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The Analysis of Artificially Flavored Coffees

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Longwood Senior Thesis Proposal

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Specific Aims

This project aims to determine the molecular components that give artificially flavored coffees their advertised flavor. Much work has been conducted to determine various characteristics such as antioxidant properties, phenolic compounds, flavors, and aromas found in natural coffees. In addition, there is ample literature on the results of sensory tests for a variety of commercial coffees. However, not much work has been conducted to determine the compounds found in artificially flavored coffees that give rise to their distinctive aroma and flavor. This is, perhaps, due to the proprietary nature of the flavoring process employed by commercial producers. The goal of this project is to develop the methodology to extract and quantify these externally-applied flavor components.

Introduction

Coffee is a beverage that has had significant cultural and even spiritual importance for millions of people and has been around for millennia. It brings people together, helps wake people up in the morning, comes in a variety of roasts and flavors, and can be customized and stylized to one's taste by adding a plethora of ingredients.

Coffee originates from the *Coffea arabica* plant, which is a large shrub or small tree that can grow up to twenty feet tall.[1] The trees make clusters of white flowers that give way to green then red berries. Inside the red berries are the "coffee beans" – seeds of the plant. Unlike most plants that have progressive stages where the whole plant flowers and then produces seeds, the coffee plant may have both flowers and seeds at the same time.[1] The flavors and aromas that result from roasting coffee beans vastly differ depending on the environmental conditions of the growing area (e.g., soil type, temperature, humidity, altitude).[2]

The history of coffee began over one thousand years ago. The *Coffea arabica* plant originated from the ancient forests of Ethiopia. Legend has it that the goat herder, Kaldi, discovered the beans after he noticed his goats became so energetic that they did not want to sleep at night after ingesting the beans. He reported his findings to the abbot of the local monastery, who found that the beans kept him awake through long hours of evening prayer.[3] From there, coffee spread to the Arabian Peninsula and eventually all over the world.

Arabs have cultivated coffee for both medicinal and pleasurable purposes for thousands of years. Until 1690, Arabia supplied the world's coffee, maintaining this monopoly by banning visitors from coffee plantations, forbidding removal of seeds from the plantations, and boiling or partially roasting the beans that were to be exported in order to ensure that the beans would not germinate. Despite their efforts, the seed made its way to other parts of the world as Muslims migrated, and the bean found its way in places like Java, India, and other parts of Southeast Asia. In the mid-nineteenth century, a fungal disease called Coffee Leaf Rust struck Asia, which wiped out all coffee growing in Southeast Asia, paving the way for Brazil to emerge as a dominant coffee producer due to its rich volcanic soil and climate providing perfect conditions for growing coffee. By the 1800's, Brazil had become a leading producer of coffee.

Coffee has a dark history in European colonies; indigenous populations and African slaves were forced to work on coffee plantations. Initially, only the wealthy European elites could enjoy it until prices decreased after the rise of slavery on plantations, and then coffee became more mainstream. As coffee became a more popular beverage, coffee houses popped up, and became known as places of enlightenment, as philosophers, writers, and political activists gathered. Coffee in America has been popular since the founding of the country, though it has significantly increased in popularity in the late 20th century with the introduction of commercial retailers like Starbucks, Peet's, Caribou, and Dunkin. As specialty coffee grew in popularity, new styles of coffee brewing emerged, which gave rise to a diverse range of types and flavors of coffee, including artificially flavored coffees that can be bought at specialty coffee shops to this day.

Coffee has numerous health benefits and bioactive compounds. For instance, coffee has phenolic compounds, alkaloids, triterpenes, organic acids, amino acids, hormones, and fatty acids.[6] Other compounds include nicotinic acid, tannic acid, and, of course, caffeine. The most abundant bioactive metabolites are caffeine, quinic acid derivatives (e.g., chlorogenic acid), caffeic acid, ferulic acid, p-coumaric acid, melatonin, and serotonin. Different growing locations (i.e., soils, temperature, humidity) affect the type and quantity of antioxidants and beneficial compounds, with one study observing more antioxidant properties in Colombian coffee compared to Peruvian coffee.[4]

There are multiple methods for making coffee. The most longstanding method included purchasing raw coffee beans, grinding them, and roasting by hand at home, then filtering out the grounds by either brewing in cloth bags (which could leak grounds) or putting eggshells and eel skins in the cup to settle the grounds to the bottom. This changed in 1908 when Melitta Bentz invented the coffee filter using blotting paper.[1] Currently, coffee is produced by either a hot brewing method (manual or automatic), an espresso method (where pressurized boiling water is forced through a puck of ground coffee beans to form a concentrated form of coffee), a percolated cold extraction method[5], a cold brew method (where the coffee grounds and cold water sit and "brew" over a period of 18-24 hours, then are filtered for drinking), and several other methods as a wide variety of coffee machines on the market work in different ways to prepare coffee. Different brewing times account for different levels of antioxidants, phenolic compound content, and caffeine content depending on the roast.[5] Decaffeination of coffee is a fairly recent invention in which caffeine is eliminated by either soaking the beans in liquid (either water alone or a mixture of water with solvents either directly or indirectly), or by using water and supercritical carbon dioxide.[6]

There are four main types of roasts: light, medium, medium-dark, and dark.[7] Light roasts exhibit a light brown color and the beans are not oily as they have not been roasted long enough to crack. Light roasts tend to have acidic characteristics. Medium roast coffee beans have a medium brown color with stronger flavor and a non-oily surface. This roast is preferred by Americans. Dark roast beans are rich and dark in color, have some oil on the surface, and a bittersweet aftertaste. Dark roast is popular in European countries, and the beans are black in color with an oily surface that is less acidic than previous roasts, but more bitter-tasting.

Previous research focused on the use of various instrumental techniques (including an electronic nose, Gas Chromatography-Ion Mobility Spectrometry (GC-IMS), and Chromatography Mass Spectrometry (GC-MS)) to determine compounds of interest in a variety of coffees. One study in particular involved analyzing coffee aroma in beans, powders, and brews using an electronic nose. The electronic nose was able to identify 37 volatile organic compounds (VOCs), which were used as biomarkers to distinguish coffee samples. These biomarkers included aldehydes (5), ketones (10), alcohols (8), acids (2), esters (4), furans (5), and other compounds (3).[8] Another study involved a sensory panel to determine the quality of a variety of coffee samples and how they correlated to molecular coffee data. The sensory quality focused on aroma, aftertaste, flavor, acidity, balance, body, uniformity, sweetness, clean cup, copper points, and other relevant points.[9] Overall, the research provided useful information on the natural properties of a variety of coffee types, which can be used in future sensory studies.

More work is needed to elucidate the flavor and aroma molecules found in artificially flavored coffees. It is well known that fruity artificial flavors like those found in various types of candies are typically esters. For example, candies like Circus Peanuts and Runts contain isoamyl acetate, which is responsible for the fruity, banana-like flavor. It is plausible that a flavored coffee like "Wild Mountain Blueberry" also contains fruity ester molecules. There are multiple molecules that make up the flavor compound of vanilla, with some of the main compounds including vanillin, p-hydroxybenzaldehyde, p-hydroxybenzoic acid and vanillic acid. Artificial vanilla flavor comes from artificial vanillin, which is synthesized from guaiacol or lignin derived from wood.[10] Caramel is used both as a flavor and as a coloring agent. Natural caramel comes from burnt sugar and involves the decomposition of saccharides, such as sucrose, glucose, and fructose. Caramelized sucrose contains three main products - a dehydration product (caramelan, $C_{12}H_{18}O_9$) and two polymers (caramelen, $C_{36}H_{50}O_{25}$ and caramelin, $C_{96}H_{102}O_{51}$).[11] There are a few molecules that impart nutty flavors in foods, which can vary depending on what kind of nut flavoring one wishes to incorporate into the food or beverage. In general, pyrazines are used in food industry to grant foods and beverages a nutty-roasted flavor.[12] Overall, there is a wide range of compounds used in the food and beverage industry to impart the desired flavor in the product of choice for consumption.

Proposed Experiments

Sampling – Several brands of coffee will be chosen based upon the number of artificial flavors available. Ideally, the flavored coffees chosen will have a natural “parent” coffee that can be analyzed as a reference for comparison. For example, Green Mountain Coffee produces a number of artificially flavored coffees (e.g., vanilla caramel, hazelnut, French vanilla) using a light roast coffee as the base.

Brewing Conditions –There will be three brewing conditions used, which will be the standard coffee pot, cold brew, and the espresso machine. Each of the artificially flavored coffees will be used in order to determine how the different brewing methods affect the molecular composition of each brew.

GC-MS Headspace Analysis – Gas chromatography-mass spectrometry (GC-MS) is a powerful analytical technique used to determine volatile compounds in coffee.[13] Coupling this technique with headspace analysis allows for the analysis of components that are easily volatilized. In headspace analysis (Figure 1), the sample of interest is placed in a sealed container equipped with a rubber septum. The sample is heated, which causes volatile components to move into the gas phase until an equilibrium is established. The gaseous sample occupying the headspace can be removed using a gas phase syringe for injection into the GC-MS.[14] Unbrewed coffee grounds as well as brewed coffee samples (artificially flavored and natural) will be analyzed using headspace analysis on a Shimadzu GCMS-QP2010 Ultra equipped with a 30-meter, non-polar (5% diphenyl/95% dimethylpolysiloxane) column. The inner diameter of the column is 0.25 mm, and the stationary phase film thickness is 0.25 µm.

HPLC Analysis – Brewed coffee samples will be analyzed using reverse phase High Performance Liquid Chromatography (HPLC). Method development will be necessary to determine the appropriate column and mobile phase conditions, as well as any required sample extraction, filtration, and dilution. All samples will be analyzed using a Shimadzu Prominence HPLC instrument equipped with a diode array detector. Initial analyses will utilize a 150 cm x 4.6 mm ODS column (5-micron particle size) and an acetonitrile/methanol mobile phase.

Timeline

August to September: Choose coffees for analysis and perfect brewing conditions

September to mid-October: Perform GC-MS analyses

mid-October to December: Perform HPLC analyses

December – January: Initial data analysis

January – February: Perform any follow-up experiments

February – March: Complete data analysis & begin writing process

March – April: Complete writing

April: Poster presentation

Committee Members

Dr. Sarah Porter, Professor of Chemistry, Longwood University

Dr. Tyler St. Clair, Assistant Professor of Science Education, Longwood University

Outside Member - TBD

Figures

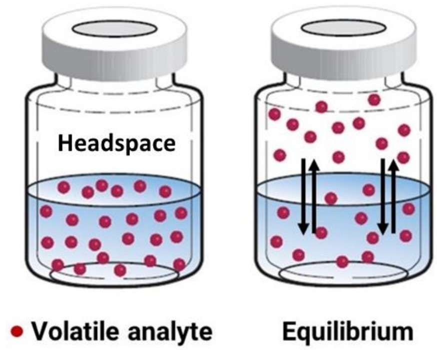

Figure 1 – Schematic representation of a volatile analyte moving out of the sample layer and establishing an equilibrium between the sample and the headspace.[14]

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