, distribution, descriptive sensory panel, sampling plan

PRACTICAL APPLICATIONS

The peanut manufacturing industry has a stated concern for fruity fermented (FF) off-flavor in peanuts purchased for use in peanut products, but there is difficulty in obtaining a truly uniform sample of all peanuts in a large lot. This study measured the variability and characterized the FF distribution among samples from bulk peanut lots, and will be used to estimate the components contributing to FF variation within peanut lots and aid in the development of sampling plans for accurate FF intensity determination. This type of research has relevance to a wide range of food factors where the factor of interest is not homogeneously distributed in a commodity or product.

INTRODUCTION

The driving force for consumer purchase and consumption of peanuts and peanut products is the roasted peanut flavor (Sanders *et al.*, 1997). In 2005, approximately 2,187 million tons of peanuts were produced in the United States (Peanut Production Statistics, 2005) and most were used in the domestic edible trade. One of the major concerns of the peanut industry, and other food industries, is the presence of off-flavors as consumers are generally not accepting of differences or variations in the flavor of products. Off-flavor development can occur during production, handling, processing, and storage. Fruity fermented (FF) off-flavor is one of the most common off-flavors in peanuts and is due to environmental factors at harvest when the early stages of peanut curing occur. Research has demonstrated that FF off-flavor may be developed during curing of immature peanuts when temperatures exceed 35°C (Sanders *et al.*, 1989a). Greene *et al.* (2006) reported that consumers were able to detect FF off-flavor in peanuts using two different sensory scaling techniques. The presence of FF off-flavor significantly lowered consumer perception of

roasted peanut flavor and negatively impacted overall liking of the product when a line scale, the most sensitive method, was used. The peanut industry has a stated concern for FF off-flavor in peanuts purchased for the manufacture of peanut products (personal communication, American Peanut Council) and the assumption can be made that data from proprietary studies forms the basis for the concern. Anecdotal information from crop years when FF was more common indicated the rejection of hundreds of tons of peanuts when FF was identified by manufacturers after delivery of commercially tested lots.

Peanut plants flower indeterminately and peanuts of different physiological maturity are found on the plant at any harvest time. Since peanuts tend to become big before they are fully mature, peanuts of each physiological maturity class are found in all grade sizes (Sanders, 1989; Sanders and Bett, 1995). Although more immature peanuts are found in smaller grade sizes, even the larger sizes contain some physiologically immature peanuts. Sanders *et al.* (1989b) reported significant differences in roast color and flavor of medium grade size peanuts from different maturity classes. Immature peanuts roast darker and are more likely to develop FF off-flavor compared to mature peanuts (Sanders *et al.* (1989a). Thus, because immature peanuts are more likely to have FF off-flavor, the distribution of the various maturity classes within a lot is correlated to the FF intensity distribution in the lot. This heterogeneous, random distribution of immature seed, that may contain FF off-flavor, results in difficulty in obtaining a truly uniform sample of all peanuts in a large lot. It is important for peanut product manufacturers to have an accurate determination of FF intensity if it is present within a single lot.

Descriptive sensory analysis by trained panelists is a powerful tool used for the detection (discrimination) and description of both the qualitative and quantitative sensory

components of a consumer product (Meilgaard *et al.*, 1999). The peanut lexicon, developed by Johnsen *et al.* (1988), includes terms such as, roasted peanut flavor, sweet aromatic, dark roast, and woody/hull/skins. Sanders *et al.* (1989b) added the FF descriptive term to the previously published peanut lexicon. The characterization of FF off-flavor has been investigated using descriptive sensory panels and can be detected at intensity levels as low as 1 using the Spectrum™ method.

Food manufacturers use acceptance sampling plans to obtain the best estimate of a wide range of factors in numerous commodities (Vandeven *et al.*, 2002). Quality factors of raw peanuts vary because of different weather conditions, seed varieties, cultural practices, and processing. Sampling plans have been used by buyers and sellers for detection of various heterogeneous contaminants, such as aflatoxin and other mycotoxins (Whitaker, 2003). Operating characteristic (OC) curves can be generated to determine how a particular sampling plan will perform, thus estimating the potential magnitude of bad lots accepted (buyer's risk) and good lots rejected (seller's risk) (Vandeven *et al.*, 2002; Whitaker and Johansson, 2005). The design of sampling plans to determine the level of aflatoxin contamination has been reported throughout the literature; however there have been no research studies on establishing the variability of FF in large lots or developing sampling plans for FF off-flavor. Determination of the variability and distribution of FF intensity among individual samples within a lot is necessary for development of sampling plans that can be used to accurately determine with high confidence the true intensity of FF in a lot (Vandeven *et al.*, 2002).

Descriptive sensory analysis of numerous samples from many large peanut lots is necessary to determine the FF distribution within lots. The objective of this study was to

measure the variability and characterize the FF distribution among samples from bulk peanut lots. This type of research has relevance to a wide range of food factors where the factor of interest is not homogeneously distributed in a commodity or product. A sampling plan that results in sufficient numbers and size of samples to provide an accurate estimate of some factor is critical to detection and/or elimination of that factor.

MATERIALS AND METHODS

Twenty, one ton lots of medium grade-size runner-type peanuts (2003 crop) were identified as having a range of FF off-flavor by a single commercial analysis. The commercial analysis is a descriptive sensory analysis using 5 highly trained panelists and a roasted, pasted sample from a random 300 g sample. The range of FF intensities within the twenty lots was reported to be from 0 to 4 and based on that analysis the lots were selected for inclusion in the study by commercial sheller personnel. Large samples (200 lbs) from the twenty different lots were obtained from the shellers and each sample was riffle-divided to obtain 20 samples of 680 g each. The 400 samples (20 lots x 20 samples) were roasted in a lab-scale roaster (Aeroglide Corporation, Raleigh, NC) at 176°C for ca 12 min to a target roast color of Hunter L = 50 ± 1 . Roasted peanuts were cooled using forced ambient air, seed coats were manually removed and 250 g of each sample was ground into a paste following the procedures of Sanders *et al.* (1989b). Peanut pastes were stored in 8 oz. glass jars at -4°C and tempered to room temperature (20°C) the day before sensory analysis.

A trained descriptive panel evaluated the flavor of the 400 peanut paste samples. The sensory panel consisted of seven members each with over 500 h of experience with descriptive sensory analysis of peanut flavor including FF. Panelists evaluated 7 samples /panel session using all the terms in the peanut lexicon (Johnsen *et al.*, 1988; Sanders *et al.*,

1989b. Samples from several different lots were randomly presented to the panel to avoid panelist bias. After each sample, panelists expectorated peanut pastes and rinsed their mouths with water and cleansed their palates with unsalted crackers. The Spectrum™ method was used for descriptive sensory analysis. This method utilizes a universal intensity scale (0 to 15), in which panelists score intensities of all attributes on the same basis (Drake and Civille, 2003). Mean, standard deviation (SD), median, and coefficient of variation (CV) of all samples from a lot were calculated.

RESULTS AND DISCUSSION

There were differences in FF off-flavor intensity of samples within and among the 20 peanut lots. Mean FF intensities for the 20 lots ranged from 0.2-2.1. Sampling issues are evident from this information alone since some of the 20 lots were originally identified by one commercial analysis as having no FF off-flavor. FF intensity among samples from a single lot was highly variable (Table 1). Lot 2816 had a FF intensity mean of 0.2 with samples ranging from 0-0.6. Trained panel threshold intensity is considered to be 1 and mean values below 1 indicate that all panelists did not identify FF in the sample. The FF off-flavor intensity mean for Lot 1022 was 0.9 and the range of sample intensities (0.2-1.9) within this lot is much wider than the range in Lot 2816. Lot 1039 had a FF mean of 2.1 and the range among samples was 1.4-2.8. Lot 1040 had a FF intensity mean of 2.1 and the range of sample intensities was 0.9-2.8. Each peanut lot had a relatively wide range of FF off-flavor intensity in individual samples which further demonstrated the difficulty associated with obtaining an accurate determination of off-flavor intensity with a single sample from a lot.

Table 1 shows the mean, median, SD, CV and range of FF intensities among the 20 lots. The SD, CV, and range of FF intensities among samples from a lot indicated variability, whereas the mean and median provide some indication of the shape of the distribution (normal vs. skewed). The mean and median FF intensities for each of the 20 lots were compared among the 20 samples to determine whether FF distributions were normal or skewed. When the FF mean of a lot is equal to the median (mean = median), the distribution among samples is normal (symmetrical). However, if the median is greater or less than the mean (median > or < mean), the FF distribution is negatively or positively skewed (nonsymmetrical). If samples from a peanut lot indicate a normal distribution 50% of the samples from that lot are less than the FF intensity mean, while the other 50% are greater than the FF intensity mean. A positively skewed distribution indicates that more than 50% of the samples from the lot have lower intensity of FF than the FF intensity mean and less than 50% of the samples are higher than the FF intensity mean. The evaluation of skewedness for the 20 peanut lots indicated that 15 of the 20 lots were positively skewed and 5 were negatively skewed, and there were zero normal distributions. Of the five negatively skewed distributions, 2 were extremely close to being normal distributions. In the 15 positively skewed lots, a single sample had a greater probability of underestimating the true FF intensity of the lots than overestimating (increased buyer's risk) and in the five negatively skewed lots there was a greater chance of overestimating the true FF intensity (increased seller's risk).

CV (standard deviation/mean x 100), which is a relative measure of variability, generally decreased as the mean FF intensity increased indicating that there is more variability among lots with low FF off-flavor (Table 1). A lot with high FF intensity is more

likely to be identified even in a small sample size. In contrast, a low level of FF intensity within a lot will be more difficult to detect. These relationships are possibly in part related to the fact that more individual seed in a high FF intensity lot have FF off-flavor and are thus more likely to be collected in an individual sample (i.e. the lot has a maturity class distribution with greater percentage of immature seed). Whereas, a sample from a low FF intensity lot, in which there are fewer seed with FF off-flavor (low percentage of immature seed) is less likely to contain a proportionate amount of the seed with FF off-flavor.

Table 1. Mean, Median, Standard Deviation, Coefficient of Variation, and Range of the Fruity Fermented Off-flavor in Twenty Peanut Lots

Lot #	Mean	Median	SD (\pm)	CV (%)	Range (low-high)
2816 ⁺	0.2	0.1	0.2	105	0.0-0.6
1075 ⁺	0.3	0.2	0.3	100	0.0-0.9
1086 ⁻	0.6	0.6	0.4	65	0.1-1.2
1093 ⁺	0.8	0.8	0.5	64	0.0-2.0
1022 ⁺	0.9	0.8	0.5	54	0.2-1.9
6363 ⁺	1.0	0.9	0.5	51	0.2-1.7
1035 ⁺	1.0	0.8	0.5	48	0.3-1.9
2821 ⁺	1.1	1.0	0.7	68	0.0-2.4
1036 ⁺	1.1	0.9	0.6	54	0.3-2.2
1087 ⁺	1.1	1.1	0.8	76	0.0-2.3
1034 ⁻	1.1	1.2	0.4	38	0.2-1.8
1020 ⁺	1.1	1.0	0.6	52	0.2-2.0
1066 ⁺	1.2	1.1	0.5	47	0.4-2.6
1041 ⁺	1.2	1.2	0.6	53	0.0-2.3
1065 ⁻	1.3	1.3	0.8	60	0.1-2.8
1063 ⁺	1.4	1.3	0.6	42	0.5-2.5
1067 ⁻	1.6	1.7	0.6	40	0.4-2.5
1064 ⁺	1.7	1.6	0.4	27	1.1-2.4
1039 ⁺	2.1	2.1	0.3	16	1.4-2.8
1040 ⁻	2.1	2.2	0.5	24	0.9-2.8

SD = standard deviation (\pm)

CV = coefficient of variation (%)

+ = positively skewed distribution

- = negatively skewed distribution

FF intensity distributions of three lots with low (Lot 2816), medium (Lot 1034), and high (Lot 1039) FF off-flavor intensity are depicted in Figure 1. Lot 2816 had the lowest FF

intensity mean (0.2) and exhibited a positively skewed distribution in which a single sample was more likely to underestimate the true lot mean (13 samples vs 7 samples). Lot 1039 had a high FF intensity mean (2.1) and was also positively skewed (13 samples vs 7 samples). Lot 1034 had a negatively skewed distribution in which a single sample was more likely to overestimate the true lot mean (9 samples vs 11 samples). Regardless of the underlying FF distribution among individual kernels in the lot, as sample size increases, the distribution becomes more normal and provides a more precise determination of the true FF intensity of the off-flavor in a lot (Whitaker and Johansson, 2005).

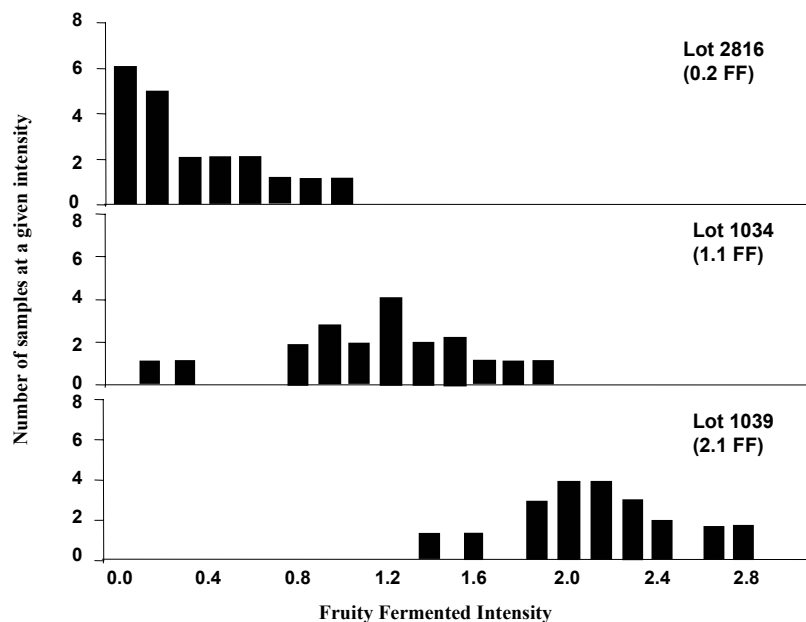


Figure 1. Fruity Fermented Off-flavor Distribution of Three Different Peanut Lots

Currently, the peanut industry uses a 300 g sample to evaluate the presence of FF off-flavor. In this study, a 250 g sample was used to determine FF off-flavor intensity and the variability should approximate the use of a single commercial analysis of 300 g. Development of sampling plans can be accomplished with data from any sample size since that involves partitioning of the source of error and eventual calculation of OC curves to

determine the effect of various sample sizes on the likelihood of obtaining a true measure of the FF intensity. Previous studies on mycotoxins have suggested that as sample size increases, the probability of accepting bad lots (false negative) and rejecting good lots (false positive) decreases (Whitaker and Johansson, 2005). An increase in sample size to identify FF off-flavor decreases the variance, which increases precision and reduces misclassification of lots. It is important for manufacturers to obtain an accurate determination of FF off-flavor to prevent the misclassification of large peanut lots. Whether the peanut lot had a low or high mean, the distribution of FF off-flavor was highly variable within a lot indicating that the current commercial method using only one 300 g sample may be inadequate to supply accurate FF data. In order to obtain a precise estimation of a factor of concern, the sample size, sample preparation, sub-sampling, and analytical methods must be investigated (Vandeven *et al.*, 2002).

CONCLUSIONS

Large peanut lots were shown to have high sample to sample variability of FF intensity. Determination of the variability and FF distribution among samples is necessary for the development of accurate sampling plans for bulk lots. The data collected in this study will be used to estimate the components contributing to FF variation within peanut lots and aid in the development of sampling plans for accurate FF intensity determination. The use of sampling plans is a cost effective way to reduce the risk of good lots being rejected (seller's risk) and bad lots being accepted (buyer's risk). The findings in this study demonstrated the critical need to develop sampling plans for a wide range of commodities in order to accurately determine the sensory quality when that quality is not homogenously distributed in the commodity or product.

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CHAPTER 6
CHARACTERIZATION OF VOLATILE COMPOUNDS IN NATURAL
FRUITY FERMENTED PEANUTS

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ABSTRACT

Roast peanut flavor is the driving force for peanut consumption; therefore, the presence of off-flavors is of major concern to the peanut industry. The most common off-flavor in peanuts is described as fruity fermented (FF) and it is developed at excessive curing temperatures ($>35^{\circ}\text{C}$). Published research has indicated that ethyl-2-methylpropanoate, ethyl-2-methylbutanoate, ethyl-3-methylbutanoate, hexanoic acid, butanoic acid, and 3-methylbutanoic acid are responsible for FF off-flavor; however these compounds were identified in samples artificially created in the laboratory. Currently, there is no published literature on the volatile compounds contributing to natural FF off-flavor in peanuts. The objective of this study was to characterize the volatile compounds contributing to natural FF off-flavor using sensory analysis, instrumental analysis, and model systems evaluation. Instrumental analysis was conducted on a no FF sample and FF sample chosen after sensory analysis of a large number of samples. Artificially created samples were prepared by curing immature peanuts at 40°C following the procedures of a previous study. Volatile compounds were extracted, concentrated, and characterized using solvent assisted flavor evaporation (SAFE), solid phase microextraction (SPME), gas chromatography-olfactometry (GC-O), and gas-chromatography-mass spectrometry (GC-MS). Aroma extract dilution analysis (AEDA) was conducted to determine the most potent aroma-active compounds. More than 100 aroma-active compounds were detected in the peanut samples. The 12 most potent compounds identified by solvent extraction were: acetoin/2,3-butanedione (buttery), 3-methylbutanal (malty/chocolate), hexanal (green/grassy), methional (potato), 2-acetyl-1-pyrroline (popcorn), 1-octen-3-one (metallic/mushroom), 2-ethyl-6-methylpyrazine (sweet), trimethylpyrazine (earthy/soil/dirt), phenylacetaldehyde (rosy/floral), 2-ethyl-3,5-

dimethylpyrazine (earthy/soil/dirt), 2,3-diethyl-5-methylpyrazine (earthy/soil/dirt), 2-methoxy-4-vinylphenol (licorice/sweet), acetic acid (vinegar/acetic acid), and butanoic acid (sweaty/musty/cheesy). There were no consistent differences between the flavor dilution factors in the no FF and FF sample nor were the previously identified esters found in these samples. SPME was used as a secondary method to distinguish differences between the samples and the results confirmed the SAFE method, in that the esters and organic acids previously identified as causing FF off-flavor were not detected in the natural FF samples; however, these compounds were detected in the artificially created samples.

INTRODUCTION

Peanuts (*Arachis hypogaea* L.) are a nitrogen-fixing legume native to South America. This valuable crop is grown in many countries with India, China, and the U.S. as the major peanut producing countries. In 2005, 2.3 million tons of peanuts were produced in the U. S. The majority of peanuts produced in the U.S. are used for human consumption and the underlying basis for consumer purchase and consumption is due to the unique roasted peanut flavor. Off-flavors are of major concern to peanut manufacturers (Sanders *et al.*, 1997). Lipid oxidation is one of the causes of off-flavors in raw and roasted peanuts because of unsaturated fatty acids in oil (Warner *et al.*, 1996). A relatively common off-flavor in peanuts is described as fruity fermented (FF). Previous research has indicated that FF off-flavor is developed when, immature peanuts are cured at temperatures that exceed 35°C (Sanders *et al.*, 1989a).

For the last 45-50 years, flavor chemists have studied the volatile compounds contributing to roasted peanut flavor. Early flavor research studies used instrumental analysis alone to hypothesize links between compounds and flavor; however, the understanding of flavor involves extensive sensory and volatile analysis. Pattee *et al.* (1965) identified formaldehyde, acetaldehyde, ethanol, acetone, isobutyraldehyde, ethyl acetate, butyraldehyde, isovaleraldehyde, 2-methyl-valeraldehyde, methyl butyl ketone, and hexaldehyde in high-temperature-cured peanuts. Walradt *et al.* (1971) and Johnson *et al.* (1971a, b) conducted research on the volatile components in roasted peanuts using gas chromatography and identified 187 compounds (142 of them, including 17 pyrazines were reported for the first time). The types of compounds reported were pyrazines, pyrroles, thiazoles, phenols, pyridines, ketones, aldehydes, terpenes, furans, esters, lactones, alcohols,

and aromatic hydrocarbons. Singleton *et al.* (1971) evaluated the volatile profiles of peanuts cured at 22°C, 35°C, 45°C, and 50°C and using gas chromatography and an untrained sensory panel. These researchers found that acetaldehyde, ethanol, and ethyl acetate increased as curing temperatures increased.

In 1973, Young investigated the volatiles produced during roasting of three types/varieties of peanuts that had been dried at 110, 135, and 160°F. Peanuts produced in Argentina and U.S. grown, Southern Runner peanuts, and Early Runner varieties increased in mercaptans, carbon dioxide, amines, and carbonyls as drying temperatures increased. Lovegren *et al.* (1982) examined raw Virginia peanuts and identified methanol, acetaldehyde, ethanol, acetone, N-methylpyrrole, hexanal, hexanol, and nonanal as the key compounds. Sensory analyses were conducted on the samples and flavor profiles were found to be in the acceptable range for normal peanuts. El-Banna *et al.* (1983) identified alkyloxazoles, alkylthiazoles, piperidines in freshly roasted peanuts. Young and Hovis (1990) examined raw and roasted peanuts using a rapid headspace analysis technique and identified sixteen compounds including N-methylpyrrole, ethanol, pentane, hexanal, 2-methyl propanol, and dimethyl disulfide.

More recent flavor research has incorporated descriptive sensory and instrumental analysis. Didzbalis *et al.* (2004) identified ethyl-2-methylpropanoate, ethyl-2-methylbutanoate, ethyl-3-methylbutanoate, hexanoic acid, butanoic acid, and 3-methylbutanoic acid as the compounds causing FF off-flavor in peanuts artificially cured at a constant temperature of 40°C. Schirack *et al.* (2006) investigated the volatile compounds responsible for off-flavor in high temperature microwave blanched peanuts and reported that

phenylacetaldehyde, guaiacol, and 2,6-dimethylpyrazine were the key compounds responsible for stale/floral and ashy off-flavors.

The majority of peanut flavor research has used instrumental analysis to identify the compounds responsible for off-flavors. Although instrumental measurements are useful, they do not differentiate between aroma-active and non aroma-active compounds. Gas-chromatography Olfactometry (GC-O) is used to determine the volatile compounds potentially responsible for flavor and assists in the identification of those compounds in the sensory threshold range (Friedrich and Acree, 1998). This technique is very useful in determining which volatile compounds are aroma-active; however, the presence and identification of a compound does not indicate a role in a particular flavor. The confirmation of the involvement of a particular compound in a particular off-flavor is accomplished by sensory panel evaluation of a model system containing the compound(s). To our knowledge, there are no reports of the compounds contributing to naturally occurring FF off-flavor in peanuts. The objective of this study was to characterize the volatile compounds contributing to natural FF off-flavor using sensory analysis, instrumental analysis, and model systems evaluation.

MATERIALS AND METHODS

Sample Preparation.

Natural FF Samples. Twenty, one ton medium grade-size runner-type peanuts previously identified as having FF off-flavor (2003 crop) were sampled to obtain 250 lbs and these samples were riffle-divided to obtain 20, 680 g subsamples. The peanut samples were roasted for 12 min at 176°C using a lab-scale benchtop roaster (Aeroglide Corporation, Raleigh, NC), cooled using forced ambient air and seed coats were manually removed.

These parameters were chosen to obtain a roast color of Hunter L= 50±1. Roasted peanuts (250 g) were processed into paste using a standard protocol (Sanders *et al.*, 1989b). Peanut pastes were stored at -4°C and tempered to room temperature prior to sensory and volatile analysis. The 400 samples (20 lots x 20 subsamples) were evaluated by a descriptive sensory panel. Instrumental analysis was conducted on two samples described by the sensory panel as having no FF off-flavor (intensity = 0) and FF off-flavor (intensity = 2.6).

Artificially Created FF Samples. One hundred pounds each (45.3 kg) of freshly harvested Georgia Green (GG) and Flavor Runner 458 (FR458) peanut varieties were obtained from USDA-ARS-National Peanut Research Laboratory (NPRL) research projects in Georgia and Texas, respectively. Samples were shipped in coolers overnight to North Carolina State University, Department of Food Science in Raleigh, NC. Peanuts were sorted into Pod Maturity Profile maturity classes by mesocarp color after removal of the exocarp (Williams and Drexler, 1981). The mesocarp colors from mature to immature are black, brown, orange B, orange A, and yellow, respectively. Black and brown colored pods (BB) were used as the mature lot and orange A, (only slightly orange) and yellow colored pods (OY) were used as the immature lot. One intermediate color, Orange B (advance orange) was not used in order to provide complete separation of mature and immature pods. BB and OY from each variety were cured at both 27 and 40°C until a moisture content of 8% was obtained following the procedures of Didzbalis *et al.* (2004). The natural samples were designated as no FF and 2.6 FF and the artificially created samples were designated as GGOY27, GGOY40, GGBB27, GGBB40, FR458OY27, FR458OY40, FR458BB27, and FR458BB40 to indicate the variety, production location, maturity class, and curing temperature.

Sensory Evaluation of Peanuts. A trained sensory panel (n=8) evaluated the samples using a lexicon developed for peanut flavor (Johnsen *et al.*, 1988). The FF descriptor was added to the lexicon by Sanders *et al.* (1989a) in studies to examine the relationship of high-temperature-curing and maturity of peanuts (Table 1). Each panelist had over 500 h of experience in descriptive sensory analysis of peanut flavor. Each panel session consisted of the evaluation of 7 samples using the Spectrum™ method. Flavor intensities were scored using the Spectrum™ method on a 15-point universal intensity scale (Meilgaard and others 1999). Panelists expectorated the peanut samples after analysis, rinsed with water, and cleansed their palates with unsalted crackers as needed. Peanut samples were evaluated in duplicate.

Table 1. Lexicon of Peanut Flavor Descriptors

<u>AROMATICS</u>	<u>DEFINITIONS</u>
Roasted Peanuty	The aromatic associated with medium-roast peanuts (about 3-4 on USDA color chips) and having fragrant character such as methylpyrazine.
Raw Bean/Peanuty	The aromatic associated with light-roast peanuts (about 1-2 on USDA color chips) and having legume-like character (specify beans or pea if possible).
Dark Roasted Peanut	The aromatic associated with dark roasted peanuts (4 + on USDA color chips) and having very browned or toasted character.
Sweet Aromatic	The aromatics associated with sweet material such as caramel, vanilla, molasses, fruit (specify type).
Woody/Hulls/Skins	The aromatics associated with base peanut character (absence of fragrant top notes) and related to dry wood, peanut hulls, and skins.
Cardboard	The aromatic associated with somewhat oxidized fats and oils and reminiscent of cardboard.
Painty	The aromatic associated with linseed oil, oil based paint.
Fruity fermented	The aromatic associated with overripe fruit.
Rotten garbage/soured	The aromatic associated with old garbage.
Burnt	The aromatic associated with very dark roast, burnt starches, and carbohydrates, (burnt toast or espresso coffee).
Green	The aromatic associated with uncooked vegetables/grasstwigs, cis-3-hexanal.
Earthy	The aromatic associated with wet dirt and mulch.
Grainy	The aromatic associated with raw grain (bran, cod liver oil, old fish).
Fishy	The aromatic associated with trimethylamine, cod liver oil, or old

Table 1 continued.

	fish.
Chemical/Plastic	The aromatic associated with plastic and burnt plastics.
Skunky/Mercaptan	The aromatic associated with sulfur compounds, such as mercaptan, which exhibit skunk-like character.

TASTES

Sweet	The taste on the tongue associated with sugars.
Sour	The taste on the tongue associated with acids.
Salty	The taste on the tongue associated with sodium ions.
Bitter	The taste on the tongue associated with bitter agents such as caffeine or quinine.

**FEELING
FACTORS**

Astringent	The chemical feeling factor on the tongue, described as puckering/dry and associated with tannins and aluminum.
Metallic	The chemical feeling factor on the tongue described as flat, metallic, and associated with iron and copper.

Adapted from Johnsen *et al.*, 1988 and Sanders *et al.*, 1989a

Solvent Extraction Techniques

Chemicals. Ethyl ether (anhydrous, 99.8%), sodium chloride (99%), sodium sulfate (99%), and 2-methyl-3-heptanone (internal standard for neutral/basic fraction) were obtained from Aldrich Chemical Co. (St. Louis, MO., USA). Internal standard (3-methylvaleric acid) for the acidic fraction was obtained from Lancaster (Windham, N.H., USA). The reference standards for aroma compounds (Table 3 and 4) were purchased from Sigma-Aldrich (St. Louis, MO., USA). The sodium bicarbonate (99.7%) and hydrochloric acid (36.5%) were obtained from Fisher Scientific (Pittsburgh, PA., USA).

Direct solvent extraction. One hundred grams of peanut pastes were weighed and divided into 4 teflon centrifuge bottles. The internal standard mixture (50 µl 2-methyl-3-heptanone and 50 µl 3-methylvaleric acid) was suspended in 5 mL of ethyl ether and added to the peanut samples (15 µl per bottle x 2 bottles per rep = 30 µl per replication). After the addition of the internal standard, 50 mL of NaCl and 50 mL ethyl ether were added to each bottle. Sample mixtures were shaken for 30 min on a Roto mix (Barnstead/Thermolyne Type

50800; Dubuque, IO., USA) at a speed of 8. The bottles were centrifuged in a Sorvall RC-5B refrigerated (3.0°C) superspeed centrifuge (DuPont Instruments) for 15 min at 3000 rpm (1207 x g) to separate the solvent phase from the peanut paste followed by the collection of the solvent from each bottle into a 400 mL glass mason jar. This procedure was repeated three more times with the addition of 50 mL of ethyl ether to each bottle each time. The combined solvent phases were combined and stored at -20°C until further analysis.

High Vacuum Distillation (Solvent Assisted Flavor Evaporation-SAFE). Volatile compounds were separated using SAFE (Figure1). The sample was loaded into the top of the SAFE apparatus and dispensed dropwise into the vacuum. The non-volatiles were collected in the round-bottom flask and the volatile compounds were collected in the first trap which was submerged in liquid nitrogen. After the sample was completely introduced into the system, the distillation was carried out for 2 h at 10^{-4} torr. The distillate was concentrated to 20 mL under a gentle stream of nitrogen gas.

Phase Separation (Neutral/Basic and Acidic Fractions). The concentrated distillate was washed with 3 mL of 0.5 M sodium bicarbonate twice and vortexed. After each wash, the bottom layer (water phase) was transferred to a screw cap tube. The distillate was washed three times with 2 mL of saturated sodium chloride solution and vortexed. The bottom layer was transferred to the same screw cap tube and was designated as the neutral/basic fraction. The pH of the collected water phase was lowered to pH 2.0-2.5 with 18% HCl and ethyl ether was added three times. After each ether addition, the ether phase (top layer) was removed and collected in another tube which was designated as the acidic fraction. Each fraction was filtered through powdered sodium sulfate tubes (3x) to remove any water in the extracts before being reduced to 0.5 mL under a stream of nitrogen gas.

Gas Chromatography-Olfactometry (GC-O) of Solvent Extracts. Peanut extracts

(neutral/basic and acidic fractions) were analyzed on an HP5890 series II gas chromatograph (Hewlett-Packard Co., Palo Alto, California, USA) equipped with a flame ionization detector (FID), a sniffer port, and a splitless injector. Two microliters of each fraction for the 2 total samples were evaluated on a nonpolar capillary column (DB-5ms, 30 m length x 0.25 mm i.d. x 0.25 μ m d_f ; J&W Scientific, Folsom, California, USA) and a polar capillary column (DB-Wax, 30 m length x 0.25 mm i.d. x 0.25 μ m film thickness d_f ; J&W Scientific, Folsom, California, USA). The column effluent was split (1:1) between the FID and sniff port. Nasal dehydration was reduced by combining 30mL/min of humidified air with the GC effluent (Van Ruth, 2001). The GC oven temperature was programmed from 40 - 200°C at a rate of 8°C/min with initial and final hold times of 5 and 20 min, respectively. Six experienced panelists evaluated neutral/basic and acidic fractions of the peanut extracts twice on the DB-5 and DB-Wax columns. As compounds eluted from the column, the panelist described the detected odor and the intensity of the odor using a 10-point numerical intensity scale (Van Ruth, 2001). The two techniques used to collect and process GCO data were postpeak intensity and aroma extract dilution analysis (AEDA). The postpeak intensity method involved the recording of a descriptor of the odor and intensity of the odor as it elutes from the column (Van Ruth, 2001). AEDA methods involve the dilution of the extract at a ratio of 1:3. The extract is subjected to GC-O analysis after each dilution until the odor is not detected. The flavor dilution (FD) factor is defined as the last dilution at which an odor is detected. Higher FD values suggest that the particular compound plays a larger role than those with lower FD values.

Gas Chromatography/Mass Spectrometry (GC/MS) of Solvent Extracts. Peanut extracts were injected on a Agilent Technologies 6890 GC/Agilent 5973 mass selective detector. Separations of peanut extracts were performed on a fused silica capillary column (DB-5ms, 30 m length x 0.25 mm i.d. x 0.25 μ m d_f, J&W Scientific, Folsom, Calif., USA) as described by Schirack *et al.* (2006). Helium was used as a carrier gas at a constant flow of 1 mL/min. Oven temperature of GC/MS was programmed from 40 - 200° C at a rate 2°C/min with initial and final hold times of 5 and 30 min, respectively. The conditions of the mass selective detector were: capillary direct interface temperature, 280°C; ionization energy, 70 eV; mass range, 33 to 330 a.m.u.; EM voltage (Atune + 200 V); scan rate, 5 scans/s. Two microliters of each extract were injected in the splitless mode in duplicate.

Headspace Extraction Techniques

Gas Chromatography/Mass Spectrometry (GC/MS) of Solidphase Microextraction.

Solid phase microextraction (SPME) was conducted to extract the highly volatile compounds from the natural and artificially created FF samples. Ten grams of peanut paste was measured into 20ml clear screw-cap vials and 5 μ l of the internal standard was added (2-methyl-3-heptanone and 2-methylvaleric acid). Headspace volatiles were analyzed in duplicate using a 50/30 μ m divinylbenzene/carboxenTM/ polydimethylsiloxane (DVB/CAR/PDMS) stableflexTM fiber on a CTC Analytics CombiPAL system autosampler. Prior to injections, the samples were agitated at 40°C (250 rpm) for 30 min. to release the volatiles into the headspace. After agitation, the fiber was exposed in the headspace for 30 min. After absorption of the volatiles by the fiber, volatiles were thermally desorbed from the fiber for 5 min. and injected onto the GC-MS. An Agilent Technologies 6890N GC/Agilent Technologies 5973 mass selective detector equipped with a fused nonpolar

capillary column (DB-5ms, 30 m length x 0.25 mm i.d. x 0.25 μ m d_f ; J&W Scientific, Folsom, California, USA) was used for separation. Oven temperatures were programmed from 40 - 250° C at a rate 8°C/min with initial and final hold times of 5 min. Helium was used as the carrier gas with a constant flow of 1ml/min. The mass selective detector (MSD) conditions were: capillary direct interface temperature, 250°C; ionization energy, 70 eV; mass range, 35 to 300 a.m.u.; EM voltage (Atune + 2211.8 V); scan rate, 5 scans/s. One microliter of each sample was injected in the splitless mode in duplicate.

Identification of Odorants. Tentative identifications were obtained by comparing mass spectra of authentic standard compounds to unknown compounds using the mass spectral database of the National Institute of Standards and Technology (NIST) (2005). Additionally, the retention indices (RI) and odor of unknown compounds were compared to the Flavornet database (<http://www.flavornet.com>) and the authentic standard compounds under the same conditions. Positive identifications were accomplished by comparing the mass spectra, RI, and odor of unknowns with those of authentic standards under identical conditions.

Retention indices (RI) were calculated using the n-alkane series as described by Van den Dool and Kratz (1963).

Quantification of Volatile Compounds. Volatiles were quantified by calculating the relative abundance of the selected compounds. The peak area and concentration of the internal standard with the peak area of the compound were used: (relative abundance= peak area IS/ peak area compound * [concentration IS]). Standard curves were generated for different groups of compounds. 2-methylbutanal, trimethylpyrazine, and hexanoic acid were quantified by analysis of standards in deodorized water using SPME and GC-MS. A 5-point standard curve was generated for each compound to validate the relative abundance data to

determine the absolute concentration of the compounds displayed in Table 7. Results indicated 2-methylbutanal and trimethylpyrazine had a linear fit of $R^2 > 0.97$ whereas hexanoic acid had $R^2 > 0.99$.

Statistical Analysis. Analysis of variance (ANOVA) using the general linear model (GLM) was used to determine differences among the sensory data. Fisher's LSD was the post-hoc tests used to determine differences among the sample means (SAS version 9.1, Cary, N.C., U.S.A).

RESULTS AND DISCUSSION

Sensory Evaluation of Peanut Pastes. There were flavor differences among the natural and artificially created FF samples. The sensory panel characterized the natural FF samples as having a sweet, overripe fruit flavor characteristic of FF off-flavor as defined in the peanut lexicon. In contrast, the panel often referred to the artificially created FF samples as having a rotten garbage/soured off-flavor. We hypothesize that this descriptor is developed when peanuts are exposed to constant high curing temperature rather than the diurnal variation that occurs in natural curing. Didzbalis *et al.* (2004) separated the terms fruity and fermented to describe flavor differences scored in the artificially created samples. For the purpose of this study, the panel scored only the term FF even though verbal descriptions was sometimes rotten garbage/soured.

The FR458OY40 had the highest FF intensity of 3.4 and was significantly ($P < 0.05$) different from the natural and GG samples (Table 2). The natural FF sample had an off-flavor intensity of 2.6 and was not significantly different ($P > 0.05$) from the FR458OY27 sample. This confirms that even at low curing temperatures (27°C), immature peanuts are more susceptible to FF off-flavor and it can develop even at ambient temperatures. Sanders

et al. (1989a) investigated the effect of curing temperature on descriptive flavor of peanuts from different maturities. Results indicated significantly higher intensities of FF, sour, and bitter notes in immature peanuts cured at ambient + 16.8°C.

The FR458BB27 sample had the highest roast peanutty intensity at 4.6 and was not significantly different from the no FF sample, FR458BB40, and FR458OY27. GGBB27 and GGBB40 had the lowest roast peanutty intensities of 2.0 and 2.2, respectively. In the present study, the natural and artificially created FR458 samples were significantly ($P<0.05$) higher in sweet aromatic and sweet taste compared to the GG samples. Among the GG samples, GGOY40 had the highest intensity of sweet aromatic (intensity = 2.3) and sweet (intensity = 1.9). For the FR458 samples, FR458OY40 had the lowest sweet aromatic (SA) intensity of 2.8. This low SA intensity may be related to the fact that FR458OY40 had rotten garbage/soured flavor (highest FF intensity) that was not described by the panel as natural FF off-flavor. In the natural samples, the no FF sample had significantly ($P<0.05$) higher sweet aromatic and sweet taste intensity.

Dark roast is associated with dark roasted peanuts having a browned or toasted character (Johnsen *et al.*, 1988). Immature peanuts tend to roast darker, have more FF off-flavor, and have a faster flavor fade than mature peanuts (McNeill and Sanders, 1998). Due to higher sugar content contributing to the Maillard reaction, FR458 samples (natural and artificial) had higher dark roast intensities than the GG samples. Additionally, GG samples had significantly ($P<0.05$) higher bitter and astringency intensities.

Table 2. Sensory analysis of natural and artificially created fruity fermented off-flavor in Georgia Green and Flavor Runner 458 peanut varieties

Sample	Roast Peanutty	Sweet Aromatic	Dark Roast	Raw Beany	Woody/ Hulls/Skins	Sweet Taste	Bitter	Astringency	Fruity fermented	Cardboardy
No FF	4.2ab	3.6ab	3.6a	1.7e	3.0bc	3.7a	2.2c	1.0d	0.0d	0.0bc
Natural FF	3.6bc	2.9cd	3.4ab	2.3d	3.2ab	2.7bc	2.9b	1.10cd	2.6b	1.1b
GGBB27	2.0e	1.7fg	2.50c	2.7a	3.3a	1.5d	3.6a	1.3b	0.0d	2.0a
GGBB40	2.2e	1.5g	2.7c	2.7a	3.3a	1.6d	3.8a	1.4a	0.0d	2.3a
GGOY27	3.6d	2.0ef	2.8bc	2.6abc	3.1ab	1.5d	3.5a	1.2bc	0.0d	1.1b
GGOY40	2.9d	2.3e	2.7c	2.6ab	3.2ab	1.9cd	3.7a	1.1cd	1.1cd	0.0cd
FR458BB27	4.6a	3.70a	3.1abc	2.2bcd	3.1ab	3.7a	2.4bc	1.0d	0.0d	0.0d
FR458BB40	4.3a	3.3bc	3.0abc	2.2cd	3.1ab	3.3ab	2.5bc	1.0d	2.2bc	0.0cd
FR458OY27	4.0ab	3.0cd	2.90bc	2.4abcd	3.2ab	3.0ab	2.9b	1.0d	2.3ab	0.0d
FR458OY40	3.2cd	2.8d	2.8bc	2.1de	2.8c	2.5bc	2.9b	1.1cd	3.4a	0.0cd

Means in the same column with different letters are significantly different ($P < 0.05$)

GG- Georgia Green

FR458- Flavor Runner 458

BB- Black and Brown (mature peanuts)

OY- Orange and Yellow (immature peanuts)

27- curing temperature (°C)

40- curing temperature (°C)

Aroma-active Volatile Compounds Determined by Solvent Extraction.

Postpeak intensity analysis is useful in determining the compounds that are present in the sensory threshold range and the compounds that are aroma-active in a sample. However, it is difficult to determine which compound(s) potentially relate to flavor because the presence of a compound is not always indicative of contribution to a particular flavor. One hundred and sixty-one aroma-active compounds were detected from the neutral/basic fractions. There were no consistent differences among the volatile profiles and odor intensities of the no FF and FF sample (data not shown). AEDA is a dilution screening technique which helps identify aroma-active compounds by focusing on those with low and high FD factor. This screening method does not correct for the loss of compounds during extraction and concentration techniques; therefore, the compounds with low and high FD factors are normally examined further (Grosch, 1993). Twelve compounds with FD factor of 5 or greater were identified in the neutral/basic fractions of the no FF (control) and the FF sample (Table 3). Among the 12 highly potent compounds, there were 4 pyrazines, 4 aldehydes, 2 ketones, 1 pyrroline, and 1 phenol. Seven compounds were positively identified and 5 were tentatively identified. The mean intensities for the majority of the compounds were higher in the control except for 1-octen-3-one (**6**), 2-ethyl-6-methylpyrazine (**7**), 2-ethyl-3,5-dimethylpyrazine (**10**). The intensity of 2,3-diethyl-5-methylpyrazine (**11**), 2-acetyl-1-pyrroline (**5**), and trimethylpyrazine (**8**) were equal for both samples. These compounds have been reported previously in roasted peanuts (Johnson *et al.* 1971a,b; Walradt *et al.* 1971; Matsui *et al.* 1998; Baker *et al.* 2003; Didzbalis *et al.* 2004; and Schirack *et al.* 2006). The FD factors of compounds from the no FF and the FF samples did not indicate meaningful differences. Acetoin/2,3-butanedione (**1**), methional (**4**), phenylacetaldehyde (**9**), and 2-ethyl-

3,5-dimethylpyrazine (**10**) had the same FD factors between the control and off-flavored sample. Hexanal (**3**), 1-octen-3-one (**6**), trimethylpyrazine (**8**), and 2-methoxy-4-vinylphenol (**12**) had higher FD factors in the control whereas 2-acetyl-1-pyrroline (**5**), 2-ethyl-6-methylpyrazine (**7**), and 2,3-diethyl-5-methylpyrazine (**11**) had higher FD factors in the FF sample. The main difference between the no FF sample and the FF sample was that the FD factors for 3-methylbutanal were 5 and <1, respectively. This indicates that 3-methylbutanal was not detected after the first dilution in the FF sample; therefore, the absence of this compound may be related to the flavor differences. In previous research, the presence of 3-methylbutanal has been identified as one of the main contributors to nutty flavor in cheddar cheese (Avsar *et al.*, 2004). Schirack *et al.* (2006) used AEDA to investigate the volatile compounds contributing to the flavor of microwave blanched peanuts. Although 2-methylbutanal was not identified as a contributor to flavor in microwave blanched peanuts, the compound was found in the control and off-flavored samples at relatively high FD factors. 3-methylbutanal was not reported in artificially created FF samples (Didzbalis *et al.*, 2004). In the acidic fractions, 2-ethyl-3,5-dimethylpyrazine (**1**), acetic acid (**2**), methional (**3**), and butanoic acid (**4**) were the compounds with FD factors of two and above (Table 4).

Didzbalis *et al.* (2004) reported that ethyl-2-methylpropanoate, ethyl-2-methylbutanoate, ethyl-3-methylbutanoate, hexanoic acid, butanoic acid, and 3-methylbutanoic acid were responsible for FF off-flavor in immature 40°C cured peanuts. In the present study, the esters were not detected in the solvent extracts from either the natural or artificially created samples. To further validate these results, different methods of concentrating the samples were evaluated to determine if they affected the presence of the esters of interest. Didzbalis *et al.* (2004) concentrated the extract to 200 µl using a

microvigreaux apparatus, whereas in our study a gentle stream of nitrogen was used to concentrate the sample to 20ml prior to phase separation in this study. Although these methods are different, both are reliable concentration methods and no differences in ester concentration or presence were observed.

Table 3. Neutral/Basic aroma-active compounds in natural fruity fermented (FF) peanuts with high flavor dilution factors as determined by aroma extract dilution analysis

No	Compound	RI	RI	Odor ^b	Mean (Log ₃ FD) ^c		Identification Method ^d
		DB-5 ^a	DB-Wax ^a		No FF (0 intensity)	FF (2.6 intensity)	
1	Acetoin/2,3-butadione	610	979	buttery/butterscotch	3.00 (4)	2.63 (4)	odor, RI
2	3-methylbutanal	642	910	malty/chocolate	3.00 (5)	2.50 (<1)	odor, RI, MS
3	Hexanal	803	1023	green/grassy	3.13 (5)	2.88 (4)	odor, RI, MS
4	Methional	921	1472	potato	4.25 (6)	3.88 (6)	odor, RI
5	2-acetyl-1-pyrroline	936	1322	popcorn	4.00 (6)	4.00 (7)	odor, RI
6	1-octen-3-one	987	1267	metallic/mushroom	3.25 (5)	3.50 (4)	odor, RI
7	2-ethyl-6-methylpyrazine	1007	1499	sweet	3.13 (2)	3.75 (3)	odor, RI, MS
8	Trimethylpyrazine	1015	1452	earthy/soil/dirt	4.00 (6)	4.00 (5)	odor, RI, MS
9	Phenylacetaldehyde	1063	1680	rosy/floral	5.38 (7)	4.63 (7)	odor, RI, MS
10	2-ethyl-3,5-dimethylpyrazine	1092	1479	earthy/soil/dirt	3.50 (9)	3.75 (9)	odor, RI, MS
11	2,3-diethyl-5-methylpyrazine	1167	1538	earthy/soil/dirt	2.00 (9)	3.50 (10)	odor, RI, MS
12	2-methoxy-4-vinylphenol	1396	ND	licorice/sweet	3.63 (5)	3.33 (6)	odor, RI

^a Retention indices on DB-5 (nonpolar) and DB-Wax (polar) columns

^b Odor described by GC-O

^c Mean intensities of experienced panelists and flavor dilution factors

^d Compounds were identified by odor, RI, and MS in comparison to the authentic standard under identical conditions

Table 4. Acidic aroma-active compounds in natural fruity fermented (FF) peanuts with high flavor dilution factors as determined by aroma extract dilution analysis

No	Compound	RI DB-5 ^a	RI DB-Wax ^a	Odor ^b	Mean (Log ₃ FD) ^c No FF (0 intensity)	Mean (Log ₃ FD) ^c FF (2.6 intensity)	Identification Method ^d
1	2-ethyl-3,5-dimethylpyrazine	1479	1092	earthy/soil/dirt	2.50 (3)	3.50 (3)	odor, RI, MS
2	Acetic acid	628	1454	vinegar/acetic acid	2.88 (3)	2.75 (3)	odor, RI, MS
3	Methional	903	1472	potato	2.63 (4)	3.38 (4)	odor, RI
4	Butanoic acid	881	1635	sweaty/musty/cheesy	1.50 (2)	2.13 (2)	odor, RI, MS

^a Retention indices on DB-5 (nonpolar) and DB-Wax (polar) columns

^b Odor described by GC-O

^c Mean intensities of experienced panelists and flavor dilution factors

^d Compounds were identified by odor, RI, and MS in comparison to the authentic standard under identical conditions

Volatile Compounds Detected by SPME.

Because there were no meaningful differences observed with solvent techniques, SPME was used as an alternative technique to identify the compounds responsible for natural and artificially created FF off-flavor. Twenty-four volatile compounds were identified by SPME (Table 5). The relative abundance was calculated for each compound identified and for the natural samples, 21 out of the 24 compounds were higher in the FF sample compared to the control. In previous research, ethanol has been used as an indicator of identifying peanuts that have been improperly cured. Pattee et al. (1965) identified ethanol, acetaldehyde, ethyl acetate, formaldehyde, acetone, isobutyraldehyde, isovaleraldehyde, 2-methylvaleraldehyde, methyl butyl ketone, and hexaldehyde in high-temperature-cured peanuts. Lovegren (1982) found higher levels of ethanol in FF samples using gas chromatography. Ethanol was not present in the no FF sample, GGOY27, and any of the BB samples; however, ethanol was detected in the FF sample and the artificially created samples. Ethanol was not present in these samples because FF is related to immaturity and high temperature curing.

The SPME results among the natural and artificially created samples were different for the three esters identified by Didzbalis et al. (2004) (Figures 2 and 3). FR458OY40 had the highest concentration of ethyl-2-methylbutanoate and ethyl-3-methylbutanoate at $2.38 \text{ ppb} \pm 0.21$ and $5.26 \text{ ppb} \pm 0.54$, respectively (Figure 2 and 3). The GGOY40 sample had lower concentrations of ethyl-2-methylbutanoate and ethyl-3-methylbutanoate whereas ethyl-2-methylpropanoate (data not shown) was not detected in the natural and artificially created samples. In the natural FF sample, ethyl-3-methylbutanoate was detected at lower levels (0.37 ± 0.05) than the artificially created FF samples and, the non-FF sample had the lowest

concentration of ethyl-3-methylbutanoate at 0.10 ± 0.02 . Esters are described as having fruity/apple-like aromas and the higher concentrations of these compounds in FR458 samples may be due to the higher sugar content. Data supporting higher sugar content in FR458 in West Texas peanuts are reported in Uniform Peanut Performance Trials (UPPT) (USDA-ARS-Market Quality and Handling Unit, 2006).

Table 5. Relative abundance of volatile compounds in natural and artificially created fruity fermented (FF) peanuts

COMPOUND	Relative abundance (ug/g) No FF	Relative abundance (ug/g) FF natural	Relative abundance (ug/g) Created Georgia Green (GG)	Relative abundance (ug/g) Created Flavor Runner (FR458)
ethanol	ND	27.57 ± 3.23	717.6 ± 7.9	2011.7 ± 5.13
2-methylbutanal	297.0 ± 25.9	381.3 ± 63.4	314.2 ± 38.7	415.7 ± 23.4
1H-pyrrole,1-methyl	150.3 ± 16.2	172.1 ± 12.6	314.2 ± 36.1	333.8 ± 25.1
hexanal	406.8 ± 63.5	280.2 ± 24.3	156.9 ± 23.6	ND
methylpyrazine	280.8 ± 27.5	331.0 ± 10.9	308.8 ± 38.1	362.5 ± 10.0
furfural	278.2 ± 28.5	296.7 ± 4.7	209.9 ± 8.6	205.3 ± 29.2
2-Furanmethanol	84.03 ± 17.8	87.4 ± 0.1	53.9 ± 7.4	101.3 ± 1.8
styrene	69.4 ± 1.8	29.52 ± 13.0	26.9 ± 38.1	61.4 ± 28.1
2,5-dimethylpyrazine	1425.0 ± 145.0	1640.2 ± 109.0	1474.1 ± 171.5	1590.3 ± 80.8
2,6-dimethylpyrazine	325.6 ± 24.7	428.4 ± 51.5	409.6 ± 53.2	544.2 ± 26.2
2,3-dimethylpyrazine	255.3 ± 18.8	313.4 ± 90.7	294.0 ± 25.2	304.5 ± 19.6
benzaldehyde	277.2 ± 39.6	323.3 ± 8.4	257.8 ± 8.7	265.2 ± 14.0
2-ethyl-5-methylpyrazine	343.7 ± 126.7	480.3 ± 36.8	409.4 ± 75.9	450.7 ± 35.3
trimethylpyrazine	791.8 ± 83.5	957.3 ± 1.1	872.6 ± 31.2	1034.6 ± 148.1
2-methylhexanoic acid	181.0 ± 56.97	1566.2 ± 1044.2	1937.4 ± 2690.7	3360.0 ± 2967.0
D-limonene	120.8 ± 56.8	ND	ND	ND
2-octenal, (E)-	ND	64.8 ± 11.6	ND	ND
acetophenone	9.8 ± 1.9	23.6 ± 3.0	ND	ND
2-acetylpyrrole	71.9 ± 32.7	85.3 ± 28.4	66.4 ± 18.5	88.1 ± 11.2
2-ethyl-3,5-dimethylpyrazine	42.4 ± 10.1	52.3 ± 2.8	76.1 ± 25.1	503.0 ± 484.7
nonanal	86.7 ± 17.2	108.3 ± 10.0	66.6 ± 7.6	97.7 ± 1.1
2-acetyl-3-methylpyrazine	35.8 ± 6.2	44.5 ± 4.5	ND	ND
2,3-diethyl-5-methylpyrazine	17.5 ± 3.9	28.60 ± 4.8	26.6 ± 2.8	63.1 ± 5.4

Table 5 continued.

2-methoxy-4-vinylphenol	208.7 ± 34.4	215.8 ± 15.6	262.6 ± 58.9	93.6 ± 0.8
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Data reported as mean ± standard deviation. DB-5 column was used to separate the volatile compounds.

The differences in volatile profiles of natural and artificially created samples may be caused by the different curing processes. Typically, when peanuts are cured they are placed in windrows for 1-3 days and moisture content decreases to 20-25%. Harvested peanuts are then normally dried with heated air in drying wagons to 10% moisture content. During this process, temperatures of >35°C for a significant amount of time generally results in natural FF development especially in immature peanuts (Sanders *et al.*, 1989a). The artificially created samples were cured at two different constant temperatures over a period of time, and these conditions cannot happen in nature. The difference in curing techniques may have affected the production of the three fruity esters.

Previous research has indicated that during high temperature curing the rate of oxygen does not diffuse into the peanut kernel efficiently which causes respiration to go from aerobic to anaerobic (Whitaker and Dickens, 1964). Esters are formed by a reaction between an alcohol and a carboxylic acid. Butanoic acid and hexanoic acid, short chain organic acids present in peanuts, may react with alcohols to produce various types of esters. Peanuts cured at 40°C generate more ethanol during anaerobic respiration; therefore, the production of esters should be more efficient. The exaggerated curing conditions used to produce the artificially created samples would result in much more ethanol production in peanuts than the natural conditions resulting in FF peanuts. Thus, less ethanol would be present in the naturally occurring FF peanuts to react with the short chain organic acids which could result in very low, non-detectable concentrations of the esters.

In comparison to the esters identified by Didzbalis *et al.* (2004), ethyl-3-methylbutanoate was the only compound detected in natural FF sample; whereas ethyl-2-methylbutanoate and ethyl-3-methylbutanoate were both detected in created samples using the SPME method. This indicates: i) natural FF off-flavor is caused by an imbalance of compounds typically found in peanuts, or ii) the esters concentrations in natural FF samples may often be below the detection limit of the instrument in natural samples.

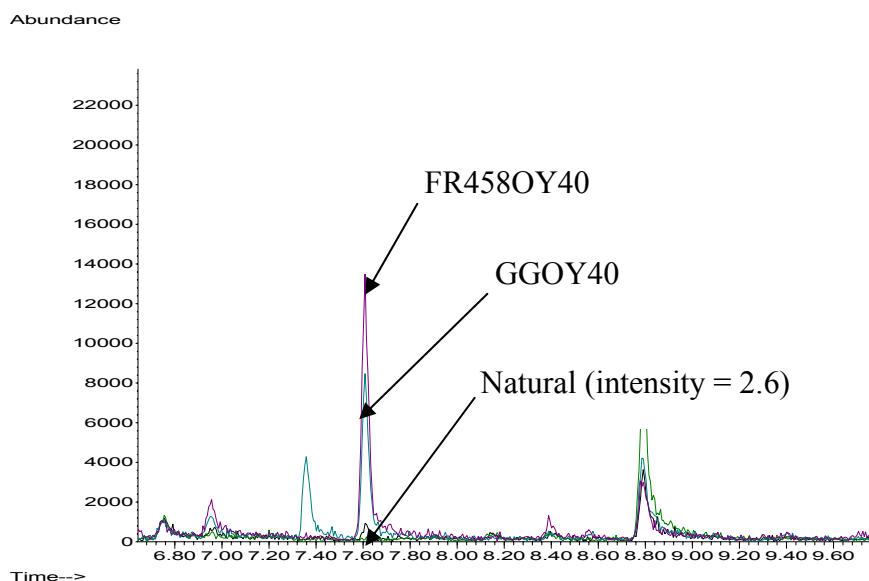


Figure 1. Ethyl-2-methylbutanoate in natural and artificially created fruity fermented samples using solid phase microextraction

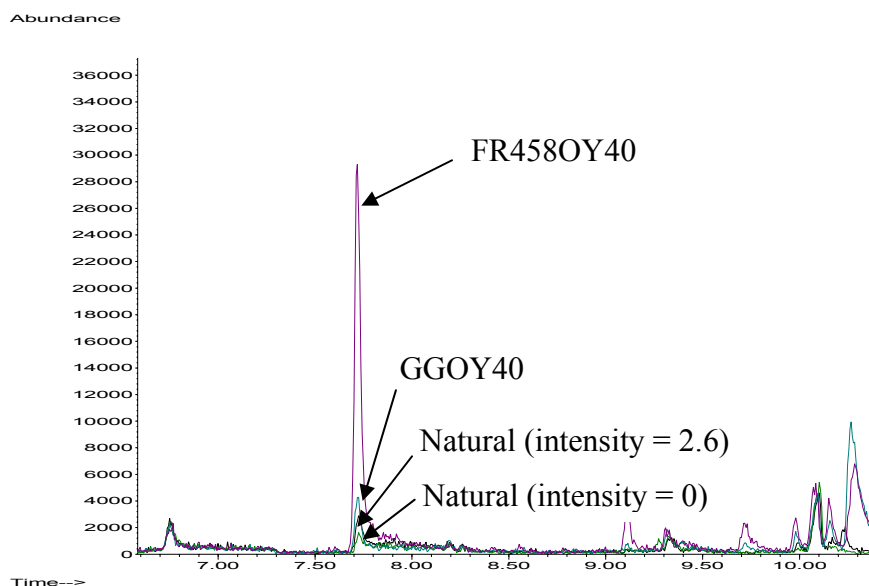


Figure 2. Ethyl-3-methylbutanoate in natural and artificially created fruity fermented samples using solid phase microextraction

Table 6. Relative abundance of esters identified in natural and artificially created fruity fermented samples

Compound	Relative Abundance (ug/g) No FF	Relative Abundance (ug/g) FF	Relative Abundance (ug/g) GGOY40	Relative Abundance (ug/g) FR458OY40
Ethyl-2-methylpropanoate	ND	ND	ND	ND
Ethyl-2-methylbutanoate	ND	0.11 ± 0.02	1.33 ± 0.19	2.38 ± 0.21
Ethyl-3-methylbutanoate	0.10 ± 0.02	0.37 ± 0.05	0.59 ± 0.06	5.26 ± 0.54

ND- not detected

Model Systems.

Model systems are an integral part of confirming that specific compounds are responsible for a certain off-flavor. Descriptive sensory analysis indicated flavor differences between natural FF and artificially created FF samples. The artificially created samples were often described by an experienced panel as having a harsh rotten garbage/soured off-flavor. Natural FF off-flavor was perceived as more sweet and associated with overripe fruit as defined in the peanut lexicon. Although the difference in flavor profiles was consistently described by the panel, they suggested that there may be a relationship or continuum of the

off-flavor found in natural and artificially created FF. Didzbalis *et al.* (2004) used ethyl-2-methylpropanoate (0.09 ppb), ethyl-2-methylbutanoate (0.13 ppb), ethyl-3-methylbutanoate (0.11 ppb), hexanoic acid (0.17 ppb), butanoic acid (0.55 ppb), and 3-methylbutanoic acid (3.04 ppb) in peanut paste to create model systems. When the concentrations reported by Didzbalis *et al.* (2004) were used, some panelists described the typical artificially created sample off-flavor. These results suggest that the compounds identified by Didzbalis *et al.* (2004) in artificially created samples are responsible for naturally occurring FF off-flavor although these compounds were not detected at high levels in naturally occurring FF off-flavor samples. Since the compounds were not detected in the natural FF samples; these results further suggest the need for caution when laboratory created samples are used to source off-flavors.

CONCLUSIONS

Volatile and sensory differences were noted in natural and artificially created fruity fermented samples. Sensory results indicated that natural and artificially created fruity fermented samples were distinct and suggested that there was a continuum of this off-flavor between the two samples. Two of the three fruity esters previously identified as causing fruity fermented off-flavor were apparently below the detection limit of the analysis methodology for natural fruity fermented samples and ethanol was not detected at low levels in natural fruity fermented samples using solvent and headspace analysis. These findings preclude the use of current analytical techniques as a rapid method of identifying peanut lots with FF off-flavor.

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CHAPTER 7:
CONCLUSIONS AND FUTURE WORK

The development of off-flavors in peanuts is influenced by environmental conditions, handling, processing, storage, and maturity stage. The research in this study investigated the most common off-flavor, fruity fermented (FF), using descriptive sensory, consumer, and instrumental analyses to characterize various FF lots, determine consumers perception of the off-flavor, evaluate the variability and FF distribution in large peanuts lots, and identify the key compound(s) responsible for FF off-flavor. Descriptive sensory analysis results of the large peanuts lots were characterized differently from lot to lot and an inverse relationship between FF off-flavor and roasted peanut flavor were established between several lots. The higher the FF intensity a reduction in roasted peanut flavor.

The characterization of an off-flavor by a trained sensory panel is often different to consumers; therefore, the second study investigated consumer's perception of FF off-flavor in peanuts using category and line scales. Results indicated that consumers were able to detect differences between FF and non-FF samples using both methodologies, and the samples with low FF intensity were shown to be more acceptable than higher FF intensity. Overall, the presence of FF off-flavor was found to negatively impact overall acceptability when the line scale, the most sensitive method, was used. Further research needs to be conducted to determine the potential impact of FF peanuts in food applications.

Peanuts indeterminate flowering pattern results in a range of maturities present at any harvest time. The third study investigated the variability and characterized the FF distribution among samples from large bulk lots. Results revealed a distribution of FF off-flavor intensity within a single lot and from lot to lot. Among the twenty lots investigated, there was a wide range of FF intensity in individual samples from a single lot which demonstrates the difficulty in obtaining an accurate determination of FF off-flavor in bulk

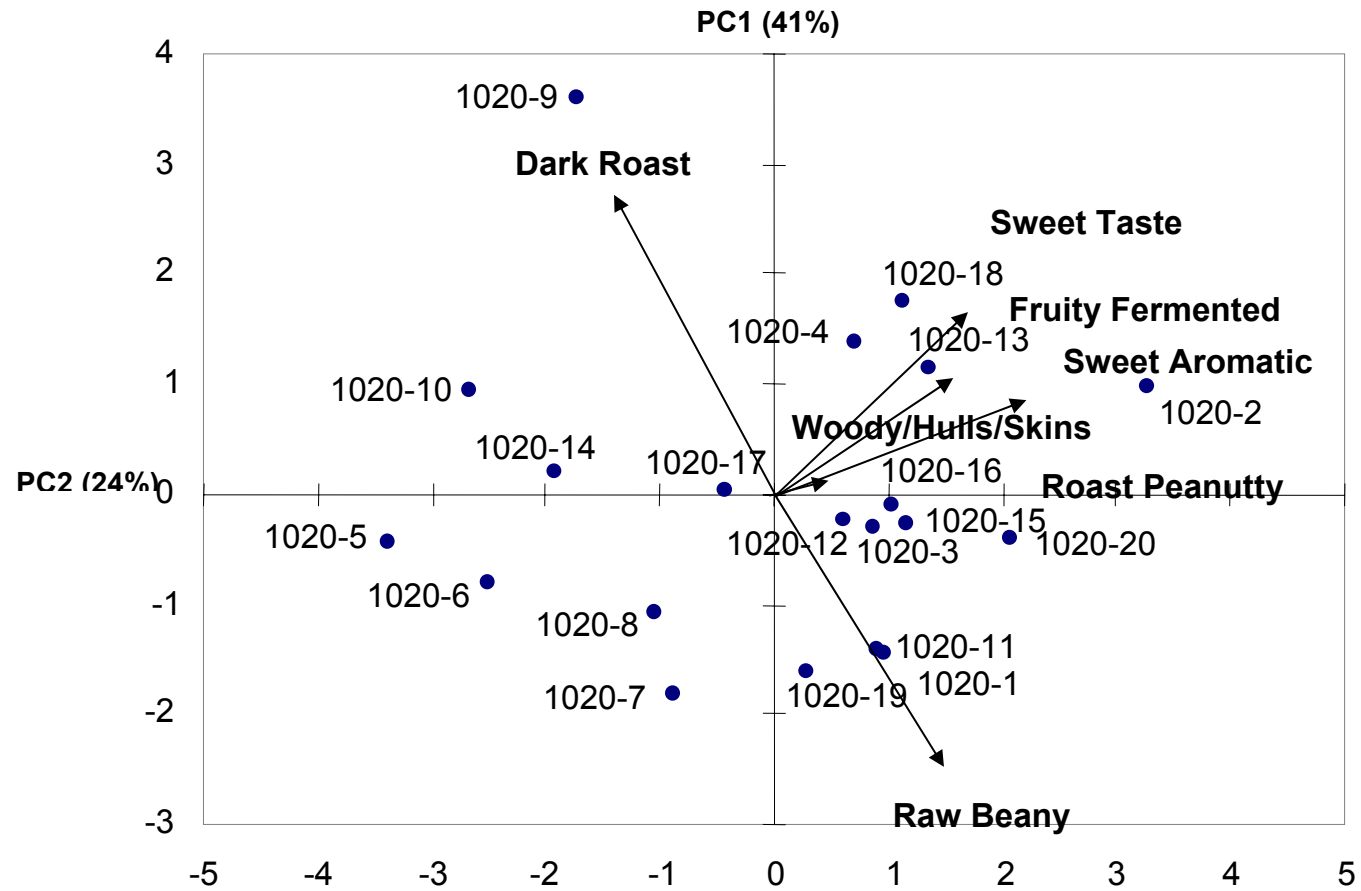
lots. The data collected in this study helped establish a sampling plan that can be used to determine the presence/absence of FF off-flavor in large peanut lots so that good lots are not rejected and bad lots are not accepted. The sampling plan developed for FF off-flavor indicated that in order to reduce variability of the test procedure is to increase the sample size. Further research would be to design parameters to evaluate FF intensity in large lots so sampling plans will not exceed specific risk levels (FF intensity > 1) for the peanut industry.

Differences in volatile and flavor profiles were revealed in natural and artificially created FF samples. Two of the three fruity esters, previously published as contributing to FF off-flavor, were not detected in natural FF samples; however, they were detected in artificially created samples in Georgia Green and Flavor Runner 458 varieties when headspace techniques were used. Ethyl-2-methylbutanoate and ethyl-3-methylbutanoate were higher in the Flavor Runner 458 samples which may be due to the higher sugar content. For many years, ethanol has been the marker for high-temperature-cured peanuts; however it was not detected in the no FF sample but was detected in the FF and artificially created samples. The esters were not detected in the solvent extracts from either the natural or artificially created samples. A sensory panel characterized the natural FF sample as sweet, overripe fruit flavor whereas the artificially created samples were described as having rotten garbage/soured off-flavor. Model system studies with a concentration range of the esters and acids produced a flavor similar to natural and artificially created FF samples.

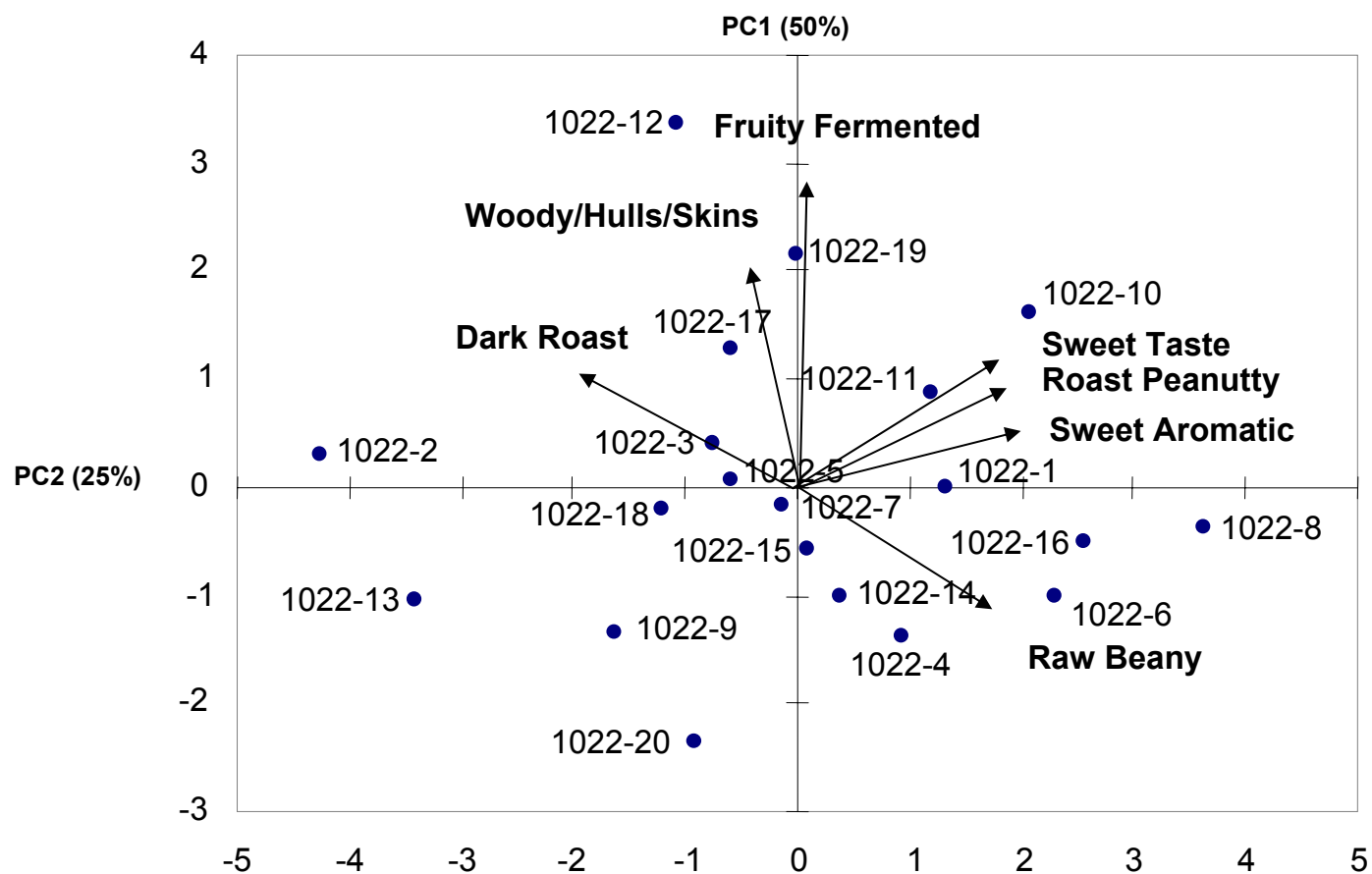
Further research needs to be conducted to determine the regional and varietal differences in the development of FF off-flavor.

APPENDICES

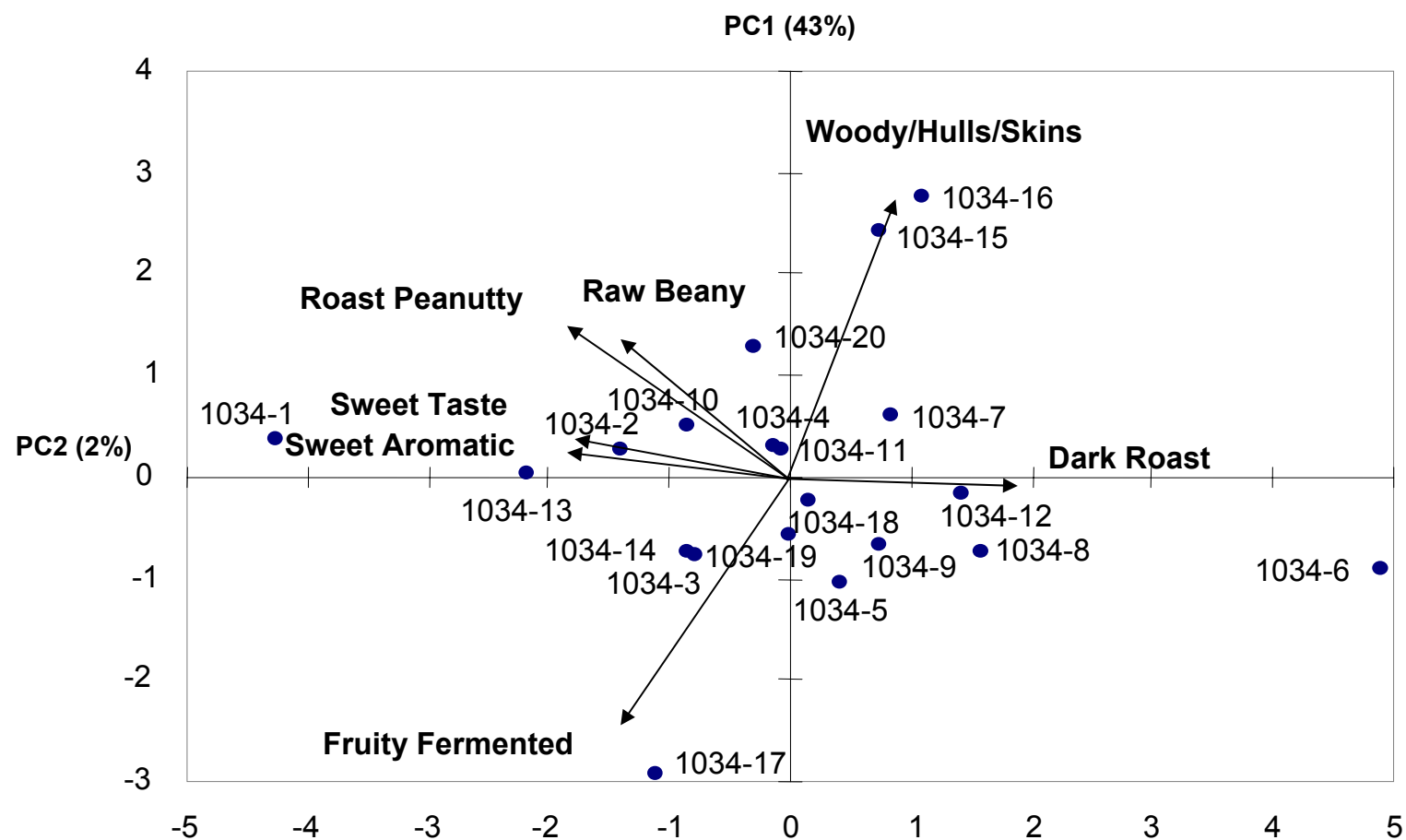
APPENDIX 1. Principal Component Biplot of Lot 1020



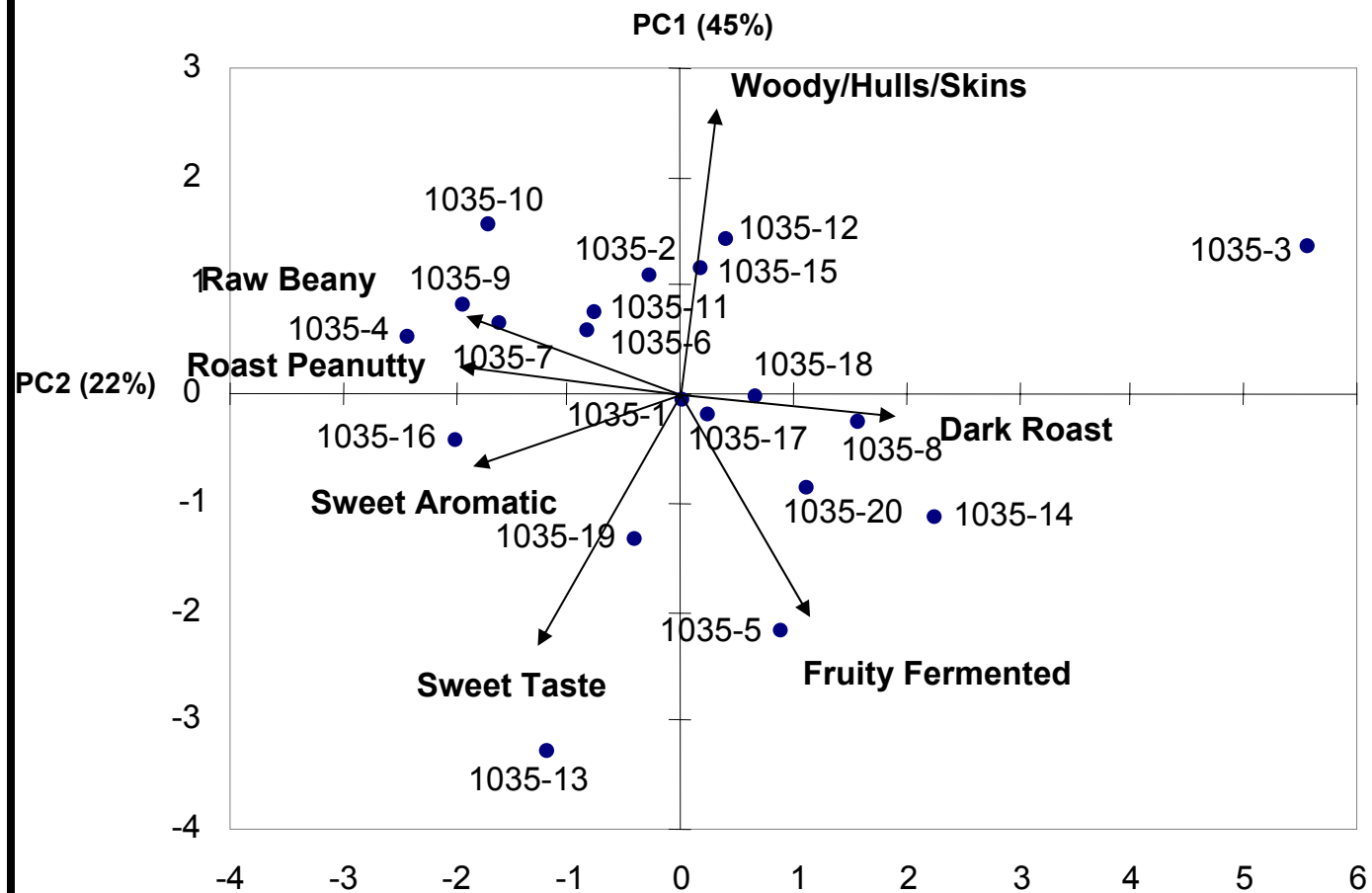
APPENDIX 2. Principal Component Biplot of Lot 1022



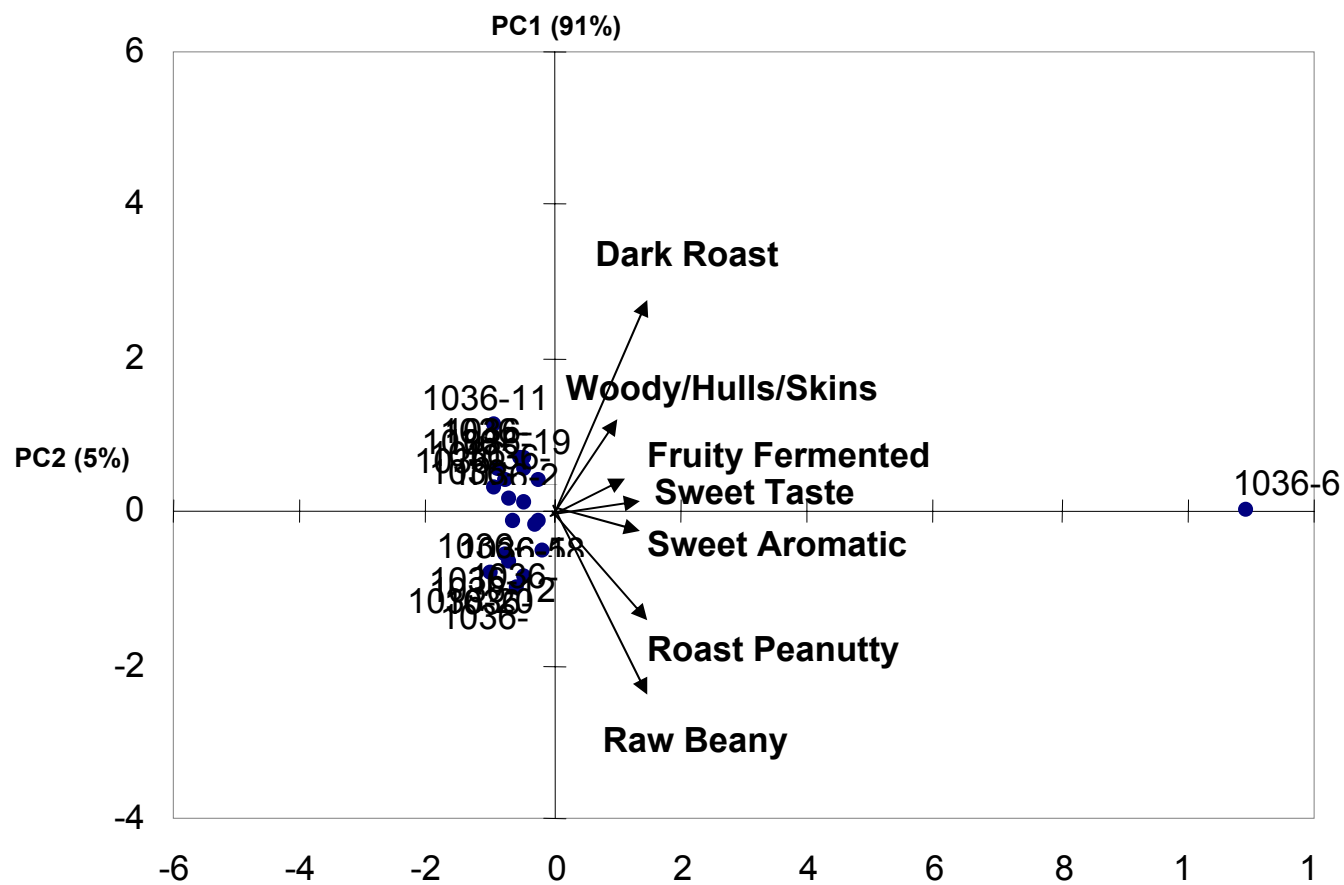
APPENDIX 3. Principal Component Biplot of Lot 1034



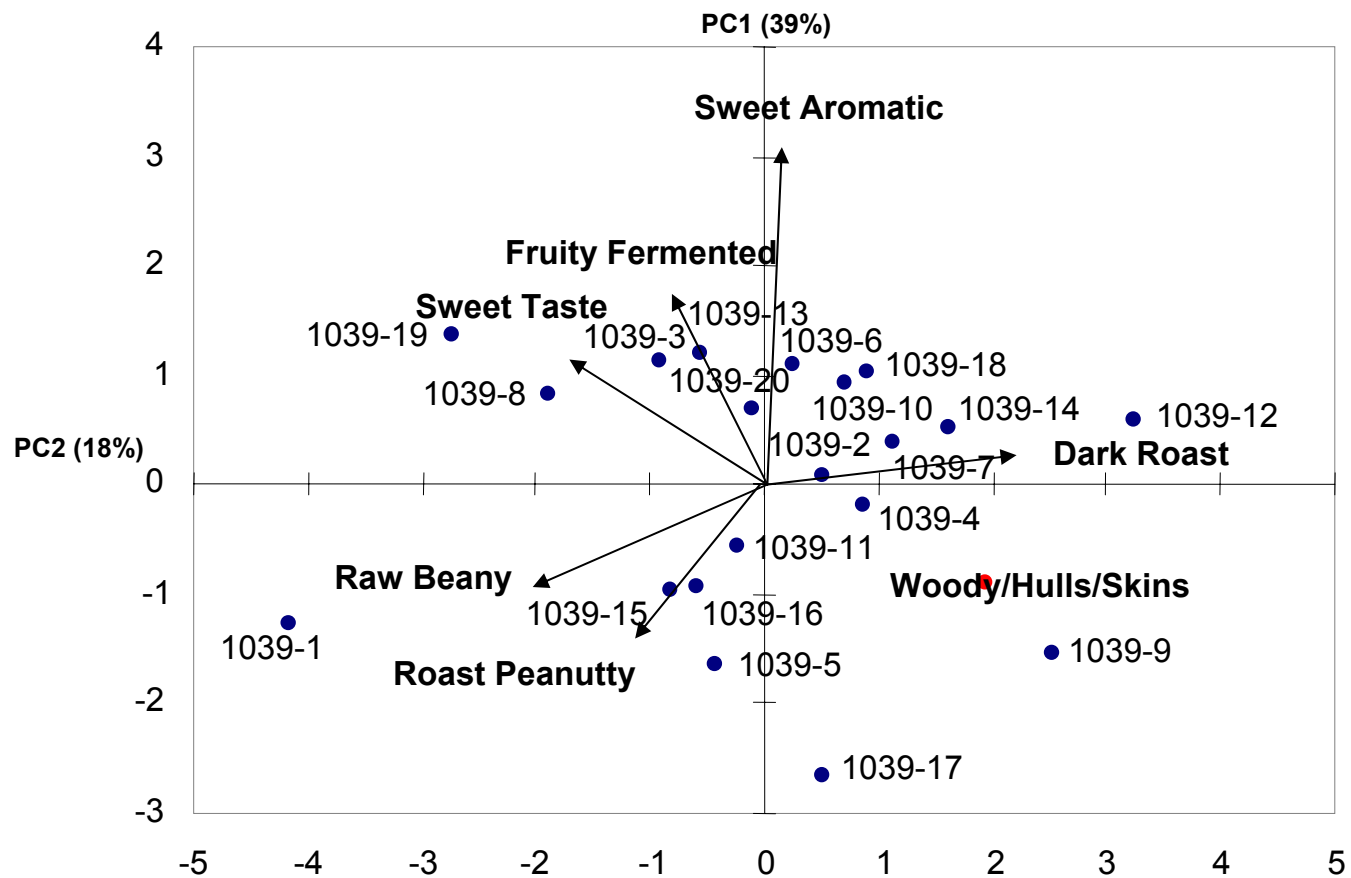
APPENDIX 4. Principal Component Biplot of Lot 1035



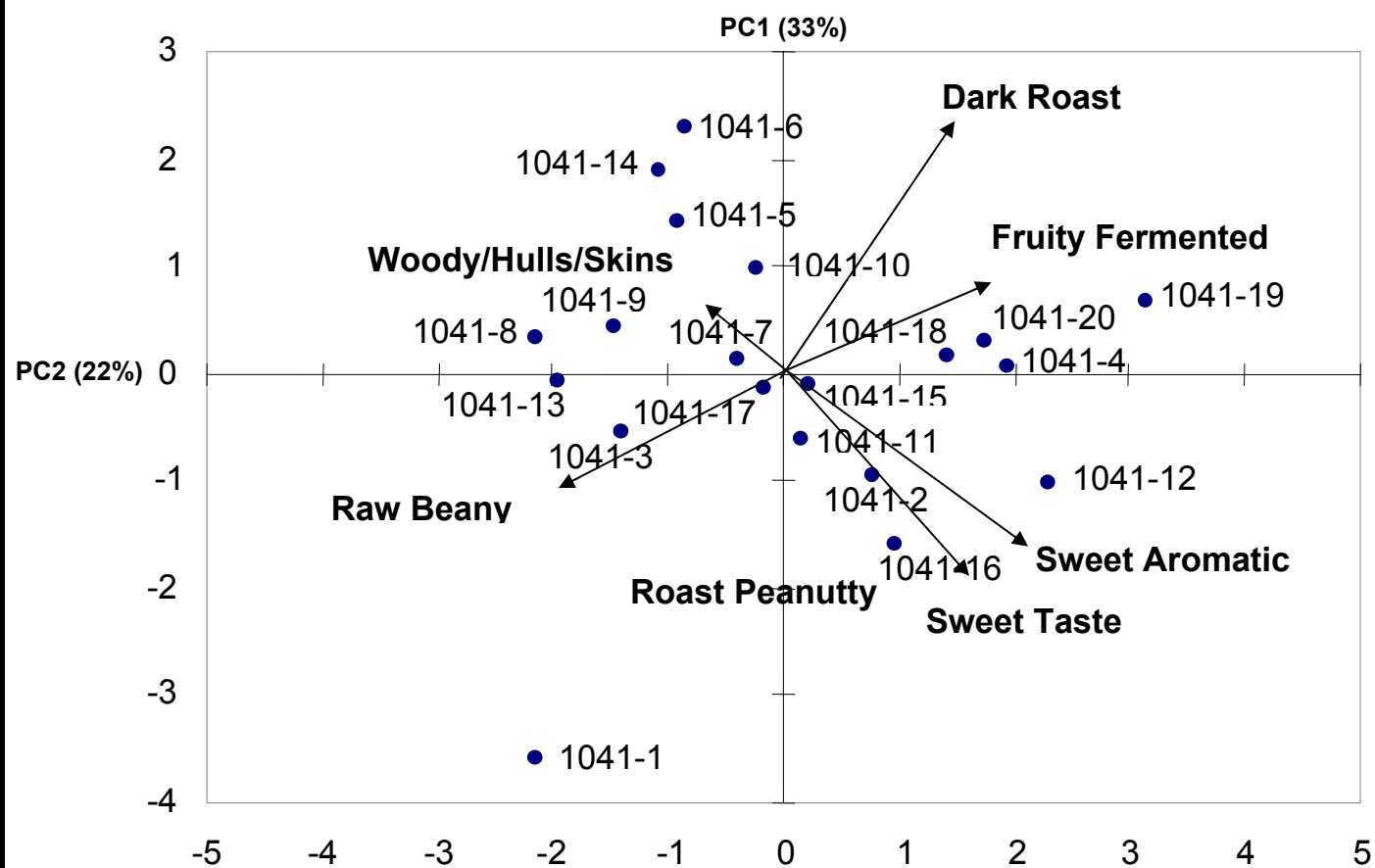
APPENDIX 5. Principal Component Biplot of Lot 1036



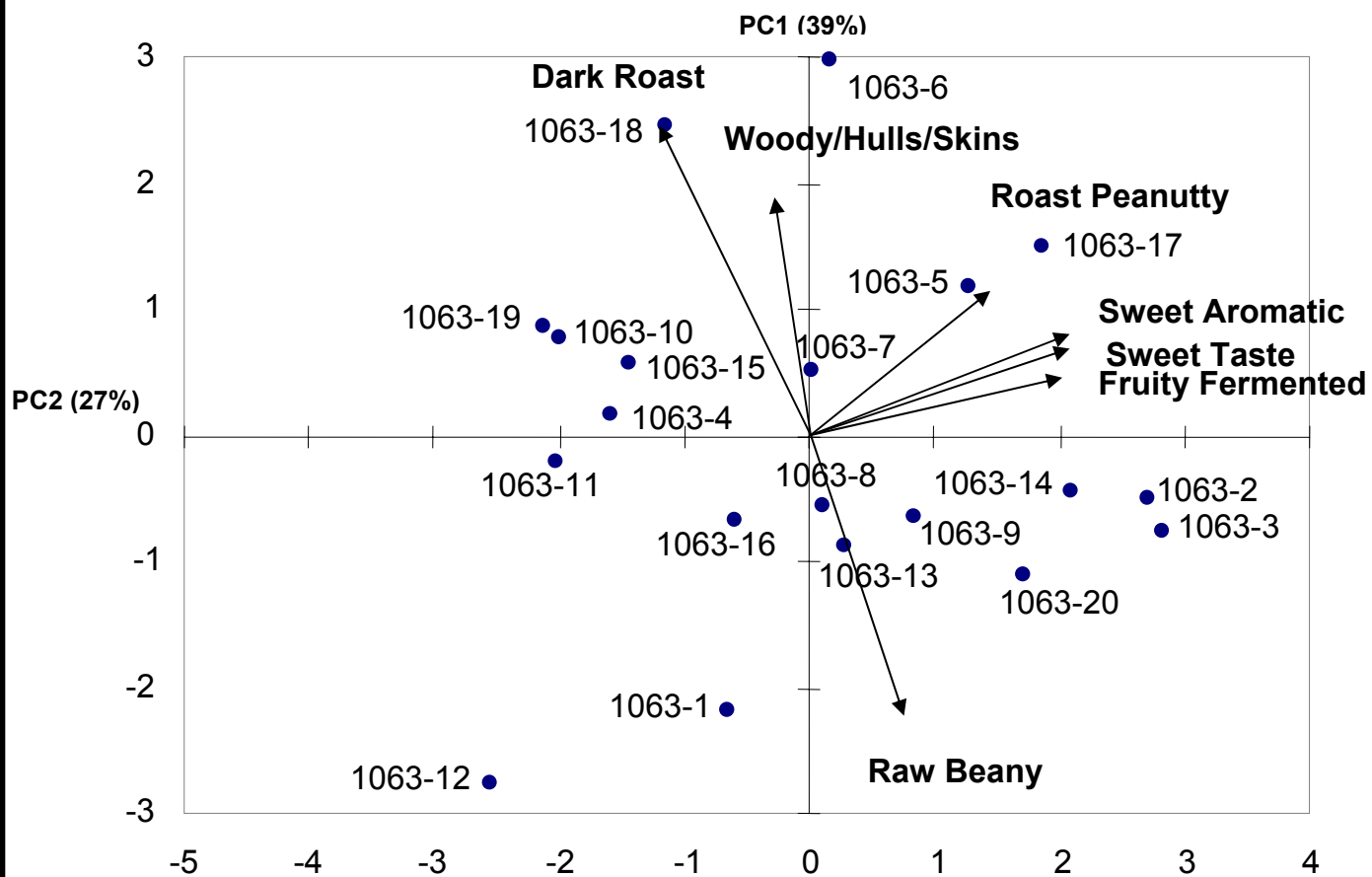
APPENDIX 6. Principal Component Biplot Lot 1039



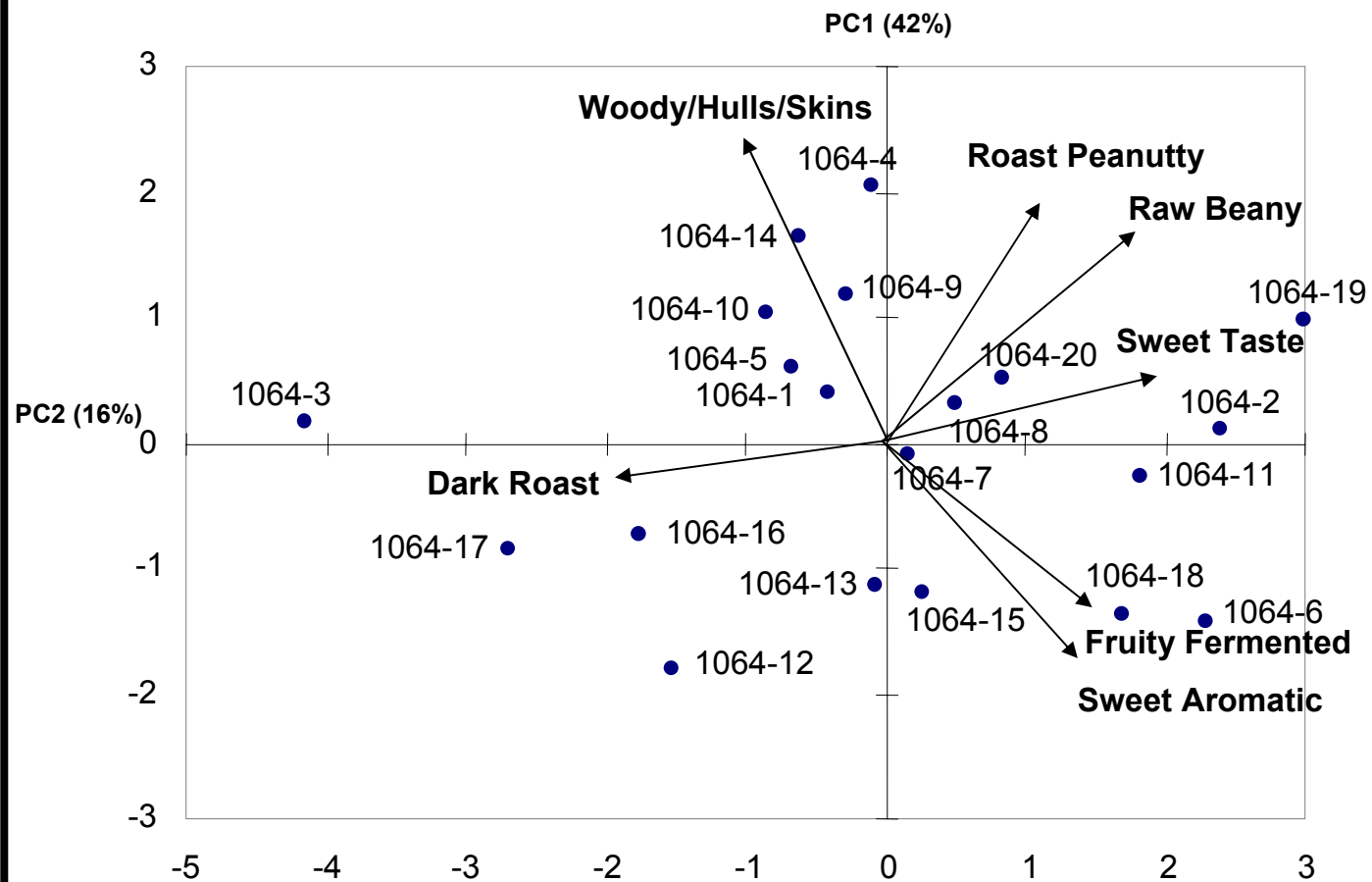
APPENDIX 7. Principal Component Biplot of Lot 1041



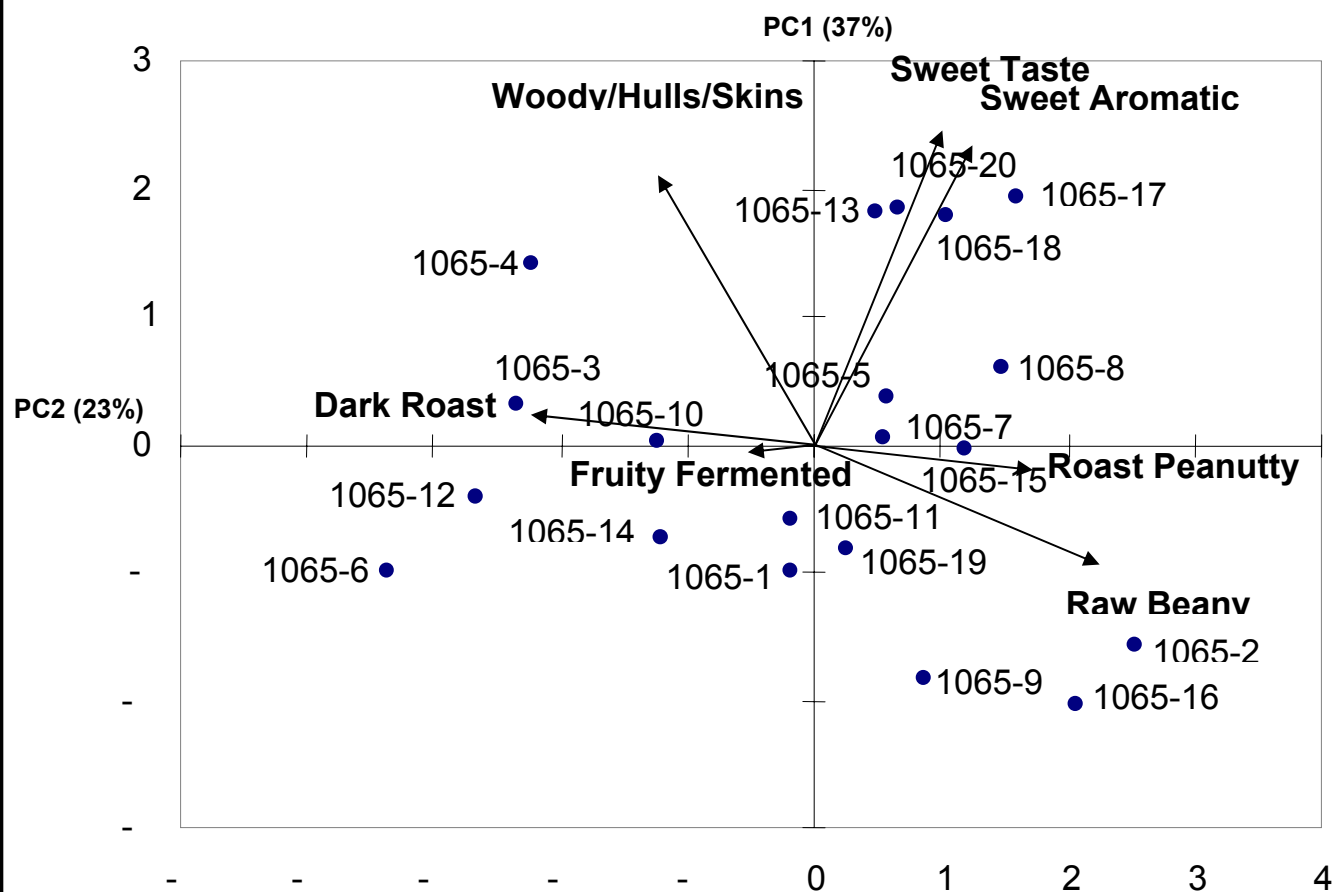
APPENDIX 8. Principal Component Biplot of Lot 1063



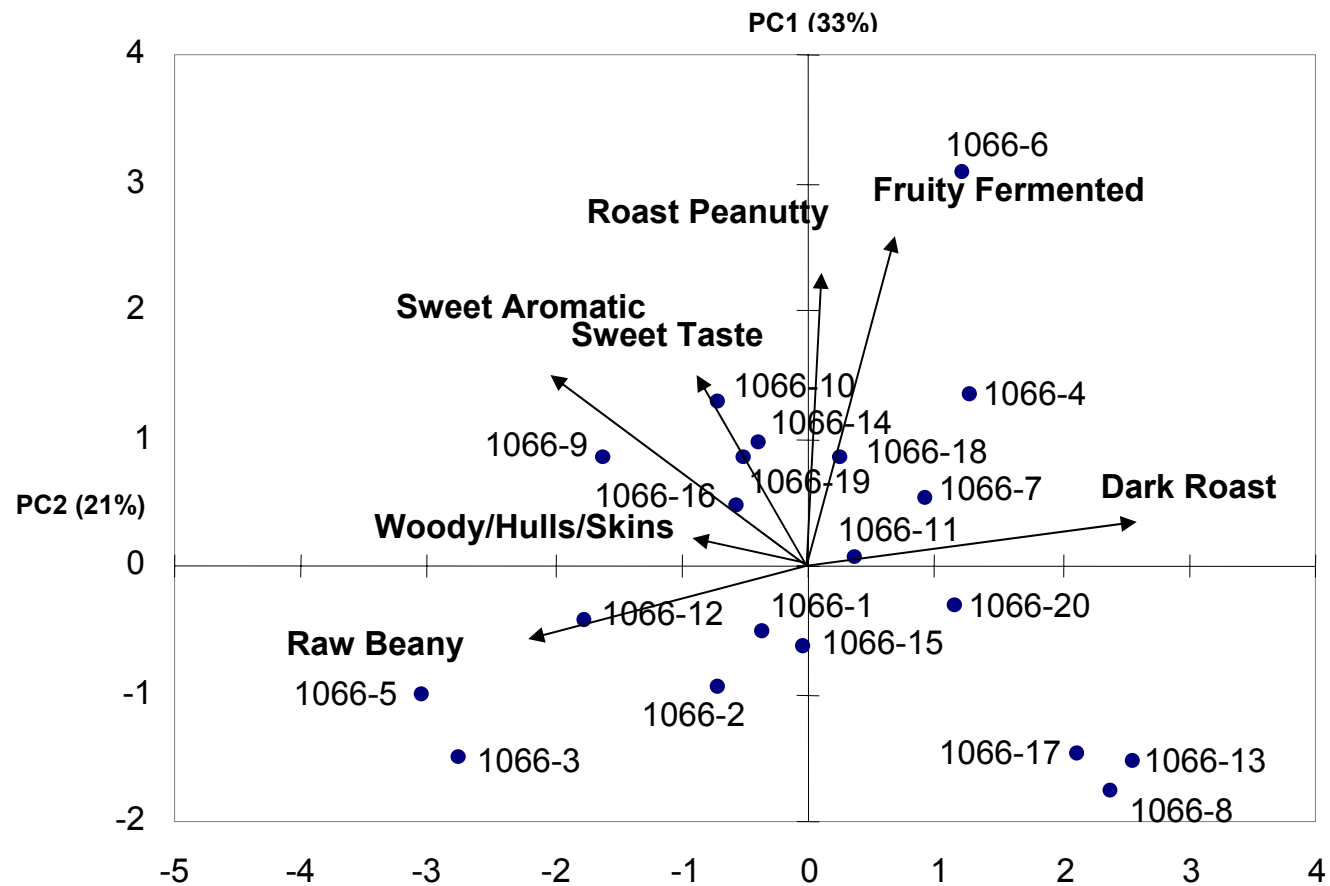
APPENDIX 9. Principal Component Biplot of Lot 1064



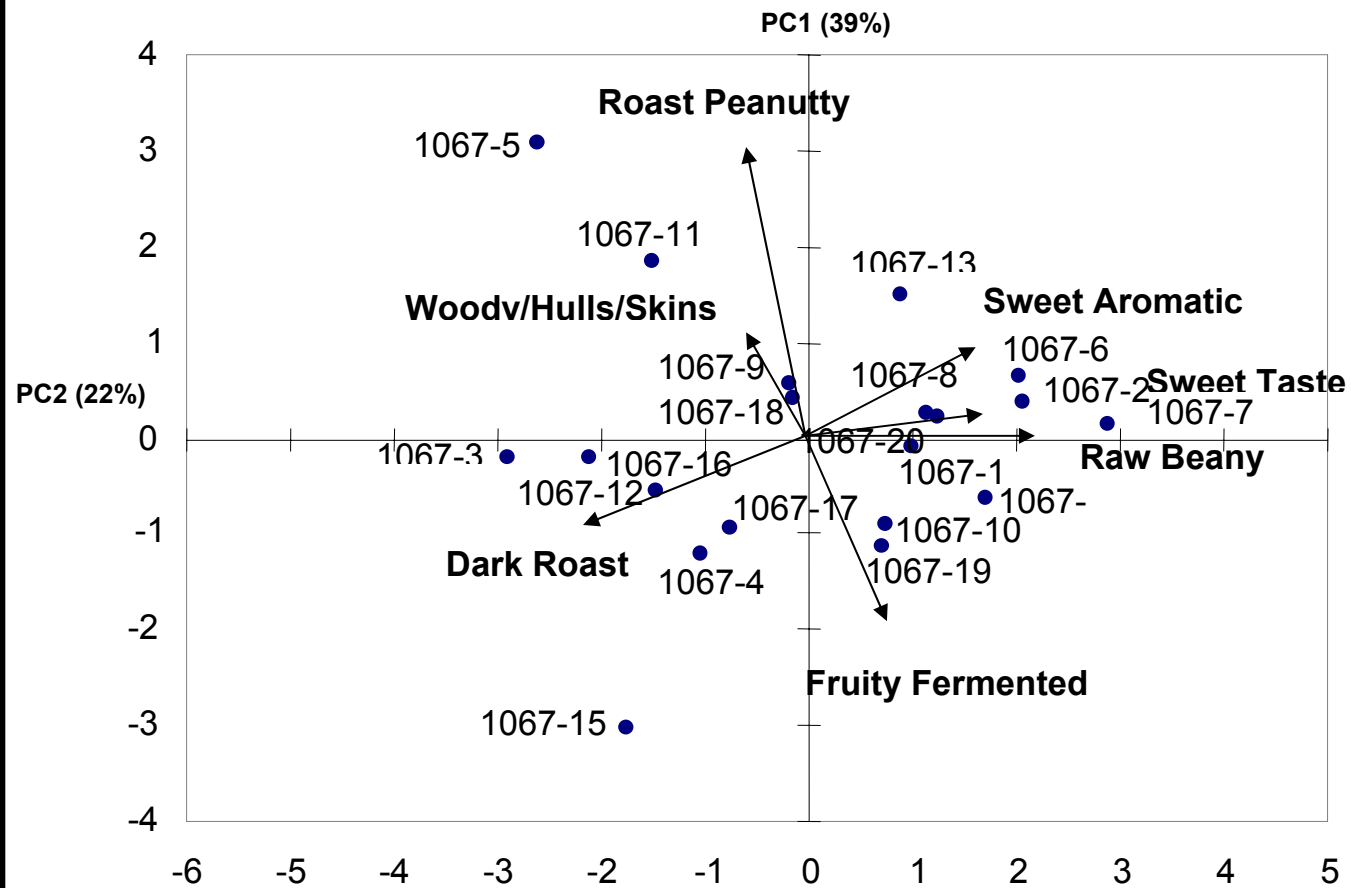
APPENDIX 10. Principal Component Biplot of Lot 1065



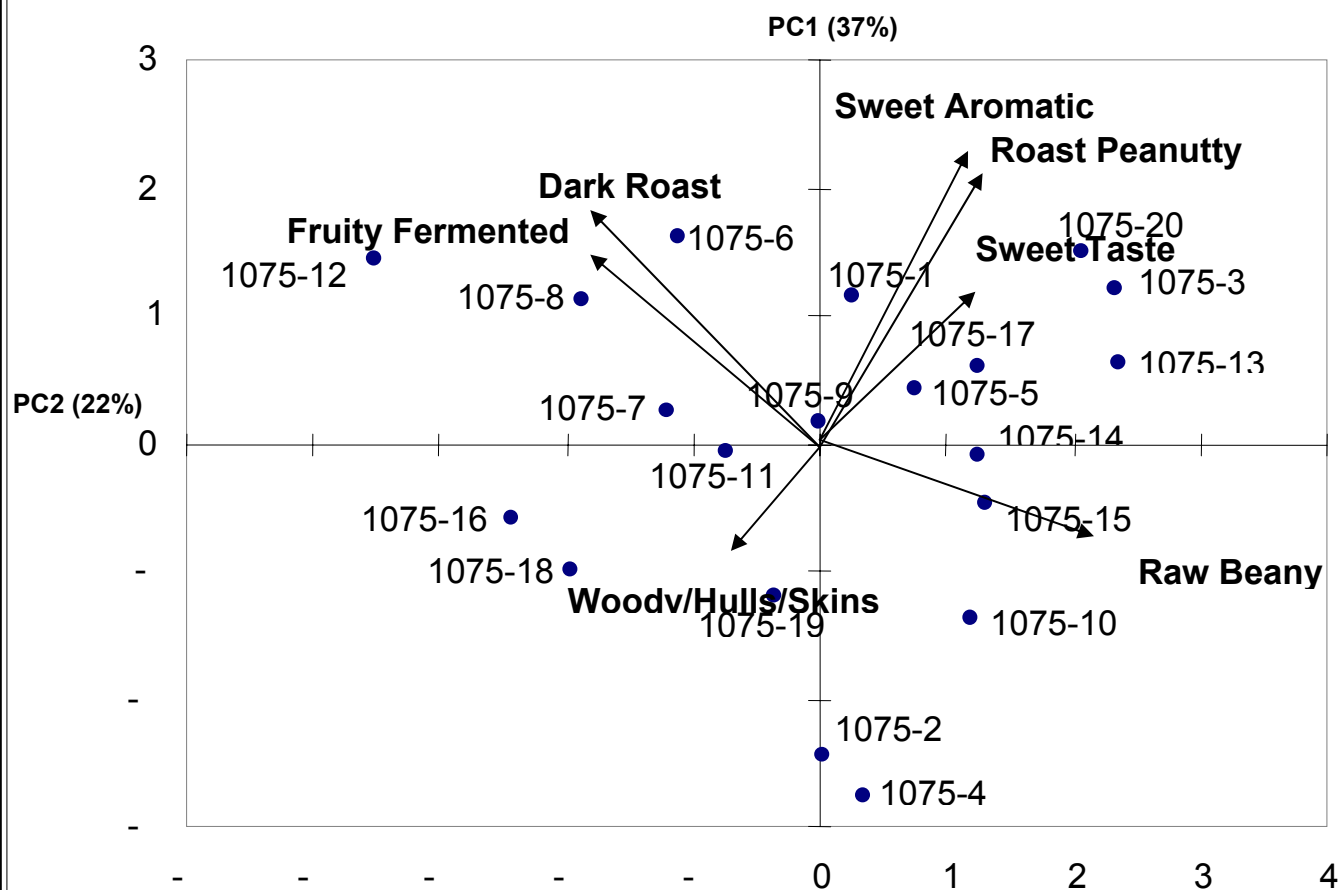
APPENDIX 11. Principal Component Biplot of Lot 1066



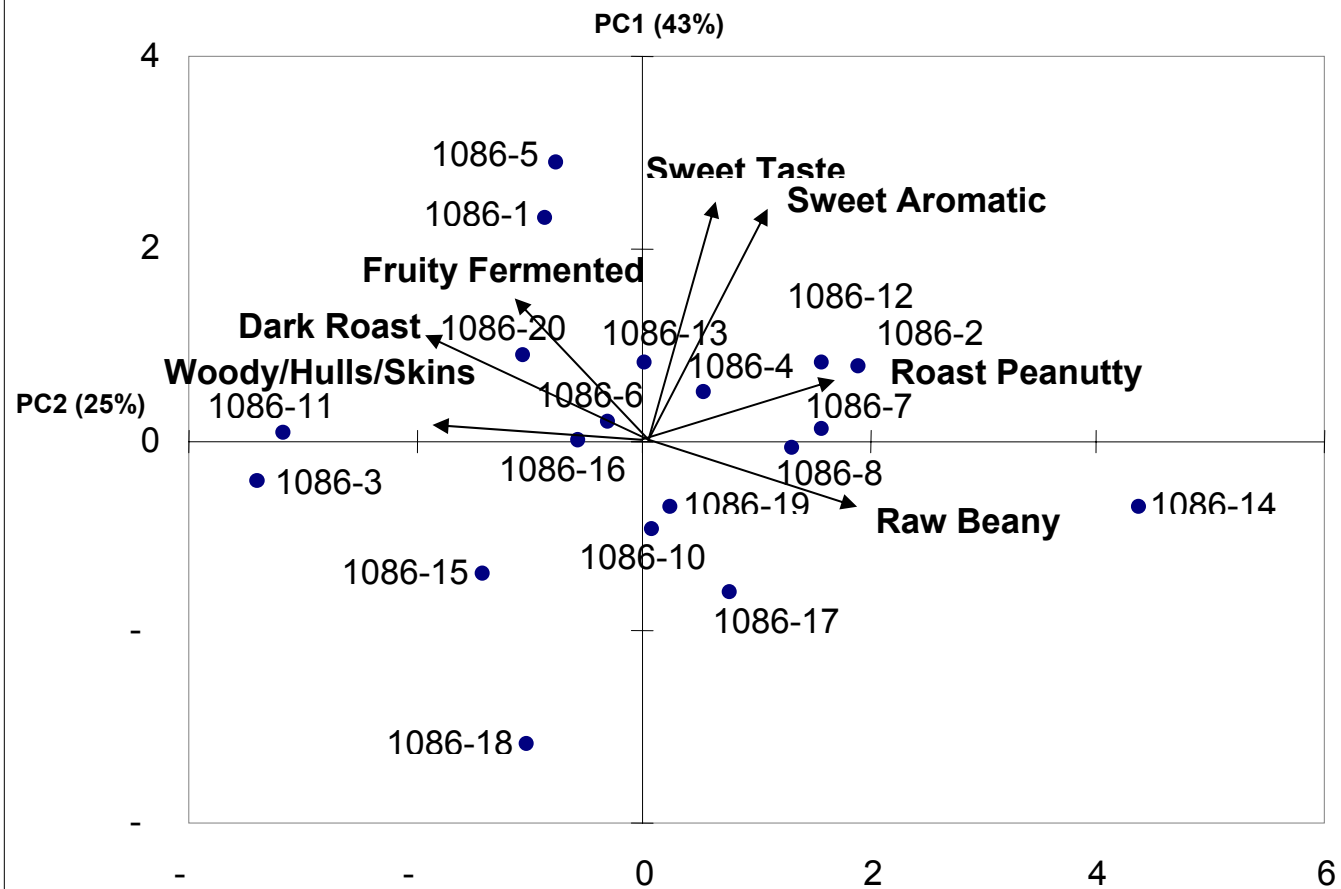
APPENDIX 12. Principal Component Biplot of Lot 1067



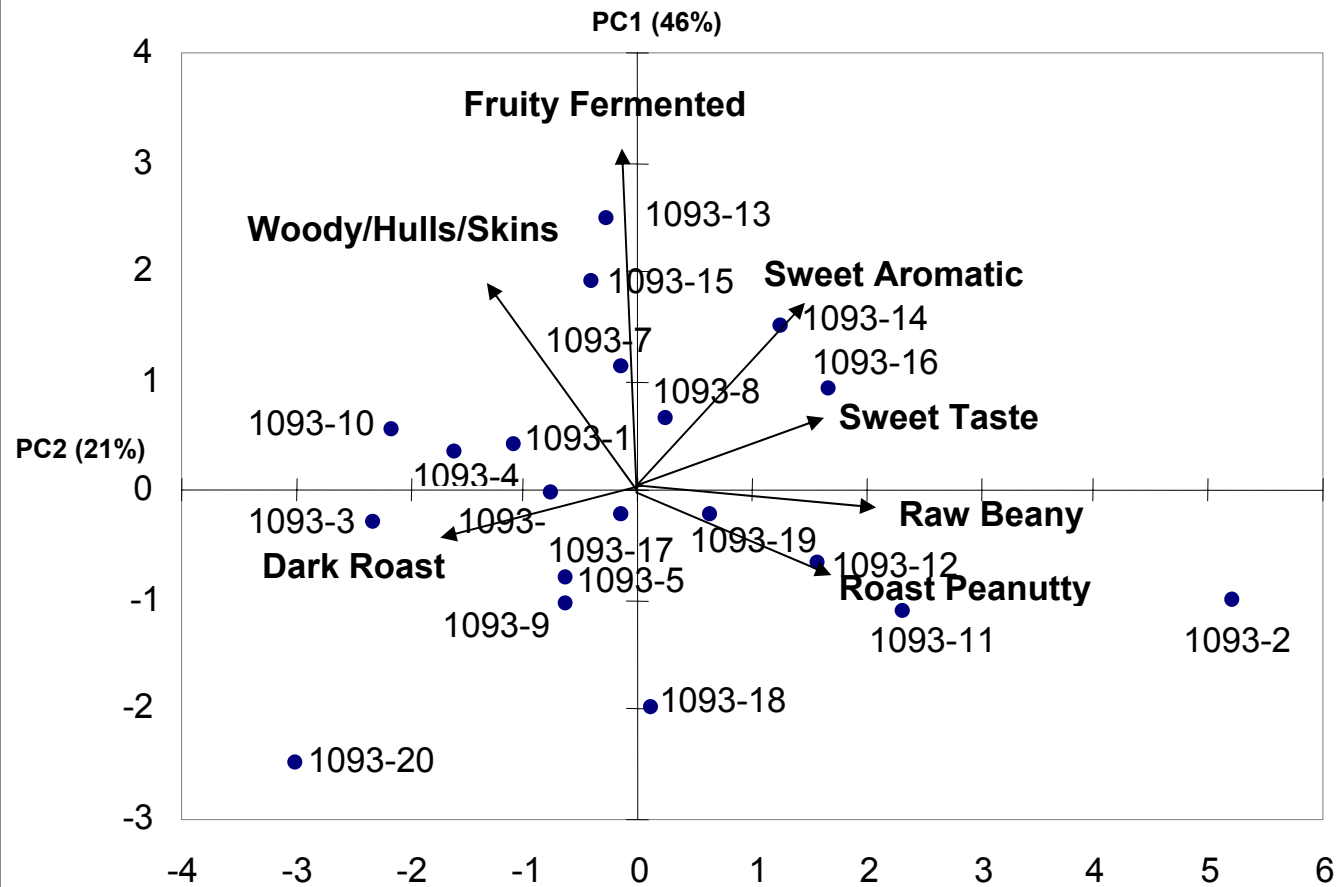
APPENDIX 13. Principal Component Biplot of Lot 1075



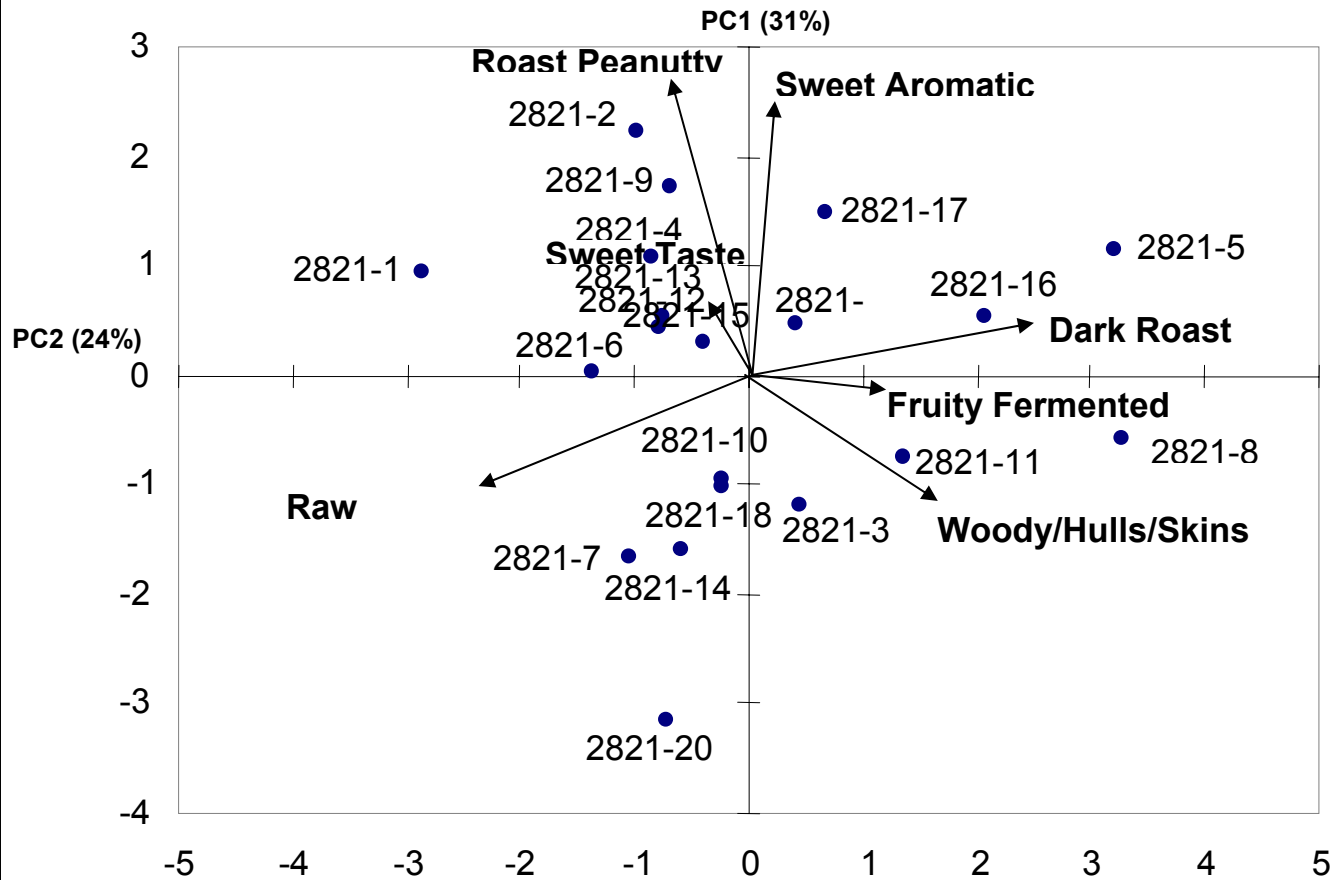
APPENDIX 14. Principal Component Biplot of Lot 1086



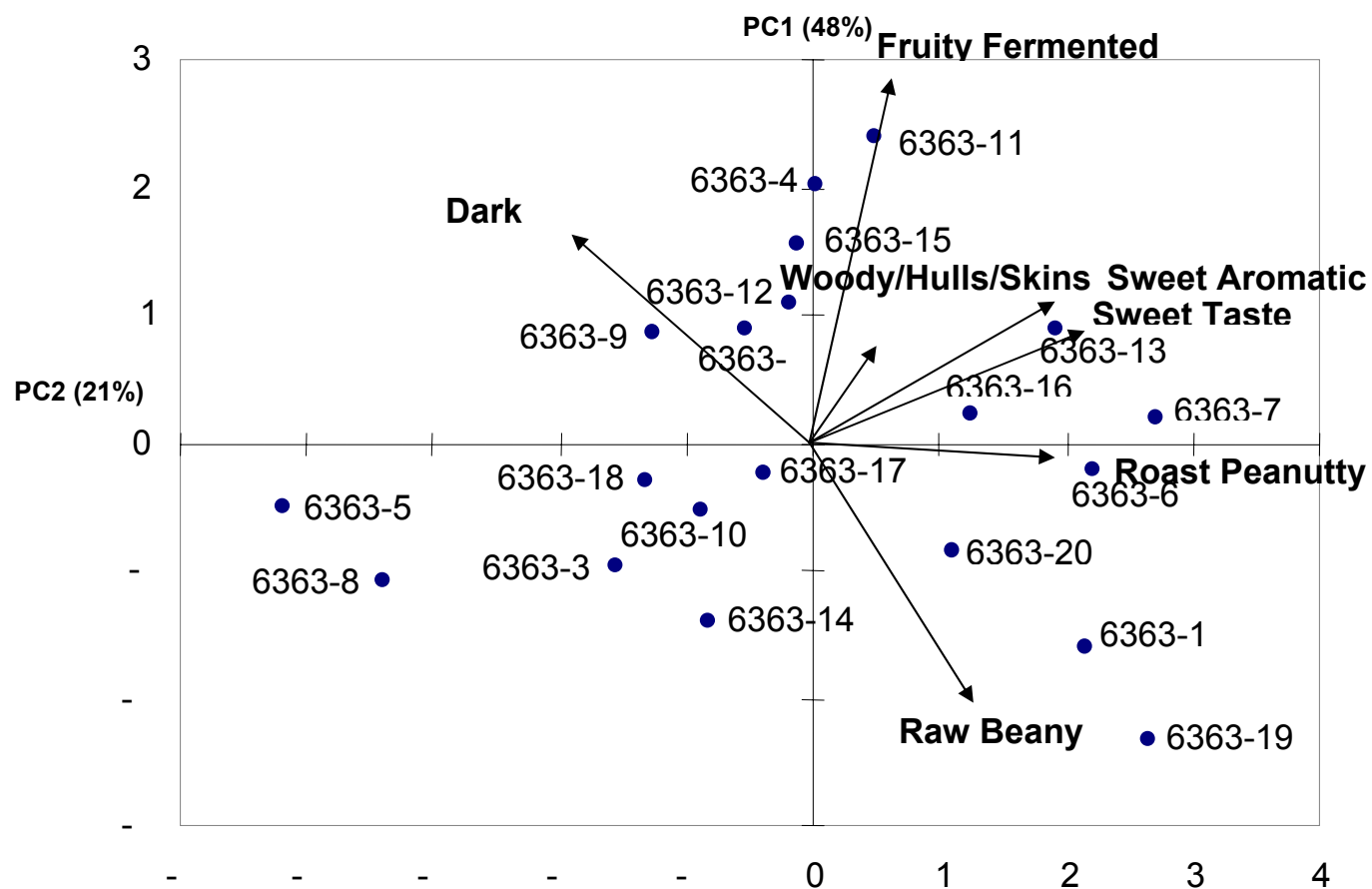
APPENDIX 15. Principal Component Biplot of Lot 1093



APPENDIX 16. Principal Component Biplot of Lot 2821



APPENDIX 17. Principal Component Biplot of Lot 6363



Appendix 18. Peanut Flavor Ballot for Descriptive Sensory Analysis

FLAVOR INTENSITY AS COMPARED TO CONTROL SAMPLE

Date:

Name:

Reference Sample: MQHR Control

Sample ID	Reference							
Attribute								
Roast Peanuttty	6.0							
Sweet Aromatic	3.5							
Other Aromatics								
Dark Roast	3.0							
Raw Beany	2.0							
Woody/Hulls/Skins	3.0							
Cardboardy/Stale								
Earthy/Musty/Wet Dirt								
Painty/Old Oil								
Plastic Chemical								
Metallic								
Fruity/Fermented								
Sweet Taste	2.5							
Sour Taste								
Bitter	3.0							
Astringency	1.0							

Reference Guide

Cooked Grape in Grape Juice	10 (Strong)
Orange in Orange Juice	7 (Moderate)
Cooked Apple in Applesauce	5 (Weak)
Baking Soda in Saltine Cracker	2 (Trace)
None Detected	0 (ND)

**Appendix 19. Line Scales Used for Consumer Study on Fruity Fermented Peanuts:
Labeled Magnitude Scale and Labeled Affective Magnitude Scale**

PEANUT PASTE CONSUMER BALLOT

PLEASE READY THE DIRECTIONS CAREFULLY.

Please taste the peanut samples in the order provided: _____, _____, _____
Record your responses below by **marking an “X” anywhere on the line** for the perceived intensity. After tasting each sample please rinse well with the water provided. **Please complete all scales and answer the questions at the end of the ballot.** If you have any questions please ask one of the panel leaders. Thank you for participating.

Roasted Peanuty Flavor

Strength / Intensity

— STRONGEST IMAGINABLE

—

—

— VERY STRONG

—

— STRONG

—

— MODERATE

—

— WEAK

—

— BARELY DETECTABLE

Liking / Disliking

— GREATEST IMAGINABLE LIKE

—

— LIKE EXTREMELY

—

— LIKE VERY MUCH

—

— LIKE MODERATELY

—

— LIKE SLIGHTLY

—

— NEITHER LIKE NOR DISLIKE

—

— DISLIKE SLIGHTLY

—

— DISLIKE MODERATELY

—

— DISLIKE VERY MUCH

—

— DISLIKE EXTREMELY

—

— GREATEST IMAGINABLE DISLIKE

Sample _____

Sweet Taste

Strength / Intensity

—	STRONGEST IMAGINABLE
—	VERY STRONG
—	STRONG
—	MODERATE
—	WEAK
—	BARELY DETECTABLE

Liking / Disliking

—	GREATEST IMAGINABLE LIKE
—	LIKE EXTREMELY
—	LIKE VERY MUCH
—	LIKE MODERATELY
—	LIKE SLIGHTLY
—	NEITHER LIKE NOR DISLIKE
—	DISLIKE SLIGHTLY
—	DISLIKE MODERATELY
—	DISLIKE VERY MUCH
—	DISLIKE EXTREMELY
—	GREATEST IMAGINABLE DISLIKE

Likes _____

Dislikes _____

Sample _____

Fresh Peanut Flavor

Strength / Intensity

Liking / Disliking

— STRONGEST IMAGINABLE

—

— VERY STRONG

—

— STRONG

—

— MODERATE

—

— WEAK

—

— BARELY DETECTABLE

— GREATEST IMAGINABLE LIKE

— LIKE EXTREMELY

— LIKE VERY MUCH

— LIKE MODERATELY

— LIKE SLIGHTLY

— NEITHER LIKE NOR DISLIKE

— DISLIKE SLIGHTLY

— DISLIKE MODERATELY

— DISLIKE VERY MUCH

— DISLIKE EXTREMELY

— GREATEST IMAGINABLE DISLIKE

Likes _____

Dislikes _____

Sample _____

Overall Liking

Liking / Disliking

—	GREATEST IMAGINABLE LIKE
—	LIKE EXTREMELY
—	LIKE VERY MUCH
—	LIKE MODERATELY
—	LIKE SLIGHTLY
—	NEITHER LIKE NOR DISLIKE
—	DISLIKE SLIGHTLY
—	DISLIKE MODERATELY
—	DISLIKE VERY MUCH
—	DISLIKE EXTREMELY
—	GREATEST IMAGINABLE DISLIKE

Please rank samples in order of preference. 1 = Most preferred 3 = Least preferred
214 _____ 545 _____ 689 _____

Please describe the differences in your least and most preferred samples.

Please rate how easy or difficult it was to use this scale. 1 = Easy 9 = Difficult
1 2 3 4 5 6 7 8 9

**Appendix 20. Category Scale Used for Consumer Study on Fruity Fermented Peanuts:
9-point Hedonic Scale**

Peanut Paste Consumer Ballot

Please taste the peanut samples in the order provided: _____, _____, _____

Record your responses below by **circling** the appropriate number for the strength/intensity and like/dislike. Make sure to rinse your mouth between each sample. **Please complete all ratings and answer the questions at the end of the ballot.**

Sample _____

Strength/Intensity	Liking/Disliking
Roasted Peanuttty Flavor: <div style="display: flex; justify-content: space-between; padding: 5px;"> 1 2 3 4 5 6 7 8 9 </div> <div style="display: flex; justify-content: space-between; padding: 5px;"> Not at all Extremely </div> <div style="display: flex; justify-content: space-between; padding: 5px;"> Peanuttty Peanuttty </div>	Roasted Peanuttty Flavor: <div style="display: flex; justify-content: space-between; padding: 5px;"> 1 2 3 4 5 6 7 8 9 </div> <div style="display: flex; justify-content: space-between; padding: 5px;"> Dislike Like </div> <div style="display: flex; justify-content: space-between; padding: 5px;"> Extremely Extremely </div>
Strength/Intensity	Liking/Disliking
Sweet Taste: <div style="display: flex; justify-content: space-between; padding: 5px;"> 1 2 3 4 5 6 7 8 9 </div> <div style="display: flex; justify-content: space-between; padding: 5px;"> Not at all Extremely </div> <div style="display: flex; justify-content: space-between; padding: 5px;"> Sweet Sweet </div>	Sweet Taste: <div style="display: flex; justify-content: space-between; padding: 5px;"> 1 2 3 4 5 6 7 8 9 </div> <div style="display: flex; justify-content: space-between; padding: 5px;"> Dislike Like </div> <div style="display: flex; justify-content: space-between; padding: 5px;"> Extremely Extremely </div>
Strength/Intensity	Liking/Disliking
Fresh Peanut Flavor: <div style="display: flex; justify-content: space-between; padding: 5px;"> 1 2 3 4 5 6 7 8 9 </div> <div style="display: flex; justify-content: space-between; padding: 5px;"> Not at all Extremely </div> <div style="display: flex; justify-content: space-between; padding: 5px;"> Fresh Fresh </div>	Fresh Peanut Flavor: <div style="display: flex; justify-content: space-between; padding: 5px;"> 1 2 3 4 5 6 7 8 9 </div> <div style="display: flex; justify-content: space-between; padding: 5px;"> Dislike Like </div> <div style="display: flex; justify-content: space-between; padding: 5px;"> Extremely Extremely </div>

Liking/Disliking
Overall Liking: <div style="display: flex; justify-content: space-between; padding: 5px;"> 1 2 3 4 5 6 7 8 9 </div> <div style="display: flex; justify-content: space-between; padding: 5px;"> Dislike Like </div> <div style="display: flex; justify-content: space-between; padding: 5px;"> Extremely Extremely </div>

Likes _____ Dislikes _____

Sample _____

Strength/Intensity										Liking/Disliking																		
Roasted Peanuttty Flavor:										Roasted Peanuttty Flavor:																		
1	2	3	4	5	6	7	8	9		1	2	3	4	5	6	7	8	9										
Not at all Peanuttty										Dislike Extremely										Like Extremely								
Strength/Intensity										Liking/Disliking																		
Sweet Taste:										Sweet Taste:																		
1	2	3	4	5	6	7	8	9		1	2	3	4	5	6	7	8	9										
Not at all Sweet										Dislike Extremely										Like Extremely								
Strength/Intensity										Liking/Disliking																		
Fresh Peanut Flavor:										Fresh Peanut Flavor:																		
1	2	3	4	5	6	7	8	9		1	2	3	4	5	6	7	8	9										
Not at all Fresh										Dislike Extremely										Like Extremely								

Liking/Disliking								
Overall Liking:								
1	2	3	4	5	6	7	8	9
Dislike								Like
Extremely								Extremely

Likes _____

Dislikes _____

Sample _____

Strength/Intensity										Liking/Disliking																			
Roasted Peanuttty Flavor:										Roasted Peanuttty Flavor:																			
1	2	3	4	5	6	7	8	9		1	2	3	4	5	6	7	8	9											
Not at all										Extremely										Dislike					Like				
Peanuttty										Peanuttty										Extremely					Extremely				
Strength/Intensity										Liking/Disliking																			
Sweet Taste:										Sweet Taste:																			
1	2	3	4	5	6	7	8	9		1	2	3	4	5	6	7	8	9											
Not at all										Extremely										Dislike					Like				
Sweet										Sweet										Extremely					Extremely				
Strength/Intensity										Liking/Disliking																			
Fresh Peanut Flavor:										Fresh Peanut Flavor:																			
1	2	3	4	5	6	7	8	9		1	2	3	4	5	6	7	8	9											
Not at all										Extremely										Dislike					Like				
Fresh										Fresh										Extremely					Extremely				

Liking/Disliking								
Overall Liking:								
1	2	3	4	5	6	7	8	9
Dislike								Like
Extremely								Extremely

Please rank the samples in order of preference. 1 = Most preferred 3 = Least preferred

617_____ 323_____ 192_____

Describe the differences in your least and most favorite samples?

Please rate how easy or difficult it was to use this scale. 1 = Easy 9 = Difficult

1 2 3 4 5 6 7 8 9

Appendix 21. Uncertainty Associated with Sampling Peanuts for Fruity Fermented Off-flavor

Uncertainty Associated with Sampling Peanuts for Fruity-Fermented Off-Flavor¹

T.B. Whitaker^{2*}, A.B. Slate³, J.L. Greene⁴, K. Hendrix⁵, and T.H. Sanders⁵

ABSTRACT

Individual peanut seed may develop a fruity fermented (FF) off-flavor if exposed to elevated temperatures after digging. Typically, high moisture, immature peanuts exposed to temperatures above 35°C either in the windrow or during artificial curing may develop the FF off-flavor. Because of the uncertainty associated with sampling and FF measurement, it is difficult to obtain a precise estimate of the true FF intensity within a bulk lot. The objectives of this study were to determine the variability associated with the sampling and measurement steps of the test procedure used to measure FF intensity in a bulk lot and to describe the FF distribution among replicated sample test results taken from the same lot. Twenty test samples of 250 g each were randomly taken from 20 medium grade lots of runner-type peanuts identified by commercial testing as having FF intensities ranging from 0.0 (no FF off flavor) to 4.0. Each test sample was prepared according to published guidelines and the FF intensity of each sample was measured by 8 members of a highly trained descriptive sensory panel. The total variability associated with the FF test procedure was partitioned into sampling and measurement variances for each lot. Each variance was a function of the FF intensity. Using the standard commercial FF test procedure (300 g sample and averaging the score of 5 panel members), the measurement and sampling variances accounted for 31.4% and 68.6% of the total error, respectively. The FF distribution among replicated sample test results tended to be positively skewed and could be described by the compound gamma distribution. The best use of resources to reduce the total variability of the FF test procedure would be to increase sample size to reduce variability of the sampling step.

Key Words: peanut, flavor, sampling, uncertainty, flavor panel.

Individual peanut seed can develop an objectionable off-flavor if exposed to certain environmental conditions. Typically, high moisture, immature peanuts exposed to temperatures above 35°C will produce a fruity-fermented (FF) off-flavor (Sanders et al., 1989a,b; Sanders et al., 1990). The intensity of FF off-flavor appears to be directly proportional to temperature, immaturity, and kernel moisture content (Whitaker and Dickens, 1964). High temperature exposure can occur in the windrow when peanuts are exposed to direct radiation from the sun or during curing when artificial heat is added to the drying air. When peanuts are exposed to these conditions, the assumption can be made that within each bulk lot of shelled peanuts, there exists a FF distribution among individual peanuts. Probably, a large percentage of peanuts in a bulk lot have no measurable FF off-flavor intensity and the remaining small percentage of peanuts have varying intensities of the FF off-flavor. If all peanuts in a lot were subjected to the same temperature, then the FF distribution among individual peanuts may be closely related to the maturity distribution among individual peanuts in the lot (Sanders, 1990; Sanders and Bett, 1995).

Currently, the peanut industry estimates the mean level of the FF attribute among all peanuts in a bulk lot by taking a 300 g sample of peanuts from the bulk lot. The test sample is roasted, blanched, and ground into a paste, a subsample of paste is removed from the comminuted test sample, and each member of a trained flavor panel scores the FF intensity. Each panel member is highly trained and experienced in evaluating peanut flavor as described by the peanut flavor lexicon (Johnsen et al., 1988; Sanders, et al., 1989b). Each panel member evaluates the intensity of the peanut flavor descriptors using standard, published sensory analysis procedures. All panel member scores are averaged and the average score is the best estimate of the true FF off-flavor intensity among all peanuts in the lot.

Customers who buy U.S. peanuts may specify in their purchase contract that the peanuts must have

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an average FF intensity below some threshold (Greene et al., 2006a; personal communication J. Leek and Associates, 2006). Occasionally, separate samples taken from the same lot by the seller and buyer will not agree when scored by their respective trained flavor panels. If a customer receives a lot that tests greater than a specified threshold, an economic hardship is created for both the buyer and seller of the lot. The lack of agreement in the FF off-flavor score is probably due to the uncertainty associated with the test procedure used by the seller and buyer of the peanuts to measure the FF intensity of peanuts in the bulk lot.

The test procedure used to estimate the FF intensity in a bulk lot consists of sampling, sample preparation, and measurement steps. Each step contributes to the overall uncertainty associated with the test procedure. Because of the uncertainty of the FF test procedure, it is not possible to determine with 100% certainty the true average FF intensity among all peanuts in the bulk lot by measuring the average FF intensity of peanuts in a sample taken from the lot.

Because of the uncertainty associated with sampling, sample preparation, and measurement steps, lots can be misclassified by a sampling plan. There is some chance that good lots (true FF intensity is below a defined tolerance) will test bad by the sampling plan (seller's risk) and some chance that bad lots (true average FF intensity is above a defined tolerance) will test good (buyer's risk) by the sampling plan. The performance (number of lots miss-classified or the buyer's and seller's risks) of a specific sampling plan can be predicted if the variability associated with sampling and measurement steps of the test procedure can be determined and if the FF distribution among replicated sample test results can be described.

The objectives of this study were to: (1) measure the total variability associated with the test procedure used to measure the FF intensity in peanuts, (2) partition the total variability associated with the FF test procedure into sampling, sample preparation, and measurement variance components, (3) measure the FF distribution among replicated samples taken from a bulk lot, and (4) demonstrate how to make best use of resources to reduce the uncertainty of the FF test procedure.

Materials and Methods

Theoretical Considerations

It was assumed that the total variability, (s_t^2) associated with the test procedure to estimate the FF intensity of peanuts in a bulk lot is the sum of

the sampling (s_s^2), sample preparation (s_{sp}^2), and measurement (s_m^2) variances (Whitaker et al., 1974).

$$s_t^2 = s_s^2 + s_{sp}^2 + s_m^2 \quad (1)$$

Sampling error occurs because the FF distribution among individual peanuts causes differences among replicated sample test results taken from the same lot. Once a sample is prepared (roasted, blanched, and ground), the FF intensity may differ among replicated subsamples of paste taken from the same comminuted sample (sample preparation error). Finally, evaluation of the FF intensity may differ among individual sensory panel members when tasting peanuts from the same sample (measurement error). It was assumed that the sample preparation error is negligible ($s_{sp}^2 = 0$) since all peanuts in the sample are ground into a homogenous paste and the FF intensity will not differ among replicated subsamples taken from the same comminuted test sample.

Experimental design

To measure the sampling and measurement variability and the FF distribution among sample test results, a balanced nested design was developed (Figure 1). Twenty bulk lots of medium runner type peanuts were identified by commercial testing as having FF off-flavor intensity ranging from 0.0 (no FF off flavor) to 4.0. A 5 kg bulk sample was removed from each identified lot. Using a riffle divider, 20 samples of 250 g each were removed from the 5 kg bulk sample. Using standard industry procedures (Greene, J.L. et al. 2006b), each 250 g sample was roasted, blanched, and ground into a paste. Each member of a highly trained descriptive sensory panel rated the FF intensity in a subsample taken from the ground 250 kg sample. Depending on the availability of panel members, each ground sample was usually rated by the same 8 panel members. All panelists used the Spectrum™ method to evaluate the intensity of all terms in the peanut lexicon (Johnson et al., 1988; Sanders et al., 1989b). Approximately 20×20×8 or 3200 FF scores, identified by panel member, sample number, and lot number, were recorded in the database for statistical analysis.

Statistical Analysis

Using Proc Mixed in SAS, an estimate of the total, sampling, and measurement variances was determined for each lot. The average FF intensity among the 160 FF off-flavor scores (8 panel member scores per sample time 20 samples per lot) was also determined for each lot. The 20 sampling and measurement variance estimates were plotted versus the average FF intensity for each lot

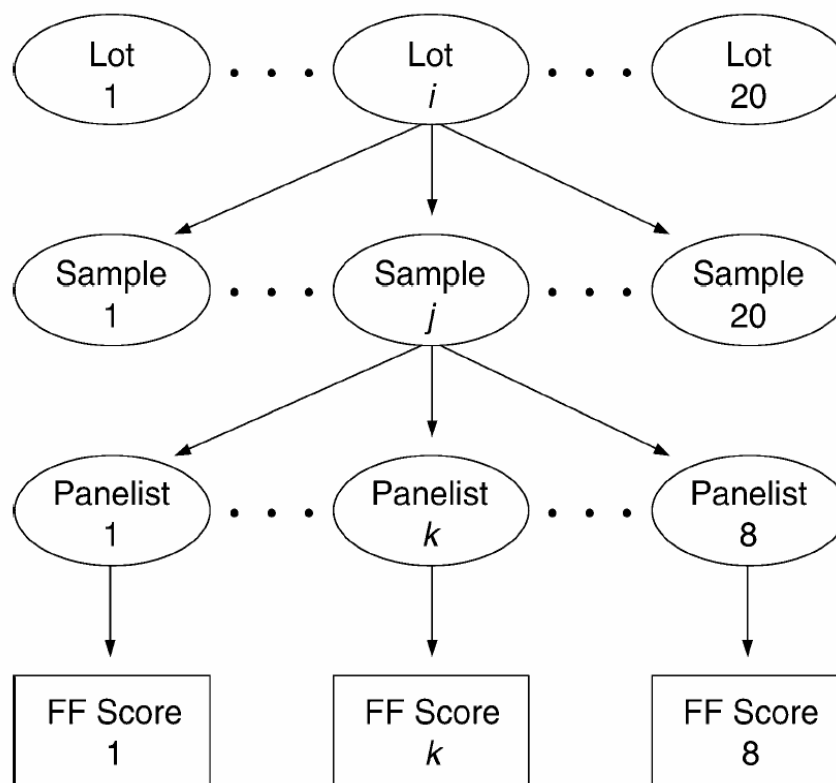


Figure 1. Nested experimental design used to determine the measurement and sampling error associated with using 250 g samples and 8 trained flavor panel members used to rate the FF intensity of samples.

to determine if each variance component was a function of the FF intensity.

Observed distribution

An observed FF distribution among the 20 sample test results for was constructed for each lot. A total of 20 observed distributions, one for each lot, were constructed. The observed cumulative FF distribution for a given lot was constructed by ranking the 20-FF sample test results from high to low. The highest FF value was assigned a cumulative probability of 1.0. The next to highest FF value was assigned a cumulative probability of $1.0 - 1/20$ or 0.95. The cumulative probability associated with each smaller FF value was reduced by $1/20$ or 0.05. The cumulative probability associated with the smallest FF value was assigned a probability of $1/20$ or 0.05.

Theoretical distribution

Four theoretical distributions, normal, lognormal, negative binomial, and compound gamma were chosen as possible models to simulate the observed FF distribution among the 20 sample test

results taken from a lot (Giesbrecht and Whitaker, 1998). These four theoretical distributions were chosen to give a broad descriptive range of distributional shapes from symmetrical (normal) to highly skewed (negative binomial) distributional shapes. Each theoretical distribution was compared to each observed FF distribution for a total of 80 comparisons.

Parameter Estimation Methods

The predicted FF distribution among sample test results was calculated from a theoretical distribution using distribution parameters computed from the mean and variance among the 20-FF sample test results. Parameters of the four theoretical distributions were estimated using the method of moments (Giesbrecht and Whitaker, 1998). The method of moments provides a direct and uncomplicated method of estimating the parameters of each theoretical distribution. Parameters of each theoretical distribution are estimated directly from the measured mean, I , and variance, S^2_t , among the 20-FF sample test results associated with each lot

(Giesbrecht and Whitaker, 1998; Whitaker et al., 1972).

Goodness of Fit

The Power Divergence (PD) test statistic, which is a conservative modification of the Chi Square GOF test, was selected as the criterion to evaluate the goodness of fit (GOF) between the theoretical and observed distributions (Read and Cressie, 1988). For a given lot, the range among the 20 sample test results is divided into 10 intervals of equal width and the number of sample test results that fell into each interval was counted. The expected number of sample test results in each interval is 2 (20 sample test results divided by 10 intervals). The PD statistics were calculated using Equation 1 and compares the observed number of sample test results in each interval to the expected number or 2.

$$PD = \frac{2}{\gamma(\gamma+1)} \sum_{i=1}^8 (observed_i) \left[\left(\frac{observed_i}{expected_i} \right)^{\gamma} - 1 \right] \quad (1)$$

where i is the interval number from 1 to 10 and γ is a coefficient equal to $2/3$. Giesbrecht and Whitaker (1998) recommended the use of PD statistics (Equation 1) with $\gamma = 2/3$ due to its reasonable power against a broad range of alternatives. If $\gamma=1$, Equation 1 would become the Chi Square GOF test. The test statistics were converted to a GOF probability where the lower the GOF probability, the better the fit. The fit between the theoretical and observed distributions was considered acceptable if the test statistic did not exceed the 95% critical value.

Results

The FF intensity for each sample and for each lot is shown in Table 1. The FF intensity associated with each sample in Table 1 is the average of all eight-panel member scores. For each lot, sample intensities are ranked from low to high to more easily view the range among sample test results within each lot. The best estimate of the true FF intensity of a lot is the average of the 160 FF scores (20 samples \times 8 panel scores per sample). The average FF intensity among the 20 lots varied from 0.2 to 2.1.

Variance

Using Proc Mixed in SAS, the mean FF intensity, total variance, sampling variance, and measurement variance for each lot is shown in Table 2. A full log plot (sometimes called a log-log plot) of the measurement variance, sampling variance, and total variance versus the average FF intensity (Table 2) is shown in Figures 2, 3, and

4, respectively. The functional relationship between variance (s^2) and FF intensity (I) was determined using a linear regression analysis on the log values. The regression results are also shown in each figure along with the measured variances. The regression equations for measurement, sampling, and total variances as a function of the FF intensity are shown in Equations 2, 3, and 4, respectively.

$$s_m^2 = 0.546 I^{0.366} \quad (2)$$

$$s_s^2 = 0.163 I^{1.179} \quad (3)$$

$$s_t^2 = 0.746 I^{0.515} \quad (4)$$

Unfortunately, the range in FF intensity among the 20 lots was not as wide as hoped. There was a clumping of mean and variance point in Figures 2, 3, and 4 and as a result the slope of the regression equations (slope in the log scale is the exponent on the I term in equations 2, 3, and 4) was determined with only 3 to 4 points. The attempt to sample peanut lots over a wide range of FF scores proved to be very difficult.

The measurement, sampling, and total variances can be predicted from Equations 2, 3, and 4, respectively, for a given FF intensity, I . For example, when measuring a lot with a true FF intensity (I) of 2.0, the measurement and sampling variances among individual panel members and among 250 g test samples are 0.704 and 0.369, respectively. The total variance of 1.073 was determined by adding the measurement and sampling variances together instead of using Equation 4. At a FF intensity of 2.0, measurement error accounts for 65.6% (0.704/1.073) of the total error and sampling error accounts for 34.4% (0.369/1.073) of the total error.

Reducing uncertainty

The measurement variance in Equation 2 reflects the variability among individual panel member scores and is specific to the particular sensory panel members used in this study. The measurement variance can be reduced by averaging the scores of 2 or more panel members. Equation 2 can be modified to predict the measurement variance associated with averaging any number of panel members (np).

$$s_m^2 = (1/np) 0.546 I^{0.366} \quad (5)$$

Because the uncertainty associated with other sensory panels was not determined, the measurement variance in Equations 2 and 5 may be more

Table 1. Average fruity fermented intensity among all panel members by lot and sample. Sample test results reflect 250 g samples and average intensity among 8 sensory panel members. Each panel member rated FF intensity to one decimal place. Blank cell indicates missing data.

Lot	Sample																				Mean	Median
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20		
2816	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.14	0.14	0.14	0.14	0.19	0.19	0.29	0.31	0.43	0.44	0.54	0.57	0.83	0.22	0.14
1075	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.13	0.14	0.17	0.20	0.29	0.38	0.46	0.57	0.63	0.64	0.71	0.75	0.86	0.30	0.18
1086	0.00	0.14	0.17	0.20	0.21	0.26	0.29	0.57	0.62	0.64	0.65	0.66	0.71	0.93	0.96	1.00	1.06	1.21	1.33		0.61	0.64
1093	0.00	0.19	0.25	0.29	0.36	0.44	0.57	0.63	0.74	0.75	0.81	0.92	0.93	0.94	0.95	1.06	1.48	1.50	1.71	2.00	0.83	0.78
1022	0.21	0.21	0.25	0.38	0.50	0.63	0.63	0.75	0.79	0.79	0.92	0.94	0.97	1.04	1.04	1.16	1.50	1.54	1.63	1.91	0.89	0.85
6363	0.21	0.25	0.38	0.44	0.50	0.63	0.67	0.75	0.84	0.87	1.05	1.07	1.26	1.28	1.55	1.60	1.60	1.66	1.67	1.68	1.00	0.96
1035	0.31	0.40	0.50	0.64	0.65	0.67	0.69	0.75	0.75	0.79	0.86	0.92	1.22	1.25	1.44	1.49	1.69	1.70	1.70	1.96	1.02	0.82
2821	0.00	0.14	0.29	0.31	0.43	0.50	0.50	0.75	0.93	1.00	1.00	1.13	1.15	1.43	1.44	1.50	2.00	2.03	2.26	2.38	1.06	1.00
1036	0.25	0.33	0.50	0.58	0.64	0.64	0.67	0.75	0.75	0.83	1.00	1.00	1.21	1.29	1.48	1.49	1.76	1.87	1.97	2.23	1.06	0.92
1087	0.00	0.13	0.14	0.17	0.25	0.29	0.45	0.57	0.73	0.96	1.16	1.23	1.25	1.79	1.95	1.97	1.98	2.19	2.20	2.29	1.08	1.06
1034	0.17	0.30	0.79	0.83	0.88	0.91	0.93	0.98	1.00	1.19	1.19	1.22	1.23	1.29	1.30	1.50	1.50	1.58	1.71	1.75	1.11	1.19
1020	0.17	0.19	0.40	0.67	0.69	0.70	0.75	0.79	0.91	0.92	1.08	1.48	1.50	1.53	1.53	1.70	1.83	1.83	1.97	2.03	1.13	1.00
1066	0.43	0.57	0.57	0.67	0.67	0.83	0.87	0.90	1.00	1.07	1.18	1.21	1.29	1.34	1.35	1.43	1.56	1.69	2.10	2.56	1.16	1.12
1041	0.00	0.00	0.59	0.64	0.79	0.86	0.97	1.00	1.06	1.06	1.30	1.38	1.47	1.54	1.54	1.71	1.75	2.05	2.06	2.29	1.20	1.18
1065	0.14	0.19	0.20	0.25	0.83	0.93	1.00	1.03	1.13	1.29	1.30	1.31	1.42	1.56	1.71	1.75	2.10	2.39	2.49	2.81	1.29	1.29
1063	0.50	0.75	0.83	0.83	0.93	1.00	1.00	1.04	1.10	1.22	1.44	1.50	1.65	1.70	1.75	1.94	2.21	2.23	2.44	2.47	1.43	1.33
1067	0.44	0.50	0.53	0.60	1.30	1.31	1.34	1.50	1.59	1.63	1.78	1.78	1.83	1.83	1.88	1.90	2.05	2.17	2.47	2.50	1.55	1.70
1064	1.13	1.17	1.20	1.20	1.25	1.38	1.38	1.40	1.43	1.50	1.63	1.65	1.67	1.80	2.00	2.08	2.30	2.30	2.35	2.42	1.66	1.57
1039	1.42	1.55	1.88	1.88	1.92	1.95	2.00	2.00	2.04	2.06	2.06	2.06	2.08	2.27	2.31	2.33	2.43	2.43	2.69	2.79	2.11	2.06
1040	0.90	1.25	1.43	1.66	1.75	1.96	2.08	2.14	2.17	2.17	2.30	2.35	2.37	2.44	2.46	2.47	2.58	2.60	2.75	2.80	2.13	2.23

or less than the uncertainty associated with other sensory panels. However, highly trained sensory panels that use the Spectrum™ method should have similar levels of uncertainty.

The sampling variance in Equation 3 is specific to a 250 g sample size. Increasing the size of the test sample taken from the lot can reduce the sampling variance. Equation 3 can be modified to reflect the

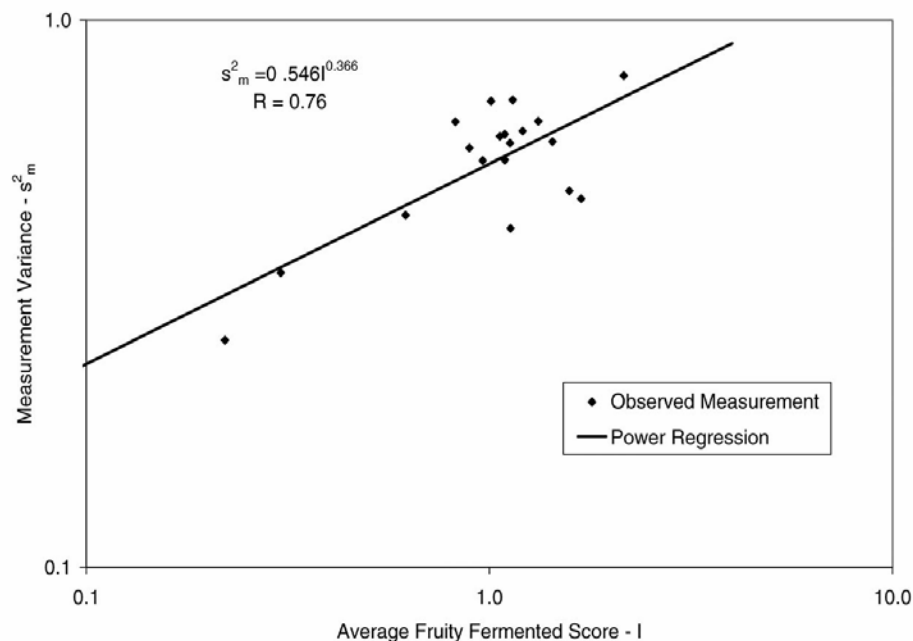


Figure 2. Observed and predicted measurement variance among individual flavor panel members. Each point represents a lot.

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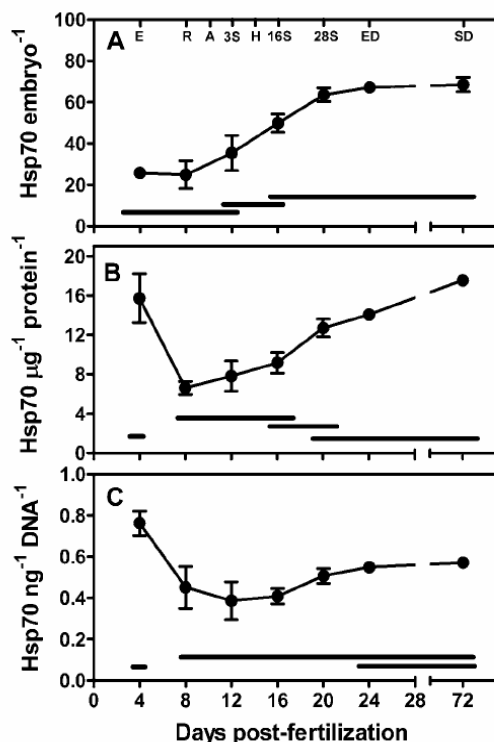


Figure 3. Observed and predicted sample variance among 250 g test samples. Each point represents a lot.

sampling variance associated with any sample size n_s in grams.

$$s_s^2 = (250/n_s)0.163 I^{1.179} \quad (6)$$

The total variance associated with a FF test procedure that averages n_p panel member scores when using a test sample of size n_s is obtained by adding Equations 5 and 6.

$$s_t^2 = (250/n_s) 0.163 I^{1.179} + (1/n_p) 0.546 I^{0.366} \quad (7)$$

As an example, the uncertainty associated with the FF test procedure used by the peanut industry to estimate the intensity of the FF off-flavor in a bulk lot can be estimated using Equation 7. The peanut industry currently uses a 300 g sample and averages the scores of 5 panel members. The measurement, sampling, and total variances associated with the current industry FF test procedure ($n_p=5$ panel members and $n_s=300$ g) when testing a lot with a true FF intensity of 2.0 is estimated from Equations 5, 6, and 7 to be 0.141, 0.308, and 0.449, respectively. The coefficient of variation (CV) associated with measurement, sampling, and

total variances are 18.8, 27.7, and 33.5%, respectively. For this example, measurement error accounted for 31.4% (0.141/0.449) of the total error and sampling accounted for 68.6% (0.308/0.449) of the total error. The measurement CV of 18.8% would appear to a reasonable level of uncertainty when comparing the ability of human taste buds to highly precise analytical equipment such as high performance liquid chromatography, which has levels of uncertainty of about 5 to 10% (Whitaker et al., 1974).

In addition, the total variance of 0.449 can be used to predict the range of sample test result one would expect when sampling a lot with a FF intensity of 2.0 using the standard peanut industry FF test procedure ($n_s=300$ g and n_p =average of 5 panelists). Assuming a normal distribution and 95% confidence limits, the FF intensity among samples would range from $[2.0 \pm (1.96 (\text{sqrt}(0.449)))]$ or range from $[2.0 \pm 1.31]$ or range from 0.69 to 3.31. The major source of uncertainty associated with the peanut industry FF test procedure is associated with the 300 g sample size (68.6% of the total uncertainty). Further reduction in the uncertainty associated with the industry FF test procedure can be achieved by increasing sample size above 300 g. For example, the measurement, sampling, and total variances associated with the FF test procedure that quantified the FF intensity in a 600 g sample by averaging 5 panel member scores are 0.141, 0.154, and 0.295, respectively (For $I = 2.0$ in Equation 7). For this example, the measurement and sampling uncertainty are about the same magnitude.

Distribution among sample score

In the above example that predicted the range among sample test results when sampling a lot with a FF intensity of 2.0 and using the standard industry FF test procedure ($n_s=300$ g and $n_p=5$ panelists), the FF distribution among sample test results was assumed to be normally distributed. However, as reported by Greene et al. (2006b), the FF distribution among the 20-sample test results for a single panel member appears to be skewed, especially for lots with low FF intensity values. The median is less than the mean for 15 of the 20 lots (Table 1) indicating that the distribution among the test results is positively skewed and not symmetrical such as the normal distribution.

Using FF intensity scores associated with one panel member (identified as panel member A), an observed cumulative FF distribution among the 20 sample test results was constructed for each lot (reflecting the uncertainty associated with Equation 7 where $n_s = 250$ g and $n_p = 1$ panel member). The 20 observed FF distributions were each compared

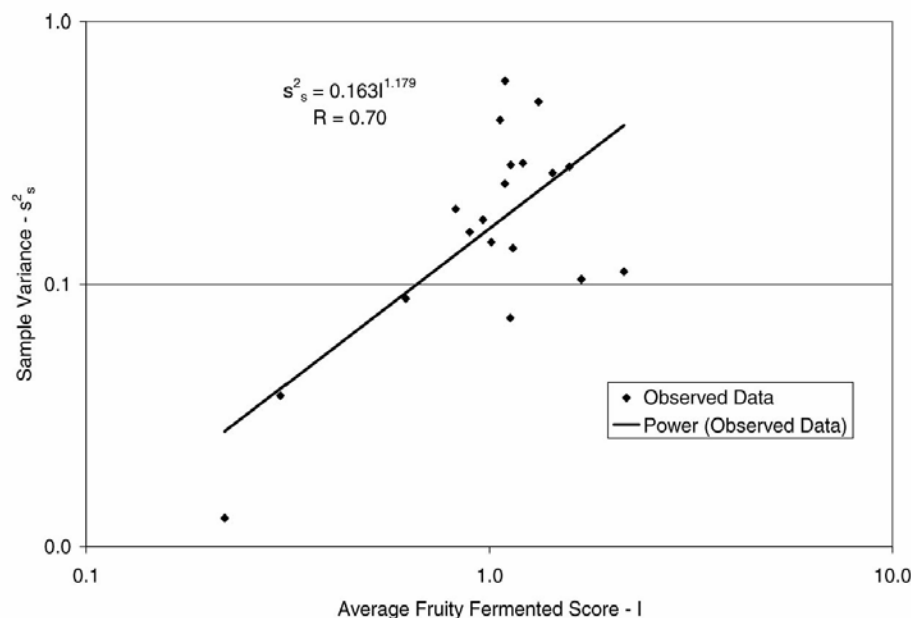


Figure 4. Observed and predicted total variance associated with the test procedure used to score FF intensity when using 250 g test samples and among individual flavor panel members. Each point represents a lot.

Table 2. Uncertainty associated with the test procedure to estimate fruity fermented score of peanuts. Sample variance reflects a 250 g sample size and measurement variance reflects variability among individual flavor panel members.

Lot	Mean	Variance Component		
		Sample	Measurement	Total
2816	0.22	0.0129	0.2600	0.2729
1075	0.30	0.0376	0.3454	0.3830
1086	0.61	0.0881	0.4402	0.5283
1093	0.83	0.1935	0.6522	0.8457
1022	0.89	0.1581	0.5837	0.7418
6363	1.00	0.1762	0.5541	0.7303
1035	1.02	0.1447	0.7113	0.8560
2821	1.06	0.4227	0.6133	1.0360
1036	1.06	0.2414	0.6185	0.8600
1087	1.08	0.5957	0.5551	1.1508
1034	1.11	0.0744	0.5963	0.6707
1020	1.13	0.2848	0.4167	0.7015
1066	1.16	0.1373	0.7148	0.8520
1041	1.20	0.2897	0.6272	0.9168
1065	1.29	0.4959	0.6535	1.1493
1063	1.43	0.2656	0.5999	0.8655
1067	1.55	0.2804	0.4877	0.7682
1064	1.66	0.1044	0.4715	0.5760
1039	2.11	0.0164	0.6573	0.6737
1040	2.13	0.1118	0.7913	0.9031
All Lots	1.14	0.2066	0.5675	0.7741

to the normal, lognormal, negative binomial, and compound gamma theoretical distributions (Giesbrecht and Whitaker, 1998). Using the method of moments, the mean and variance values computed from panel member A's FF scores for each lot were used to calculate parameters for each of the four theoretical distributions (Read and Cressie, 1988). A suitable fit occurred when the probability associated with the fit statistic was 0.95 or less. Goodness of fit tests (Table 3) indicated that the compound gamma provided the highest number of suitable fits to each of the 20 FF distributions. An example of the observed and theoretical distributions for lot 2821 is shown in Figure 5.

The distribution among sample test results can be predicted for specified sample size (n_s) and use of a specified number of panel members (n_p) using variance Equation 7 and the compound gamma distribution. In future studies, a model will be developed using the compound gamma distribution and variance Equation 7 to predict the probability of accepting a lot with a given FF intensity using a given FF test procedure.

Summary and Conclusions

This study indicated that the measurement, sampling, and total variances associated with the

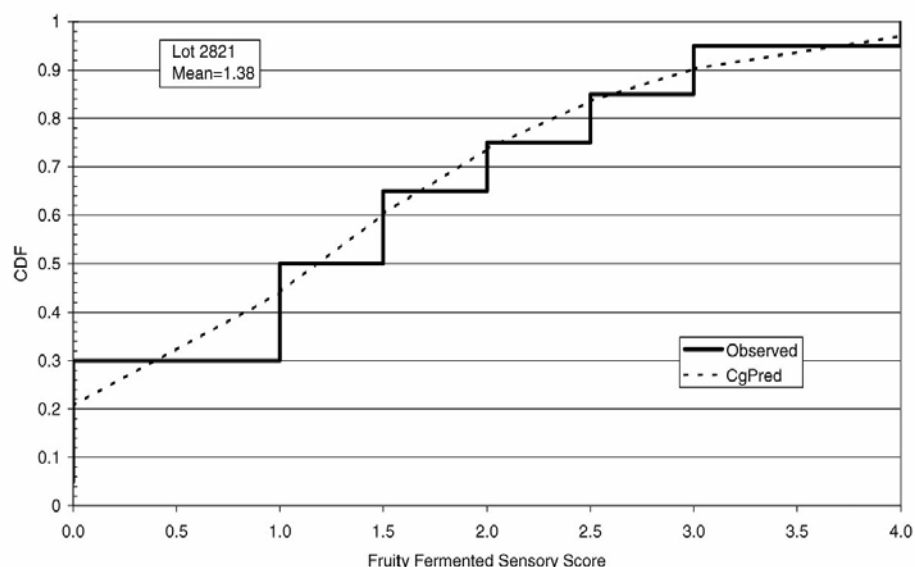


Figure 5. Cumulative observed (solid line) and predicted (dashed line) FF distributions (CDF) among FF intensity values for panelist A from lot 2821. The predicted cumulative FF distribution was calculated using the compound gamma distribution with mean and variance parameters shown in Table 3 for lot 2821.

standard industry test procedure (300 g sample and average of 5 panels member scores) used to score a bulk lot with a true FF score of 2.0 were predicted to be 0.141, 0.308, and 0.449, respectively. For this example, measurement error accounted

for 31.4% (0.141/0.449) of the total error and sampling accounted for 68.6% (0.308/0.449) of the total error. Since there is a different cost associated with reducing sampling and measurement uncertainty, the best use of resources to reduce the total

Table 3. Goodness of fit summary when comparing the compound gamma ($\alpha=7.0$), negative binomial, normal and 2-parameter lognormal distributions to the observed FF distribution among sample scores for panel member A.

Lot	N	Mean FF	Variance	Compound Gamma		Negative Binomial		Normal		2 Parameter LogNormal	
				Pd Statistic	Prob	Pd Statistic	Prob	Pd Statistic	Prob	Pd Statistic	Prob
2816	20	0.15	0.2275	0.00	0.00	10.06	0.81	23.25	1.00	23.25	1.00
1075	20	0.28	0.3369	0.00	0.00	30.83	1.00	16.93	0.98	21.16	1.00
1086	20	0.45	0.5725	1.01	0.01	70.43	1.00	22.50	1.00	30.82	1.00
1022	20	0.60	0.9400	4.78	0.31	64.06	1.00	21.70	1.00	21.70	1.00
1034	20	0.73	0.4619	30.36	1.00	49.21	1.00	35.78	1.00	38.12	1.00
1093	20	0.85	0.9275	3.35	0.15	43.92	1.00	18.52	0.99	33.30	1.00
1020	20	0.93	0.7569	12.31	0.91	37.07	1.00	17.72	0.99	34.60	1.00
1035	20	0.93	0.7069	12.52	0.92	41.93	1.00	17.94	0.99	34.60	1.00
6363	20	1.15	0.9275	23.38	1.00	31.70	1.00	23.38	1.00	34.04	1.00
1036	20	1.25	1.2375	7.27	0.60	26.60	1.00	11.61	0.89	32.50	1.00
1066	20	1.28	0.8619	14.27	0.95	26.32	1.00	23.63	1.00	49.83	1.00
1063	20	1.33	1.1819	13.62	0.94	21.94	1.00	13.62	0.94	32.61	1.00
1065	20	1.33	1.7819	4.36	0.26	26.27	1.00	12.83	0.92	31.81	1.00
2821	20	1.38	1.3719	7.59	0.63	19.26	0.99	10.94	0.86	34.04	1.00
1041	20	1.43	1.0319	14.79	0.96	20.93	1.00	20.93	1.00	40.49	1.00
1064	20	1.50	0.8000	27.15	1.00	27.15	1.00	27.15	1.00	37.07	1.00
1067	20	1.55	1.0475	16.85	0.98	16.85	0.98	16.85	0.98	35.39	1.00
1087	20	1.58	1.6569	12.83	0.92	31.70	1.00	12.83	0.92	35.71	1.00
1039	20	2.35	0.6025	25.05	1.00	25.05	1.00	25.05	1.00	25.05	1.00
1040	20	2.58	0.9819	16.18	0.98	15.51	0.97	16.18	0.98	36.36	1.00
Total Acceptable Fits (prob <= 0.95)					13		1		5		0

variability associated with estimating the true FF off-flavor of a bulk lot may be to increase sample size. The variance and distributional information among sample test results will be used to develop a model to predict the performance of FF sampling plans for peanuts. With the evaluation model, the effect of sample size and the number of panels member used to evaluate the FF intensity in a sample on the chances of accepting bad lots (buyer's risk) and the chances of rejecting good lots (seller's risk) can be determined. Sampling plan design parameters such as sample size and number of panel members used to evaluate the FF intensity in bulk peanut lots can be investigated so that sampling plans developed for the peanut industry will not exceed specified risk levels.

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Appendix 22. Table 1: Neutral/Basic and Acidic Aroma-active Compounds Found in No Fruity Fermented Peanuts Detected by Gas Chromatography-Olfactometry (0 intensity)

No.	Phase ^a NB/AC	Odor Property ^b	RI ^c DB5	RI ^d DBWax	Odor ^e Intensity
1	NB	old/gas/oxidized	602		3.0
2	NB	butterscotch	619		3.0
3	NB	sweet/chocolate/malty	643		3.0
4	NB	green/grassy/cooked	647		3.5
5	NB	rotten/old	648		2.5
6	NB	chives/garlic/onion/sweaty	656		3.0
7	NB	sweet/burnt/cooked	687		1.5
8	NB	buttery/butterscotch	690		2.8
9	NB	caramel/sweet/rich/buttery	697		2.0
10	NB	sickening/repulsive	719		4.2
11	NB	natural gas/gas	752		2.7
12	NB	onion	760		2.0
13	NB	rubber/tires	762		1.5
14	NB	garlic/onion/sweet	763		3.0
15	NB	gas	770		2.5
16	NB	burnt plastic	772		1.5
17	NB	rotten/dirty	793		1.5
18	AC	sweet		798	1.0
19	NB	grassy	800		3.1
20	AC	brothy		803	1.0
21	NB	chives/garlic/onion	811		3.1
22	NB	stale/sweet	816		3.0
23	NB	potato	818		1.5
24	NB	cooked/fatty	819		3.0
25	NB	skunk	833		1.5
26	AC	cooked milk/potato		836	1.2
27	NB	sweaty/musty	837		2.0
28	NB	fruity/sweet	851		2.1
29	NB	sulfur	873		1.5
30	NB	celery	881		2.5
31	NB	rotten/sulfur/bad eggs	885		2.6
32	NB	chives/garlic/onion/spicy	895		2.0
33	NB	sweet/veggies	904		3.0
34	NB	sweet/sweaty	904		3.0
35	NB	sweet	907		2.0
36	AC	butterscotch/sweet		909	1.5
37	NB	urine/sweaty/musty	910		3.1
38	NB	broth/meaty	913		3.0
39	AC	scorched milk		916	1.5
40	NB	potato	920		4.2

41	NB	rosy/floral	924		2.0
42	NB	popcorn	930		4.0
43	NB	roasted/nutty/spicy	941		3.2
44	NB	rubbery/burnt	947		2.0
45	NB	roasted/nutty/cooked	950		4.0
46	AC	sickening/repulsive		952	1.5
47	NB	meaty/brothy	953		3.0
48	NB	roasted/veggie	953		2.0
49	NB	meaty/brothy	962		3.1
50	NB	earthy/soil	968		3.0
51	NB	brine/meaty	970		3.5
52	NB	fatty	970		2.0
53	NB	meaty/brothy	975		3.0
54	NB	sweet/roasted	977		3.5
55	AC	cooked milk/cooked		977	1.2
56	NB	skunk/weeds	979		6.0
57	NB	cabbage/dimethyltrisulfide	981		4.5
58	NB	burnt/repulsive/earthy	982		3.5
59	NB	burnt	983		3.0
60	NB	mushroom/metallic	987		3.2
61	NB	sulfur	987		2.5
62	NB	earthy/dirt	995		3.5
63	NB	roasted/sweet	997		2.0
64	NB	sweet/fruity/citrus	1008		3.1
65	AC	butterscotch		1009	1.0
66	NB	earthy/spice	1013		4.0
67	NB	skins/plastic	1013		4.5
68	NB	fatty	1017		4.0
69	NB	chlorine	1023		2.5
70	NB	sweet/roasted	1025		2.5
71	NB	nutty	1034		3.0
72	AC	green		1034	1.0
73	NB	popcorn	1038		3.0
74	NB	cooked corn	1039		2.0
75	NB	aftershave	1040		3.5
76	NB	shaving cream/permanent marker	1043		3.5
77	NB	roasted	1049		2.0
78	NB	peanut	1050		2.5
79	NB	plastic	1054		3.0
80	NB	solventy/sweet	1054		2.0
81	NB	fresh/pungent	1059		2.0
82	NB	fatty	1062		1.5
83	NB	oxidized/grassy	1064		2.5
84	NB	rosy/floral	1065		5.3
85	NB	roasted nuts	1073		4.0
86	NB	fatty	1075		2.0

87	NB	skins/sweet	1075		4.0
88	NB	peanut	1076		4.0
89	NB	oxidized/lotion	1080		3.5
90	NB	green/grassy	1082		3.5
91	NB	earthy/soil/skins	1093		3.5
92	AC	popcorn		1093	1.0
93	NB	fatty/sharp	1094		3.0
94	AC	skunk		1094	1.5
95	NB	cleanser	1095		3.5
96	NB	sweet/fresh	1098		2.5
97	NB	bell pepper	1099		6.0
98	NB	skins/earthy/soil	1100		3.7
99	NB	grassy/weedy	1102		3.0
100	NB	peanut/roasted/sweet	1106		3.0
101	AC	burnt/rubber		1111	1.5
102	AC	gas stove/rubber		1111	3.0
103	NB	cotton candy	1112		3.0
104	NB	nutty/sweet	1114		3.0
105	NB	nutty	1116		4.0
106	AC	clean clothes/laundry		1116	1.5
107	NB	oxidized	1118		2.0
108	NB	nutty/sweet/peanuts	1123		3.0
109	AC	floral/fresh		1126	1.0
110	NB	maple/spicy	1127		3.0
110	NB	nutty	1136		3.0
111	NB	earthy/soil	1139		4.0
112	NB	peanuts/roasted	1140		2.7
113	AC	burnt/dusty		1141	1.0
114	NB	sweet/fruity/plastic	1145		3.0
115	NB	kool-aid/grape	1147		3.5
116	AC	clean clothes/laundry		1150	1.5
117	NB	spicy/sweet	1151		2.0
118	NB	plastic	1155		3.5
119	NB	phenolic/barn/farm	1159		3.3
120	AC	musty/green		1161	1.0
121	NB	rosy/floral	1165		5.0
122	NB	skunk	1170		3.5
123	NB	earthy	1171		2.0
124	NB	sweet/fruity	1173		3.0
125	NB	oxidized/fatty	1174		3.0
126	AC	gas stove/rubber		1177	1.0
127	NB	roasted/oxidized	1181		2.0
128	NB	carpet	1182		4.0
129	NB	dirty/brothy	1187		2.5
130	NB	plastic	1190		3.0
131	NB	burnt/licorice/grass	1192		3.5

132	NB	vitamin/nutty	1192		3.0
133	NB	oxidized lotion	1195		3.0
134	NB	licorice/nuts/cooked	1198		4.0
135	NB	stale/grass/weeds	1202		3.0
136	NB	garlic	1203		2.0
137	NB	nutty	1216		2.5
138	AC	celery/cucumbers		1223	3.5
139	AC	chemical/acid		1225	2.0
140	NB	roasted nuts	1225		3.5
141	NB	rosy/metallic	1225		2.0
142	AC	earthy		1230	1.0
143	AC	citrus/fruity		1232	1.5
144	NB	earthy/soil	1232		3.0
145	NB	spicy	1236		3.0
146	NB	soil/earthy	1239		2.0
147	NB	nutty/roasted/sweet	1246		3.0
148	NB	fatty	1248		6.0
149	AC	cooked milk		1258	2.5
150	AC	cooked/old/oxidized		1259	2.5
151	NB	roasted/nutty	1259		2.5
152	AC	peanuts/burnt		1261	1.5
153	NB	oxidized/lotion	1265		3.5
154	AC	nutty		1268	2.0
155	NB	licorice/sweet	1271		2.0
156	AC	chemical/raid/bug spray		1273	2.0
157	NB	weedy/green/spicy	1278		2.5
158	AC	popcorn		1282	1.7
159	NB	licorice/sweet	1297		2.5
160	NB	cilantro	1301		3.0
161	AC	litterbox/catty/phenolic		1308	3.0
162	AC	rubber/dirty		1308	2.5
163	NB	oatmeal/sweet	1322		2.5
164	AC	sweet/citrus		1328	1.5
165	NB	books/carpet	1330		3.5
166	NB	vitamins	1331		3.0
167	NB	sweet/fruity	1341		3.3
168	NB	malty/grainy/oatmeal	1346		3.5
169	NB	licorice/sweet	1352		4.0
170	NB	plastic/sweet	1352		2.0
171	AC	brothy/popcorn		1360	2.0
172	AC	nutty/cooked		1361	2.0
173	AC	sour/vinegar/acetic acid		1373	2.8
174	NB	corn tortilla	1373		4.0
175	NB	sweet/fruity	1373		3.0
176	NB	hay/tortilla	1381		2.6
177	AC	charred		1382	2.5

178	AC	burnt/coffee		1385	2.2
179	NB	licorice	1392		3.3
180	AC	potato		1398	2.6
181	NB	sweet/licorice/cinnamon	1401		3.5
182	NB	cooked/rich/corn	1411		2.0
183	NB	lentils/rice/grains/licorice	1423		3.0
184	AC	burnt/brothy		1427	2.0
185	NB	cooked/buttery	1429		2.2
186	AC	earthy/dirt/soil		1437	2.5
187	NB	beans/dried fruit	1437		4.0
188	NB	cooked/sausage/jumbo/redbeans	1439		4.5
189	NB	beans	1445		5.0
190	NB	sweet/metallic/grainy	1453		3.0
191	NB	beans/dried fruit	1462		4.2
192	AC	metal		1466	2.5
193	AC	dirty		1466	1.5
194	AC	metal		1492	2.5
195	AC	nutty		1515	1.5
196	NB	caramelized/sweet	1518		1.5
197	NB	lactone	1532		2.0
198	NB	burnt/mothballs/nuts	1557		1.8
199	AC	vinegar		1565	1.0
200	AC	cheesey		1576	1.5
201	AC	floral		1600	1.8
202	AC	sweaty/cheesey		1608	3.7
203	AC	sweaty/slightly cheesy		1612	3.0
204	NB	cooked corn/popcorn/roasted	1626		1.5
205	AC	cheesy/sweaty/dirty socks		1638	2.3
206	NB	peanuts/roasted	1660		1.5
207	NB	burnt/nuts	1672		2.0
208	AC	sweaty/cheesy		1683	1.5
209	AC	sweet		1692	1.5
210	NB	sweet/burnt	1716		1.5
211	NB	nuts/sweet	1847		1.5
212	AC	sweet/sugar/burnt		1877	2.5
213	NB	carpet	1900		1.5
214	AC	acidic/musty		1989	1.0
215	AC	sweet/burnt sugar		2015	1.0
216	AC	sweet/burnt sugar		2059	2.0

^a Phase fraction in which the odor was detected; NB= neutral/basic, AC= acidic

^b Odors detected by experienced sniffers during a gas-chromatography-olfactometry run

^c Retention indices of neutral/basic compounds on a DB5 (nonpolar) column

^d Retention indices of acidic compounds on a DBWax (polar) column

^e Mean odor intensities of the aroma-active compounds

Appendix 23. Table 2: Neutral/Basic and Acidic Aroma-active Compounds Found in Fruity Fermented Peanuts Detected by Gas Chromatography-Olfactometry (2.6 intensity)

No.	Phase ^a NB/AC	Odor Property ^b	RI ^c DB5	RI ^d DBWax	Odor ^e Intensity
1	NB	butterscotch/buttery	612		2.6
2	NB	sweaty	640		3.5
3	NB	sweet/green/grassy	642		3.0
4	NB	malty/chocolate	651		2.5
5	NB	sweaty	651		3.5
6	NB	garlic/onion	656		3.0
7	NB	acidic	661		1.5
8	NB	butterscotch/buttery	691		2.5
9	NB	fruity	716		2.5
10	NB	sickening/repulsive	717		4.0
11	NB	catty	724		1.5
12	NB	sweet/fruity	746		1.8
13	NB	plastic/solvent	751		2.0
14	NB	old/stale/oxidized	752		3.0
15	NB	brothy/cardboardy	753		2.0
16	NB	green/grassy/oxidized	760		2.0
17	NB	rubbery/plastic	762		2.5
18	NB	old/stale/oxidized	768		2.0
19	NB	grassy/weedy/dirty	774		1.5
20	NB	green/grassy	801		2.8
21	NB	garlic/onion	810		3.1
22	AC	grainy/nutty		815	2.0
23	NB	cooked	817		3.2
24	NB	dusty/cleanser	819		2.0
25	NB	roasted	822		3.5
26	NB	fruity/sweet/cherry/kool-aid	847		2.8
27	NB	sickening/fatty/plastic/foul	878		3.5
28	NB	fruity/sour	879		1.5
29	NB	skunk/rubbery	880		2.5
30	NB	sickening/rotten	884		2.0
31	NB	nutty	891		2.0
32	NB	garlic/onion	896		2.0
33	NB	sweet/musty/sweaty	903		3.2
34	NB	green/plastic	906		1.5
35	NB	oxidized/fatty	908		2.5
36	NB	sweaty/body odor	910		3.5
37	AC	butter/sweet		911	1.6
38	NB	weedy	911		2.0
39	NB	cooked/sweaty	915		2.5
40	NB	potato	920		3.8

41	NB	roasted/beef	927		4.0
42	NB	popcorn	930		4.0
43	NB	peanut/roasted	932		4.5
44	AC	popcorn		935	1.5
45	NB	cheesy	935		1.5
46	NB	roasted beef/nutty	938		4.5
47	NB	meaty/brothy	945		3.0
48	NB	minty	948		2.0
49	AC	catty/sulfur		953	1.5
50	NB	meaty/brothy	956		3.3
51	NB	peanut skins/green/grassy	965		3.0
52	NB	cabbage/vomit	967		3.5
53	NB	nutty	968		3.5
54	NB	brothy/meaty/nutty	971		3.0
55	NB	green/grassy	975		3.5
56	AC	grassy		975	1.0
57	NB	solvent/plastic	980		2.0
58	NB	burnt	980		3.0
59	NB	cabbage/dmts	981		4.5
60	NB	metallic/mushroom	986		3.5
61	NB	brothy	987		2.5
62	NB	sweet/metallic	990		2.7
63	NB	plastic	994		3.0
64	NB	metal/metallic	995		3.5
65	NB	sweet/fruity	1005		3.7
66	AC	rancid/sweet		1006	1.0
67	AC	buttery		1008	1.0
68	NB	nutty/hazelnuts	1011		3.0
69	NB	skins/earthy	1012		4.0
70	NB	roasted	1012		3.0
71	NB	fruity	1014		2.5
72	NB	solvent	1017		3.0
73	NB	chemical	1022		2.5
74	NB	roasted	1033		2.0
75	NB	popcorn/savory	1039		2.5
76	NB	minty	1042		2.2
77	NB	popcorn/roasted	1045		2.5
78	NB	sweet/fruity/rosy/floral/coconut	1052		3.0
79	AC	green/grassy		1054	1.0
80	NB	cheesy/brothy	1055		3.0
81	NB	chlorine	1064		3.0
82	NB	rosy	1066		4.6
83	NB	green/grassy	1069		3.5
84	NB	roasted peanuts	1074		3.5
85	NB	dirt/roasted/earthy	1075		4.0
86	NB	nutty	1079		3.0

87	AC	roasted/nutty		1080	1.5
88	NB	oxidized/fatty	1080		3.0
89	NB	skunk	1082		3.5
90	AC	grainy		1084	1.5
91	NB	dirt/sweet/skins/earthy/soil	1094		3.7
92	NB	roasted	1096		2.0
93	NB	solvent	1099		2.0
94	NB	earthy/legumes	1100		4.0
95	NB	raw potato	1103		3.0
96	AC	cooked/malty		1106	2.5
97	AC	sickening/sulfur/rubber/plastic		1108	2.5
98	NB	garlic/onion	1109		3.2
99	AC	bug spray/plastic		1110	3.0
100	AC	old/stale/oxidized		1112	2.5
101	NB	peanuts	1114		3.5
102	NB	cotton candy	1115		4.0
103	NB	sweet/roasted	1116		3.5
104	NB	old/oxidized	1128		2.7
105	NB	skins/burnt	1131		2.5
106	NB	roasted/nut-like	1137		2.5
107	AC	rubbery/burnt		1139	1.0
108	NB	sweet/burnt	1143		3.0
109	NB	sweet/grape kool-aid	1146		2.6
110	AC	sweet		1147	1.0
110	NB	barn/farm	1152		3.5
111	NB	burnt/sweet	1152		2.0
112	NB	barn/old/oxidized	1159		3.5
113	AC	green/dusty		1159	1.5
114	AC	cooked milk/oil		1168	1.5
115	AC	sweet/cooked		1168	1.0
116	NB	skunky	1168		3.0
117	NB	green	1169		3.0
118	AC	old/stale/oxidized		1170	2.0
119	NB	phenolic	1172		2.5
120	AC	plastic/dusty		1173	2.0
121	NB	carpet/cake	1175		3.0
122	NB	sweet/fruity	1176		3.2
123	NB	brothy	1183		2.5
124	NB	earthy/soil/dirt	1185		3.5
125	NB	plastic	1193		2.5
126	NB	sweet/cooked/licorice	1194		4.0
127	AC	green		1203	1.0
128	AC	nutty		1204	1.0
129	NB	spicy/medicinal/grass	1207		2.5
130	NB	sweet/skunky	1212		3.5
131	AC	stale		1214	1.0

132	AC	green pepper/green		1219	3.0
133	NB	roasted/nutty	1220		2.2
134	AC	sickening/gasoline/sulfur/sickening		1223	3.0
135	NB	roasted/spicy	1238		2.0
136	NB	corn chips	1241		3.0
137	AC	popcorn		1245	2.5
138	NB	oatmeal/grainy	1246		3.5
139	NB	nutty/burnt/peanuts	1251		3.0
140	NB	spicy/herb	1253		2.0
141	NB	stale/beer/malty	1256		2.5
142	AC	cooked/milky		1258	2.0
143	AC	nutty/roasted/broth		1261	2.0
144	NB	oxidized/fatty	1265		2.0
145	AC	sweet		1267	1.0
146	NB	cilantro	1270		3.0
147	NB	floral	1274		1.5
148	AC	chemical/solvent/acetone		1276	2.1
149	NB	herb	1285		2.0
150	NB	plactic/rubber	1292		2.5
151	NB	cilantro	1296		2.5
152	NB	sweet	1301		1.5
153	NB	cilantro/herbs	1306		2.0
154	AC	phenolic/catty/litterbox		1306	2.8
155	AC	old/skunky		1311	3.0
156	NB	oatmeal/grainy	1313		2.2
157	NB	burnt/plastic	1317		3.0
158	NB	oatmeal/grainy	1327		2.5
159	NB	cooked	1330		2.5
160	AC	sweet/candy		1334	1.5
161	AC	sulfur/acidic		1336	2.0
162	NB	sweet	1337		4.0
163	NB	fecal	1337		1.5
164	AC	sulfur/dmts/cabbage		1341	2.0
165	NB	sweet/fruity/licorice	1349		4.0
166	AC	sweet		1354	2.0
167	NB	coconut/sweet	1359		1.5
168	NB	corn chips	1360		3.0
169	AC	vinegar/acetic acid		1361	2.7
170	NB	baked/grainy/malty/oatmeal	1368		2.5
171	AC	peanut skins		1370	3.0
172	AC	sour/vinegar/acetic acid/tuna		1374	3.5
173	AC	burnt/nutty/roasted		1382	2.8
174	NB	tortilla/corn tortilla	1384		3.6
175	NB	licorice	1393		3.6
176	AC	potato		1398	3.3
177	NB	nutty	1417		2.5

178	AC	earthy/potato/soil		1421	3.2
179	NB	beans/dried fruity	1429		4.0
180	NB	roasted	1430		1.5
181	AC	potato/raw		1434	3.0
182	NB	dusty	1436		3.5
183	AC	cooked/peanut skins		1439	2.5
184	NB	roasted/brothy	1450		2.0
185	AC	earthy/soil/potato		1451	3.5
186	NB	beans	1457		4.0
187	NB	meat/sausage/spicy/jambalaya	1464		4.5
188	AC	stale		1471	2.0
189	AC	earthy/soil		1488	1.5
190	NB	beans	1494		4.0
191	AC	green		1503	1.5
192	NB	nutty	1527		2.0
193	AC	earthy/soil		1544	1.5
194	NB	burnt/nutty	1563		2.5
195	AC	cheesy		1573	2.1
196	NB	roasted/peanuts	1587		2.0
197	AC	floral/sweaty/stale		1596	4.0
198	AC	cheesy/sweet		1598	1.7
199	AC	sweaty/cheesy/wet dog		1602	4.0
200	AC	nutty/roasted		1606	1.5
201	NB	burnt/plastic/poopy	1616		2.5
202	AC	cheesy/sweaty/musty		1637	2.7
203	AC	rosy		1668	2.5
204	AC	wet dog/moldy		1669	1.0
205	AC	brothy/meaty		1674	3.0
206	AC	sweet		1745	1.5
207	AC	burnt/fishy		1868	2.0
208	AC	roasted peanuts/nutty		1897	1.7
209	AC	meaty/sausage/gumbo		1939	3.0
210	AC	burnt sugar		2012	2.0
211	AC	sweet/burnt sugar		2099	1.5
212	AC	sugar/roasted peanuts		2102	2.0
213	AC	sweet/burnt sugar		2116	2.0
214	AC	corn chips/sweet		2138	2.0
215	AC	sweet/burnt sugar		2166	1.5
216	AC	cooked/roasted/brothy		2167	1.5
217	AC	sweet/burnt sugar		2173	1.5
218	AC	sweet/coconut		2187	1.5
219	AC	burnt sugar		2206	1.5
220	AC	cooked		2233	1.0

^a Phase fraction in which the odor was detected; NB= neutral/basic, AC= acidic

^b Odors detected by experienced sniffers during a gas-chromatography-olfactometry run

^c Retention indices of neutral/basic compounds on a DB5 (nonpolar) column

^d Retention indices of acidic compounds on a DBWax (polar) column
^e Mean odor intensities of the aroma-active compounds