IDENTIFICATION AND EXPLORATION OF THE COMPONENTS OF A DESIRABLE PECAN FLAVOR

by

BRENDAN T. KELLY

B. S., Biochemistry, Miami University – Oxford, 2014

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Food Science

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2016

Approved by:

Major Professor
Dr. Kadri Koppel

Abstract

The pecan, [*Carya illinoinensis* (Wangenh) K. Koch], has a long history of cultivation and economic value. Knowledge of the compositional differences that exist between cultivars is important to the marketing of pecan varieties. The objectives of this study were to A) profile flavors for various pecans, B) determine flavor differences attributed to preparation method, C) find characteristics of acceptable pecan flavor, and D) evaluate sources of pecan flavor variation through chemical profiling. The flavor profiles of eight pecan cultivars ('Chetopa,' 'Giles,' 'Kanza,' 'Lakota,' 'Major,' 'Maramec,' 'Pawnee,' and 'Witte') were evaluated using descriptive sensory analysis under raw, roasted, and candied preparation methods. A trained panel evaluated samples for 21 flavor attributes. Five of these attributes differed significantly ($p \leq 0.05$) between cultivars, while the preparation method significantly affected 17 attributes. Unique profiles were exhibited for each sample, with the 'Pawnee' and 'Lakota' samples displaying outlying characteristics for certain attributes. These results were used to select cultivars with varied but desirable pecan flavor. 102 nut consumers evaluated 'Kanza,' 'Maramec,' 'Pawnee,' and 'Witte' pecans under raw and roasted conditions for liking and flavor intensity. All samples were met with generally positive consumer acceptance, but three consumer segments were formed based on *Overall Flavor Liking* scores. Segment 1 was driven by cultivar differences, segment 2 by preparation method, and segment 3 by a combination of these factors. The largest drivers of consumer liking related to the roasting process. Chemical differences between cultivars under raw and roasted preparation methods were explored through fatty acid profiling (8 cultivars) and volatile olfactory compound profiles ('Kanza,' 'Maramec,' 'Pawnee,' and 'Witte'). Fatty acid profile variation could generally be attributed to cultivar differences, not changing much with the roasting process. Linoleic, palmitic, and stearic acids were correlated with more roasted-type

attributes while linolenic acid was associated with dry, unfavorable attributes. 51 compounds with olfactory contribution were tentatively identified, 33 of which were found in all samples. Chemical profiles were unique to each sample, but some trends were apparent. The roasted ‘Pawnee’ sample, having many desirable flavor attributes, being met with great consumer acceptance, and having a composition that is associated with preferential attributes, may serve as a good standard for flavor.

Table of Contents

List of Figures

List of Tables

Acknowledgements

This has been a long and rewarding process. I am very grateful for the assistance and support I have received throughout. I would like to thank Dr. William Reid for providing the pecans utilized in this study from Kansas State University's Pecan Experiment Field. Thanks is also due to the trained panelists of Kansas State University's Sensory Analysis Center as well as the students of the center, who aided in preparation of samples and helped bring the experiment to fruition. The shelling process would not have been possible without the generous help of the graduate students of the center. The statistics help from Uyen Phan, Curtis Maughan, Federica Higa, and especially Sirichat Chanadang was *greatly* appreciated.

A special thanks is to be given to Dr. Kadri Koppel, who helped in the development of the experiment and provided guidance on methods and instrumentation, and additionally to Dr. Edgar Chambers IV, whose direction in analysis was greatly valued.

Chapter 1 - Literature Review

Pecans

The pecan [*Carya illinoinensis* (Wangenh) K. Koch] is a species indigenous to North America. From initial years of husbandry to the widespread cultivation and production of today, this crop has continually grown in popularity, measurable by its commercial cultivation and widespread sales in an international market (Santerre 1994). A valuable and sometimes recreational food source historically in Native American culture, it has a long history of use in North America. Naturally found in proximity to major waterways, pecans were heavily utilized by precolonial residents even before their formal cultivation (Santerre 1994). The size and longevity of the tree, its bounty, and the minimal requirements for successful growth make pecan trees an ideal low-input orchard tree (Reid 2000). Its success and importance has been realized by Kansas State University, with an entire research park (Kansas State University's Pecan Experiment Field) devoted to the examination and experimentation of pecans, currently 35 different cultivars strong (Reid 2016). Though pecans have a long history of use, further research is nonetheless necessary to further understand and optimize this valuable crop.

The Value of Pecans

Several compounds found in pecans have been shown to possess antioxidant properties, namely y-tocopherol and flavan-3-ol among others, in vitro (Hudthagosol et al. 2011). In addition, experimental evidence supports that these same components have high bioavailability and contribute to antioxidant defenses within the human system as well.

One of the reasons why pecans serve as large contributors of antioxidants is because of their high tannin content. Tannins, water-soluble polyphenols found in many plant-based foods

and beverages, are found in high quantities in products such as tea and nuts (Chung et al. 1998). Many studies have supported a link between tannin consumption and low cancer rates as well as a reduction in blood pressure, a decrease in serum lipid levels, and an improved immune-response among other health benefits. One study, in attempt to better the marketing of pecans for health benefit, profiled five varieties of pecan for several known antioxidant components, including various tannins and phenolic compounds (Lombardini et al. 2009). The findings indicated that although the different varieties of pecans had similar compounds present, the extractible content varied significantly, indicating that certain cultivars of pecans have higher prospect for use in the nutraceutical market. The anticarcinogenic and antioxidant potentials are only a few of the many health benefits that pecan consumption may provide.

Although the pecan is notoriously high in fat content, its lipid profile is favorable for long-term health (Alasalvar et al. 2009). Regular consumption of pecans and other tree nuts has been linked to lower plasma cholesterol as well as a reduced risk for diabetes and cardiovascular disease through the high volume of 'healthy fats' obtained. Although the method is not completely understood, the phytosterols present in pecans and other tree nuts interfere with the absorption of cholesterol, resulting in a reduction in low-density lipoprotein cholesterol or serum LDL cholesterol. The large concentration of sphingolipids, lipids broken down through the gastrointestinal tract that are essential for cellular function, contribute to overall health at a cellular level.

One of the most promising aforementioned disease-prevention uses of pecans is in cardiovascular disease. The mechanisms that facilitate this cardio-protective effect are not well understood, but research suggests that pecan consumption enhances antioxidant capacity, lowering the oxidation of lipids linked to cardiovascular complications (Preedy et al. 2011).

Despite the missing explanation, there is a multitude of evidence that supports the contributions of pecans to heart health.

In addition to providing long-term health benefits, pecans and other tree nuts have not shown negative effects in terms of pressing short-term health concerns. Although energy rich and high in fat, the regulated consumption of tree nuts has not shown a net gain in body weight when used as a replacement food (Feldman 2002). The contradiction between caloric content of this fatty food and lack of weight gain has not been fully explained, however several hypotheses attempt to make a connection (Garcia-Lorda et al. 2003). One study observed an increase in fat content in the stool of subjects upon the increased intake of pecans, suggesting incomplete absorption and lipid digestion, while other hypotheses propose an increase in metabolic rate and a satiating effect in tree nuts that decrease appetite and other food intake (Garcia-Lorda et al. 2003).

Another concern that arises with high-fat diets is the detrimental effects on glucose homeostasis (Garcia-Lorda et al. 2003). Although much of the research is preliminary, evidence supports that not only does nut consumption not affect glucose homeostasis adversely, but it may help to regulate glycemic control in diabetic patients and even aid in reducing the risk for developing diabetes.

Containing many health-beneficial components such as arginine, folate, and fiber in addition to its basic high-energy components, tree nuts meet ample immediate nutritional needs without many of the shortcomings of similar high-energy foods (Alasalvar et al. 2009). Further, the scale of the evidence supporting the long-term disease-preventative and antioxidant effects of pecans makes them invaluable to the field of nutraceuticals.

Cultivation and Maintenance

Through selective breeding, different cultivars of pecans can be created. Cultivars may be bred for a variety of reasons: maturation period, water necessity, disease and pest resistance, tree structure for specific weather conditions, pecan size, shell thickness or hardness, amount of nuts produced, and nut flavor among other traits (Reid 2016). Because of the large variety of cultivars available, many of the cultivation and maintenance techniques must be uniquely defined for certain varieties. However, in maintaining an orchard, many of the methods developed and growth observations of the trees can apply to a wide array of the cultivars.

One of the most prevalent cultivars of pecans is *Kanza*, initially bred in 1951 but not formally released as a cultivar until 1996 (Reid 2015). This cultivar originated as a cross between Major, a northern cultivar with scab resistance, a thick firm husk, early ripening, and great flavor, and Shoshoni, a southern large, thin-shelled cultivar with great shelling ability. This was done with the hopes of creating a pecan that had the best qualities of both parents. Although the nuts have been deemed too small for the market, accepting only the largest of nuts, *Kanza*'s superior flavor and ease of cracking and maintenance make it one of the most popular among growers (Reid 2015).

The root system of the pecan tree is rather unique. The spread of the root system of pecan trees is about twice that of the branches (Woodroof et al. 1934). The roots tend to stay near the surface, rarely extending beyond 5 feet under the soil, with a high concentration of roots very near the surface, where they are repeatedly killed by freezes, droughts, and tilling and replaced. This system allows for the trees to readily soak up water as soon as it becomes available, which is especially important during the kernel filling phase of pecan development.

With an increase in water cost and a decrease in availability, maintaining a proper irrigation system without wasting water is of upmost importance (Garrot et al. 1993). Studies with the aim to find optimal water delivery and timing have been conducted to prevent a loss in profit. Within a single orchard, the water retention and use by the trees can vary greatly and a standard method of delivery is not advised across all areas.

Water availability and timing is of the upmost importance to pecan success (Reid 2012). Shortly before maturation, the pecans enter a water stage, which is a point during the nutmeat development where the endosperm is a liquid within the fully sized shell and kernel seed coat (Reid 2012). If not enough water is available, the kernels will not fill out well and the nutmeat will be less than ideal. A shortage of water early on in the growing season will lead to a smaller nut, while drought toward the end of the growing season will leave the nut shriveled (Reid 2000). When the kernels are not filled, the nuts may appear shorter than the shell and have airspace within the kernel (Reid 2013). This may lead to a stale, cardboard flavor due to the general lack of oil and mature nutmeat.

Kernel fuzz may be one result from this lack of water. Kernel fuzz, often mislabeled as a defect or disease, can be the harmless result of lack of water during the kernel filling phase or the lowering of temperature and shortening of sunlight exposure before kernel filling is complete (Reid 2012). Without the water necessary to fill the kernel, the nutmeat does not exert the pressure necessary to compress the packing material surrounding the kernel between it and the shell wall, resulting in a loosely packed, fuzzy coat on portions of the kernel. This occurs similarly when pecans are undergoing crucial growing phases in the midst of the shortening daylight and cooler temperatures of Autumn (Reid 2012).

Beyond the availability and timing, the salinity of the water available to pecan trees may be of importance. In response to stunted growth of pecan trees grown in more clay-based soil, the effect of salinity on tree growth has been explored (Miyamoto et al. 1986). As the salinity of the water increases, the growth rate of pecan trees becomes stunted. The higher sodium availability of clay-like silt-based soil should be taken into consideration when maintaining a pecan orchard.

Often, cover crops are introduced to young pecan orchards to promote beneficial insects and aid in nitrogen and organic material content in soil (Foshee et al. 1995). Generally, the incorporation of other plant materials in the early stages of a pecan orchard can be advantageous, but these cover crops may adversely affect the orchard trees by competing for nutrients and water. Pecan trees grown with cover crops nearby, but not in the immediate area have been shown to thrive and are much bigger than their counterparts grown in conjunction with cover crops.

The beneficial insects that come with the use of cover crops is vital for pest control in the early stages of development. Pecan trees, both young and old, face a host of potential pests each growing season, including various arthropods and nematodes, birds and rodents, weeds, and numerous pathogens (Harris 1983). To combat the problem, a variety of pesticides have been implemented. However, due to high cost of maintaining treatment, environmental concerns, and fear of the emergence of resistance, in recent years pesticide use has been limited to an as-needed basis. This has required growers to be much more diligent in monitoring their orchards, but in the long term has economic benefit.

One of the most prevalent arthropods of concern for pecan growth interference is the pecan weevil, *Curculio caryae* (Criswell et al. 1975). The weevil emerges from their underground pupil cases during the pecan maturation season before ascending the tree to feeding,

mating, and oviposition sites. The female weevil punctures the nut during the gel stage of kernel development, burrowing and consuming the nutmeat for up to a week before oviposition. The larvae will consume the kernel and drill an exit hole from which they fall to the ground and pupate.

Another large threat comes from disease and parasitic fungal infections. *Phyllosticta carya, Cercospora fusca, Glomerella cingulata,* and *Coniothyrium caryogenum* are a few of the species of fungus pecans are susceptible to (Rand 1914). These lead to diseases such as nursery-blight and brown leaf-spot, which affect the leaves and consequent stunting of growth in the tree, and pecan anthracnose and kernel-spot, which affect both the leaves and the nuts, altering the flavor, texture, and even color of the nuts in an unfavorable fashion. However, the most prevalent and devastating disease in pecans is pecan scab, a fungal infection that inhibits kernel fill and affects the shuck. This disease is caused by *Cladosporium caryigenum* and can be very serious to both the nut and the foliage (Reid 2002). High humidity and excess water are correlated with a higher prevalence of pecan scab and related fungal diseases. In order to manage pecan scab, pruning may be required to allow for improved air movement for quicker foliage drying. Additionally, the spacing of trees may be important to reduce the spread, as well as the sensible use of fungicides. Because of its devastating effects, pecan scab resistance may be an important factor when deciding which cultivars of pecan to grow and breed when creating new cultivars.

Pecan growers must use a full range of tools in the combatting of pests and must have extensive knowledge of the ecological system within their orchards, including pest biology, crop phenology, and pest behavior (Reid 2002). A system of managing pests, the integrated pest management system (IPM), has been developed to minimize the overuse of pesticides. This

system covers several different methods. Pest detection, identification, and monitoring is vital for effective control, so routine surveys are of upmost importance. Monitoring of weather will help to determine when application of pesticides, most importantly fungicides, should be applied and when their use will not be effective. The conservation of chief predators, such as spiders and certain predaceous insects, will also help to minimize pest infection within the orchard.

Economics and Market Trends

Pecans have long existed in the market as an article with high economic value, dating back to the late 18th century in sales by French and Spanish colonists (Santerre 1994). In the winters of 1886-87 and shortly after in the winters of 1894-96, devastating freezes destroyed much of the citrus populations, leaving a need for orchard crops to be filled by pecans. The increase in production furthered the growth of the industry, which continued to grow until the mid-1980s, when the industry met a cost-price squeeze, where the over-flooding of the market led to decreased sale price and lower profits than required input. During this period, the market made a move toward higher quality nuts, encouraging the development of better cultivars (Santerre 1994).

According to the 2014 summary of noncitrus fruits and nuts released by the United States Department of Agriculture (2015), between 2012 and 2014, the production of pecans decreased for the country as a whole as well as for a large majority of the individual states where pecans were grown. However, the unit price dramatically increased, moving from $1.57 per pound in 2012, averaged across the states, to $1.96 per pound in 2014. This decrease in supply and subsequent increase in price leaves a gap open in the market for the pecan industry to grow.

Nuts grown in different parts of the United States have different growing standards and sale potential. Pecans have long existed in southern states, but a new market has appeared in recent years for pecans grown in more northern states (Reid et al. 2000). These northern pecans have adapted to shorter growing seasons and more intensive winter, which comes with some additional benefits. The shorter growing season means that less pesticide applications are required. Furthermore, the sale price for native northern cultivars is higher than that of southern grown cultivars. Between the reduced production cost and the higher sale price, a larger profit is possible for pecan growers in the north, predominately in Kansas, Missouri, and Illinois.

One method of increasing profit from pecan orchards is through the use of silvopastures. Silvopasture practice combines forestry with the use of grazing animals on the same land to provide economic benefits and potentially turn the ecosystem in way that is beneficial to both practices. The use of this system has been shown to be beneficial to maintaining soil integrity, reducing phosphorus runoff and increasing carbon retention, as well as promoting a favorable environment for natural biological pest controls (Ares 2006). The cost of mowing and weed maintenance is significantly cut and the dual purpose of the land has huge economic advantages. This system works well after the trees of a young orchard have had the chance to develop, limiting its use to more long-established orchards.

Pecan Production and Industry

Before modern means of harvesting pecans, the nuts are allowed to cure and develop on the trees before falling naturally to the ground (Heaton et al. 1975). This was followed by hand collection of the nuts and prompt drying and refrigeration. This method exposed many of the pecans that matured early on to adverse weather and prolonged time on the ground with various

pests and dangers of decomposition. Modern mechanical methods allow for a much more controlled system, reducing the risk of pecan loss. The advancement of pecan production has allowed for the industry to develop into what it is today.

The pecan industry, evolving from wild tree status to commercial fields over the past century, has seen immense changes in scale and spread. Recently, there is a move toward concentration of production in larger fields, with farms operating with 25 or less acres declining in pecan production (Wood 2001). This suggests movement toward industrialization. This applies predominantly to orchards that have long existed in southern states of the United States. An emergence of farms in more northern states indicates a demographic shift in the industry as well. Between price and production characteristics, location of farms, and trends toward industrialization, it is clear that the pecan industry is undergoing an evolutionary change toward growth.

Most cultivars of pecans grown commercially began as selections from the wild (Reid et al. 2000). Over time, growers select trees that have high pecan output, taking quality, size, and resilience into account, for their fields. Experimentation with new cultivar creation is liberally shared within the industry and the most successful are propagated. With the new emergence of northern pecan demand, the industry will continue to grow. Struggling farmers may find pecan growing to be a profitable alternative between nutmeat and wood sales.

Consumer Interpretation

Tree nuts have long been a food of hot commodity, being of high quality and typically high price while remaining commonplace in traditional cooking and baking. Pecans in particular have held a place in the American food market, making their way toward becoming a ‘household

item.' A study conducted by Gold et al. (2004) found that, in a poll of 232 consumers, 90% consume pecans 2-6 times per year, with 50% consuming pecans on a weekly or monthly basis. The study also found that only 1.4% of the participants had not tried pecans in the past. When asked about factors that influence buying decisions with pecans, taste and quality were shown to have the most influence over purchasing whereas price was the lowest decision-making factor in purchasing. This information provides some insight into consumer perception of pecans.

Beyond taste value, many consumers of pecans make purchases based on their knowledge of their nutritional attributes. A study was conducted by Lombardini et al. (2008) to determine consumer knowledge and interpretation of nutritional facts relating to pecans. A large portion of the consumers surveyed had a good knowledge of the basic nutritional facts of pecans, being able to identify them as good sources of fats, protein, antioxidants, and vitamin E. Additionally, in several cases, sugars and minerals were correctly identified as being constituents of pecan nutmeat. One concerning misconception, however, was that pecans could lead to increased LDL cholesterol levels when the contrary is supported by numerous studies. Overall, the study supported the idea that pecans are perceived as a heart-healthy food and a food of an overall healthy lifestyle, which can be a useful base for marketing and sales.

The consumption of pecans has remained fairly stable over the past half-decade, averaging just under half of a pound per capita in the United States annually (Wolfe et al. 2007). In attempts to increase per capita purchasing, a study conducted in 2007 by Wolfe et al. attempted to profile to average pecan consumer. Their findings, through extensive analysis of consumer demographic information, indicate that the average consumer is on the higher end of the age range of 35 to 54. The average purchaser is more likely to be female, which may be explained by the higher likelihood of females to be the ones to purchase groceries in a

partnership. The average household income was also significantly higher for pecan consumers than the median income in the United States, suggesting that pecan purchasers tend to be more affluent. The survey results showed that in every region of the United States, pecans were largely bought from grocery stores. With this information, new products may be created to increase pecan sales, specifically marketing toward a younger audience through more on-the-go type products which can be sold by retailers beyond the grocery store.

Descriptive Sensory Analysis

Sensory analysis, using human means to quantify sensory impressions, is often a necessary step in understanding how different products, formulations, and/or time points of a single product relate to one another. This can be applied to the study of nuts, specifically pecans, allowing for appearance, aromatic, flavor, texture, and aftertaste differences to be determined and interpreted between various cultivars of the same species through descriptive sensory analysis (Suwonsichon et al. 2012). Through numerous statistical analyses, product sensory profiles can be compared, from which conclusions about outlying products, similar products, and distinguishing sensory variations can be drawn and interpretations applied in the industry.

For nuts, sensory analysis is necessary for developing a lexicon in order to better describe and understand the differences that lie between cultivars and nuts under several conditions. Creating a language to describe a product helps in determining defects, identifying unique distinguishing attributes, and creating a picture to market to consumers. Limited research has been performed on pecan sensory profiling. Although available research is limited in scope, descriptive sensory analysis has been used to study different aspects of pecan flavor in a few instances. One study examined the effects of irradiation treatment on pecan sensory qualities as

well as vitamin E content (Taipina et al. 2009). The study found that small doses of irradiation, 1 kGy, did not show a significant effect on appearance, texture, flavor, or aroma, broadly characterized, on pecans when compared to a control. Another study performed by Oro et al. (2009) examined the effects of storage time on *Apparent Homogeneity, Pecan Nut Aroma, Vegetable Oil Aroma, Pecan Nut Taste, Oxidized Taste,* and *Bitterness* of pecan oil, finding that 60 days of storage had little effect on sensory qualities, with *Oxidized Taste* and *Bitterness* increasing significantly beyond 90 days. A similar study examined storage time and humidity on *Crunchiness, Internal Lightness, Rancid Aroma,* and *Rancid Flavor* of pecan nutmeat (Erickson et al. 1994). These studies, though useful in comparing pecan products under different conditions, do little to provide a flavor profile for pecans. One study performed by Magnuson et al. (2016), however, describes flavor profiles of pecans on a large scale, from key characteristics of several pecans examined to character notes of individual cultivars. A lexicon of 20 flavor attributes was used to evaluate different cultivars of pecans under different conditions. These attributes were *Pecan ID, Overall Nutty, Nutty-Woody, Nutty-Grainlike, Nutty-Buttery, Brown, Caramelized, Acrid, Burnt, Musty/Earthy, Woody, Roasted, Overall Sweet, Oily, Rancid, Oxidized, Astringent, Bitter, Sour,* and *Sweet*.

Similar to pecans, many other nuts, such as black walnuts, which have long been big players in the nut industry, until recently, have not had a developed lexicon to describe them. A list of aroma and flavor attributes has now been developed to profile some of the sensory attributes of black walnuts. This list of attributes includes, black walnut ID, overall nuttiness, nutty-woodiness, nutty-grain-like, nutty-buttery, brown, caramelized, acrid, burnt, floral/fruity, fruity-dark, piney, musty/dusty, musty/earthy, woodiness, overall sweetness, oiliness, rancidity, astringency, bitterness, sourness, and sweetness (Miller and Chambers, 2013). With this

compiled list, nut growers are able to better market their products based on the specific profiles of their nuts, allowing for consumers to compare different varieties and cultivars based using a standardized terminology.

Beyond describing differences between products, sensory analysis can be helpful in describing and explaining some of the changes that occur during the natural growing process. A recent study, serving the dual purpose of developing a lexicon to describe mangos and to determine differences between different mangos at different stages in the ripening process, exhibits this use (Suwonsichon et al. 2012). Though obvious textural and flavor changes occur during the maturation of the mango, some of the nuances can be easily lost without the use of sensory evaluation. Some of the attributes found to change the most dramatically as each mango cultivar ripened were viney, green, firmness, cohesiveness of mass, astringency, particle amount, and particle size. Without the terminology to describe these attribute differences, many of these would not be accounted for or would be grouped with a broader descriptor.

Sensory evaluation can be very useful in supplementing and supporting data gained from instrumental measurements. The converse is also true. In one study conducted by Ocon et al. (1995), sensory evaluations of the texture of pecans were compared to data collected using various instrumental means of measuring hardness, flexibility, and crispness. The aim of this research was to determine the best methods of instrumental evaluation to approximate the human experience of eating pecans. In industry, it is difficult and rare to utilize a panel of evaluators for quality control in the context of sensory attributes, so much of the quality control methods in place rely on instrumentation to ensure that products hold up to the company and industry standards. With trends established from quantified sensory texture attributes obtained from the panel, different means of instrumental evaluation, including 50% compression, Texture Profile

Analysis, puncture, and blending methods, were used on the same samples and the results were compared. Puncture and 50% compression methods gave the best instrumental approximations of sensory characteristics, varying the least from the corresponding sensory data, however this study supports the irreproducibility of data obtained from sensory analysis.

The Roasting Process

Although studies conducted using descriptive sensory analysis are useful for products under many different conditions, helping to note differences between products in every stage of production, it is often important to note the condition in which products will be received by consumers for commercialized products. In a study conducted by Tsantilli et al. (2010), this was taken into account in researching pistachio nuts. Previous research had been conducted on the physical, compositional, and sensory properties of the nuts in fresh form, but little had been performed on dried and salted pistachios. In commercial pistachios, the nuts are dried down to a moisture content below 5%, which has significant effects on the compositional and sensory properties.

One method that is efficient in bringing down the moisture content of commercial nuts, and which provides desirable sensory modifications, is the process of roasting. Roasting can significantly affect the flavor, color, texture, appearance, etc. of nuts, causing them to become more brittle and giving the product an enhanced flavor (Nikzadeh and Sedaghat 2008). Previous research has shown significant sensory differences between samples under raw versus roasted conditions. A study performed by Magnuson et al. (2016) examined raw and roasted pecan samples of eight different cultivars for 20 different flavor attributes. Of these, 10 were found to be significantly different between raw and roasted samples of the same cultivar, including *Pecan*

ID, Overall Nutty, Nutty-Woody, Nutty-Grainlike, Nutty-Buttery, Brown, Caramelized, Roasted, Overall Sweet, and Sweet. Significant differences have also been seen in textural qualities of raw versus roasted nuts. One study examined the various effects of roasting on pistachio nuts, looking at moisture content, hardness, fracture force, and firmness, obtained from sensory data, under different roasting conditions (Nikzadeh and Sedaghat 2008). With higher roasting temperatures, the pistachios were shown to have lower moisture content, lower hardness with higher brittleness, decreased fracture force, and higher firmness. Another factor that needs to be considered for commercial products is storage conditions and storage time. This study further examined these pistachio nuts over a 3 month storage period. As pistachios were stored for longer, moisture content initially increased before leveling off, the hardness increased, the fracture force increased, and the firmness also increased.

The roasting process may have different factors beyond roasting temperature that affect the sensory characteristics of nuts. Buckholz et al. (1980) studied the effects of roasting time on the intensity and desirability of aroma and flavor attributes of Spanish peanuts. Statically significant differences were found between peanuts under slightly different roasting times, suggesting that even slight variations in time for the roasting process can affect the profile of nuts, potentially making them more or less desirable. This study also was able to identify some of these differences on a chemical level, finding a correlation between roasting time and gas chromatographic profiles. Further, Buckholz et. al. were able to develop an equation to predict the strength and quality of flavor from the data collected with gas chromatography.

Another factor thought to play a role in the changes that arise during the roasting process is the medium in which the roasting occurs. A study performed by Kita and Figiel in 2006 examined the effects of roasting time and temperature on walnuts, in a similar fashion to studies

with other nuts. However, additionally, the study focused on changes that occur under air versus oil roasting conditions. The findings of this study were similar to that of nut studies performed by Nikzadeh and Sedaghat (2008), showing decreased moisture content and hardness with increasing temperatures and roasting times. However, the medium in which the roasting occurred did not have a significant effect on the textural properties evaluated through instrumental means.

The thickness of the layer of nuts being dried may also affect the roasting process. With a thicker layer of nuts under convection air roasting, those nuts exposed to the surface receive a higher level of heating through radiation and undergo further roasting than those nuts buried beneath a layer. Those nuts exposed to the surface of the pan or tray used to hold the nuts for roasting also receive a higher degree of heating and subsequent roasting from contact with a hot surface through conduction. For even roasting, thin layer drying is imperative (Ozdemir and Devres 1999).

Shelf Life and Oxidation

One of the biggest concerns with foods containing high concentrations of fats in terms of storage and preservation is oxidation. Oxidation of phospholipids present in pecans may result in undesirable flavor, color, and aroma changes (Erickson et al. 1994). A university study investigated this effect with storage time on four flavor qualities of pecans, concluding that rancid flavor increases steadily with time and sweetness generally decreases (Magnuson et al. 2015). This process often occurs in commercial settings because pecans are predominantly kept at an ambient temperature. Many factors affect the degree and rate of oxidation that occur in pecans, notably the moisture content of the kernels, the presence of antioxidants, the exposure to

air, treatment by roasting, and the storage temperature. Optimizing storage conditions for pecans greatly helps in improving their shelf life by reducing the oxidation that takes place.

As pecans age and reach the end of their storage stability, the compounds present in the system begin to change and degrade due to oxidation and an array of other factors. Phenolic acid integrity in particular has been shown to correspond with pecan sensory quality. Most significant are dihydroxy and trihydroxy benzoic acids (Senter et al. 2006). As the concentrations of these decrease, the sensory quality of the nuts similarly decreases, implicating them as important factors in storage stability of pecans. Another group of compounds detected in pecans that have a large role in maintaining pecan integrity through storage is tocopherols. Tocopherols serve as antioxidants in the nutmeat, serving to preserve the nut and delay the onset of rancidity resulting from oxidation of fatty acids. One study performed by Yao et al. (2006) looked at the relationship between tocopherol concentration and kernel quality over the storage process. In several cultivars of pecans different tocopherols were identified, with gamma-tocopherol being the primary form, as having a positive relationship with shelf stability. A positive correlation between tocopherol presence and kernel quality was found, as well as a decrease in total tocopherols over time, linking tocopherol degradation to increased oxidation.

Many steps have been taken in the past to decrease the oxidation of unsaturated fatty acids in pecans. Among these is the use of refrigeration to slow the oxidation process. This method, though effective, contributes a significant cost to the marketing, shipping, and storage of pecans and is not often used in the commercialized system. However, other methods are under development to increase the shelf life of these nuts. One method that has been studied by Baldwin and Wood (2006), which has shown potential for increased preservation at room temperature, is the utilization of a coating to limit oxygen exposure and regulate nutmeat

moisture content. During their experiment, pecans were coated with either hydroxypropyl cellulose or carboxymethyl cellulose with a variety of different additives and evaluated at different time points for textural, flavor, and appearance sensory attributes as well as for accumulation of hexanal, which builds up with more oxidation. The coated pecans showed significantly less oxidation when compared to those not coated and fewer off-notes at each time point. Those pecans coated with carboxymethyl cellulose coatings containing alpha-tocopherol were shown to have the fewest signs of oxidation, including having minimal sensory changes and the least accrual of hexanal. The findings of this study provide a potential alternate solution to refrigeration, allowing for more cost effective means of extending shelf life.

Chemistry

Pecans, like many other seeds, nuts, and other products of plant reproduction, are extremely complex on a chemical level. Even through extensive chemical analyses, the exact nature of many of the compounds present in pecans is not apparent. Modern methods of analysis, such as gas chromatography-mass spectrometry (GC-MS), allow for a wider view of the compounds that make up pecan nutmeat, but falls short of complete profiling, despite the long history of analysis. Nonetheless, research on the composition of pecans has dated back before these methods were available. One study in particular that attempted to characterize the chemical makeup of pecans before modern means, performed in 1946 by Hammar and Hunter, examined the changes in basic chemical composition that take place during the maturation process. The study looked at the formation of oils and protein in the nutmeat as well as the distribution of various minerals, including nitrogen, phosphorus, potassium, calcium, magnesium, between the shuck, the shell, and the kernel. Their findings suggested that the majority of the kernel and its

chemical components are formed within a very short window, during which rapid movement of minerals and building materials occurs from the shuck into the kernel through the shell, preceded by an accumulation of potassium in the shuck and the kernel. Although specifics of the compounds formed during this process are not specified, their research nonetheless was able to describe some of the chemical framework and movement that is present in pecans.

There are several different classifications of compounds that can be found in pecans. Using combined gas chromatography-mass spectrometry as a means of analysis, Wang and Odell (1972) reported several compounds identified in roasted pecans, broken down into carbonyls, pyridine, pyrazines, acids, alcohols, and lactone. The carbonyls identified and confirmed include ethanol, propanol, butanol, pentanal, hexanal, heptanal, octanal, 2-hexanal, 2-heptanal, 2-decanal, 2-undecanal, acrolein, 2,4-heptadienal, 2,4-decadienal, furfural, glyoxal, pyruvaldehyde, diacetyl, and 2,3-pentanedione. Pyridine as well as the pyrazines 2-methylpyrazine, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, 2,3-dimethylpyrazine, 2-ethyl-6-methylpyrazine, 2-ethyl-5-methylpyrazine, 2,3,5-trimethylpyrazine, and 2,5-dimethyl-3-ethylpyrazine, all basic compounds, were further identified. Several acids, including acetic acid, propionic acid, pentanoic acid, 4-methyl-pentanoic acid, hexanoic acid, heptanoic acid, and octanoic acid, were found. Additionally, ethanol, 1-pentanol, 1-hexanol, 1-heptanol, and 1-octanol, all alcohols, were seen as present in roasted pecans. Finally, gamma-octalactone was identified. These were the major compounds identified, while many other peaks were present but not explored in Wang's and Odell's research.

A study similar to Wang's and Odell's was performed on black walnuts in order to determine volatile compounds. Lee et al. (2011) tentatively identified 34 compounds contributing to black walnut aromatics of light, medium, and dark samples across three different

cultivars. The study found that many identified compounds likely originated from amino acid metabolism, determined to be associated with fruity and floral notes. Furans contributed nutty characteristics while aldehydes and alcohols were associated with rancid and acrid aromatics. Hexanal, additionally, was associated with rancid and acrid notes as well as musty/earthiness. Higher concentrations of these aldehydes, alcohols, and hexanal specifically were found in darker nuts, contributing to the 'lower quality.' Many of the desirable ester, benzene derivatives, and furans were present in higher amounts in the lighter nuts, providing a more desirable flavor.

The presence of high quantities of phenolic compounds in pecans is one of the reasons pecans are linked to having health benefits. Some of these compounds include gallic acid, catechol, catechin, epicatechin, *m*-coumaric acid, chlorogenic acid, ellagic acid, and caffeic acid (Malik et al. 2009). Also associated with 'health foods' is an organic status. One study performed by Malik et al. aimed to see if organically grown pecans do indeed have higher health benefits by comparing their phenolic compound composition to that of conventionally grown pecans. For one of the cultivars studied, the concentration of some of the phenolic compounds as well as the total oil content was significantly higher in the organically grown pecan, while minimal differences were observed for the other cultivars studied. As a whole, these findings suggest that, for most cultivars, organically grown pecans do not have a more significant health benefit over conventionally grown pecans when it comes to phenolic compound content.

Through time and different thermal and oxidative processes, the chemical composition of the nuts is prone to change, contributing a variety of sensory modifications. One of the changes that play a large role in pecan oil becoming rancid is the oxidation of linoleic acid (Rudolph et al. 1992). This change is followed by an increase of rancidity products and a discoloration of the

oils, when isolated in particular, from yellow to a darker reddish color and eventually resulting in a change to a colorless oil.

Oil Content

The largest constituents of pecan kernels are lipids, making up 70-79% of the kernel by weight (Toro-Vazquez and Perez-Briceno 1998). Of this, oleic (18:1) acid makes up between 50 and 75 percent of the lipid weight. Beyond oleic acid, several other fatty acids are found in significant amounts in pecan oils, including palmitic (16:0), stearic (18:0), linoleic (18:2), and linolenic (18:3) acids. Of these, oleic, linoleic, and linolenic acids make up the unsaturated components. Toro-Vazquez and Perez-Briceno found that a relationship exists between the concentrations of these unsaturated fatty acids. As the concentration of oleic acid increases in pecans, those of linoleic acid and linolenic acid decreased proportionally. This increase in concentration of oleic acid resulted in a decrease in degree of unsaturation in the total lipids, yielding pecan oil that is less susceptible to oxidation. Further research has confirmed that this relationship is a function of age, with lower oleic acid content and subsequent higher linoleic and linolenic acid content observed in older pecan trees (Toro-Vazquez et al. 1999).

Because the oleic-acid content plays such an important role in oxidative stability, nut cultivars with a high oleic acid trait may be of particular interest. These nuts may have an improved shelf-life as well as a later onset of off-notes in terms of sensory impressions. A study of the effects of these high-oleic traits in peanuts was performed, hypothesizing a link between a high oleic acid content and higher sensory quality (Isleib et al. 2006). The research findings did not report a major impact of the high-oleic acid content on sensory quality, however. Despite this, these findings suggest that the utilization of high-oleic peanuts may be utilized for improved

oxidative stability, and even though the sensory quality may not improve, the sensory profile of the product can remain unchanged.

Analysis of Volatile Compounds

Gas chromatography-olfactometry (GCO) is one way in which sensory and instrumental data can be used simultaneously, each supporting the other. This system is used to detect the olfactory contribution of various chemical compounds within a product, most frequently in food and beverage products as well as flavoring agents (Van Ruth 2001). Four methods of gas chromatography-olfactometry are frequently used, including dilution analysis, detection frequency methods, posterior intensity methods, and time-intensity methods, each which have their advantages and disadvantages (Van Ruth 2001). Achieving effective results relies on accurate human interpretation and elimination of bias among a variety of other factors, including extraction method, instrumental method conditions, and environmental conditions (Delahunty 2006). Data collected from this type of analysis have important applications in flavor development as well as determining chemical and sensory differences between products or treatments.

In the flavor industry, determining the composition of a raw material in the context of flavor and aromatics is key to creating successful flavors. Identifying compounds and using synthetic components for characterization of natural flavors may be extremely cost effective and allow for a flavor to be applied on a larger scale, not needing a potentially limited supply of raw materials. In determining which volatile molecules detectible in different raw materials are important in the development of a character flavor, gas chromatography-olfactometry is imperative for seeing which compounds have olfactory contribution, i.e. which compounds are

aroma-active (Zellner et al. 2007). Because of the importance of these analyses to the creation of flavors, much of the research performed using this method is proprietary.

For all of its usefulness, at times the utilization of gas chromatography-olfactometry may prove to be difficult to analyze. For many raw products, not having undergone any extraction or purification procedures, the magnitude of chemical compounds volatilized and detected by the GCO system often is tremendous. The detection of so many compounds may complicate the interpretation of the results. Often, peaks of detected compounds overlap and are very close together, making it difficult to assign olfactory impressions to such compounds. For instance, one study of wine (Cullere et al. 2004) encountered this problem, where several chemical and olfactory differences between different wine samples could not be established due to areas of the chromatogram being too complicated.

Research Objectives

After a careful literature review, it is apparent that research of pecans has been predominantly focused on preservation and nutritional characteristics while flavor characterization has not been thoroughly explored. Several studies have identified and explored many of the compounds found in tree nuts, including pecans, but have predominately presented their results from a health perspective. This research aimed to determine which attributes are desired and which cultivars are generally more appealing to consumers, allowing for those cultivating pecans to understand which cultivars will have more sucessful sales. By pairing instrumental data and sensory data and applying the result to consumer preference, further understanding of optimal pecan flavoring and composition can be reached.

The main goal of this study was to determine which chemical compounds characterize certain flavor attributes and comprise a desirable pecan flavor. This was achieved through four objectives: 1) compare descriptive sensory profiles of cultivars under different preparation methods to determine similarities between cultivar profiles and identify potential flavor defects; 2) determine consumer acceptance of raw and roasted pecan cultivars; 3) compare of fatty acid profiles of pecan cultivars under raw and roasted conditions; and 4) identify flavor and odor active compounds using GCO analysis of pecan cultivars in raw and roasted conditions.

References

Alasalvar, C. & Shahidi, F. (2009) *Tree Nuts: Composition, Phytochemicals, and Health Effects.* Boca Raton, FL: Taylor & Francis Group.

Ares, A., Reid, W., & Brauer, D. (2006). Production and economics of native pecan silvopastures in central United States. *Agroforestry Systems,* 66(3): 205-215.

Baldwin, E. & Wood, B. (2006). Use of edible coating to preserve pecans at room temperature. *Hortscience,* 41(1): 188-192.

Buckholz, L., Daun, H., Stier, E., & Trout, R. (1980). Influence of roasting time on sensory attributes of fresh roasted peanuts. *Journal of Food Science,* 45(3): 547-554.

Chung, K., Wong, T., Wei, C., Huang, Y., & Lin, Y. (1998). Tannins and human health: a review. *Critical Reviews in Food Science and Nutrition,* 38(6): 421-464.

Criswell, J., Boethel, D., Morrison, R., & Eikenbary, R. (1975). Longevity, puncturing of nuts, and ovipositional activities by the pecan weevil on three cultivars of pecans. *Journal of Economic Entomology*, 1(28): 173–77.

Cullere, L., Escudero, A., Cacho, J., & Ferreira, V. (2004) Gas chromatography-olfactometry and chemical quantitative study of the aroma of six premium quality Spanish aged red wines. *Journal of Agricultural and Food Chemistry,* 52(6): 1653-1660

Delahunty, C., Eyres, & G., Dufour, J. (2006). Gas chromatography-olfactometry. *Journal of Separation Science,* 29(14): 2107-2125.

Erickson, M., Santerre, C., & Malingre, E. (1994). Oxidative stability in raw and roasted pecans: chemical, physical, and sensory measurements. *Journal of Food Science,* 59(6): 1234-1238.

Feldman, E. (2002). The scientific evidence for a beneficial health relationship between walnuts and coronary heart disease. *The Journal of Nutrition,* 132(5): 10625-11015.

Foshee, W., Goff, W., Patterson, M., & Ball, D. (1995). Orchard floor crops reduce growth of young pecan trees. *HortScience*, 30(5): 979-980.

Garcia-Lorda, P., Rangil, I., Salas-Salvado, J. (2003). Nut consumption, body weight, and insulin resistance. *European Journal of Clinical Nutrition,* 57(1): S8-S11.

Garrot, D., Kilby, M., Fangmeier, D., Husman, S., & Ralowicz, A. (1993). Production, growth, and nut quality in pecans under water stress based on the crop water stress index. *Journal of the American Society for Horticultural Science*, 118(6): 694-698,

Gold, M., Cernusca, M., & Godsey, L. (2004). Consumer preferences for chestnuts, eastern black walnuts, and pecans. *HortTechnology,* 14(4): 583-589.

Hammar, H. & Hunter, J. (1946). Some physical and chemical changes in the composition of pecan nuts during kernel filling. *Plant Physiology,* 21(4): 476–491.

Harris, M (1983). Integrated pest management of pecans. *Annual Reviews of Entomology*, 1(28): 291–318.

Heaton, E., Worthington, R., & Shewfelt, A. (1975). Pecan nut quality: effect of time of harvest on composition, sensory, and quality characteristics. *Journal of Food Science,* 40(6): 1260-1263.

Hudthagosol, C., Haddad, E., McCarthy, K., Wang, P., Oda, K., & Sabate, J. (2011). Pecans acutely increase plasma postprandial antioxidant capacity and catechins and decrease LDL oxidation in humans. *The Journal of Nutrition*, 141(1): 56-62.

Isleib, T., Pattee, H., Sanders, T., Hendrix, K., & Dean, L. (2006). Compositional and sensory comparisons between normal- and high-oleic peanuts. *Journal of Agricultural and Food Chemistry*, 54(5): 1759-1763.

Kita, A. & Figiel, A. (2006). The effect of roasting on the texture of walnuts. *Acta Agrophysica*, 7(1): 87-97.

Lee, J., Vazquez-Araujo, L., Adhikari, K., Warmund, M., & Elmore, J., (2011). Volatile compounds in light, medium, and dark black walnut and their influence on the sensory aromatic profile. *Journal of Food Science*, 76(2): C199-C204.

Lombardini, L., Villarreal-Lozoya, J., & Cisneros-Zevallos, L. (2009). Antioxidant properties of pecan kernels. *Acta Horticulture*, 841: 91-96.

Lombardini, L., Waliczek, T., & Zajicek, J. (2008). Consumer knowledge of nutritional attributes of pecans and factors affecting purchasing behavior. *HortTechnology*, 18(3): 481-488.

Magnuson, S., Kelly, B., Koppel, K., & Reid, W. (2016). A comparison of flavor differences between pecan cultivars in raw and roasted forms. *Journal of Food Science*, 81(5): S1243-S1253.

Magnuson, S., Koppel, K., Reid., W., Chambers IV, E. (2015). Pecan flavor changes during storage. *Journal of the American Pomological Society*, 69(4): 206-214.

Malik, N., Perez, J., Lombardini, L., Cornacchia, R., Cisneros-Zevallos, L., & Braford, J. (2009). Phenolic compounds and fatty acid composition of organic and conventional grown pecan kernels. *Journal of the Science of Food and Agriculture*, 89(13): 2207-2213.

Miller, A. & Chambers, D. (2013). Descriptive analysis of flavor characteristics for black walnut cultivars. *Journal of Food Science*, 78(6): S887-S893.

Miyamoto, S., Riley, T., Gobran, G., & Petticrew, J. (1986). Effects of saline water irrigation on soil salinity, pecan tree growth, and nut production. *Irrigation Science*, 7(2): 83-95.

Nikzadeh, V. & Sedaghat, N. (2008). Physical and sensory changes in pistachio nuts as affected by roasting temperature and storage. *American-Eurasian Journal of Agricultural and Environmental Science*, 4(4): 478-483.

Ocon, A., Alzaldua-Morales, A., Quintero, A., & Gastelum, G. (1995). Texture of pecans measured by sensory and instrumental means. *Journal of Food Science,* 60(6): 1333-1336.

Oro, T., Bolini, H., Arellano, D., & Block, J. (2009). Physichemical and sensory quality of crude Brazilian pecan nut oil during storage. *Journal of the American Oil Chemists' Society*, 86(10): 971-976.

Ozdemir, M. & Devres, Y. (1999). The thin layer drying characteristics of hazelnuts during roasting. *Journal of Food Engineering*¸ 42(4): 225-233.

Preedy, V., Watson, R., & Patel, V. (2011). *Nuts and Seeds in Health and Disease Prevention.* San Diego, CA: Academic Press.

Rand, F. (1914). Some diseases of pecans. *Journal of Agricultural Research,* 1(4): 303–44.

Reid, W. (2000, October 12). Growing pecans in Missouri. Retrieved from https://mospace.umsystem.edu/xmlui/bitstream/handle/10355/8380/GrowingPecansMissouri.pdf?sequence=3&isAllowed=y. (accessed July 4, 2016)

Reid, W. (2002). Current pest management systems for pecan. *HortTechnology*, 12(4): 633–39.

Reid, W. (2012). *Fuzzy pecan kernels.* Retrieved from http://northernpecans.blogspot.com/2012/01/fuzzy-pecan-kernels.html (accessed July 4, 2016)

Reid, W. (2012). *Pecans in the water stage.* Retrieved from http://northernpecans.blogspot.com/2012/08/pecan-in-water-stage.html (accessed July 4, 2016)

Reid, W. (2013) *Cultivars that ran out of time to fill kernel*. Retrieved from http://northernpecans.blogspot.com/2013/12/cultivars-that-ran-out-of-time-to-fill.html (accessed July 4, 2016)

Reid, W. (2015). *A Kanza story*. Retrieved from http://northernpecans.blogspot.com/2015/11/a-kanza-story.html (accessed July 4, 2016)

Reid, W. (2016). *Pecan Cultivars*. Retrieved from http://northernpecans.blogspot.com/p/blog-page.html. (accessed July 4, 2016)

Reid, W. & Hunt, K. (2000). Pecan production in the northern United States. *HortTechnology*, 10(2): 298-301.

Rudolph, C., Odell, G., Hinrichs, H., Thomson, H., & Kays, S. (1992). Chemical changes in pecan oils during oxidation. *Journal of Food Quality*, 15(4): 279-293.

Santerre, C. (1994). *Pecan Technology*. New York, NY: Chapman & Hall.

Senter, S., Horvat, R., & Forbus, W. (1980). Relation between phenolic acid content and stability of pecans in accelerated storage. *Journal of Food Science,* 45(5): 1380-1382.

Suwonsichon, S., Chambers IV, E., Kongpensook, V., & Oupadissakoon, K. (2012). Sensory lexicon for mango as affected by cultivars and stages of ripeness. *Journal of Sensory Studies,* 27(3): 148-160.

Taipina, M., Lamardo, L., Rodas, M., Del Mastro, N. (2009). The effects of gamma irradiation on the vitamin E content and sensory qualities of pecan nuts (Carya illinoensis). *Radiation Physics and Chemistry*, 78(7): 611-613.

Toro-Vazquez, J. & Perez-Briceno, F. (1998). Chemical and physiochemical characteristics of pecan (Carya illinoensis) oil native of the central region of Mexico. *Journal of Food Lipids,* 5(3): 211-231.

Toro-Vazquez, J., Charo-Alonso, M., & Perez-Briceno, F. (1999). Fatty acid composition and its relationship with physiochemical properties of pecan (Carya illinoensis) oil. *Journal of the Americal Oil Chemists' Society*, 76(8): 957-965.

Tsantill, E., Takidelli, S., Christopoulos, M., Lambrinea, E., Rouskas, D., & Roussos, P. (2010). Physical, compositional, and sensory differences in nuts among pistachio (Pistachia vera L.) varieties. *Scientia Horticulturae,* 125(4): 562-568.

United States Department of Agriculture (2015). *Noncitrus Fruits and Nuts.* Retrieved from http://usda.mannlib.cornell.edu/usda/current/NoncFruiNu/NoncFruiNu-07-17-2015.pdf (accessed July 4, 2016)

Van Ruth, S. (2001). Methods for gas chromatography-olfactometry: a review. *Biomolecular Engineering*, 17(4): 121-128.

Wang, P. & Odell, G. (1972). Characterization of some volatile constituents of roasted pecans. *Journal of Agricultural and Food Chemistry,* 20(2): 206-210.

Wolfe, K., Daniels, J., Swickard, K., & McKissick, J. (2007). *U. S. pecan consumer profile results from national survey.* Retrieved from http://athenaeum.libs.uga.edu/xmlui/bitstream/handle/10724/18765/CR-07-11.pdf?sequence=1 (accessed July 4, 2016)

Wood, B. (2001). Production unit trends and price characteristics within the United States pecan industry. *HortTechnology,* 11(1): 110-118.

Woodroof, J. & Woodroof, N. (1934). Pecan root growth and development. *Journal of Agricultural Research,* 49(6): 511-530.

Yao, F., Dull, G., & Eitenmiller, R. (2006). Tocopherol quantification by HPLC in pecans and relationship to kernel quality during storage. *Journal of Food Science,* 57(5): 1194-1197.

Zellner, B., Dugo, P., Dugo, G., & Mondello, L. (2008). Gas chromatography-olfactometry in food flavour analysis. *Journal of Chromatography A,* 1186(1): 123-143.

Chapter 2 - A Sensory Comparison of Pecan Cultivars in Raw, Roasted, and Candied Forms

Abstract

The objectives of this study were to compare flavor profiles of eight cultivars of pecans ('Chetopa,' 'Giles,' 'Kanza,' 'Lakota,' 'Major,' 'Maramec,' 'Pawnee,' and 'Witte') under different preparation methods (raw, roasted, and candied) as well as to determine the effect of this preparation method on flavor profiles. The cultivars were collected from the 2014 growing season at Kansas State University's Pecan Experiment Field. A panel of eight highly trained evaluators from Kansas State University's Sensory Analysis Center evaluated each of the cultivars under each of the preparation methods in duplicate for 21 flavor attributes using a hybrid descriptive sensory analysis method. Five attributes were significantly different between the cultivars (*Pecan ID, Nutty-Buttery, Caramelized, Acrid,* and *Astringent*), while 17 attributes were affected significantly by the preparation method. These included *Pecan ID, Overall Nutty, Nutty-Woody, Nutty-Grainlike, Nutty-Buttery, Brown, Caramelized, Acrid, Musty/Earthy, Woody, Roasted, Overall Sweet, Oily, Bitter, Sour, Sweet,* and *Salt* attributes. Each of the samples exhibited unique profiles, with some cultivars displaying outlying characteristics for certain attributes, such as the caramelized, buttery features of 'Pawnee' and the high astringency of the 'Lakota' cultivar. The candying process was shown to have a masking effect on certain attributes, namely *Nutty-Woody, Acrid, Musty/Earthy, Woody,* and *Bitter* attributes. The results from this experiment will help pecan growers to understand how different preparation treatments affect different pecan varieties, as well as to learn which cultivars may exhibit profiles with more

desirable attribute intensities, allowing for better marketing and subsequent application of their pecans in the market.

Introduction

The pecan, *Carya illinoinensis,* is one of the few plant species native to North America that has a history of importance as an agricultural crop. Within the past century, the cultivation and production of the tree nut has experienced gradual growth, its success evidenced by the amount of commercial production that has spread beyond United States borders (Mexico, Australia, South Africa, Brazil, Israel, etc.) (Santerre 1994). In 2013, in the United States, sales of pecan nutmeat exceeded 460 million dollars with 106,569,000 pounds of nutmeat produced (NASS 2015). The following year saw increased revenue with 101,858,000 pounds of pecan nutmeat generating $593,591,000 (NASS 2015). The crop's value is clear, with continual efforts to optimize production, maintenance, and cultivation made. A total of 161 cultivars, or varieties, of pecan are patented and utilized in the United States, each with unique resistances to detrimental conditions, growing periods, and textural and flavor profiles, giving way to a wide range of applications (Grauke and Thompson 2016).

The pecan tree is valued for its many uses including its nutmeat, which can be used in a wide variety of culinary application, as well as for its wood. The demand for pecan wood has shown an increase in recent decades, being used for furniture, cabinetry, veneer, and other woodwork, having good machining properties (Adams and Thielges 1977). The incorporation of pecans into traditional dishes, whether characterizing the food culture of the southern United States in pecan pie or being used in holiday foods such as sweet potato casserole, has also impacted the growth of the pecan industry. Pecans are used in baking, confections, and are a

common additive to salads and similar dishes. They are served both raw and prepared, often undergoing processing such as candying, roasting, or chocolate coating (Wood 2001; Lombardini et al. 2008). This additional processing may play a significant role in the sensory properties of these nuts.

Roasting, a process often seen in the nut industry, involves prolonged exposures to high temperatures, which affects the moisture content of the nuts and may have a significant effect on the flavor, color, texture, aroma, appearance, and other attributes of the nuts (Nikzadeh and Sedaghat 2008). The time of exposure, medium of roasting, and thickness of the layer of nuts during the roasting process have all been shown to have an effect on these attributes (Buckholz et al. 1980; Kita and Figiel 2006; Ozdemir and Devres 1999). Understanding the changes that occur on a sensory level during the roasting and treatment process is important to the marketing of these nuts, especially given the spread of different cultivars that are in the industry.

With the majority of pecan research being limited in the past to oxidative stability studies and investigation of nutritional and long term health effects (Erikson et al. 1994; Lombardini et al. 2009; Alasalvar and Shahidi 2009; etc.), creating a vocabulary and describing sensory differences that occur during the pecan preparation process may be helpful to pecan growers in marketing their pecans in application. Further, noting sensory differences between pecan cultivars under different preparation methods will help growers to see which cultivars have similar profiles, which have unique profiles, and which may have flavor defects within each treatment. The objectives of this study were to address these needs in comparing the sensory profiles of eight pecan cultivars under three different preparation means: raw, roasted, and candied.

Materials and Methods

Samples

Eight cultivars of pecan were obtained from Kansas State University's Pecan Experiment Field (Chetopa, KS, USA) from the 2014 growing season. These cultivars included: 'Chetopa,' 'Giles,' 'Kanza,' 'Lakota,' 'Major,' 'Maramec,' 'Pawnee,' and 'Witte.' All samples were kept under frozen conditions (-18° C ± 1° C) before and after shelling. After the shelling process, all samples were vacuum sealed in 3.79 L FoodSaver vacuum seal bags using a FoodSaver Heat-Seal Vacuum Sealing System (Sunveam Products Inc., Boca Raton, FL, USA). Refrigeration and vacuum sealing procedures were undergone to limit contamination, preserve moisture content and freshness, and minimize the effects of oxidation (Reid 2011). The shelling took place over an approximate 90-day period after the receiving of the nuts using a Duke Pecan Walnut Cracker (Duke Pecan Company, West Point, MS, USA), a Davebilt Nutcracker (Davebilt Company, Lakeport Calif., USA), and Channel Lock model number 436, 15.24 cm cutting pliers (Channel Lock Inc., Meadville, Pa., USA) to remove the nutmeat from the shells. The samples were stored frozen (–18 °C) until analysis. The initial percent moisture was measured using a Mettler Toledo HE 73/03 Moisture Analyzer (Mettler-Toledo AG, Greifensee, Switzerland) to ensure that each of the cultivars fell within industry standard for sale with a moisture content below 4.5% (Nelson et al. 1992). Table 2-1 lists average initial percent moisture of the cultivars.

The pecans in this experiment were evaluated under three different preparation methods: raw, roasted, and candied. The pecans used in the raw evaluation were removed from the freezer one day prior to testing and left to thaw at room temperature (23 °C ± 1 °C) in sealed 92 g cups (Solo Cup Company, Lake Forest, IL, USA) overnight. The samples used for the roasted and candied evaluations were removed from the freezer two days prior to evaluation and, similarly,

left to thaw at room temperature (23 °C ± 1 °C) overnight in their vacuum sealed bags. The roasted samples were prepared one day prior to evaluation. 100 g of sample was placed in a single layer on a baking tray and roasted at 176 °C for 10 minutes, with stirring at 5 and 8 minutes to prevent burning and uneven roasting. After the samples were removed from the oven, they were left to cool at room temperature (23 °C ± 1 °C) for 30 minutes on aluminum baking trays before being placed in sealed 92 g plastic cups overnight. The pecans used in the candied evaluation underwent the same roasting process, with a glaze applied after removal from the oven. The glaze consisted of 18.75 g granulated sugar (C&H Sugar, Crockett, CA, USA), 7.5 g deionized water, 0.78 g vanilla extract (McCormick, Sparks, MD, USA), and 0.98 g salt (Morton Salt Inc., Chicago, IL, USA) per 100 g sample. The ingredients for the glaze were mixed prior to addition of the pecans and the pecans were added to the glaze mixture immediately after removal from the oven. The glaze and pecans were mixed in a bowl for approximately 2 minutes until an even coat was applied. The candied pecans were spread out on parchment paper and left to dry and cool at room temperature (23 °C ± 1 °C) overnight, covered by an additional piece of parchment paper. The morning of evaluation, candied samples were placed in sealed 92 g plastic cups.

Table 2-1. Average initial percent moisture in the pecan cultivars

Cultivar	Percent Moisture % ± StDev
Giles	3.20 ± 0.11
Chetopa	2.48 ± 0.06
Kanza	2.37 ± 0.05
Lakota	3.59 ± 0.16
Major	2.45 ± 0.07
Maramec	2.97 ± 0.09
Pawnee	2.71 ± 0.10
Witte	3.01 ± 0.10

Descriptive Sensory Analysis

Eight panelists (6 female, 2 male) from the Sensory Analysis Center at Kansas State University (Manhattan, KS, USA) were selected for the evaluation of the samples for this study. All of these panelists had completed more than 120 hours of general training in descriptive sensory analysis and at least 2000 hours of evaluation experience with a wide variety of food, beverage, and non-food items, including nut-related items. Three days of orientation were used by the panel, during which a list of key attributes was determined and definitions and references for these attributes were established to maintain consistency throughout the evaluation. During this period, panelists also familiarized themselves with the samples and practiced evaluation. Twenty-one flavor attributes were evaluated using descriptive sensory analysis (Table 2-2). Similar methods have been utilized in several other recently published research (Magnuson et al. 2016, Suwonsichon et al. 2012, Miller and Chambers 2013, Cherdchu and Chambers 2014).

Table 2-2. Flavor attributes, definitions, and references for descriptive analysis of pecans*

Attribute	Definition	Reference
Pecan ID	The aromatics commonly associated with pecans which include musty/earthy, piney, woody, brown, sweet, buttery, oily, astringent, and slightly acrid aromatics. Other aromatics may include musty/dusty, floral/fruity, and/or fruity-dark.	Ground Pecan pieces = 7.0 (flavor) Preparation: Measure out 1 tbsp. of various cultivars into a food processor and blend for 30 seconds. Pour into 1 oz. cups.
Overall Nutty	A measurement that reflects the total of the nutty characteristics and the degree to which these characteristics fit together. These nutty characteristics are: sweet, oily, light brown, slightly musty and/or buttery, earthy, woody, astringent, bitter, etc. Examples: nuts, wheat germ, certain whole grains.	Gold Medal Whole Wheat Flour = 4.5 (flavor) Kretschmer Wheat Germ = 7.5 (flavor) Mixture of Diamond Slivered Almonds and Kroger Chopped Hazelnuts = 7.5 (flavor) Diamond Shelled Walnuts = 8.0 (flavor) Diamond Pecan Halves = 9.0 (flavor) Preparation: Puree the almonds and hazelnuts separately in blenders for 45 seconds on high speed. Combine equal amounts of the chopped nuts. Serve in individual 1 oz. cups. Serve pecans and walnuts in 1 oz cups.
Nutty-Woody	A nutty aromatic characterized by the presence of woodiness, increased musty/dustiness, brown, astringent and bitter.	Diamond Pecan Halves = 7.5 (flavor) Diamond Shelled Walnuts = 7.5 (flavor) Preparation: Serve pecans and walnuts in 1 oz cups.
Nutty-Grainlike	A nutty aromatic characterized by the presence of a grainy aromatic, increased musty/dustiness and brown.	Gold Medal Whole Wheat Flour = 4.5 (flavor) Kretschmer Wheat Germ = 7.5 (flavor)
Nutty-Buttery	A nutty aromatic characterized by a buttery impression, and/or increased fatty aromatics and musty/earthy character.	HyVee Dry Roasted and Salted Macadamia Nuts = 5.0 (flavor) Preparation: Serve macadamia nuts in a 1 oz cup.

Brown	A rich, full aromatic impressions always characterized with some degree of darkness generally associated with attributes (i.e. toasted, nutty, sweet).	Bush's Best Pinto Beans (Canned) = 5.0 (flavor) Kretschmer Wheat Germ = 7.5 (flavor) Preparation: Drain beans and rinse with de-ionized water. Serve in 1 oz. cups.
Caramelized	A round, full-bodied, medium brown aromatic.	Caramelized Sucrose dissolved in water (diluted by half) = 3.0 (flavor) Caramelized Sucrose dissolved in water = 6.0 (flavor) Preparation: Dissolve 5g and 10g caramelized sucrose in 80g water.
Acrid	The sharp/acrid, charred flavor note associated with something over baked or excessively browned in oil.	Alf's Natural Nutrition Puffed Red Wheat Cereal = 3.0 (flavor)
Burnt	A dark, brown, somewhat sharp, over-baked grain aromatic.	Alf's Natural Nutrition Puffed Red Wheat Cereal = 4.0 (flavor)
Musty/Earthy	Humus-like aromatics that may or may not include damp soil, decaying vegetation, or cellar like characteristics.	Bush's Best Pinto Beans (Canned) = 5.0 (flavor) Sliced Button mushroom = 10.5 (f) Preparation: Serve chopped mushroom in 1 oz cups.
Woody	The sweet, brown, musty, dark, dry aromatics associated with the bark of a tree.	Diamond Shelled Walnuts = 4.0 (flavor)
Roasted	Dark brown impression characteristic of products cooked to a high temperature by dry heat. Does not include bitter or burnt notes.	Reference: Planters Dry Roasted Unsalted Peanuts= 5.0 (flavor)
Overall Sweet	An aromatic associated with the impression of sweet substances.	Post Shredded Wheat = 1.5 (flavor) General Mills Wheaties = 3.0 (flavor) Lorna Doone Cookie = 4.5 (flavor)

Oily	The light aromatics associated with vegetable oil such as corn or soybean oil.	Kroger Slivered and Blanched Almonds = 4.0 (flavor) HyVee Dry Roasted and Salted Macadamia Nuts = 9.0 (flavor)
Rancid	An aromatic commonly associated with oxidized fat and oils.	Wesson Vegetable Oil = 2.5 (flavor) Preparation: Microwave 1/3 cup of oil on high power for 2 1/2 minutes. Let cool and serve in individual covered cups.
Oxidized	The aromatic associated with aged or highly used oil and fat.	Microwave Oven Heated Wesson Vegetable Oil = 6.0 (flavor) Preparation: Add 300ml of oil from a newly purchased and opened bottle of Wesson Vegetable Oil to a 1000ml glass beaker. Heat in the microwave oven on high power for 3 minutes. Remove from microwave and let sit at room temperature to cool for approximately 25 minutes. Then heat another 3 minutes, let cool another 25 minutes, and heat for one additional 3 minute interval. Let beaker sit on counter uncovered overnight. Serve in 1 oz cup.
Astringent	A feeling of a puckering or a tingling sensation on the surface and/or edge of the tongue and mouth. Reference:	0.030% Alum solution = 1.5 0.050% Alum solution = 2.5 0.075% Alum solution = 3.5 0.10% Alum solution = 5.0
Bitter	A fundamental taste factor of which caffeine is typical.	0.010% Caffeine Solution = 2.0 0.020% Caffeine Solution = 3.5 0.035% Caffeine Solution = 5.0
Sour	A fundamental taste factor of which citric acid is typical.	0.015% Citric Acid Solution = 1.5 0.025% Citric Acid Solution = 2.5

Sweet	A fundamental taste factor of which sucrose is typical.	1% Sucrose Solution = 1.0 2% Sucrose Solution = 2.0 4% Sucrose Solution = 4.0 6% Sucrose Solution = 6.0
Salt	A fundamental taste factor of which sodium chloride is typical.	0.15% Sodium Chloride Solution = 1.5 0.20% Sodium Chloride Solution = 2.5 0.25% Sodium Chloride Solution = 3.5
*0-15 point numeric scale with 0.5 increments was used to rate the intensities of the samples and references.		

Test Design and Sample Evaluation

Microsoft Excel software (Microsoft ©, Redmond, WA, USA) was used to produce a randomized test design (Appendix B) and paper ballots (Appendix D) were used for data collection. Approximately 10 g of sample were served in plastic 92 g cups with plastic lids (Solo Cup Company, Lake Forest, IL, USA) to each panelist for each evaluation. The samples were labeled with random 3-digit blinding codes produced in Microsoft Excel (Microsoft ©, Redmond, WA, USA). The evaluation was performed under ambient temperature (23 °C ± 1 °C) and lighting conditions. The panelists were given 10 minutes to evaluate each sample, assigning attribute intensities to the attributes listed in Table 2-2 using a 0 to 15 point numerical scale with 0.5 increments, where 0.0 = absence of attribute and 15.0 = highest possible intensity. The panelists each received definition and reference sheets (Table 2-2) and a tray of references corresponding with the attributes. As palate cleansers, reverse osmosis, de-ionized water (both room temperature (23 °C ± 1 °C) and heated to approximately 90 °C), 1.27 cm low moisture – part skim mozzarella cheese cubes (Kroger Company, Cincinnati, OH, USA), 0.32 cm peeled cucumber slices, and 0.64 cm peeled carrot slices were used. Five minutes were taken between sample evaluations for palate cleansing.

Eight samples were evaluated each day, with raw, roasted, and candied samples intermixed within each day for the first half of evaluations, and all samples within a preparation method evaluated in one day for the second half of the study. All samples were evaluated in duplicate, each cultivar being analyzed in raw, roasted, and candied forms by each panelist two times. Each replicate was performed over a 3-day period with 120 minute evaluation sessions. The project took place over 8 days, with two days of orientation and 6 days of sample evaluation being utilized.

Statistical Analysis

Analysis of variance (ANOVA) was performed initially to test significant differences between the individual samples (each cultivar under each preparation method) for each attribute, using sample as a fixed effect and replication and panelist as random effects. This was followed by 2-way ANOVA, using cultivar and preparation method as well as the interaction between cultivar and preparation method as fixed effects and rep and panelist as random effects. This was done to test significant differences in attribute intensities between samples across preparation method and cultivar. All ANOVA was carried out at the 5% level of significance. Using Fisher's protected least significant difference (LSD) test, *post hoc* means separations were also analyzed at the 5% level of significance. Statistical analysis was performed using SAS® statistical software (SAS® version 9.3, SAS Institute Inc., Cary, NC, USA) using PROC GLIMMIX and PROC GLM.

Principal Components Analysis (PCA) was additionally performed using the covariance matrix to evaluate the relationships among the cultivars and preparation methods. The PCA biplot allowed for visualization of how the attributes relate to one another as well as to individual samples and helps to explain the variation between the different cultivars and different preparation methods. PCA was performed using all samples and was additionally applied within individual preparation methods (raw, roasted, and candied) to gain further insight into cultivar differences. R software (R version 3.1.1, Ihaka R. and Gentleman, R., Aukland, New Zealand) was used to perform this analysis.

Results

Flavor Variations among Samples

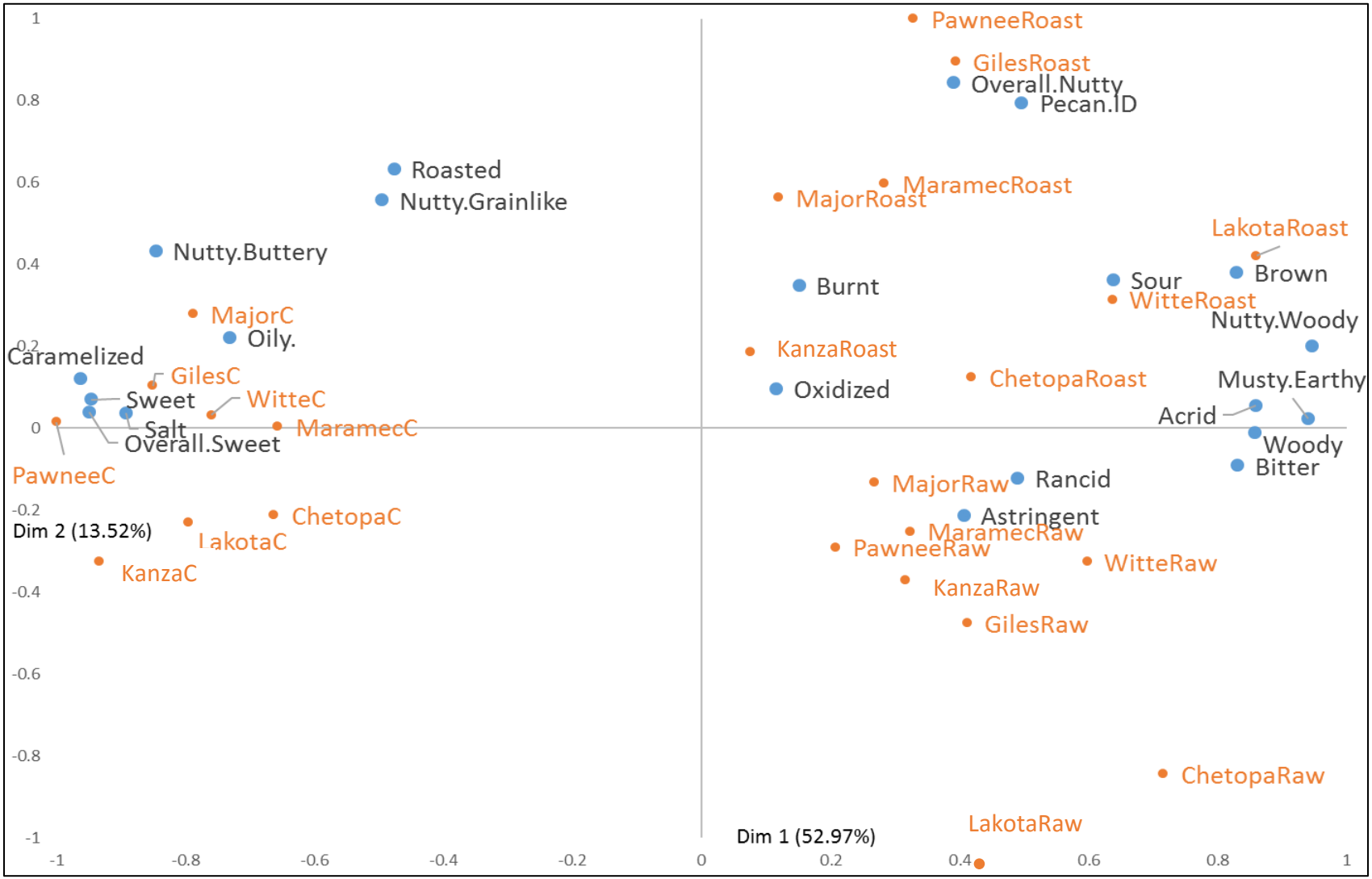
In order to illustrate overarching flavor profile trends, sample profiles were compared to one another, treating each cultivar under each preparation method as individual samples. Spider plots gave visual representation to flavor profiles for each cultivar (Figures 2-2 through 2-9). One-way ANOVA was performed, which revealed that, of the 21 attributes evaluated, 14 attributes showed significant differences ($p \leq 0.05$) in intensity between samples. These attributes were *Pecan ID, Overall Nutty, Nutty-Woody, Nutty-Buttery, Brown, Caramelized, Acrid, Musty/Earthy, Woody, Roasted, Overall Sweet, Bitter, Sweet,* and *Salt* attributes. *Nutty-Grainlike, Burnt, Oily, Rancid, Oxidized, Astringent,* and *Sour* attributes did not show significant differences between the samples at a 5% significance level. Many of these attributes that did not show significant differences between samples had negligible intensities, including the attributes *Burnt, Rancid,* and *Oxidized.*

Principal Components Analysis was performed, using each cultivar under each preparation method as individual samples, to visualize relationships between samples and attributes (Figure 2-1). PCA allowed for extrapolation about the sources of variation between the samples in terms of the evaluated attributes. In this experiment, looking at the samples individually, principal component 1 explained 52.97% of the variation among samples. This component was highly correlated to *Sweet, Overall Sweet, Salt,* and *Caramelized* attributes at one end of the spectrum and *Nutty-Woody, Acrid, Bitter, Woody,* and *Musty/Earthy* lying on the opposing end. Principal component 2, which explained 13.52% of the variation between samples, was more highly correlated with the attributes *Astringent, Bitter,* and *Woody* on one end and *Overall Nutty, Pecan ID,* and *Roasted* on the other. Samples within each preparation method fell

within the same region. The raw and candied samples were close to one another within their preparation method with the exception of the raw 'Chetopa' and the raw 'Lakota' samples, which were slightly further away from the other raw samples. The roasted samples, however, exhibited a larger spread. Several attributes were closely associated with each of these groups. *Nutty-Buttery, Oily, Overall Sweet, Sweet, Salt,* and *Caramelized* attributes were all within the same region as the candied samples; *Brown, Musty/Earthy, Woody, Nutty-Woody, Bitter,* and *Acrid* attributes were found within the same region, between the raw and roasted samples; *Astringent* and *Rancid* attributes were closely related to the raw samples; and *Roasted, Pecan ID,* and *Overall Nutty* attributes were found with the roasted samples.

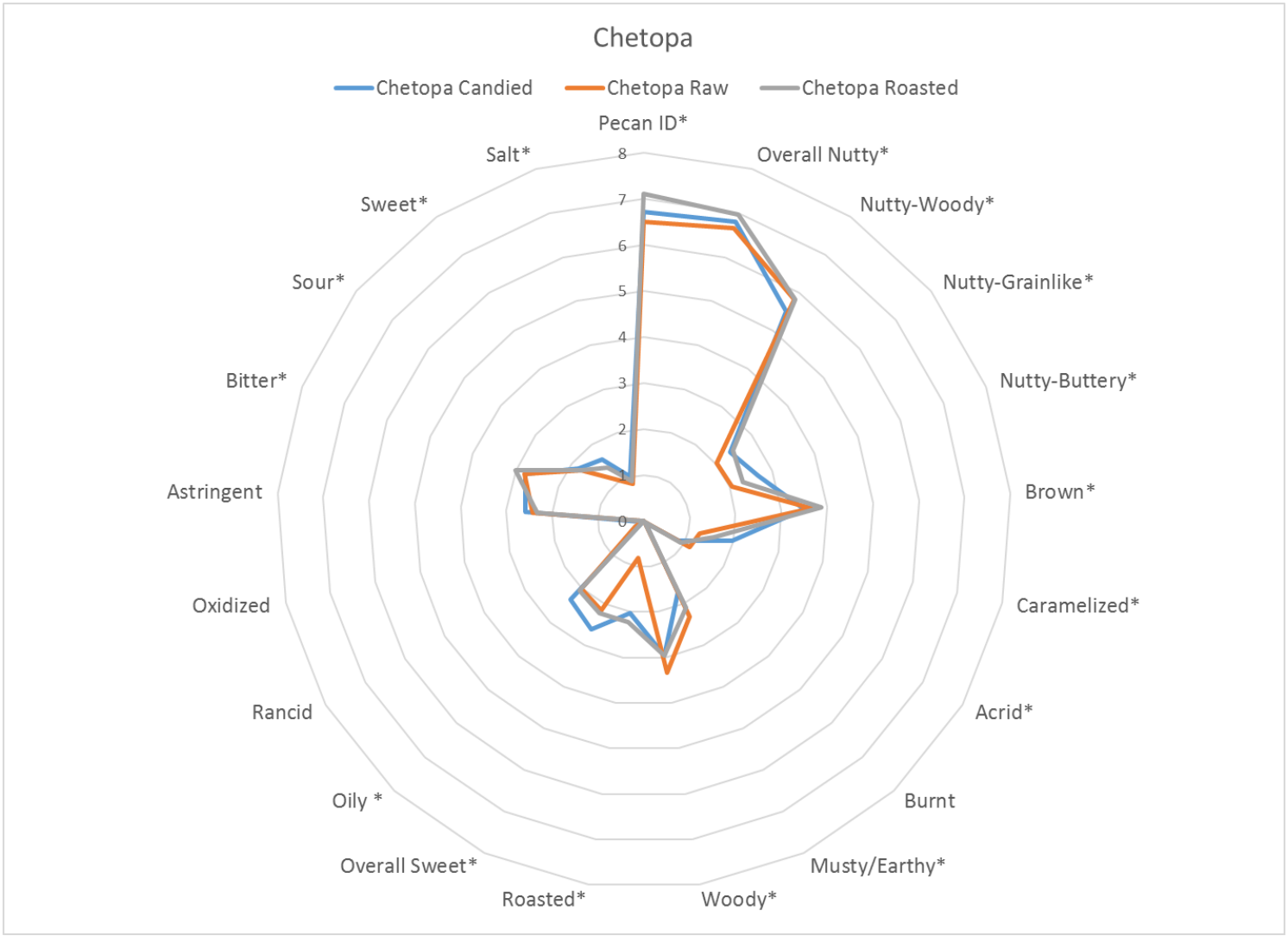
To obtain further insight into the variations between samples, 2-way ANOVA was performed across all flavor attributes, taking into account both cultivar variations and preparation method variations, as well as an interaction between cultivar and method. Through this analysis, significant differences in attribute intensities across cultivars and across preparation methods were able to be identified (Table 2-3). Cultivar significantly affected *Pecan ID, Nutty-Buttery, Caramelized, Acrid,* and *Astringent* attributes, while the preparation method significantly affected *Pecan ID, Overall Nutty, Nutty-Woody, Nutty-Grainlike, Nutty-Buttery, Brown, Caramelized, Acrid, Musty/Earthy, Woody, Roasted, Overall Sweet, Oily, Bitter, Sour, Sweet,* and *Salt* attributes. The interaction between the cultivar effect and the preparation method effect was not significant for any attributes.

Figure 2-1. Principal Components Analysis of descriptive attributes across all cultivars and preparation methods *



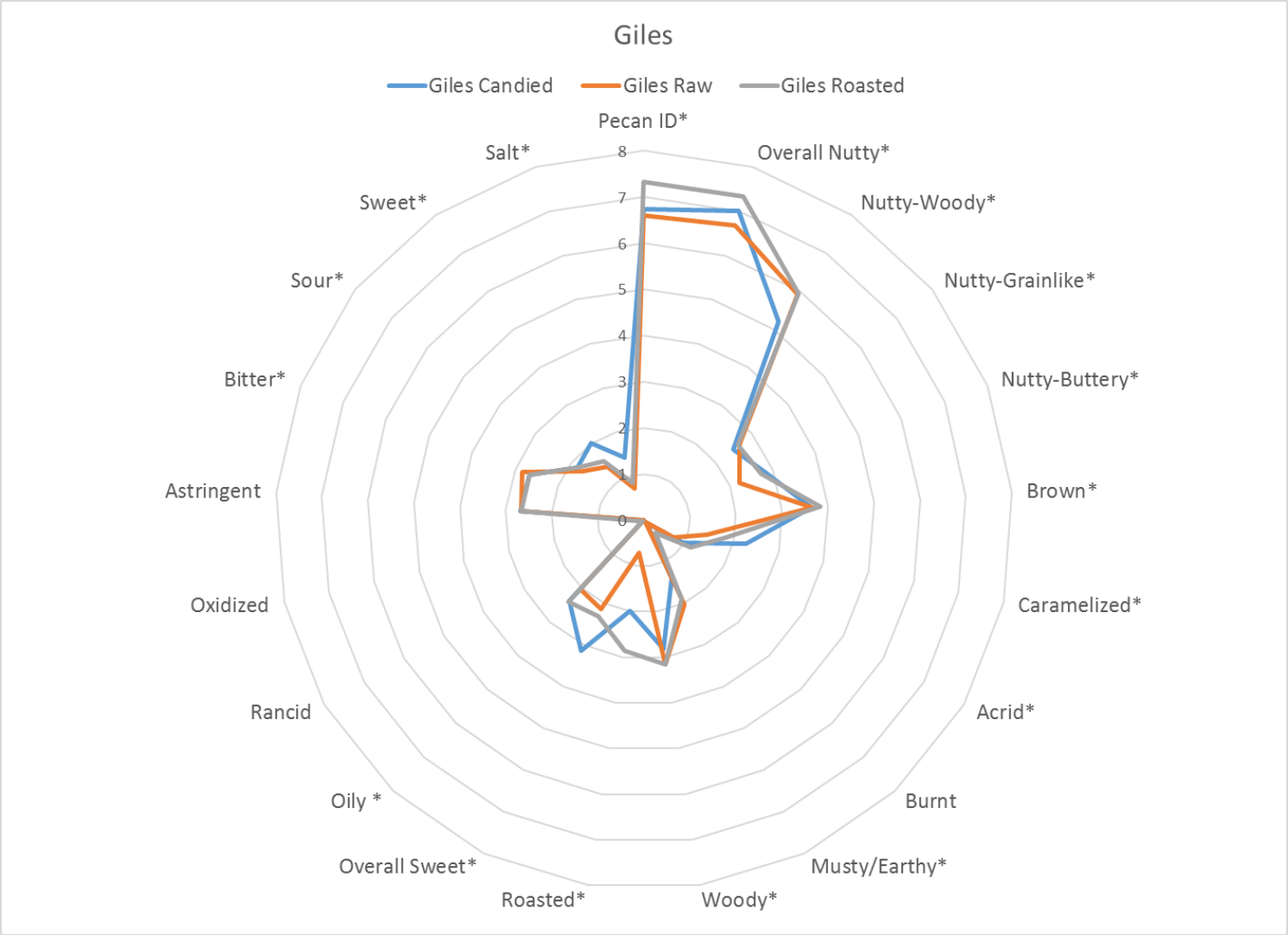
* Sample names consist of cultivar and preparation method (Raw = raw; Roast = roasted; C = candied)

Figure 2-2. Spider plot of 'Chetopa' cultivar [a]



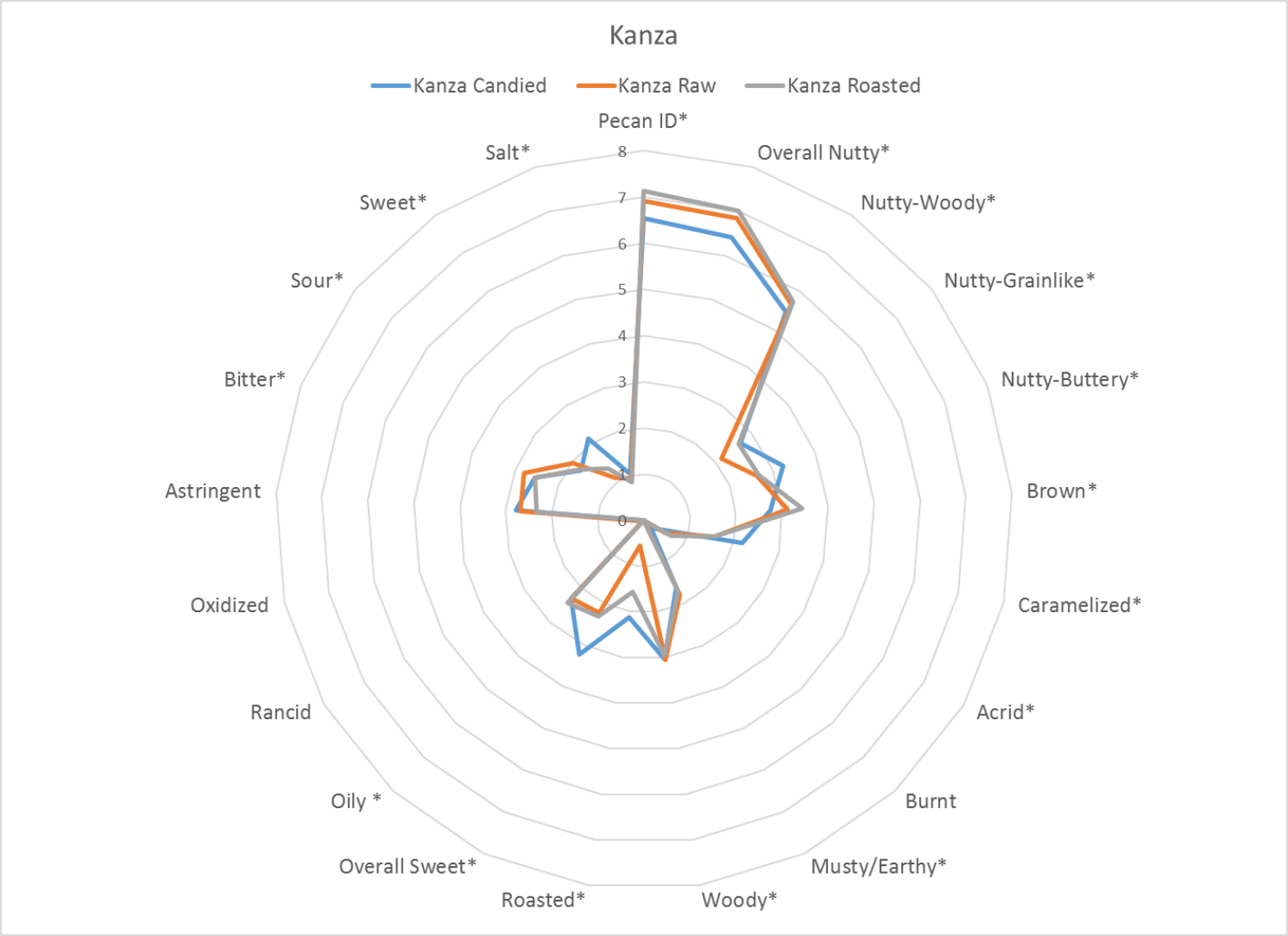
[a] All attribute intensities were measured on a scale of 0 to 15 with 0.5 increments
*Statistically significant difference between preparation methods (raw, roasted, and candied) ($p \leq 0.05$)

Figure 2-3. Spider plot of 'Giles' cultivar [a]



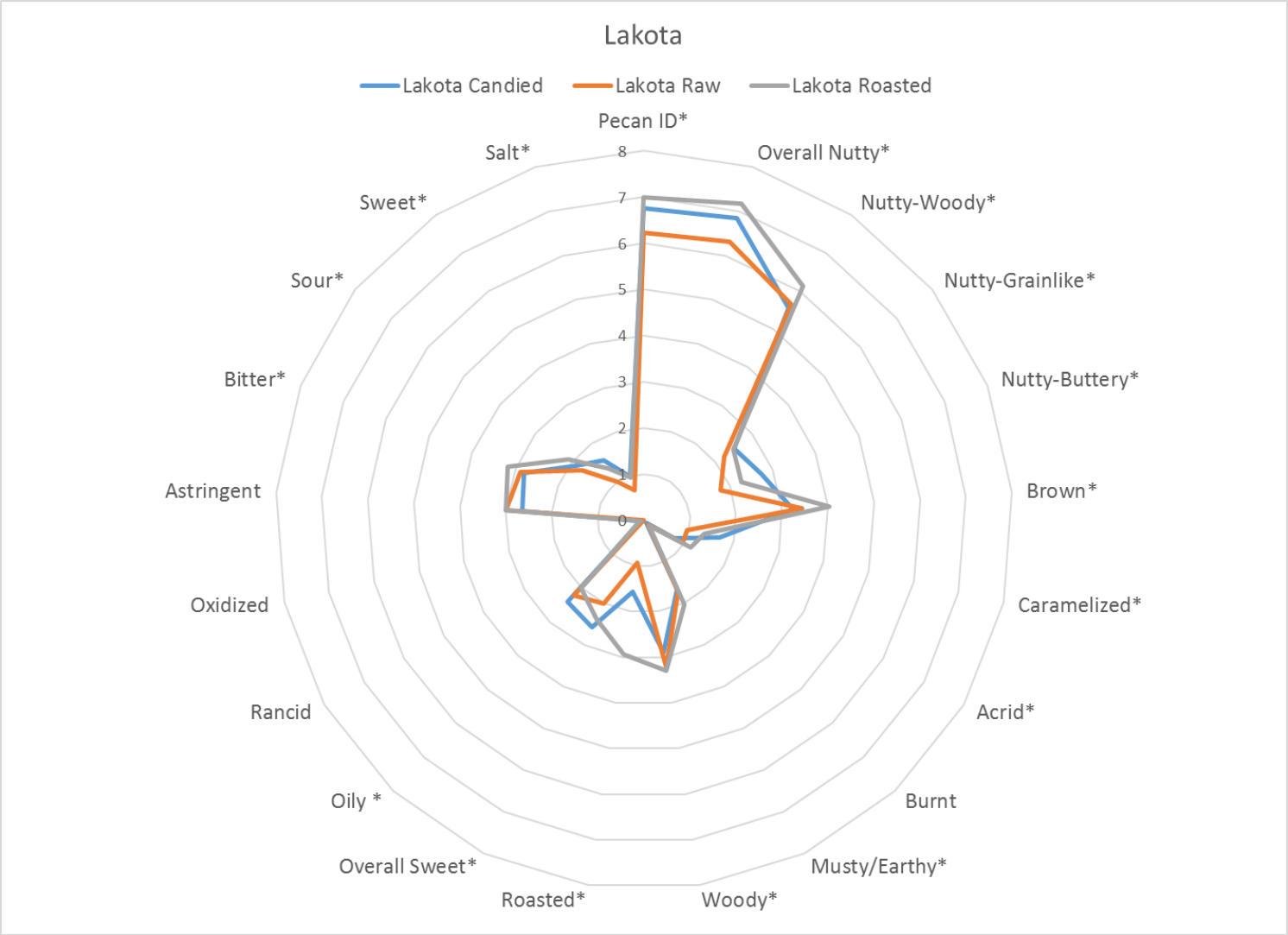
[a] All attribute intensities were measured on a scale of 0 to 15 with 0.5 increments
*Statistically significant difference between preparation methods (raw, roasted, and candied) ($p \leq 0.05$)

Figure 2-4. Spider plot of 'Kanza' cultivar [a]



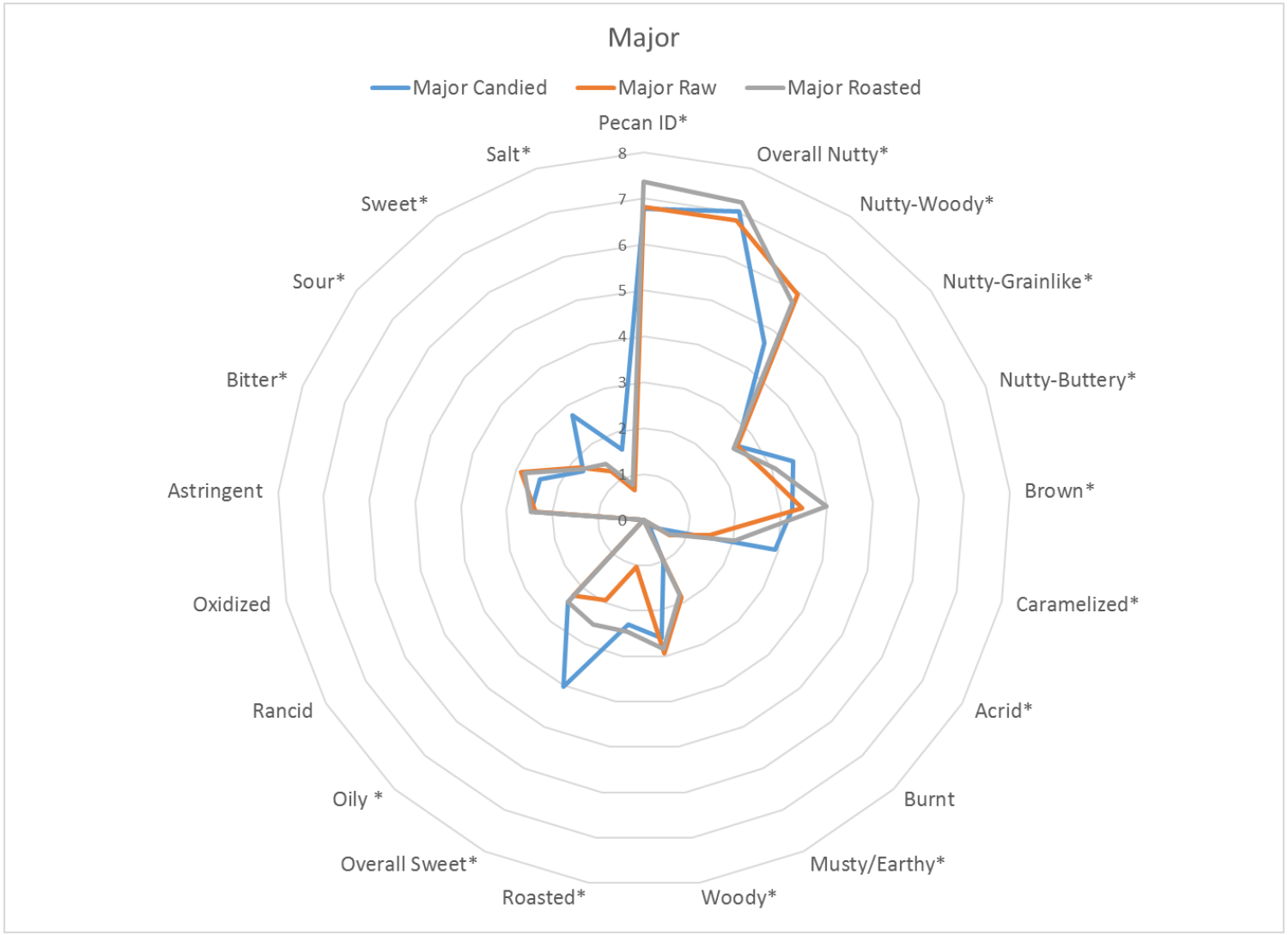
[a] All attribute intensities were measured on a scale of 0 to 15 with 0.5 increments
*Statistically significant difference between preparation methods (raw, roasted, and candied) ($p \leq 0.05$)

Figure 2-5. Spider plot of 'Lakota' cultivar [a]



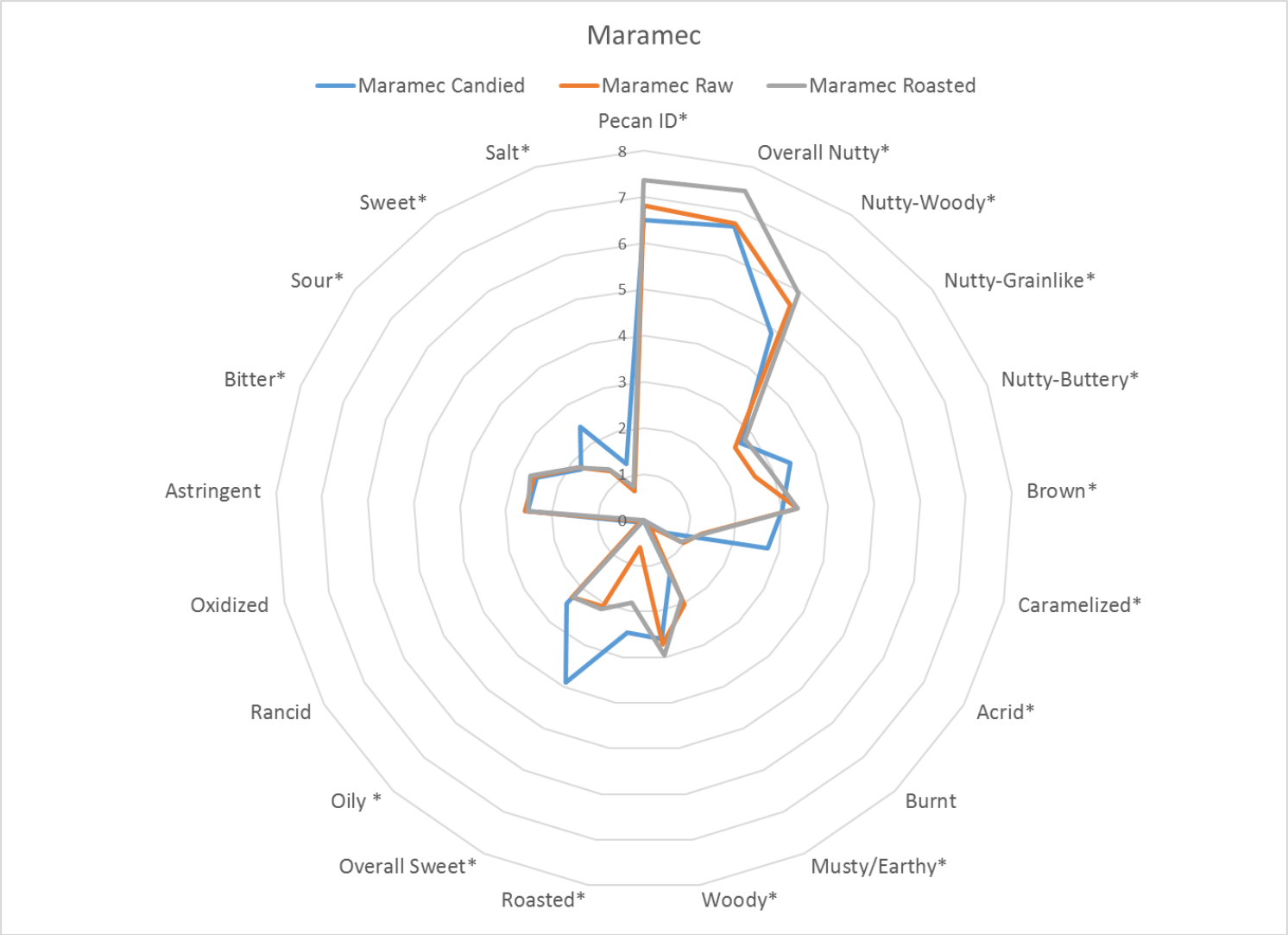
[a] All attribute intensities were measured on a scale of 0 to 15 with 0.5 increments
*Statistically significant difference between preparation methods (raw, roasted, and candied) ($p \leq 0.05$)

Figure 2-6. Spider plot of 'Major' cultivar [a]



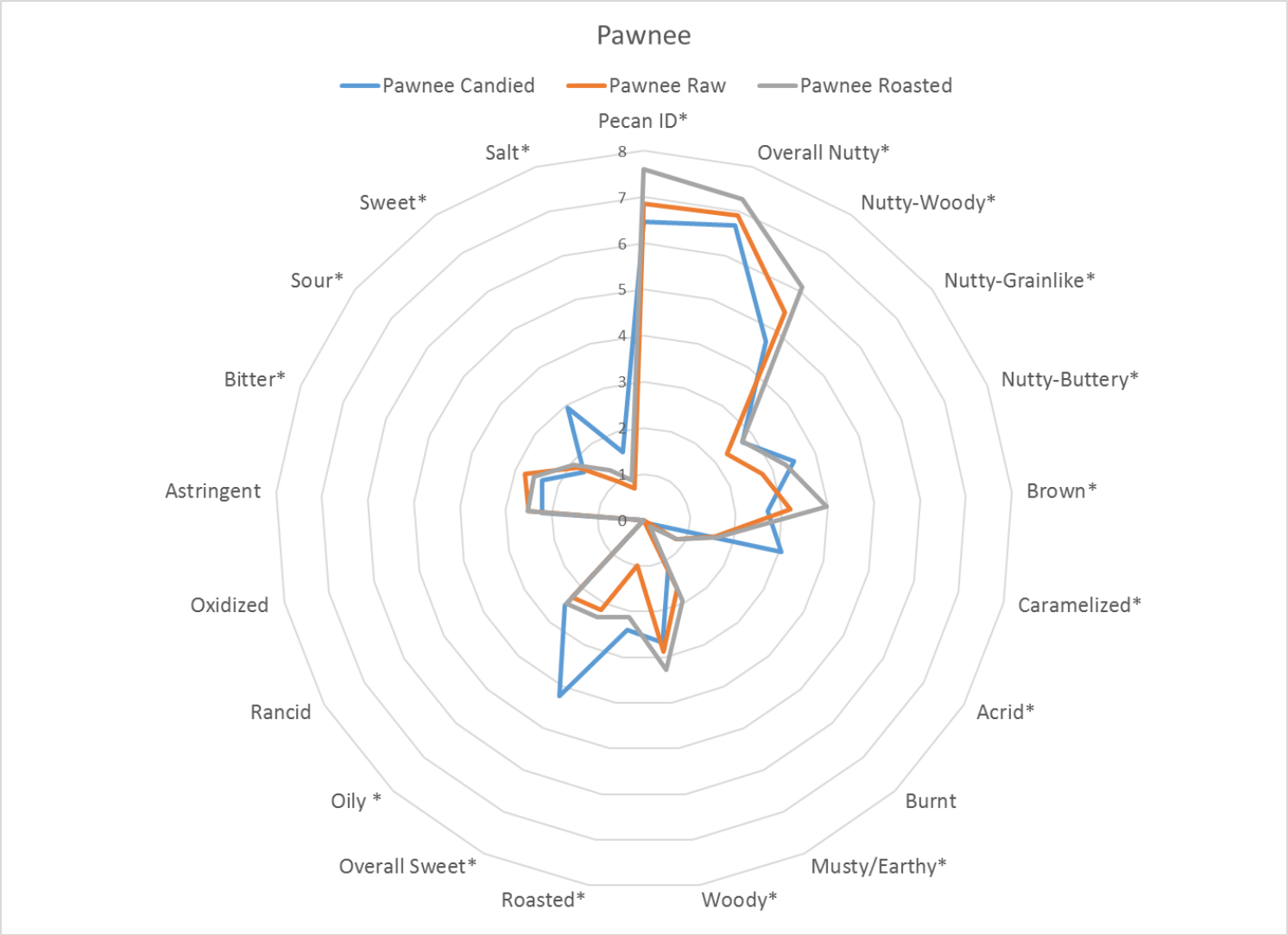
[a] All attribute intensities were measured on a scale of 0 to 15 with 0.5 increments
*Statistically significant difference between preparation methods (raw, roasted, and candied) ($p \leq 0.05$)

Figure 2-7. Spider plot of 'Maramec' cultivar [a]



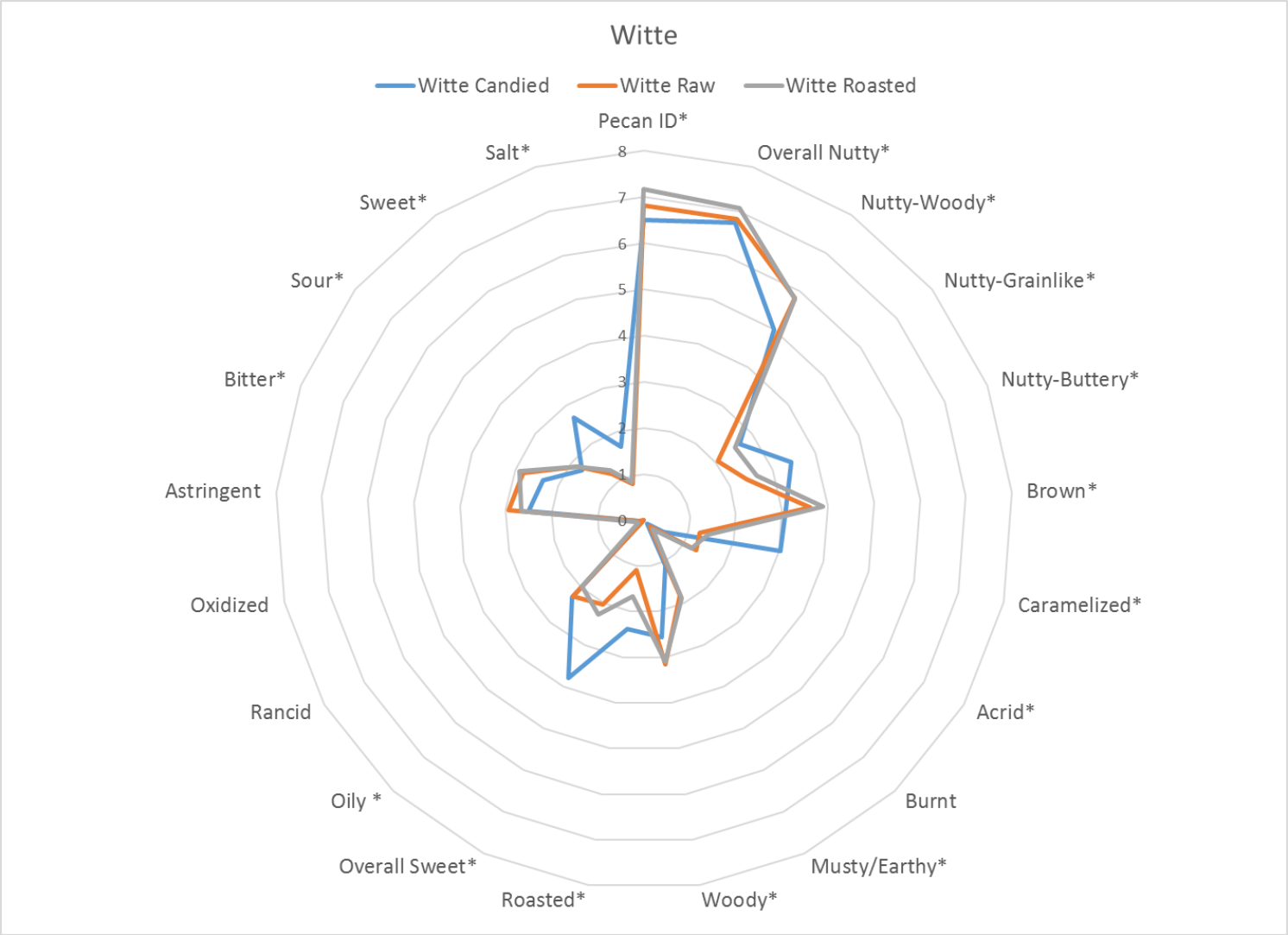
[a] All attribute intensities were measured on a scale of 0 to 15 with 0.5 increments
*Statistically significant difference between preparation methods (raw, roasted, and candied) ($p \leq 0.05$)

Figure 2-8. Spider plot of 'Pawnee' cultivar [a]



[a] All attribute intensities were measured on a scale of 0 to 15 with 0.5 increments
*Statistically significant difference between preparation methods (raw, roasted, and candied) ($p \leq 0.05$)

Figure 2-9. Spider plot of 'Witte' cultivar [a]



[a] All attribute intensities were measured on a scale of 0 to 15 with 0.5 increments
*Statistically significant difference between preparation methods (raw, roasted, and candied) ($p \leq 0.05$)

Table 2-3. P-values of individual factors and factor interactions from ANOVA for flavor attributes [a]

Flavor Attribute	Cultivar	Method	Cultivar*Method
Pecan ID	**0.0277**	**<0.0001**	0.9377
Overall Nutty	0.3362	**<0.0001**	0.9354
Nutty-Woody	0.9870	**<0.0001**	0.9734
Nutty-Grainlike	0.7086	**0.0172**	0.9482
Nutty-Buttery	**<0.0001**	**<0.0001**	0.6402
Brown	0.3107	**<0.0001**	0.9611
Caramelized	**0.0218**	**<0.0001**	0.8844
Acrid	**0.0002**	**<0.0001**	0.7309
Burnt	0.1832	0.5950	0.6641
Musty/Earthy	0.5145	**<0.0001**	0.9287
Woody	0.0569	**<0.0001**	0.7581
Roasted	0.1274	**<0.0001**	0.4900
Overall Sweet	0.4398	**<0.0001**	0.7479
Oily	0.6355	**0.0273**	0.8822
Rancid	0.4946	0.2040	0.0763
Oxidized	0.1531	0.9414	0.4789
Astringent	**0.0054**	0.2167	0.6072
Bitter	0.0998	**<0.0001**	0.8767
Sour	0.9587	**0.0065**	0.6095
Sweet	0.1245	**<0.0001**	0.7775
Salt	0.4105	**<0.0001**	0.8860

[a] Significance taken at $p \leq 0.05$

[b] Bolded p-values indicate a significant difference (5% level of significance) between samples for the given attribute

Effect of Preparation Method

Seventeen attributes differed significantly ($p \leq 0.05$) with preparation method as a factor (Table 2-3). These were *Pecan ID, Overall Nutty, Nutty-Woody, Nutty-Grainlike, Nutty-Buttery, Brown, Caramelized, Acrid, Musty/Earthy, Woody, Roasted, Overall Sweet, Oily, Bitter, Sour, Sweet,* and *Salt.* A similar study examined hazelnuts under raw and roasted conditions, evaluating for sixteen flavor attributes: aftertaste, bitter, bunt, coffee/chocolate-like, caramel-like, fruity, green/grassy, nutty, oily, painty, pungent, rancid, roasty, sour, sweet, and woody, finding that the roasting process had a significant effect on half of the attributes (Alasalvar et al. 2003). In this study, however, only four attributes were not significantly affected by preparation method: *Burnt, Rancid, Oxidized,* and *Astringent*. *Burnt, Rancid,* and *Oxidized* attributes showed trivial intensities for all samples, also not showing the factor of cultivar to have a significant effect.

For *Pecan ID, Nutty-Buttery, Brown, Caramelized, Overall Sweet,* and *Sweet* attributes, all three preparation methods yielded significantly different ($p \leq 0.05$) attribute intensities. Candied samples had the highest intensities, followed by the roasted samples, then the raw samples for *Nutty-Buttery, Caramelized, Overall Sweet,* and *Sweet* attributes, each significantly different from other preparation methods. Similarly, roasted samples had significantly higher attribute intensities for *Pecan ID* and *Brown,* followed by the raw samples, then the candied samples (Figures 2-2 through 2-9).

Several attributes were only significantly different ($p \leq 0.05$) for one preparation method when compared to the other methods. These were *Overall Nutty, Nutty-Woody, Nutty-Grainlike, Acrid, Musty/Earthy, Woody, Roasted, Oily, Bitter, Sour,* and *Salt.* Raw samples had significantly lower intensities of *Nutty-Grainlike* and *Roasted* attributes than both the roasted and

the candied samples. Roasted samples showed significantly higher ($p \leq 0.05$) *Overall Nutty* intensity than both raw and candied samples and significantly higher *Sour* intensity than the candied samples. Candied pecans yielded significantly higher *Oily* intensity than the raw samples, significantly higher *Salt* intensities than the raw or roasted samples, and significantly lower *Nutty-Woody*, *Acrid*, *Musty/Earthy*, *Woody*, and *Bitter* intensities than the raw or roasted samples.

Cultivar Effect

In looking at Principal Components Analysis with every sample (Figure 2-1), it is clear that the majority of the variation among samples is due to differences in attributes relating to the preparation method, yielding a plot with samples grouped by preparation method. Though the main focus of this experiment was on flavor profile differences due to the effects preparation method, several differences worth noting were found due to cultivar variation. In order to obtain further insight on cultivar differences, PCA was performed within each preparation method (Figures 2-10 through 2-12), allowing for visualization of which attributes contribute the most to cultivar variation.

PCA performed among raw samples explained 49.77% of the variation between the samples (Figure 2-10). Principal component 1, which explained 32.27% of the cultivar variation for raw samples, was closely linked to *Woody* versus *Nutty*-type attributes. *Astringency* was highly correlated to principal component 2, which explained 17.50% of the sample variation. The majority of the attributes were not highly associated with one another, exhibiting a predominantly even spread across the plot. However, *Pecan ID, Nutty-Buttery, Overall Nutty,* and *Caramelized* attributes were closely associated. Additionally, raw ‘Pawnee’ and raw ‘Kanza’

samples were closely aligned with one another. The ‘Giles’ and ‘Chetopa’ cultivars were more pronounced in *Musty/Earthy* and *Nutty-Woody* attributes, the raw ‘Witte’ samples were more pronounced in *Roasted, Burnt,* and *Astringent* notes, ‘Major’ showed association with *Sweet* and *Nutty-Grainlike* attributes, the ‘Maramec’ cultivar was close to the *Overall Sweet* flavor attribute, and the ‘Kanza’ and ‘Pawnee’ samples showed *Sour, Pecan ID,* and *Nutty-Buttery* association. The raw ‘Lakota’ cultivar was not closely associated with any attributes, but was highly driven by *Astringency* and *Woodiness*.

Among the roasted samples, PCA explained 58.21% of sample variation, with principal component 1 explaining 39.60% of the variation and principal component 2 explaining the remaining 18.61% (Figure 2-11). In principal component 1, variation in sensory attributes was associated to differences in *Astringent* and *Acrid* attributes versus *Oily, Nutty,* and *Caramelized* attributes. In principal component 2, *nuttiness* versus *bitterness* explained differences among samples. *Pecan ID, Nutty-Buttery, Nutty-Grainlike,* and *Oily* attributes were associated with one another. Similarly, *Nutty-Woody, Woody, Sour, Astringent, Acrid, Burnt, Roasted, Musty/Earthy, Brown, Salt, Rancid, Oxidized,* and *Bitter* attributes were found within the same region. The roasted ‘Pawnee’ sample showed high association with *Overall Nuttiness*, ‘Maramec’ was aligned with *Nutty-Grainlike* and *Nutty-Buttery* attributes, the ‘Major’ and ‘Kanza’ samples were associated with the *Caramelized* and *Sweet* attributes, ‘Witte’ was more pronounced in *Brown* and *Oxidized* notes, and the ‘Lakota’ cultivar was highly driven by attributes relating to *Astringency* and *Acrid*. The ‘Chetopa’ cultivar did not show a link with any specific attributes, however was driven by principal component 2 with *Bitterness*. The ‘Giles’ cultivar also did not show a close connection to specific flavor attributes, however was more closely associated with the *Nuttiness* of principal component 2 than the *Bitterness.*

Looking at samples under the candied preparation method, Principal Components Analysis revealed additional cultivar differences (Figure 2-12). Principal component 1 (34.10%) is linked to *Sweetness* and *Saltiness,* attributes related to the candying process. Principal component 2, explaining 22.83% of sample variation, was linked to sample oiliness. Aligning with the candying process, *Sweet, Overall Sweet, Salt, Caramelized, Nutty-Buttery, Pecan ID,* and *Overall Nutty* attributes were associated with one another. The 'Pawnee,' 'Giles,' and 'Major' cultivars were more prominent in *Overall Sweet, Sweet, Salt, Caramelized,* and *Nutty-Buttery* attributes, 'Kanza' was associated with *Overall Nuttiness*, 'Maramec' was aligned with *Oxidized* and *Nutty-Woody* notes, and the 'Chetopa' samples showed a connection with *Acrid, Astringent,* and *Nutty-Woody* attributes. The 'Witte' and 'Lakota samples were not associated with any specific attributes, however 'Witte' was slightly driven by principal component 2 in lower *Oiliness* and higher *Musty/Earthiness* and the 'Lakota' sample was strongly driven by both principal components 1 and 2 with high *Oiliness* and low candied-type notes.

In each of these PCA plots, the Lakota cultivar was an obvious outlier. It was not closely associated with any of the other cultivars, but was highly driven by *Astringent, Bitter, Woody,* and *Acrid* attributes, falling on the more extreme ends of factors related to these attributes.

Two-way ANOVA shed additional light onto cultivar differences. For five attributes, the cultivar was a significant factor for attribute intensity differences ($p \leq 0.05$; Table 2-3). These were *Pecan ID, Nutty-Buttery, Caramelized, Acrid,* and *Astringent.* For *Pecan ID*, 'Major' and 'Pawnee' showed significantly higher intensities than 'Chetopa' and 'Lakota' cultivars. *Nutty-Buttery* intensities were significantly lower in 'Chetopa' and 'Lakota' samples as well. 'Major' and 'Pawnee' samples also showed significantly higher *Caramelized* intensities than 'Maramec' and 'Lakota' cultivars. For the *Acrid* attribute, 'Witte,' 'Chetopa,' and 'Lakota' samples yielded

significantly higher intensities than ‘Pawnee,’ ‘Major,’ and ‘Kanza.’ Finally, *Astringent* intensities were significantly higher in the ‘Lakota’ samples than in all other samples except for the ‘Witte’ cultivar.

Figure 2-10. Principal Components Analysis of all cultivars within the raw preparation method

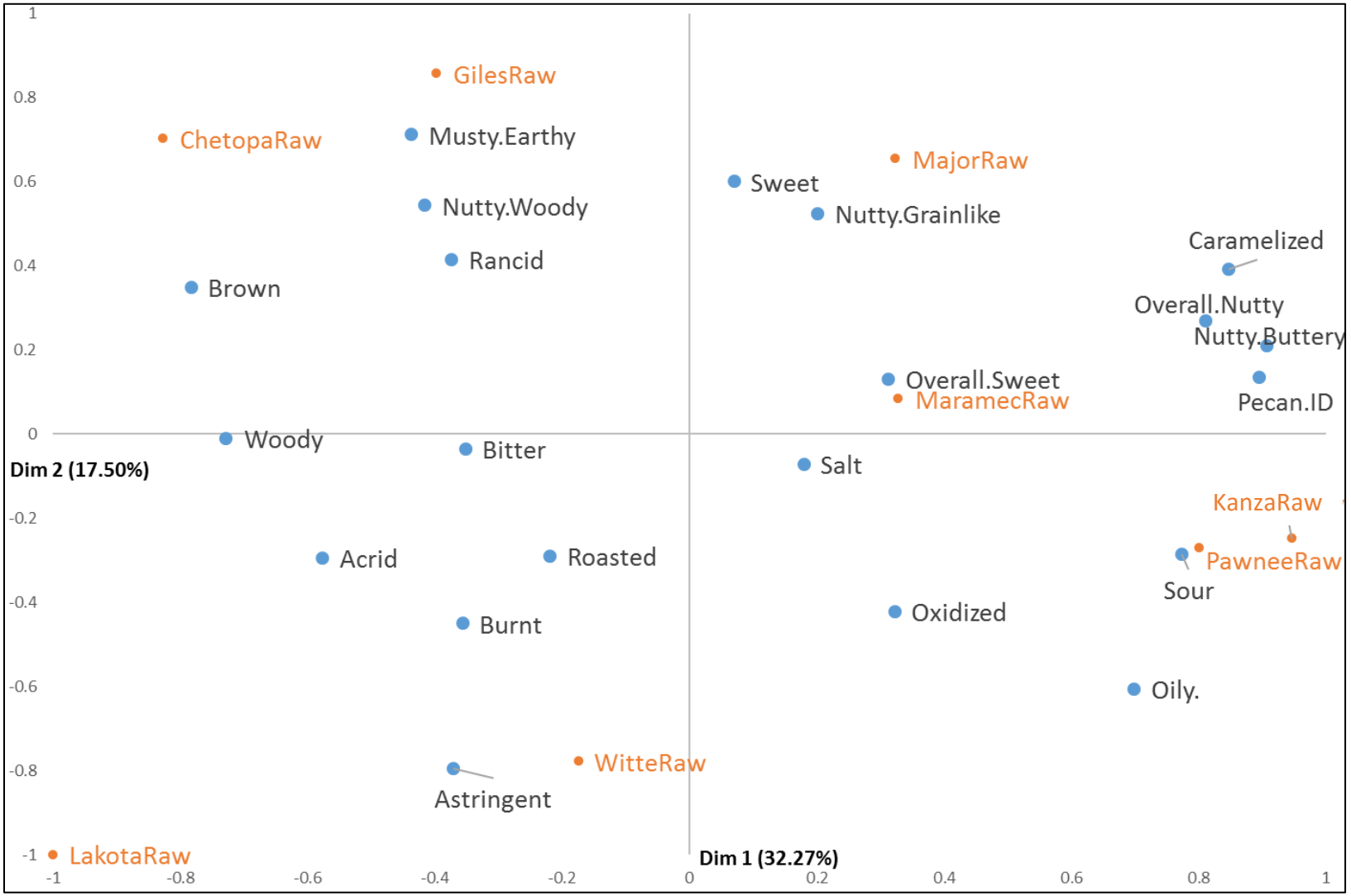


Figure 2-11. Principal Components Analysis of all cultivars within the roasted preparation method

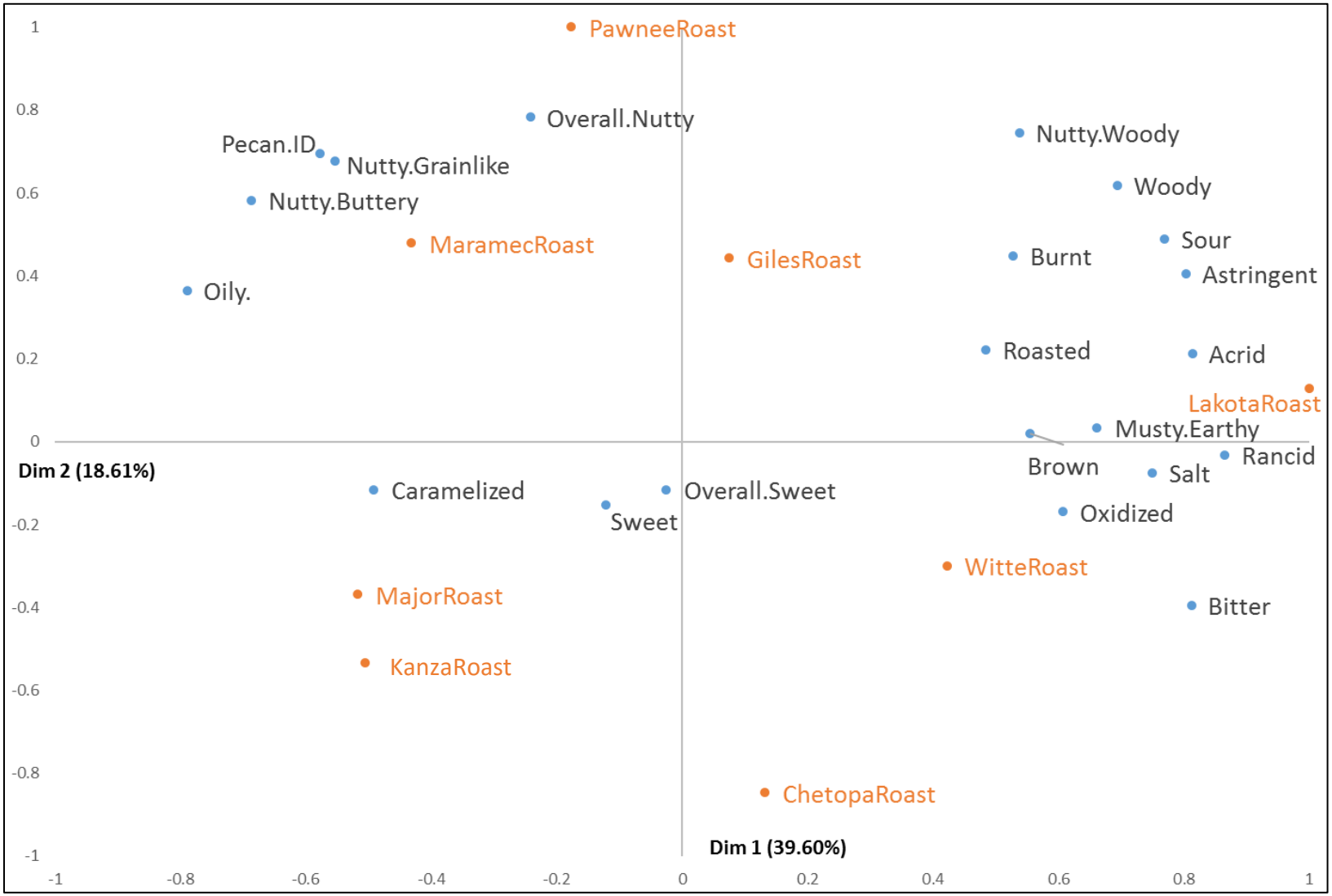
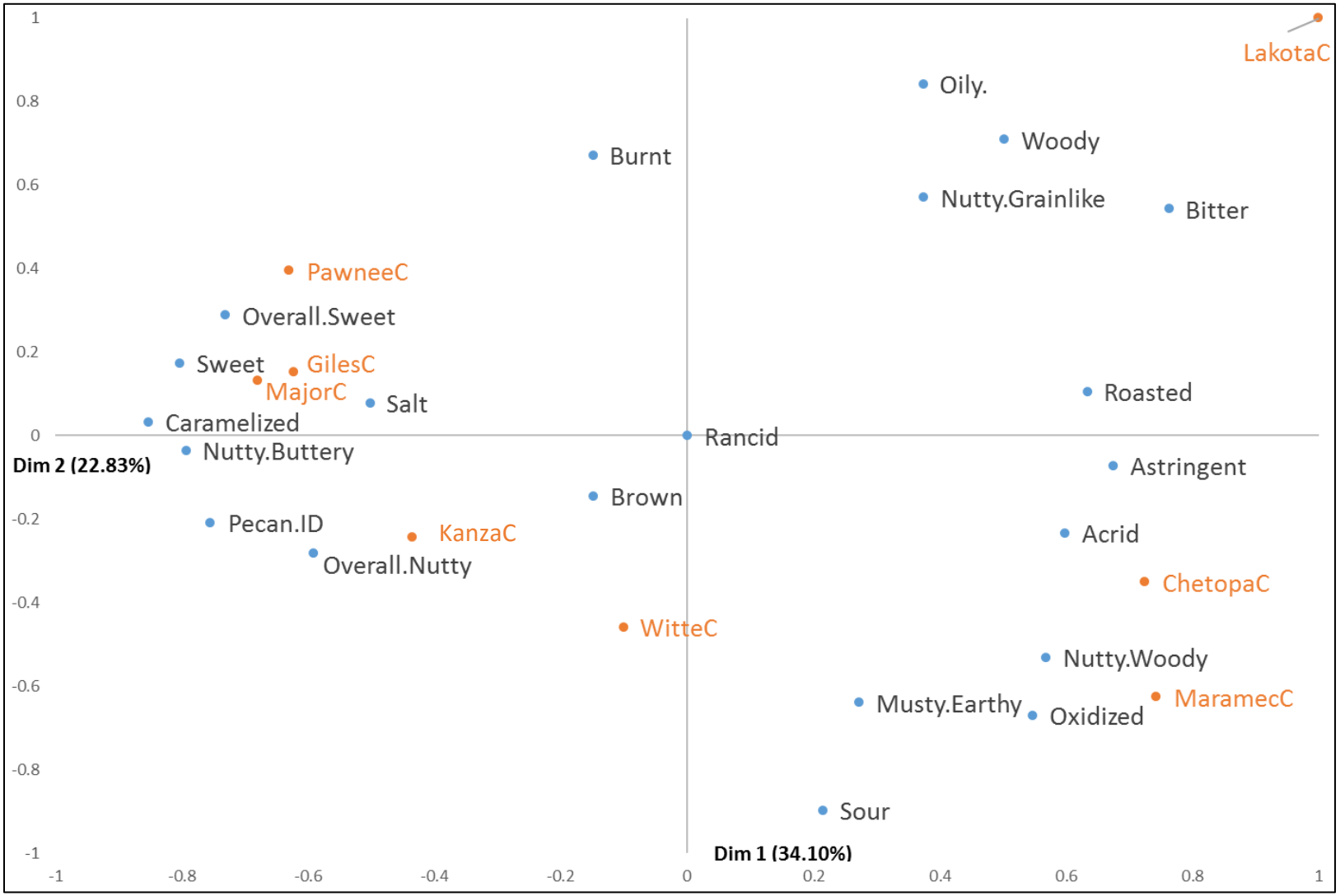


Figure 2-12. Principal Components Analysis of all cultivars within the candied preparation method



Discussion

Many of the samples yielded similar flavor profiles, an expected result with each of the cultivars being of the species *Carya illinoinensis*. However, profile variations were also expected and exhibited by experimental results. Several factors contributed to these sensory differences. Each cultivar's unique chemical composition and concentrations of flavor characterizing chemicals stimulate different responses in the tasting situation. Fatty acids, comprising 70-79% of nutmeat (wt/wt), are found in varying levels between different cultivar samples and certain fatty acids may be more prone to oxidation than others, affecting flavor (Toro-Vazquez and Perez-Briceno 1998, 1999). Moisture content may also play a role in flavor variation. A variety of factors may affect moisture content, including storage conditions, rainfall during the growth stage of the nutmeat, and composition of the cultivar. In an industry setting, moisture content is maintained below 4.5% in pecans and similar nuts to limit potential for spoilage through bacterial and yeast contamination. However, a low moisture content may affect flavor. In this experiment, pecans were examined under consumer-available conditions, exhibiting a range of moisture content below 4.5%, and moisture content was not altered for individual cultivars. Additional processing, in this case roasting and candying, may affect cultivars, each with a unique chemical makeup, in different ways.

Although many of the profiles exhibited similar trends in the visually representative spider plots (Figures 2-2 through 2-9), clear differences were largely present within each cultivar between samples with different preparation methods as well as between the cultivars themselves. Examining all samples individually (each cultivar under each preparation method) revealed three groupings of samples, corresponding with the three preparation methods. This was confirmed in PCA (Figure 2-1), with factor 1 corresponding to attributes related to the candying process and

factor 2 corresponding with attributes associated with the roasting process. The samples within each preparation method fell within the same region of the PCA plot, indicating that the preparation method has a large impact on flavor profile. Raw and candied samples were closely associated to other samples within their preparation methods but roasted samples exhibited a wider spread, indicating that cultivar differences had a larger impact on the roasted sample variation than within the candied or raw samples. *Pecan ID, Brown, Overall Nutty, Sour* and *Nutty-Grainlike* attributes were brought out by the roasting process. The candying process enhanced several attributes (*Nutty-Grainlike, Nutty-Buttery, Caramelized, Overall Sweet, Sweet, Salt, Oily*) and masked others (*Nutty-Woody, Acrid, Musty/Earthy, Woody, Bitter*).

Because this study examined samples from only one growing season, conclusions on cultivar differences are limited in scope; results may not entail all variation that would be captured in a study covering multiple growing seasons. However, in this experiment, cultivar variation played a significant role in attribute intensity differences in several cases. Several cultivars consistently showed higher intensities of certain attributes. Most notably, the 'Lakota' cultivar exhibited a stronger association with undesirable attributes such as *Astringent, Bitter, Woody,* and *Acrid* and a low association with any other cultivar. The 'Lakota' samples also exhibited low intensities of more desirable attributes, such as *Pecan ID, Nutty-Buttery,* and *Caramelized.* Conversely, the 'Pawnee' cultivar showed generally higher intensities of desirable attributes, such as *Pecan ID, Nutty-Buttery,* and *Caramelized,* and lower intensities of undesirable attributes such as *Acrid*. Within each preparation method, cultivar variation was explained by similar attribute differences across preparation methods. However, for the candied samples, a large portion of the variation can be explained by attribute intensity variation related

to the candying process, suggesting that the variation is largely explained by the amount of grooves and creases present within each cultivar in which the glaze accumulated.

Though the presence of pecan flavor research is limited, similar findings were surmised by one study performed by Magnuson et al. (2016). Flavor profiles of 'Chetopa,' 'Giles,' 'Kanza,' 'Lakota,' 'Major,' 'Maramec,' 'Pawnee,' and 'Witte' pecans were compared under raw and roasted preparation methods. Flavor profiles unique to each cultivar under each preparation method were found, some exhibiting outlying characteristics such as high *Astringency, Bitter,* and *Woody* characteristics in the 'Lakota' cultivar and the *Oily, Nutty* nature of the 'Pawnee' cultivar. The roasting process was found to significantly affect *Pecan ID, Overall Nutty*, *Nutty-Woody, Nutty-Grainlike, Nutty-Buttery, Brown, Caramelized, Roasted, Overall Sweet,* and *Sweet* attributes. These conclusions drawn from Magnuson's research corroborate the findings of this study, similar trends being found for the 'Lakota' and 'Pawnee' cultivars and each of these attributes being significantly affected by preparation method across the studies.

This study included 8 cultivars of pecans grown in Chetopa, Kansas obtained from a single growing season. The incorporation of further cultivars within and outside of the region could lead to additional flavor profiles and profile variations. An additional growing season would furthermore explain some of the profile variation that is due to seasonal variation. Because only a single procedure was used for each of the preparation methods, optimal flavor profiles may not have been achieved. Future research should study different roasting and candying methods and their effects on flavor profiles. However, studying the flavor profiles for different cultivars under different preparation methods opens opportunities for further study of pecan flavor.

Conclusions

While many similarities existed among the samples, across cultivars and preparation methods, such as negligible *Burnt, Rancid,* and *Oxidized* attribute intensities, each cultivar constructed a unique profile. Some of these profiles were more unique than others, such as the high *Pecan ID* and *Caramelized* notes and low *Astringent* and *Acrid* attribute intensities of the 'Pawnee' and 'Major' cultivars and 'Lakota's' low *Pecan ID, Caramelized,* and *Nutty*-Buttery intensities and a high association with *Acrid, Astringent,* and related attributes. Despite this, sample variation was largely driven by attributes linked to the preparation method used. The attributes closely linked to the candying process can be broadly categorized into sweetness, saltiness, and buttery, while the roasting process was linked to nutty and roasted type attributes. The raw samples were closely associated with musty, bitter, and woody type attributes. A closer examination revealed that 17 attributes were significantly affected by the preparation method effect, while only 5 attributes were significantly affected by the cultivar effect. Of those attributes with cultivar being a significant factor, only *Astringent* is affected by cultivar but not preparation method, indicating that cultivar variation was the predominant source of astringency variation in this experiment. The interaction term between cultivar and preparation method was not significant for any of the attributes, meaning that the preparation method affected each of the attributes for each of the cultivars in similar ways.

A secondary conclusion that can be drawn from this experiment is that the candying process may be useful in masking certain attributes. *Nutty-Woody, Acrid, Musty/Earthy, Woody,* and *Bitter* attributes were shown to have significantly lower intensities in candied samples when compared to raw and roasted samples.

Future research will focus on consumer acceptance of cultivars under different preparation methods as well as on compositional differences between these samples. This, in conjunction with the findings of this experiment, will allow for better marketing and increased application of pecans.

References

Adams, J. and Thielges, B. (1977). Research underway on pecan timber improvement. *Louisiana Agriculture,* 20(2): 14-15.

Alasalvar, C. & Shahidi, F. (2009) *Tree Nuts: Composition, Phytochemicals, and Health Effects.* Boca Raton, FL: Taylor & Francis Group.

Alasalvar, C., Shahidi, F., & Cadwallader, K. (2003). Comparison of natural and roasted Turkish tombul hazelnut (Corylus avellana L.) volatiles and flavor by DHA/GC/MS and descriptive sensory analysis. *Journal of Agricultural Food Chemistry,* 51:5067–72.

Buckholz, L., Daun, H., Stier, E., & Trout, R. (1980). Influence of roasting time on sensory attributes of fresh roasted peanuts. *Journal of Food Science,* 45(3): 547-554.

Cherdchu, P. & Chambers, E. (2014). Effect of carriers on descriptive sensory characteristics: a case study with soy sauce. *Journal of Sensory Studies,* 29: 272–284.

Erickson, M., Santerre, C., & Malingre, E. (1994). Oxidative stability in raw and roasted pecans: chemical, physical, and sensory measurements. *Journal of Food Science,* 59(6): 1234-1238.

Grauke, L. and Thompson, T. Pecan breeding program: national collection of genetic resources for pecans and hickories. ARS-USDA. Available from: http://cgru.usda.gov/carya/. (accessed July 4, 2016).

Kita, A. & Figiel, A. (2006). The effect of roasting on the texture of walnuts. *Acta Agrophysica,* 7(1): 87-97.

Lombardini, L., Villarreal-Lozoya, J., & Cisneros-Zevallos, L. (2009). Antioxidant properties of pecan kernels. *Acta Horticulture,* 841: 91-96.

Magnuson, S., Kelly, B., Koppel, K., & Reid, W. (2016). A comparison of flavor differences between pecan cultivars in raw and roasted forms. *Journal of Food Science,* 81(5): S1243-S1253.

Miller, A. & Chambers, D. (2013). Descriptive analysis of flavor characteristics for black walnut cultivars. *Journal of Food Science*, 78(6): S887-S893.

NASS, United States Department of Agriculture (2015). Non citrus fruits and nuts: 2014 summary.

Nelson, S., Lawrence, K., Kandala, C. (1992). Sensing moisture in peanut and pecan kernels by RF impedance measurements. *Journal of Microwave Power and Electromagnetic Energy*, 27:171–4.

Nikzadeh, V. & Sedaghat, N. (2008). Physical and sensory changes in pistachio nuts as affected by roasting temperature and storage. *American-Eurasian Journal of Agricultural and Environmental Science*, 4(4): 478-483.

Ozdemir, M. & Devres, Y. (1999). The thin layer drying characteristics of hazelnuts during roasting. *Journal of Food Engineering*¸ 42(4): 225-233.

Reid, W. (2011). Storage conditions for black walnut kernels. *Personal Communication.* August 2011.

Santerre, C. (1994). *Pecan Technology*. New York, NY: Chapman & Hall.

Suwonsichon, S., Chambers, E. IV, Kongpensook, V., & Oupadissakoon, C. (2012). Sensory lexicon for mango as affected by cultivars and stages of ripeness. *Journal of Sensory Studies*, 27:148–160.

Toro-Vazquez, J. & Perez-Briceno, F. (1998). Chemical and physiochemical characteristics of pecan (Carya illinoensis) oil native of the central region of Mexico. *Journal of Food Lipids*, 5(3): 211-231.

Toro-Vazquez, J., Charo-Alonso, M., & Perez-Briceno, F. (1999). Fatty acid composition and its relationship with physiochemical properties of pecan (Carya illinoensis) oil. *Journal of the Americal Oil Chemists' Society*, 76(8): 957-965.

Tsantill, E., Takidelli, S., Christopoulos, M., Lambrinea, E., Rouskas, D., & Roussos, P. (2010). Physical, compositional, and sensory differences in nuts among pistachio (Pistachia vera L.) varieties. *Scientia Horticulturae*, 125(4): 562-568.

Chapter 3 - Determination of Optimal Raw and Roasted Pecan Flavor

Abstract

In the marketing of pecans, understanding driving factors of consumer preference is vital for successful sale and incorporation into the market. The objective of this study was to gain insight into these driving factors through the pairing of consumer evaluation with descriptive sensory data. Four cultivars of pecans, 'Kanza,' 'Maramec,' 'Pawnee,' and 'Witte,' were evaluated by 102 nut consumers under raw and roasted preparation methods. Consumers evaluated each of the cultivars under each of the preparation methods for *Overall Flavor Liking, Overall Flavor Intensity, Pecan Flavor Liking, Pecan Flavor Intensity,* and *Overall Liking.* Additionally, after evaluating all raw and roasted samples, participants were asked for preference between raw pecans and roasted pecans. Based on data collected, three distinct consumer segments were determined using *Overall Flavor Liking* responses. One cluster of consumers (n=29) showed significantly higher acceptance of all cultivars over 'Maramec' for all liking evaluations and assigned significantly lower flavor intensity scores for this 'Maramec' cultivar at 4.8 for raw samples and 5.2 for roasted samples on a scale of 1 to 9 (1 = dislike extremely, 9 = like extremely), compared to the next lowest flavor liking score of 6.9 in the raw 'Witte' sample. Another cluster (n=38) liked all of the samples, but gave higher acceptance scores for the roasted samples over the raw, regardless of cultivar. The third cluster (n=35) assigned neutral to slight-positive *Overall Flavor Liking* scores to all samples, with only *Overall Flavor Intensity* showing any significant effect in liking or intensity scores from cultivar or preparation method differences. Across all of the participants, roasted samples were generally met with higher acceptance. However, when asked for preference, consumers had equal split (n=51 : n=51)

between raw and roasted pecans. With the incorporation of descriptive sensory analysis data, the largest drivers of consumer liking were found to be related to the roasting process. The roasted 'Pawnee' and 'Witte' cultivars were met with the highest consumer acceptance and their flavor profiles may serve as good standards for highly accepted pecan flavor.

Introduction

In recent years, the pecan (*Carya illinoinensis* [*Wangenh*] K. Koch) has become a staple household food item. A survey of 232 consumers indicated that only 1.4% of consumers had not tried pecans, while 90% of consumers consumed pecans 2-6 times per year and 50% ate pecans on a weekly or monthly basis (Gold et al. 2004). Its extensive use in baking, confectionary application, and cooking have maintained the popularity of the pecan in the American market. This is evidenced by a steady half pound of pecan nutmeat consumed annually per capita within the past half-decade in the United States (Wolfe et al. 2007). The commonplace status of the pecan in traditional cooking and baking paired with the higher price typically found with tree nuts gives pecans high economic potential. This potential extends beyond the modern market, dating back to the late 18th century with sales by Spanish and French colonists to North America (Santerre 1994). Recent sales trends of pecans reveal an increased demand for the nuts as well. According to a 2014 summary of noncitrus fruits and nuts released by the Department of Agriculture (2015), in potential response to decreased production of pecans, the unit price of pecan nutmeat increased dramatically between 2012 and 2014, moving from $1.57 per pound to $1.96 per pound.

The popularity of the nut, as well as its unique nutritional qualities, has made the pecan a focus of many studies on its impact on health. Several compounds found in pecans have been

shown to possess antioxidant properties (Hudthagosol et al. 2011). The consumption of such antioxidants has been linked to a decreased risk of degenerative diseases and may have anti-carcinogenic potential (Mertens-Talcott and Percival 2005, Tam et al. 2006). In pecans, the majority of the antioxidant content is due to the presence of tannins, water-soluble polyphenols found in plant-based food items such as teas and nuts (Chung et al. 1998). These tannins and compounds exhibiting similar antioxidant properties are found in varying levels between different varieties of pecan, although similar compounds are found in each (Lombardini et al. 2009). This could mean that different cultivars of pecan could exhibit different antioxidant potentials. Beyond anti-carcinogenic potential, the consumption of high antioxidant containing pecans has been linked to increased cardiovascular health (Preedy et al. 2011). The consumption of pecans is suggested to increase antioxidant capacity, resulting in a lowering of oxidation of lipids linked to cardiovascular complications. Additionally, studies have supported a connection between the high levels of unsaturated fats found in pecans and a reduced risk for heart disease (Rajaram et al. 2000, Rajaram et al. 2001).

These health benefits are generally known and understood by consumers, with consumers being able to identify pecans as good sources of fats (predominantly unsaturated), protein, sugars, antioxidants, and vitamin E (Lombardini et al. 2008). Pecans are perceived as being heart-healthy foods and associated with a healthy lifestyle. Despite this knowledge, taste and quality have been shown to have the largest impact on purchasing decisions (Gold et al. 2004). Several studies have examined pecan flavor. Oro et al. (2009) looked at several flavor and other sensory attributes of pecans under different storage times, finding that the shelf-life of pecans under ambient conditions was around 90 days for flavor and sensory preservation. Erickson et al. (1994) performed a similar study, additionally examining humidity as a factor in rancid flavor

development. Magnuson et al. (2016) compiled flavor profiles of different cultivars of pecans under raw and roasted conditions, finding unique profiles for each of the 8 cultivars under each condition. Despite the available, though limited, research on pecan flavor, consumer studies on optimal pecan flavor have yet to be performed. Because of this, the objectives of this research were to A) understand cultivar and preparation acceptability by standard pecan consumers of four cultivars of pecans in raw and roasted forms and B) relate these results to flavor differences. This will help to determine the market potential of different cultivars and provide a standard for an optimal pecan flavor.

Materials and Methods

Samples

Four cultivars of pecans, selected for varied pecan flavor and an absence of any extreme unfavorable flavor attribute intensities based on the findings of Chapter 2, were used in this study. These cultivars were 'Kanza,' 'Maramec,' 'Pawnee,' and 'Witte.' All samples were obtained from Kansas State University's Pecan Experiment Field from the 2014 growing season. Samples were kept under frozen conditions (-18° C ± 1° C) before and after the removal of the nutmeat from the pecan shells. After the shelling process, samples were additionally vacuum sealed using a FoodSaver Heat-Seal Vacuum Sealing System (Sunbeam Products Inc. Boca Raton, FL, USA) in 3.79 L FoodSaver vacuum seal bags to prevent contamination and limit oxidation, as well as to preserve the sample moisture content (Reid 2011). The pecan shelling was performed using a Duke Pecan Walnut Cracker (Duke Pecan Company, West Point, MS, USA), a Davebilt Nutcracker (Davebilt Company, Lakeport Calif., USA), and Channel Lock model number 436, 15.24 cm cutting pliers (Channel Lock Inc., Meadville, Pa., USA), removing

the kernels from the shell and cleaning debris away from the nutmeat. Initial moisture content was analyzed using a Mettler Toledo HE 73/03 Moisture Analyzer (Mettler-Toledo AG, Greifensee, Switzerland). This was done to ensure that all samples had a moisture content below the industry maximum of 4.5% (Nelson et al. 1992). The average initial percent moisture for each of the cultivars can be found in Table 3-1. Samples were stored under frozen conditions (-18° C ± 1° C) until preparation and evaluation.

In this experiment, 'Kanza,' 'Maramec,' 'Pawnee,' and 'Witte' cultivars were evaluated under two preparation methods: raw and roasted. The pecans that were evaluated under the raw preparation method were removed from the freezer one day prior to testing and sealed in 92 g plastic containers with plastic lids (Solo Cup Company, Lake Forest, IL, USA) to thaw at room temperature (23 °C ± 1 °C) overnight. The pecans used in the roasted evaluations were removed from the freezer two to three days prior to evaluation and left in their vacuum sealed bags overnight to thaw at room temperature (23 °C ± 1 °C). The roasted pecans were prepared one to two days prior to evaluation. 100 g of sample was roasted in a single layer on a baking tray at 176 °C for 10 minutes, with stirring at 5 and 8 minutes to ensure even roasting and to prevent burning. After the roasting process, samples were left to cool on aluminum trays at room temperature (23 °C ± 1 °C) for 30 minutes prior to being placed in 92 g sealed plastic cups (Solo Cup Company, Lake Forest, IL, USA) overnight.

Table 3-1. Average percent moisture for each cultivar

Cultivar	Percent Moisture %
Kanza	2.37 ± 0.05
Maramec	2.97 ± 0.09
Pawnee	2.71 ± 0.10
Witte	3.01 ± 0.10

Descriptive Sensory Analysis

Descriptive sensory data was collected for each of the cultivars examined in this study under each of the preparation methods. A sensory panel analyzed each of the samples in duplicate for 21 flavor attributes (Appendix C). The panel was comprised of 8 members (2 male, 6 female), each of whom had completed more than 120 hours of general training in descriptive sensory analysis as well as at least 2000 hours of evaluation experience with a range of products, including nuts. Panelists completed 3 days of orientation, during which the list of key attributes, definitions, and references were determined and practice evaluations were performed. Products were evaluated across a six-day period, using a 15-point scale with 0.5 increments to evaluate attribute intensities.

Consumer Recruitment

Consumers were recruited using RedJade Sensory Software Suite (RedJade®, Redwood Shores, CA, USA) in conjunction with a consumer database of active consumer participants collected by Kansas State University's Sensory Analysis Center. Prospective participants in the Manhattan, Kansas area were screened for several factors and eliminated if any of the disqualifying responses were selected (Appendix E). An approximately equal distribution of male and female participants was desired, with a 40% minority gender represented. However, due to a system malfunction, 74.77% of participants were female and only 25.23% of the participants were male. Participants were asked about their age category, ensuring that all participants were above 18 years of age. Participants were eliminated if they had any dietary restrictions or food allergies, if they had any affiliation with market research or food manufacturing companies, or if they were not consumers of nuts. Additionally, participants were

eliminated if they had participated in a consumer research study within the past 30 days. After qualifying as nut consumers, prospective participants were asked about nut consumption frequency and willingness to eat certain nuts, being disqualified if they did not eat nuts at least once every three months and/or if they were not willing to consume pecans. Those consumers that qualified to participate in this study then registered for one of twelve sessions using RedJade® software, spanning a four-day period. A total of 111 consumers were recruited for this study and 102 consumers participated.

Test Design and Sample Evaluation

RedJade Sensory Software Suite (RedJade®, Redwood Shores, CA, USA) was used to create a balanced test design (Appendix H). Randomized block design was used, with the four raw samples being served first followed by the four roasted samples, the samples randomized within their block. Even pair tallies and position distribution was utilized, minimizing bias.

Consumers participated in one 60-minute session, although each session only required approximately 30 minutes, spanning a four-day period. Upon arrival participants were asked to sign in and be seated at one of the prepared evaluation stations before a short explanation of the purpose and guidelines of the study was given by a moderator (Appendix F). At each station, water and unsalted crackers (Kroger Company, Cincinnati, OH, USA) were provided for palate cleansing alongside an expectoration cup. Computer tablets equipped with internet capabilities to access RedJade® online software were provided for data collection.

Before beginning the study, participants were required to electronically sign an informed consent form, stating that they understood the conditions of the study and were participating of their own free will, free to withdraw at any point (Appendix G). Before evaluation of the raw

samples, consumers were told that they would be evaluating eight pecan samples and that the first four samples were fresh pecans. After completing this first phase of the study, sample evaluation could begin.

Approximately 10 g of each sample was served to each consumer in 92 g sealed plastic cups (Solo Cup Company, Lake Forest, IL, USA) under randomized four-digit code (Appendix H). These blinding codes were provided by RedJade® software. Participants were given approximately three minutes to evaluate each sample, served one at a time using a sequential monadic design to minimize interaction between products. Each sample was evaluated for *Overall Flavor Liking, Overall Flavor Intensity, Pecan Flavor Liking, Pecan Flavor Intensity,* and *Overall Liking* (Appendix I). To evaluate liking scores, a nine-point hedonic scale was used, with a score of 1 being 'dislike extremely' and 9 being 'like extremely.' Similarly, a nine-point scale was used to evaluate *Overall Flavor* and *Pecan Flavor* intensities, 1 indicating 'not at all flavorful' and 9 indicating 'extremely flavorful.'

Between evaluation of raw and roasted samples, participants were given a short five-minute break. Before initiating the roasted sample evaluation, participants were told that the fresh sample evaluation had been completed and that the following four samples would be roasted pecans. Participants evaluated the roasted products using the same method, scales, and questions as the raw samples. After completion of the sample evaluation, consumers were reminded that they saw four fresh samples first, followed by four roasted samples, and asked which set they preferred overall. Finally, demographic information was collected in a short survey. Age and gender demographics are presented in Table 3-2. When the participants completed the session, they were compensated for their time.

Table 3-2. Demographic information of consumer study participants

	Gender		Age					
	Male	**Female**	**18-24**	**25-35**	**36-45**	**46-55**	**56-65**	**66 or older**
Number of Participants	24	78	7	28	10	26	29	2

Statistical Analysis

Topline analysis was calculated with *X*LStat statistical software (Addinsoft©, New York, NY, USA), providing mean responses for *Overall Flavor Intensity* and *Pecan Flavor Intensity* with Fisher's protected least significant difference *post-hoc* test results at the 5% significance level. Liking response distribution was also provided for *Overall Flavor Liking, Pecan Flavor Liking,* and *Overall Liking*.

Agglomerated hierarchical clustering was performed based on *Overall Flavor Liking* scores using *X*LStat statistical software (Addinsoft©, New York, NY, USA) under Ward's agglomeration method. This was done to better understand what factors drive liking and flavor intensity scores for different segments of consumers. 2-way analysis of variance (ANOVA) at the 5% significance level was performed using SAS® statistical software (SAS® version 9.3, SAS Institute Inc., Cary, NC, USA) to determine if significant differences exist between liking and intensity scores of different cultivars under different preparation methods. PROC GLIMMIX and PROC MIXED codes were used. Fisher's protected least significant difference *post-hoc* means were used to discern significantly different cultivars and preparation methods at the 5% significance level. This was performed within each cluster.

Partial least squares regression analysis was performed at the 5% significance level with the aid of The Unscrambler® software (CAMO Software, Oslo, Norway). Mean attribute

intensities from previously collected descriptive sensory analysis were incorporated as supplementary information into regression analysis to determine sensory factors that contribute to consumer acceptance of pecan samples.

Results

Topline Sample Variation

For sample liking evaluations, a hedonic scale from 1 to 9 was utilized, with scores of 1 to 4 indicating negative acceptance of varying degrees, a score of 5 indicating a neutral response, and scores of 6 through 9 indicating positive acceptance of varying degrees. Participants gave generally positive liking scores (scores above 5) to evaluated pecan samples (Table 3-3). For *Overall Flavor Liking*, neutral or negative responses (scores at or below 5) were seen in, at most, 34% of consumers, received by both the raw and roasted 'Maramec' samples, followed by a large gap with only 22% of the consumers giving neutral or negative liking scores to the next lowest scored sample, the raw 'Pawnee' cultivar (Table 3-4). Roasted 'Maramec' samples also received the most neutral or negative responses for *Pecan Flavor Liking* at 36%, closely followed by the raw 'Maramec' samples at 34% (Table 3-5). This was followed by another large gap, with 'Witte' raw samples receiving the next highest neutral or negative responses at 26%. Finally, neutral or negative responses were only seen in 37% and 36% of respondents in 'Maramec' raw and roasted samples respectively (Table 3-6). 'Witte' roasted samples received the most positive responses for *Overall Flavor Liking*, with 86% of consumers giving positive liking responses, while roasted 'Pawnee' samples received the most positive responses for *Pecan Flavor Liking* and *Overall Liking*, with 82% and 83% of participants assigning positive scores respectively.

Roasted pecans were given significantly higher intensity scores for *Overall Flavor Intensity* than raw samples across all cultivars (Table 3-3). Roasted sample generally received higher *Pecan Flavor Intensity* scores than raw samples as well. Raw ‘Maramec’ pecans received significantly lower *Pecan Flavor Intensity* scores than all roasted cultivars.

Table 3-3. Mean liking and intensity scores and *post-hoc* separation for intensity scores

	Kanza Raw	Kanza Roasted	Maramec Raw	Maramec Roasted	Pawnee Raw	Pawnee Roasted	Witte Raw	Witte Roasted
Overall Flavor Liking	6.7**	6.9	5.9	6.1	6.6	6.7	6.6	6.9
Pecan Flavor Liking	6.8	6.8	6.0	6.0	6.5	6.6	6.5	6.7
Overall Liking	6.6	6.7	5.9	5.9	6.4	6.6	6.4	6.7
Overall Flavor Intensity	4.4^{B***}	4.9^{A}	4.0^{B}	4.9^{A}	4.4^{B}	4.9^{A}	4.1^{B}	5.1^{A}
Pecan Flavor Intensity	4.3^{ABC}	4.7^{A}	3.9^{C}	4.5^{AB}	4.1^{BC}	4.7^{A}	4.1^{BC}	4.7^{A}

* Mean liking scores and intensities of 102 responses

** Scores based on 9-point hedonic scales (liking: 1 = dislike extremely, 9 = like extremely; intensity: 1 = not at all flavorful, 9 = extremely flavorful)

*** Fisher's protected least significant difference *post-hoc* test at 5% level of significance for intensities; Means with different superscripts within a row are significantly different ($P<0.05$)

Table 3-4. Overall flavor liking response distribution*

Liking Response	Kanza Raw	Kanza Roasted	Maramec Raw	Maramec Roasted	Pawnee Raw	Pawnee Roasted	Witte Raw	Witte Roasted
Like Extremely (= 9)	9%	14%	5%	10%	7%	10%	10%	14%
Like Very Much (= 8)	24%	27%	17%	20%	25%	22%	22%	30%
Like Moderately (= 7)	30%	27%	25%	25%	23%	31%	27%	23%
Like Slightly (= 6)	17%	12%	20%	13%	24%	21%	21%	19%
Neither Like nor Dislike (= 5)	10%	9%	10%	3%	11%	5%	8%	5%
Dislike Slightly (= 4)	9%	6%	9%	17%	9%	9%	8%	4%
Dislike Moderately (= 3)	1%	4%	8%	7%	1%	2%	5%	6%
Dislike Very Much (= 2)	1%	1%	5%	6%	1%	1%	0%	0%
Dislike Extremely (= 1)	0%	0%	2%	1%	0%	0%	0%	0%

***** Percentage taken of 102 responses

Table 3-5. Pecan flavor liking response distribution*

Liking Response	Kanza Raw	Kanza Roasted	Maramec Raw	Maramec Roasted	Pawnee Raw	Pawnee Roasted	Witte Raw	Witte Roasted
Like Extremely (= 9)	6%	16%	6%	11%	6%	9%	8%	16%
Like Very Much (= 8)	30%	24%	16%	14%	24%	23%	20%	24%
Like Moderately (= 7)	30%	22%	26%	26%	25%	28%	29%	18%
Like Slightly (= 6)	14%	19%	19%	14%	21%	22%	18%	19%
Neither Like nor Dislike (= 5)	11%	11%	12%	8%	13%	5%	11%	15%
Dislike Slightly (= 4)	6%	4%	7%	12%	10%	8%	8%	4%
Dislike Moderately (= 3)	2%	5%	8%	10%	1%	4%	7%	5%
Dislike Very Much (= 2)	1%	1%	6%	5%	1%	2%	0%	1%
Dislike Extremely (= 1)	0%	0%	1%	1%	0%	0%	0%	0%

***** Percentage taken of 102 responses

Table 3-6. Overall liking response distribution*

Liking Response	Kanza Raw	Kanza Roasted	Maramec Raw	Maramec Roasted	Pawnee Raw	Pawnee Roasted	Witte Raw	Witte Roasted
Like Extremely (= 9)	7%	12%	5%	10%	5%	11%	8%	15%
Like Very Much (= 8)	29%	29%	16%	16%	25%	18%	18%	25%
Like Moderately (= 7)	27%	18%	25%	25%	22%	29%	32%	20%
Like Slightly (= 6)	14%	21%	19%	15%	23%	25%	17%	18%
Neither Like nor Dislike (= 5)	10%	8%	10%	6%	11%	3%	6%	10%
Dislike Slightly (= 4)	8%	6%	13%	14%	12%	6%	14%	6%
Dislike Moderately (= 3)	2%	6%	8%	6%	2%	7%	5%	6%
Dislike Very Much (= 2)	3%	1%	6%	9%	1%	1%	1%	1%
Dislike Extremely (= 1)	0%	0%	0%	1%	0%	0%	0%	0%

***** Percentage taken of 102 responses

Consumer Segmentation

To further understand sample variation in the context of flavor, additional analysis was performed on *Overall Flavor Liking* scores to determine if any consumer segments existed. Rather than incorporating additional sensory factors seen in *Overall Liking* evaluation, *Overall Flavor Liking* scores were used for additional analyses to maintain a focus on flavor, although *Overall Liking* acceptance scores showed very similar results (Tables 3-4 and 3-6). Hierarchical cluster analysis was utilized through which three clusters of participants were found, each with unique demographics (Table 3-7). Cluster 1 consisted of 29 consumers (28.44%), cluster 2 had 38 consumers (37.25%), and the remaining 35 participants were in cluster 3 (34.31%). Of the males that participated, 29.17%, 37.50%, and 33.33% fell into clusters 1, 2, and 3 respectively. Similarly, 28.21% of female participants were in cluster 1, 37.18% in cluster 2, and 34.62% in cluster 3. The age distribution between clusters is displayed in Table 3-8. Cluster 1 had a larger percentage of the 25-35 year-old participants while cluster 2 had a majority of the consumers aged 18 to 24 and a larger percentage of the 56-65 year-old participants. Cluster 3 had a majority of the 36-45 year-old consumers and a large portion of the 46-55 year-old participants. The two participants aged above 65 years of age were split between clusters 2 and 3.

Cluster 1 respondents had a distribution of liking scores across cultivars and preparation means (Table 3-9), ranging from 4.8, assigned to the raw ‘Maramec’ sample, to 7.6, assigned to both ‘Witte’ and ‘Kanza’ roasted samples. Cluster 2 gave higher liking and intensity scores across all cultivars and preparation methods, using only a small portion of the scale, around 7.5 on a 9-point hedonic scale. The lowest average liking score was 7.2 in the raw ‘Witte’ sample while the highest was 7.8 in the roasted ‘Kanza’ sample. Similarly, cluster 3 used only a small window of scores, around 5.5, across all preparation methods and cultivars, the lowest exhibiting

a range between 5.0 (roasted ‘Maramec’) and 5.9 (roasted ‘Pawnee’). Segmenting further analysis by cluster, or consumer segment, allowed for better determination of cultivar and preparation method differences based on the scale used for evaluation within each segment.

Table 3-7. Demographic data across clusters*

	Gender %		Age %					
	Male	**Female**	**18-24**	**25-35**	**36-45**	**46-55**	**56-65**	**66+**
All	23.53	76.47	6.86	27.45	9.80	25.49	28.43	1.96
Cluster 1	24.14	75.86	3.45	41.38	3.45	24.14	27.59	0.00
Cluster 2	23.68	76.32	10.53	26.32	7.89	21.05	31.58	2.63
Cluster 3	22.86	77.14	5.71	17.14	17.14	31.43	25.71	2.86

***** All (n=102), Cluster 1 (n=29), Cluster 2 (n=38), Cluster 3 (n=35)

Table 3-8. Total age distribution across clusters*

	Cluster 1	**Cluster 2**	**Cluster 3**
18-24	14.29%	57.14%	28.57%
25-35	42.86%	35.71%	21.43%
36-45	10.00%	30.00%	60.00%
46-55	26.92%	30.77%	42.31%
56-65	27.59%	41.38%	31.03%
66+	0.00%	50.00%	50.00%

***** 18-24 (n=7), 25-35 (n=28), 36-45 (n=10), 46-55 (n=26), 56-65 (n=29), 66+ (n=2)

Table 3-9. Mean overall flavor liking responses*

	Pawnee		Maramec		Witte		Kanza	
	Raw	**Roasted**	**Raw**	**Roasted**	**Raw**	**Roasted**	**Raw**	**Roasted**
Cluster 1	7.1	6.9	4.8	5.2	6.9	7.6	7.1	7.6
Cluster 2	7.3	7.3	7.3	7.7	7.2	7.7	7.4	7.8
Cluster 3	5.4	5.9	5.4	5.0	5.7	5.6	5.6	5.2

***** Scores based on a 9-point hedonic scale (1 = dislike extremely, 9 = like extremely)

Cluster One

Cluster 1 consisted of 24.14% male and 75.86% female respondents, mostly of the age groups 25-35, 46-55, and 56-65, 41.38%, 24.14%, and 27.59% respectively (Table 3-7). A total 42.86% of participants aged between 25 and 35 fell into this consumer segment (Table 3-8).

Within cluster 1, 2-way ANOVA revealed that, for *Overall Flavor Liking*, *Pecan Flavor Liking*, and *Overall Liking*, cultivar differences had a significant effect ($p \leq 0.05$) on liking (Table 3-10). 'Maramec' pecans were given significantly lower liking scores than the other three cultivars. The preparation method also had a significant effect on *Overall Flavor Liking*, with the roasted samples receiving significantly higher scores.

Intensity scores were also significantly affected by cultivar and preparation method for cluster 1 (Table 3-10). The 'Maramec' cultivar received significantly lower *Overall Flavor Intensity* and *Pecan Flavor Intensity* scores than other cultivars. Raw samples additionally received significantly lower intensity scores for both *Overall Flavor Intensity* and *Pecan Flavor Intensity* than the roasted samples.

The interaction term between cultivar and preparation method was not significant for any of the liking or intensity evaluations, meaning that each of the cultivars was affected similarly by the roasting process (Table 3-10).

Cluster Two

Cluster 2, similar to cluster 1, was comprised of 23.68% male participants and 76.32% female participants (Table 3-7). The age of participants was more evenly distributed. However, due to the size of the cluster (n=38) and the limited number of participants in certain age groups,

the majority (57.14%) of participants aged 18-24, half of those aged 66 and above, and nearly half (41.38%) of participants between 56 and 65 years old fell into this cluster (Table 3-8)

Limited scores were used for evaluation of liking and flavor intensity within cluster 2 (7.2 to 7.8). Despite that, preparation method was shown to have a significant effect on *Overall Flavor Liking*, *Pecan Flavor Liking*, and *Overall Liking* evaluations as well as on *Overall Flavor Intensity* and *Pecan Flavor Intensity* (Table 3-11). For each of the liking and intensity evaluations, roasted samples were rated significantly higher than raw samples across all cultivars. The interaction term was not significant between cluster and preparation method.

Cluster Three

Cluster 3 had 22.86% male participants and 77.14% female, like the other two segments. The age distribution was fairly even between the 25-35 age group through the 26-65 age group, with less than 17% of participants present only with the 18-24 year-old age group and the 66 and older age group (Table 3-7). Like cluster 2, the large size of the cluster (n=35) meant that a fairly high percentage of each age group was represented in cluster 3 (Table 3-8). Notably, 60.00% of 36-45 year-olds and 42.31% of 46-55 year-olds, as well as half of those participants above 65 years of age, were represented.

For cluster 3, neither preparation method nor cultivar had a significant effect ($p \leq 0.05$) on liking scores (*Overall Flavor Liking*, *Pecan Flavor Liking*, *Overall Liking*) (Table 3-12). Similarly, *Pecan Flavor Intensity* scores were not affected by preparation method or cultivar. Only *Overall Flavor Intensity* showed significant effect by a factor, with raw samples given significantly lower intensity scores than roasted samples. Interaction terms were not significant for any liking or intensity evaluations.

Table 3-10. P-values of individual factors and factor interactions from ANOVA for liking and intensity evaluations in cluster 1 [a]

	Cultivar	Preparation Method	Cultivar*Preparation Method
Overall Flavor Liking	**<0.0001**	**0.0483**	0.3757
Overall Flavor Intensity	**<0.0001**	**<0.0001**	0.1763
Pecan Flavor Liking	**<0.0001**	0.3775	0.2625
Pecan Flavor Intensity	**<0.0001**	**0.0034**	0.6503
Overall Liking	**<0.0001**	0.3885	0.3112

[a] Significance taken at $p \leq 0.05$
[b] Significant factor or interaction for given attribute

Table 3-11. P-values of individual factors and factor interactions from ANOVA for liking and intensity evaluations in cluster 2[a]

	Cultivar	Preparation Method	Cultivar*Preparation Method
Overall Flavor Liking	0.2541	**0.0031**	0.5969
Overall Flavor Intensity	0.5411	**<0.0001**	0.6165
Pecan Flavor Liking	0.0511	**0.001**	0.9447
Pecan Flavor Intensity	0.2103	**0.0001**	0.8871
Overall Liking	0.2044	**0.0021**	0.8666

[a] Significance taken at $p \leq 0.05$
[b] Significant factor or interaction for given attribute

Table 3-12. P-values of individual factors and factor interactions from ANOVA for liking and intensity evaluations in cluster 3[a]

	Cultivar	Preparation Method	Cultivar*Preparation Method
Overall Flavor Liking	0.3436	0.5699	0.4624
Overall Flavor Intensity	0.0574	**0.0238[b]**	0.4871
Pecan Flavor Liking	0.4952	0.1023	0.5231
Pecan Flavor Intensity	0.8524	0.5150	0.5819
Overall Liking	0.4921	0.4710	0.3178

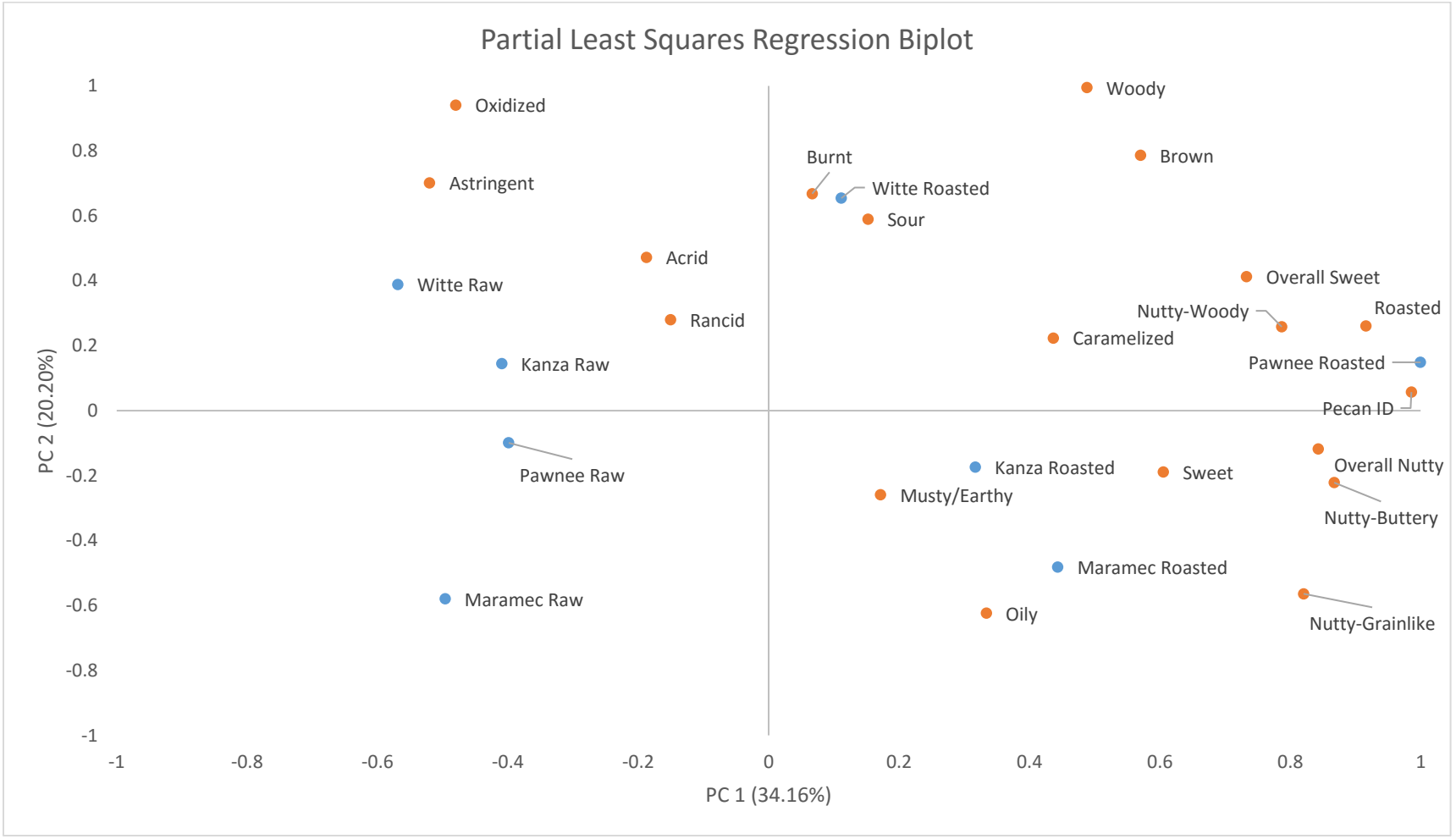
[a] Significance taken at $p \leq 0.05$
[b] Significant factor or interaction for given attribute

Relating Consumer Evaluation to Descriptive Sensory Analysis

Pairing the consumer data with sensory data, a connection between *Overall Flavor Liking* and flavor attributes could be made. A partial least squares regression biplot explained 54.56% of the variation between samples in terms of *Overall Flavor Liking* (Figure 3-1). Principal component 1, which explained 34.16% of sample variation, is linked to differences in samples related to astringency and versus roasted, nutty, and sweet type attributes. Oiliness, versus more dry and woody type attributes, is highly correlated to principal component 2, which explained 20.20% of the variation in *Overall Flavor Liking* scores between samples.

Liking for the raw samples was correlated to astringent characters and showed some connection to acrid and roasted notes. *Overall Flavor Liking* for the roasted 'Witte' sample was driven by burnt and sour type attributes, while the roasted 'Kanza' sample was related to musty/earthy flavor. The roasted 'Maramec' sample liking was connected to oiliness. The driving factors of *Overall Flavor Liking* for the roasted 'Pawnee' sample were related to roasted and nutty type attributes.

Figure 3-1. Partial least squares regression of descriptive sensory analysis results with consumer evaluation of *Overall Flavor Liking*



Consumer Interpretation of Preparation Method

Although liking response distribution indicated that roasted pecans were assigned higher acceptance for overall flavor (Table 3-4) and analysis of variance showed a clearly higher acceptance for roasted pecans in terms of *Overall Flavor Liking, Pecan Flavor Liking,* and *Overall Liking* within cluster 2, the post evaluation question about fresh pecan versus roasted pecan preference showed contradictory results. Exactly 50% of respondents indicated that they preferred the fresh samples while the other 50% preferred the roasted pecans (Table 3-13). A closer examination within each cluster revealed that clusters 1 and 2 preferred the roasted samples, albeit less substantially than indicated from ANOVA results. Cluster 1 showed a split of 41.38% and 58.62% for raw versus roasted preference. Similarly, cluster 2 revealed a 42.11% to 57.89% split between raw and roasted sample preference. Cluster 3, however, contrary to ANOVA results, had a much larger percentage of consumers preferring the raw samples (65.71%) than the roasted samples (34.29%) as a whole.

Table 3-13. Fresh versus roasted pecan preference within each consumer segment *

	Percentage of Consumers that Prefer Raw Samples	Percentage of Consumers that Prefer Roasted Samples
All	50.00%	50.00%
Cluster 1	41.38%	58.62%
Cluster 2	42.11%	57.89%
Cluster 3	65.71%	34.29%

***** All (n=102), Cluster 1 (n=29), Cluster 2 (n=38), Cluster 3 (n=35)

Discussion

Examining consumer responses collectively, the generally positive acceptance (receiving a liking score above 5 on a scale of 1 to 9) of the pecans samples across cultivars and preparation methods emphasized that pecans as a whole were generally liked by nut consumers and can be very successful in the nut market. However, neutral or negative liking scores were received by a much higher percentage of consumers for the 'Maramec' cultivar when compared to other cultivars, indicating that its sensory qualities were less desirable than the other cultivars examined in this study. The intensities of these well-received flavors were additionally shown to be enhanced by the roasting process, producing general higher intensities in roasted samples than raw across all cultivars. Because of the range of consumer perceptions, however, these results reveal only surface-level results.

Many factors may have an effect on consumer preference and interpretation, including sensitivity to certain taste sensations and previous exposure. One study performed by Wolfe et al. (2007) attempted to profile the average pecan consumer. Their findings suggested that pecan consumers tend to be between the ages of 35 and 54, have higher average incomes than the average American household, and are more likely to be female. In this study, many of the participants fell within this demographic. Although some of these consumers may in fact be more regular consumers of pecans, it is important to keep in mind that not all participants, inside and outside of the 'average pecan consumer' demographic, have different interpretations and experiences with pecans. Previous research has shown the importance of defining consumer segments in order to determine different factors that drive acceptance and product perception (Murray et al. 2000). This was apparent from the three consumer segments revealed from hierarchical cluster analysis.

Across all three consumer segments, gender was evenly distributed, with approximately 24% of participants being male and 76% of participants being female in each cluster. This suggests that gender played a minimal role in defining the segments. Cluster 1 was highly influenced by the cultivar, assigning higher liking and intensity scores to all cultivars over 'Maramec.' Additionally, this cluster perceived the *Overall Flavor Intensity* and *Pecan Flavor Intensity* as lower in raw samples. This cluster had a higher distribution of liking scores, utilizing a larger portion of the scale, indicating thoughtful responses. Cluster 1 was comprised of a large portion of the 25-35 year-olds, suggesting that this age group may be more sensitive to cultivar differences than other age groups and may be more thoughtful in its responses. Cluster 2 was predominantly influenced by the roasting process, assigning higher liking and intensity scores to roasted samples over their raw counterparts. This was further confirmed by partial least squares regression analysis (Figure 3-1), where the samples that received the higher *Overall Flavor Liking* scores were revealed to be correlated with dry, roasted, nutty, and sweet-type attributes, which are generally associated with roasted pecans. Cluster 2 was comprised of a large portion of the 18-24 and the 56-65 year-old participants and half of the 65 and older participants, suggesting that roasted samples and associated sensory properties may be met with the most success for the oldest and youngest age groups. Finally, cluster 3, comprised of the majority of 36-45 year-olds and a large portion of 46-55 year-olds as well as the other half of those above 65 years of age, was only significantly influenced by the preparation method for *Overall Flavor Intensity*, assigning higher intensity scores for the roasted samples. When paired with sensory data, the *Overall Flavor Liking* scores of this cluster was shown to be influenced by a lack of oiliness. Both clusters 2 and 3 utilized small windows of the scales for *Overall Flavor Liking* across all samples, suggesting some bias, potentially from timidity or lack of motivation. This

was especially apparent in cluster 3, which used scores around the neutral score of 5 and did not vary much across preparation methods nor cultivars. The age groups that are largely represented in this cluster, 36-45 and 46-55, seem to be most prone to these biases.

Across the participants, roasted samples were met with the most acceptance. A higher percentage of participants assigned top-two-box scores (scores of 8 [Like Very Much] or 9 [Like Extremely]) to each of the roasted samples than their raw counterparts (Table 3-4). For all cultivars but 'Pawnee', the roasted samples received top two box scores by at least 8% more of the participants than the raw. Despite different drivers of liking for *Overall Flavor Liking* among the consumer segments, the roasted samples collectively showed higher acceptance scores than the raw samples. The roasted 'Pawnee' sample specifically was well-received across all clusters of consumers. However, examining the results of the post-evaluation question reveals contradictory information. The reason for the discrepancy between acceptance results from the liking evaluations and the preference response results could be due to the use of the term 'fresh.' Studies have shown that people generally associate certain terminology, for example 'organic' and 'whole grain,' with a healthier product (Just and Wansink 2009). The use of the term 'fresh' may have had a psychological effect on consumers, with 'fresh' products generally having a higher association with health, quality, and a lack of 'unnatural treatment,' whereas the use of the term 'roasted' may have incited thoughts of processing and human manipulation. The high imbalance of raw over roasted sample preference in cluster 3 may indicate that this psychological effect played a large role in responses to direct inquiry about preparation method preference for this consumer segment. The participants in cluster 3 had a large representation of the total 36-45 year-olds (60.00%) and of those aged between 46 and 55 (42.31%). This suggests that consumers

aged between 36 and 55 years old may be slightly more susceptible to bias by psychological factors than other age groups.

With only 4 cultivars of pecans examined, results were limited in scope. Other cultivars than those included may reveal higher acceptance and should be considered for further research. Moreover, questions about more specific components of pecan flavor may show a more in-depth picture of the driving factors of pecan liking. Conversely, participants may have been overwhelmed by the amount of samples and questions presented, contributing to some of the bias seen. Samples were examined using a block design, with raw samples and roasted samples segmented, additionally contributing to evaluation bias. Further research should examine some of the suspected psychological biases from this study, both in evaluation methods and those caused by terminology used, and the roles they play on pecan acceptance. Additional demographic information, such as educational background and socio-economic status may shed light into differences in consumer segments as well.

Conclusions

Although all four cultivars of pecans ('Kanza,' 'Maramec,' 'Pawnee,' and 'Witte') were well-received by consumers under both raw and roasted preparation methods, with a minimum of 63% of consumers assigning positive liking scores for every sample. The 'Maramec' cultivar and the raw preparation method were generally assigned lower *Overall Flavor Liking, Pecan Flavor Liking,* and *Overall Liking* scores. This trend was carried into intensity scores, with *Overall Flavor Intensity* and *Pecan Flavor Intensity* receiving generally lower intensity scores than other cultivars or the roasted preparation method. The degree of the cultivar and preparation method effect varied between consumers, with three distinct consumer segments formed. Although the

different segments showed slightly different preferences, attributes related to the roasting process (*Brown, Nutty-Woody, Sweet, Roasted, Woody, Sour, Pecan ID, Overall Nutty,* etc.) were correlated with samples that received higher acceptance scores, exhibiting that these attributes were generally preferred by the majority of consumers. Psychological bias likely played a role in consumer preference based on the terminology under which samples were presented when consumers are directly asked about product preference.

The roasted 'Pawnee' and 'Witte' cultivars were met with the highest acceptance with 84% and 86% positive *Overall Flavor Liking* scores (scores above 5 of 9) respectively and their flavor profiles may serve as good standards for the future of pecan flavor. Future research will focus on understanding compositional differences that may explain sensory profile variation and different levels of consumer acceptance of different cultivars under different preparation methods.

References

Chung, K., Wong, T., Wei, C., Huang, Y., & Lin, Y. (1998). Tannins and human health: a review. *Critical Reviews in Food Science and Nutrition,* 38(6): 421-464.

Erickson, M., Santerre, C., & Malingre, E. (1994). Oxidative stability in raw and roasted pecans: chemical, physical, and sensory measurements. *Journal of Food Science,* 59(6): 1234-1238.

Gold, M., Cernusca, M., & Godsey, L. (2004). Consumer preferences for chestnuts, eastern black walnuts, and pecans. *HortTechnology,* 14(4): 583-589.

Hudthagosol, C., Haddad, E., McCarthy, K., Wang, P., Oda, K., & Sabate, J. (2011). Pecans acutely increase plasma postprandial antioxidant capacity and catechins and decrease LDL oxidation in humans. *The Journal of Nutrition*, 141(1): 56-62.

Just, D. and Wansink, B. (2009). Better school meals on a budget: using behavioral economics and food psychology to improve meal selection. *Choices,* 24(3): 1-6.

Lombardini, L., Villarreal-Lozoya, J., & Cisneros-Zevallos, L. (2009). Antioxidant properties of pecan kernels. *Acta Horticulture*, 841: 91-96.

Lombardini, L., Waliczek, T., & Zajicek, J. (2008). Consumer knowledge of nutritional attributes of pecans and factors affecting purchasing behavior. *HortTechnology*, 18(3): 481-488.

Magnuson, S., Koppel, K., Reid., W., Chambers IV, E. (2016). Pecan flavor changes during storage. *Journal of the American Pomological Society*, 69(4): 206-214.

Mertens-Talcott, S. U., & Percival, S. S. (2005). Ellagic acid and quercetin interact synergistically with resveratrol in the induction of apoptosis and cause transient cell cycle arrest in human leukemia cells. *Cancer Letters*, 218(2), 141-151.

Murray, J. and Delahunty, C. (2000). Mapping consumer preference for the sensory and packaging attributes of cheddar cheese. *Food Quality and Preference*, 11(5), 419-435.

Nelson, S., Lawrence, K., Kandala, C. (1992). Sensing moisture in peanut and pecan kernels by RF impedance measurements. *Journal of Microwave Power and Electromagnetic Energy*, 27:171–4.

Oro, T., Bolini, H., Arellano, D., & Block, J. (2009). Physichemical and sensory quality of crude Brazilian pecan nut oil during storage. *Journal of the American Oil Chemists' Society*, 86(10): 971-976.

Preedy, V., Watson, R., & Patel, V. (2011). *Nuts and Seeds in Health and Disease Prevention.* San Diego, CA: Academic Press.

Reid, W. (2011). Storage conditions for black walnut kernels. *Personal Communication.* August 2011.

Rajaram, S., Burke, K., Connell, B., Myint, T., & Sabaté, J. (2001). A monounsaturated fatty acid–rich pecan-enriched diet favorably alters the serum lipid profile of healthy men and women. *The Journal of Nutrition*, 131(9), 2275-2279.

Rajaram, S., Myint, T., Connell, B., Burke, K., & Sabaté, J. (2000). Effect of pecan rich diet on serum lipids and lipoproteins in healthy men and women. *Faseb Journal*, 14(4), A293-A293

Santerre, C. (1994). *Pecan Technology*. New York, NY: Chapman & Hall.

Tam, N. N., Nyska, A., Maronpot, R. R., Kissling, G., Lomnitski, L., Suttie, A., ... & Ho, S. M. (2006). Differential attenuation of oxidative/nitrosative injuries in early prostatic neoplastic lesions in TRAMP mice by dietary antioxidants. *The Prostate*, 66(1), 57-69.

United States Department of Agriculture (2015). *Noncitrus Fruits and Nuts.* Retrieved from http://usda.mannlib.cornell.edu/usda/current/NoncFruiNu/NoncFruiNu-07-17-2015.pdf (accessed July 4, 2016)

Wolfe, K., Daniels, J., Swickard, K., & McKissick, J. (2007). *U. S. pecan consumer profile results from national survey.* Retrieved from http://athenaeum.libs.uga.edu/xmlui/bitstream/handle/10724/18765/CR-07-11.pdf?sequence=1 (accessed July 4, 2016)

Chapter 4 - A Comparison of Fatty Acid Profiles of Eight Pecan Cultivars in Raw and Roasted Forms

Abstract

The pecan is a nutrient-dense food item with a high level of lipids. Five fatty acids comprise the bulk of the lipid content: palmitic acid, stearic acid, oleic acid, linoleic acid, and linolenic acid. Understanding the profiles of these fatty acids for different pecan samples and how they relate to sensory characteristics may provide a means of predicting success of new and existing cultivars in the market. Further, fatty acid profile analysis offers an explanation for flavor defects that may exist in certain cultivars of pecans. The objective of this study was to examine and compare fatty acid profiles of eight cultivars of pecans, 'Chetopa,' 'Giles,' 'Kanza,' 'Lakota,' 'Major,' 'Maramec,' 'Pawnee,' and 'Witte,' under raw and roasted preparation methods. Additionally, the fatty acids' association with sensory attributes was examined. Percentages of palmitic, stearic, oleic, linoleic, and linolenic acids to total fatty acid content were determined using gas chromatography. Similar trends were seen across samples, with oleic acid comprising the majority of the total fatty acids and linolenic acid comprising the smallest percentage. The raw and roasted samples within a cultivar predominantly had very similar profiles with the exception of the Giles cultivar, which differed significantly in oleic acid and linoleic acid percentages of total fatty acid content between raw and roasted samples. The majority of fatty acid profile differences was derived from the cultivar effect, with significant differences present across all of the fatty acids between different cultivars. Some cultivars exhibited higher percentages of certain fatty acids when compared to the other cultivars, for example 'Pawnee's' higher linoleic and palmitic acids and the higher percentage of linolenic acid found in the 'Lakota' cultivar. When paired with sensory data, linoleic, palmitic, and stearic

acids were associated with roasted-type flavors. Linolenic acid, however, was significantly correlated with *Astringent, Burnt,* and *Woody* attributes and strongly correlated with *Bitter, Acrid,* and *Oxidized* attributes, showing a connection to more dry and typically undesirable flavor qualities. The high linolenic acid content of 'Lakota' may explain many of its undesirable flavor qualities, while the fatty acid profile of 'Pawnee' pecans may serve as a good standard for the industry.

Introduction

The pecan, *Carya illinoinensis* (Wangenh) K. Koch, a largely popular tree nut native to North America, is unique in its nutritional content, with high caloric value and a high concentration of nutrients, when compared to other products within the niche of health foods. One of the most valuable components of pecans is their extremely high lipid content, consisting of 70-79% of the kernel by weight (Toro-Vazquez and Perez-Briceno 1998). This lipid content is largely comprised of five fatty acids: stearic acid, palmitic acid, oleic acid, linoleic acid, and linoleic acid. Of these, oleic, linoleic, and linolenic acids make up the unsaturated components. The total amount and individual concentrations of each of these unsaturated fats can vary greatly between different cultivars, or varieties, of pecans and may be affected by a variety of factors such as additional processing, environmental factors during the kernel growth phase, or even tree age. Research supports that oleic acid content is higher in younger trees, while linoleic and linolenic acids are present in higher concentrations in older trees (Toro-Vazquez et al. 1999). Despite the range that exists between these fatty acids, the general high unsaturated fat content of pecans makes them desirable for a variety of health applications.

Although pecans do have a high fat content, their lipid profile, consisting largely of these unsaturated fats, may be favorable for long-term health, with the regular consumption of pecans being linked to lower plasma cholesterol and a reduced risk for cardiovascular disease from the high volume of 'healthy fats' ingested (Alasalvar et al. 2009). Several studies support a significant effect on pecan and similar nut consumption on decreased levels of total cholesterol, low-density lipoprotein, and high-density lipoprotein as well as an association with a decreased risk of coronary heart disease on the order of 30-50% (Fraser 1999; Morgan and Clayshulte 2000). Additionally, consumption of pecans has not shown negative effects for short-term health. Despite having a high caloric content and being very nutrient-dense, regular consumption of pecans has not shown a net gain in body weight when used as a replacement food (Feldman 2002). Although the lack of weight gain is not completely understood, it has been hypothesized that there is incomplete absorption and lipid digestion, based on higher fat content in stool upon increased consumption of pecans, and that the consumption of such nutrient dense foods has a satiating effect that decreases appetite, among other theories (Garcia-Lorda et al. 2003). Research suggests that consumers know and understand the health benefits of consuming pecans regularly, and capitalizing on the health factors associated with their unsaturated fat content may be useful for marketing purposes (Lombardini et al. 2008).

Beyond connections to health, storage conditions and shelf-life of pecans are largely contingent on fatty acid content. Moisture content, the presence of antioxidants, exposure to air, and storage temperature are all factors that affect the rate of oxidation of lipids found in pecans, a process that may result in undesirable sensory changes in pecans (Erickson et al. 1994). To optimize the shelf-life and preservation of pecans, it is necessary to recognize the

fatty acids present and their susceptibility to oxidation. Several methods are in place to limit lipid oxidation, including refrigeration, pecan coating, and vacuum packaging, each having their benefits and drawbacks (Baldwin and Wood 2006). Without proper precautions, these changes that occur during the oxidation process can lead to unfavorable flavor changes. Research has found that, over time, the flavor profiles of pecan samples develop off characteristics, specifically with a decrease in sweetness and an increase in more undesirable attributes, such as bitterness, sourness, and rancidity (Magnuson et al. 2016).

Taking all of these factors into account, understanding the fatty acid profiles of different cultivars of pecans under different preparation methods is important to understanding how different cultivars can be used in application. Despite the information available about pecans from nutritional, storage, and developmental perspectives, minimal research has been performed on these fatty acids present in pecans and the sensory differences between pecan samples. The objectives of this research were to A) compare fatty acid profiles of eight different cultivars of pecans in raw and roasted forms, and B) compare these differences to those in flavor profiles obtained through descriptive sensory analysis.

Materials and Method

Samples

Eight cultivars of pecans, obtained from Kansas State University's Pecan Experiment Field in Chetopa, KS (USA), were used in this experiment. These cultivars, all grown in the 2014 growing season, include 'Chetopa,' 'Giles,' 'Kanza,' 'Lakota,' 'Major,' 'Maramec,' 'Pawnee,' and 'Witte.' Upon arrival, all samples were stored in-shell under frozen conditions (-18° C ± 1° C). Over a 90-day period, pecans were shelled using a Duke Pecan Walnut Cracker (Duke Pecan

Company, West Point, MS, USA), a Davebilt Nutcracker (Davebilt Company, Lakeport Calif., USA), and Channel Lock model number 436, 15.24 cm cutting pliers (Channel Lock Inc., Meadville, Pa., USA) to remove the kernels from the shell and clean away debris. Following the shelling process, pecan samples were additionally vacuum sealed in 3.79 L FoodSaver vacuum seal bags using a FoodSaver Heat-Seal Vacuum Sealing System (Sunbeam Products Inc. Boca Raton, FL, USA) before being returned to frozen conditions (-18° C ± 1° C). This was done to limit oxidation, retain moisture in the nutmeat, and minimize contamination (Reid 2011). Initial moisture content was measured using a Mettler Toledo HE 73/03 Moisture Analyzer (Mettler-Toledo AG, Greifensee, Switzerland), ensuring that all samples fell below the industry standard for moisture content at 4.5% (Nelson et at. 1992). Average initial percent moisture levels can be found in Table 4-1. Samples were maintained under frozen conditions (-18° C ± 1° C) until preparation and evaluation.

Each cultivar was profiled under raw and roasted conditions. Those that were analyzed raw were shelled and cleaned before the extraction process. The roasted pecans were removed from the freezer and allowed to thaw at room temperature (23° C ± 1° C) overnight. Approximately 100 g of sample was spread out in a single layer on an aluminum baking tray atop parchment paper and baked at 176 ° C for ten minutes. The samples were stirred at 5 and 8 minutes to prevent uneven roasting or burning. After removal from the oven, samples were allowed to cool at room temperature (23° C ± 1° C) for 30 minutes before being vacuum sealed and returned to the freezer (-18° C ± 1° C).

Samples were removed from the freezer one to three days prior to fatty acid extraction and ground to a paste using a frozen (-18° C ± 1° C) pestle and mortar. Ground samples were

vacuum sealed and returned to the freezer after processing and kept at frozen conditions (-18° C ± 1° C) until lipid extraction.

Table 4-1. Average initial percent moisture of the cultivars

Cultivar	Percent Moisture %
Giles	3.20 ± 0.11
Chetopa	2.48 ± 0.06
Kanza	2.37 ± 0.05
Lakota	3.59 ± 0.16
Major	2.45 ± 0.07
Maramec	2.97 ± 0.09
Pawnee	2.71 ± 0.10
Witte	3.01 ± 0.10

Descriptive Sensory Analysis

Descriptive sensory data was collected for each of the samples. A panel of evaluators, 2 male and 4 female, each having 120 hours of general descriptive training and at least 2000 hours of evaluation experience, evaluated each of the samples in duplicate. During a two-day orientation process, a list of 21 key flavor attributes as well as attribute definitions and references was decided upon (Appendix C) and practice evaluations were performed. This was followed by a 6-day evaluation period, each replicate being completed over three days. Attribute intensities were assigned using a 15-point scale with 05 increments.

Fatty Acid Extraction

For lipid profile evaluation, lipids from each cultivar under each preparation method were extracted in triplicate. A total of 48 extractions were performed. For each extraction

approximately 20 mg of ground sample was used. Samples were heated in 1.0 mL 75° C isopropanol (Fisher Scientific, Waltham, MA, USA) with 0.01% butylated hydroxytoluene (BHT) (Sigma Aldrich ®, St. Louis, MO, USA) for 15 minutes to inactivate phospholipase enzymes. Samples were then homogenized completely in solution using a Corning PYREX ® Tissue Grinder, glass pestle (PYREX ®, Greencastle, PA, USA), using 1.0 mL chloroform (Fisher Scientific, Waltham, MA, USA) and 1.0 mL methanol (Fisher Scientific, Waltham, MA, USA) to rinse instrumentation. HPLC-grade water (Fisher Scientific, Waltham, MA, USA) was added to the mixture, 0.8 mL, and 1.0 mL of both chloroform and methanol were added. The resulting solution was well mixed and transferred to a glass vial for centrifugation. Samples were centrifuged at approximately 6,000 rpm for 5 minutes. A total of four extractions were performed, using 1.0 mL of chloroform for each extraction, and the bottom layer containing the chloroform and lipids was extracted and placed in a new glass vial. 0.5 mL 1 M potassium chloride (Sigma Aldrich ®, St. Louis, MO, USA) was added to the extracted samples (chloroform and lipid), well mixed, and once again separated through centrifugation. The water layer on top was discarded, and 1.0 mL of HPLC-grade water was added to the extraction solution to collect any proteins or carbohydrates that may have carried through the extraction. After thorough mixing, centrifugation, and extraction of the bottom layer, containing the chloroform and lipids, to a new vial, samples were thoroughly dried under nitrogen gas, weighed, and redissolved in 1000 µL of chloroform in 2.0 mL Teflon-lined screw cap glass vials. Samples were stored at -40° C ± 1° C before preparation for gas chromatography.

Fatty Acid Methylation for Gas Chromatography

In preparation for gas chromatography (GC), each sample had to be methylated for detection. Approximately 1 mg of lipid was used for each run on GC. As an internal standard 50 nmol pentadecanoic acid (Sigma Aldrich ®, St. Louis, MO, USA) was added to each sample. In a glass vial, 1 mg of lipid in chloroform solution (Fisher Scientific, Waltham, MA, USA) and the internal standard were added to 1 mL 3M methanolic hydrochloric (Sigma Aldrich ®, St. Louis, MO, USA) acid and bubbled with nitrogen gas, because oxygen in the vial prevents the reaction between the methanolic hydrochloric acid and the lipids from occurring. The vials were heated for 30 minutes to allow for the reaction to complete its course. Samples were cooled back to room temperature (23° C ± 1° C) and 2.0 mL of HPLC-grade water (Fisher Scientific, Waltham, MA, USA) and 2.0 mL hexane:chloroform (4:1, v/v) (Fisher Scientific, Waltham, MA, USA) were thoroughly mixed into the solution. The aqueous phase was separated and extracted through centrifugation at approximately 6,000 rpm for 5 minutes. This was followed by three additional extractions using 2.0 mL hexane:chloroform. The samples were dried down completely under nitrogen gas and redissolved in hexane in 2.0 mL glass vials with Teflon-lined screw caps with glass inserts.

Gas Chromatography Methodology and Fatty Acid Identification

The analysis of the extracted lipid samples was performed at the Kansas State Lipodomics Research Center (Manhattan, KS, USA), using the center's database and expertise. Analysis was performed on an Agilent Technologies 6890N GC coupled with a flame ionization detector (FID) (Agilent Technologies, Santa Clara, CA, USA). The column used was a HP-88 capillary column with a bis-cyanopropyl-polysiloxane stationary phase with a column length of

100 m, internal diameter of 250 µm, and a film thickness of 0.20 µm. Helium was used as a carrier gas at a flow rate of 1.6 mL/minute. The inlet was under a pressure of 51.61 psi and 275° C.

1 µL of sample was injected in the splitless mode using an Agilent Technologies 7683 autosampler (Agilent Technologies, Santa Clara, CA, USA). The initial oven temperature was set to 150° C and held for 1 minute before increasing at 10° C/minute to 175°C. This was held for 10 minutes then increased at 5° C/minute to 210° C and held for 5 minutes. Finally, the temperature was increased at 5° C/minute to a final temperature of 230° C and held for 7 minutes. The total run time was 36.5 minutes. The FID was operated at 260° C using a flow of 30mL/minute of hydrogen and a flow rate of 400 mL/minute of air. A sampling rate of 20 Hz was utilized by the FID.

All data for gas chromatography was processed using ChemStation software (Agilent Technologies, Santa Clara, CA, USA). Individual fatty acids were identified through the use of a standard mixture (37-component FAME mixture; Supelco Inc., Bellfonte, PA, USA) of fatty acids run at the time of instrumental analysis. Results were transformed into percentage of total fatty acids for analysis, following the methods of similar studies (Van Nieuwenhove et al. 2014, Stefanova et al. 2011, Pavithra et al. 2012).

Statistical Analysis

After gas chromatography data was collected, fatty acid profiles were compiled using Microsoft Excel software (Microsoft ©, Redmond, WA, USA). 2-way analysis of variance was performed using SAS® statistical software (SAS® version 9.3, SAS Institute Inc., Cary, NC, USA) to determine any significant differences in fatty acid content of different cultivars under

different preparation methods. PROC GLIMMIX and PROC MIXED methods were used for analysis. Fisher's protected least significant difference *post-hoc* means were used to distinguish between significantly different cultivars and preparation methods at the 5% significance level.

*X*LStat statistical software (Addinsoft©, New York, NY, USA) was used to perform agglomerative hierarchical clustering under Ward's agglomeration method. Pearson's principal components analysis was utilized to visualize relationships between sample fatty acid profiles and explain variance due to specific fatty acids. This was done using a correlation biplot. Partial least squares regression and Pearson's correlation test were also performed using *X*LStat statistical software at the 5% level of significance. This was done using mean attribute intensities for each of the samples from prior descriptive sensory research as supplementary quantitative data to find correlations between fatty acid profile differences and sensory attributes.

Results

Fatty Acid Profile Variation

The fatty acid profiles of many of the samples showed similar trends. In all eight cultivars under both raw and roasted conditions, oleic acid comprised more than 50% of the total fatty acid content. This was followed by linoleic acid, making up between 25 and 38 percent of the lipid content. Palmitic acid showed the next greatest concentration at between 6 and 7.25 percent. Finally, stearic acid then linolenic acid comprised the following highest concentrations respectively, both at around 1.5% to 2%. This order of concentration, oleic, linoleic, palmitic, stearic, then linolenic acids, was seen across all cultivars across both preparation methods. Fatty acid profiles for each sample are presented in Table 4-2.

Within each cultivar, little difference in fatty acid profile could be seen between raw and roasted samples, with the exception of the 'Giles' cultivar. The 'Giles' cultivar showed large differences in fatty acid profile due to the preparation method. Despite this case, most of the sample variation could be attributed to cultivar differences, supported by the results of 2-way ANOVA. Table 4-3 depicts the significance ($p \leq 0.05$) of the cultivar effect, the preparation method effect, and the interaction between these two factors on concentration of each of the fatty acids. For palmitic acid, stearic acid, and linolenic acid, cultivar had a significant effect on concentration. For palmitic acid, 'Pawnee' had a significantly higher percentage than 'Major,' 'Kanza,' 'Witte,' 'Chetopa,' and 'Maramec' cultivars, regardless of preparation method. Conversely, 'Maramec' exhibited a significantly lower concentration of palmitic acid than 'Pawnee,' 'Giles,' 'Lakota,' and 'Major' cultivars. Stearic acid comprised a significantly higher percentage of total fatty acids in all cultivars over 'Major,' and 'Pawnee' and 'Kanza' cultivars showed significantly higher percentage than 'Maramec' and 'Major.' In concentration of linolenic acid, 'Lakota,' 'Giles,' and 'Witte' were all significantly higher than 'Kanza,' 'Chetopa,' 'Major,' and 'Maramec.'

For oleic and linoleic acids, there was a significant interaction between cultivar and preparation method (Table 4-3). This meant that the roasting process had a different effect on the fatty acid profile between cultivars. Figures 4-1 and 4-2 illustrate the effects of these factors for oleic acid and linoleic acid, respectively, showing that the preparation method affected the cultivars in different ways. In both oleic and linoleic acids, the 'Giles' cultivar exhibited significantly different ($p \leq 0.05$) percentages between the raw and roasted preparation method, disrupting the trends displayed by the other cultivars.

Examining the samples individually allowed for a better understanding of the variation trends between different cultivars under different preparation methods (Tables 4-4 and 4-5). Within the oleic fatty acid, samples generally had similar concentrations between raw and roasted samples, with the 'Major' cultivar yielding the highest concentration of oleic acid, followed by 'Witte,' 'Maramec,' 'Chetopa,' 'Kanza,' then the raw 'Giles' sample, followed by 'Lakota' and 'Pawnee' and finally the roasted 'Giles' sample. The 'Giles' cultivar did not follow this trend, with a significant difference between the raw and roasted samples. 'Major' was shown to have a significantly higher percentage than all other cultivars but 'Witte,' regardless of preparation method. The 'Pawnee' samples had significantly lower percent of total fatty acids for oleic acid than all samples but the roasted 'Giles' and the roasted 'Lakota' samples. Similar trends were seen with linoleic acid for the 'Giles' cultivar, with the roasted sample having a significantly higher percentage than the raw sample but other cultivars generally having the same concentration between preparation methods. The 'Witte' and 'Major' cultivars were shown to have significantly lower percentage of linoleic acid than all of the other cultivars. Additionally, 'Pawnee' was significantly higher in linoleic acid concentration than all of the other cultivars but the 'Lakota' cultivar and the roasted 'Giles' sample.

A principal components analysis biplot allowed for further explanation of the variation between samples (Figure 4-3). The plot produced was able to explain 85.37% of the variation between the samples. Principal component 1, explaining 67.62% of the sample variation, was highly correlated to the oleic acid percentage of the total fatty acid content. Principal component 2, which explained 17.75% of the variation between samples, is more correlated with linolenic and palmitic acids versus linoleic acid and stearic acid content. In order to determine which samples were highly correlated to each other, and to better explain the trends in fatty acid profile,

hierarchical cluster analysis was utilized to find three distinct clusters of samples. These clusters are incorporated into Figure 4-3. The first cluster of samples consisted of roasted ‘Lakota’ and ‘Giles’ samples and the ‘Pawnee’ cultivar in both raw and roasted forms. This cluster was characterized by a low level of oleic acid, but exhibited a range of other fatty acid concentrations. The second cluster, consisting of raw ‘Lakota,’ ‘Giles,’ ‘Kanza,’ ‘Chetopa,’ and ‘Maramec’ samples and roasted ‘Kanza,’ ‘Chetopa,’ and ‘Maramec’ samples, was associated with a mid-range percentage of oleic acid, and an association with higher linoleic acid and stearic acid concentrations. The final cluster was comprised of the ‘Witte’ and ‘Major’ cultivars under both preparation means. This cluster had a higher correlation with linolenic and palmitic acids and higher concentrations of oleic acid.

Some cultivars showed a high association with certain fatty acids. The ‘Lakota’ cultivar showed a high association with linolenic acid, ‘Pawnee’ was aligned with linoleic acid and palmitic acid, and the ‘Major’ and ‘Witte’ cultivars were closely tied with oleic acid.

Table 4-2. Fatty acid profiles of pecan samples

Cultivar	Preparation Method	% of Total Fatty Acids ± StdDev				
		Palmitic	Stearic	Oleic	Linoleic	Linolenic
Chetopa	Raw	6.18 ± 0.36	2.11 ± 0.06	58.32 ± 1.47	32.11 ± 1.74	1.29 ± 0.07
	Roasted	6.55 ± 0.61	2.24 ± 0.15	58.73 ± 2.30	31.17 ± 1.15	1.31 ± 0.15
Giles	Raw	6.66 ± 0.39	2.00 ± 0.24	56.78 ± 0.73	33.05 ± 1.20	1.52 ± 0.10
	Roasted	7.13 ± 0.73	2.34 ± 0.72	50.41 ± 1.79	38.38 ± 1.51	1.74 ± 0.58
Kanza	Raw	6.62 ± 0.43	2.19 ± 0.11	57.62 ± 2.05	32.22 ± 1.61	1.35 ± 0.08
	Roasted	6.64 ± 0.27	2.33 ± 0.12	57.12 ± 0.37	32.61 ± 0.41	1.30 ± 0.03
Lakota	Raw	6.73 ± 0.19	1.96 ± 0.01	56.47 ± 1.69	33.16 ± 1.44	1.69 ± 0.09
	Roasted	7.04 ± 0.42	2.21 ± 0.40	54.96 ± 1.89	34.10 ± 1.36	1.69 ± 0.19
Major	Raw	6.72 ± 0.24	1.76 ± 0.07	64.87 ± 0.90	25.30 ± 0.60	1.36 ± 0.06
	Roasted	6.70 ± 0.24	1.60 ± 0.05	62.97 ± 0.37	27.48 ± 0.13	1.24 ± 0.03
Maramec	Raw	6.19 ± 0.05	2.03 ± 0.03	59.31 ± 1.09	31.27 ± 1.03	1.20 ± 0.11
	Roasted	6.35 ± 0.11	1.94 ± 0.05	59.59 ± 0.38	30.95 ± 0.29	1.17 ± 0.01
Pawnee	Raw	7.25 ± 0.12	2.38 ± 0.11	53.61 ± 0.57	35.22 ± 0.67	1.54 ± 0.01
	Roasted	7.12 ± 0.29	2.30 ± 0.31	52.68 ± 0.20	36.48 ± 0.54	1.42 ± 0.15
Witte	Raw	6.48 ± 0.07	2.01 ± 0.05	62.04 ± 2.91	27.85 ± 2.75	1.62 ± 0.22
	Roasted	6.60 ± 0.12	2.27 ± 0.16	60.75 ± 0.40	28.82 ± 0.27	1.56 ± 0.06

Table 4-3. P-values of individual factors and factor interactions from ANOVA across fatty acids[a]

Fatty Acid	Cultivar	Preparation Method	Cultivar*Preparation Method
Palmitic Acid	**0.0006[b]**	0.0902	0.7323
Stearic Acid	**0.0012[b]**	0.1419	0.4411
Oleic Acid	**<0.0001[b]**	**0.0012[b]**	**0.0093[b]**
Linoleic Acid	**<0.0001[b]**	**0.0021[b]**	**0.0077[b]**
Linolenic Acid	**0.0001[b]**	0.7670	0.7970

[a] Significance taken at $p \leq 0.05$
[b] Significant factor or interaction for given attribute

Figure 4-1. Interaction between cultivar and preparation method across samples for oleic acid percent total fatty acids

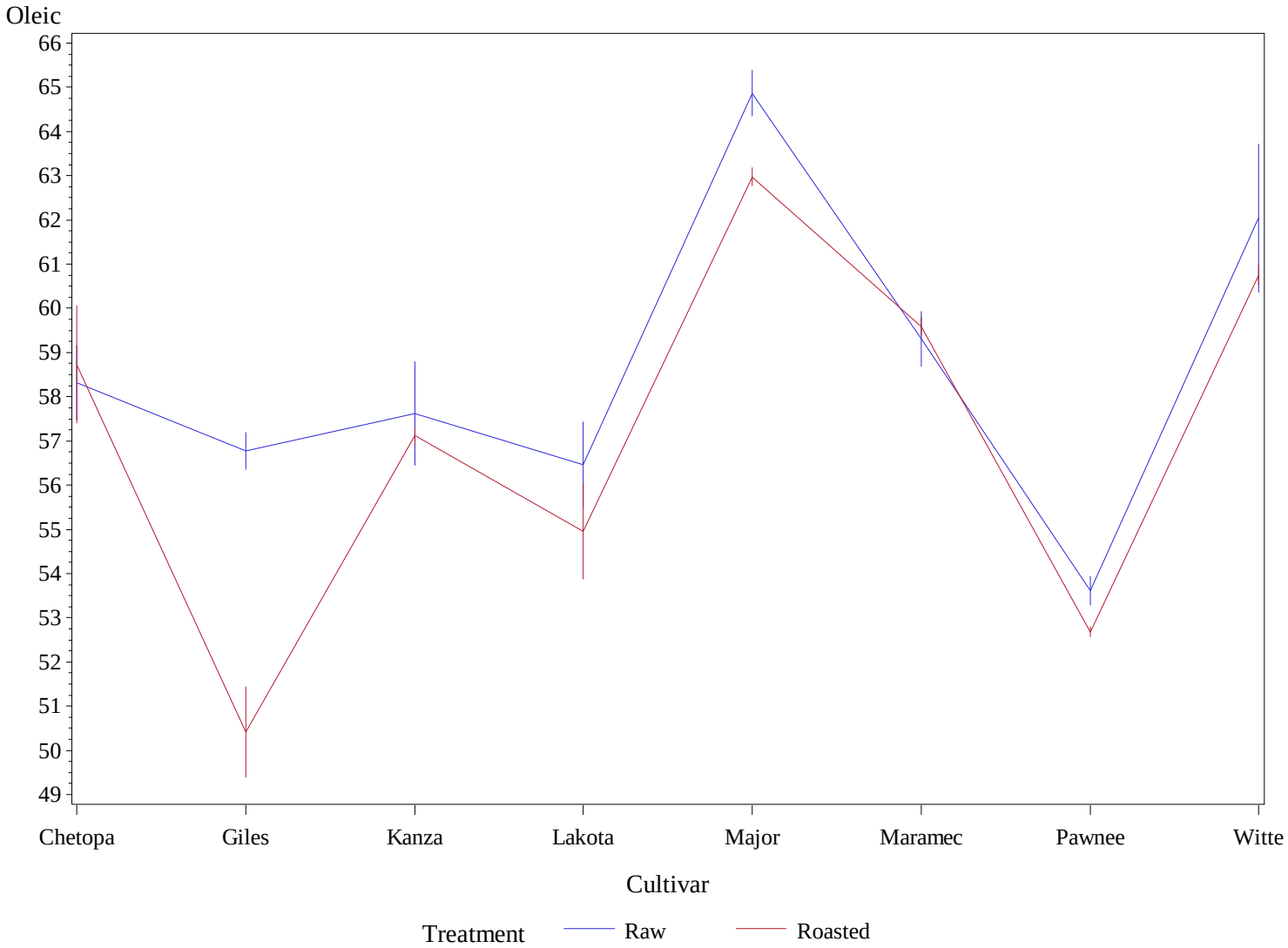


Figure 4-2. Interaction between cultivar and preparation method across samples for linoleic acid percent total fatty acids

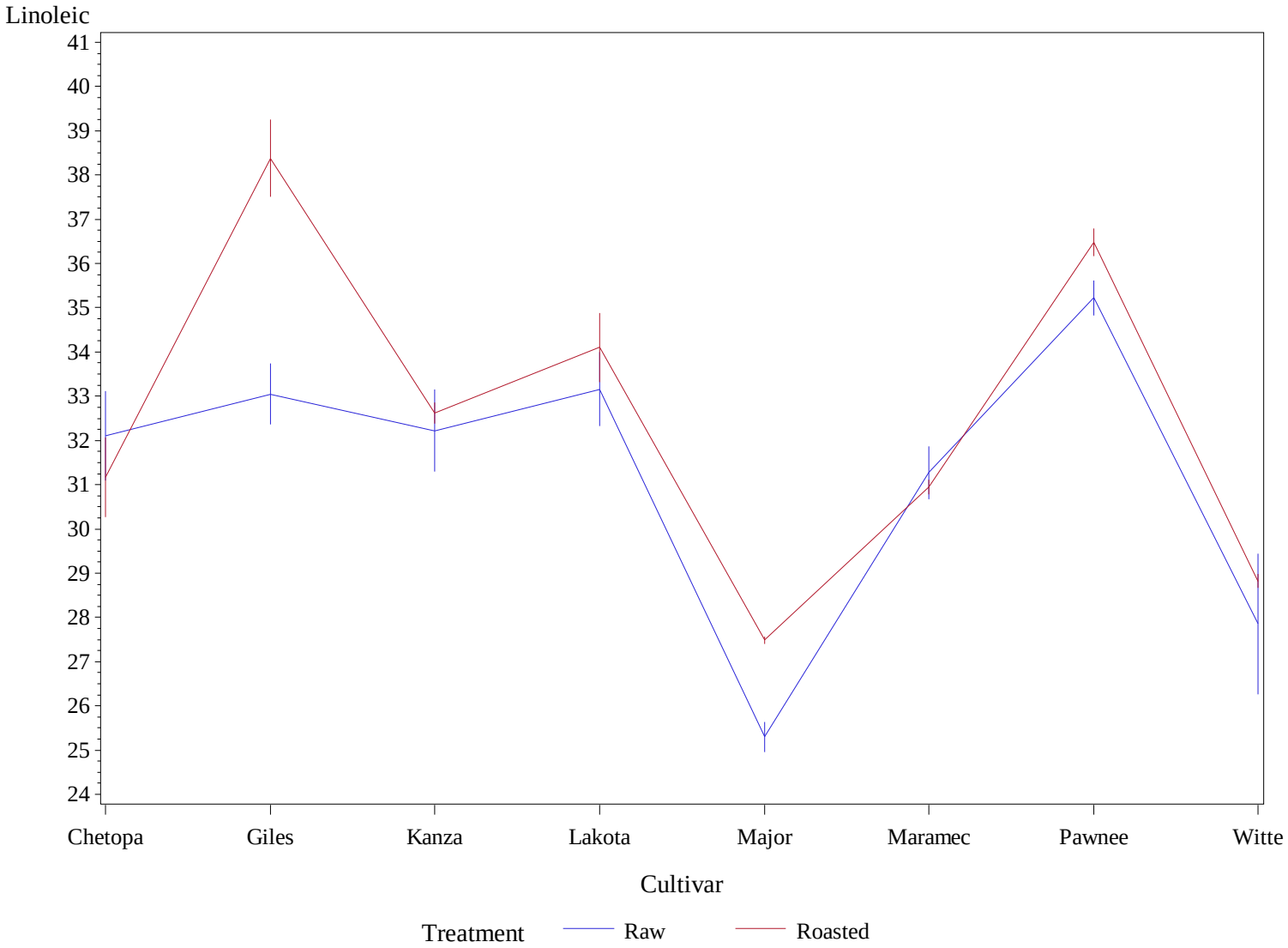


Table 4-4. Mean percent of total fatty acids for oleic acid and separation for all cultivars in raw and roasted forms *

Cultivar	Preparation Method	Percent of Total Fatty Acid
Chetopa	Raw	58.32 ± 1.47 ^{DEF}
Chetopa	Roasted	58.73 ± 2.30 ^{CDEF}
Giles	Raw	56.78 ± 0.73 ^{FG}
Giles	Roasted	50.41 ± 1.79 ^{I}
Kanza	Raw	57.62 ± 2.05 ^{DEF}
Kanza	Roasted	57.12 ± 0.37 ^{EFG}
Lakota	Raw	56.47 ± 1.69 ^{FG}
Lakota	Roasted	54.96 ± 1.89 ^{GH}
Major	Raw	64.87 ± 0.90 ^{A}
Major	Roasted	62.97 ± 0.37 ^{AB}
Maramec	Raw	59.31 ± 1.09 ^{CDE}
Maramec	Roasted	59.59 ± 0.38 ^{CD}
Pawnee	Raw	53.61 ± 0.57 ^{H}
Pawnee	Roasted	52.68 ± 0.20 ^{HI}
Witte	Raw	62.04 ± 2.91 ^{B}
Witte	Roasted	60.75 ± 0.40 ^{BC}

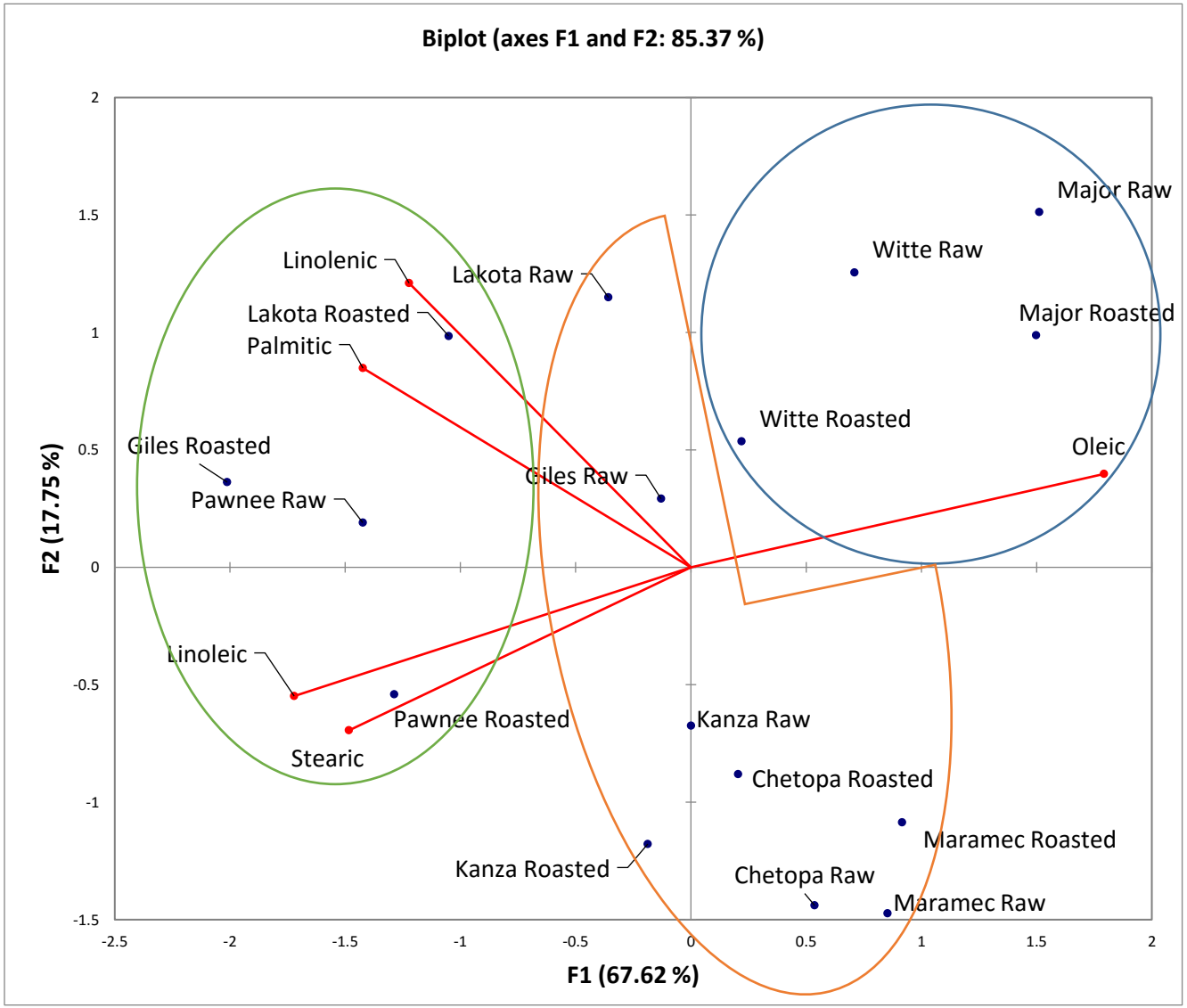
* Means with different superscripts are significantly different ($p \leq 0.05$) according to Fisher's protected least significant difference (LSD) test

Table 4-5. Mean percent of total fatty acids for linoleic acid and separation for all cultivars in raw and roasted forms *

Cultivar	Preparation Method	Percent of Total Fatty Acid
Chetopa	Raw	32.11 ± 1.74 ^{DEF}
Chetopa	Roasted	31.17 ± 1.15 ^{EF}
Giles	Raw	33.05 ± 1.20 ^{DEF}
Giles	Roasted	38.38 ± 1.51 ^{A}
Kanza	Raw	32.22 ± 1.61 ^{DEF}
Kanza	Roasted	32.61 ± 0.41 ^{DEF}
Lakota	Raw	33.16 ± 1.44 ^{CDE}
Lakota	Roasted	34.10 ± 1.36 ^{CD}
Major	Raw	25.30 ± 0.60 ^{H}
Major	Roasted	27.48 ± 0.13 ^{G}
Maramec	Raw	31.27 ± 1.03 ^{EF}
Maramec	Roasted	30.95 ± 0.29 ^{F}
Pawnee	Raw	35.22 ± 0.67 ^{BC}
Pawnee	Roasted	36.48 ± 0.54 ^{AB}
Witte	Raw	27.85 ± 2.75 ^{G}
Witte	Roasted	28.82 ± 0.27 ^{G}

* Means with different superscripts are significantly different ($p \leq 0.05$) according to Fisher's protected least significant difference (LSD) test

Figure 4-3. Principal components analysis of fatty acids across all cultivars under raw and roasted preparation methods with cluster results*



*Samples within each shape fell within one cluster

The Role of Fatty Acids in Flavor

Partial least squares analysis illustrated the relationship between individual fatty acids and sensory characteristics using data from Chapter 2 (Figure 4-4). Oleic acid did not show strong associations with any specific flavor attributes. Linoleic acid, stearic acid, and palmitic acid were closely related and showed some connection with more of the roasted-type attributes, such as *Roasted, Overall Sweet,* and *Overall Nutty*. These fatty acids and sensory characteristics were closely related to the 'Pawnee' cultivar in both raw and roasted forms. A connection was present between the linolenic acid content and attributes relating to more undesirable flavor attributes, such as *Astringent, Acrid, Oxidized, Bitter, Burnt,* and *Woody*. The 'Lakota' cultivar exhibited these sensory qualities and had an association with the linolenic acid. Pearson's correlation analysis further supported the connection between linolenic acid and higher intensities of these sensory attributes, depicted in Table 4-6. A significant correlation was found between linolenic acid and *Burnt, Woody,* and *Astringent* attributes and a strong correlation with *Acrid, Oxidized,* and *Bitter* attributes in addition.

Figure 4-4. Partial least squares correlation plot between fatty acid and flavor profiles for all cultivars in raw and roasted forms

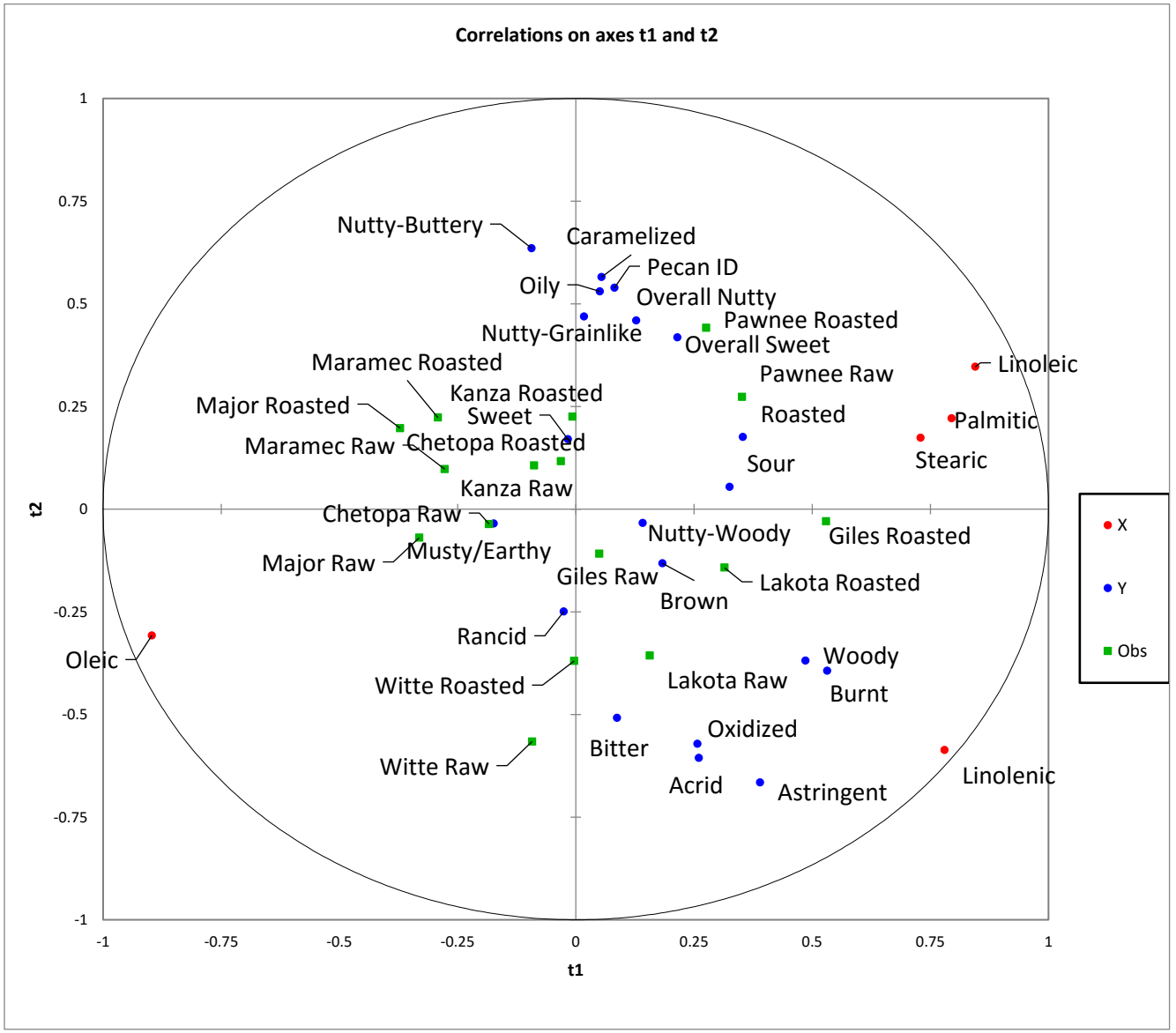


Table 4-6. Correlation matrix between fatty acids and sensory attributes, part 1 [a]

Variables	Palmitic	Stearic	Oleic	Linoleic	Linolenic
Pecan ID	0.310	0.185	-0.144	0.133	-0.226
Overall Nutty	0.319	0.157	-0.181	0.171	-0.138
Nutty-Woody	0.161	-0.018	-0.110	0.101	0.162
Nutty-Grainlike	0.219	-0.006	-0.118	0.125	-0.220
Nutty-Buttery	0.302	-0.029	0.030	-0.040	-0.376
Brown	0.239	-0.009	-0.072	0.045	0.269
Caramelized	0.419	0.015	-0.085	0.066	-0.208
Acrid	-0.124	0.251	-0.137	0.123	0.484
Burnt	0.253	0.355	-0.403	0.373	**0.620**
Musty/Earthy	-0.424	-0.003	0.012	0.038	-0.204
Woody	0.188	0.287	-0.403	0.386	**0.567**
Roasted	0.478	0.158	-0.298	0.268	0.233
Overall Sweet	0.352	0.181	-0.273	0.265	-0.048
Oily	0.298	-0.065	-0.165	0.173	-0.206
Rancid	-0.324	0.168	-0.008	0.027	0.029
Oxidized	0.021	0.383	-0.014	-0.037	0.476
Astringent	0.160	-0.002	-0.194	0.164	**0.721**
Bitter	0.141	-0.111	0.180	-0.232	0.426
Sour	0.358	0.319	-0.229	0.190	0.233
Sweet	0.104	-0.081	-0.009	0.010	-0.078

[a] Bolded values indicate a significant correlation at the 5% level of significance

Discussion

While different samples of pecans have been shown to yield highly diverse profiles in sensory studies, most notably between raw and roasted preparation methods (Magnuson et al. 2016), the fatty acid composition between cultivars were revealed to have similar trends, with sample variation due almost exclusively to the cultivar effect and the preparation method having little effect on the variation. Only in the 'Giles' cultivar was significant variation seen between raw and roasted samples. This may have been due to sampling error and the nature of the cultivar, being more shriveled and having a larger ratio of kernel surface to nutmeat than the other cultivars. Further research is necessary to determine if these qualities have a significant effect on fatty acid profile. Despite this irregularity seen in the 'Giles' samples, compositional trends were seen across the pecans; in all samples, the bulk of the total fatty acids was comprised of oleic and linoleic acids, with stearic acid and linolenic acid present in comparatively small amounts.

One of the important applications of understanding fatty acid profiles for different pecan samples is in flavor. Several studies have been performed to further understanding of the sensory effects of different fatty acids and fatty acid concentrations, namely in the impact on flavor of oleic acid (Isleib et al. 2006; Pattee et al. 2002). The findings of this study indicated that oleic acid did not have a significant effect on flavor, but was linked to higher oxidative stability. In this study, palmitic, stearic, oleic, linoleic, and linolenic acid profiles were related to descriptive sensory profiles of each of the cultivars in raw and roasted forms. Linoleic acid, stearic acid, and palmitic acid were closely related to attributes such as *Roasted, Overall Sweet,* and *Overall Nutty* – the roasted-type attributes. Linoleic and palmitic acids were seen in higher concentrations for the 'Pawnee' cultivar, showing that this cultivar has a composition aligned with these more

desirable characteristics. Conversely, less desirable attributes such as *Astringent, Acrid, Oxidized, Bitter, Burnt,* and *Woody* were found to be correlated with linolenic acid, which was found in significantly higher concentrations in the 'Lakota' cultivar, followed by the 'Witte' and 'Giles' cultivars. The presence of high amounts of linoleic acid may serve as a good indicator for these undesirable attributes and may be an efficient way of predicting the success of a cultivar in the market. Oleic acid, though not strongly associated with specific flavor attributes, was closely tied to 'Major' and 'Witte' cultivars. Based on the findings of Isleib et al. (2006) and Pattee et al. (2002), these cultivars may be more successful in maintaining their flavor profiles for longer storage periods than others, due to the higher oxidative stability linked to high oleic acid content.

This study examined 8 cultivars of pecans in three replicates from a single growing season. The inclusion of additional replicates may reveal clearer distinctions between samples. Also, the incorporation of different cultivars may show fatty acid trends not found in this study. Growing season may have a significant effect on the fatty acid profiles and should be the focus of future research. Sampling error was likely present in this study due to methods used, predominantly in limited initial homogenization from grinding with mortar and pestle and incomplete breakdown of skin on the kernel surface. The effect of the ratio of kernel skin to nutmeat on the fatty acid profile should be examined in future studies to see if more kernel skin leads to higher presence of specific fatty acids.

Conclusions

The fatty acid profiles of different cultivars of pecans under raw and roasted conditions showed several similarities, following the same order of highest to lowest concentration fatty acids and containing very similar percentages of each fatty acid. However, many differences

existed that distinguish cultivars from one another. Most of these differences were due to the effect of cultivar. The roasting process did not have a large effect on the fatty acid profiles of each cultivar. The small differences caused by the roasting process affected each of the cultivars in a similar way. The exception to this is with the 'Giles' cultivar, which showed significantly higher percentage of linoleic acid and significantly lower percentage of oleic acid in the roasted samples than the raw. When compared to the other samples, palmitic acid was exhibited in higher percentage in 'Pawnee' pecans and in lower concentrations in 'Chetopa' and 'Maramec' cultivars. Stearic acid was found in lower amounts of total lipid in the 'Major' cultivar. 'Major' and 'Witte' exhibited high percentage of oleic acid while 'Pawnee,' 'Giles,' and 'Lakota' cultivars had lesser percentages. 'Pawnee' pecans had higher concentrations of linoleic acid, with 'Major' and 'Witte' showing low concentrations. Finally, linolenic acid was found in higher percentages of total fatty acids in 'Lakota' and roasted 'Giles' samples and in lower percentages in 'Maramec' pecans.

The examination of the relationship between fatty acid profile and sensory attributes showed a significant correlation between linolenic acid and attributes associated with dryness, namely *Astringency*, *Woody*, and *Burnt* attributes. The additional strong correlation with *Bitter*, *Acrid*, and *Oxidized* attributes suggested that higher concentrations of linolenic acid are linked to higher intensities of undesirable attributes. Based on their association with the 'Pawnee' cultivar as well as the results of partial least squares analysis, linoleic acid and palmitic acid may play a large role in the presence and intensity of roasted-type attributes. Given these results, pecan samples with higher concentrations of linolenic acid can be avoided for cultivation for the market, with focus on those with higher linoleic acid and palmitic acid content.

References

Alasalvar, C. & Shahidi, F. (2009) *Tree Nuts: Composition, Phytochemicals, and Health Effects.* Boca Raton, FL: Taylor & Francis Group.

Baldwin, E. & Wood, B. (2006). Use of edible coating to preserve pecans at room temperature. *Hortscience,* 41(1): 188-192.

Erickson, M., Santerre, C., & Malingre, E. (1994). Oxidative stability in raw and roasted pecans: chemical, physical, and sensory measurements. *Journal of Food Science,* 59(6): 1234-1238.

Fraser, G. (1999). Nut consumption, lipids, and risk of a coronary event, *Clinical Cardiology,* 22(S3): 11-15.

Feldman, E. (2002). The scientific evidence for a beneficial health relationship between walnuts and coronary heart disease. *The Journal of Nutrition,* 132(5): 10625-11015.

Garcia-Lorda, P., Rangil, I., Salas-Salvado, J. (2003). Nut consumption, body weight, and insulin resistance. *European Journal of Clinical Nutrition,* 57(1): S8-S11.

Isleib, T., Pattee, H., Sanders, T., Hendrix, K., & Dean, L. (2006). Compositional and sensory comparisons between normal- and high-oleic peanuts. *Journal of Agricultural and Food Chemistry,* 54(5): 1759-1763.

Lombardini, L., Waliczek, T., & Zajicek, J. (2008). Consumer knowledge of nutritional attributes of pecans and factors affecting purchasing behavior. *HortTechnology,* 18(3): 481-488.

Magnuson, S., Koppel, K., Reid., W., Chambers IV, E. (2016). Pecan flavor changes during storage. *Journal of the American Pomological Society*, 69(4): 206-214.

Morgan, W. & Clayshulte B. (2000). Pecans lower low density lipoprotein cholesterol in people with normal lipid levels. *Journal of the American Dietetic Association,* 100(3): 312-318.

Nelson, S., Lawrence, K., Kandala, C. (1992). Sensing moisture in peanut and pecan kernels by RF impedance measurements. *Journal of Microwave Power and Electromagnetic Energy,* 27:171–4.

Pavithra, H., Gowda, B., Rajesh Kumar, K., Prasanna, K., & Shivanna, M. (2012). Oil, fatty acid profile and karanjin content in developing Pongamia pinnata (L.) pierre seeds. *Journal of the American Oil Chemists' Society*, 89(12): 2237-2244.

Pattee, H., Isleib, T., Gorbet, D., Moore, K., Lopez, Y., Baring, M., & Simpson, C. (2002). Effect of the high-oleic trait on roasted peanut flavor in backcross-derived breeding lines. *Journal of Agricultural Food Chemistry,* 50(25): 7362-7365.

Reid, W. (2011). Storage conditions for black walnut kernels. *Personal Communication.* August 2011.

Stefanova, R., Toshkov, S., Vasilev, N., Vasilev, N., & Marekov, I. (2011). Effect of gamma-ray irradiation on the fatty acid profile of irradiated beef meat. *Food Chemistry*, 127(2): 461-466.

Toro-Vazquez, J. & Perez-Briceno, F. (1998). Chemical and physiochemical characteristics of pecan (Carya illinoensis) oil native of the central region of Mexico. *Journal of Food Lipids,* 5(3): 211-231.

Toro-Vazquez, J., Charo-Alonso, M., & Perez-Briceno, F. (1999). Fatty acid composition and its relationship with physiochemical properties of pecan (Carya illinoensis) oil. *Journal of the Americal Oil Chemists' Society*, 76(8): 957-965.

Van Nieuwenhove, C., Perez, M., Hernandez, M., & Luna Pizzaro, P. (2014). Characterization of gross composition, energy value, and fatty acid profile of milk from lowland tapir (Tapirus terrestris) during different lactation periods. *Canadian Journal of Zoology*, 902(9): 803-809.

Chapter 5 - Detection of Volatile Flavor Compounds in Four Cultivars of Pecans in Raw and Roasted Forms

Abstract

The chemical composition of pecans, though complex, has been studied through many years of research. Despite this, the chemical profile of the pecan is not fully described. The objectives of this study were to gain insight into the chemical makeup and compositional changes that occur during preparation in order to better understand the sensory qualities of pecans as well as differences that exist between sensory profiles. In this study, 'Kanza,' 'Maramec,' 'Pawnee,' and 'Witte' pecans, obtained from Kansas State University's Pecan Experiment Field from the 2014 growing season were profiled under raw and roasted conditions. Gas chromatography-olfactometry was used to determine chromatographic peaks that had olfactory contribution and sensory descriptors that characterized them. The compounds corresponding to these peaks were tentatively identified using gas chromatography-mass spectrometry and semi-quantitative data was collected for analysis. 45 compounds, broadly categorized into aromatic hydrocarbons, nitriles, ethers, aldehydes, terpenes, acids, alcohols, and pyridines, were tentatively identified as olfactory contributors, 29 of which were present across all cultivars and preparation methods. One compound was found in only half of the cultivars, one compound was unique to the raw preparation method, and the roasted preparation method had two unique compounds. Additionally, two compounds were unique to the roasted 'Maramec' sample. 1R-alpha-pinene, identified as an olfactory contributor in all samples but raw 'Kanza' and raw 'Witte' and described as *Woody, Brown, Earthy, Buttery, Caramel,* and *Musty,* likely has the most influence on pecan character notes across the samples. Each sample had

concentrations of compounds associated with a mix of desirable and undesirable attributes. However, the ‘Pawnee’ cultivar, in particular the roasted ‘Pawnee’ sample, exhibited higher concentrations of compounds that were described with more desirable attributes. This roasted ‘Pawnee’ sample may serve as a good standard for the development of pecan flavor.

Introduction

The pecan is extremely complex on a chemical level, as are many other plant reproductive products such as fruit pits, seeds, and beans. The exact nature of many of the compounds found in pecans and similar products is not completely understood, despite years of research and countless studies spanning the past century. However, several pieces have been put together about the composition and general behavior of these components through the years. One study performed by Hammer and Hunter in 1946 attempted to identify the components that make up the pecan and how they change throughout the development of the pecan kernel, examining the oil formation and protein development as well as the movement of several minerals such as nitrogen, phosphorus, potassium, calcium, and magnesium between the shuck, shell, and nutmeat. Although limited in scope, this research was able to describe some of the chemical framework of the pecan.

More modern methods of analysis allow for a deeper understanding of the compositional makeup of pecans, but still fall short of complete profiling. Many compounds have nonetheless been identified. One study performed in 1972 identified several compounds in roasted pecans, broken down into carbonyls, pyridine, pyrazines, acids, alcohols, and lactones (Wang and Odell 1972). A total of 41 compounds were identified across pecan samples. Another study performed by Malik et al. (2009) further identified several phenolic compounds, including gallic acid,

catechol, catechin, epicatechin, *m*-coumaric acid, chlorogenic acid, ellagic acid, and caffeic acid, which have been linked to many of the health benefits associated with pecan consumption, finding these compounds in both organically grown and traditionally grown pecan samples.

The knowledge of the chemical makeup and the changes that occur to pecan composition under different preparation methods is vital to understanding the sensory profiles and provides an explanation for sensory differences that exist between samples. For examples, research has shown a connection between the oxidation of linoleic acid and the development of rancid off-flavors (Rudolph et al. 1992). Gas chromatography-olfactometry is one method that allows for the identification of chemical components while simultaneously collecting sensory data in order to gain an understanding of the olfactory contributions of various chemical compounds within a product. This process if frequently used in the flavoring industry to identify the compounds necessary to characterize a flavor, allowing for larger application and potentially the development of more cost effective ways of synthesizing and applying flavors (Zellner et at. 2007). Achieving effective results, however, may be difficult due to a range of factors, including limitations in human accuracy, bias, inefficient extraction of volatiles, and environmental conditions (Delanunty 2006). Because of this, proper training and preparation methods for panelists may have a significant effect on a meaningful outcome (Vene et al. 2013).

The objective of this study is to A) identify chemical compounds that have olfactory contribution and B) compare the chemical profiles that were defined between four different cultivars of pecans under raw and roasted conditions. The understanding of these chemical differences will provide further explanation of sensory differences that exist between the samples.

Materials and Methods

Samples

In this study, four cultivars of pecans were examined: 'Kanza,' 'Maramec,' 'Pawnee,' and 'Witte.' All samples were obtained from Kansas State University's Pecan Experiment Field in Chetopa, KS (USA) from the 2014 growing season. Samples were kept under frozen conditions (-18° C ± 1° C) after being received. All samples were shelled over a 90-day period, using a Duke Pecan Walnut Cracker (Duke Pecan Company, West Point, MS, USA), a Davebilt Nutcracker (Davebilt Company, Lakeport, CA, USA), and Channel Lock model number 436, 15.24 cm cutting pliers (Channel Lock Inc., Meadville, PA, USA) to remove the nutmeat from the shell and clean away debris. Samples were returned to frozen conditions (-18° C ± 1° C) after the shelling process, additionally being vacuum sealed in 3.79 L FoodSaver vacuum seal bags using a FoodSaver Heat-Seal Vacuum Sealing System (Sunbeam Products Inc. Boca Raton, FL, USA) to preserve freshness through maintaining moisture, limiting oxidation, and minimizing contamination (Reid 2011). A Mettler Toledo HE 73/03 Moisture Analyzer (Mettler-Toledo AG, Greifensee, Switzerland) was used to ensure that the initial moisture content of all samples fell below the industry standard of 4.5% moisture, preventing any major flavor alterations and limiting micro-organismal growth (Nelson et al. 1992). The initial percent moisture values for each cultivar are displayed in Table 5-1. Samples were stored frozen until preparation and evaluation (-18° C ± 1° C).

Each of the cultivars were analyzed under both raw and roasted conditions. Those samples evaluated under the raw preparation method were shelled and cleaned before evaluation. Samples were prepared under the roasted preparation method for each cultivar as well. These pecans were removed from the freezer and left at room temperature (23° C ± 1° C) in their

vacuum-sealed bags to thaw overnight. Approximately 100 g of sample was placed on sheet of parchment on an aluminum baking tray in a single layer and roasted for ten minutes at 176° C. Stirring of the samples was done at five and eight minutes to ensure an even roast. After the samples were removed from the oven, they were left to cool at room temperature (23° C ± 1° C) for 30 minutes before being resealed in vacuum seal bags and returned to the freezer to await evaluation. The roasting process was performed in triplicate to minimize the effects of preparation error or sampling bias.

Each of the cultivars under each of the preparation methods was extracted and analyzed in triplicate for both gas chromatography-olfactometry (GCO) and gas chromatography-mass spectrometry (GC-MS).

Table 5-1. Average initial percent moisture for the cultivars

Cultivar	Percent Moisture %
Kanza	2.37 ± 0.05
Maramec	2.97 ± 0.09
Pawnee	2.71 ± 0.10
Witte	3.01 ± 0.10

Gas Chromatography-Olfactometry Sample Preparation

Samples were removed the day of evaluation and approximately 4 pecan halves were ground to a paste using a frozen (-18° C ± 1° C) pestle and mortar. 1.00 g (± 0.05 g) sample was transferred to a 10.0 mL screw-cap glass vial with a polytetrafluoroethylene–silicone septum with 0.50 mL water. Samples were then incubated for 1 hour at 36° C in a Gyromax™ Orbital Incubator Shaker, Model 747R (Amerex Instruments Inc., Concord, CA, USA), at 18.0 rpm with a Supelco solid-phase microextraction (SPME) portable field sampler (Supelco, Bellfonte, PA,

USA) to extract volatile compounds. The SPME field sampler was coated with a polydimethylsiloxane/divinylbenzene matrix. The extracted volatiles were injected into the GC injection port within 20 minutes of extraction.

Gas Chromatograph-Olfactometry Instrumental Method

Gas chromatography-olfactometry analysis was performed on an Agilent Technologies G1530A GC coupled with a flame ionization detector (FID) and a sniffing port (Agilent Technologies, Santa Clara, CA, USA). A Stabilwax® (Crossbond® Carbowax® polyethylene glycol) column (Restek US, Bellefonte, PA, USA; 30 m × 0.32 mm × 1 μm film thickness) was used with helium as a carrier gas.

Samples were injected through the incubated SPME injector at 240° C under a pressure of 10.88 psi with a total flow of 45.1 ml/min using splitless mode. The initial oven temperature was set to 36° C and held for 3 min. The temperature was then increased at a rate of 7° C per minute until reaching a temperature of 240° C and undergoing a holding period of 10 minutes. The total run time was 42.14 minutes. The FID was operated at 280° C using a flow rate of hydrogen of 35 mL/min and a 300 mL/min flow of air.

Samples were simultaneously exuded through a sniff port and analyzed by trained panelists. All data for gas chromatography was processed using ChemStation software (Agilent Technologies, Santa Clara, CA, USA).

Panel Training and Sample Evaluation

Four highly-trained panelists from the Sensory Analysis Center (Manhattan, KS, USA) participated in evaluation. Each panelist had undergone more than 120 hours of general training

in sensory analysis and had at least 2000 hours of descriptive sensory evaluation experience with a wide variety of food, beverage, and non-food items, including nut-related items. Prior to evaluation, an orientation period spanning 3 days took place. During this period, the panelists familiarized themselves with the odor detection method, developed and refined a list of attributes used to identify aromatics, and practiced with sample evaluation. The list of attributes used for evaluation is depicted in Table 5-2.

Samples were evaluated randomly across panelists in a random order, each cultivar under each preparation method being evaluated in triplicate. Evaluation took place over a 5-week period, with each panelist evaluating no more than one sample per day to minimize fatigue.

During evaluation, as aromatic compounds were exuded from the sniff port, if the compound had an olfactory response, panelists selected the corresponding attribute and assigned an intensity to the aroma on a scale of 0 to 100, with 0 being undetected and 100 indicating highest possible intensity. This process was repeated for each detected aromatic and the compiled sensory responses were overlaid atop the corresponding GC chromatogram obtained through ChemStation software (Agilent Technologies, Santa Clara, CA, USA). All sensory data was collected through AromaTrax software (Micron©, Round Rock, TX, USA).

Table 5-2. Gas chromatography-olfactometry list of attributes for aromatic evaluation

List of Attributes			
Acrid	Brown	Burnt	Buttery
Caramel	Cereal	Earthy	Fatty
Floral	Foul	Fruity	Fusel Oil
Grainy	Green	Musty	Nutty
Oily	Overall Sweet	Oxidized	Pecan
Rancid	Resiny	Roasted	Skunky
Stale	Sweet	Woody	Dill
	Metallic	Unknown	

Gas Chromatography-Mass Spectrometry Sample Preparation and Methods

The methods used in GC-MS were very similar to that of GCO. Samples were removed from the freezer the day of evaluation, with approximately 4 pecan halves being ground down using a frozen (-18° C ± 1° C) pestle and mortar. A total of 1.00 g (± 0.05 g) of each sample was weighed into a 10.0 mL screw-cap glass vial with a polytetrafluoroethylene–silicone septum and 0.50 mL water was added. Additionally, 0.01 mL of 100 ppm 1,3-dichlorobenzene in methanol was added as an internal standard to each vial with a final concentration of 6.6 µg kg^{-1}.

To extract volatile compounds, a divinylbenzene– carboxen–polydimethylsiloxane fiber was exposed to the sample headspace for one hour at 36° C in an autosampler (Pal system, model CombiPal, CTC Analytics, Switzerland) at 18 rpm agitation. The extracted volatiles were then injected into the gas chromatograph injection port at 240° C under a pressure of 10.88 psi with a total flow of 45.1 ml/min using splitless mode, using helium as a carrier. The column used was a Stabilwax® (Crossbond® Carbowax® polyethylene glycol) column (Restek US, Bellefonte, PA, USA; 30 m × 0.25 mm × 0.5 µm film thickness). The oven temperature began at

36° C and was held for 3 minutes, then was increased at a rate of 7° C per minute until reaching 240° C. This final temperature was held for 10 minutes for a total run time of 42.14 minutes. This was performed using an identical column to that used for GCO analysis. All GC-MS analysis was performed on a Varian gas chromatograph (GC CP3800; Varian Inc., Walnut Creek, CA, USA), coupled with a Varian mass spectrometer detector (Saturn 2000). Compounds were tentatively identified using a mass spectral library (NIST/EPA/NIH Mass Spectral Library, Version 2.0, 2005) and semi-quantitative data was collected using the internal standard for approximation.

All samples were extracted and analyzed in triplicate. Analysis was split into two days, with 12 samples being evaluated each day.

Data Analysis

Data was analyzed using Microsoft Excel software (Microsoft ©, Redmond, WA, USA) and *X*LStat statistical software (Addinsoft©, New York, NY, USA). In examining GCO data, detection frequency method was used, being advantageous for its simplicity and being repeatable, not requiring high levels of panelist training (Van Ruth 2001). The responses were quantified using nasal impact frequency (NIF), which depicts the percentage of evaluations a particular compound was identified as having olfactory contribution within a sample, indicating which compounds have a larger influence on the odor of the given sample (Pollien et al. 1997).

Results

Identification of Odor-Contributing Compounds

Synthesizing GCO and GC-MS data, a total of 44 compounds were tentatively identified as having olfactory contribution, semi-quantified and listed alongside sensory descriptors in Table 5-3, Table 5-4, and Table 5-5. The descriptors used to define olfactory impacts across identified contributors were *Green, Earthy, Grainy, Nutty, Woody, Buttery, Musty, Unknown, Resiny, Stale, Brown, Caramel, Buttery, Pecan, Overall Sweet, Skunky, Metallic, Roasted, Foul, Rancid, Floral, Sweet, Oxidized,* and *Oily.* Descriptors assigned to chromatographic peaks were obtained and compiled from GCO output collected across all cultivars and preparation methods in triplicate (Appendix J). Tentative peak identification and semi-quantitative data were collected from GC-MS analysis across all cultivars under both preparation methods, also in triplicate (Appendix K). The compounds identified in this study can be broadly categorized into aromatic hydrocarbons, nitriles, ethers, aldehydes, terpenes, acids, alcohols, and pyridines. The total concentration of volatile compounds contributing to aroma was relatively low, ranging from 11.86 μg kg^{-1} in in the raw 'Witte' sample to 32.58 μg kg^{-1} in the raw 'Maramec' sample. Several compounds were found in very low concentrations, including aristolene, 1R-alpha-pinene, cyclobutanol, and 2-butanone, while others had concentrations closer to that of the internal standard, such as acetic acid, ethylbenzene, and 2-methyl-cyclopentanol. Some detected compounds were more unique to certain cultivars, preparation methods, or samples, such as 2,4-dimethylpentanal, (3)-2-decanal, oleic acid, 2,3-butanediol, 9-octadecenoic acid (Z)-[phenylmethyl ester], and 3-furaldehyde. However, the majority of compounds, though only detected by the panelists in a few instances, were present across all samples in varying amounts.

Many of the compounds identified in this study have been previously found in pecans, including pentanal, octanal, pyridine, acetic acid, pentanoic acid, hexanoic acid, heptanol and octanol (Wang and Odell 1972). A similar study performed on black walnuts also found 2,3-butanediol, hexanoic acid methyl ester, and 1,2,3-trimethyl-benzene (Lee et al. 2011). Similar odor descriptors have been reported for the compounds identified in the pecan samples (Table 5-6 through 5-8).

Detection Frequency and Nasal Impact Frequency

As a whole, more compounds were detected as olfactory contributors more frequently in roasted samples than in raw samples (Table 5-9). Although some compounds were unique to certain samples, cultivars, or preparation method, 18 of the 44 identified compounds were detected by panelists in three or more instances. 1R-alpha-pinene in particular was detected with high frequency, being detected at least once in every sample but raw 'Kanza' and raw 'Witte' samples.

Several compounds were detected in 2 out of 3 of evaluations for one sample. For the raw 'Kanza' sample, this was ethylbenzene, for the raw 'Maramec' sample, acetic anhydride, heptanol, and 4-methyl-3-pentanoic acid, for the raw 'Pawnee' sample, 1R-alpha-pinene and propylbenzene, and for the raw 'Witte' sample, 2-decanol and benzaldehyde. For the roasted 'Kanza' sample, 2,5,6-trimethyldecane, 1R-alpha-pinene, ethylbenzene, indane, 2-nonen-1-ol, acetic acid, and benzaldehyde were detected in 66.66% of evaluations. This high detection rate (66.66%) was found in the roasted 'Maramec' sample for o-decyl-hydroxylamine, 1,2,4-trimethylbenzene, 2-nonen-1-ol, 1-octen-3-ol, acetic acid, and benzaldehyde. For the roasted 'Pawnee' sample, 2 of 3 evaluations found 1R-alpha-pinene, 1,3,5-cycloheptatriene, 3-

furaldehyde, and benzaldehyde to be olfactory contributors. Roasted 'Witte' sample had 1,2,4-trimethylbenzene, 2-(9-octadecanyloxy) ethyl ester stearic acid, and linalyl isobutyrate detected in 2 out of 3 sample evaluations. The high detection rate of these compounds for each of these samples suggested that these compounds had a high influence on the sample aromatics.

Sample Variation

The chemical profiles of many of the samples showed similar results; the majority of compounds were not unique to any one cultivar, preparation method, or specific sample. Of the total 44 identified, 29 compounds were found in variable amounts in every sample (Tables 5-3 through 5-5). Some compounds, however, were unique. 2,4-dimethylpentanal was only found in the 'Witte' and 'Maramec' cultivars while (3)-2-decanal and oleic acid were only detected in the roasted 'Maramec' sample. 2,3-butanediol was only present in raw samples, while only roasted samples contained 9-octadecenoic acid (Z)- [phenylmethyl ester] and 3-furaldehyde.

Analysis of variance gave further insight into differences between olfactory chemical profiles, using semi-quantitative data to determine significant differences. Only 11 compounds did not exhibit significant differences between cultivars, preparation methods, or show a significant interaction between cultivar and preparation method at the 5% level of significance (Table 5-10, Table 5-11, and Table 5-12). These were 2-butanone, 2-decanol, 3-ethyl-2-methyl heptane, 1,3,5-cycloheptatriene, toluene, o-xylene, ethylbenzene, propylbenzene, 1,2,3-trimethylbenzene, aristolene, and pentanoic acid. The remaining 33 compounds were significantly different across samples. These differences are illustrated in Table 5-13, Table 5-14, and Table 5-15. For 21 compounds, the interaction between the cultivar effect and preparation method effect was significant. These compounds were cyclobutanol, 2,3-butanediol, 2,5,6-

Trimethyldecane, 1R-alpha-pinene, methyl ester hexanoic acid, 1-ethyl-3-methyl-benzene, 1,2,4-trimethyl-benzene, isopropylbenzene, tetradecane, (Z)-phenylmethyl 9-octadecenoic acid, isopropylbenzene, (1-methylbutyl)-oxirane, indane, 1-octen-3-ol, 3-furaldehyde, ethyl ester 2-(9-octadecanyloxy) stearic acid, benzaldehyde, (3)-2-decanal, oleic acid, ethyl ester 4-hydroxymandelic acid, and (E)-cinnamaldehyde. Many of these compounds were found in significantly higher amounts ($p \leq 0.05$) in the Maramec or Pawnee cultivars, with the roasting process affecting each of the samples in different ways. For those without a significant interaction between factors, 3 compounds were significantly affected by both the cultivar effect and the preparation method. Acetic anhydride was found in high amounts in the 'Maramec' and 'Witte' cultivars and the raw preparation method. O-decyl-hydroxylamine was found in higher amounts in the 'Pawnee' cultivar and the roasted samples. The 'Maramec' cultivar and the roasted samples showed higher amounts of acetic acid.

Six compounds were significantly different among samples due to only the cultivar effect. These were 2,4-dimethyl pentanal, 2-methyl-cyclopentanol, 2,4,5-trimethoxymandelic acid, octenal, 1-methyl-4-(1-methylethyl)-benzene, and heptanol. Of these, 2,4-dimethyl pentanal was not present in 'Kanza' or 'Pawnee' samples, 1-methyl-4-(1-methylethyl)-benzene was seen in higher amounts in the 'Kanza' cultivar, and the other compounds were seen in higher amounts in the 'Maramec' cultivar

Four compounds were only significantly different amongst samples due to preparation method, including 2,3-dioxo-dioxime-o,o'-diacetyl-butanenitrile, 2-nonen-1-ol, linalyl isobutyrate, and benzeneethaneamine. In each of these, higher amounts were exhibited in roasted samples.

Table 5-3. Tentatively identified aromatic compounds (µg kg^{-1}) and associated olfactory attributes, part 1 [ab]

Detected Compound	Time (min)	Kanza Raw	Kanza Roasted	Maramec Raw	Maramec Roasted	Pawnee Raw	Pawnee Roasted	Witte Raw	Witte Roasted	Descriptor
Acetic Anhydride	2.39	0.051 ± 0.027	0.041 ± 0.018	0.145 ± 0.048	0.074 ± 0.036	0.069 ± 0.012	0.038 ± 0.011	0.114 ± 0.012	0.082 ± 0.020	Green
Cyclobutanol	2.65	0.017 ± 0.009	0.017 ± 0.004	0.070 ± 0.040		0.025 ± 0.008	0.045 ± 0.001	0.056 ± 0.012	0.032 ± 0.011	Earthy, Grainy
Butanenitrile, 2,3-dioxo-, dioxime-, o,o'-diacetyl-	3.67	0.286 ± 0.067	0.893 ± 0.587	0.750 ± 0.140	1.097 ± 0.159	0.374 ± 0.050	1.432 ± 0.185	0.519 ± 0.032	0.599 ± 0.450	Nutty, Earthy
2-Butanone	4.89	0.021 ± 0.004	0.020 ± 0.013	0.018 ± 0.003	0.023 ± 0.003	0.024 ± 0.012	0.029 ± 0.006	0.018 ± 0.006	0.025 ± 0.004	Woody
Pentanal, 2,4-dimethyl	5.24			0.038 ± 0.032	0.021 ± 0.004			0.027 ± 0.015	0.023 ± 0.008	Nutty, Earthy
2,3-butanediol	5.60	0.209 ± 0.072		0.334 ± 0.088		0.072 ± 0.037		0.141 ± 0.052		Nutty
Decane, 2,5,6-Trimethyl	5.81		0.110 ± 0.053	0.292 ± 0.091	0.088 ± 0.017		0.193 ± 0.028	0.110 ± 0.016	0.151 ± 0.008	Buttery, Woody
2-Decanol	6.07	0.036 ± 0.014	0.027 ± 0.010	0.045 ± 0.002	0.018 ± 0.003	0.024 ± 0.012	0.027 ± 0.004	0.020 ± 0.001	0.097 ± 0.090	Nutty, Musty
Heptane, 3-ethyl-2-methyl	6.78	0.322 ± 0.095	0.352 ± 0.149	0.295 ± 0.049	0.272 ± 0.046	0.414 ± 0.135	0.413 ± 0.072	0.210 ± 0.039	0.375 ± 0.27	Green, Resiny, Stale, Earthy
1R-alpha-pinene	7.40	0.063 ± 0.001	0.058 ± 0.032	0.196 ± 0.068	0.031 ± 0.008	0.221 ± 0.094	0.061 ± 0.005	0.016 ± 0.005	0.088 ± 0.010	Woody, Brown, Earthy, Buttery, Caramel, Musty
1,3,5-Cycloheptatriene	7.98	2.560 ± 0.574	2.007 ± 1.012	1.605 ± 0.693	1.360 ± 0.020	1.076 ± 0.571	2.132 ± 0.257	1.937 ± 0.353	2.608 ± 0.122	Nutty, Buttery, Pecan, Brown, Overall Sweet
Toluene	8.24	0.128 ± 0.055	0.121 ± 0.062	0.092 ± 0.036	0.065 ± 0.011	0.131 ± 0.103	0.159 ± 0.021	0.136 ± 0.029	0.168 ± 0.018	Overall Sweet, Earthy
Cyclopentanol, 2-methyl-	8.91	0.210 ± 0.050	0.506 ± 0.24	5.541 ± 4.891	4.954 ± 2.677	0.065 ± 0.022	0.729 ± 0.138	0.137 ± 0.023	2.512 ± 0.071	Woody, Green
2,4,5-trimethoxymandelic acid	9.31	0.355 ± 0.141	0.485 ± 0.103	0.601 ± 0.185	0.521 ± 0.039	0.146 ± 0.053	0.312 ± 0.074	0.187 ± 0.046	0.286 ± 0.026	Musty
o-Xylene	9.92	0.954 ± 0.179	0.773 ± 0.393	0.806 ± 0.389	0.607 ± 0.012	0.631 ± 0.212	0.836 ± 0.122	0.888 ± 0.161	1.013 ± 0.073	Earthy, Stale

[a] Olfactory sensory attributes obtained from GCO analysis; elution time identified per GC-MS
[b] Elution time recorded per GC-MS

Table 5-4. Tentatively identified aromatic compounds (µg kg^{-1}) and associated olfactory attributes, part 2 [ab]

Detected Compound	Time (min)	Kanza Raw	Kanza Roasted	Maramec Raw	Maramec Roasted	Pawnee Raw	Pawnee Roasted	Witte Raw	Witte Roasted	Descriptor
Ethylbenzene	10.05	2.407 ± 0.588	2.003 ± 0.987	1.736 ± 0.823	1.973 ± 0.339	1.328 ± 0.486	1.786 ± 0.201	1.746 ± 0.259	2.622 ± 0.083	Woody, Brown, Nutty, Green, Musty
Hydroxylamine, o-decyl-	10.55	0.441 ± 0.001	0.178 ± 0.060	0.145 ± 0.064	0.086 ± 0.036	0.169 ± 0.046	0.421 ± 0.071	0.024 ± 0.008	0.189 ± 0.025	Green, Brown
Hexanoic Acid, methyl ester	10.98	1.128 ± 0.426	0.913 ± 0.416	3.191 ± 0.840	0.772 ± 0.021	0.713 ± 0.522	0.642 ±0.050		1.431 ± 0.104	Brown
Benzene, propyl	11.44	0.227 ± 0.054	0.201 ± 0.093	0.211 ± 0.088	0.161 ± 0.016	0.178 ± 0.051	0.224 ± 0.053	0.177 ± 0.035	0.238 ± 0.020	Green, Floral, Overall Sweet
Benzene, 1-ethyl-3-methyl-	11.73	0.977 ± 0.218	0.792 ± 0.390	1.056 ± 0.353	0.985 ± 0.014	0.218 ± 0.030	1.268 ± 0.116	0.275 ± 0.061	1.747 ±0.079	Musty, Pecan, Overall Sweet
Benzene, 1,2,4-trimethyl	12.21	0.244 ± 0.030	0.178 ± 0.081	0.260 ± 0.840	0.220 ± 0.017	0.576 ± 0.184	0.292 ± 0.044	0.693 ± 0.110	0.267 ± 0.030	Stale, Musty, Skunky
Isopropylbenzene	12.78		0.289 ± 0.131	0.256 ± 0.103	0.229 ± 0.070	0.195 ± 0.105	0.278 ± 0.049	0.162 ± 0.057	0.519 ± 0.019	Caramel, Metallic
Tetradecane	13.00	0.266 ± 0.000	0.156 ± 0.000		0.050 ± 0.000	0.052 ± 0.000		0.128 ± 0.040	0.529 ± 0.166	Buttery
Benzene, 1,2,3-trimethyl-	13.13	1.035 ± 0.214	0.955 ± 0.481	1.083 ± 0.282	0.920 ± 0.138	0.699 ± 0.302	1.286 ± 0.243	0.896 ± 0.160	1.401 ± 0.042	Stale
Octanal	13.33		0.163 ± 0.072	0.804 ± 0.601	1.212 ± 0.798		0.261 ± 0.074		0.397 ± 0.027	Woody
9-octadecenoic acid (Z)-, phenylmethyl ester	13.77		0.098 ± 0.019		0.095 ± 0.026		0.147 ± 0.027		0.171 ± 0.019	Green
Isopropylbenzene	14.47	0.258 ± 0.075	0.260 ± 0.112	0.294 ± 0.074	0.270 ± 0.024	0.194 ± 0.069	0.462 ± 0.038	0.250 ± 0.056	0.423 ± 0.021	Buttery, Musty
Oxirane, (1-methylbutyl)-	14.77	0.180 ± 0.049		4.262 ± 1.756	1.242 ± 0.412	0.121 ± 0.069	0.232 ± 0.115	0.156 ± 0.055	0.444 ± 0.023	Metallic, Musty
Benzene, 1-methyl-4-(1-methylethyl)-	14.87	0.110 ± 0.052	0.167 ±0.075	0.162 ± 0.015	0.102 ± 0.075	0.046 ± 0.014	0.082 ± 0.007	0.051 ± 0.015	0.114 ± 0.018	Woody
Indane	15.09	0.161 ± 0.083	0.112 ± 0.041	0.091 ± 0.028	0.076 ± 0.030	0.128 ± 0.055	0.309 ± 0.022	0.155 ± 0.044	0.157 ± 0.004	Earthy
2-nonen-1-ol	15.47	0.374 ± 0.105	1.046 ± 0.382	0.901 ± 0.519	1.945 ± 0.605	0.380 ± 0.074	1.897 ± 0.712	0.293 ± 0.086	1.905 ± 1.054	Roasted, Buttery, Overall Sweet, Nutty, Pecan
1-octen-3-ol	16.54	0.064 ± 0.000	0.100 ± 0.069	0.254 ± 0.148	0.274 ± 0.126	0.066 ± 0.021	0.365 ± 0.047		0.303 ± 0.059	Grainy, Nutty
Heptanol	16.67			0.420 ± 0.293	0.382 ± 0.223		0.107 ± 0.026		0.131 ± 0.019	Earthy, Nutty

[a] Olfactory sensory attributes obtained from GCO analysis
[b] Elution time recorded per GC-MS

Table 5-5. Tentatively identified aromatic compounds (µg kg^{-1}) and associated olfactory attributes, part 3 [ab]

Detected Compound	Time (min)	Kanza Raw	Kanza Roasted	Maramec Raw	Maramec Roasted	Pawnee Raw	Pawnee Roasted	Witte Raw	Witte Roasted	Descriptor
Acetic Acid	16.91	1.318 ± 0.700	1.609 ± 0.901	2.458 ± 0.074	2.528 ± 0.905	0.916 ± 0.527	2.170 ± 0.274	0.669 ± 0.256	2.093 ± 0.326	Buttery, Skunky, Metallic, Earthy, Rancid
3-Furaldehyde	17.09		0.276 ± 0.136		0.259 ± 0.051		0.615 ± 0.045		0.388 ± 0.033	Pecan, Roasted, Stale
Stearic acid, 2-(9-octadecanyloxy) ethyl ester	17.56	0.101 ± 0.013		0.072 ± 0.039	0.112 ± 0.018	0.033 ± 0.015	0.147 ± 0.037	0.038 ±0.016	0.152 ± 0.048	Pecan, Caramel, Buttery
Benzaldehyde	18.28	0.155 ± 0.039	0.155 ± 0.062	0.155 ± 0.019	0.284 ± 0.077	0.145 ± 0.063	0.404 ± 0.032	0.148 ± 0.034	0.278 ± 0.042	Pecan, Nutty, Roasted, Grainy, Earthy, Stale
Linalyl isobutyrate	18.51	0.144 ± 0.077	0.196 ± 0.118	0.390 ± 0.260	0.534 ± 0.405	0.116 ± 0.031	1.219 ± 0.611	0.103 ± 0.029	0.755 ± 0.352	Nutty, sweet
Aristolene	19.31	0.020 ± 0.010	0.021 ± 0.014	0.045 ± 0.052	0.034 ± 0.031	0.011 ± 0.005	0.431 ± 0.453	0.008 ± 0.001	0.063 ± 0.045	Floral, Green
3-Pentanoic acid, 4-methyl-	19.59	0.395 ± 0.097	0.641 ± 0.070	1.034 ± 0.264	1.412 ± 0.482	0.813 ± 0.322	1.178 ± 0.159	0.039 ± 0.004	0.290 ± 0.057	Brown, Green, Earthy, Sweet, Unknown, Musty, Oily
2-Decanal, (3)-	20.31				0.120 ± 0.069					Earthy
Oleic Acid	22.26				0.047 ± 0.022					Nutty
4-Hydroxymandelic acid, ethyl ester	24.04	0.058 ± 0.009	0.133 ± 0.032	0.074 ± 0.007	0.078 ± 0.012	0.056 ± 0.026	0.060 ± 0.017	0.036 ± 0.025	0.133 ± 0.018	Musty, Green
Pentanoic Acid	24.61			0.286 ± 0.000	0.082 ± 0.001				0.136 ± 0.069	Oxidized
Benzeneethaneamine	24.80	0.308 ± 0.131	0.619 ± 0.241	0.359 ± 0.136	0.384 ± 0.039	0.340 ± 0.123	0.524 ± 0.084	0.271 ± 0.034	0.515 ± 0.147	Caramel, Woody
Cinnamaldehyde, (E)-	27.90	0.045 ± 0.026	0.034 ± 0.008	0.161 ± 0.035	0.024 ± 0.003	0.043 ± 0.030	0.037 ± 0.009	0.069 ± 0.002	0.062 ± 0.037	Floral, Oily
Total Contributing Volatile Compound Concentration		16.225	18.199	32.584	27.269	11.873	25.755	11.861	27.797	

[a] Olfactory sensory attributes obtained from GCO analysis
[b] Elution time recorded per GC-MS

Table 5-6. Reported odor descriptors for identified compounds, part 1

Compound		*The Good Scents Company*	*Flavornet*	Vera et al.	Zeng et al.
Acetic Anhydride	*Green*	Acidic			
Cyclobutanol	*Earthy, Grainy*				
Butanenitrile, 2,3-dioxo-, dioxime-, o,o'-diacetyl-	*Nutty, Earthy*				
2-Butanone	*Woody*	Ethereal, fruity, green			
Pentanal, 2,4-dimethyl	*Nutty, Earthy*	Green, ethereal	Almond, malt, puntent		Woody, fruity
2,3-butanediol	*Nutty*		Fruit, onion		
Decane, 2,5,6-Trimethyl	*Buttery, Woody*				
2-Decanol	*Nutty, Musty*		Fat		
Benzoic acid, 2,4-bis(trimethylsiloxy)-, methyl ester	*Unknown*				
Heptane, 3-ethyl-2-methyl	*Green, Resiny, Stale, Earthy*				
1R-alpha-pinene	*Woody, Brown, Earthy, Buttery, Caramel, Musty*	Herbal**,** terpene	Pine**,** turpentine		
1,3,5-Cycloheptatriene	*Nutty, Buttery, Pecan***,** *Brown, Overall Sweet*				
Toluene	*Overall Sweet, Earthy*	Sweet	Paint	Paint	
Cyclopentanol, 2-methyl-	*Woody, Green*				
2,4,5-trimethoxymandelic acid	*Musty*				
o-Xylene	*Earthy, Stale*	Geranium	Geranium	Sweet	
Ethylbenzene	*Green*				
Hydroxylamine, o-decyl-	*Woody, Brown, Nutty, Green, Musty*				
Hexanoic Acid, methyl ester	*Green, Brown*	Fruity, pineapple, ethereal			Sweet**,** cheesy
Benzene, propyl	*Brown*				
Benzene, 1-ethyl-3-methyl-	*Green, Floral, Overall Sweet*				
Benzene, 1,2,4-trimethyl	*Musty, Pecan, Overall Sweet*	Plastic			
Isopropylbenzene	*Stale, Musty, Skunky*				
Tetradecane	*Caramel, Metallic*	Mild, waxy		Alkane	

Table 5-7. Reported odor descriptors for identified compounds, part 2

Compound	Descriptor from GCO Analysis	*The Good Scents Company*	*Flavornet*	Vera et al. 2012	Zeng et al. 2009
Benzene, 1,2,3-trimethyl-	*Buttery*				
Octanal	*Stale*	Aldehydic, waxy, citrus, orange peel, green, fatty	Fat, soap, lemon, green		Green, citrus-like
9-octadecenoic acid (Z)-, phenylmethyl ester	*Woody*				
Isopropylbenzene	*Green*				
Oxirane, (1-methylbutyl)-	*Buttery, Musty*				
Benzene, 1-methyl-4-(1-methylethyl)-	*Metallic, Musty*	Fresh, citrus, terpene, woody, spice			
Indane	*Woody*				
2-nonen-1-ol	*Earthy*	Sweet, fatty, melon, cucumber, vegetable			
1-octan-3-ol	*Musty, Green, Nutty, Pecan, Unknown, Foul*	Mushroom, earthy, green, oily, fungal, raw, chicken, vegetable, brothy	Mushroom		
Heptanol	*Buttery, Skunky, Metallic, Earthy, Rancid*	Musty, leafy, violet, herbal, green, sweet, woody, fruity, fermented, nutty	Herb		Green, fruity
Acetic Acid	*Pecan, Roasted, Stale*	Sharp, pungent, sour, vinegar	Sour	Sour, vinegar-like	
3-Furaldehyde	*Pecan, Caramel, Buttery*	Sweet, woody, almond, brown, bready, caramellic, burnt			
Stearic acid, 2-(9-octadecanyloxy) ethyl ester	*Floral*	Mild, fatty			
Benzaldehyde	*Nutty, sweet*	Strong, sharp, sweet, bitter, almond, cherry	Almond, burnt sugar		Nutty, bitter

Table 5-8. Reported odor descriptors for identified compounds, part 3

Compound	Descriptor from GCO Analysis	*The Good Scents Company*	*Flavornet*	Vera et al. 2012	Zeng et al. 2009
Linalyl isobutyrate	*Musty*	Light, fruity, lavender, woody, bergamot	Sweet, pear		
Aristolene	*Brown, Green, Earthy, Sweet, Unknown, Musty, Oily*				
3-Pentanoic acid, 4-methyl-	*Earthy*	Animal, sharp, acidic, cheesy, green, fruity, sweaty			
2-Decanal, (3)-	*Stale, Earthy, Green, Unknown, Musty*	Sweet, aldehydic, waxy, orange peel, citrus, floral	Soap, orange peel, tallow		Green, soapy, waxy
Oleic Acid	*Musty, Green*	Fatty, waxy, lard, fried, vegetable	Fat		
4-Hydroxymandelic acid, ethyl ester	*Oxidized*				
Pentanoic Acid	*Caramel, Woody*	Acidic, sweaty, rancid, cheesy, fruity	Sweat		
Benzeneethaneamine	*Floral, Oily*	Ammoniacal, fishy			
Cinnamaldehyde, (E)-	*Buttery*	Sweet, spice, cinnamon, warm	Cinnamon, paint		

Table 5-9. Nasal impact frequency of all samples [a]

Compound	Kanza		Maramec		Pawnee		Witte	
	Raw	Roasted	Raw	Roasted	Raw	Roasted	Raw	Roasted
Acetic Anhydride			2*					
Cyclobutanol			1				1	
Butanenitrile, 2,3-dioxo-, dioxime-, o,o'-diacetyl-		1		1				
2-Butanone								1
Pentanal, 2,4-dimethyl								1
2,3-butanediol			1					1
Decane, 2,5,6-Trimethyl		2*						
2-Decanol			1				2*	
Heptane, 3-ethyl-2-methyl	1	1	1	1		1		
1R-alpha-pinene		2*	1	1	2*	2*		1
1,3,5-Cycloheptatriene	1		1	1		2*	1	1
Toluene			1				1	
Cyclopentanol, 2-methyl-		1				1		
2,4,5-trimethoxymandelic acid			1					
o-Xylene		1						1
Ethylbenzene	2*	2*	1	1	1			1
Hydroxylamine, o-decyl-			1	2*				1
Hexanoic Acid, methyl ester				1				
Benzene, propyl		1	1		2*			
Benzene, 1-ethyl-3-methyl-				1		1		1
Benzene, 1,2,4-trimethyl		1		2*			1	2*
Isopropylbenzene		1					1	
Tetradecane								1
Benzene, 1,2,3-trimethyl-			1					
Octanal				1				
9-octadecenoic acid (Z)-, phenylmethyl ester								1
Isopropylbenzene		1					1	
Oxirane, (1-methylbutyl)-				1				1
Benzene, 1-methyl-4-(1-methylethyl)-						1		
Indane		2*	1					
2-nonen-1-ol		2*		2*		1		1
1-octen-3-ol		1		2*				
Heptanol			2*	1				
Acetic Acid	1	2*		2*	1			
3-Furaldehyde						2*		1
Stearic acid, 2-(9-octadecanyloxy) ethyl ester						1		2*
Benzaldehyde		2*	1	2*		2*	2*	
Linalyl isobutyrate								2*
Aristolene						1		
3-Pentanoic acid, 4-methyl-	1	1	2*	1		1	1	1
2-Decanal, (3)-				1				
Oleic Acid				1				
4-Hydroxymandelic acid, ethyl ester						1		
Pentanoic Acid				1				
Benzeneethaneamine	1		1					
Cinnamaldehyde, (E)-			1	1				

[a] Number of times detected out of three replicates

[b] Total observations (n= 24)

* Compound detected in at least 66.66% of olfactory evaluations

Table 5-10. P-values of individual factors and factor interactions from ANOVA across detected compounds, part 1 [a]

Detected Compound	Cultivar	Treatment	Cultivar*Treatment
Acetic Anhydride	**0.008**[b]	**0.014** [b]	0.447
Cyclobutanol	0.133	**0.028** [b]	**0.006** [b]
Butanenitrile, 2,3-dioxo-, dioxime-, o,o'-diacetyl-	0.166	**0.002** [b]	0.133
2-Butanone	0.504	0.240	0.824
Pentanal, 2,4-dimethyl	**0.003**[b]	0.358	0.693
2,3-butanediol	**0.000**[b]	**< 0.0001** [b]	**0.004** [b]
Decane, 2,5,6-Trimethyl	**0.003**[b]	0.111	**< 0.0001** [b]
2-Decanol	0.658	0.610	0.261
Heptane, 3-ethyl-2-methyl	0.220	0.363	0.491
1R-alpha-pinene	**0.028** [b]	**0.009** [b]	**0.003** [b]
1,3,5-Cycloheptatriene	0.066	0.361	0.115
Toluene	0.173	0.791	0.787
Cyclopentanol, 2-methyl-	**0.013** [b]	0.513	0.777
2,4,5-trimethoxymandelic acid	**0.001** [b]	0.155	0.387
o-Xylene	0.278	0.900	0.355
Ethylbenzene	0.307	0.293	0.422

[a] Significance taken at $p \leq 0.05$

[b] Significant factor or interaction for given attribute

Table 5-11. P-values of individual factors and factor interactions from ANOVA across detected compounds, part 2 [a]

Detected Compound	Cultivar	Treatment	Cultivar*Treatment
Hydroxylamine, o-decyl-	**0.038** [b]	**0.050** [b]	0.117
Hexanoic Acid, methyl ester	**0.004** [b]	0.354	**0.000** [b]
Benzene, propyl	0.842	0.742	0.283
Benzene, 1-ethyl-3-methyl-	0.236	**< 0.0001** [b]	**< 0.0001** [b]
Benzene, 1,2,4-trimethyl	**0.000** [b]	**0.000** [b]	**0.012** [b]
Isopropylbenzene	**0.002** [b]	**< 0.0001** [b]	**0.001** [b]
Tetradecane	**< 0.0001** [b]	**0.024** [b]	**0.002** [b]
Benzene, 1,2,3-trimethyl-	0.733	0.089	0.078
Octanal	**0.010** [b]	0.118	0.958
9-octadecenoic acid (Z)-, phenylmethyl ester	**0.016** [b]	**< 0.0001** [b]	**0.016** [b]
Isopropylbenzene	0.184	**0.002** [b]	**0.005** [b]
Oxirane, (1-methylbutyl)-	**0.000** [b]	0.050	**0.009** [b]
Benzene, 1-methyl-4-(1-methylethyl)-	**0.023** [b]	0.189	0.084
Indane	**0.004** [b]	0.182	**0.008** [b]
2-nonen-1-ol	0.292	**0.000** [b]	0.534
1-octen-3-ol	**0.012** [b]	**0.000** [b]	**0.037** [b]

[a] Significance taken at $p \leq 0.05$

[b] Significant factor or interaction for given attribute

Table 5-12. P-values of individual factors and factor interactions from ANOVA across detected compounds, part 3 [a]

Detected Compound	Cultivar	Treatment	Cultivar*Treatment
Heptanol	**0.004** [b]	0.475	0.784
Acetic Acid	**0.012** [b]	**0.005** [b]	0.126
3-Furaldehyde	**0.001** [b]	**< 0.0001** [b]	**0.001** [b]
Stearic acid, 2-(9-octadecanyloxy) ethyl ester	**0.025** [b]	**0.003** [b]	**0.001** [b]
Benzaldehyde	**0.034** [b]	**0.000** [b]	**0.020** [b]
Linalyl isobutyrate	0.181	**0.006** [b]	0.088
Aristolene	0.302	0.179	0.245
3-Pentanoic acid, 4-methyl-	**< 0.0001** [b]	**0.011** [b]	0.950
2-Decanal, (3)-	**0.007** [b]	**0.027** [b]	**0.007** [b]
Oleic Acid	**0.001** [b]	**0.010** [b]	**0.001** [b]
4-Hydroxymandelic acid, ethyl ester	0.071	**0.000** [b]	**0.004** [b]
Pentanoic Acid	0.055	0.251	0.181
Benzeneethaneamine	0.770	**0.013** [b]	0.496
Cinnamaldehyde, (E)-	**0.012** [b]	**0.003** [b]	**0.002** [b]

[a] Significance taken at $p \leq 0.05$

[b] Significant factor or interaction for given attribute

Table 5-13. Significant differences between samples across detected compounds, part 1[a]

Detected Compound	*Descriptor [b]*	*Cultivar*	*Treatment*	*Cultivar*Treatment*
Acetic Anhydride	Green	Maramec & Witte significantly higher than Pawnee and Kanza	Raw samples significantly higher than roasted samples	
Cyclobutanol	Earthy, Grainy			Raw Maramec sample significantly higher than raw Pawnee & Kanza and roasted Witte, Kanza, & Maramec
Butanenitrile, 2,3-dioxo-, dioxime-, o,o'-diacetyl-	Nutty, Earthy		Roasted samples significantly higher than raw samples	
2-Butanone	Woody			
Pentanal, 2,4-dimethyl	Nutty, Earthy	Not present in Kanza or Pawnee samples		
2,3-butanediol	Nutty			Raw Maramec sample significantly higher than all other samples; not present in roasted samples
Decane, 2,5,6-Trimethyl	Buttery, Woody			Raw Maramec sample significantly higher than all other samples; not present in raw Kanza or Pawnee samples
2-Decanol	Nutty, Musty			
Heptane, 3-ethyl-2-methyl	Green, Resiny, Stale, Earthy			
1R-alpha-pinene	Woody, Brown, Earthy, Buttery, Caramel, Musty			Raw Pawnee & Maramec samples significantly higher than all other samples
1,3,5-Cycloheptatriene	Nutty, Buttery, Pecan, Brown, Overall Sweet			
Toluene	Overall Sweet, Earthy			
Cyclopentanol, 2-methyl-	Woody, Green	Maramec significantly higher than all other cultivars		
2,4,5-trimethoxymandelic acid	Musty	Maramec & Kanza significantly higher than Witte & Pawnee		
o-Xylene	Earthy, Stale			
Ethylbenzene	Green			

[a] Significance taken at $p \leq 0.05$

[b] Olfactory sensory attributes obtained from GCO analysis

Table 5-14. Significant differences between samples across detected compounds, part 2[a]

Detected Compound	*Descriptor* [b]	*Cultivar*	*Treatment*	*Cultivar*Treatment*
Hydroxylamine, o-decyl-	Woody, Brown, Nutty, Green, Musty	Pawnee significantly higher than Maramec & Witte	Roasted samples significantly higher than raw samples	
Hexanoic Acid, methyl ester	Green, Brown			Raw Maramec sample significanly higher than all other samples; not present in raw Witte sample
Benzene, propyl	Brown			
Benzene, 1-ethyl-3-methyl-	Green, Floral, Overall Sweet			Roasted Witte sample significantly higher than all other samples; raw Witte & Pawnee samples significantly lower than all other samples
Benzene, 1,2,4-trimethyl	Musty, Pecan, Overall Sweet			Raw Witte & Pawnee samples significantly higher than all other samples
Isopropylbenzene	Stale, Musty, Skunky			Roasted Witte sample significantly higher than all other samples; raw Kanza sample significantly lower than all other samples
Tetradecane	Caramel, Metallic			Roasted Witte sample significantly higher than all other samples; raw Kanza sample significantly lower than all other samples; not present in raw Maramec or roasted Pawnee samples
Benzene, 1,2,3-trimethyl-	Buttery			
Octanal	Stale	Maramec significantly higher than all other cultivars		
9-octadecenoic acid (Z)-, phenylmethyl ester	Woody			Not present in raw samples; roasted Witte & Pawnee samples significantly higher than roasted Maramec & Kanza samples
Isopropylbenzene	Green			Roasted Pawnee & Witte samples significantly higher than all other samples
Oxirane, (1-methylbutyl)-	Buttery, Musty			Roasted Maramec sample significantly higher than all other samples; Maramec cultivar higher than all other cultivars
Benzene, 1-methyl-4-(1-methylethyl)-	Metallic, Musty	Kanza significantly higher than Witte & Pawnee		
Indane	Woody			Roasted Pawnee sample significantly higher than all other samples
2-nonen-1-ol	Earthy		Roasted samples significantly higher than raw samples	
1-octen-3-ol	Musty, Green, Nutty, Pecan, Unknown, Foul			Roasted Pawnee, Witte, & Maramec samples significantly higher than raw Pawnee, Kanza, & Witte samples

[a] Significance taken at $p \leq 0.05$

[b] Olfactory sensory attributes obtained from GCO analysis

Table 5-15. Significant differences between samples across detected compounds, part 3[a]

Detected Compound	*Descriptor* [b]	*Cultivar*	*Treatment*	*Cultivar*Treatment*
Heptanol	Buttery, Skunky, Metallic, Earthy, Rancid	Maramec significantly higher than all other cultivars; not found in Pawnee		
Acetic Acid	Pecan, Roasted, Stale	Maramec significantly higher than all other cultivars	Roasted samples significantly higher than raw samples	
3-Furaldehyde	Pecan, Caramel, Buttery			Not present in raw samples; roasted Pawnee sample significantly higher than all other samples
Stearic acid, 2-(9-octadecanyloxy) ethyl ester	Floral			Roasted Pawnee & Witte samples significantly higher than all other samples
Benzaldehyde	Nutty, sweet			Roasted Pawnee sample significantly higher than all other samples; roasted samples generally higher than raw samples
Linalyl isobutyrate	Musty		Roasted samples significantly higher than raw samples	
Aristolene	Brown, Green, Earthy, Sweet, Unknown, Musty, Oily			
3-Pentanoic acid, 4-methyl-	Earthy	Maramec & Pawnee significantly higher than Witte & Kanza; Witte significantly lower than all other cultivars	Roasted samples significantly higher than raw samples	
2-Decanal, (3)-	Stale, Earthy, Green, Unknown, Musty			Only present in roasted Maramec sample
Oleic Acid	Musty, Green			Only found in roasted Maramec sample
4-Hydroxymandelic acid, ethyl ester	Oxidized			Roasted Witte & Kanza samples significantly higher than all other samples
Pentanoic Acid	Caramel, Woody			
Benzeneethaneamine	Floral, Oily		Roasted samples significantly higher than raw	
Cinnamaldehyde, (E)-	Buttery			Raw Maramec sample significantly higher than all other samples

[a] Significance taken at $p \leq 0.05$
[b] Olfactory sensory attributes obtained from GCO analysis

Discussion

Due to the magnitude of volatized chemical compounds detected by the system, the occasional incomplete peak separation, and the variability of human processing time between volatile compound detection and olfactory assignment, identification of compounds with olfactory contribution proved difficult. Often, raw, unpurified products such as nuts produce intricate chemical profiles that are difficult to analyze. In this study, one compound in particular, detected as having olfactory contribution in multiple samples, had a retention time very similar to that of the internal standard and did not have consistent and identifiable peak separation from the standard. One similar study of wine (Cullere et al. 2004) encountered this problem with a highly complex chromatogram, making identification of individual compounds difficult. Nonetheless, a tentative profile of several compounds and their odor characteristics was constructed.

Pecan flavor research has been limited in the past with much of the research performed under lock and key as industry secrets. One study performed by Wang and Odell (1972) identified 41 volatile compounds characteristic of roasted pecans, discussing possible contributions to pecan flavor. However, gas chromatography-olfactometry research was not available to confirm olfactometry contributions. Raw and roasted pecan chemical flavor profiles have not been explored in a research setting until now.

Some trends could be drawn for each of the cultivars and preparation methods. The roasted samples tended to have higher approximate concentrations of compounds attributed with caramel, buttery, nutty, brown, musty, oily, pecan, roasted, and earthy type sensory descriptors while the raw samples generally had higher alignment with compounds associated with green, musty, and oily type attributes. The ‘Maramec’ cultivar showed a chemical profile that aligned

with the descriptors *Green*, *Earthy*, *Grainy*, *Woody*, *Musty*, and *Stale*, generally more undesirable characteristics in a pecan. The 'Kanza' cultivar generally did not show outlying chemical concentrations, however the 'Witte' cultivar showed larger concentrations of compounds that characterized a mix of desirable and undesirable attributes with green, floral, overall sweet, musty, stale, and woody type aromatics. The 'Pawnee' cultivar generally had a chemical profile that could be considered favorable, characterizing sweet, nutty, floral, caramel, buttery, woody, green, and musty type aromatics. This was especially true for the roasted 'Pawnee' sample, showing significantly higher values of 3-furanaldehyde and benzaldehyde, which have been attributed with nutty, sweet, pecan, caramel, and buttery type attributes. The threshold for these compounds and the interactions between them, however, requires further research, and these conclusions based on semi-quantitative data were limited.

The higher detection rate of olfactory contributors in roasted samples than raw samples suggested that the aromatics of pecans are more developed by the roasting process. However, the compounds identified in roasted samples were present in nearly all of the raw counterparts in similar amounts. This indicated that those compounds unique to each preparation method (2,3-butanediol for raw samples; 9-octadecenoic acid (Z)-[phenylmethyl ester] and 3-furaldehyde for roasted samples) as well as those significantly higher in each preparation method (acetic anhydride in raw samples; 2,3-dioxo-dioxime-o,o'-diacetyl-butanenitrile, o-decylhydroxylamine, 2-nonen-1-ol, acetic acid, linalyl isobutyrate, 4-methyl-3-pentanoic acid, and benzeneethaneamine in roasted samples) were largely responsible for explaining the flavor development during the roasting process. Nasal impact frequency analysis further showed acetic anhydride (*Green)* identified as an olfactory contributor only in raw samples while 9-octadecenoic acid (Z)-[phenylmethyl ester], 2,3-dioxo-dioxime-o,o'-diacetyl-butanenitrile, and

linalyl isobutyrate, collectively described as *Woody*, *Floral*, *Green*, *Nutty*, *Earthy*, and *Musty*, were only identified in roasted samples. The unique identification within one preparation method was especially true for 3-furaldehyde and 2-nonen-1-ol, described as *Pecan*, *Caramel*, and *Buttery* and as *Earthy* respectively, with these compounds identified frequently in roasted samples but not in raw samples. These two compounds may have the largest impact on roasted pecan flavor.

Because only 4 cultivars of pecans from a single growing season, all from one growing field, were utilized in this study, application of these results may be limited to a small set of pecan samples. The inclusion of additional cultivars, inside and outside of the Midwest growing region, across growing seasons may lead to more general conclusions about pecan chemical profiles. Additional research should further include additional replicates and references for aromatic attributes should be considered to minimize the effects of bias and human error. Sampling error may have come into play, with sample homogenization being limited to mortar and pestle. Future research should investigate the correlation between chemical concentrations and corresponding attribute intensities to examine the impact of individual compounds on flavor intensity.

Conclusions

While the chemical profiles of olfactory contributing compounds were similar between cultivars under different preparation methods, generally being comprised of the same compounds at varying levels, many differences were present that gave samples unique chemical, and subsequent olfactory, profiles. 2,4-dimethylpentanal, which characterized nutty and earthy type attributes, was present in only ‘Witte’ and ‘Maramec’ cultivars. Only the raw samples contained

2,3-butanediol, which was described as *Nutty,* while only roasted samples contained 9-octadecenoic acid (Z)- [phenylmethyl ester] (*Woody*) and 3-furaldehyde (*Pecan, Caramel,* and *Buttery*). Finally, only the roasted 'Maramec' sample had a presence of (3)-2-decanal, which was described as *Stale, Earthy, Green, Unknown,* and *Musty*, and oleic acid, which was attributed with musty and green type descriptors. These unique components were character notes that helped define each sample.

A total of 44 compounds were identified as olfactory contributors, 29 of which were found across all samples. One high aromatic contributor was 1R-alpha-pinene, associated with woody, brown, earthy, buttery, caramel, and musty type characters, which was seen in every sample and detected in all but raw 'Kanza' and raw 'Witte' samples. This compound, though only detected at between 0.016 and 0.221 μg kg^{-1}, likely has the most influence on pecan character notes across the samples.

Significant differences were found between cultivars and/or preparation methods or there was a significant interaction between cultivar and preparation method for all of the compounds detected but 11. These significant differences revealed that the 'Maramec' cultivar generally had higher concentrations of compounds associated with undesirable attributes, while the 'Pawnee' cultivar, in particular the roasted 'Pawnee' sample, had a chemical profile that aligns with more desirable olfactory attributes. The association with compounds that characterize desirable aromatic attributes may make the roasted 'Pawnee' sample a good standard for the development of pecan flavors.

References

Cullere, L., Escudero, A., Cacho, J., & Ferreira, V. (2004) Gas chromatography-olfactometry and chemical quantitative study of the aroma of six premium quality Spanish aged red wines. *Journal of Agricultural and Food Chemistry,* 52(6): 1653-1660

Delahunty, C., Eyres, & G., Dufour, J. (2006). Gas chromatography-olfactometry. *Journal of Separation Science,* 29(14): 2107-2125.

Flavornet. Available online: www.flavornet.org (accessed July 5, 2016).

Hammar, H. & Hunter, J. (1946). Some physical and chemical changes in the composition of pecan nuts during kernel filling. *Plant Physiology,* 21(4): 476–491.

Lee, J., Vazquez-Araujo, L., Adhikari, K., Warmund, M., & Elmore, J., (2011). Volatile compounds in light, medium, and dark black walnut and their influence on the sensory aromatic profile. *Journal of Food Science,* 76(2): C199-C204.

Malik, N., Perez, J., Lombardini, L., Cornacchia, R., Cisneros-Zevallos, L., & Braford, J. (2009). Phenolic compounds and fatty acid composition of organic and conventional grown pecan kernels. *Journal of the Science of Food and Agriculture,* 89(13): 2207-2213.

Nelson, S., Lawrence, K., Kandala, C. (1992). Sensing moisture in peanut and pecan kernels by RF impedance measurements. *Journal of Microwave Power and Electromagnetic Energy,* 27:171–4.

Pollien, P., Ott, A., Montigon, F., Baumgartner, M., Munoz-Box, R., and Chaintreau, A. (1997). Hyphenated headspace-gas chromatography-sniffing technique: screening of impact odorants and quantitative aromagram comparisons. *Journal of Agricultural Food Chemistry,* 45(7): 2630-2637.

Reid, W. (2011). Storage conditions for black walnut kernels. *Personal Communication.* August 2011.

Rudolph, C., Odell, G., Hinrichs, H., Thomson, H., & Kays, S. (1992). Chemical changes in pecan oils during oxidation. *Journal of Food Quality*, 15(4): 279-293.

The Good Scents Company. Available online: www.thegoodscentscompany.com (accessed July 5, 2016).

Van Ruth, S. (2001). Methods for gas chromatography-olfactometry: a review. *Biomolecular Engineering*, 17(4): 121-128.

Vene, K., Seisonen, S., Koppel, K., Leitner, E., & Palmee, T. (2013) A method for GC-Olfactometry panel training. *Chemical Perceptions*, 6: 179-189.

Vera, P., Uliaque, B., Canellas, E., Escudero, A., & Nerin, C. (2012). Identification and quantification of odorous compounds from adhesives used in food packaging materials by headspace solid phase extraction and headspace solid phase microextraction coupled to gas chromatography–olfactometry–mass spectrometry. *Analytica Chimica Acta*, 7(45): 53-63.

Wang, P. & Odell, G. (1972). Characterization of some volatile constituents of roasted pecans. *Journal of Agricultural and Food Chemistry,* 20(2): 206-210.

Zellner, B., Dugo, P., Dugo, G., & Mondello, L. (2008). Gas chromatography-olfactometry in food flavour analysis. *Journal of Chromatography A,* 1186(1): 123-143.

Zeng, Z., Zhang, H., Zhang, T., Tamogami, S., & Chen, J. (2009). Analysis of flavor volatiles of glutinous rice during cooking by combined gas chromatography–mass spectrometry with modified headspace solid-phase microextraction method. *Journal of Food Composition and Analysis*, 24(4): 347-353.

Appendix A - SAS® Codes

SAS® Codes for Analyzing Descriptive Sensory Data

```
ods rtf;
data (data name);
input cultivar$ treatment$      Rep$    Panelist$       atr1 atr2 atr3 atr4 atr5 atr6 atr7 atr8 atr9
atr10 atr11 atr12 atr13 atr14 atr15 atr16 atr17 atr18 atr19 atr20 atr21;
datalines;
(input raw data here)
;
proc sort;
by cultivar treatment;
run;

proc print; run;

ods rtf;
proc means;
var     atr1 atr2 atr3 atr4 atr5 atr6 atr7 atr8 atr9 atr10 atr11 atr12 atr13 atr14 atr15 atr16 atr17
atr18 atr19 atr20 atr21;
by cultivar treatment;
run;

proc glimmix;
        class cultivar treatment rep panelist;
        model atr#  = cultivar treatment cultivar*treatment/ddfm=sat;
        random rep panelist;
        lsmeans cultivar treatment cultivar*treatment/ lines;
        run;

proc mixed;
```

```
class Cultivar Treatment rep panelist;
model atr# = cultivar  treatment       cultivar*treatment/ddfm=sat;
random          rep panelist;
lsmeans cultivar treatment cultivar*treatment;
run;

symbol1 interpol=STD1MJ v=none l=1;
symbol2 interpol=STD1MJ v=none l=2;
proc gplot;
plot atr#*cultivar=treatment;
run;

ods rtf close; quit;
```

Notes

1) The PROC GLIMMIX and PROC MIXED procedures were repeated for each attribute resulting in 21 individual codes.
2) "atr#" is replaced by "atr1" , "atr 2", …, "atrXX" for each of the attributes.

SAS® Codes for Consumer Study

```
ods rtf;
data (data name);
input consumer$ cultivar$ treatment$          atr1 atr2 atr3 atr4 atr5;
datalines;
(input raw data here)
;
proc sort;
by cultivar treatment;
run;

proc print; run;

ods rtf;
proc means;
var      atr1 atr2 atr3 atr4 atr5;
by cultivar treatment;
run;

proc glimmix;
        class consumer cultivar treatment;
        model atr#  = cultivar treatment cultivar*treatment/ddfm=sat;
        random consumer;
        lsmeans cultivar treatment cultivar*treatment/ lines;
        run;

proc mixed;
        class consumer cultivar treatment;
        model atr# = cultivar   treatment        cultivar*treatment/ddfm=sat;
        random          consumer;
        lsmeans cultivar treatment cultivar*treatment;
```

run;

symbol1 interpol=STD1MJ v=none l=**1**;
symbol2 interpol=STD1MJ v=none l=**2**;
proc gplot;
plot atr#*cultivar=treatment;
run;

ods rtf close; **quit**;

Notes

1) The PROC GLIMMIX and PROC MIXED procedures were repeated for each liking and intensity evaluation, resulting in 5 individual codes.
2) “atr#” is replaced by “atr1” , “atr 2”, …, “atrXX” for each of the liking and intensity evaluation.
3) The code was repeated for each consumer segment (total 3 times).

SAS® Codes for Fatty Acid Profile

```
ods rtf;
data (data name);
input cultivar$ treatment$ rep$          atr1 atr2 atr3 atr4 atr5;
datalines;
(input raw data here)
;
proc sort;
by cultivar treatment;
run;

proc print; run;

ods rtf;
proc means;
var       atr1 atr2 atr3 atr4 atr5;
by cultivar treatment;
run;

proc glimmix;
        class cultivar treatment rep;
        model atr#  = cultivar treatment cultivar*treatment/ddfm=sat;
        random rep;
        lsmeans cultivar treatment cultivar*treatment/ lines;
        run;

proc mixed;
        class cultivar treatment rep;
        model atr# = cultivar   treatment         cultivar*treatment/ddfm=sat;
        random            rep;
        lsmeans cultivar treatment cultivar*treatment;
```

run;

symbol1 interpol=STD1MJ v=none l=**1**;
symbol2 interpol=STD1MJ v=none l=**2**;
proc gplot;
plot atr#*cultivar=treatment;
run;

ods rtf close; **quit**;

Notes

1) The PROC GLIMMIX and PROC MIXED procedures were repeated for each fatty acid, resulting in 5 individual codes.
2) “atr#” is replaced by “atr1” , “atr 2”, …, “atrXX” for each of the fatty acids.

Appendix B - Descriptive Sensory Analysis Codes and Serving Order

				Replicate 1			Replicate 2		
Serving Time		Orientation 1	Orientation 2	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
11:20	Serving 1	458	606	58	765	81	335	454	102
11:35	Serving 2	604	223	402	759	152	862	723	388
11:50	Serving 3	897	342	192	602	357	202	78	451
12:05	Serving 4	556	674	265	452	905	409	98	514
12:20	Serving 5	617	839	976	439	654	806	185	238
12:35	Serving 6	394	951	620	478	347	70	20	973
12:50	Serving 7			303	882	279	14	595	986
1:05	Serving 8			200	8	249	675	407	990

				Replicate 1			Replicate 2		
Pecans	**Orientation Raw**	**Orientation Roasted**	**Orientation Candied**	**Raw**	**Roasted**	**Candied**	**Raw 2**	**Roasted 2**	**Candied 2**
Giles	606			765	452	905	14	454	451
Konza	342		604	249	81	620	70	185	973
Major		458		58	402	759	335	723	338
Witte			839	303	654	347	675	407	514
Chetopa		394		152	192	439	862	78	102
Lakota	556		223	478	200	8	202	595	990
Maramec			617	357	279	602	806	20	986
Pawnee	897	674	951	976	882	265	409	98	388

Appendix C - Pecan Definition and Reference Sheets for Descriptive Analysis

Cleanout: Crackers and Deionized Water
Panelists: Use 1 piece for evaluation. Please swallow at least one sample during evaluation.

FLAVOR

Pecan ID: The aromatics commonly associated with pecans which include musty/earthy, piney, woody, brown, sweet, buttery, oily, astringent, and slightly acrid aromatics. Other aromatics may include musty/dusty, floral/fruity, and/or fruity-dark.
Reference: Ground Pecan pieces = 7.0 (flavor)
Preparation: Measure out 1 tbsp. of various cultivars into a food processor and blend for 30 seconds. Pour into 1 oz. cups.

Overall Nutty: A measurement that reflects the total of the nutty characteristics and the degree to which these characteristics fit together. These nutty characteristics are: sweet, oily, light brown, slightly musty and/or buttery, earthy, woody, astringent, bitter, etc.
Examples: nuts, wheat germ, certain whole grains.

Reference: Gold Medal Whole Wheat Flour = 4.5 (flavor)
Kretschmer Wheat Germ = 7.5 (flavor)
Mixture of Diamond Slivered Almonds and Kroger Chopped Hazelnuts = 7.5 (flavor)
Diamond Shelled Walnuts = 8.0 (flavor)
Diamond Pecan Halves = 9.0 (flavor)
Preparation: Puree the almonds and hazelnuts separately in blenders for 45 seconds on high speed. Combine equal amounts of the chopped nuts. Serve in individual 1 oz. cups. Serve pecans and walnuts in 1 oz cups.

Nutty-Woody: A nutty aromatic characterized by the presence of woodiness, increased musty/dustiness, brown, astringent and bitter.
Reference: Diamond Pecan Halves = 7.5 (flavor)
Diamond Shelled Walnuts = 7.5 (flavor)
Preparation: Serve pecans and walnuts in 1 oz cups.

Nutty-Grain-like: A nutty aromatic characterized by the presence of a grainy aromatic, increased musty/dustiness and brown.
Reference: Gold Medal Whole Wheat Flour = 4.5 (flavor)
Kretschmer Wheat Germ = 7.5 (flavor)

Nutty-Buttery: A nutty aromatic characterized by a buttery impression, and/or increased fatty aromatics and musty/earthy character.
Reference: HyVee Dry Roasted and Salted Macadamia Nuts = 5.0 (flavor)
Preparation: Serve macadamia nuts in a 1 oz cup.

Brown: A rich, full aromatic impressions always characterized with some degree of darkness generally associated with attributes (i.e. toasted, nutty, sweet).
Reference: Bush's Best Pinto Beans (Canned) = 5.0 (flavor)
Kretschmer Wheat Germ = 7.5 (flavor)
Preparation: Drain beans and rinse with de-ionized water. Serve in 1 oz. cups.

Caramelized: A round, full-bodied, medium brown aromatic.
Reference: Caramelized Sucrose dissolved in water (diluted by half) = 3.0 (f)
Caramelized Sucrose dissolved in water = 6.0 (f)
Preparation: Dissolve 5g and 10g caramelized sucrose in 80g water.

Acrid: The sharp/acrid, charred flavor note associated with something over baked or excessively browned in oil.
Reference: Alf's Natural Nutrition Puffed Red Wheat Cereal = 3.0 (flavor)

Burnt: A dark, brown, somewhat sharp, over-baked grain aromatic.
Reference: Alf's Natural Nutrition Puffed Red Wheat Cereal = 4.0 (flavor)

Musty/Earthy: Humus-like aromatics that may or may not include damp soil, decaying vegetation, or cellar like characteristics.
Reference: Bush's Best Pinto Beans (Canned) = 5.0 (flavor)
Sliced Button mushroom = 10.5 (f)
Serve chopped mushroom in 1 oz cups.

Woody: The sweet, brown, musty, dark, dry aromatics associated with the bark of a tree.
Reference: Diamond Shelled Walnuts = 4.0 (flavor)
Preparation: Serve walnuts in a 1 oz cup.

Roasted: Dark brown impression characteristic of products cooked to a high temperature by dry heat. Does not include bitter or burnt notes.

Reference: 'Planters Dry Roasted Unsalted Peanuts= 5.0 (f)

Overall Sweet: An aromatic associated with the impression of sweet substances.
Reference: Post Shredded Wheat = 1.5 (flavor)
General Mills Wheaties = 3.0 (flavor)
Lorna Doone Cookie = 4.5 (flavor)

Oily: The light aromatics associated with vegetable oil such as corn or soybean oil.
Reference: Kroger Slivered and Blanched Almonds = 4.0 (flavor)
HyVee Dry Roasted and Salted Macadamia Nuts = 9.0 (flavor)
Preparation: Serve macadamia nuts in a 1 oz cup.

Rancid: An aromatic commonly associated with oxidized fat and oils.
Reference: Wesson Vegetable Oil = 2.5
Preparation: Microwave 1/3 cup of oil on high power for 2 1/2 minutes. Let cool and serve in individual covered cups.

Oxidized: The aromatic associated with aged or highly used oil and fat.
Reference: Microwave Oven Heated Wesson Vegetable Oil = 6.0
Preparation: Add 300ml of oil from a newly purchased and opened bottle of Wesson Vegetable Oil to a 1000ml glass beaker. Heat in the microwave oven on high power for 3 minutes. Remove from microwave and let sit at room temperature to cool for approximately 25 minutes. Then heat another 3 minutes, let cool another 25 minutes, and heat for one additional 3 minute interval. Let beaker sit on counter uncovered overnight. Serve in 1 oz cup.

Astringent: A feeling of a puckering or a tingling sensation on the surface and/or edge of the tongue and mouth.
Reference: 0.030% Alum solution = 1.5
0.050% Alum solution = 2.5
0.075% Alum solution = 3.5
0.10% Alum solution = 5.0

Bitter: A fundamental taste factor of which caffeine is typical.
Reference: 0.010% Caffeine Solution = 2.0
0.020% Caffeine Solution = 3.5
0.035% Caffeine Solution = 5.0

Sour: A fundamental taste factor of which citric acid is typical.
Reference: 0.015% Citric Acid Solution = 1.5

0.025% Citric Acid Solution = 2.5

Sweet: A fundamental taste factor of which sucrose is typical.
Reference: 1% Sucrose Solution = 1.0
2% Sucrose Solution = 2.0
4% Sucrose Solution = 4.0
6% Sucrose Solution = 6.0

Salt: A fundamental taste factor of which sodium chloride is typical.
Reference: 0.15% Sodium Chloride Solution = 1.5
0.20% Sodium Chloride Solution = 2.5
0.25% Sodium Chloride Solution = 3.5

Appendix D - Descriptive Sensory Analysis Ballot

Panelist _____ **Sample _____** **Date _____**

Flavor																															
Pecan ID	**0**	**0.5**	**1**	**1.5**	**2**	**2.5**	**3**	**3.5**	**4**	**4.5**	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
Overall Nutty	**0**	**0.5**	**1**	**1.5**	**2**	**2.5**	**3**	**3.5**	**4**	**4.5**	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
Nutty-Woody	**0**	**0.5**	**1**	**1.5**	**2**	**2.5**	**3**	**3.5**	**4**	**4.5**	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
Nutty-Grain-like	**0**	**0.5**	**1**	**1.5**	**2**	**2.5**	**3**	**3.5**	**4**	**4.5**	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
Nutty-Buttery	**0**	**0.5**	**1**	**1.5**	**2**	**2.5**	**3**	**3.5**	**4**	**4.5**	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
Brown	**0**	**0.5**	**1**	**1.5**	**2**	**2.5**	**3**	**3.5**	**4**	**4.5**	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
Caramelized	**0**	**0.5**	**1**	**1.5**	**2**	**2.5**	**3**	**3.5**	**4**	**4.5**	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
Acrid	**0**	**0.5**	**1**	**1.5**	**2**	**2.5**	**3**	**3.5**	**4**	**4.5**	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
Burnt	**0**	**0.5**	**1**	**1.5**	**2**	**2.5**	**3**	**3.5**	**4**	**4.5**	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
Musty/Earthy	**0**	**0.5**	**1**	**1.5**	**2**	**2.5**	**3**	**3.5**	**4**	**4.5**	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
Woody	**0**	**0.5**	**1**	**1.5**	**2**	**2.5**	**3**	**3.5**	**4**	**4.5**	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
Roasted	**0**	**0.5**	**1**	**1.5**	**2**	**2.5**	**3**	**3.5**	**4**	**4.5**	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
Overall Sweet	**0**	**0.5**	**1**	**1.5**	**2**	**2.5**	**3**	**3.5**	**4**	**4.5**	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
Oily	**0**	**0.5**	**1**	**1.5**	**2**	**2.5**	**3**	**3.5**	**4**	**4.5**	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
Rancid	**0**	**0.5**	**1**	**1.5**	**2**	**2.5**	**3**	**3.5**	**4**	**4.5**	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
Oxidized	**0**	**0.5**	**1**	**1.5**	**2**	**2.5**	**3**	**3.5**	**4**	**4.5**	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
Astringent	**0**	**0.5**	**1**	**1.5**	**2**	**2.5**	**3**	**3.5**	**4**	**4.5**	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
Bitter	**0**	**0.5**	**1**	**1.5**	**2**	**2.5**	**3**	**3.5**	**4**	**4.5**	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**

Sour	**0**	**0.5**	**1**	**1.5**	**2**	**2.5**	**3**	**3.5**	**4**	**4.5**	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
Sweet	**0**	**0.5**	**1**	**1.5**	**2**	**2.5**	**3**	**3.5**	**4**	**4.5**	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**
Salt	**0**	**0.5**	**1**	**1.5**	**2**	**2.5**	**3**	**3.5**	**4**	**4.5**	**5**	**5.5**	**6**	**6.5**	**7**	**7.5**	**8**	**8.5**	**9**	**9.5**	**10**	**10.5**	**11**	**11.5**	**12**	**12.5**	**13**	**13.5**	**14**	**14.5**	**15**

Appendix E - Consumer Study Screening Ballot, Performed through RedJade® online software

1) Please indicate your gender:

   Male Female

   (approximate even distribution; quota 40:60 minimum)

2) Which of the following best describes your age?

17 or younger (disqualify) 18 - 24 25 - 35 36 – 45 46 - 55

56 - 65 66 or older

(age distribution)

3) Do you have any known food allergies or dietary restrictions?

   Yes (disqualify) No

4) Do you or any of your immediate family work for a market research firm, advertising firm, or food manufacturing company?

   Yes (disqualify) No

5) Which of the following foods do you eat? (select all that apply)

   Beans Nuts Yogurt Rice Cereal

   (must select "Nuts" to proceed to following questions)

6) You have indicated that you eat nuts. How often do you eat nuts of any kind?

Every day

At least once every 1-2 weeks

At least once a month

Once every 2-3 months

Once every 6 months (disqualify)

Once a year (disqualify)

7) Which of the following nuts/legumes would you be willing to eat?

Peanuts Black Walnuts Black Beans Almonds Pecans

Walnuts Pinto Beans

(must select pecans to qualify for study)

Appendix F - Consumer Study Moderator Guide

- Today you will be seeing a total of 8 samples of pecans, 4 fresh samples and 4 roasted samples.
- You will be given 2 minutes to evaluate each sample.
- In front of you is a slip of paper with the serving order for your samples along with your participant number. Be sure that the code on the samples served to you match that on your serving sheet.
- We ask that you do not discuss any of your answers or the samples with anyone during or after the study.
- We have provided you with water and crackers to use between samples to cleanse your palate.
- At 12:30, please hit “try again” highlighted in blue under the “no project scheduled” bar, sign in using the participant number on the sheet in front of you, and read through the consent form.
- Once you have completed the study, please come to the front of the room to collect your payment, submit your social security number, and fill out our sign-in sheet.
- If you have any questions during the study, please raise your hand.
- Thank you for your time and enjoy the samples.

Appendix G - Consumer Study Informed Consent Form

Informed Consent Statement
Sensory Analysis Center
Kansas State University
Ice Hall 136
Manhattan, KS 66502

1. I, (print your name) _____, agree to participate as a panelist for research at the Kansas State University Sensory Analysis Center.

2. I understand that the purpose of this research is to participate in a taste test evaluating eight samples of candied pecans.

3. I understand that if I have any food allergies I should not participate in the study.

4. For this test, I will receive $10 when I complete this 45 minute study.

5. I understand that my performance as an individual will be treated as research data and will in no way be associated with me for identification purposes, thereby assuring confidentiality of my performance and responses.

6. I understand that I do not have to participate in research, and that if I choose not to participate, there will be no penalty.

7. I understand that I may withdraw from this research at any time.

8. If I have any questions concerning this study, I understand that I may contact Brendan Kelly, 136 Ice Hall, Kansas State University, Manhattan, KS at 785-532-0144, or Kadri Koppel at 785-532-0163.

9. If I have questions about my rights as a consumer or about the manner in which this research was conducted, I may contact Rick Scheidt, Chair, Committee on Research Involving Human Subjects, at 203 Fairchild Hall, or Gerald Jaax, Associate Vice-provost for Research, 1 Fairchild Hall (785-532-2334).

I understand the above statements (Participant must sign):

_____

Signature **Date**

Appendix H - Consumer Evaluation Test Design

Serving Set	*Serving 1*	*Serving 2*	*Serving 3*	*Serving 4*	*Serving 5*	*Serving 6*	*Serving 7*	*Serving 8*
1	3125	7761	4787	6819	5531	1890	9546	4183
2	4787	3125	6819	7761	9546	5531	4183	1890
3	6819	4787	7761	3125	4183	9546	1890	5531
4	7761	6819	3125	4787	1890	4183	5531	9546
5	7761	3125	4787	6819	4183	5531	9546	1890
6	4787	7761	6819	3125	1890	9546	5531	4183
7	3125	6819	7761	4787	5531	1890	4183	9546
8	6819	4787	3125	7761	9546	4183	1890	5531
9	3125	7761	6819	4787	9546	5531	1890	4183
10	6819	3125	4787	7761	4183	1890	5531	9546
11	4787	6819	7761	3125	5531	4183	9546	1890
12	7761	4787	3125	6819	1890	9546	4183	5531
13	3125	6819	4787	7761	1890	5531	4183	9546
14	7761	4787	6819	3125	5531	9546	1890	4183
15	4787	3125	7761	6819	9546	4183	5531	1890
16	6819	7761	3125	4787	4183	1890	9546	5531
17	4787	7761	3125	6819	9546	1890	5531	4183
18	6819	3125	7761	4787	1890	4183	9546	5531
19	7761	6819	4787	3125	5531	9546	4183	1890
20	3125	4787	6819	7761	4183	5531	1890	9546
21	3125	4787	7761	6819	4183	9546	5531	1890
22	7761	3125	6819	4787	1890	5531	9546	4183
23	4787	6819	3125	7761	5531	4183	1890	9546
24	6819	7761	4787	3125	9546	1890	4183	5531
25	3125	4787	7761	6819	4183	1890	9546	5531
26	4787	6819	3125	7761	1890	5531	4183	9546
27	6819	7761	4787	3125	9546	4183	5531	1890
28	7761	3125	6819	4787	5531	9546	1890	4183
29	4787	6819	7761	3125	5531	4183	9546	1890
30	3125	7761	6819	4787	1890	9546	4183	5531
31	7761	4787	3125	6819	9546	5531	1890	4183
32	6819	3125	4787	7761	4183	1890	5531	9546
33	7761	4787	6819	3125	9546	5531	4183	1890
34	3125	6819	4787	7761	4183	9546	1890	5531
35	4787	3125	7761	6819	1890	4183	5531	9546
36	6819	7761	3125	4787	5531	1890	9546	4183
37	7761	3125	4787	6819	4183	5531	9546	1890
38	6819	4787	3125	7761	9546	4183	1890	5531
39	3125	6819	7761	4787	5531	1890	4183	9546
40	4787	7761	6819	3125	1890	9546	5531	4183

41	4787	3125	6819	7761	5531	4183	1890	9546
42	3125	7761	4787	6819	9546	1890	4183	5531
43	6819	4787	7761	3125	1890	5531	9546	4183
44	7761	6819	3125	4787	4183	9546	5531	1890
45	7761	6819	4787	3125	5531	9546	4183	1890
46	4787	7761	3125	6819	4183	5531	1890	9546
47	6819	3125	7761	4787	9546	1890	5531	4183
48	3125	4787	6819	7761	1890	4183	9546	5531
49	3125	4787	6819	7761	1890	5531	4183	9546
50	6819	3125	7761	4787	5531	9546	1890	4183
51	7761	6819	4787	3125	4183	1890	9546	5531
52	4787	7761	3125	6819	9546	4183	5531	1890
53	4787	3125	6819	7761	4183	9546	5531	1890
54	7761	6819	3125	4787	1890	5531	9546	4183
55	6819	4787	7761	3125	9546	1890	4183	5531
56	3125	7761	4787	6819	5531	4183	1890	9546
57	7761	3125	6819	4787	9546	5531	1890	4183
58	6819	7761	4787	3125	1890	9546	4183	5531
59	3125	4787	7761	6819	5531	4183	9546	1890
60	4787	6819	3125	7761	4183	1890	5531	9546
61	7761	4787	6819	3125	5531	9546	4183	1890
62	6819	7761	3125	4787	4183	5531	1890	9546
63	4787	3125	7761	6819	9546	1890	5531	4183
64	3125	6819	4787	7761	1890	4183	9546	5531
65	6819	4787	3125	7761	1890	4183	5531	9546
66	4787	7761	6819	3125	5531	1890	9546	4183
67	7761	3125	4787	6819	9546	5531	4183	1890
68	3125	6819	7761	4787	4183	9546	1890	5531
69	6819	3125	4787	7761	4183	5531	9546	1890
70	3125	7761	6819	4787	9546	4183	1890	5531
71	7761	4787	3125	6819	1890	9546	5531	4183
72	4787	6819	7761	3125	5531	1890	4183	9546
73	7761	3125	4787	6819	1890	5531	4183	9546
74	6819	4787	3125	7761	5531	9546	1890	4183
75	3125	6819	7761	4787	9546	4183	5531	1890
76	4787	7761	6819	3125	4183	1890	9546	5531
77	7761	4787	3125	6819	1890	9546	4183	5531
78	4787	6819	7761	3125	9546	5531	1890	4183
79	3125	7761	6819	4787	4183	1890	5531	9546
80	6819	3125	4787	7761	5531	4183	9546	1890
81	7761	3125	6819	4787	9546	4183	1890	5531
82	6819	7761	4787	3125	4183	5531	9546	1890
83	4787	6819	3125	7761	1890	9546	5531	4183
84	3125	4787	7761	6819	5531	1890	4183	9546
85	7761	6819	3125	4787	1890	5531	9546	4183

86	6819	4787	7761	3125	5531	4183	1890	9546
87	4787	3125	6819	7761	4183	9546	5531	1890
88	3125	7761	4787	6819	9546	1890	4183	5531
89	4787	3125	7761	6819	9546	5531	4183	1890
90	3125	6819	4787	7761	5531	1890	9546	4183
91	6819	7761	3125	4787	4183	9546	1890	5531
92	7761	4787	6819	3125	1890	4183	5531	9546
93	7761	6819	4787	3125	4183	5531	1890	9546
94	4787	7761	3125	6819	9546	1890	5531	4183
95	3125	4787	6819	7761	5531	9546	4183	1890
96	6819	3125	7761	4787	1890	4183	9546	5531
97	3125	7761	6819	4787	4183	9546	1890	5531
98	7761	4787	3125	6819	1890	4183	5531	9546
99	6819	3125	4787	7761	5531	1890	9546	4183
100	4787	6819	7761	3125	9546	5531	4183	1890
101	6819	4787	7761	3125	9546	1890	5531	4183
102	4787	3125	6819	7761	4183	5531	1890	9546
103	3125	7761	4787	6819	5531	9546	4183	1890
104	7761	6819	3125	4787	1890	4183	9546	5531
105	3125	4787	6819	7761	5531	4183	9546	1890
106	7761	6819	4787	3125	1890	9546	4183	5531
107	6819	3125	7761	4787	9546	5531	1890	4183
108	4787	7761	3125	6819	4183	1890	5531	9546
109	7761	4787	6819	3125	5531	1890	4183	9546
110	6819	7761	3125	4787	1890	9546	5531	4183
111	3125	6819	4787	7761	9546	4183	1890	5531
112	4787	3125	7761	6819	4183	5531	9546	1890
113	7761	3125	4787	6819	4183	9546	5531	1890
114	6819	4787	3125	7761	5531	4183	1890	9546
115	4787	7761	6819	3125	1890	5531	9546	4183
116	3125	6819	7761	4787	9546	1890	4183	5531
117	4787	6819	3125	7761	5531	9546	1890	4183
118	7761	3125	6819	4787	4183	1890	9546	5531
119	6819	7761	4787	3125	9546	4183	5531	1890
120	3125	4787	7761	6819	1890	5531	4183	9546

Sample	*Sample Code*
Raw Kanza	6819
Raw Maramec	7761
Raw Pawnee	4787
Raw Witte	3125
Roasted Kanza	5531
Roasted Maramec	4183
Roasted Pawnee	1890
Roasted Witte	9546

Appendix I - Consumer Study Ballot

Introductory Screen

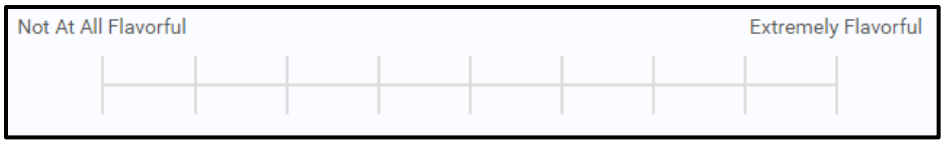
Today, you will be seeing 8 samples of pecans. The first 4 samples are FRESH pecans.

Sample Evaluation

Please taste the sample and answer the following questions. Retaste as necessary.

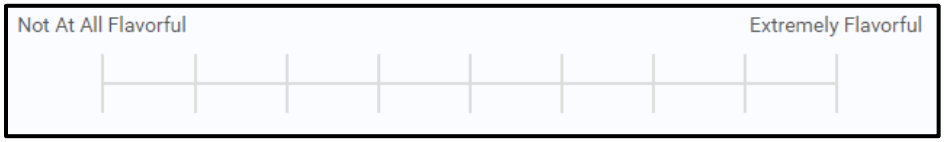
1) How much do you LIKE or DISLIKE the OVERALL FLAVOR of this sample?

2) Please rate the INTENSITY of the OVERALL FLAVOR of this sample.



3) How much do you LIKE or DISLIKE the PECAN FLAVOR of this sample?

4) Please rate the INTENSITY of the PECAN FLAVOR of this sample.



5) How much do you LIKE or DISLIKE the sample OVERALL?

Mid Study

You have now completed the portion of the study using FRESH pecans. The final 4 samples are ROASTED pecans.

Post Sample Evaluation

You have now seen 8 samples of pecans, 4 FRESH and 4 ROASTED. Please select which set of pecans you prefer overall:

FRESH ROASTED

You have now completed this study. Please press the final "next" button and proceed to the front of the room. If you have not submitted your social security number in the indicated box, please do so before collecting your payment. Thank you for your time and we hope to see you soon in another of the studies here at the Sensory Analysis Center.

Demographic Information

1) Please indicate your gender:

   Male Female

2) Which of the following best describes your age?

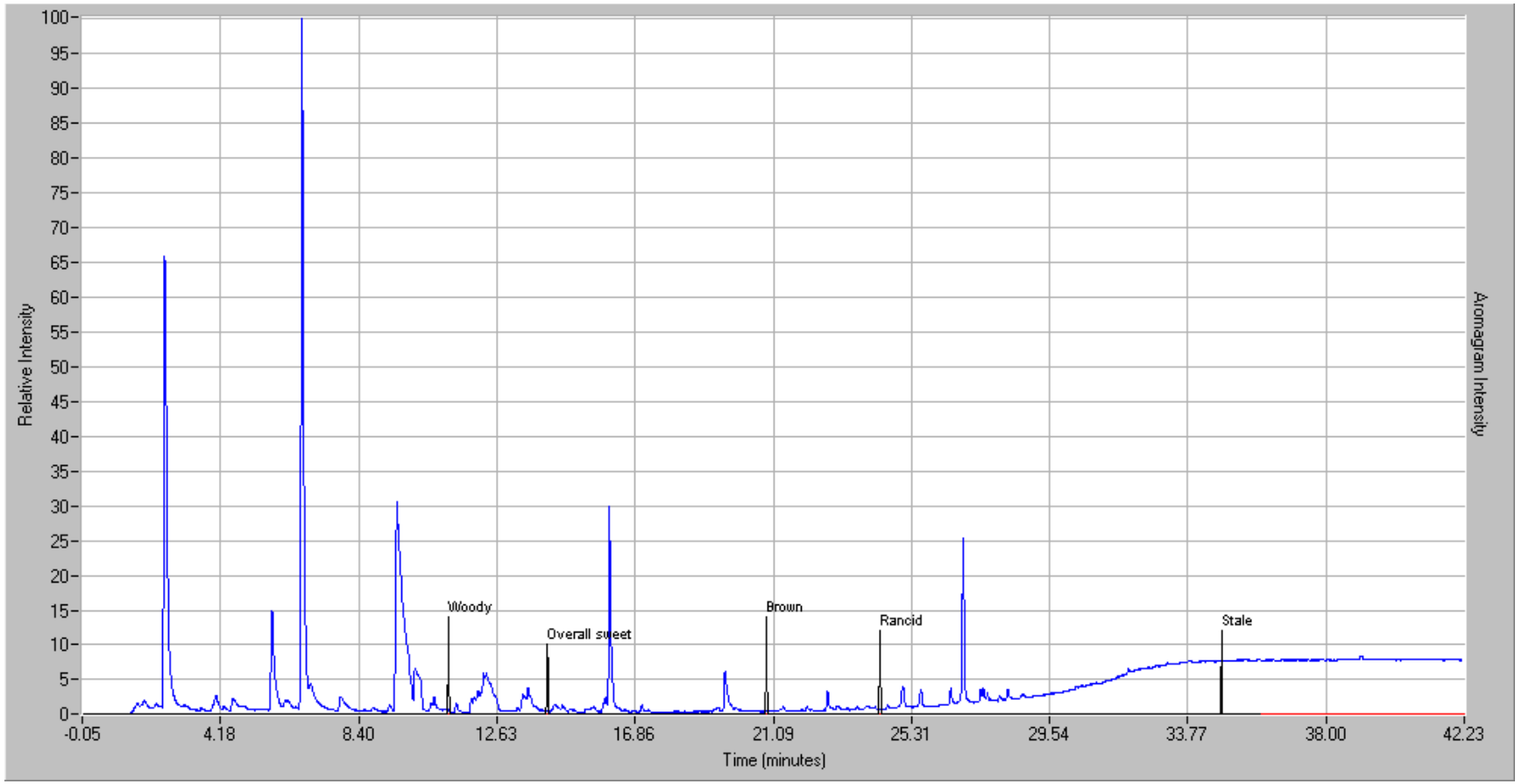
18-24 25-35 36-45 46-55 56-65 66 or older

Notes:

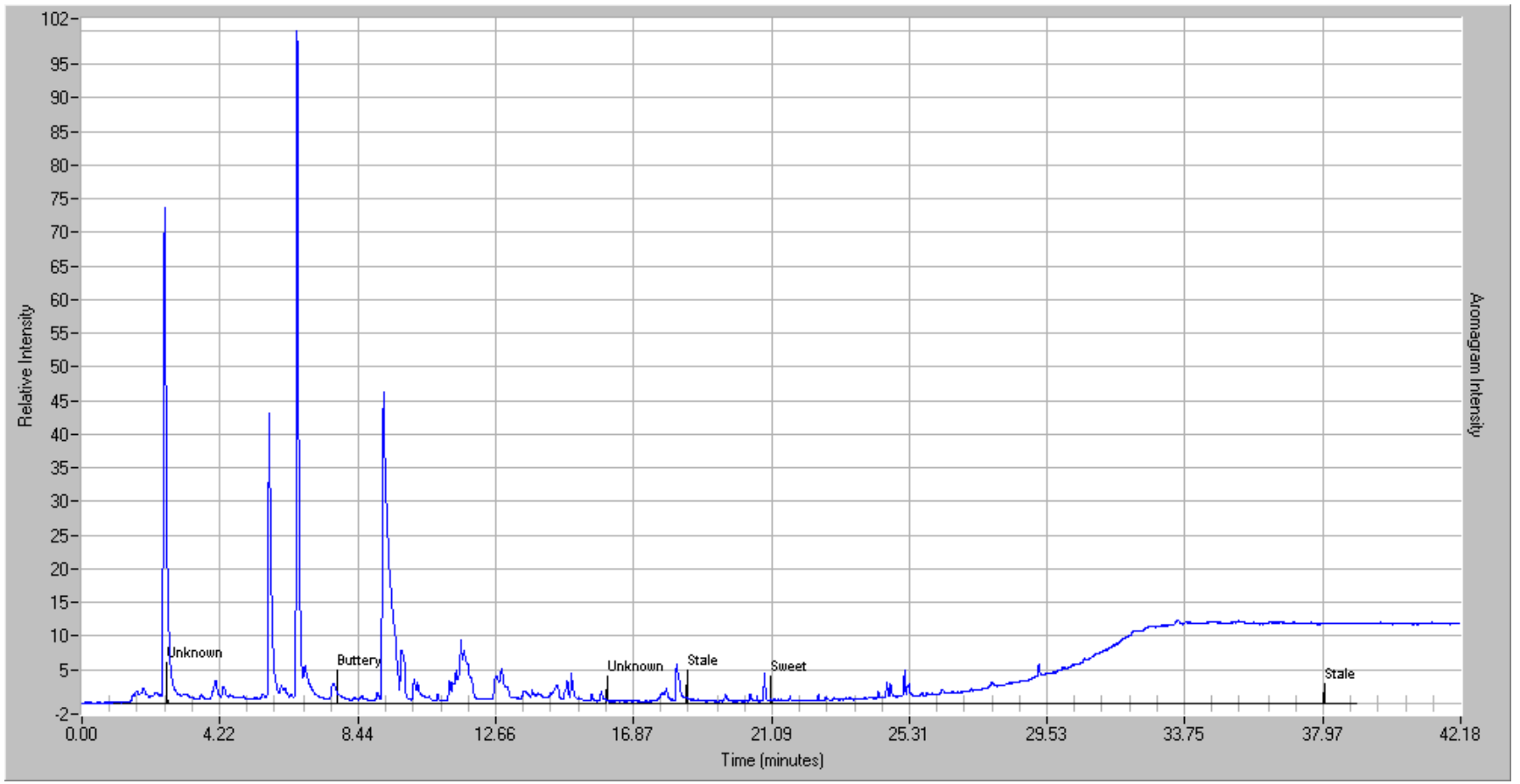
1) The Sample Evaluation survey was completed with each sample (total of 8 samples)

Appendix J - Gas Chromatography-Olfactometry Output

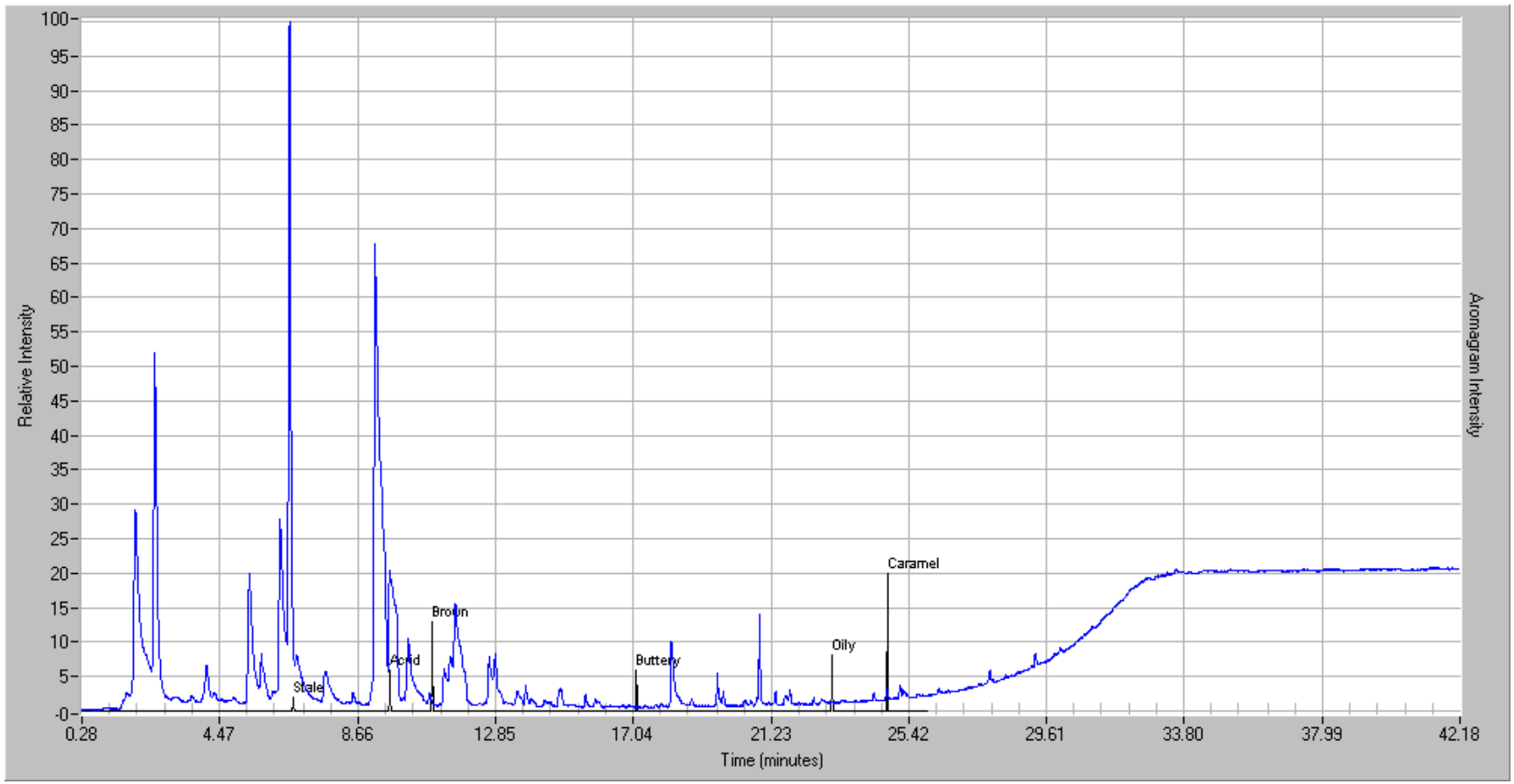
Gas chromatography-olfactometry chromatogram for raw Kanza sample (rep 1) performed by panelist 1



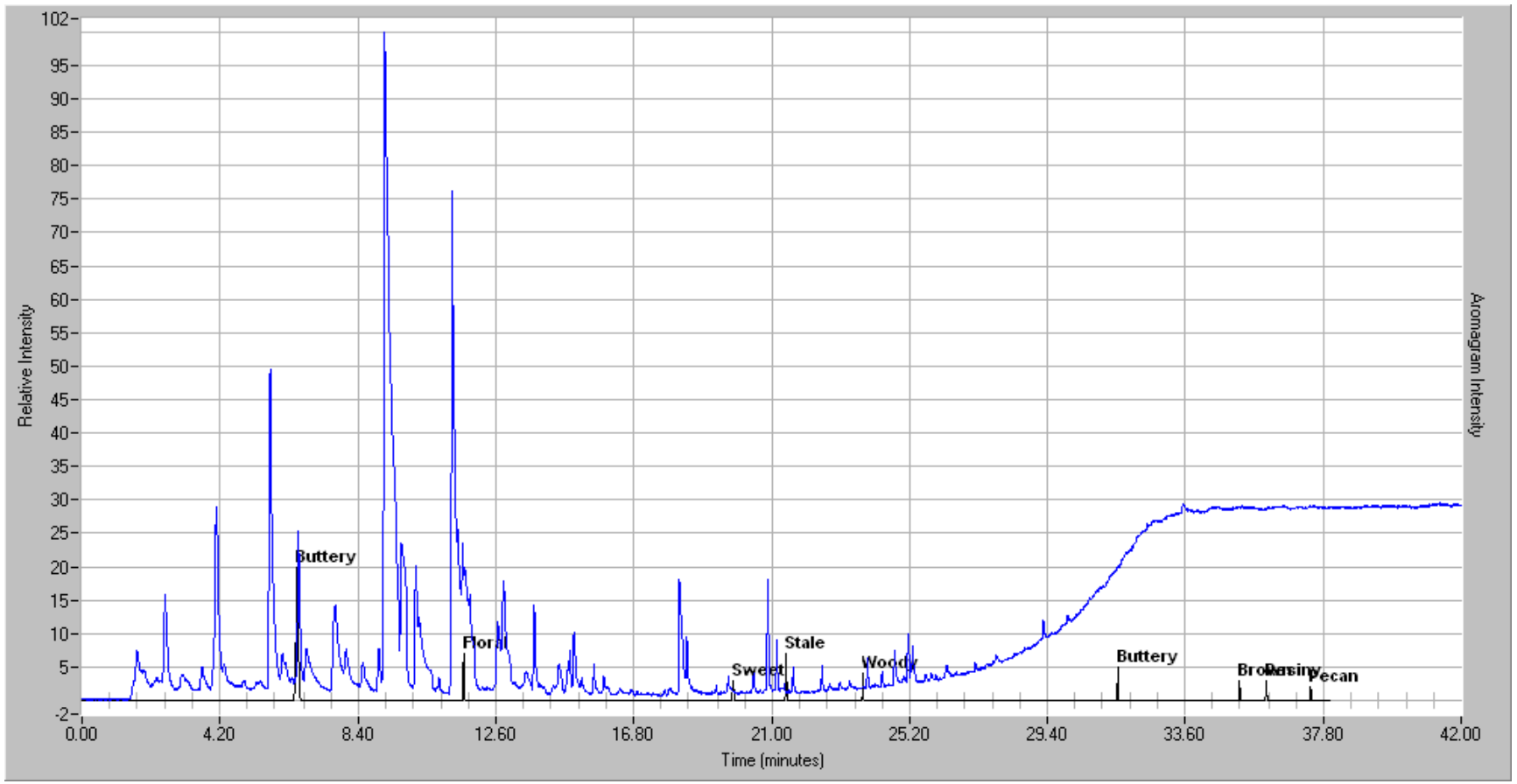
Gas chromatography-olfactometry chromatogram for raw Kanza sample (rep 2) performed by panelist 3



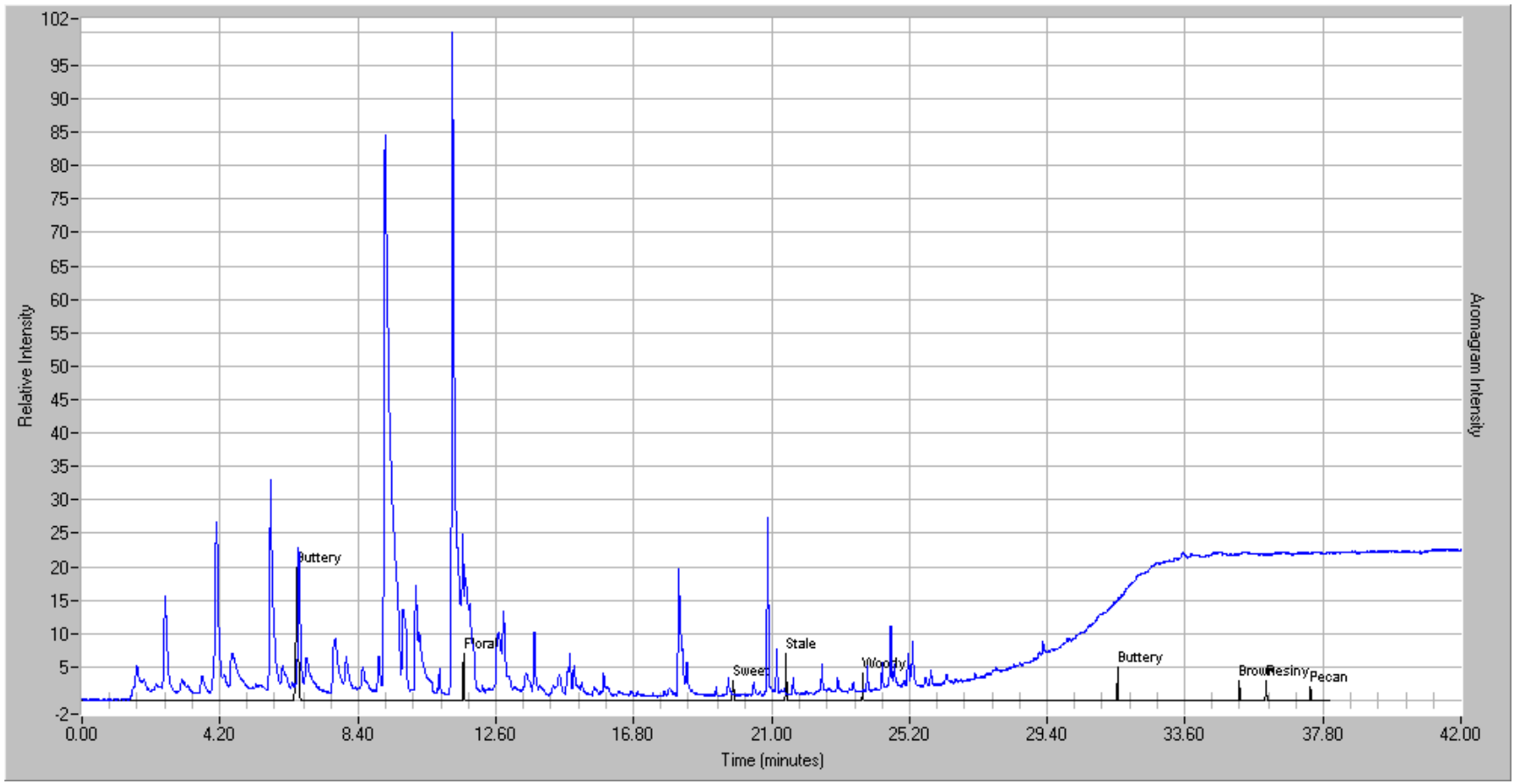
Gas chromatography-olfactometry chromatogram for raw Kanza sample (rep 3) performed by panelist 2



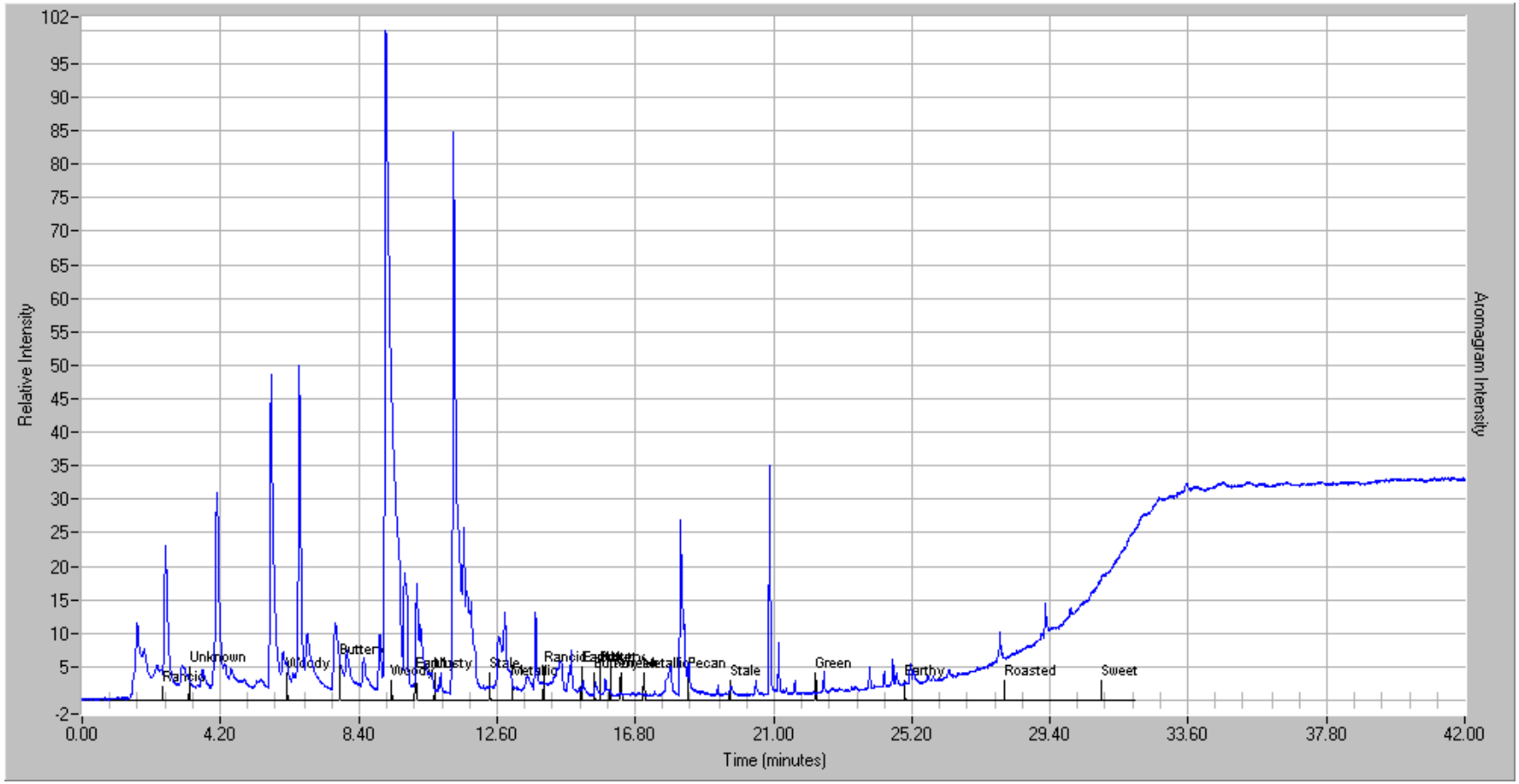
Gas chromatography-olfactometry chromatogram for roasted Kanza sample (rep 1) performed by panelist 2



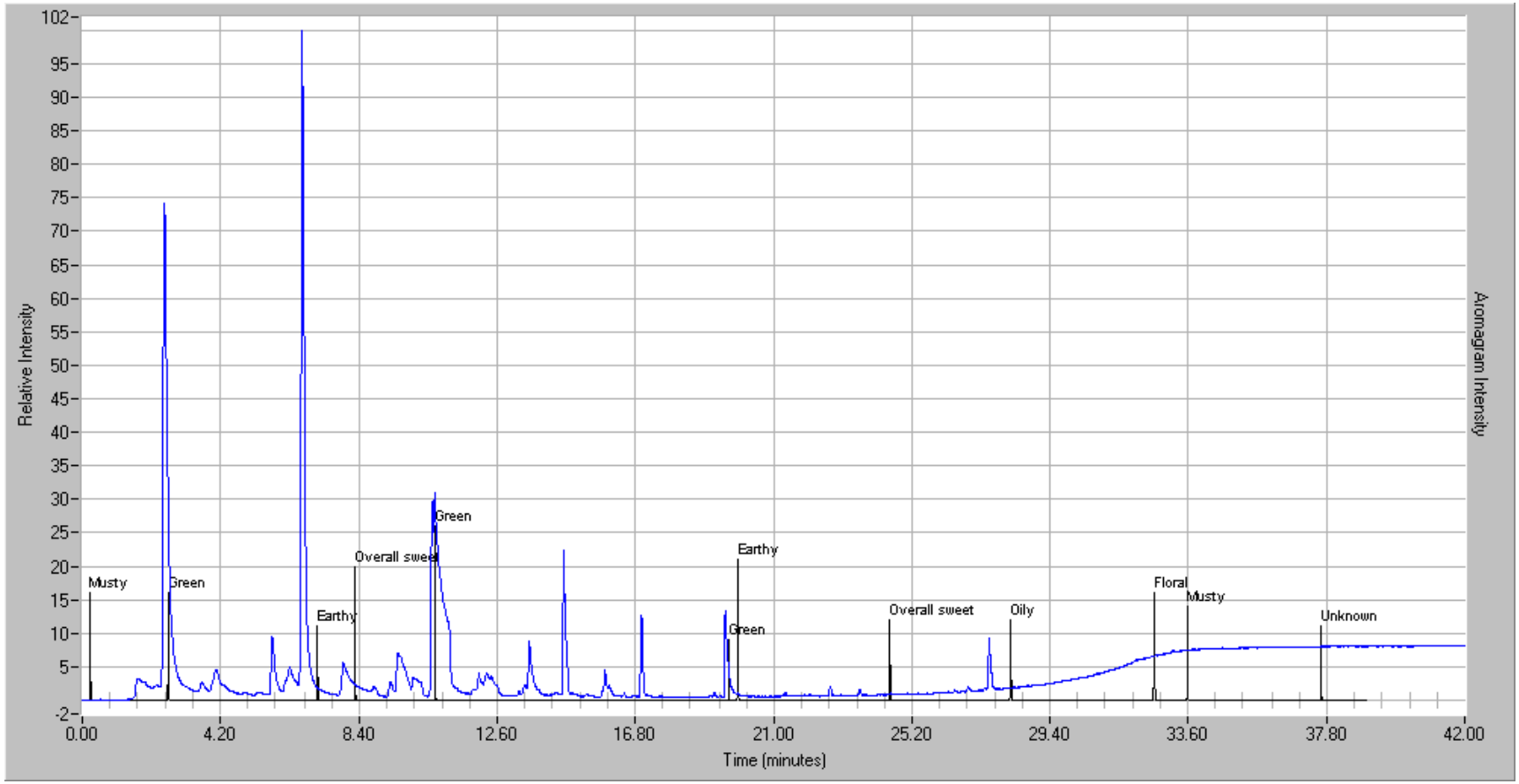
Gas chromatography-olfactometry chromatogram for roasted Kanza sample (rep 2) performed by panelist 3



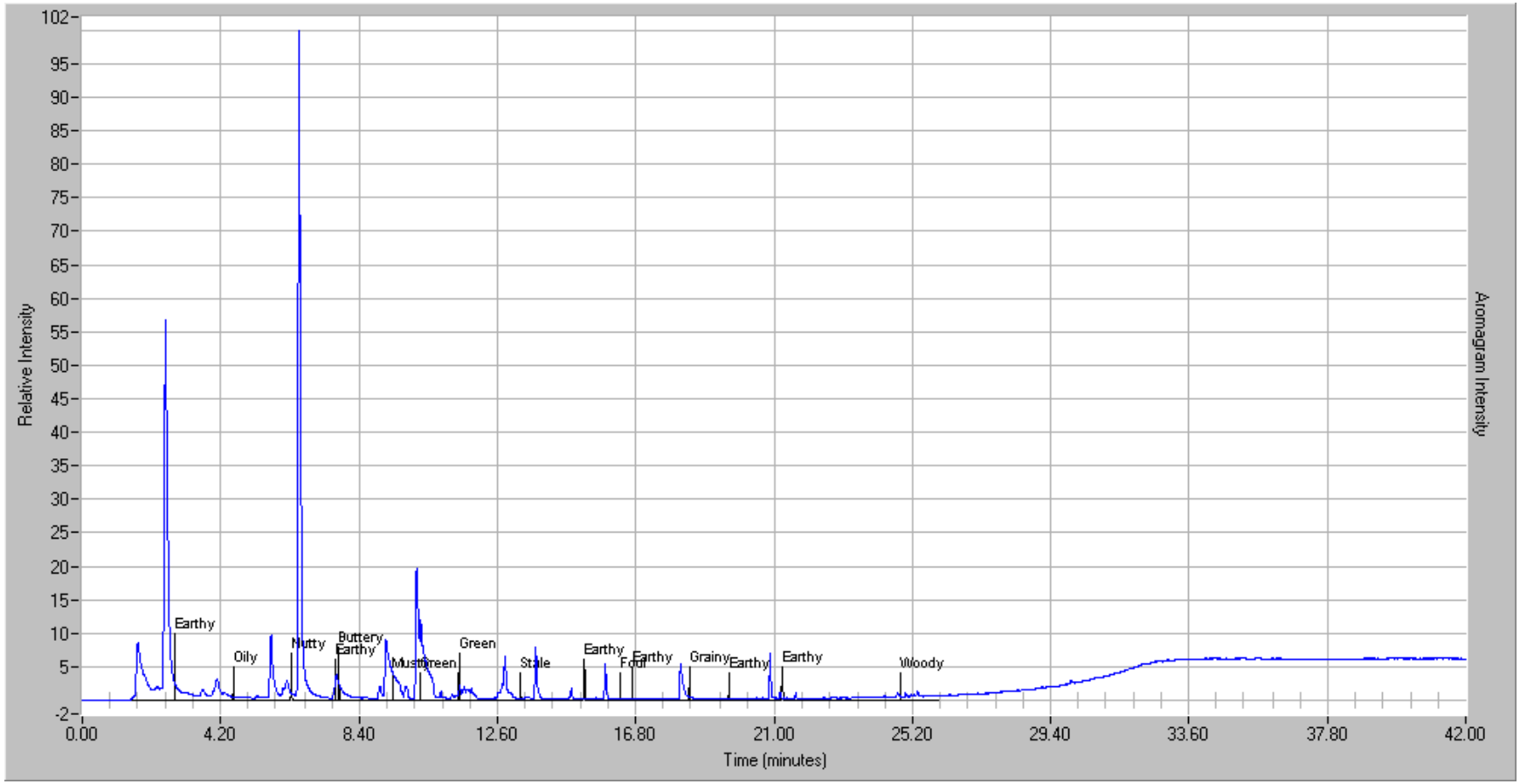
Gas chromatography-olfactometry chromatogram for roasted Kanza sample (rep 3) performed by panelist 3



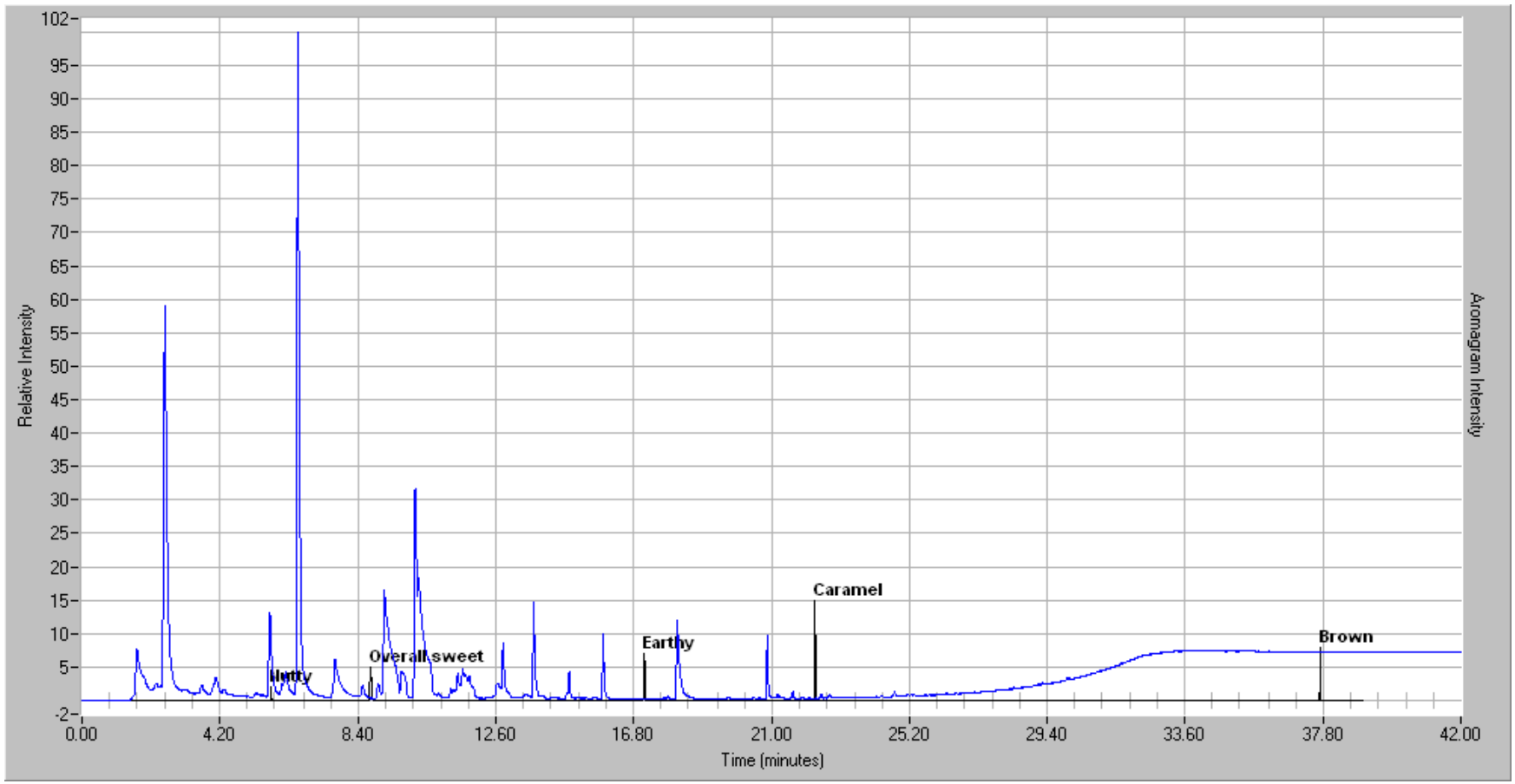
Gas chromatography-olfactometry chromatogram for raw Maramec sample (rep 1) performed by panelist 4



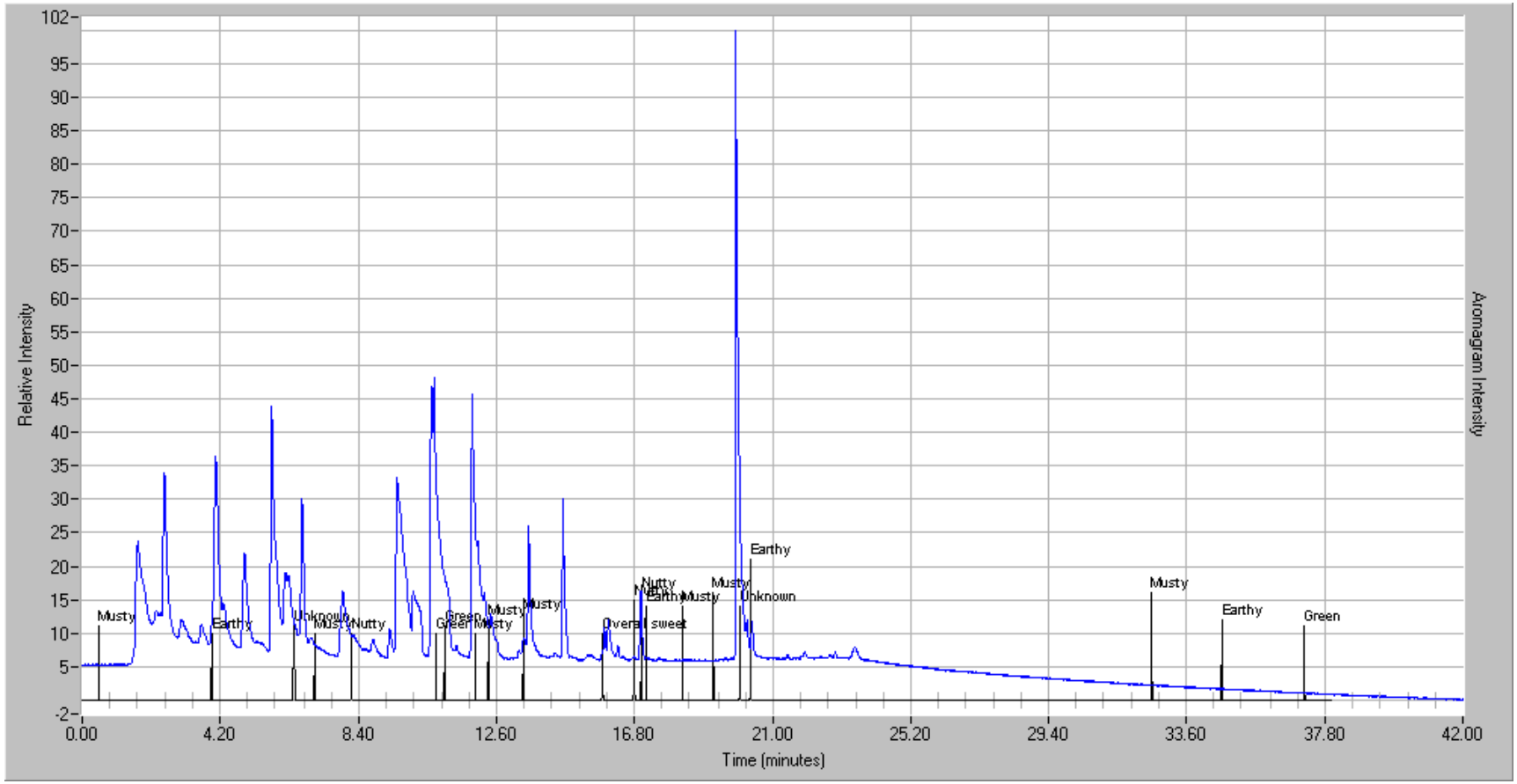
Gas chromatography-olfactometry chromatogram for raw Maramec sample (rep 2) performed by panelist 3



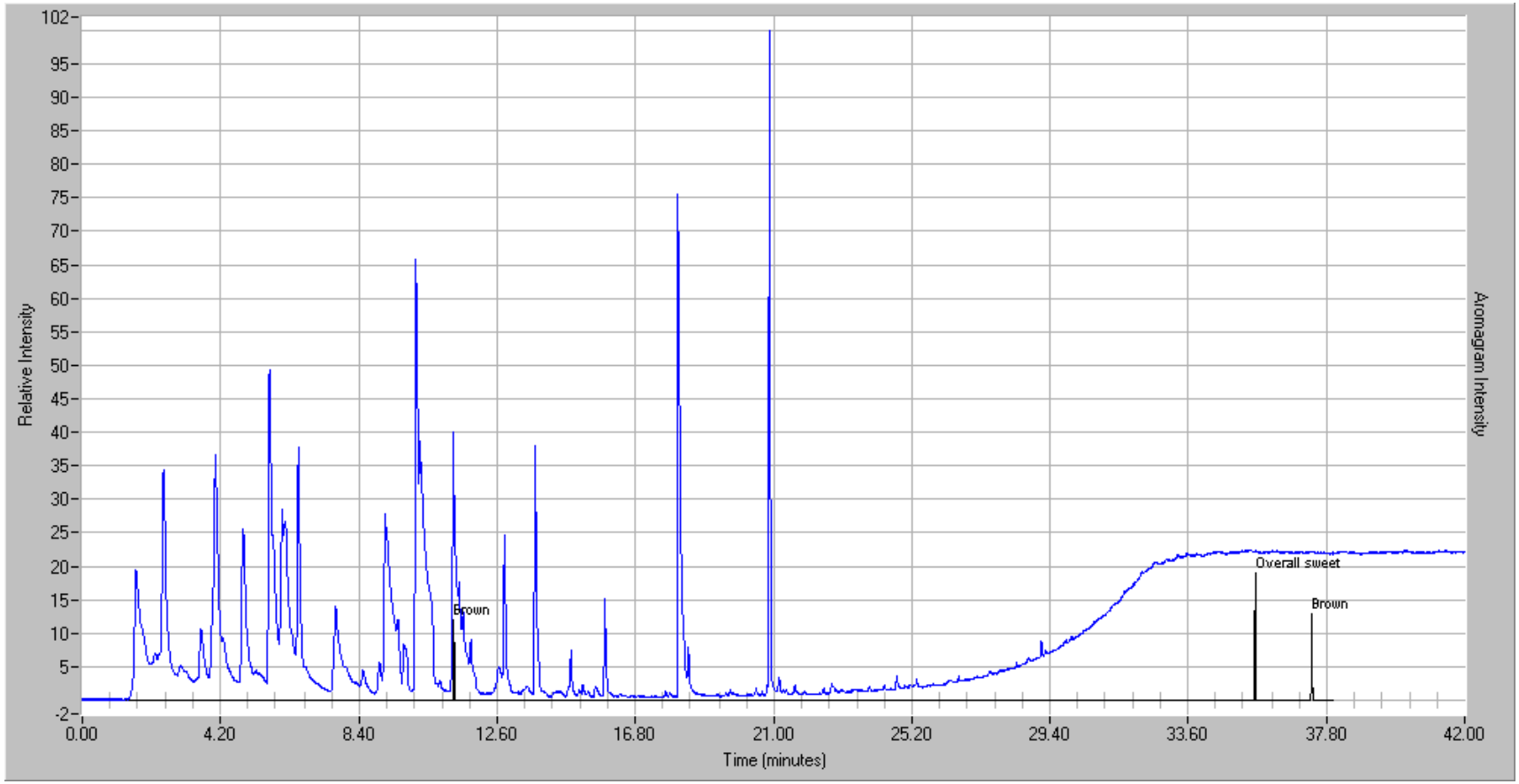
Gas chromatography-olfactometry chromatogram for raw Maramec sample (rep 3) performed by panelist 2



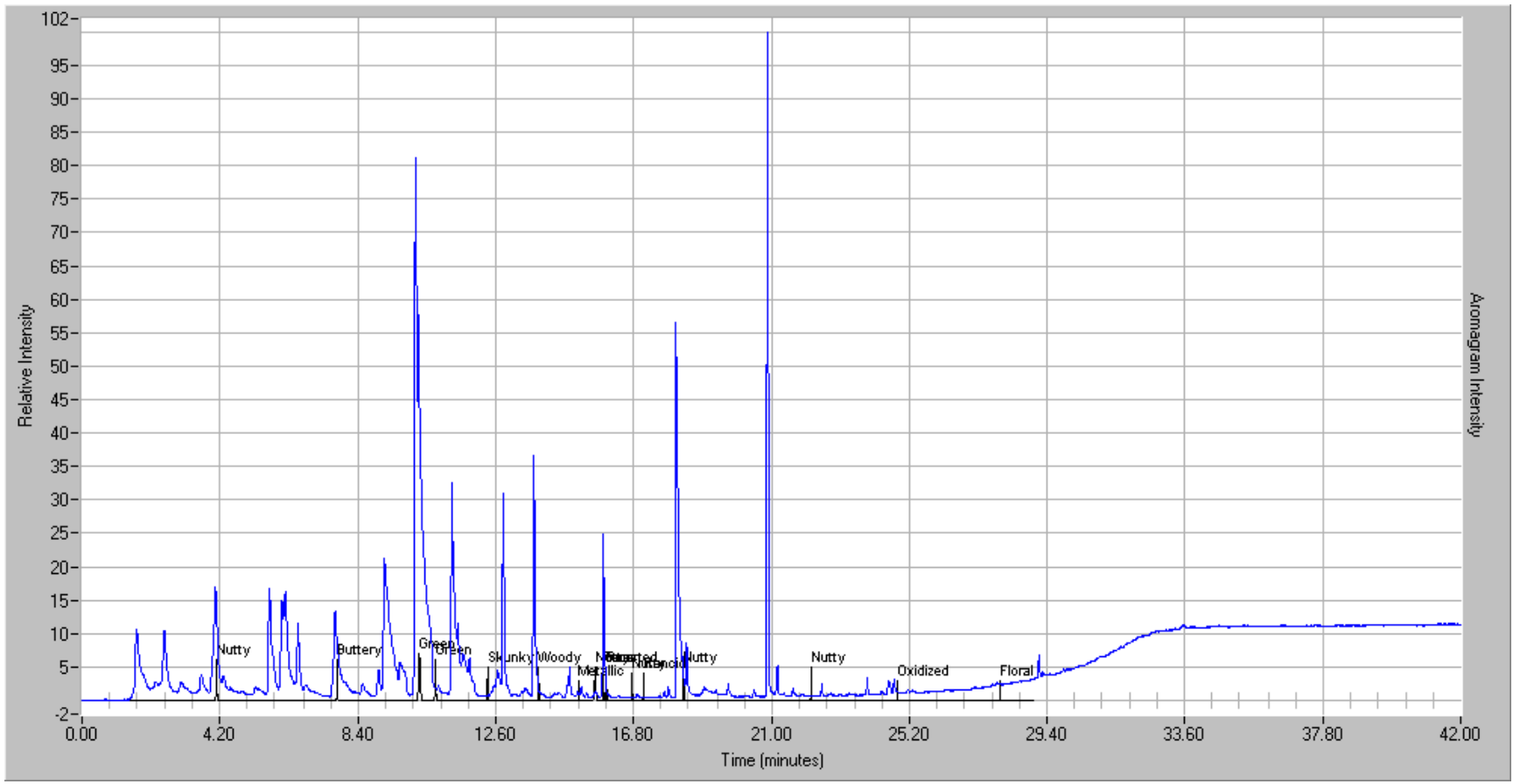
Gas chromatography-olfactometry chromatogram for roasted Maramec sample (rep 1) performed by panelist 4



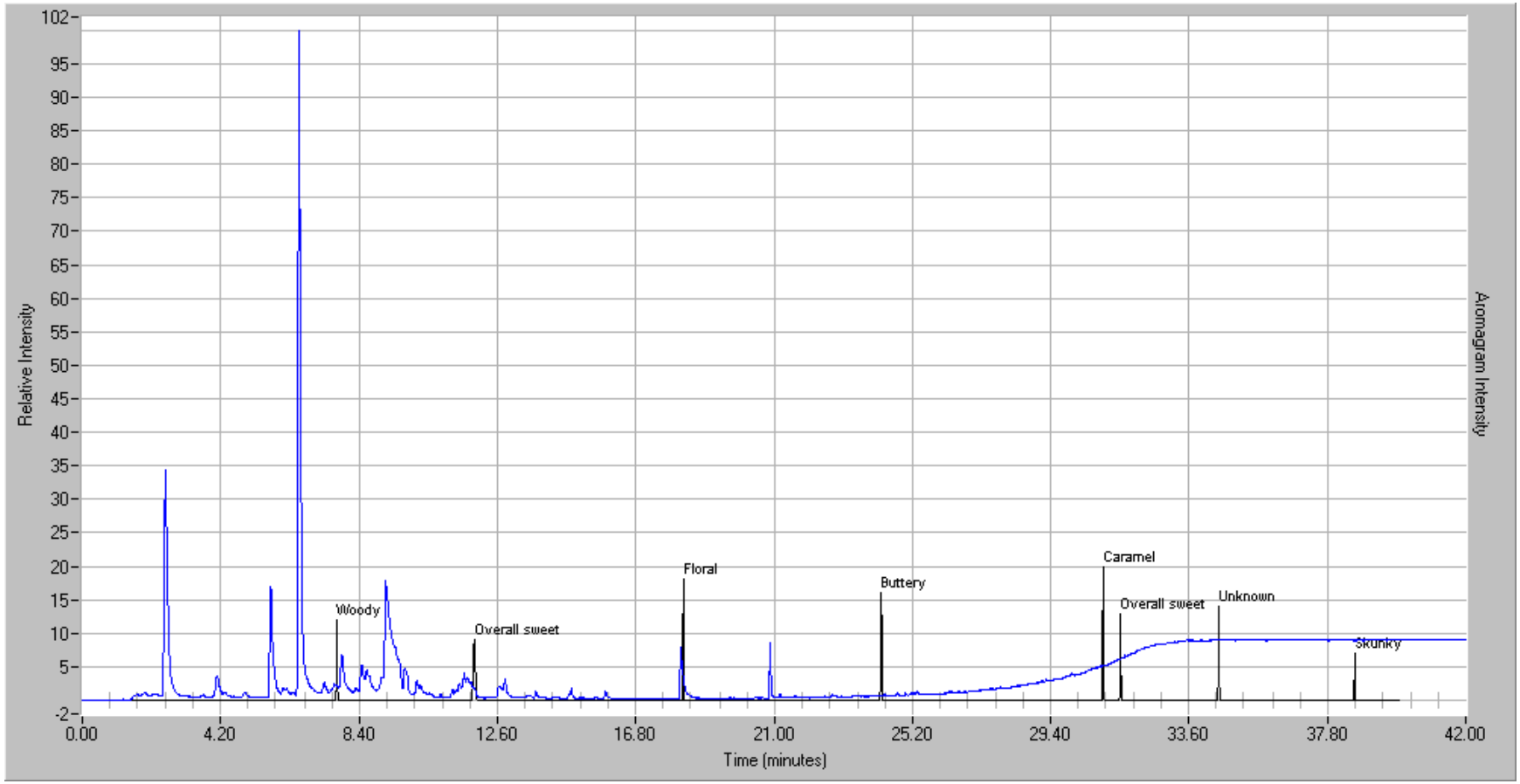
Gas chromatography-olfactometry chromatogram for roasted Maramec sample (rep 2) performed by panelist 2



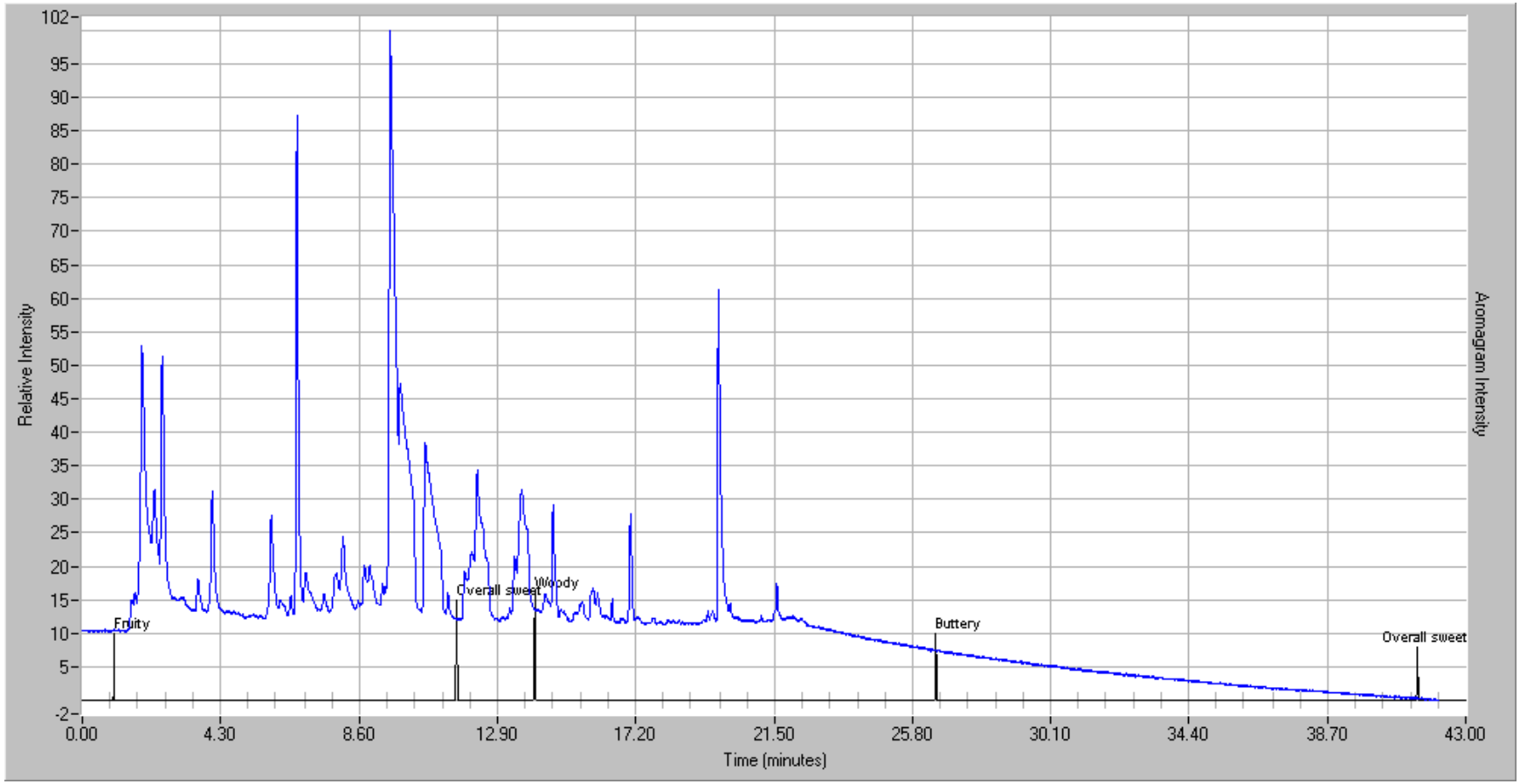
Gas chromatography-olfactometry chromatogram for roasted Maramec sample (rep 3) performed by panelist 3



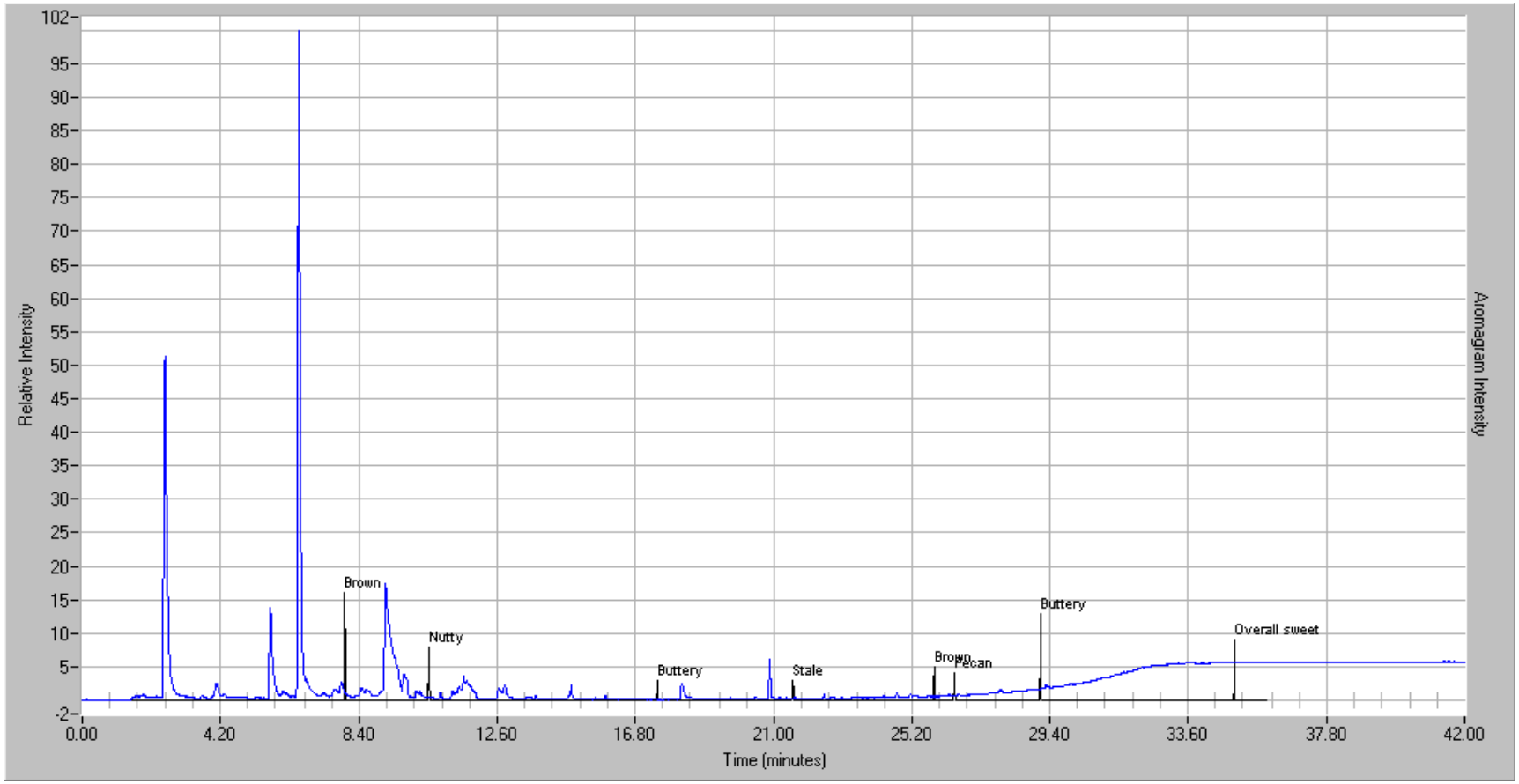
Gas chromatography-olfactometry chromatogram for raw Pawnee sample (rep 1) performed by panelist 1



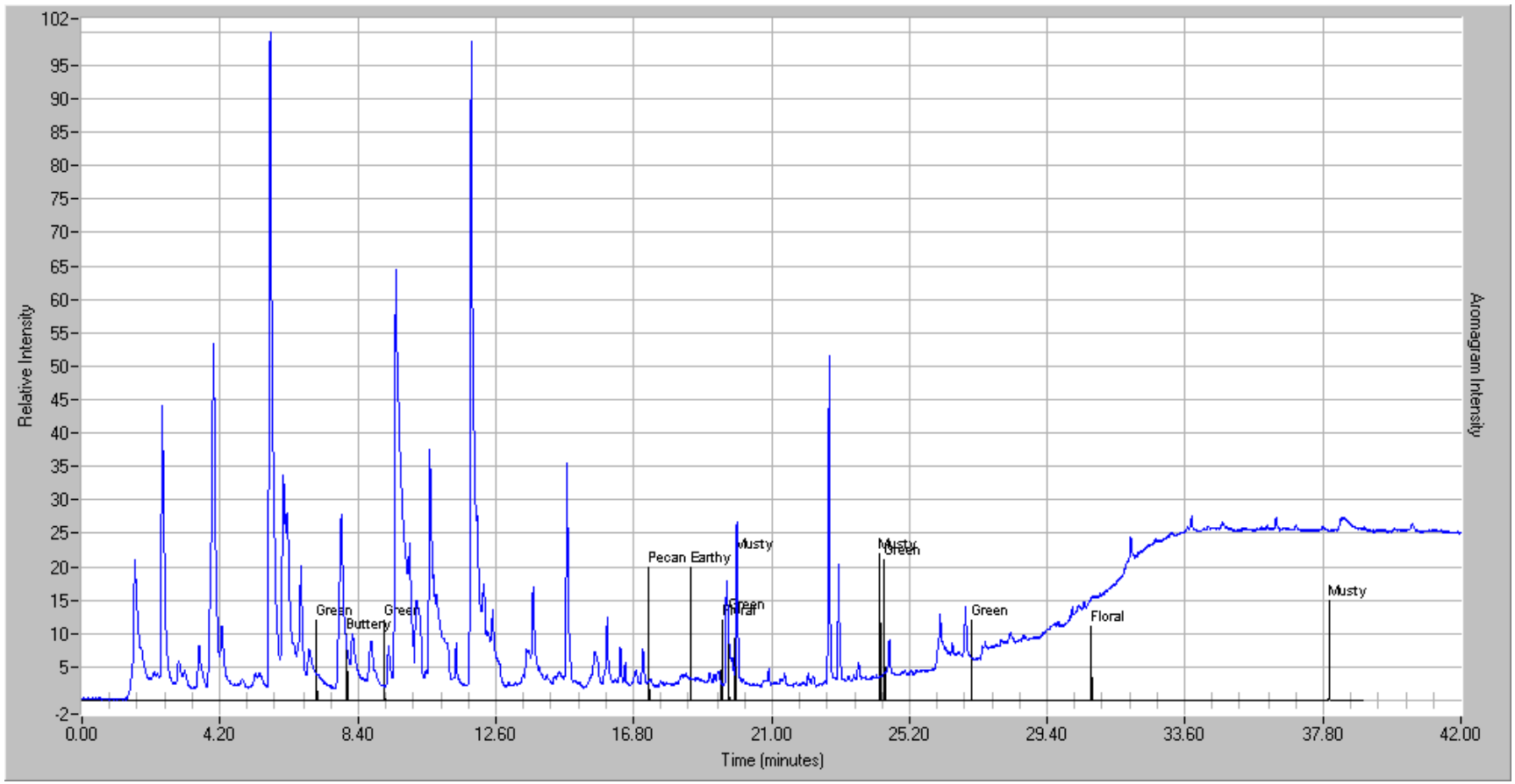
Gas chromatography-olfactometry chromatogram for raw Pawnee sample (rep 2) performed by panelist 1



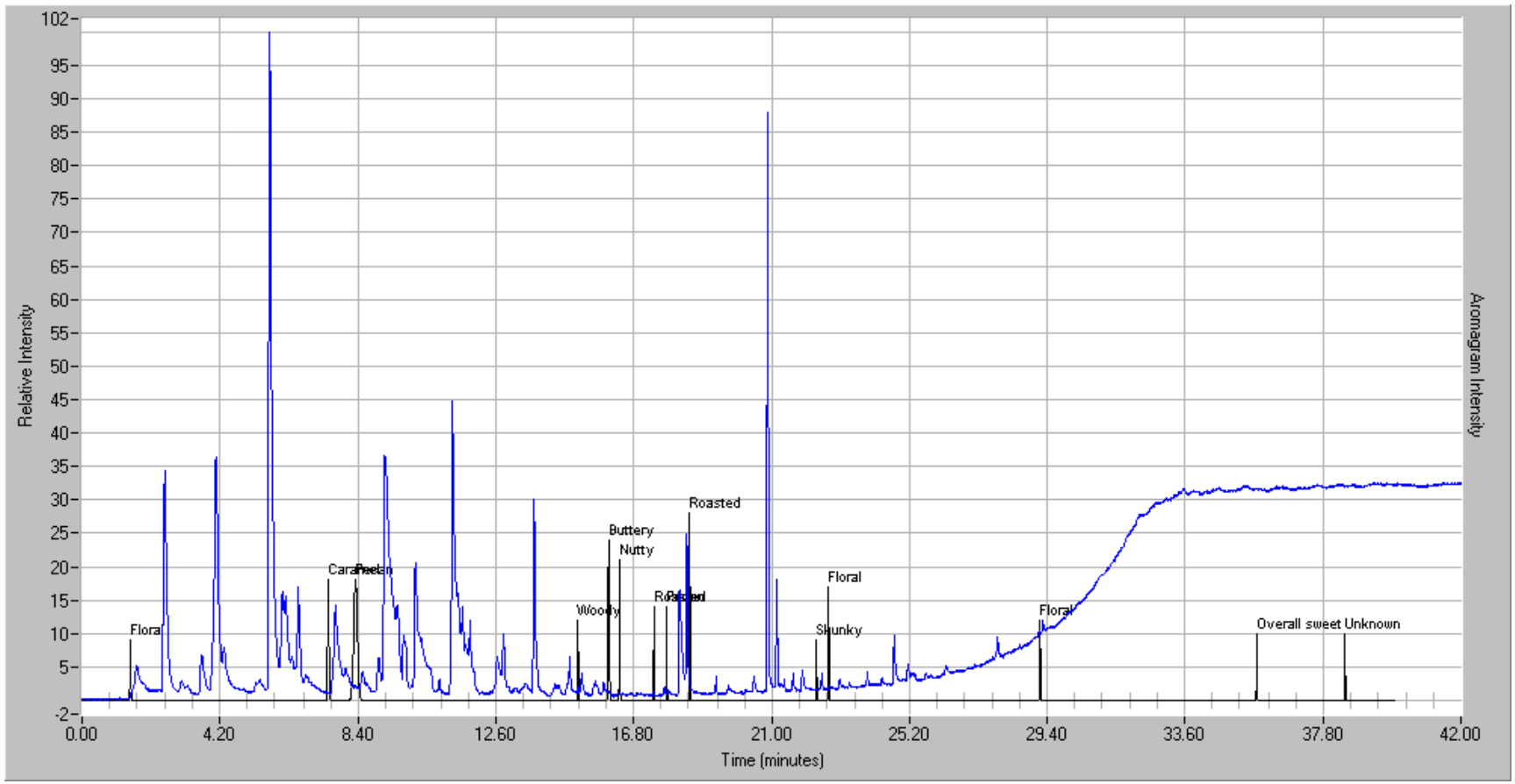
Gas chromatography-olfactometry chromatogram for raw Pawnee sample (rep 3) performed by panelist 2



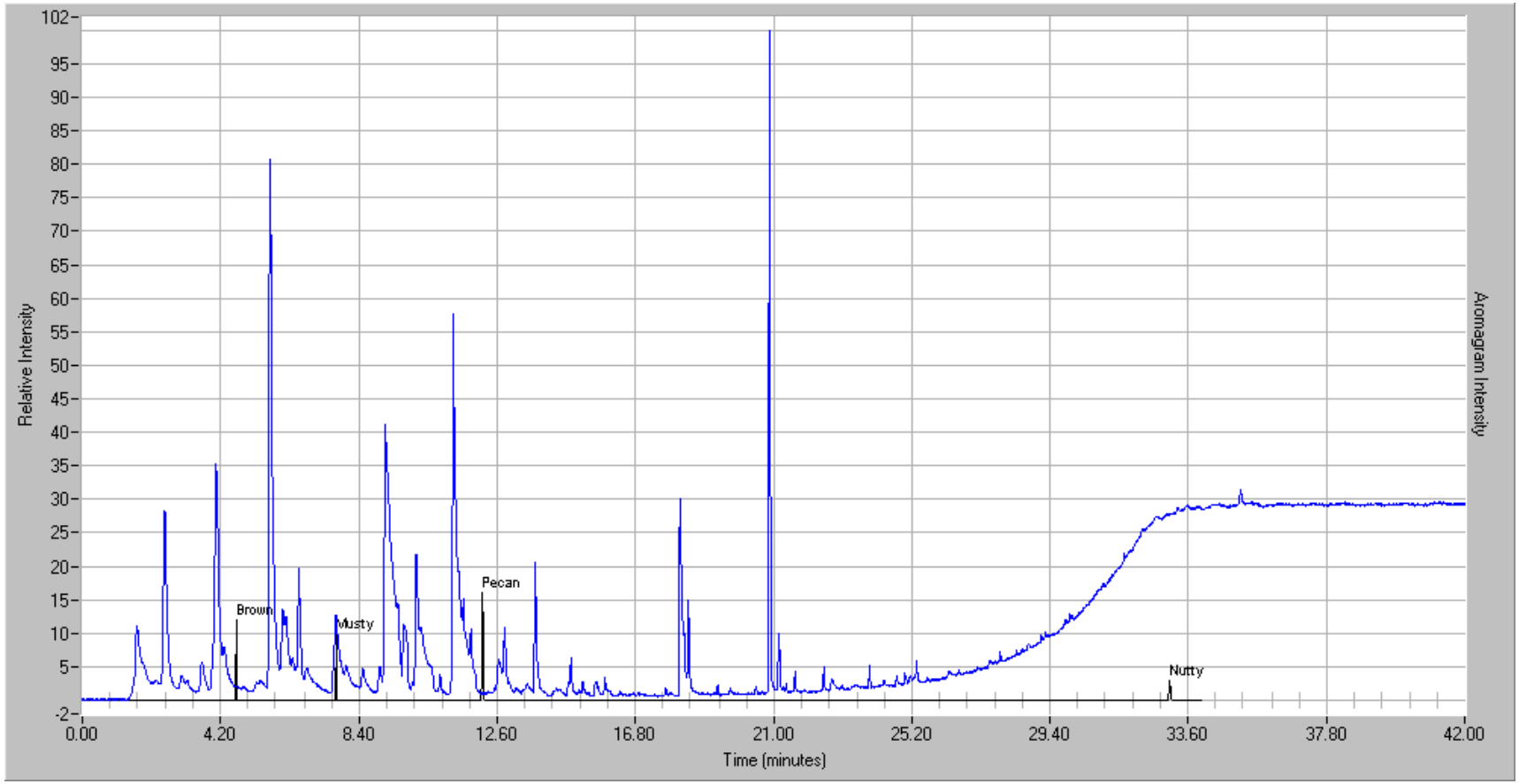
Gas chromatography-olfactometry chromatogram for roasted Pawnee sample (rep 1) performed by panelist 4



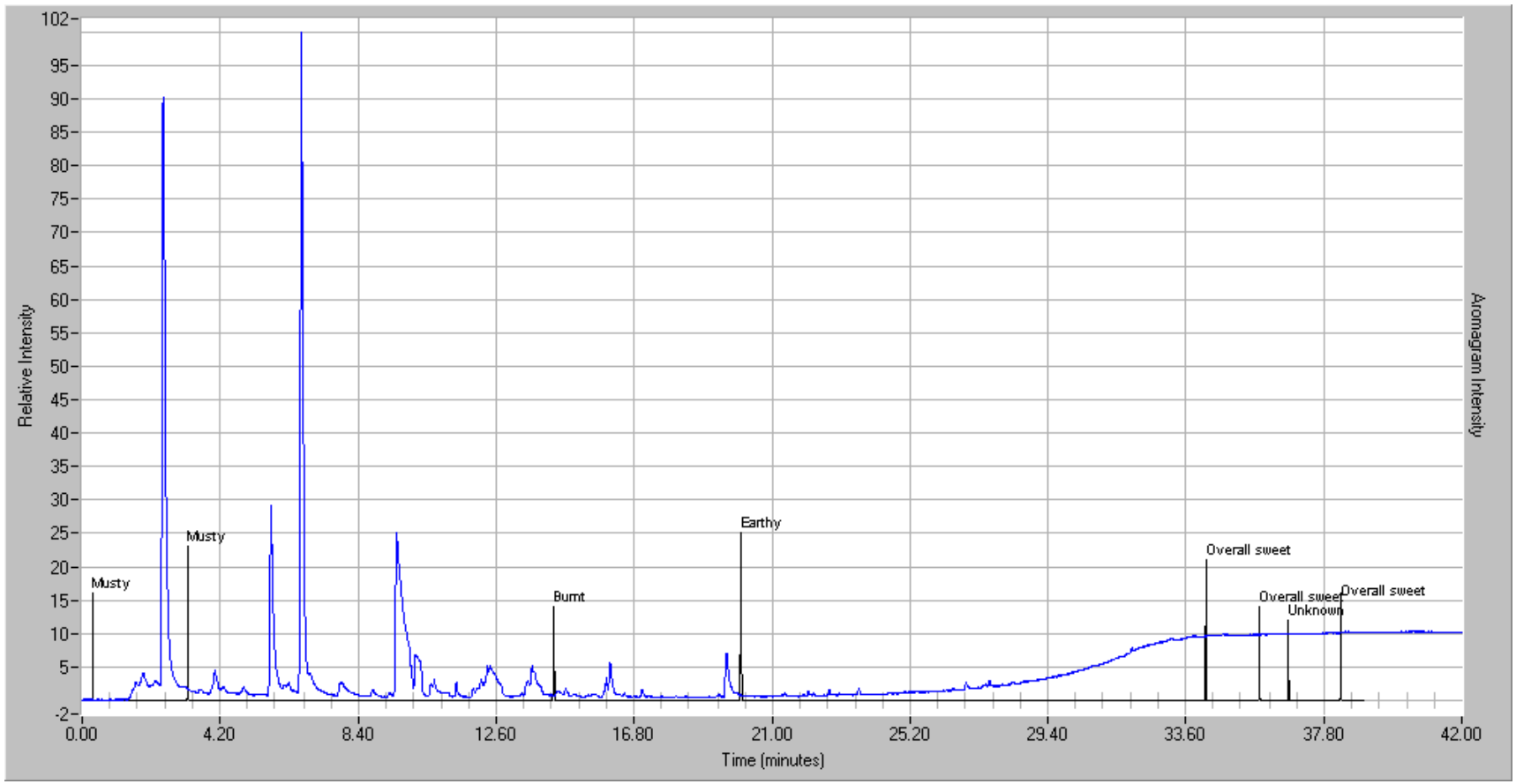
Gas chromatography-olfactometry chromatogram for roasted Pawnee sample (rep 2) performed by panelist 1



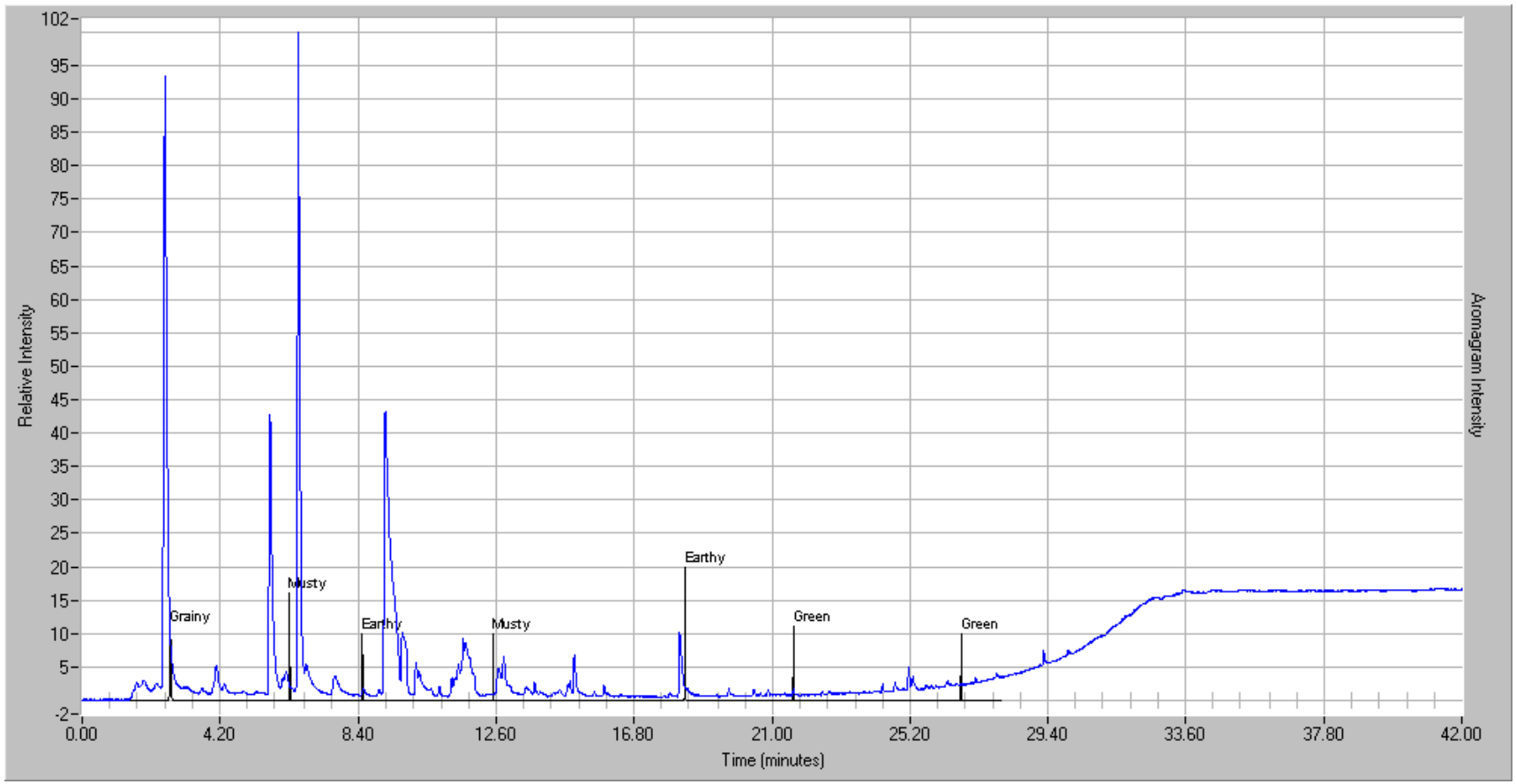
Gas chromatography-olfactometry chromatogram for roasted Pawnee sample (rep 3) performed by panelist 2



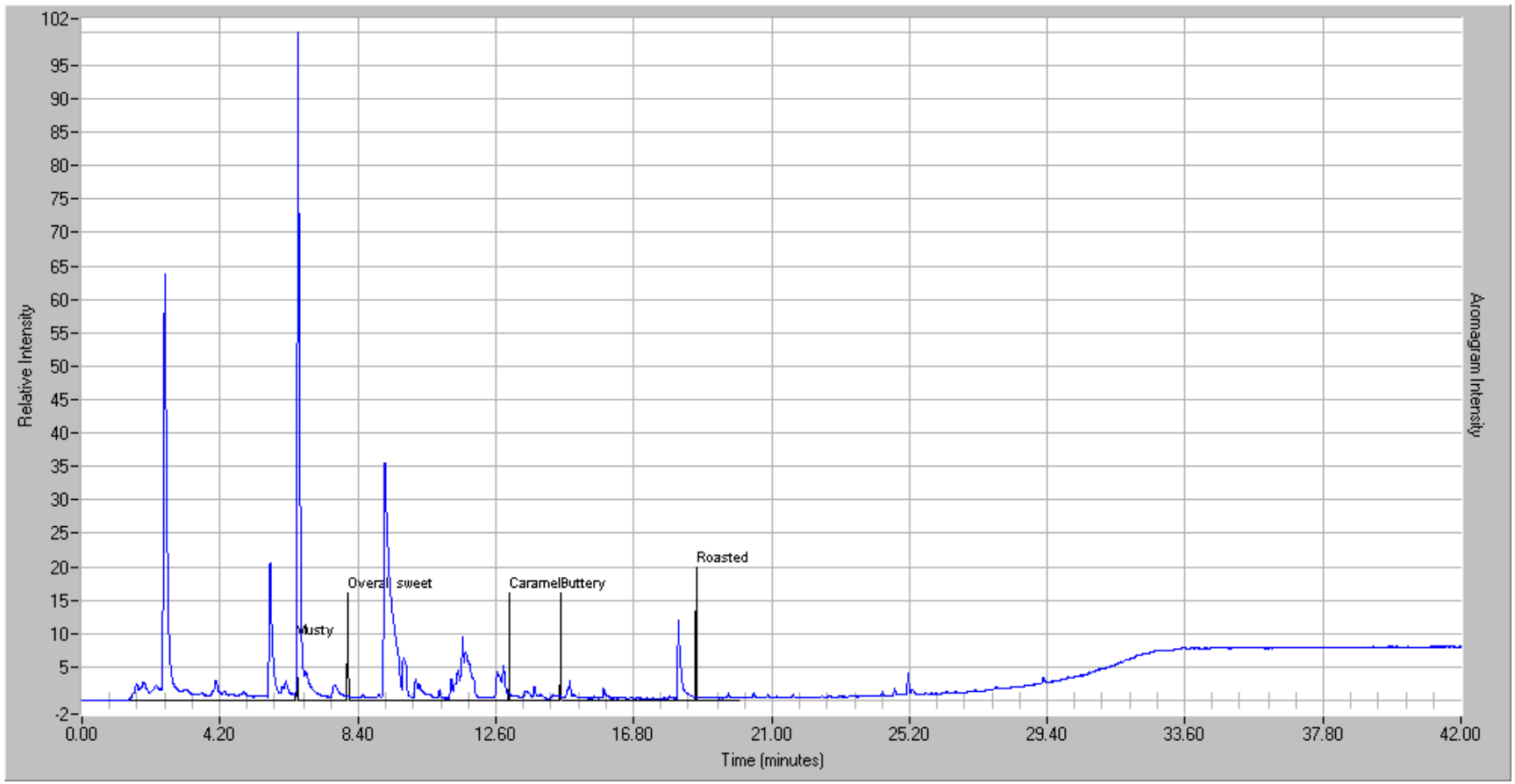
Gas chromatography-olfactometry chromatogram for raw Witte sample (rep 1) performed by panelist 4



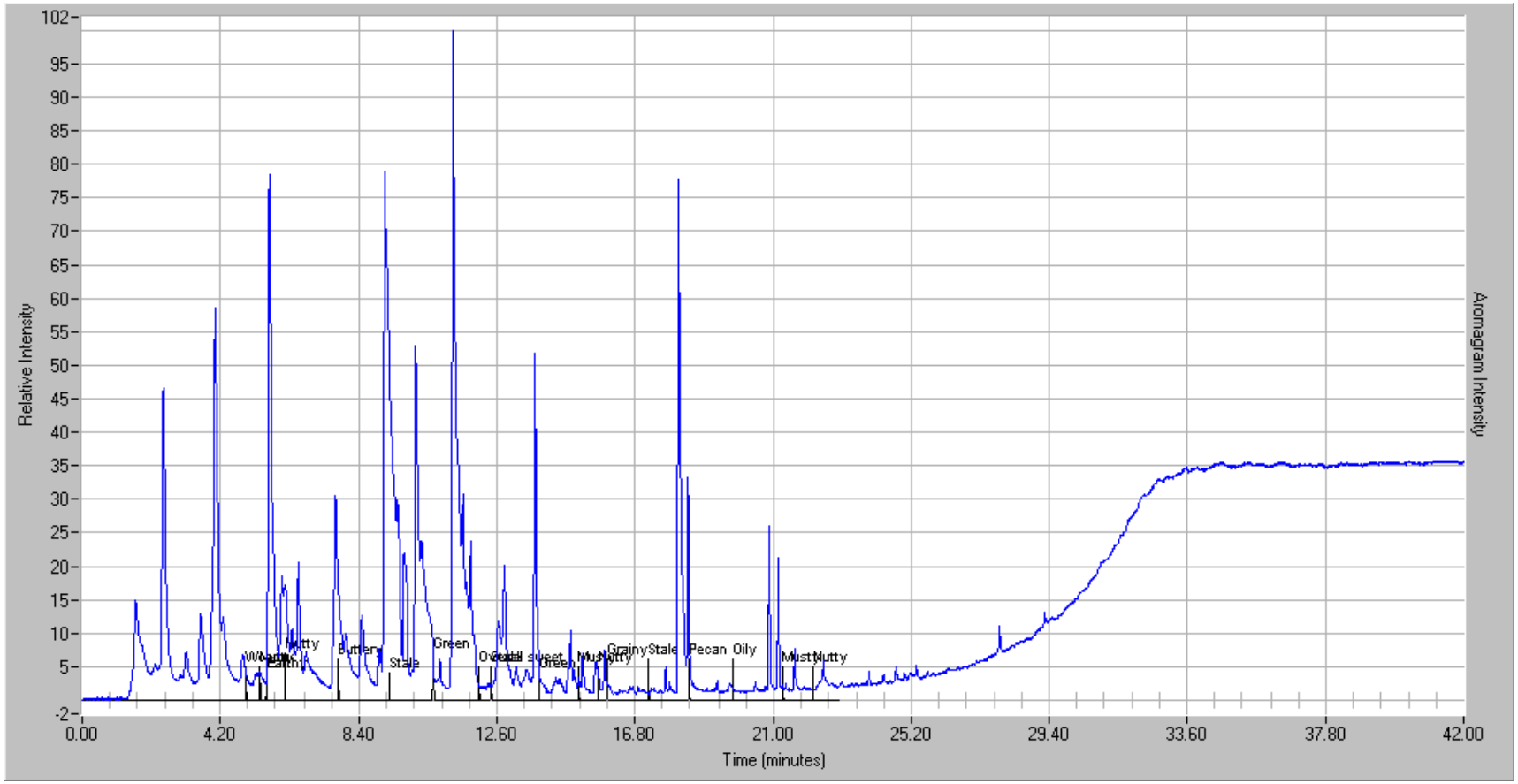
Gas chromatography-olfactometry chromatogram for raw Witte sample (rep 2) performed by panelist 4



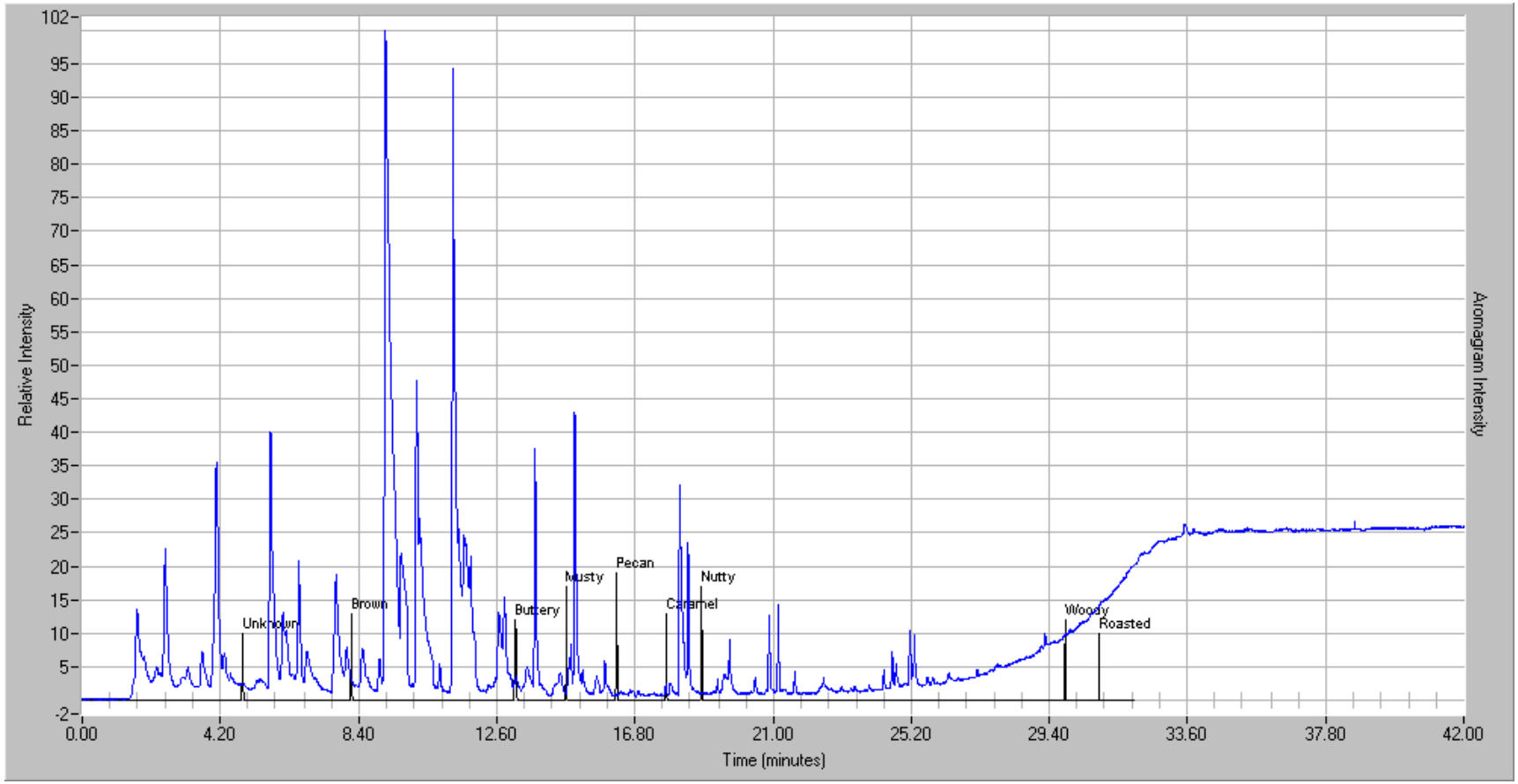
Gas chromatography-olfactometry chromatogram for raw Witte sample (rep 3) performed by panelist 1



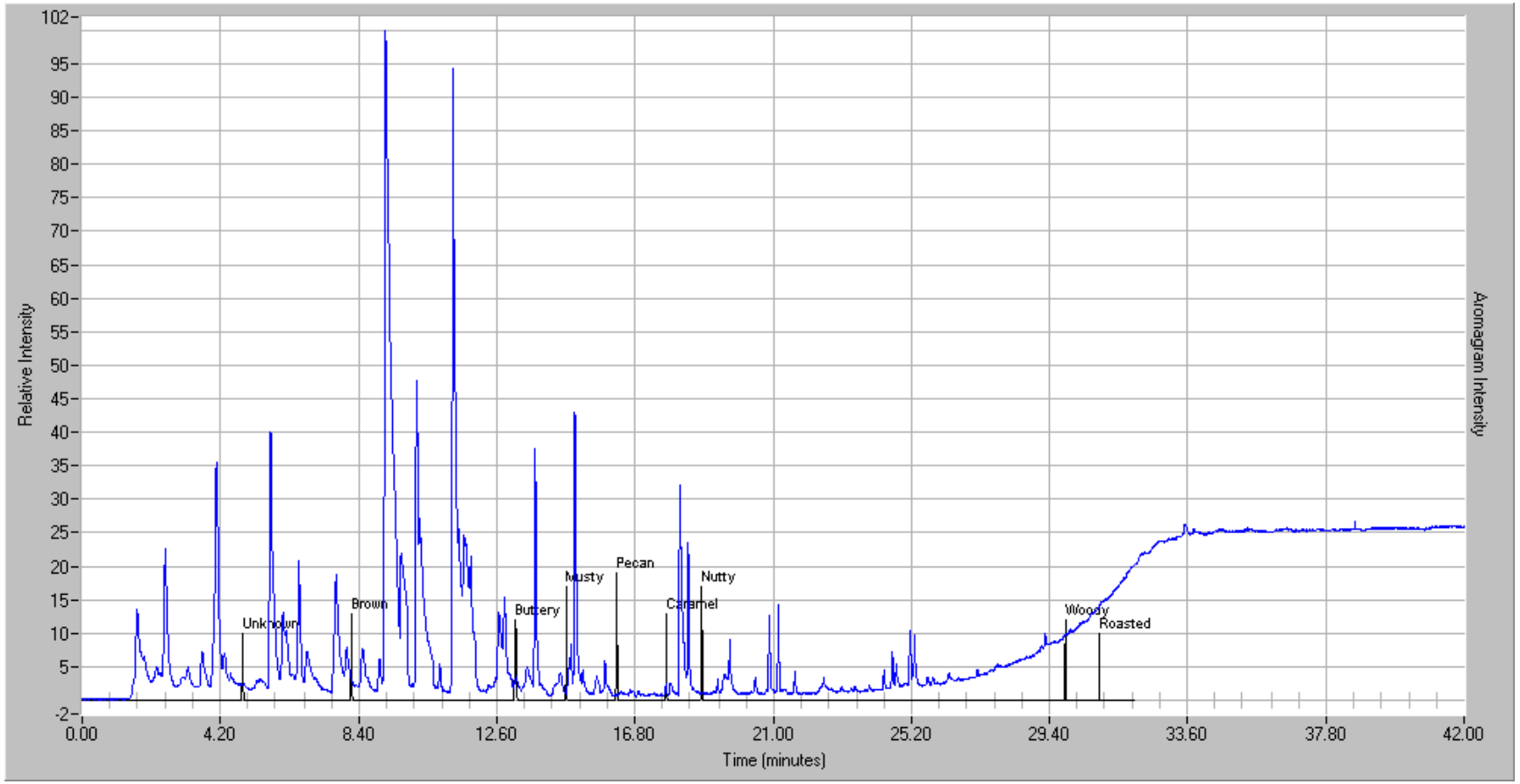
Gas chromatography-olfactometry chromatogram for roasted Witte sample (rep 1) performed by panelist 3



Gas chromatography-olfactometry chromatogram for roasted Witte sample (rep 2) performed by panelist 1

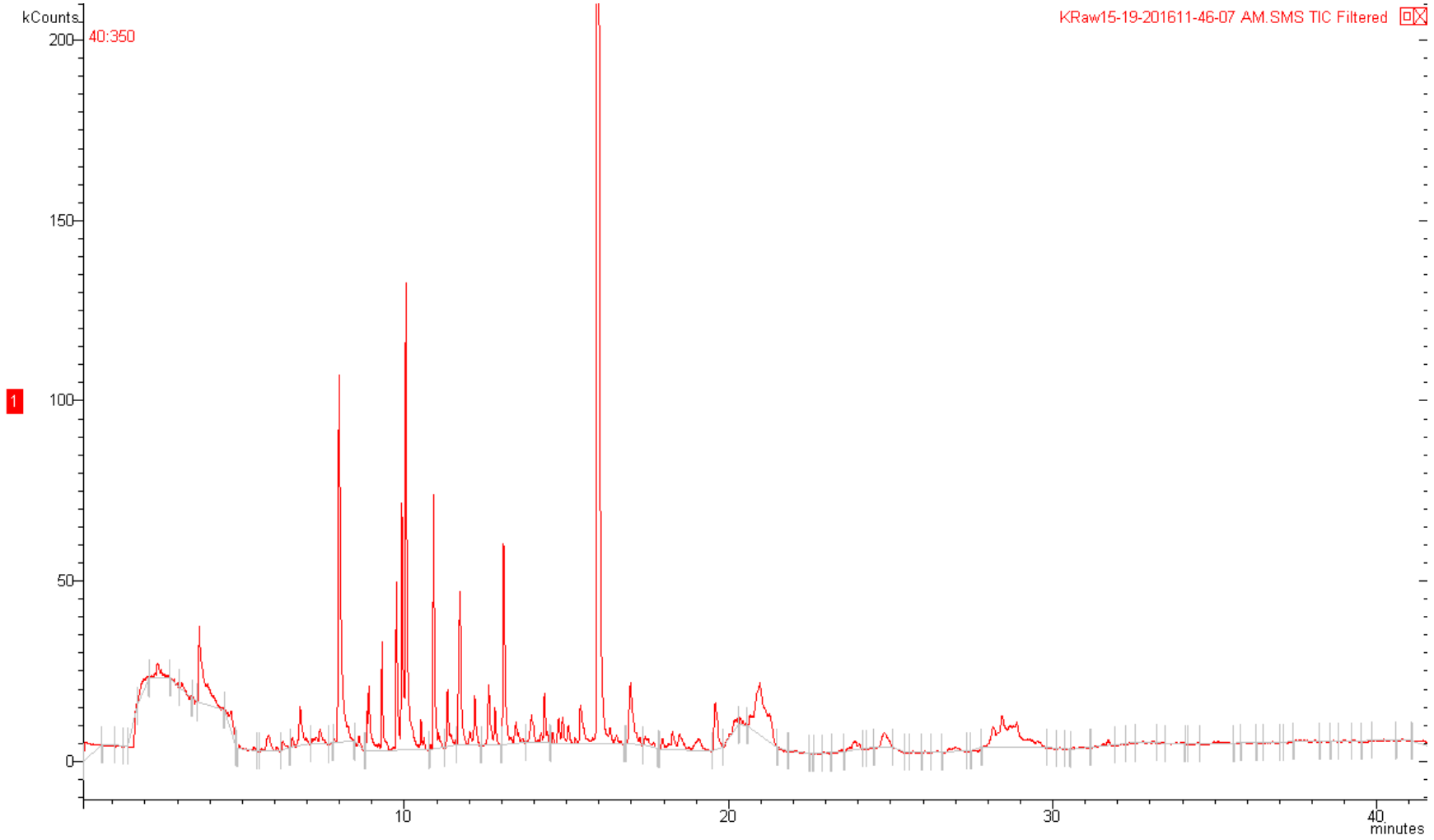


Gas chromatography-olfactometry chromatogram for roasted Witte sample (rep 3) performed by panelist 2

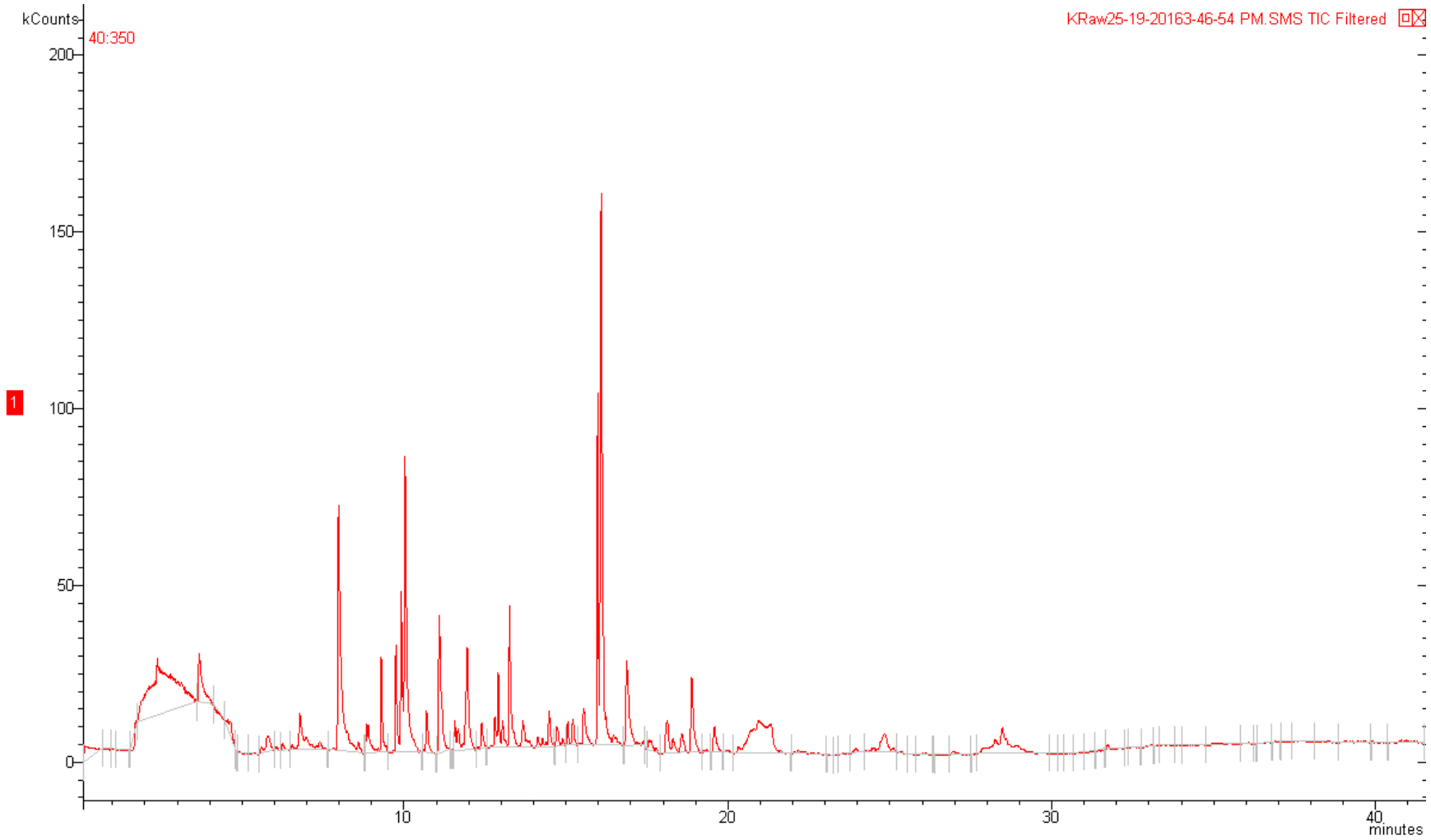


Appendix K - Gas Chromatography-Mass Spectrometry Output

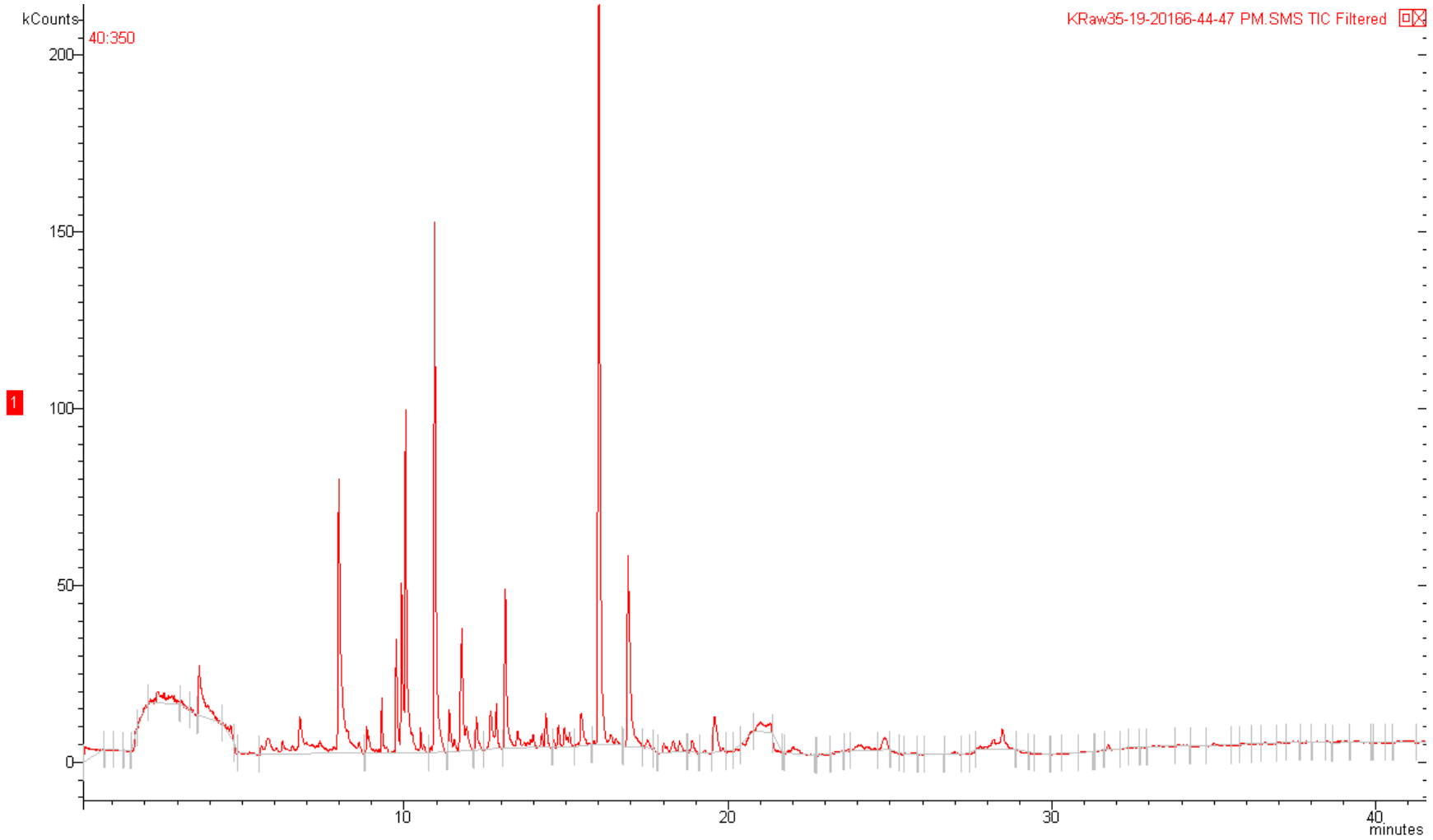
Gas chromatography-mass spectrometry chromatogram for raw Kanza sample (rep 1)



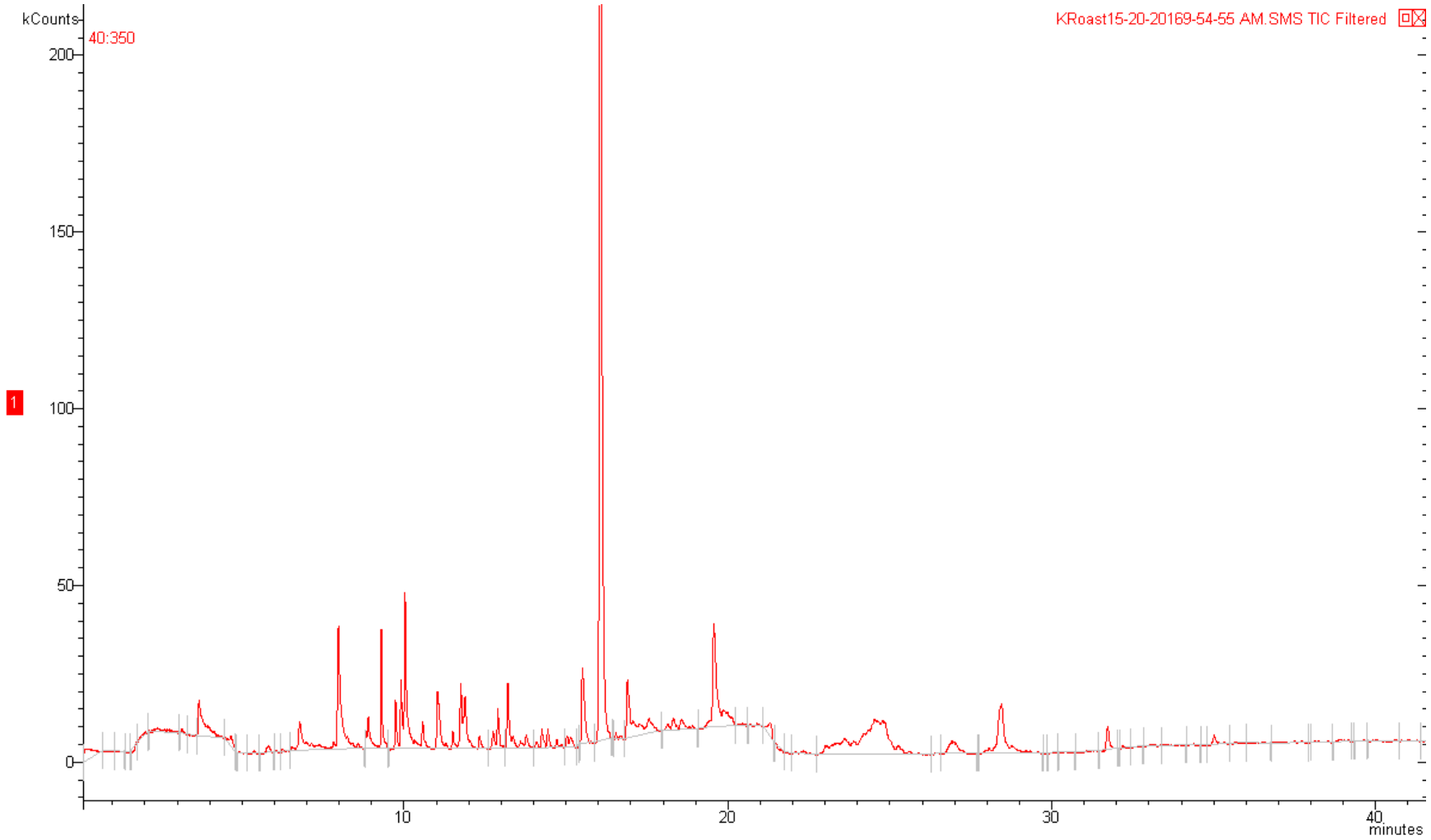
Gas chromatography-mass spectrometry chromatogram for raw Kanza sample (rep 2)



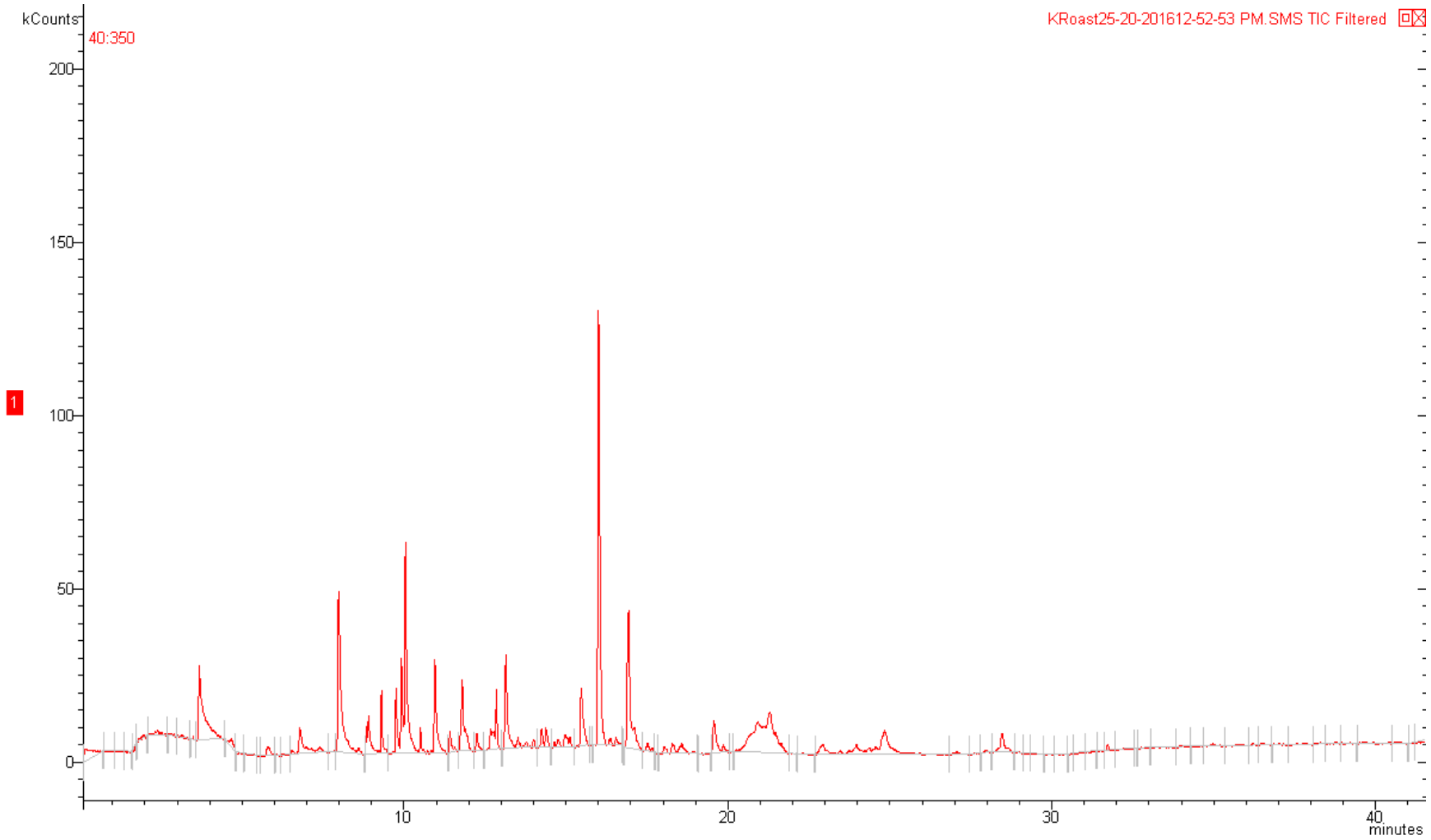
Gas chromatography-mass spectrometry chromatogram for raw Kanza sample (rep 3)



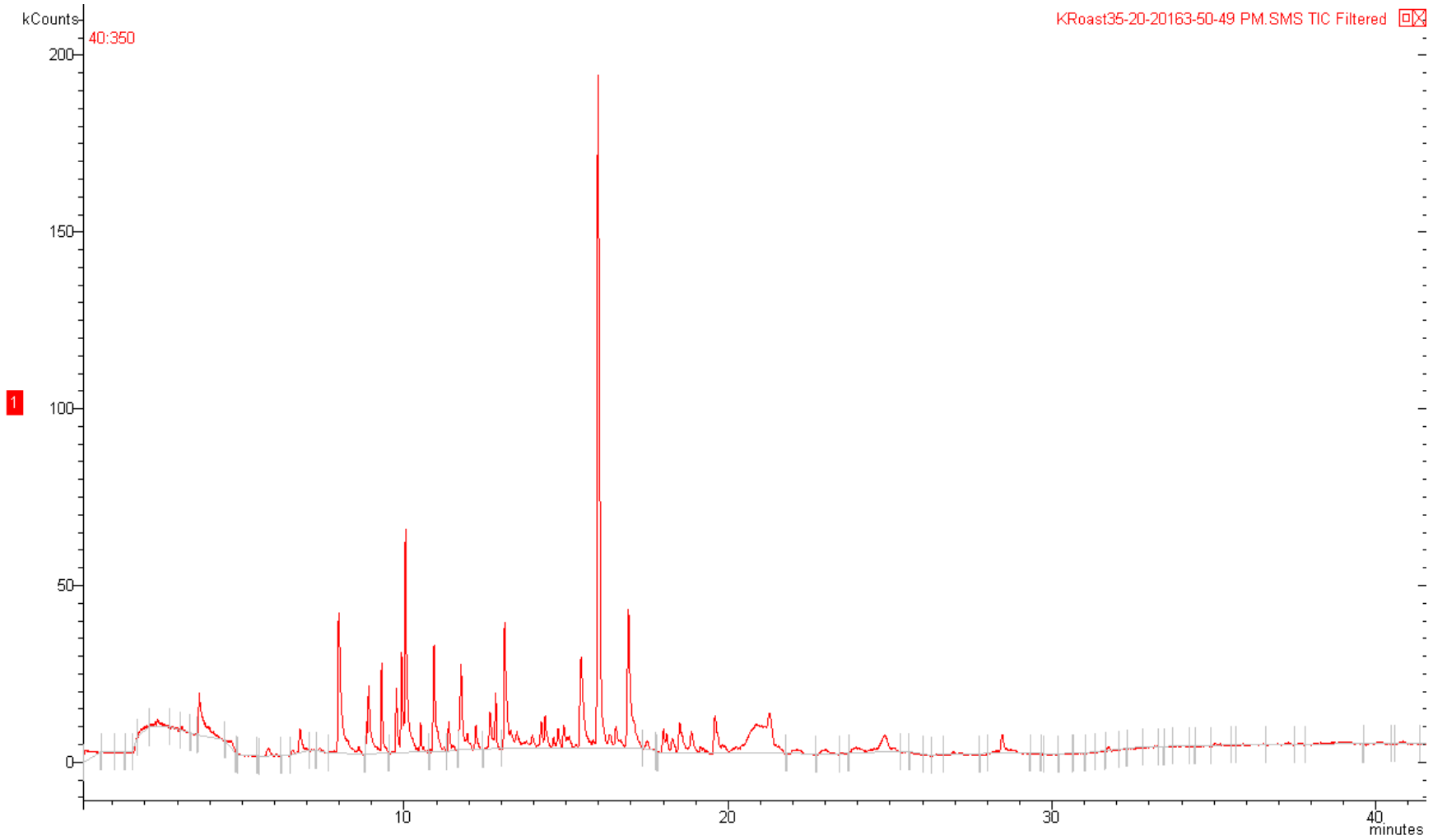
Gas chromatography-mass spectrometry chromatogram for roasted Kanza sample (rep 1)



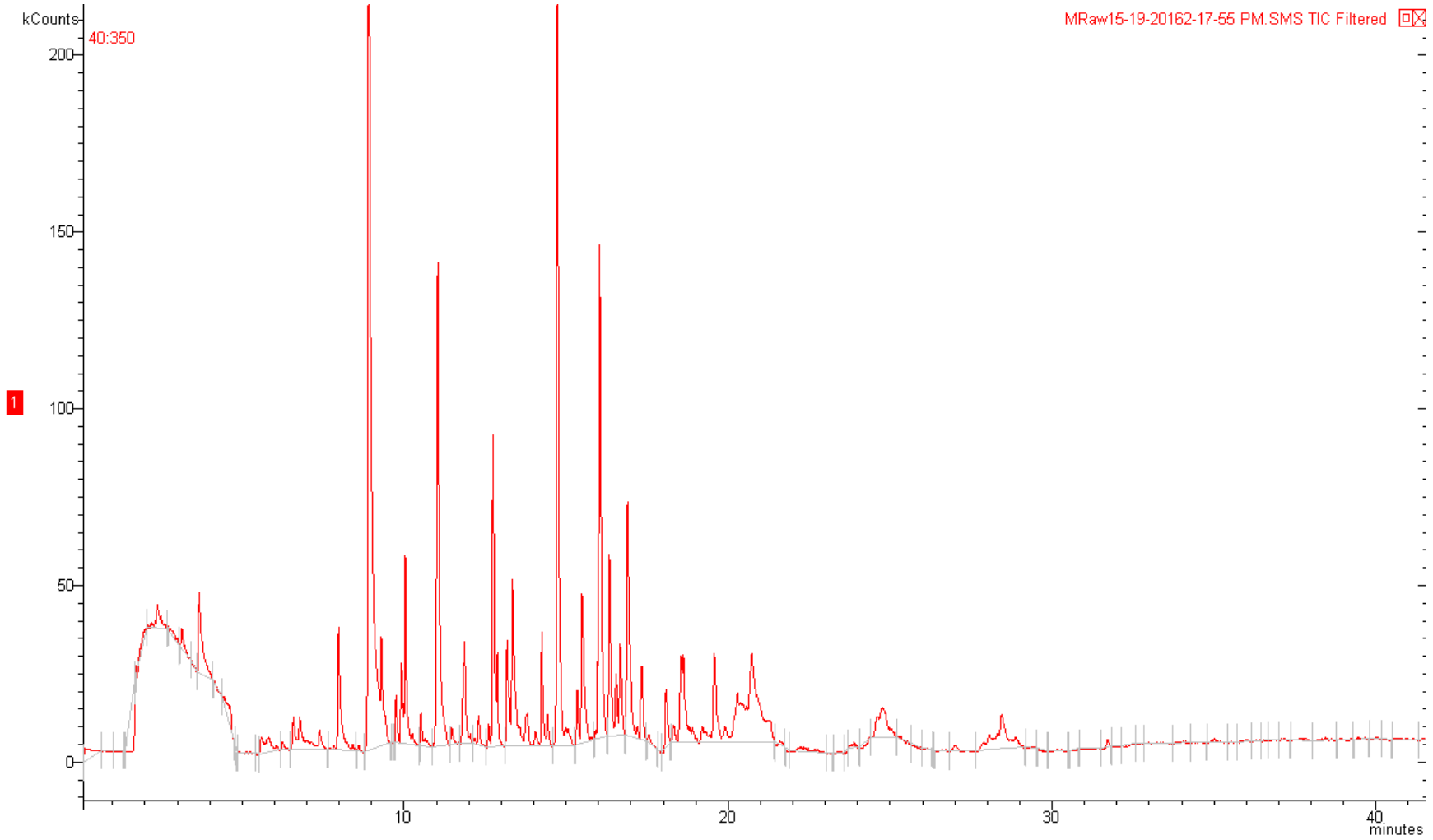
Gas chromatography-mass spectrometry chromatogram for roasted Kanza sample (rep 2)



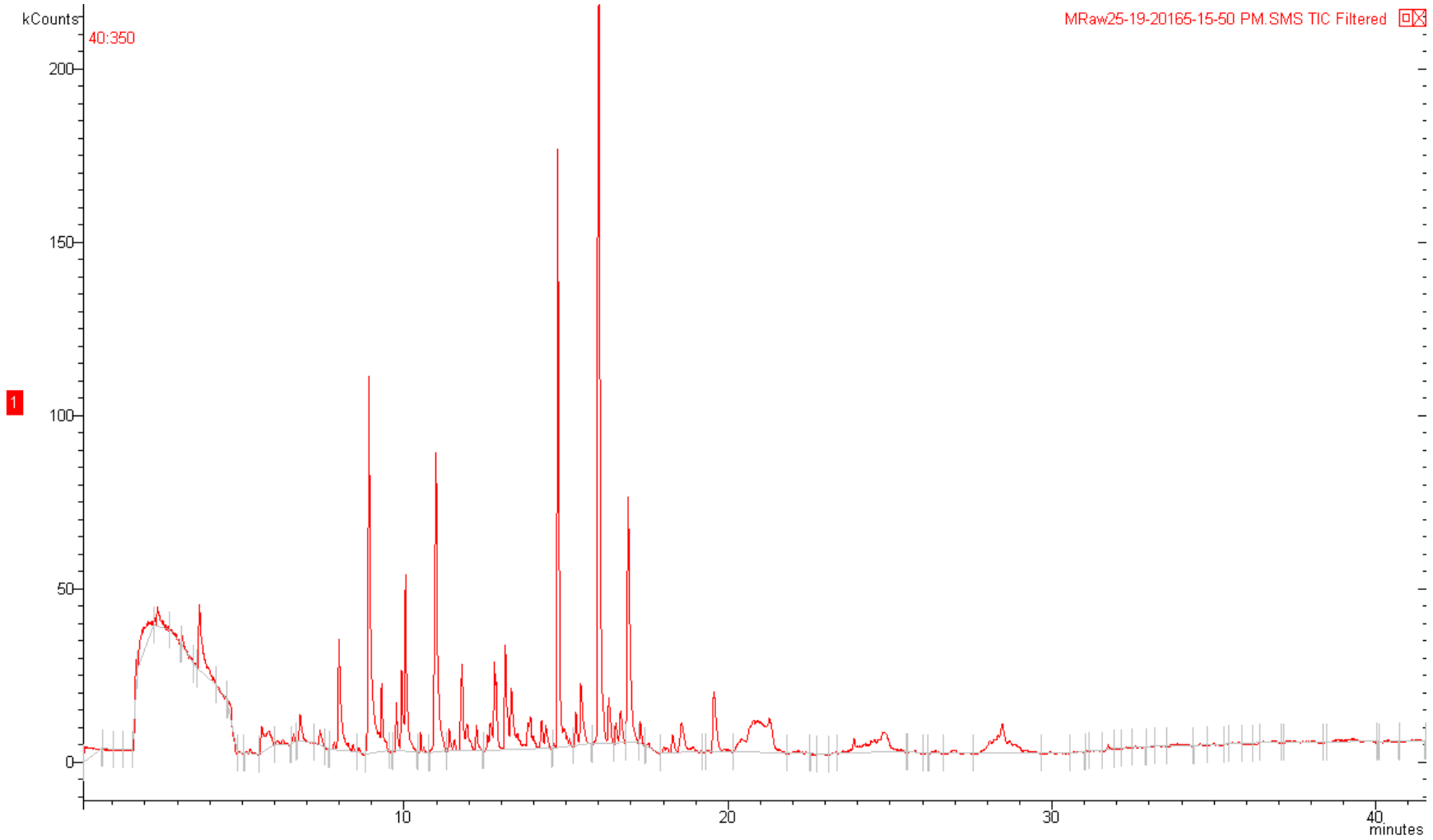
Gas chromatography-mass spectrometry chromatogram for roasted Kanza sample (rep 3)



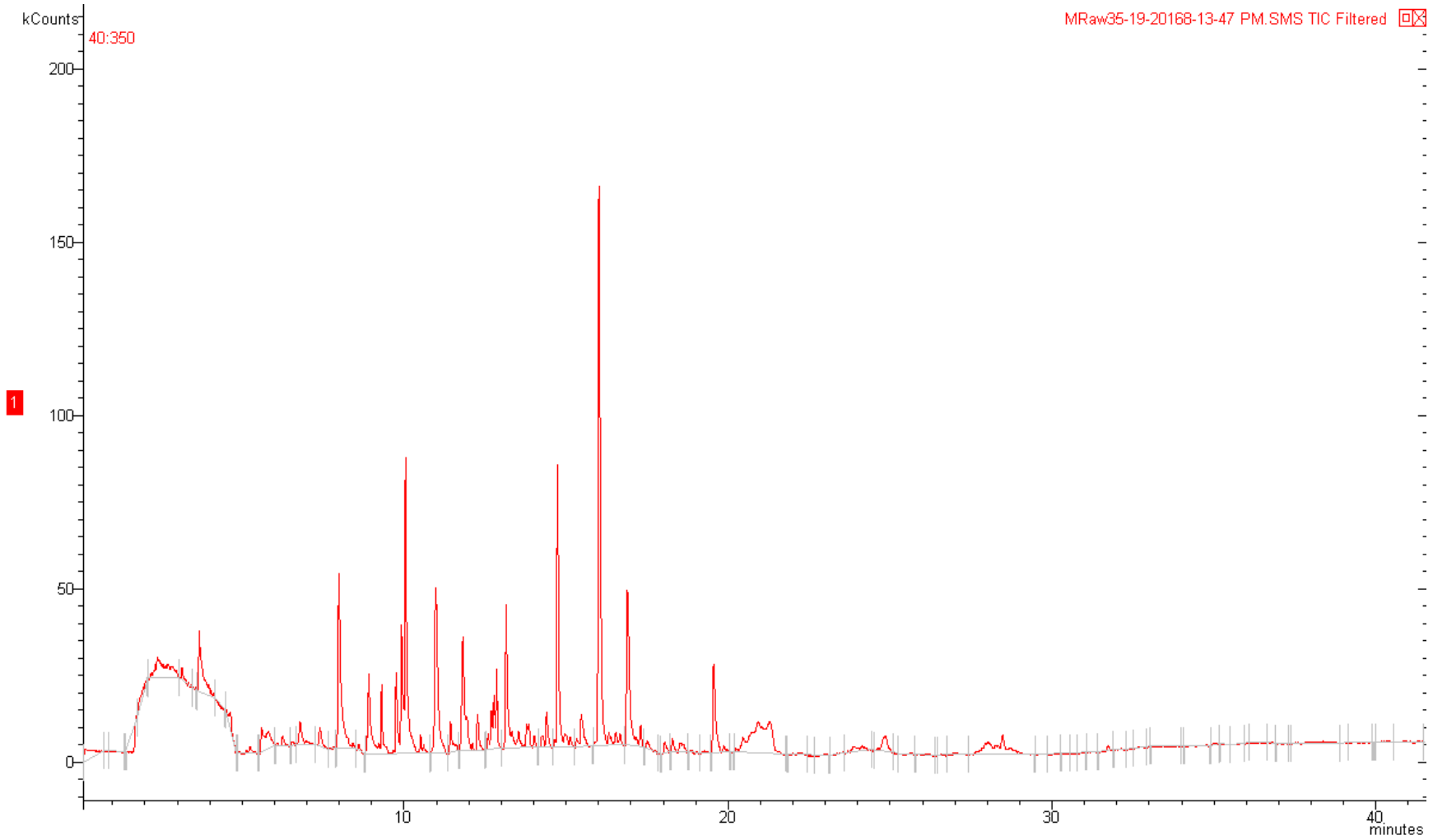
Gas chromatography-mass spectrometry chromatogram for raw Maramec sample (rep 1)



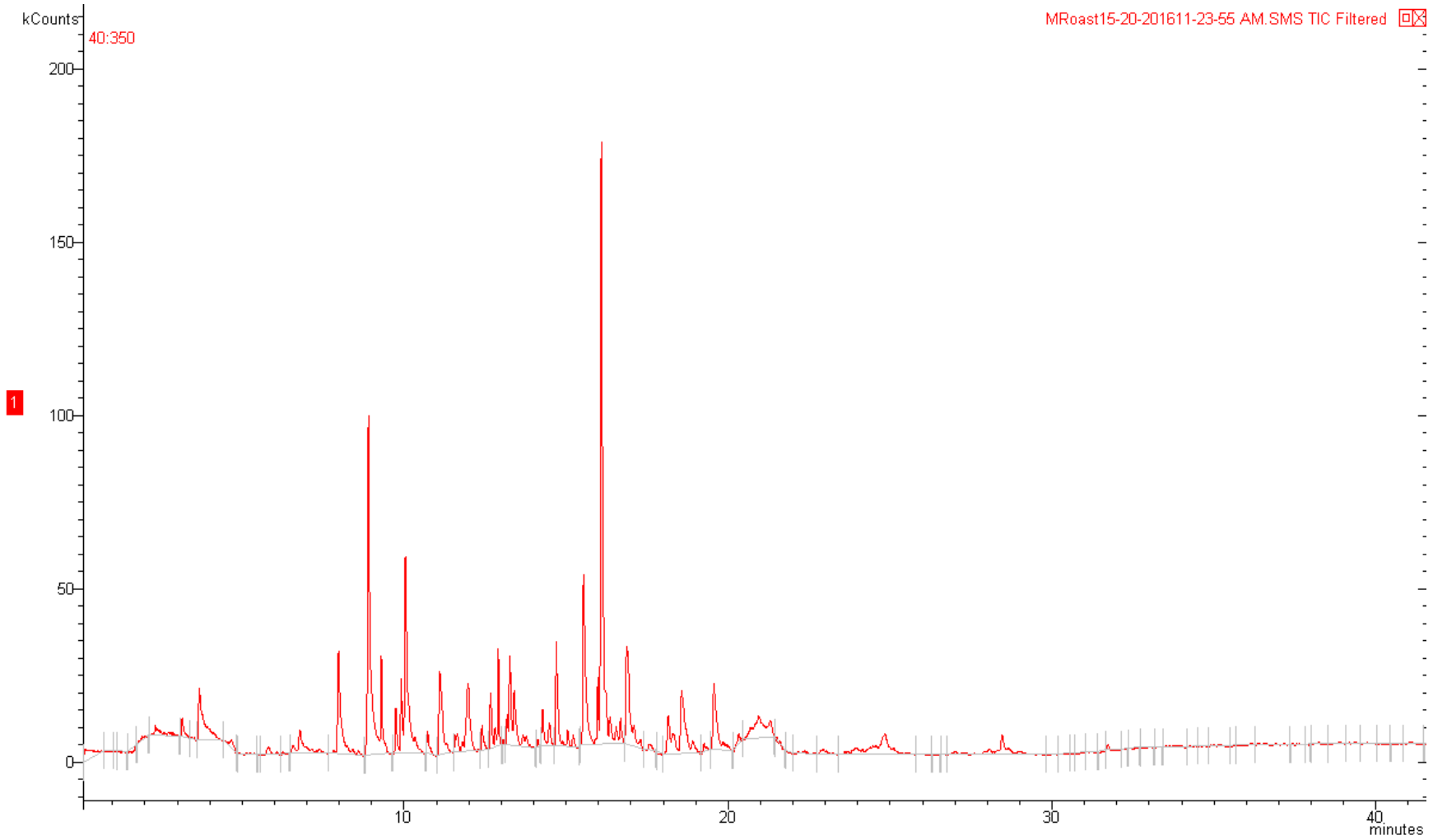
Gas chromatography-mass spectrometry chromatogram for raw Maramec sample (rep 2)



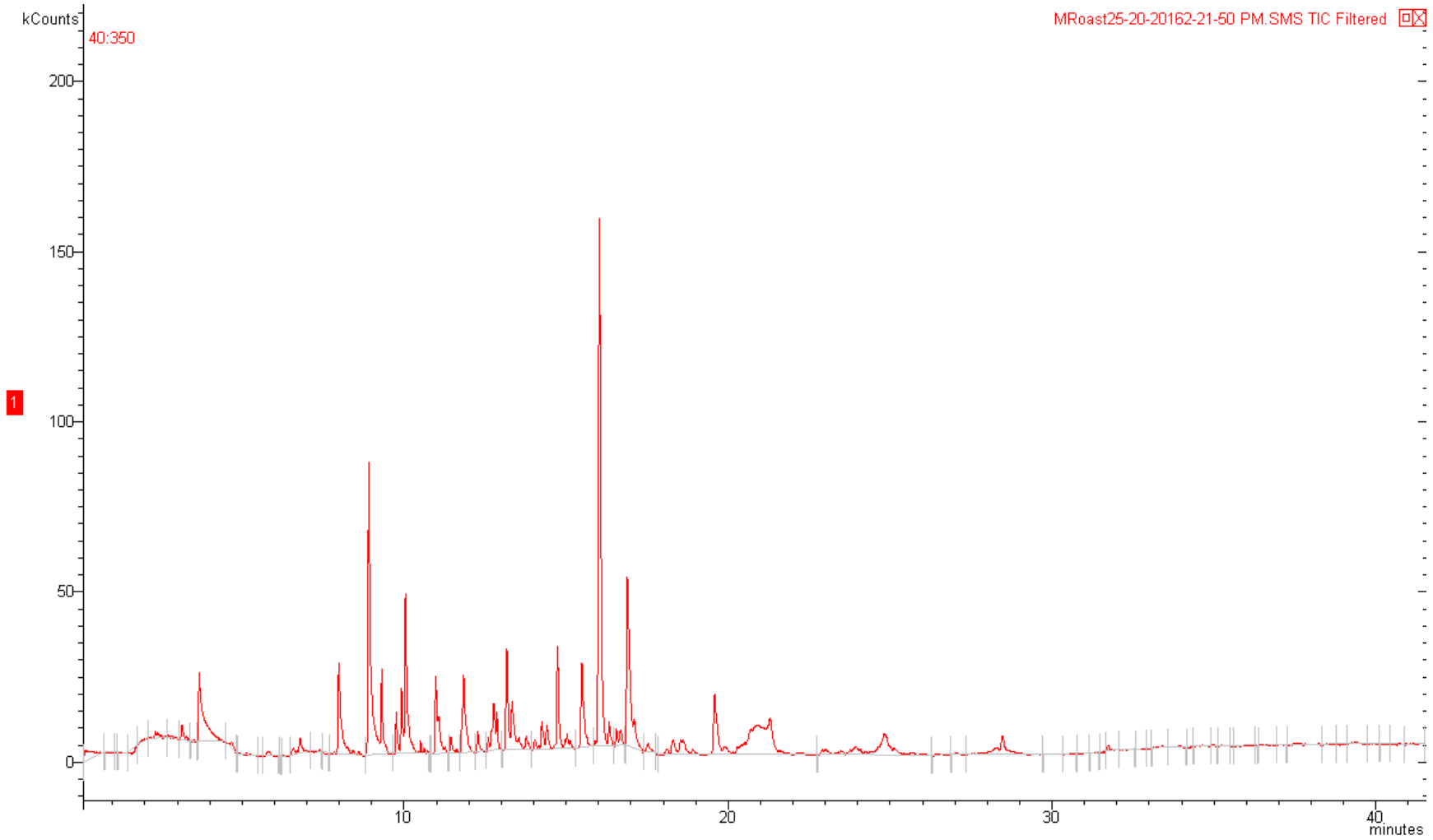
Gas chromatography-mass spectrometry chromatogram for raw Maramec sample (rep 3)



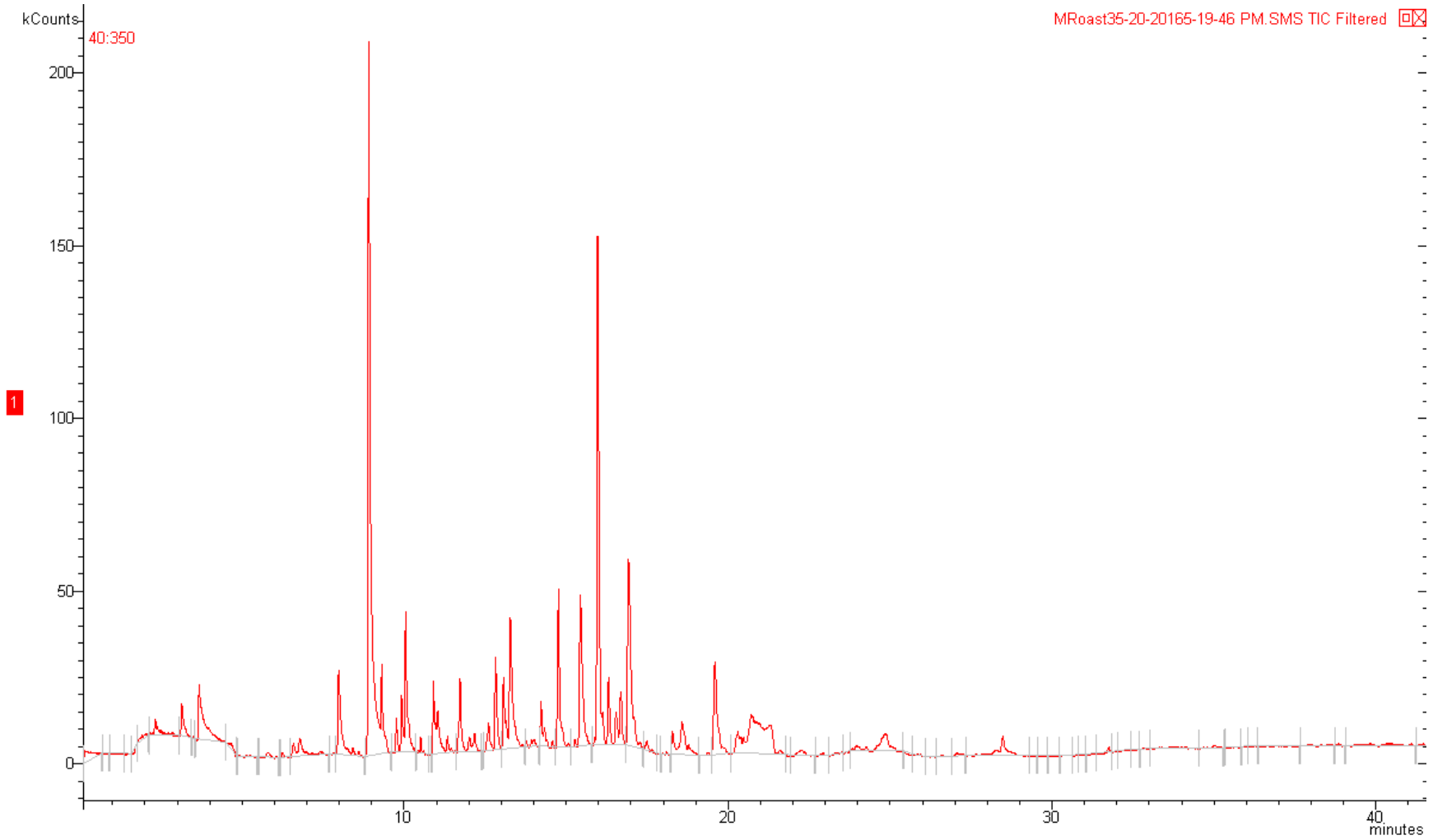
Gas chromatography-mass spectrometry chromatogram for roasted Maramec sample (rep 1)



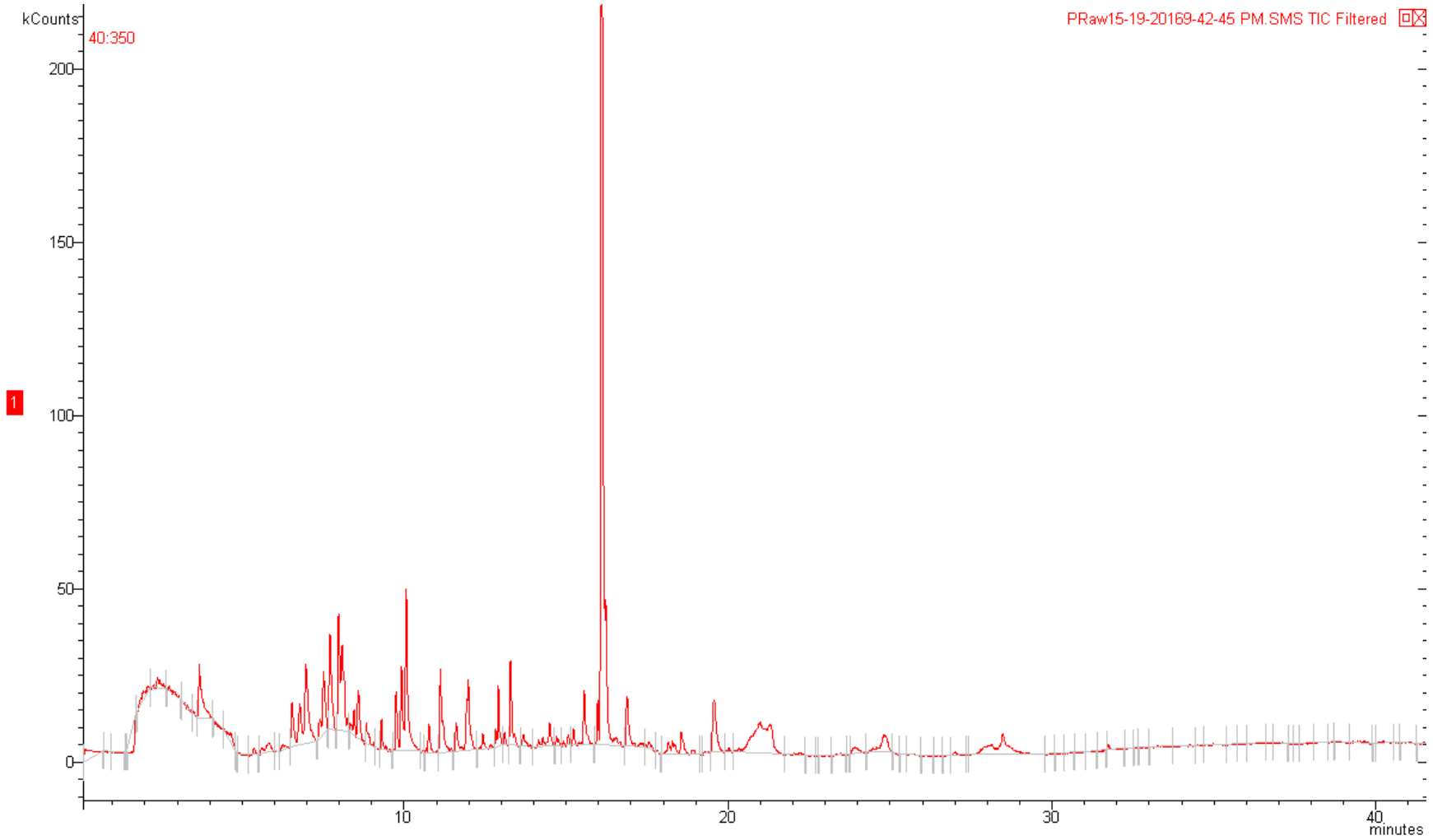
Gas chromatography-mass spectrometry chromatogram for roasted Maramec sample (rep 2)



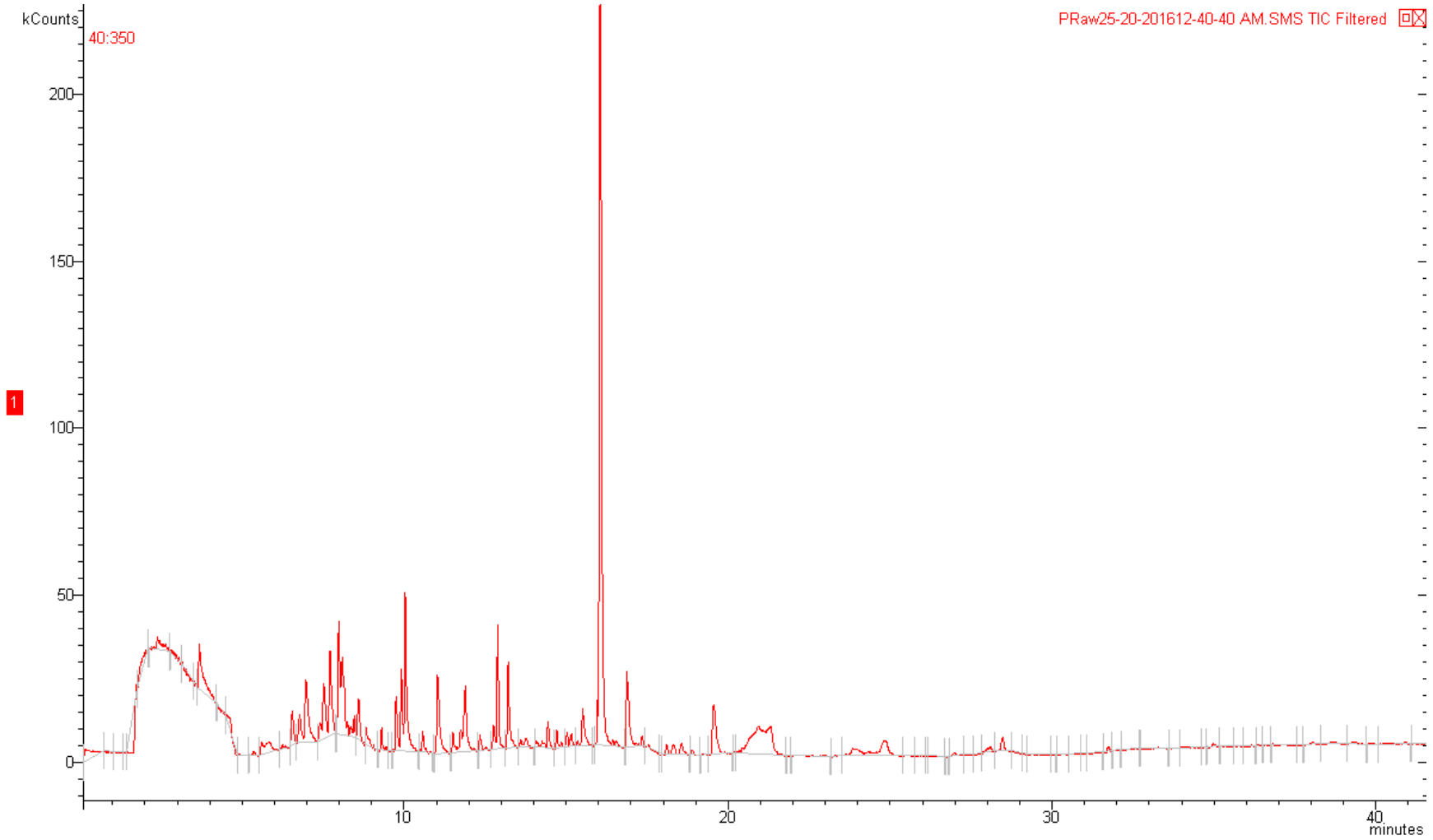
Gas chromatography-mass spectrometry chromatogram for roasted Maramec sample (rep 3)



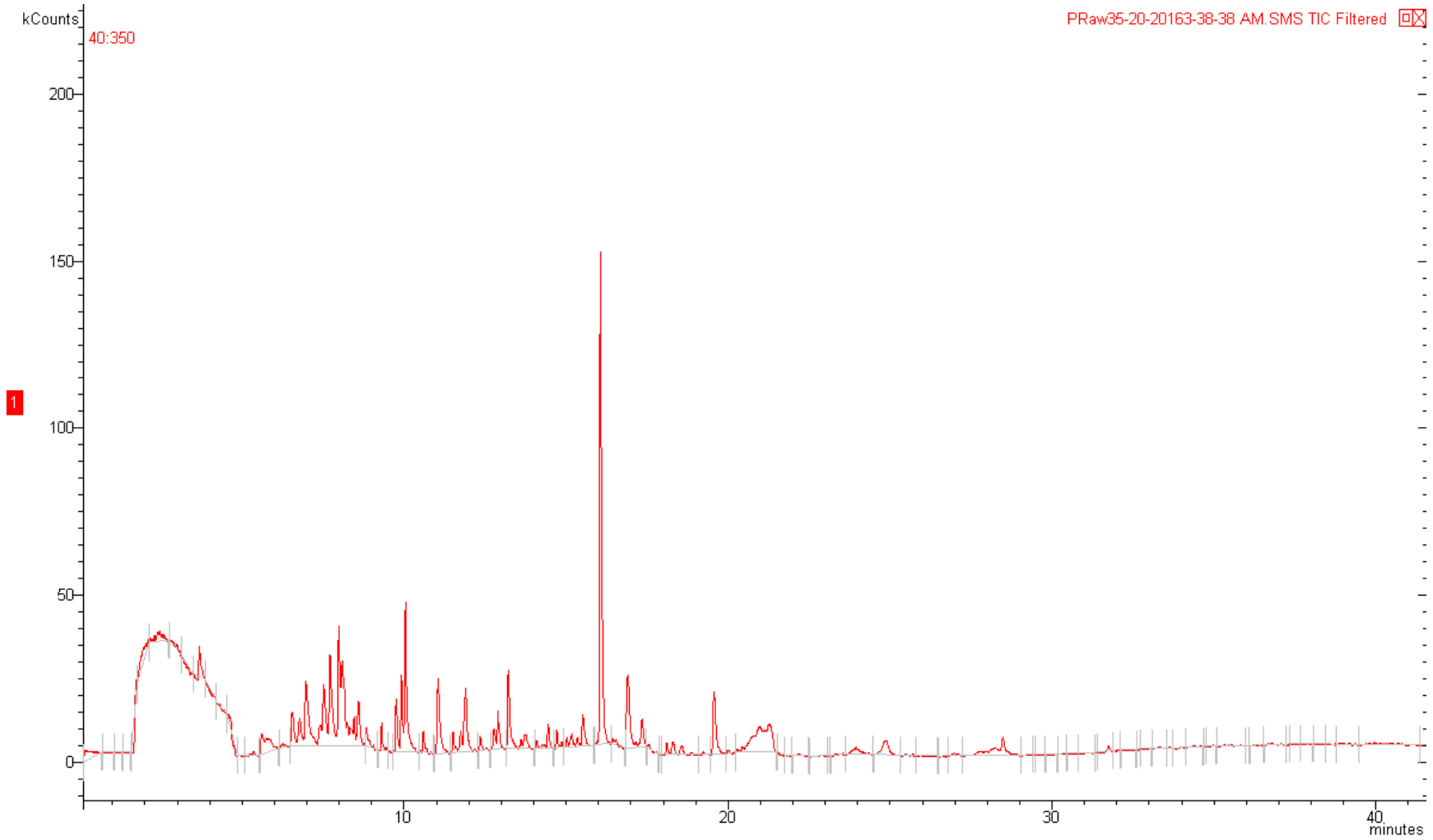
Gas chromatography-mass spectrometry chromatogram for raw Pawnee sample (rep 1)



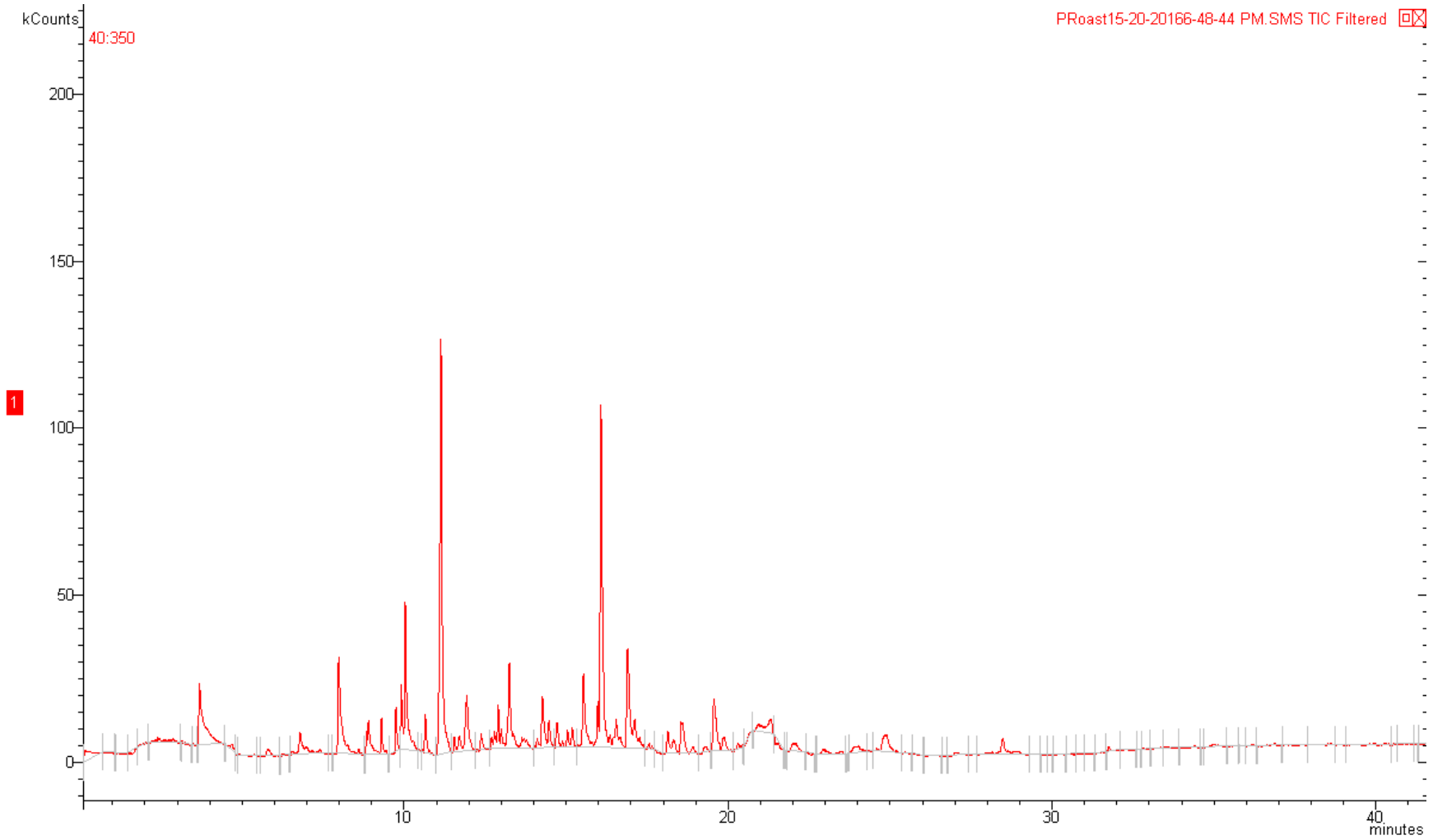
Gas chromatography-mass spectrometry chromatogram for raw Pawnee sample (rep 2)



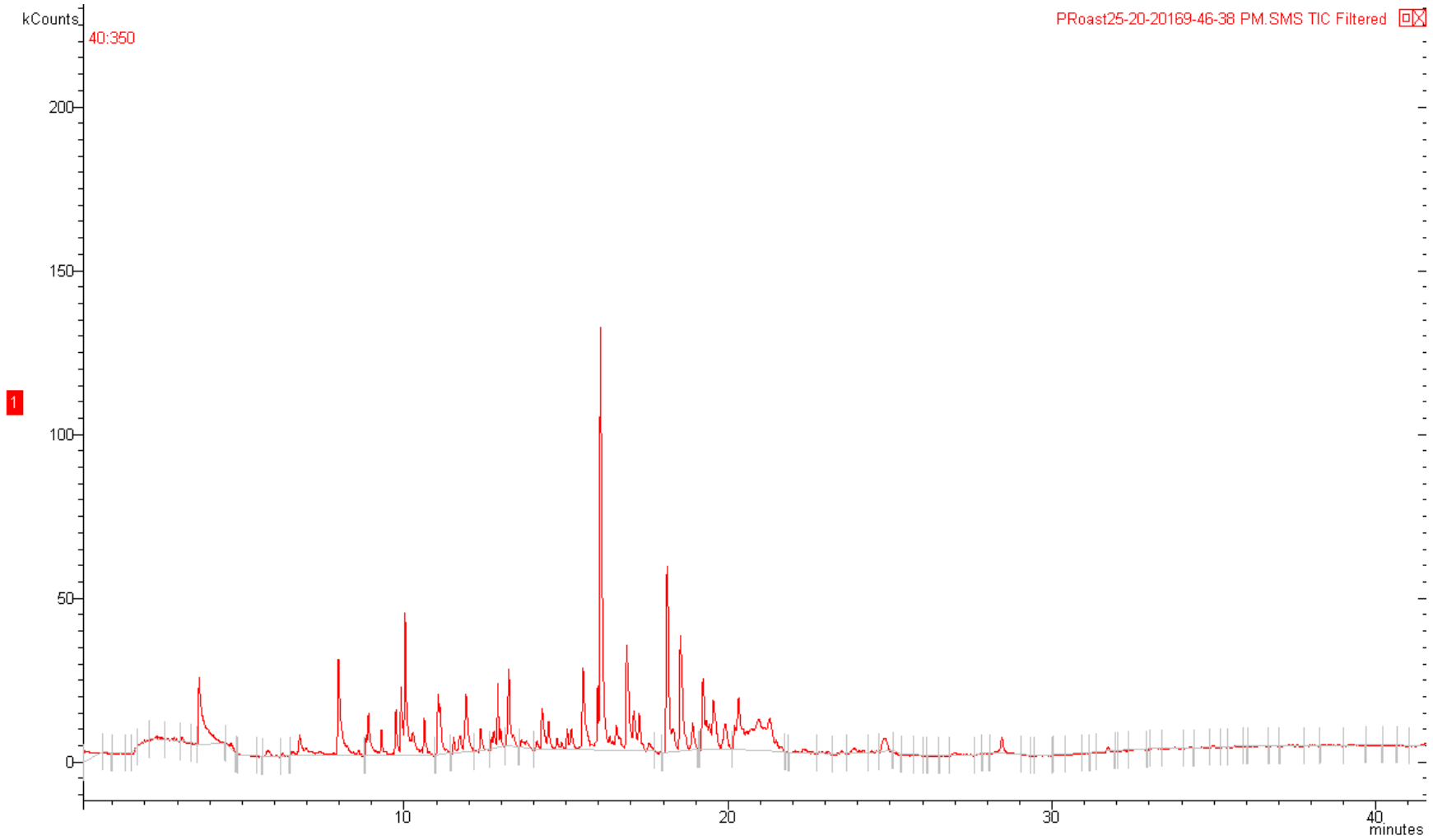
Gas chromatography-mass spectrometry chromatogram for raw Pawnee sample (rep 3)



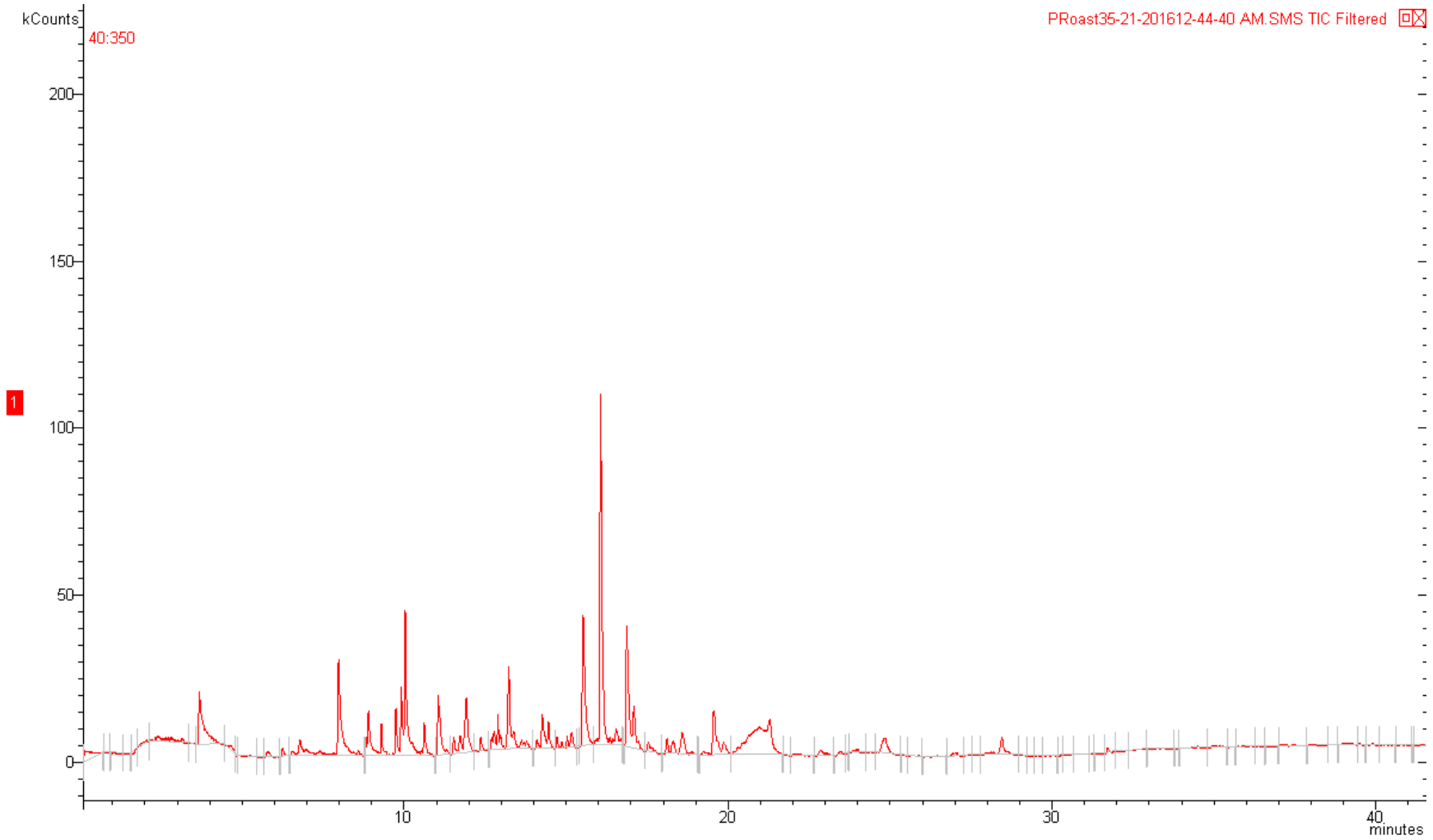
Gas chromatography-mass spectrometry chromatogram for roasted Pawnee sample (rep 1)



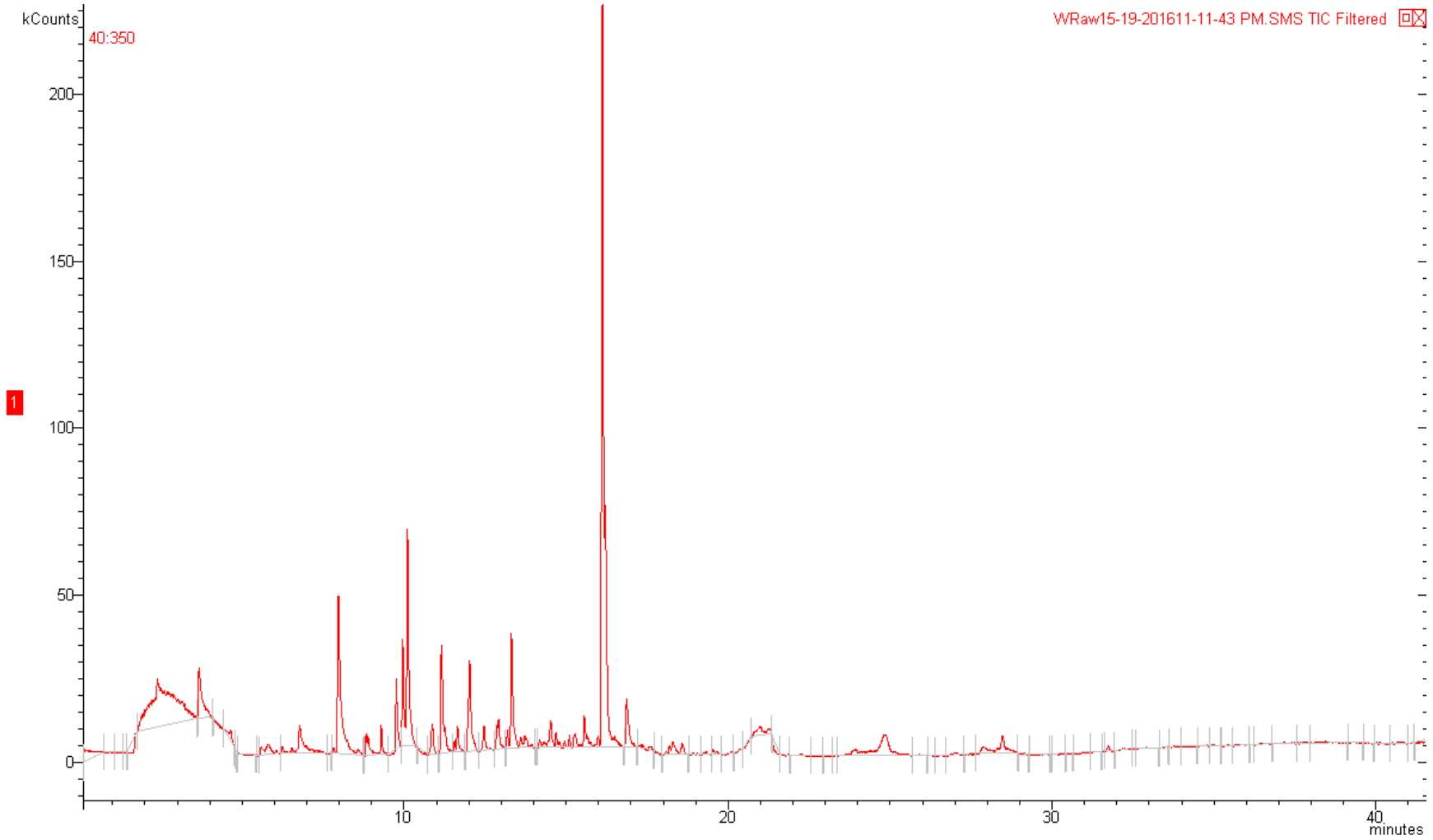
Gas chromatography-mass spectrometry chromatogram for roasted Pawnee sample (rep 2)



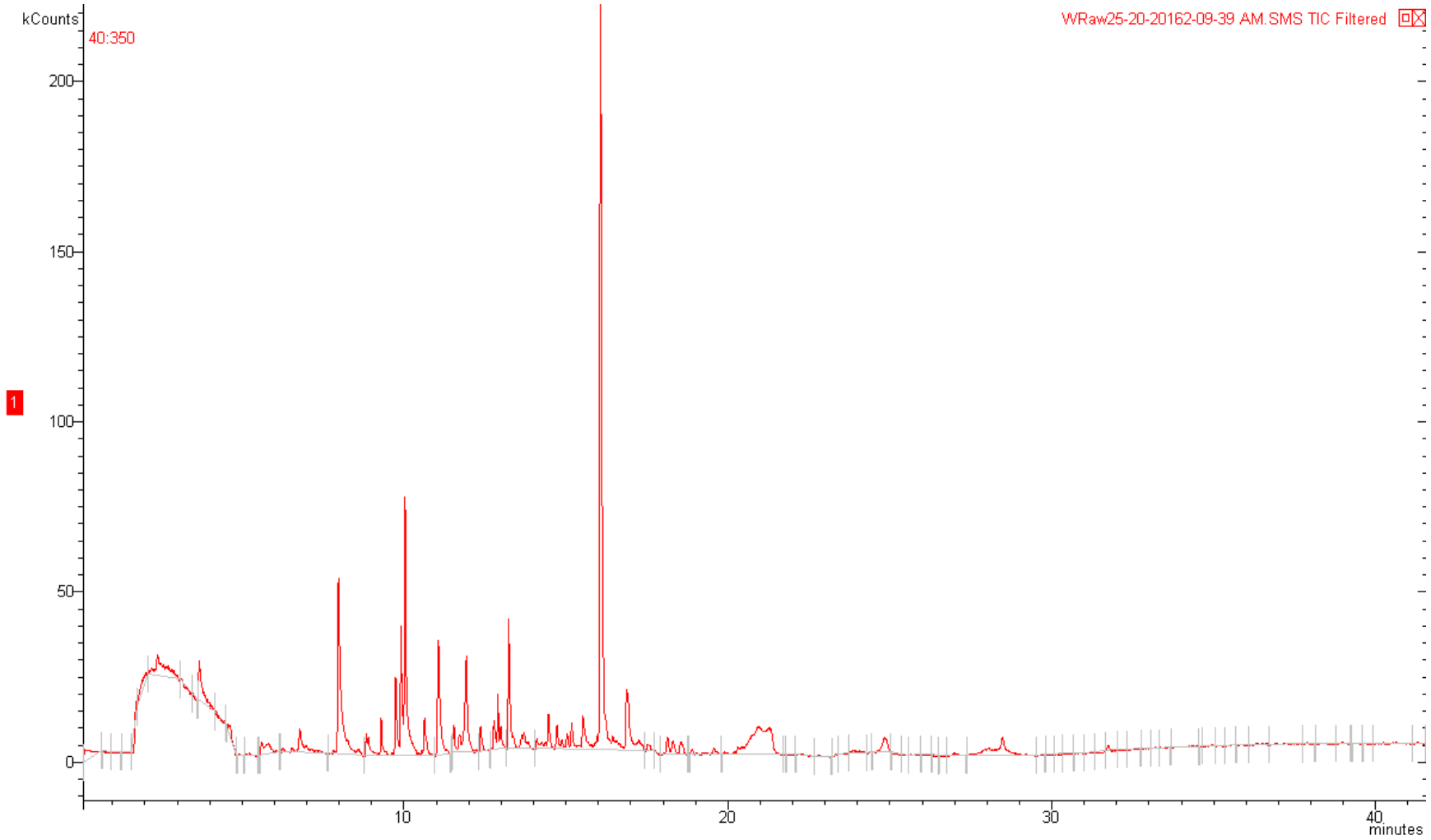
Gas chromatography-mass spectrometry chromatogram for roasted Pawnee sample (rep 3)



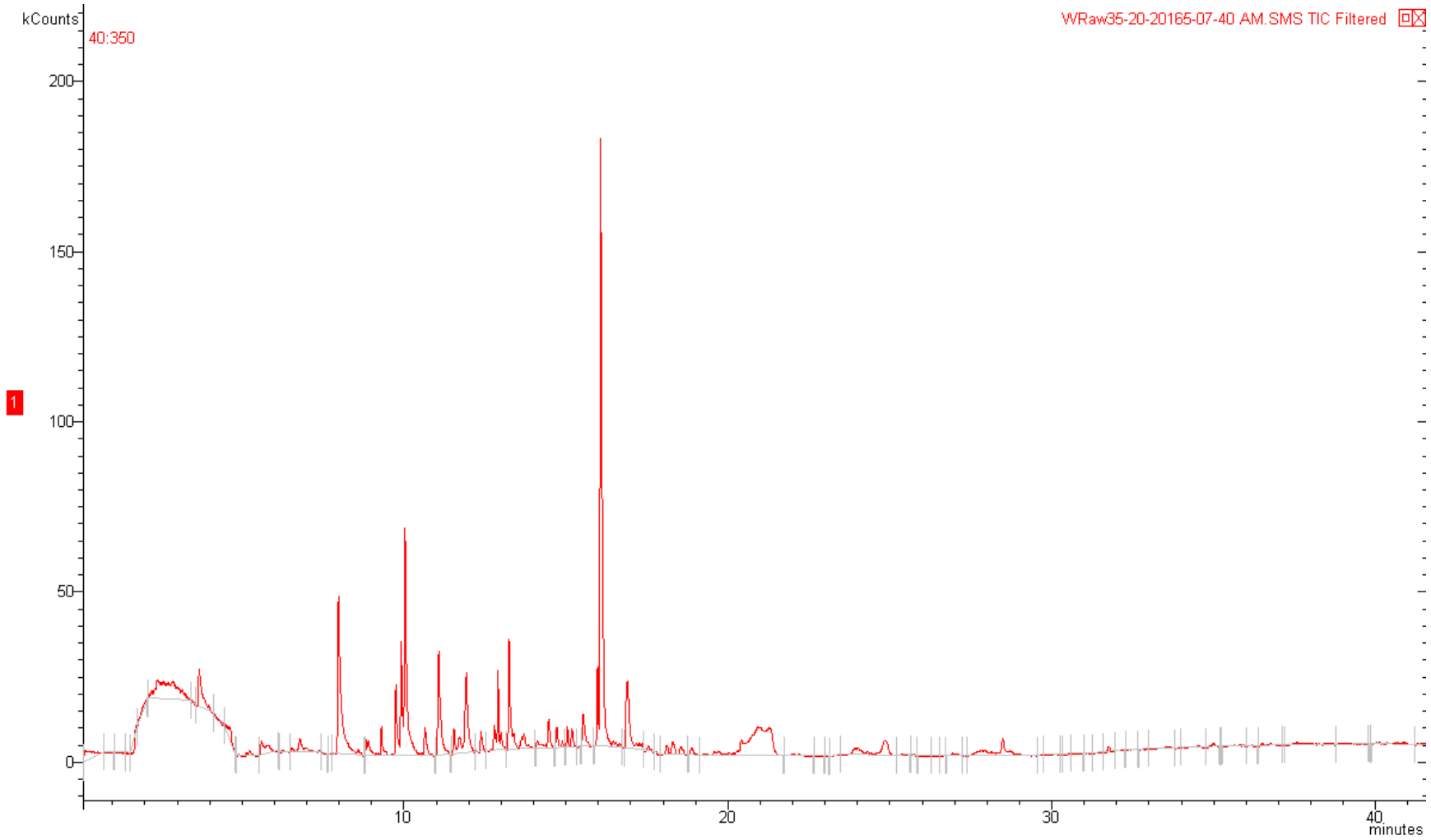
Gas chromatography-mass spectrometry chromatogram for raw Witte sample (rep 1)



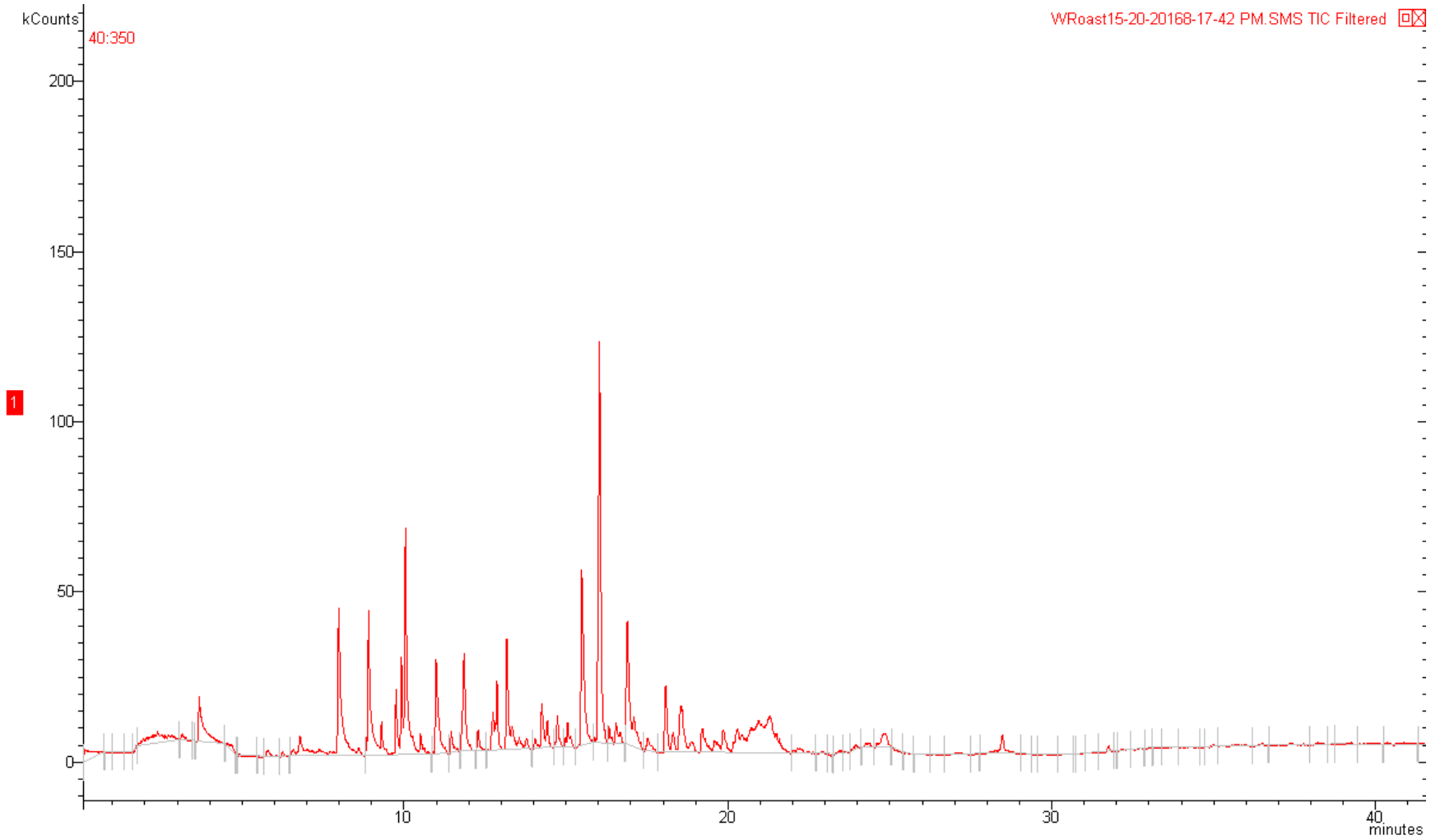
Gas chromatography-mass spectrometry chromatogram for raw Witte sample (rep 2)



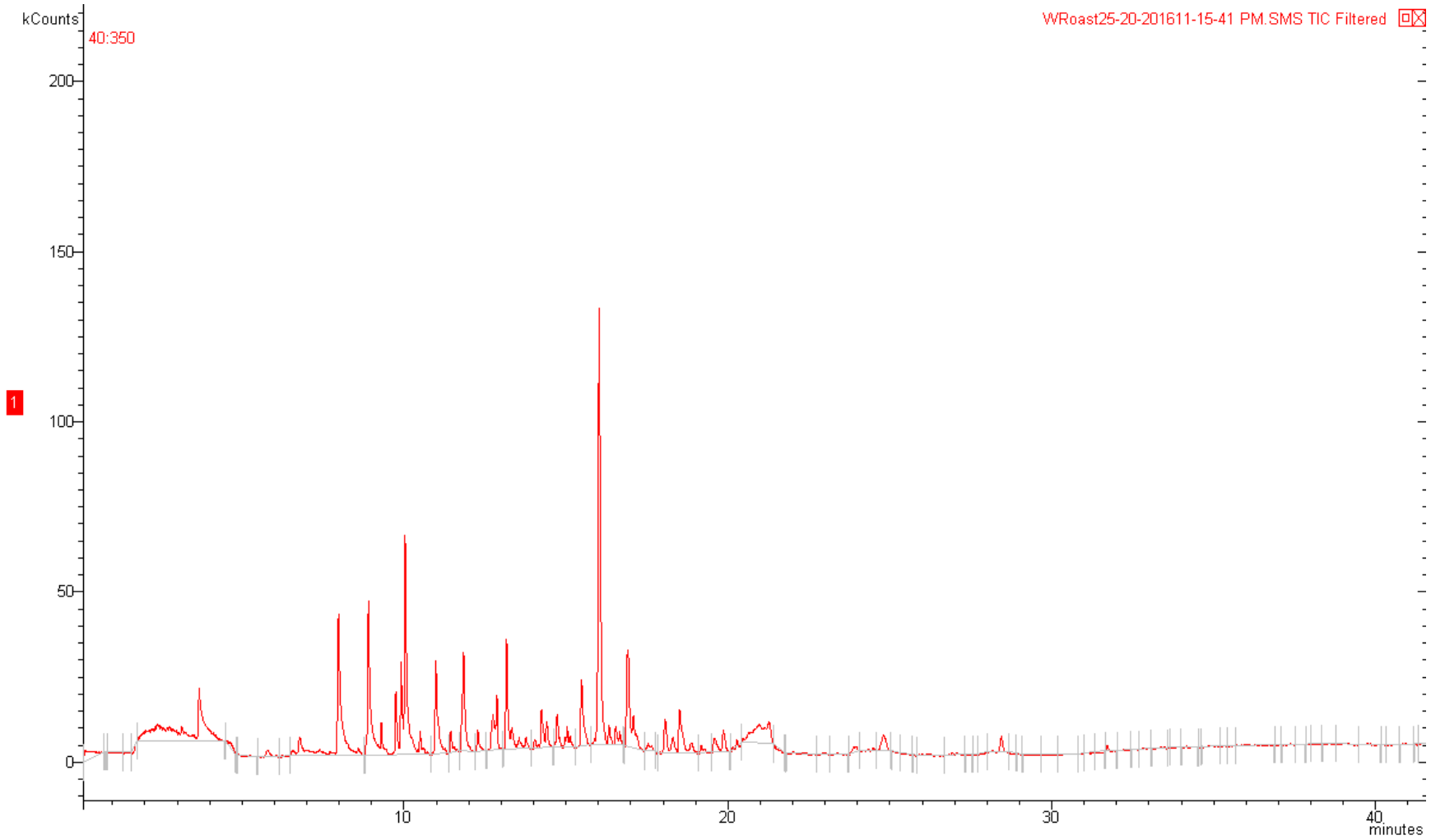
Gas chromatography-mass spectrometry chromatogram for raw Witte sample (rep 3)



Gas chromatography-mass spectrometry chromatogram for roasted Witte sample (rep 1)



Gas chromatography-mass spectrometry chromatogram for roasted Witte sample (rep 2)



Gas chromatography-mass spectrometry chromatogram for roasted Witte sample (rep 3)