ABSTRACT

LEBLANC, CORRINE. Factors Affecting the Quality and Shelf Life of Specialty *Coffea arabica* Green Coffee. (Under the direction of Dr. Gabriel Keith Harris).

Specialty grade green coffee is coffee that receives a score of > 80 on the 100-point Specialty Coffee Association (SCA) scale due to unique flavor attributes. Specialty coffee receives a higher price per unit weight than commodity coffee, as coffee price is directly related to quality. The market demand for specialty coffee is also increasing. Producing specialty coffee requires great care both pre- and post-harvest. One of the key factors for maintaining quality is controlling water in green coffee. Water in green coffee can be quantified in terms of moisture content (MC) or water activity (a_w), and both should be controlled to avoid quality degradation and microbial spoilage. Moisture sorption isotherms (MSIs) can be used to depict the relationship between MC and a_w in green coffee. The aim of this thesis was threefold: (1) to use the static method to create working MSIs for specialty *Coffea arabica* green coffee at relevant temperatures (20, 30, and 40 °C) and over two production years (2019 and 2020); (2) to assess thermodynamic properties of green coffee, namely net isosteric heat of sorption (H_s) and monolayer moisture content (m_o), to correlate physical and chemical characteristics of specialty green coffee with MSI and thermodynamic data; and, (3) to attempt to identify rapid, accessible predictors of quality changes that may be useful to the specialty coffee industry. Green coffee was found to follow a Type II isotherm. The m_o values estimated using the GAB equation from the working isotherm data were 6.17±0.18% (dry weight basis), slightly higher than literature data for m_o determined from adsorption or desorption isotherms. The m_o is technically the MC where the product is the most stable but storing green coffee at such a low MC is impractical. Net isosteric heat of sorption (H_s) is a measure of the binding energy of water where higher values indicate water is more tightly bound. H_s was found to be significantly greater below the

m_o (1241.65 cal/mol at 9% MC vs. 6720.23 cal/mol at 3% MC). Intermediate a_w values from MSI construction (0.33, 0.54, and 0.75 a_w) corresponded to suboptimal (0.33 a_w, 6% MC and 0.75 a_w, 14% MC) and optimal (0.54 a_w, 10% MC) MC values for green coffee. Physical and chemical changes were further explored at these a_w ranges over a three-month storage period (analyzed every 3 weeks for 3, 6, 9, and 12 weeks) in the dark at 20°C ± 1°C. Physical analyses included weight change, bulk density, MC, a_w, and color (L*a*b*). Chemical analyses included caffeine, 3-caffeoylquinic acid (3-CQA), total ochratoxin (OT), total aflatoxin (AF), and total phenolic content quantification. Green coffee beans stored at 75% humidity increased % MC (to 14%), a_w, L*, and weight (presumably from water) over 12 weeks. Bulk density decreased, indicating bean size increased. Most weight gain, a_w change, and %MC change happened by week 3. Physical data showed that maintaining a_w of around 0.54 produced the most stable product (no changes in weight, MC, a_w, color). No significant differences in caffeine content were identified between RH treatments or time intervals. The same was true for 3-CQA, total phenolic content, and OT and AF content. OT and AF were identified in all samples. Additionally, cup scores and fade rating of green coffee followed no trend and seemed to be independent of any variable explored. Specialty coffee quality is likely a multi-dimensional variable that may not be correlated to any simple, rapid analytical parameter, but storage at 0.54 a_w was identified as the most stable environment for green coffee.

Factors Affecting the Quality and Shelf Life of Specialty *Coffea arabica* Green Coffee

by
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DEDICATION

To my Nana—for teaching me to love life, knowledge, conversation, and food.

BIOGRAPHY

Corrine Anastasia LeBlanc spent most of her youth split between eastern North Carolina and New Hampshire. She began her higher education at Middlebury College after graduating from Croatan High School in 2013. Throughout her final year of high school and freshman year of undergraduate studies, she worked at cafés, in dining hall and restaurant bakeries, and joined cooking and food education clubs. Eventually, she realized food and coffee were more than just hobbies and decided to transfer to the Food, Bioprocessing, and Nutrition Sciences department at North Carolina State University (NCSU) in 2016 to pursue a degree in food science. She worked as a research fellow at the NCSU Center for Marine Science and Technology (CMAST) the summer before she transferred to NCSU and continued pursuing research opportunities throughout the rest of her undergraduate career. In 2018, she led a 6-week research project as a summer scholar in the lab of Dr. Gabriel Keith Harris. In December 2018, she graduated Summa Cum Laude with her bachelor's in food science and began her Master's work in January 2019 under the direction of Dr. Harris.

Throughout her time at NCSU, Corrine was an active member of the Food Science Club, serving as a co-chair for the Dairy Bar fundraiser the club runs at the NC State Fair and holding an executive board position as club treasurer. She also worked part-time for a retail shop near downtown Raleigh, was an active member of the Institute of Food Technologists (IFT), and worked as a teaching assistant for many food science courses throughout her graduate school career. In addition, she has presented research posters at the Specialty Coffee Expo, run by the Specialty Coffee Association (SCA), and at an international conference run by the Association for Science and Information on Coffee (ASIC). She was nominated as an Outstanding Graduate Student for the 2019-2020 academic year. She held an internship with S&D Coffee and Tea in

Concord, NC as a product development assistant during the final year of her master's research and has gone on to work as an Associate Scientist for Keurig Dr. Pepper in Texas. Corrine's hobbies include trying new restaurants, drinking coffee, reading fiction, listening to exercise science podcasts, lifting weights, rock climbing, trail running, hiking, cycling, and riding her motorcycle. She plans to spend the rest of her life engaging in coffee research, learning, and experiencing all life has to offer.

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Chapter 1. Literature Review

1.1 An Introduction to Coffee

Coffee, both the bean and beverage, is widely known for its energizing and rejuvenating physical effects. These effects have been apocryphally referenced as early as the 10th century when Ethiopian natives first discovered the plant. By the 17th century, Arabian and then European traders had contributed to the global rise of the coffee market. Since its discovery, coffee has increased in popularity around the globe. In 2019, approximately 87 million 60-kilogram bags of coffee were consumed, compared to approximately 73 million 60-kg bags in 2009 and 68 million in 1999 (International Coffee Organization (ICO)). In terms of coffee exports, ICO reporting countries exported 131 million 60-kg bags of coffee (all forms) in 2019, versus 96 million 60-kg bags of coffee in 2009 and 86 million 60-kg bags in 1999 (ICO).

The modern-day coffee industry can be divided into two main categories: commodity and specialty coffee. Commodity coffee, which makes up the bulk of the coffee market, can be loosely defined as a mass-produced good with a standard quality used to identify the commodity as coffee. Specialty coffee, a term coined in 1974 by Erna Knutsen, can be defined as high-quality coffee (Stack, 2018). High-quality coffee is: (a) coffee from ideal and unique climates with distinct flavor attributes, (b) coffee that has met specific quality standards, including low defect counts, set by the Specialty Coffee Association (SCA), and (c) coffee that scores above 80 points on the 100-point SCA of America (SCAA) scale, which must be scored by Coffee Quality Institute-certified graders (SCAA, 2012; Traore, Wilson, & Fields, 2018; Poltronieri & Rossi, 2016).

In recent decades, the market demand for coffee has begun to change. Specifically, the specialty coffee market share of the coffee industry has begun to grow. This change may be

primarily driven by the third wave of coffee movement, which was a push by both consumers and producers for flavorful, high-quality coffee. In the United States in 2014, 55% of coffee shops were specialty, meaning they were independent shops with three or fewer locations (Specialty Coffee Association, 2015). According to Adroit Market Research, the market for specialty coffee will reach $83.5 billion by 2025 (Adroit Market Research, 2019). Specialty coffee is produced and maintained by controlling various pre- and post-harvest factors to produce a high-quality final product. These factors, as well as how they affect coffee quality, will be explored below.

1.2 The Genus *Coffea*

Coffee is produced by harvesting the fruit of trees belonging to the botanical family Rubiaceae, genus *Coffea*. More than one hundred species are in the *Coffea* genus, but the most commonly traded species are *Coffea arabica* and *Coffea canephora*. *C. arabica* is referred to as 'Arabica coffee,' while *C. canephora* is called 'Robusta coffee.' In general, Robusta coffee beans contain more caffeine, more phenolic compounds, and less sugar than Arabica beans, are more resistant to pests, diseases, and mechanical stress, and produce more coffee beans per tree (Ferreira, Shuler, & Guimarães, 2019; Alonso-Salces, Serra, Reniero, & Héberger, 2009). However, Arabica coffee is the dominant species produced today due to its superior flavor attributes. During the 2018 coffee production year, approximately 100 million 60-kg bags of Arabica were produced worldwide, compared to 70 million 60-kg bags of Robusta (ICO).

Most coffee is grown in the Torrid Zone, between the Tropic of Cancer and the Tropic of Capricorn. Production countries include, but are not limited to, Brazil, Colombia, Ethiopia, Kenya, Costa Rica, Panama, Tanzania, and Indonesia. Brazil was the global leader in coffee production during the 2018 production year (ICO). According to ICO statistics, the largest

coffee-producing region was South America, followed by Asia & Oceania, Mexico & Central America, and finally Africa (ICO). It is estimated that 70% of coffee is produced on smallholder farms, which are small-scale coffee operations.

Coffees from different geographical regions have distinct flavor attributes, but other variables also influence the final flavor and quality of the coffee. Altitude, climate, shade amount, and cultivar selection are just a few of these factors (others that will not be discussed include fertilizer type, fertilizer timing, and soil quality) (Bertrand, et al., 2012). *C. arabica* is most productive in areas with annual temperatures between 18°C and 22°C and at elevations of 3,000 to 6,000 feet (1000 to 2000 meters). Below 3,000 feet, coffee quality generally decreases. *C. canephora* can be grown at lower altitudes and higher temperatures. Unlike *C. canephora*, *C. arabica* is less tolerant to variations outside of optimal conditions. Both key species of the *Coffea* genus are not tolerant to frost. Table 1.1 below compares some critical differences between coffee species.

Table 1.1 Key Differences Between Economically Relevant Coffee Species.

Species	Growth Conditions				Time to Ripening (months)	Avg. Price August 2020 (US cents/ lb)	% Of Market
	Altitude (m)	Temperature (°C)	Rainfall (cm)	Relative Humidity (%)			
Coffea arabica	1200 – 1950+	18 – 22	120 – 180	60 - 75	6 – 9	114.78	~70
Coffea canephora	50 – 500+	22 – 30	120+	80 – 90	10 – 11	72.68	~30

Specialty coffee typically results from fruit development from higher altitudes. Research shows this is because higher elevations slow the development of the coffee fruit, producing a more dense and flavorful coffee (Tassew, Yadessa, Bote, & Obso, 2021). Of course, there are exceptions to this; for example, shade-grown coffee ripens at a slower rate, so coffee can be

grown at lower altitudes in the shade and still produce a good quality coffee (Vaast, Bertrand, Perriot, Guyot, & Génard, 2005). Another exception is Kona coffee, a *C. arabica* variety typically grown outside of the Torrid Zone at 2,000 feet of elevation, but the distance from the equator and the soil composition work together to produce an excellent quality bean. Specialty coffee can also originate from areas of a country, region, or even plantation, that have unique microclimates. Microclimates are areas that have slight variations in certain variables like sun exposure, temperature, rainfall, soil nutrients, and harvest time and can result in a better-than-average cup of coffee for the area.

Each genus-species of coffee has multiple cultivars, or varieties, which differ by production region, pest resistance, flavor attributes, and a multitude of other factors. Cultivars or varieties may be naturally occurring from genetic mutations, the result of selective breeding, or the result of lab-made hybrids. Cultivar selection is another consideration for specialty coffee production. Cultivar selection is also used to select varieties of green coffee that can withstand climate change. Taxonomically classifying coffee cultivars is complex, but work is being done to map the phylogeny of relevant coffee species and their common cultivars. Some economically relevant cultivars are compared in Table 1.2 below. Cultivar species of origin for *C. arabica* and *C. canephora* in Table 1.2 may be oversimplified.

Table 1.2 Some Economically Relevant Coffee Varietals and Cultivars.

Name	Growth Region	Disease resistance*	Geographical Origin	Quality Potential**	Bred vs. Natural Mutation
Typica	Peru, Dominican Republic, Jamaica (Jamaican Blue Mountain)	Very susceptible	Ethiopia	Very Good	Selective Breeding

Table 1.2 (continued)

Name	Growth Region	Disease resistance*	Geographical Origin	Quality Potential**	Bred vs. Natural Mutation
Bourbon	El Salvador, Guatemala, Honduras, Peru	Very susceptible	Yemen	Very Good	Selective Breeding
Maragogipe	Bahia, Brazil	Very susceptible	Bahia, Brazil	Very Good	Wild Typica Mutation
Geisha/ Gesha	Panama Anywhere at very high elevation	Resistant	Gesha, Ethiopia	Exceptional	Wild
Pacas	El Salvador, Honduras	Very susceptible	EL Salvador	Good	Bourbon Mutation
Pache	Guatemala	Very susceptible	Guatemala	Good	Typica Mutation
Pacamara	El Salvador	Very susceptible	El Salvador	Exceptional	related to Bourbon & Typica
Java	Panama, Costa Rica	Tolerant	Ethiopia	Very Good	Selective Breeding
Casiopea	Costa Rica, El Salvador, Guatemala, Honduras	Very susceptible	Central America	Exceptional	Hybrid (F1 breed to increase genetic variety)

*Resistance to Coffee Leaf Rust and Coffee Borer Disease
**Based on growth at high altitude, SCA grading, and reported for WCR
***source: World Coffee Research

The *Coffea* genus comprises many different species, with *C. arabica* and *C. canephora* being the two most well-known and traded species. Many pre-harvest factors, such as climate and shade amounts, can vary during the growth and development of the coffee plant and

contribute to the final quality of the coffee bean. The next step in the coffee production process that influences coffee quality is harvesting.

1.3 Harvesting Coffee

Coffee trees begin producing fruit between three and five years after they have been planted. Coffee tree flowering is triggered by rainfall after a period of moisture deficit. After flowering, the coffee fruit will set and begin to develop. It usually takes between six to nine months after coffee flowers bloom for the coffee fruit to develop and fully ripen; however, this period varies depending on the species of coffee. Table 1.1 above compares the time to ripening of economically relevant coffee species. Coffee trees will produce fruit for an average of fifty years, but quality and yield generally decrease as the tree ages.

1.3.1 The Coffee Fruit

The coffee fruit is commonly referred to as a coffee ‘cherry,’ as it is botanically a drupe, like the cherry from the *Prunus* genus. From the outside in, there is a fleshy exocarp and mesocarp, and then a hard endocarp surrounding the seed. The exocarp may be referred to as the skin, the mesocarp the pulp and mucilage, and the endocarp the parchment. The exocarp, mesocarp, and endocarp together compose the pericarp. Beneath the endocarp is the spermoderm (also called the perisperm or integument), often referred to as the silverskin or chaff.

Table 1.3 A Comparison of Common Coffee Fruit Nomenclature.

Botanical Term		**Industry Term**
Exocarp	Pericarp	Skin
Mesocarp		Pulp, Mucilage
Endocarp		Parchment, Hull
Spermoderm/ perisperm/ integument		Silverskin, Chaff
Endosperm	Coffee Seed	Coffee bean
Embryo		

The coffee cherry typically contains two seeds, referred to as coffee beans, after harvesting and processing. Coffee seeds are composed of endosperm and an embryo. The embryo is located near the surface of the coffee seed and is reliant on the endosperm for nutrients if germination is to occur. The endosperm contains flavor precursors, macronutrients, and other compounds essential in developing coffee flavors during roasting. Table 1.3 above compares the botanical vs. industry terms for the different parts of the coffee fruit.

Occasionally, in about 5-10% of a coffee crop, only one seed is found in the coffee cherry. This is called peaberry coffee; the bean that results is usually rounder and smaller than a typical coffee bean (Suhandy, Yulia, & Kusumiyati, 2018). Three or more seeds have also been found in the coffee cherry, but this is even less common. Microscopic analysis of green coffee has emphasized the heterogeneity of the seed, both on a macro and cellular level (Ramírez-Martínez, et al., 2013).

1.3.2 Harvesting

Coffee cherries are ideally harvested when ripe, usually indicated by a deep red fruit color. Harvesting coffee cherries can be done manually or mechanically. Two types of manual harvesting are common: strip picking and selective picking. Strip picking, otherwise known as

stripping or milking, means all cherries are removed from a branch at one time. Selective picking relies on individuals to examine coffee fruits and only harvest those that are ripe. This is a useful method because it is common to have fruits at different stages of ripeness on the same tree or branch. Selective picking may be more expensive and less productive because it is more time and labor-intensive, but it allows for greater quality control and thus is used frequently for harvesting specialty coffee. A combination of selective and strip picking may also be used when feasible. Some areas, such as regions of Brazil where coffee plantations are large and flat, may rely on mechanical harvesting. Mechanical harvesting applies stripping methodologies on a larger scale and is mainly reserved for commodity coffee or Robusta varieties (Sivetz & Foote, 1963). The spacing of the coffee trees, rough terrain typical of coffee plantations, worker wages and expertise, desired final bean quality, and variety of ripeness' on a single tree all contribute to the difficulties in harvesting that may affect the harvesting method used.

Generally, the main coffee crop is harvested during the hemispheric winter. Coffee-producing countries in the Northern Hemisphere typically harvest from September to March, while in the Southern Hemisphere, harvest occurs from April to August. Some countries have climates that allow for more than one harvest a year, such as Kenya or Colombia, although secondary crops are distinct from the main crop and are smaller in volume. After harvesting, the coffee must be processed to separate the bean from the cherry and create a dry, transportable product.

1.4 Post-Harvest Processing of Coffee

After coffee is harvested, it is brought to a facility for post-harvest processing. These processing facilities may be specific to a single coffee plantation, they may be a central processing plant for multiple farms in a geographical region, forming a cooperative, or they may

be independently owned by a large company. Regardless of the processing location, coffee fruits must be sorted and processed as quickly as possible after harvesting. Both specialty and commodity coffee rely on rapid processing after harvesting to maintain quality (Poltronieri & Rossi, 2016). There are many steps involved in the post-harvest processing of coffee. Sorting, fermentation, and drying will be discussed below.

1.4.1 Sorting

Sorting coffee fruits is commonly done using water flotation, where ripe fruits sink and overripe fruits float on top and are removed and discarded, or processed separately. Plant matter and rocks are also sorted out via floatation. Under-ripe coffee fruits are harder to separate because their densities are closer to ripe fruits (Brando, 2004). When necessary, sorted coffee fruits may be sorted again by stage of ripeness, using fruit color as an indicator before they are processed further. Sorting is an important quality control step for high-quality coffee as under-ripe or overripe fruits can negatively impact the final quality of an entire lot of coffee (Brando, 2004). Winnowing is another sorting step that may be used to remove stems, leaves, and other miscellaneous plant matter that may have been accidentally collected during harvesting. For commodity coffee harvested mechanically, winnowing is very common. After the coffee fruits have been sorted, the seeds must be separated from the rest of the fruit and dried before they can be stored, exported, and roasted.

1.4.2 Fermentation and Drying: Post-Harvest Processing Methods

To produce raw coffee—an exportable commodity also called green coffee—the coffee fruit must undergo fermentation and drying. The drying step is crucial for mitigating the growth of mold and fungus during storage and transport (Taniwaki, Pitt, Teixeira, & Iamanaka, 2003; Ramírez-Martínez, et al., 2013). Drying time depends on various factors, including the moisture

level of the coffee fruit after harvesting, ambient temperature, sun exposure, relative humidity of the air, oxygen availability, and processing method. Coffee is typically dried in the sun in flat layers or via mechanical drying in tumblers. It may be dried with or without the pericarp present, depending on the post-harvest processing method employed.

Two common post-harvest processing methods are the washed and natural processes. Washed coffee, also known as wet-processed coffee, is coffee that is dried after the outer pulp (the exocarp and mesocarp) has been separated from the remaining parts of the fruit, usually done mechanically. Then, the coffee is fermented and dried to produce washed green coffee. Natural coffee, also called dry-processed coffee, is produced by leaving the coffee fruit whole to ferment and dry; then, the whole pericarp, including the parchment, is removed from the bean to produce the final green coffee. In between wet and natural processing is a method that involves partial removal of the pericarp, leaving mucilage behind during fermentation. This method of processing has many names and variations, including honey processing and pulped natural processing. Processing methods and harvesting methods are sometimes inter-connected. Natural Arabica coffee is commonly harvested using stripping methods, while washed coffees may be selectively harvested (Brando, 2004; Sivetz & Foote, 1963). While not specific to different origins, species, or varieties, processing methods may also correlate with these variables. For example, almost all beans from *C. canephora* are processed using the natural process (Brando, 2004).

Research suggests processing method affects the sensory profile of green coffee. During drying and fermentation, flavor development can depend on the sugar concentration, acidity, fruit ripeness before processing, and moisture of the fruit, as well as other variables (Selmar, Kleinwächter, & Bytof, 2015). Processing method also significantly affects the chemical

composition of green coffee (Bytof, Knopp, Scheiberle, Teutsch, & Selmar, 2005; Knopp, Bytof, & Selmar, 2006; Mintesnot & Dechassa, 2018; Kleinwächter & Selmar, 2010), except for fatty acid composition (Rendón, Salva, & Bragagnolo, 2014). Chemical composition has been shown to be affected by processing methods (Poltronieri & Rossi, 2016).

During the drying process, coffee beans naturally ferment, allowing for unique flavor compounds to develop. Fermentation also allows for easier removal of the pulp and mucilage from the coffee bean because enzymes present during the fermentation step break down complex carbohydrates in the coffee fruit, such as pectin (Poltronieri & Rossi, 2016). Processing methods affect the bacterial and fungal cultures that colonize the coffee during fermentation. For example, wet-processed coffee has been uniquely found to contain lactic acid bacteria (Hamdouche, et al., 2016). When the coffee pulp and mucilage are removed before fermentation and drying, as in washed coffee, endophytic bacteria and endogenous bacteria from the environment are responsible for fermentation. Research has been conducted to inoculate coffee beans with desirable bacteria and yeast cultures to speed up the fermentation process and favor desirable organoleptic properties in the final coffee beverage (Wang, Sun, Lassabliere, Yu, & Lui, 2020; Lee, et al., 2017).

If drying takes too long or if the ambient temperature is too high, causing the drying to occur too quickly, there may be a detrimental effect on final cup quality (Sivetz & Foote, 1963). Evidence for this phenomenon is indicated by stress reactions induced in the coffee seed during the drying process, where stress can be identified by γ-aminobutyric acid (GABA) accumulation (Kramer, Breitenstein, Kleinwächter, & Selmar, 2010; Rendón, Salva, & Bragagnolo, 2014; Bown & Shelp, 1997). Other evidence that time influences cup quality is indicated by changes in fermentation substrates during drying. As complex carbohydrates are used up during

fermentation, yeasts dominate the fermentation process (Poltronieri & Rossi, 2016). This can have a negative effect on final cup quality if yeasts dominate for too long. During coffee processing, coffee seeds are stored for conditioning after drying and fermentation but before packaging and final green coffee storage (International Trade Center, 2011). After coffee beans have been harvested and processed into green coffee, the green coffee beans can be graded, packaged, stored, and exported.

1.4.3 Grading Green Coffee

Grading and classification of green coffee are essential processes as the price of green coffee is directly affected by its quality. There are multiple steps during the production of green coffee where bean sorting and grading may occur. The first post-harvest processing step, discussed previously, is separation via flotation before drying. After beans are dried and ready to be packaged, they may go through separation via density, color, and/or size. Failure to separate out beans with unwanted sizes, shapes, colors, densities, or other unwanted attributes will impact final cup quality throughout the storage life of the green coffee. Beans of different sizes, such as peaberry coffee and elephant seeds, may be separated out to be processed or sold separately. Coffee cultivar, production altitude, and cup quality are other factors that are considered when grading. There are different specifications, such as the allowable number of defects and range of bean size, for different coffee cultivars, processing methods, and regions of coffee production. There are set limits on the number of defects allowed to be present in a batch of green coffee if that coffee is to be sold as specialty green coffee.

Green coffee defects include, but are not limited to, broken beans, quakers, and black beans. Defective beans may be separated out along with foreign materials such as pods, sticks, and leaves. Beans with discolored patches may have insect damage from borer insects and

should be separated out if possible. Ideal coffee beans are blue to grey-green in color. Bleached, white, or pale beans are usually the result of poor storage or processing and are typically removed. Brown beans may be the result of improper drying, overripe cherries, or old crop coffee; although, coffee beans processed using the natural method are browner in color because of the bean contacting the cherry during drying, causing enzymatic browning reactions to occur on the surface (Wintgens, Green Coffee Defects, 2001). Pale yellow bean colors are from immature coffee cherries and have been shown to illicit nutty/grassy flavors in the coffee when roasted (Sivetz & Foote, 1963). If defective beans are not removed, there may be a significant effect on the cup quality of the coffee after roasting (Agresti, Franca, Oliveira, & Augusti, 2008). Defective green coffee has also been shown to have smaller quantities of desirable health compounds than non-defective beans (n.d. on significance) (Ramalakshmi, Kubra, & Rao, 2007). Thus, grading and sorting out defective beans is an important quality control step. Once beans are roasted, they may be sorted by color again to ensure a uniform roast degree in the finished product. Cupping will then be done to grade the coffee on its organoleptic qualities.

1.5 Packaging, Storage, and Shipment of Green Coffee

After harvesting and processing, green coffee beans are usually packaged into bags in 60-70 kg quantities (net bag weight varies by country of origin). Commodity coffee is often packaged in “big bags,” also called super sacks, which can hold up to 1,000 60-kg bags of green coffee at a time. Maintaining green coffee quality post-harvesting and post-processing is essential for specialty coffee. Packaging, storage, and shipment and their effect on green coffee quality will be discussed below.

1.5.1 Packaging

Packaging is an important consideration for maintaining green coffee quality. One goal of packaging is to prevent moisture reuptake by green coffee that would result in quality losses (Ribeiro, et al., 2011; Harris & Miller, 2008). Packaging also provides protection from pests, oxygen in some cases, and UV light; however, the amount of protection depends on the material. Bag materials may include jute, sisal (a fiber produced from agave), hermetic plastic (GrainPro® or similar), and paper. Foil pouches with either white or silver-colored lining may also be used (Borém, et al., 2019). Jute bags are inexpensive, easy to sample from, and are the most traditional packaging material. Storage in jute bags has been associated with green coffee deterioration, which can be indicated by bean whitening (Ribeiro, et al., 2011; Júnior & Corrêa, 2003; Tripetch & Borompichaichartkul, 2019), increased moisture content (Tripetch & Borompichaichartkul, 2019), and decreased free fatty acid content (Borém, et al., 2019). Ribeiro et al. also tested potassium lixiviation (soluble mineral extraction from the solid coffee bean used as an indicator of cell membrane damage) and found that, after both three and twelve months, beans stored in jute bags had higher potassium lixiviation levels indicating more cell membrane damage (Ribeiro, et al., 2011). Cell membrane damage has been correlated with quality losses, providing more evidence that jute bags are not effective at preventing quality loss. Furthermore, storage in jute bags over twelve months caused a 6-point decrease in quality on the SCAA scale (from 79.5 to 74) (Ribeiro, et al., 2011). However, the most considerable quality losses occur in green coffee beans stored in permeable paper packaging (Abreu, Borém, Oliveira, & Alves, 2019).

Hermetic plastic bags are impermeable to nitrogen, oxygen, and carbon dioxide when sealed appropriately and are made to keep moisture, pests, and other contaminants from

contacting the product. Hermetic storage bags have been widely implemented in the specialty coffee industry to maintain product quality (Brody, 2017), but are less common for the storage of commodity coffee. After 18-months of storage, both paper packaging with a high vapor barrier and vacuum packaging maintained green coffee quality at specialty grade (above 80 points) (Abreu, Borém, Oliveira, & Alves, 2019; Ribeiro, et al., 2011). Modified atmospheres can be utilized in tandem with hermetic packaging and may maintain green coffee at the specialty coffee level (Borém, et al., 2013; Ribeiro, et al., 2011). It should be noted that green coffee has been found to sorb carbon dioxide from modified atmospheres during storage (Borém, et al., 2013), and the extent to which this influences quality needs to be considered. There are a variety of packaging materials to choose from for green coffee, but research suggests beans should be packaged in hermetic storage bags to maintain a specialty coffee grade (Donovan, Foster, & Salinas, 2020).

1.5.2 Storage

Packaging is one factor responsible for maintaining the safety and quality of green coffee, but storage conditions need to be considered as well. Green coffee may be stored in several warehouses before it is roasted. Storage warehouses can be personal, private, community, or centralized. Storage warehouse locations may include the harvesting site, the shipping port, the roaster, or alternative locations. The journey of coffee from the place where it is processed to the place where it is roasted is often very complicated and time-consuming. In addition, coffee is a seasonal commodity with constant demand, meaning storage times vary widely and can sometimes reach up to three years. Steps must be taken to slow degradation rates and prevent microbial spoilage during storage (Selmar, Bytof, & Knopp, The Storage of Green Coffee (Coffea arabica): Decrease of Viability and Changes of Potential Aroma Precursors, 2007).

Whether packaged in super sacks or in individual bags, these steps may include eradicating pests, preventing moisture exposure, monitoring/ controlling temperature and humidity, removing dust, and reducing heat accumulation.

Known factors for maintaining quality during storage and distribution are temperature, time, and moisture content. Other factors to consider are light and humidity. Exposure to heat may cause bean bleaching, protein denaturation, and flavor degradation depending on how the beans were processed and stored (Rojas, 2004). Green coffee has noticeable flavor degradation when exposed to temperatures around 90°F for any longer than a week (Sivetz & Foote, 1963). Quality losses are common when good quality coffee is stored at tropical seaports if the temperature is more than 80°F and the relative humidity is more than 50% (Sivetz & Foote, 1963). The moisture content of the beans may rise during shipment if they are not stored properly, usually via direct contact with shipping container walls causing adsorption, or absorption, of water from condensation on the container walls (International Trade Center, 2011). Storage recommendations for green coffee include relative humidity ranges of 50-70% and temperatures below 26°C (Bucheli, Meyer, Pittet, Vuataz, & VIani, 1998).

Green coffee needs to be stored with heat dissipation in mind. Green coffee is a live commodity that is respiring during storage. Respiration, an exothermic process that will be discussed in a later section, may cause the ambient temperature around the beans to rise. Heat dissipation is commonly achieved through warehouse design, where there are guidelines for the number of bags of coffee that can be stacked on top of one another and the distance between pallets of coffee bags. These guidelines allow airflow between bags and pallets and reduce the risk of pest infestations. Aeration during storage has been tested for heat dissipation with the goal

of improving the shelf life of green Robusta coffee and has been found to be successful (Bucheli, Meyer, Pittet, Vuataz, & VIani, 1998).

Regardless of how careful one is, green coffee will inevitably degrade over time as compounds in green coffee are metabolized or oxidized during storage, affecting flavor and seed viability. During storage, free amino acids have been found to decrease (Pokorný, Côń, Šmidrkalová, & Janíček, 1975). Free amino acids may be used up by Maillard browning reactions with reducing sugars, causing browning of the beans, or by interactions with lipid or polyphenolic oxidation byproducts (Pokorný, Côń, Šmidrkalová, & Janíček, 1975). Reducing sugars in green coffee decrease during storage as well, likely because they are being used in Maillard browning reactions (Pokorný, Côń, Šmidrkalová, & Janíček, 1975). Research indicates lipid oxidation occurs during storage as well, contributing to off-note development (Selmar, Bytof, & Knopp, The Storage of Green Coffee (Coffea arabica): Decrease of Viability and Changes of Potential Aroma Precursors, 2007; Rendón, Salva, & Bragagnolo, 2014; Speer & Kölling-Speer, 2006). As compounds like free amino acids and reducing sugars change during storage, the aroma potential of green coffee is reduced, and cup quality flattens. Even though these are changes are challenging to prevent, when paired with adequate packaging materials, proper storage techniques can prolong green coffee shelf life and quality.

1.5.3 Shipment

Green coffee may be roasted and consumed domestically, but the majority is shipped to importing countries. Germany and the US are two major importers; the EU alone imported 80,057 thousand 60-kg bags of green coffee in 2019 (ICO). Green coffee is usually shipped at least a month after harvesting and processing. For example, coffee harvested in Brazil in June-July may ship to North America, Asia, and Europe between the months of September and

January (Palacios-Cabrera, et al., 2007; International Trade Center, 2011). Maritime shipment length varies from around two to three weeks (Palacios-Cabrera, et al., 2007). As discussed above, proper packaging and storage conditions are essential for maintaining green coffee quality, but shipping variables need to be considered as well.

Green coffee is typically shipped via maritime transport. Green coffee that is being shipped should not exceed a moisture content of 12.5% (International Trade Center, 2011). Research has shown that moisture content can increase during shipment (Palacios-Cabrera, et al., 2007), especially at the top of shipping containers; thus, the moisture content of the beans must be below 12.5% to account for potential increases. If the moisture content is at or above 12.5%, mold and fungal growth and quality losses during transport become a risk. Coffee is traded on a wet weight basis, where pallets for shipment usually total around 1.5 tons (Palacios-Cabrera, et al., 2007). Because of this, sellers of green coffee may try to keep green coffee as close to the upper limit of moisture content as possible to get the highest purchase price for their product. This is a risky practice and may result in losses from mold or fungal growth, or quality degradation that is noticeable by importers when they conduct quality control cuppings on the product.

Green coffee trade often depends on the scale of the purchase. For specialty coffee, coffee procuring operations may be farmer direct, meaning the coffee roaster buys directly from the coffee plantation or co-op. Both commodity and specialty coffee can also rely on international trade houses and dealers to procure coffee for them. Substantial roasting operations may have their own industry-direct, in-house trade groups (International Trade Center, 2011). The scale of the purchase influences the shipment method, the shipment volume, and how long it is held at origin or at a warehouse in the destination country. Regardless of the volume, once

green coffee has reached the importing country, receiving and discharging should occur as quickly as possible to reduce the risk of mold growth or quality losses (Palacios-Cabrera, et al., 2007). Maintaining the safety and quality of green coffee post-harvest requires controlling many variables when packaging, storing, and shipping the coffee.

1.5.4 Green Coffee Respiration during Storage & Transport

Green coffee is alive and respiring post-harvest, processing, and during the early stages of storage. Respiration is a biological process that supports growth and life through the exchange of atmospheric gases and is a potential pathway for green coffee quality degradation (Songer & Associates, Inc., 2013). Oxygen availability, moisture content, and ambient and bean temperatures are all factors that regulate respiration and germination (Ribeiro, et al., 2011). As mentioned above, the respiration of green coffee – an exothermic process – can cause ambient temperatures to rise during storage if beans are stored too close together. When exposure to oxygen is limited, coffee bean respiration rates decline. Modified atmosphere and vacuum sealing may be used to reduce oxygen availability and prevent respiration, thereby reducing quality losses (Rojas, 2004). This practice is more common for smaller lots of specialty coffee, as large volumes of coffee are difficult to vacuum seal.

Respiration is a necessary process for seed germination. Germination can be prevented by processing seeds to a state of dormancy. Some seeds need to have a period of dormancy before they can germinate, but coffee beans do not need this dormant period. Germination can be triggered by high humidity, air temperatures of 30-35°C, or soil temperatures of 28-30°C (Sivetz & Foote, 1963). Germination rates are lower in beans that were dried at high drying temperatures. As discussed in the processing section, drying temperature also affects quality. It is best to germinate green coffee after harvesting, but mid-processing (before drying has finished).

One indicator of respiration in green coffee is reducing sugar content (Ribeiro, et al., 2011). Glucose is a reducing sugar present in green coffee at low concentrations. Glucose content may be an indicator for respiration, as glucose is a by-product of respiration-related reactions and may increase as respiration occurs. Glucose concentration has been linked to woody/rubbery notes in green coffee, which may be related to quality degradation (Bucheli, Meyer, Pittet, Vuataz, & VIani, 1998; Songer & Associates, Inc., 2013).

1.5.5 Green Coffee Viability

Green coffee viability may be linked to final cup quality (Sivetz & Foote, 1963). Viability, in the context of seeds, is the ability of that seed to germinate. Coffee seeds are metabolically active and viable from before the coffee fruit has fully ripened to a few months into their storage life (Joët, et al., 2010; Eira, et al., 2006; Selmar, Bytof, & Knopp, 2002). The percent of viable green coffee post-processing is between 80% and 93% (Rendón, Salva, & Bragagnolo, 2014; Selmar, Bytof, & Knopp, 2007; Wintgens, 2012). During storage, green coffee viability decreases. After approximately a year in storage, all Arabica viability is lost (Rendón, Salva, & Bragagnolo, 2014; Rojas, 2004; Selmar, Bytof, & Knopp, 2007). Robusta coffee loses viability more rapidly than Arabica coffee (Rojas, 2004; Wintgens, 2012). Parchment coffee remains viable longer than hulled coffee, even though parchment is not a major diffusion barrier for gases around coffee beans (Selmar, Bytof, & Knopp, The Storage of Green Coffee (Coffea arabica): Decrease of Viability and Changes of Potential Aroma Precursors, 2007). Research needs to be done to determine why parchment coffee has an extended viability window. It is possible that reduced exposure to mechanical damage because of skipping the hulling step is a factor in the prolonged viability of parchment coffee.

It has been of interest to find chemical indicators of viability. Research conducted by Selmar et al. concluded that sugar composition might not be a reliable indicator of green coffee viability, even if it does indicate respiration (2007). Conflicting data exists on whether free fatty acid losses may be correlated with viability losses (Selmar, Bytof, & Knopp, The Storage of Green Coffee (Coffea arabica): Decrease of Viability and Changes of Potential Aroma Precursors, 2007; Rendón, Salva, & Bragagnolo, 2014). Reactive oxygen species from lipid and protein oxidation during drying are related to viability changes (Rendón, Salva, & Bragagnolo, 2014). Post-mortem reactions may be used as indicators for green coffee that has yet to experience quality degradation but has been stored for an extended period (Selmar, Bytof, & Knopp, 2007).

In general, low humidity and temperatures lengthen the window of green coffee viability. Storage recommendations for the longevity of coffee seeds are 10-11% moisture content, wet-basis, and between 10 and 15°C (Rosa, Carvalho, McDonald, Pinho, & Silva, 2011; Abreu, et al., 2017). Some research has suggested freezing to maintain seed viability, but conflicting research suggests that freezing green coffee damages viability. Green coffee that is viable but not respiring is likely to have good cup quality and should be stored to facilitate these conditions.

1.5.6 Safety Considerations during Coffee Processing, Storage, and Shipment

All green coffee, like other natural and fermented products, is covered in mold and fungal spores that will germinate given proper conditions. Mold growth, and toxin formation as a result, is a hazard that must be controlled during the processing, storage, and shipment of green coffee. It is the primary food safety concern related to coffee and is also a concern for maintaining harvest quality and loss prevention. Fungal growth on green coffee may result in the production

of mycotoxins. The primary mycotoxins of concern are ochratoxins (OT), specifically ochratoxin A (OTA), although ochratoxin B, C, and TA may also be found.

OTA is produced by the fungal genera *Aspergillus* and *Penicillium* and is nephrotoxic, immunotoxic, teratogenic, and carcinogenic (Suárez-Quiroz, et al., 2004; Höhler, 1998; Pfohl-Leszkowicz & Manderville, 2007; Cabañes, Bragulat, & Castellá, 2010). OTA can be produced during post-harvest processing, specifically the drying step, or during storage, if green coffee is not stored properly (Bucheli, Meyer, Pittet, Vuataz, & VIani, 1998; Taniwaki, Pitt, Teixeira, & Iamanaka, 2003; Urbano, Taniwaki, Leitão, & Vicentini, 2001; Poltronieri & Rossi, 2016; Barcelo & Barcelo, 2018). Re-wetting because of improper storage or drastic increases in relative humidity during storage also influences OTA production (Palacios-Cabrera, Taniwaki, Menezes, & Iamanaka, 2004). Parchment also reduces the risk of OTA development by reducing contamination of the beans by *Aspergillus ochraceus* (Ramírez-Martínez, et al., 2013). Parchment is also less hygroscopic than other regions of the coffee bean, adding more protection from OTA development in the event of moisture exposure (Ramírez-Martínez, et al., 2013). Allowed levels of OTA in coffee vary by country but fall between 5-20 ppb (Mutua, 2000). ICO guidelines have been established to reduce the risk of OTA production. Guidelines include maintaining moisture content levels below 12.5%, taking steps during transport to reduce the risk of re-wetting, and inspecting holding containers for damage (2002). The application of Hazard Analysis and Critical Control Points (HACCP) in coffee production has been explored as a mitigation strategy for OTA production (Lopez-Garcia, Augusto Mallmann, & Pineiro, 2008). Conflicting evidence exists on whether coffee is a major source of OTA or other mycotoxins in the diet. Reported contamination levels vary between 0.2 and 360 ppb (Poltronieri & Rossi, 2016; Mutua, 2000). Current accepted knowledge is that coffee is not a significant source of

OTA in the diet, as OTA is degraded during roasting; however, one study found that some samples of roasted coffee remained above five ppm after roasting (Barcelo & Barcelo, 2018).

Another mycotoxin of concern is aflatoxin (AF), which has types B1, B2, G1, and G2 and is produced by *Aspergillus flavus*. AFs are the most toxic mycotoxin, and AFB1 – a carcinogen classified by the International Agency for Research on Cancer (IARC) as Group 1, or carcinogenic to humans – has been identified in green coffee. The European Food Safety Authority has set a limit of 4 ng/g of total AF in food, and recent research has estimated total aflatoxin exposure from food in European adults at 0.036 ng/kg bodyweight/day (Food, et al., 2020; Khayoon, Saad, Salleh, Manaf, & Latiff, 2014). Limited research has been conducted on AF in green coffee, but AFs have been identified at concentrations ranging from 4.28 to 17.45 µg/kg of green coffee (Soliman, 2002; Jeszka-Skowron, Zgoła-Grześkowiak, Waśkiewicz, Stępień, & Stanisz, 2017; Al-Ghouti, AlHusaini, Abu-Dieyeh, Elkhabeer, & Alam, 2020). Preventative methods for AF production are typically storage-related, but other methods to reduce AF production have been explored, such as inoculating green coffee with probiotic bacteria (Florina, Popescu, Rotariu, Cozma, & Butnariu, 2018). Research conducted on canned coffees had no detectable levels of AFs, suggesting limited exposure from finished-good coffee beverages (Khayoon, Saad, Salleh, Manaf, & Latiff, 2014).

The most effective method for preventing mycotoxin production is preventing fungal growth, indicating environmental controls for temperature and relative humidity to control moisture content are essential for green coffee safety (Garcia, Ramos, Sanchis, & Marın, 2009). Mycotoxin production during storage of green coffee can occur between 10°C and 35°C, water activity (a_w) between 0.80 and 0.99 (Suárez-Quiroz, et al., 2004; Palacios-Cabrera, Taniwaki, Menezes, & Iamanaka, 2004; Gil-Serna, et al., 2014; Pardo, Ramos, Sanchis, & Marín, 2005). A

green coffee moisture content of 12.5% or below and a relative humidity environment below 75% is necessary to avoid spoilage of green coffee due to fungal growth, although some research set limits at 14% moisture content instead of 12.5% (Bucheli, Meyer, Pittet, Vuataz, & VIani, 1998). The limit of 12.5% moisture content and 75% relative humidity is especially relevant when the green coffee is stored in jute sacks (Broissin-Vargas, Snell-Castro, Godon, González-Ríos, & Suárez-Quiroz, 2018). It is worthwhile to continue to quantify mycotoxin levels in green coffee to ensure the product is being handled and stored in such a way as to prevent fungal growth, as conditions that may spur fungal growth are also conditions that can cause quality degradation.

1.6 The Relationship between Water and Shelf Life of Green Coffee

Thus far, green coffee production, packaging, storage, transportation, and how these steps relate to green coffee quality, have been explored. These steps rely on a shared variable: water. From rainfall affecting tree flowering to moisture exposure during transport eliciting mold growth, water is an essential quality determinant for green coffee. Green coffee beans are hygroscopic materials shipped and stored at intermediate moisture. Quantifying and controlling the amount of water in green coffee is essential for the longevity of raw, specialty-grade green coffee beans.

1.6.1 Moisture Content

Water has an essential role in the physical and textural nature of a product, as well as stability. The amount of water present in a product is a strong predictor of shelf life. Water in a product can be quantified in terms of water activity or moisture content. Both measurements are necessary to understand the states of water in a food. In green coffee production and trade, moisture content is commonly used as a quality and safety indicator.

Moisture content is a measure of the total amount of water in a product and is usually reported as a percentage of the total weight of a known amount of the product. Moisture content can either be reported on a wet (wb) or dry weight basis (db). Moisture content on a wet basis is the ratio of the weight of water in the sample (weight of dry sample subtracted from weight of wet sample) to the total weight of the sample (Mauer & Bradley Jr., 2017). Moisture content on a wet basis falls between 0 and 100 percent. Wet weight basis MC is the most common reporting method. Green coffee is traded by weight, with moisture being measured on a wet weight basis. Moisture content on a dry basis is the ratio of the weight of water in the sample to the weight of the dried sample, thus db can range from 0 to greater than 100 percent (Mauer & Bradley Jr., 2017). Dry weight basis MC is often used to describe changes in moisture during drying.

Methods for determining moisture content can be generally grouped into direct and indirect methods. Rapid methods exist for both groups. Direct methods often involve quantifying the weight change of a sample before and after water has been evaporated from the sample via heating. Common direct methods for determining coffee bean moisture content are oven methods following ISO 6673 (Rendón, Salva, & Bragagnolo, 2014; Gautz, Smith, & Bittenbender, 2008; Oliveira, Corrêa, Oliveira, Baptestini, & Vargas-Elías, 2017; Pittia, Nicoli, & Sacchetti, Effect of Moisture and Water Activity on Textural Properties of Raw and Roasted Coffee Beans, 2007; Goneli, Corrêa, Oliveira, & Júnior, 2013; Corrêa, Goneli, Júnior, Oliveira, & Valente, 2010; Ribeiro, et al., 2011). Moisture methods rely on gravimetric values obtained pre- and post-drying at low temperatures (70°C to 103°C) with vacuum, convection, or forced-air ovens. Oven-based moisture methods are gold standard methods for moisture quantification. Another direct method for measuring moisture content is the Karl Fischer titration, a chemical method. The Karl Fischer titration is used for food products, such as roasted coffee, that lose their integrity during heating

or when they are exposed to vacuum environments (Mauer & Bradley Jr., 2017). Rapid direct methods rely on instruments to continuously monitor the weight change of a sample as water is driven off, usually via microwave or infrared drying, or a combination of both. Rapid direct methods may reduce handling errors as the sample is left alone, but there are limiting factors, such as the sample size necessary to produce an accurate measurement. Rapid methods must also be validated for their precision and accuracy using conventional direct methods if they are to be used as substitutes for direct methods. Both direct methods and rapid direct methods are destructive.

Indirect methods rely on water-related physical properties of food (i.e., conductivity or density) to quantify moisture content and are often nondestructive. Methods that rely on electric currents to determine moisture content are called dielectric methods. Since water is conductive, it is possible to measure changes in conductivity when a sample is subjected to a current and relate these changes to moisture content. Indirect methods may also rely on properties such as density and refractive index to determine moisture content, but these methods often require liquid samples. Much like rapid methods, indirect methods rely on calibration using conventional direct methods. One indirect method for moisture content determination in coffee beans utilizes infrared (IR) spectroscopy. IR methods take advantage of the ability of oxygen-hydrogen bonds in water to absorb energy from IR radiation, stretch, and produce characteristic peaks at specific wavelengths on a given spectrum. IR methods are both rapid and indirect. Near-infrared spectroscopy (NIRS) can also be used for rapid prediction of moisture content, although it is more expensive and complicated (Adnan, Hörsten, Pawelzik, & Mörlein, 2017). Rapid IR moisture analysis can be done in the field by coffee growers and purchasers or used by coffee roasters as a quality measurement. One barrier for use is the cost of this equipment, thus more

accessible rapid methods may be desirable. Hyperspectral imaging (HSI) has also been used as an indirect method for moisture content prediction (Caporaso, Whitworth, Grebby, & Fisk, 2018). However, capacitance- and microwave-based rapid indirect methods are the most common and affordable (Reh, Gerber, Prodolliet, & Vuataz, 2006).

Rapid and conventional direct and indirect methods may be used for determining the moisture content of coffee beans. When possible, rapid methods save time and produce less waste as they are nondestructive. Rapid methods and indirect methods must be calibrated with conventional direct methods, such as the oven method, to ensure measurements are accurate. As discussed in the sections above, the moisture content of green coffee should fall between 9% and 12-13%, where specialty coffee moisture content is usually between 9% and 11%.

1.6.2 Water Activity (a_w)

Another value used to describe water in coffee is water activity (a_w). A_w is a thermodynamic property and a measure of the energy status of water in a system, not just the weight of water in a system. It is sometimes explained as a measure of the amount of water available to participate in chemical and biological reactions. It is related to moisture content but is a more accurate predictor of stability as it directly correlates to microbial growth, chemical and enzymatic reactions, and changes in physical properties. A_w is derived from the relationship between the thermodynamic principles of chemical potential and fugacity, where fugacity is a measure of a substance's tendency to escape a system (Reid, 2007).

Mathematically, a_w is the ratio of vapor pressure of water in a sample to the vapor pressure of pure water, where the sample and pure water must be at the same temperature and pressure (Equation 1). A_w values are unitless and always fall between 0 (no water whatsoever) and 1 (pure water). A_w values also rely on the assumption that the system and the sample are at

equilibrium. Theoretically, a_w applies only to ideal, equilibrium systems. Most systems are not ideal or completely in equilibrium. The mathematical derivation of a_w relies on vapor pressure to approximate fugacity. Relative vapor pressure (RVP) is used synonymously with a_w due to their relationship on psychrometric charts. Because moisture content is not directly related to equilibrium vapor pressure, moisture content will never provide a complete understanding of water in a system, making a_w superior to moisture content and allowing a_w to remain a common quality standard even with theoretical problems (Reid, 2007). Equilibrium relative humidity (ERH) can also be used as an expression of a_w. The relationship between a_w, fugacity, RVP, and ERH is shown below in Equation 1.

Equation 1

$$a_w = RVP = \frac{\%ERH}{100} = \frac{f_w}{f_w^0} = \frac{p_w}{p_w^0}$$

A_w is the driving force behind moisture migration in food products because water will move from regions of higher water activity to regions of lower water activity in a food system to reach equilibrium (Mauer & Bradley Jr., 2017). A_w is influenced by three major variables in food products: capillary effects, colligative properties, and surface interactions. Capillary effects are caused by water in pores and capillaries present in the microstructure of food products. More or narrower pores or capillaries cause a_w reduction. This is because the vapor pressure in the space above the pores and capillaries is lower than the vapor pressure of pure water. As mentioned above, vapor pressure is related to the amount of water that can escape into the headspace (fugacity). Thus, it is more difficult for water molecules to escape into the headspace, causing the reduced a_w. Colligative properties are governed by ionic, dipole, and hydrogen bonding between solutes (such as salt and sugar) and water. These properties include boiling point, freezing moisture, and (most importantly for a_w) vapor pressure. The more interactions between solutes

and water, the lower the vapor pressure of the water, the lower the a_w. Surface interactions are caused by direct interactions (hydrogen bonds, ionic bonds, etc.) between water molecules and large compounds (starches and proteins, for example) in a food product. If more water is bound to these ingredients, more energy is needed to free them; thus, the escaping tendency of water decreases, vapor pressure decreases, and water activity decreases.

As discussed above, food products containing water 'bound' via surface and solute interactions and water trapped in small pores or capillaries maintain a low a_w when stored properly, meaning less water is available to participate in chemical and biological reactions, increasing product stability and shelf life. Of course, when exposed to drastic temperature or moisture changes, products can rehydrate and then dehydrate, altering the properties of the material. This is one of the reasons why storage is so important for food products, especially green coffee. The interactions between a_w and moisture content in food products will be discussed in the following section.

In general, green coffee beans have an unusual geometry and a heterogeneous, compact structure with small pores that affect water transport, adsorption, and desorption in the bean (M. Kamal, Sobolik, Kristiawan, Mounir, & Allaf, 2008; Pittia, et al., 2011). However, the role of colligative properties, capillary effects, and surface interactions and their effect on a_w have not been well described in green coffee, although they have been explored in roasted coffee.

One common method for obtaining green coffee a_w is the use of a water activity meter, such as the Water's group AquaLab® meters. Dew point cells in AquaLab® meters utilize chilled mirror dew point sensors to detect condensation on a temperature-controlled mirror. The meter measures the relative humidity of the headspace over the sample, which is equivalent to the a_w of the sample at equilibrium, at the point when condensation occurs on the mirror and

records this as sample a_w. Mathematically, the system determines water vapor pressure (p_o) by taking the temperature of the sample and then headspace vapor pressure (p) from the dew point temperature (temperature where condensation occurs), then calculates a_w by dividing p by p_o (Equation 1 above). To get accurate and precise data from a_w meters, the sample and the instrument chamber must be at the same temperature and pressure and equilibrated at the time the final measurement is taken. Sample-chamber equilibration can take a minimum of five minutes up to a few hours. A_w meters rely on calibration with salt solution standards for accurate measurements. The importance of salt solutions for a_w determination will be discussed later.

A_w is reliant on a system in equilibrium and will continue to change in response to changes in the system around the material. Green coffee is hygroscopic and readily absorbs moisture out of the environment if not sealed in hermetic packaging. Thus, green coffee is vulnerable to frequent changes in a_w because of changes in the environment around it, such as from weather events or during off-loading at import docks (Palacios-Cabrera, Taniwaki, Menezes, & Iamanaka, 2004). Because of this, green coffee a_w can be highly variable day to day. This is one of the reasons why parts of the coffee industry have yet to adopt a_w and, instead, rely on moisture content. Red Fox Coffee Merchants, a United States specialty coffee importer, has successfully incorporated a_w into their roasting protocol (Edwards, 2016). The SCA requires green coffee to have an a_w at or below 0.70 to be considered specialty (SCA, 2018). Café Imports published a white paper with over 25,000 specialty coffee a_w values (almost half were longitudinal) over six years with the goal of correlating a_w with the shelf life of specific flavors noted during cupping trials and found the following: (a) mean observed a_w was 0.554; (b) a_w data alone was not a good predictor of MC; (c) a_w alone was not a good predictor of off-flavors; (d)

storage conditions should be below 60% RH and 65°F-70°F; and (e) green coffee equilibrates to 0.525 – 0.550 a_w over time when stored properly (Fretheim, 2019).

A_w is an important value for predicting quality and safety; many guidelines set by food regulatory agencies include acceptable a_w products. It is evident that more work needs to be done to define how a_w can be used more effectively to predict shelf life and quality of specialty green coffee.

1.6.3 The Relationship Between A_w and Moisture Content: Moisture Sorption Isotherms

The relationship between the amount of water present in a food (moisture content) and the chemical availability of that water (a_w) can be used to predict product stability, shelf life, and quality using moisture sorption isotherms (MSIs). MSIs reveal how food products adsorb and desorb water, meaning they help answer the following question: at a given a_w/ERH, how much water (moisture content) will a product hold? MSIs are product-specific, temperature-dependent plots of water activity or ERH (independent variable) vs. moisture content (dependent variable). MSIs rely on sample equilibration to constant and known a_w, where at equilibrium, the a_w (or ERH) of the environment is equal to the a_w (ERH) of the product. Once equilibrium is reached, then the moisture content can be referred to as equilibrium moisture content (EMC). Moisture content values used in MSI construction must be reported on a db.

To construct MSIs, a closed system with a constant, known a_w/ERH must be created. These environments are constructed using saturated salt solutions with excess salt, called salt slurries. The ERH of different salt slurries are well defined at a range of temperatures and the ERH is not affected by gain or loss of water in the system given the temperature of the environment remains constant and there is extra salt in the slurry to compensate for any gains or losses (Greenspan, 1976; Reid, 2007). Slurries provide a constant, known ERH to a closed

system. Salt slurries are used for construction of static MSIs, where static isotherms are a series of six or more individual measurements taken at a known and constant temperature and ERH at the EMC. Dynamic isotherms rely on machinery to collect many a_w and MC points at a constant temperature but varying ERH values, producing a Dynamic Dewpoint Isotherm (DDI) (Schmidt & Lee, 2012). When applied to green coffee, DDIs produced very different isotherms from the static isotherm method, indicating the hydration of green coffee is highly time-dependent (Iaccheri, et al., 2015). Thus, until the DDI method can be validated for green coffee, the static isotherm is likely more accurate.

There are three standard methods for MSI development: adsorption, desorption, and working (Labuza, 2000). Adsorption MSIs involve completely drying a food product and then allowing the product to adsorb water in a series of known ERH environments. This is the most common type of MSI in coffee literature. Desorption MSIs start with the product in an entirely hydrated state (as close to an a_w of 1 as possible) and then the product is stored in a series of lower ERH environments to track moisture loss. Working MSIs start with the product "as is", usually at an intermediate a_w, and data on adsorption and desorption is collected (AquaLab, 2011-2012). For green coffee, desorption MSIs have been used to track post-harvest drying of coffee cherries and seeds, as well as drying after re-wetting, and explore drying kinetics (Corrêa, Goneli, Júnior, Oliveira, & Valente, 2010; Goneli, Corrêa, Oliveira, & Júnior, 2013; Ramírez-Martínez, et al., 2013). Adsorption MSIs have also been used to evaluate water binding and kinetics, as well as to identify plasticization a_w and temperature, characterize textural changes, predict product stability, and predict OTA production (Pittia, Nicoli, & Sacchetti, 2007; Oliveira, Corrêa, Oliveira, Baptestini, & Vargas-Elías, 2017; Nilnont, et al., 2012; Rocculi, et al., 2011; Palacios-Cabrera, Taniwaki, Menezes, & Iamanaka, 2004; Iaccheri, et al., 2015; Iaccheri, et al.,

2019). To the author's knowledge, there has only been one isotherm made on coffee "as is." This study examined various ambient conditions in industrial green coffee silos and the effect of these conditions on various physical and chemical properties, as well as OTA production, in non-specialty green coffee (Bucheli, Meyer, Pittet, Vuataz, & VIani, 1998). Furthering green coffee knowledge using working isotherms may be of interest to the coffee industry because they provide practical data on green coffee behavior during storage.

If adsorption and desorption MSIs for a given product at a constant temperature were put onto the same graph, there would be a difference in the curves of these MSIs. This difference is called hysteresis. It is typical for the desorption curve to be above the adsorption curve, meaning as water is desorbed, there is more moisture being held at the same water activity when compared to adsorption (Labuza & Altunakar, 2007). The concept of hysteresis goes against the laws of thermodynamics (the a_w vs. EMC of a product should not depend on the 'path' taken), but there is currently no generally accepted explanation for this phenomenon. Many factors can affect the degree of hysteresis, including the rate of desorption, physical changes of the food during adsorption or desorption, and temperature. One implication of hysteresis may be different rates of microbial growth depending on whether a product is being adsorbed or desorbed. There is limited literature on green coffee hysteresis during storage or rewetting, but one study found no significant hysteresis for parchment and green coffee when using a prototype for a rapid ERH determination (Gough, 1975).

MSIs reveal how MC is affected by a_w changes. However, the relationship between MC and a_w is not linear. Instead, sorption profiles for products are often described by the Brunauer, Deming, Deming, and Teller (BDDT) classification (Brunauer, 1945). The three main classifications of MSIs following BDDT are type I, type II, and type III. Type I isotherms are

typical of anticaking agents and hygroscopic materials. These products hold large amounts of water while maintaining low a_w because of their high binding energy and non-swelling capillaries. Once all binding sites for water and capillaries have been filled, a_w increases rapidly with small increases in MC. Amorphous solids, proteins, seeds, and polysaccharides are just a few products that follow the type II isotherm classification. The heterogeneous chemical makeup of the food products, where each part has varying affinities for water, causes two bends on the isotherm, usually between 0.2 and 0.4 a_w and 0.65 and 0.75 a_w. The type II isotherm rises slowly at low a_w, then levels off around moderate a_w, then rises again at high a_w values, creating a semi-sigmoidal shape. Crystalline materials form type III, sometimes called J-type, isotherms. These products increase in a_w with very little increase in MC until the crystal structure is disrupted by water molecules, at which point the MC will rapidly increase. Research suggests that green coffee follows a type II isotherm (Iaccheri, et al., 2015; Corrêa, Goneli, Júnior, Oliveira, & Valente, 2010; Pittia, Nicoli, & Sacchetti, 2007).

As previously stated, green coffee is a heterogeneous, hygroscopic natural material of intermediate moisture. MSIs can be used to predict the a_w or MC of a product at a known temperature. Furthering research on green coffee MSIs, specifically using the coffee "as is" by following the working isotherm methodology, may provide practical data to the coffee industry on coffee behavior during storage.

1.6.4 Mathematical Modeling of MSIs

To predict moisture sorption behavior, determine MC at known a_w, and plot MSIs, mathematical models are used. Over 100 models have been proposed for modeling the sorption behavior of various products. The models can generally be classified as theoretical (kinetic) or empirical (Labuza & Altunakar, 2007). Theoretical models are based on monolayer or multilayer

sorption and rely on numerical constants defined by the material's physical properties. Monolayer sorption is based on the monolayer value (m_o). M_o is the moisture content where, at or below the m_o, a product is the most stable. This is because, at the m_o, each polar and ionic group throughout a product (on surfaces and in capillaries) is interacting with a water molecule, forming a thin, stable monolayer (Taoukis & Richardson). As temperature increases, m_o decreases because the number of available binding sites decreases (Welti-Chanes, Pérez, Guerrero-Beltrán, Alzamora, & Vergara-Balderas). M_o is usually between 0.1 and 0.4 a_w and is the starting point of many theoretical MSI models (Welti-Chanes, Pérez, Guerrero-Beltrán, Alzamora, & Vergara-Balderas). For reference, the m_o of roasted and ground coffee is approximately 3.5% (db). There is no generally referenced m_o for green coffee to the author's knowledge.

Examples of theoretical MSI models that are frequently used in green coffee MSI prediction are the Brunauer-Emmet-Teller (BET) and Guggenheim-Anderson de Boar (GAB) models. The BET model (Equation 2) is a two-parameter model that can be transformed into a linear equation and is applicable between 0 and 0.55 a_w. The BET equation is:

Equation 2

$$\frac{a_w}{(1-a_w)m} = \frac{1}{m_o} + \left[\frac{c-1}{m_o c}\right] a_w$$

where m is the EMC (db) at a known a_w and temperature, T. The model provides two constants: m_o, the monolayer moisture value, and the energy constant, c, calculated from Equation 3:

Equation 3

$$c = e^{Q_s/RT}$$

where Q_s, in cal/mol, is excess heat of sorption, T is temperature in Kelvin, and R is the gas constant (1.987 cal/mol*K).

The GAB model, in comparison to the BET model, is a three-parameter equation with a nonlinear solution. It was created as an improved version of the BET and can be applied between 0 and 0.95 a_w. A minimum of five a_w points are necessary to solve the GAB model. To solve the GAB model, the equation can be rearranged into a polynomial and solved stepwise, or a nonlinear regression program can be used. The GAB model can also be used to determine the monolayer moisture content of a product (m_o). The model formula is:

Equation 4

$$m_{eq} = \frac{Ckm_o a_w}{(1 - ka_w)(1 - ka_w + Cka_w)}$$

where m_{eq} is the EMC (db, in g/100 g dry solids), C and k are dimensionless constants related to water binding and partitioning, a_w is the water activity, and m_o is the monolayer moisture content. The constant C is a measure of the binding strength of water in the monolayer and is usually between 1 and 20. K is a ratio of the amount of water that exists as bulk water in a product to the amount of water bound in a multilayer above the monolayer. A larger k value indicates more water is behaving like free water (Labuza & Altunakar, 2007).

Empirical models for graphing MSIs are traditional linear models with two or three parameters and are solvable without computer-aided curve fitting. In today's computer age, they are often used to verify sorption data in combination with GAB or BET models. Modifications of empirical models exist as well, such as the modified Henderson model.

MSI data can be used to solve the Clausius-Clapeyron equation for net isosteric heat of sorption (H_s). The equation is:

Equation 5

$$ln\frac{a_2}{a_1} = \frac{H_s}{R}(\frac{1}{T_1} - \frac{1}{T_2})$$

where a_1 is the a_w at T_1 (Kelvin, K), a_2 is the a_w at T_2 (K), R is the gas constant (1.987 cal/mol*K), and H_s is the heat of sorption (cal/mol). H_s can be used to estimate product stability as it is a measure of how strongly bound water is to food particles. The Clausius-Clapeyron equation can also be used to predict isotherms at any temperature if isotherms at more than one temperature have been constructed. H_s values paired with MSI data can be transformed into practical tools for the coffee industry to predict a_w from any temperature/MC combination, allowing insight into product stability and shelf life.

1.6.5 Problems with Isotherm Development

One major problem that may be encountered while constructing an isotherm is mold growth. Mold growth at high a_w (> ~0.80) is a common problem in static isotherm development. To combat mold growth, researchers have explored a variety of options. Schmidt et al. exposed dent cornstarch to four different treatments for preventing *A. niger* growth: irradiating with cobalt-60, mixing with 1% potassium sorbate, mixing with 7% sodium acetate, and exposing to toluene vapor (2008). The treatment effects on mold growth, isotherm performance, and physical properties were analyzed. Irradiating with cobalt-60 did not affect isotherm performance and did prevent mold growth, but it slightly altered the properties of the cornstarch. The sodium acetate and potassium sorbate solutions were not effective at preventing mold growth without affecting the isotherm performance. Toluene inhibited mold growth but negatively impacted the isotherm performance. Methods that prevent mold growth but do not impact the isotherm or affect the physical properties of the product have yet to be identified for static isotherm development.

One sterilization method used to study green coffee germination could be explored and modified for use in green coffee MSI development. This method involved soaking the coffee in 0.4% chlorine solution and drying it under UV light (Suárez-Quiroz, et al., 2004). The antifungal

effect of UV-C radiation on roasted coffee was also explored. It was found to be successful at reducing some *Aspergillus* species growth (Byun, Park, Lee, Chun, & Ha, 2020). UV-C has also been used to control mold and mildew on strawberry plants and mushrooms (Europe Patent No. EP1940222A1, 2008; Janisiewicz, et al., 2016; Jin, et al., 2017; Short, Janisiewicz, Takeda, & Leskey, 2018). UV-C, or other methods, may be able to be used to prevent mold growth at high a_w and should be explored to improve the reliability and accuracy of MSIs.

1.6.6 Using A_w to Determine a Material's Physical State: Glass Transition, Water Transport, and Texture Analysis

MSIs can be paired with physical data to understand the mechanisms behind product stability, identify critical a_w or MC values to optimize shelf life, and predict changes in the behavior of a product. For green coffee, MSI research has been coupled with glass transition, water transport, and texture analysis to achieve these objectives.

A_w, a temperature-dependent, equilibrium-based property, is complementary to the process of glass transition. Glass transition is when a food product goes from behaving like a glassy solid to a rubbery structure, or vice versa. Glass transition affects the macro and microstructure of a food, diffusion rates within a food, and other food properties. Amorphous foods, foods that are disordered and heterogeneous, undergo glass transition at a_w-dependent temperatures. The temperature at which glass transition occurs is called the glass transition temperature (T_g). Below the T_g, the physical structure of a product is rigid and glass-like, but above the T_g, the product has more molecular mobility and is described as rubbery. Differential scanning calorimetry (DSC) is one method employed to identify the T_g of green coffee. The T_g of green coffee has been found to be 48.76°C and 34.89°C at 0.115 and 0.512 a_w, respectively (Iaccheri, et al., 2019). Increases in a_w and MC caused the T_g of green coffee to decrease. The

main components contributing to the glass transition in green coffee were identified as water and glycerol (Iaccheri, et al., 2019).

Glass transition to the rubbery phase can be described as plasticization, where water molecules interact with hydrophilic parts of amorphous solids and cause the structure of the product to relax and act more fluid-like. Water often acts as a plasticizer (a substance that can enhance the workability, flexibility, ductility, and extensibility of a polymer or product). In green coffee, water may act as a plasticizer above a critical MC or a_w value at a known temperature. Critical MC and a_w values can be determined for amorphous food systems and indicate the a_w or MC at which the product is liable to plasticize and deteriorate rapidly.

The movement of water from one region of a product to another region is called water transport (or moisture migration). Water transport is governed by thermodynamics and has been explored in green coffee to understand the behavior of water in each part of the bean during drying. Water transport has also been used to identify regions where mold growth or toxin production may occur (Ramírez-Martínez, et al., 2013; Taniwaki, Pitt, Teixeira, & Iamanaka, 2003). This was accomplished by determining the water transport coefficient for the parchment, silver skin, and endosperm of the green coffee bean. Research suggests silverskin may impact fungal development in the crease of the bean and parchment may reduce the risk of *A. ochraceus* contamination. There is limited research on the thermodynamics of green coffee water transport, but the existing data is useful for identifying processing and drying methods to prevent quality and safety problems.

A_w influences the macro and microstructure of a food product. These changes may influence the texture of that food product, and if the critical a_w is exceeded, then the product can soften, causing a decrease in the shelf stability of the product. In food science, textural properties

are determined by rheological methods and are measured using force, deformation, and flow. Key parameters for texture analysis are force (F, in Newtons), stress (Pascals (Pa) or force/ area), and strain (dimensionless). Applying this data to a food product can be difficult as the relationship between how humans perceive texture and how it is analyzed via an instrument is complicated. Nevertheless, texture analysis can be used for a variety of applications, including determining how easy or hard it is to break a product or how well liquids flow. Anecdotal information provided by coffee purchasers who have traveled to coffee-producing countries indicates that coffee farmers will often bite down on green coffee during coffee drying post-harvest as a rapid method to check for doneness, an art form much like a chef pressing on a steak. The rheological test that most applies to this situation is a compression test, specifically a uniaxial compression test (force is only applied from one direction). This method has been applied to roasted coffee in an attempt to characterize the brittleness of the roasted product, and thus the efficiency of grinding the roasted product (Pittia, Nicoli, & Sacchetti, 2007; Gabriel-Guzmán, Rivera, Cocotle-Ronzón, García-Díaza, & Hernandez-Martinez, 2017); texture analysis has also been applied to coffee cherries a green coffee (Ismail, Anuar, & Shamsudin, 2014). Uniaxial compression tests have been used to create force-displacement curves, from which fracture force (N), fracture energy (J, area under the force-displacement curve up to the first failure event), and strain at fracture (%) can be determined. Fracture force, energy, and strain were all higher for green coffee than roasted coffee. Beans stored at a_w above 0.52 had force-deformation curves indicative of a product that had undergone plasticization. Changes in the force-deformation curve shape occurred at a lower a_w (0.44) for raw than roasted coffee (Pittia, Nicoli, & Sacchetti, 2007).

Green coffee at low a_w values experience a progressively greater stiffness as moisture increases, a phenomenon in opposition to the plasticization effects of water discussed above (Pittia, Nicoli, & Sacchetti, 2007; Seow, Cheah, & Chang, 1999; Rocculi, et al., 2011; Pittia & Sacchetti, 2008). This suggests that below a critical a_w, moisture can act as an antiplasticizer in green coffee, and above the critical a_w, water behaves normally as a plasticizer. Pittia et al. suggested this critical a_w was between 0.538 and 0.760 for green coffee, and another study identified this a_w at 0.64 (2007; Rocculi, et al., 2011). The application of texture analysis for green coffee stability seems to be an under-explored area and could be useful for understanding the quality and shelf life of specialty-grade green coffee.

1.7 Compounds in Green Coffee & their Changes during Storage and Quality Loss

Green coffee growth, harvesting, and processing is a complicated multi-step process with many possible variations, all of which have an influence on the final quality of the product. Water, specifically moisture content and occasionally a_w, is the most frequently controlled variable to maintain coffee quality and it can be used to explain various mechanisms for quality degradation throughout the production process. This section is dedicated to looking at quality losses during post-harvest storage and transport. The influence of some major components of green coffee on quality, common techniques used to quantify these components, and their changes during the shelf life of green coffee, will be explored.

1.7.1 Caffeine & Chlorogenic Acids

Caffeine is a widely known psychoactive compound in coffee that also serves as a natural pesticide for the coffee plant. Research suggests it also prevents mycotoxin formation (Suárez-Quiroz, et al., 2004; Leitão, 2019; Akbar, Medina, & Magan, 2016; Buchanan, Tice, & Marino, 1982; Tsubouchi, Terada, Yamamoto, Hisada, & Sakabe, 1985). Caffeine is a purine alkaloid

and methylxanthine (xanthine alkaloid). Nitrogen fertilization has been found to increase caffeine content in coffee plants (Gonthier, Witter, Spongberg, & Philpott, 2011). Caffeine is heat-liable, as it decreases during roasting (Farah, 2012). *C. arabica* contains 0.9% to 2.5% (9-25 g/kg) of caffeine on average. For Ethiopian specialty coffee, caffeine content varies between approximately 14 and 18 g/kg (Tolessa, D'heer, Duchateau, & Boeckx, 2017). Caffeine can be used to differentiate *C. arabica* from *C. canephora* (Bicho, Leitão, Ramalho, Alvarenga, & Lidon, 2013). There is little discussion of caffeine content changes during green coffee storage, but one study examined caffeine in organic roasted coffee and reported a significant increase during storage (non-significant increase in conventional roasted coffee) (Król, Gantner, Tatarak, & Hallmann, 2020).

Chlorogenic acids (CGAs) are phenolic acids that behave as antioxidants and are the most dominant group of acids in green coffee; coffee is also the most dominant source of CGAs in the diet (Fukushima, et al., 2009; Rice-Evans, Miller, & Paganga, 1996; Gonthier, Verny, Besson, Rémésy, & Scalbert, 2003; Clifford, 1999; Xu, Hu, & Liu, 2012; Sato, et al., 2011). They are esters formed between *trans*-cinnamic acids, such as caffeic acid and quinic acid, other acids found in green coffee. CGAs are heat-liable and decrease during roasting. The most common CGA in coffee is 5-O-caffeoylquinic acid (5-CQA following IUPAC nomenclature), but thirteen classes of CGA have been identified in green coffee. Abbreviations for CGAs vary by source, so it is best to identify nomenclature rules (i.e., IUPAC) being followed when specifying CGA type (Clifford, 1999). CGAs are important to the organoleptic qualities of coffee and typically compose between 4.1-11.3% (41-113 g/kg) of the green coffee bean (Worku, Mohammed, Meulenaer, Duchateau, & Boeckx, 2018; Tolessa, D'heer, Duchateau, & Boeckx, 2017; Rendón, Salva, & Bragagnolo, 2014; Bobková, et al., 2020). CGAs have been identified as

a major storage compound during coffee seed development, along with sucrose, lipids, galactomannans, and proteins, and CGA concentration correlates with, and may be significantly affected by, altitude and mean daily temperatures, although whether the effect is positive or negative is unclear (Worku, Mohammed, Meulenaer, Duchateau, & Boeckx, 2018; Tolessa, D'heer, Duchateau, & Boeckx, 2017; Joët, et al., 2010). However, research has identified CGAs as potential discriminating compounds between coffees from different origins and processing methods (Alonso-Salces, Serra, Reniero, & Héberger, 2009; Bicho, Leitão, Ramalho, Alvarenga, & Lidon, 2013). Like caffeine, CGA changes during storage are not well documented, but one especially relevant study by Rendón et al. on Brazilian natural and semi-washed found CGA decreased from 4.9%-5.2% to 4.6% (db); however, no statement was made on the statistical significance of this change (2014). More research is necessary to determine the changes of caffeine and CGAs during storage.

High-performance liquid chromatography (HPLC) is commonly used to quantify caffeine and CGAs in coffee. Extraction methods from the literature include, but are not limited to, the following: 1) boiling 1 g of ground (495 µm), roasted coffee with 100 mL DI water for 6 minutes (Król, Gantner, Tatarak, & Hallmann, 2020); 2) grinding and freeze-drying green coffee before extracting via direct solvent extraction (10 mL methanol/water/acetic acid with ascorbic acid) (Alonso-Salces, Serra, Reniero, & Héberger, 2009); 3) freezing overnight, griding in hammer mill to < 0.7 mm in size, then extracting 0.5 g in 70% v/v aqueous methanol (Clifford, Johnston, Knight, & Kuhnert, 2003); 4) extracting ground green coffee in 70% (v/v) aqueous methanol at 60°C for 60 min, agitating every 10 min (Rendón, Salva, & Bragagnolo, 2014); 5) 100 mg green coffee ground to < 0.5 mm extracted with 10 mL methanol, water, acetic acid + ascorbic acid (2 mg/mL) in an ultrasonic bath for 15 minutes (modification of (3) above) (Worku, Mohammed,

Meulenaer, Duchateau, & Boeckx, 2018; Tolessa, D'heer, Duchateau, & Boeckx, 2017); and 6) 1 g ground green coffee samples extracted in 250 mL Erlenmeyer flask containing 100 mL methanol- water (70/30) and 0.5% Na_2SO_3 shaken overnight at 4°C in darkness and then treated with Carrez reagents before HPLC analysis (Bertrand, et al., 2003; Ky, Noirot, & Hamon, 1997). Method 6 above was the best procedure from five tested CGA purification methods (Bertrand, et al., 2003; Ky, Noirot, & Hamon, 1997). Percent yields for CGA extraction were found to be higher in isopropyl alcohol than methanol (Siva, Rajikin, Haiyee, & Ismail, 2016), but other literature has cited methanol as more effective. There are many extraction methods with good caffeine and CGA recovery for green coffee analysis, but methods used may vary based on reagent accessibility, funding limitations, analysis method, or other variables considered during the experimental design phase of research. Non-extraction-based caffeine and CGA quantification methods are currently being explored. One rapid method for quantifying caffeine content in green coffee is the use of Fourier transform infrared spectroscopy in combination with attenuated total reflectance (FT-IR-ATR) (Weldegebreal, Redi-Abshiro, & Chandravanshi, 2017). In general, it may be worthwhile to examine if and how caffeine and CGAs change in specialty grade green coffee during storage as quality degrades.

1.7.2 Polyphenols & Antioxidants

As mentioned above, CGAs are polyphenols that are present in relatively large quantities in green coffee; coffee drinkers may ingest anywhere from 500-1000 mg of CGA a day. There are many positive health effects from polyphenols in coffee, including anti-inflammatory effects and regulatory effects on metabolism (Takatoshi Murase, 2011; Ae-Sim Cho, 2010; Fukagawa, et al., 2017). It is for these reasons that CGAs and polyphenols are commonly quantified in roasted coffee. However, polyphenols and antioxidants may play a role in preserving green

coffee quality during storage. This is because CGAs have been shown to slow lipid oxidation, a mechanism for green coffee quality degradation (Santana-Galvez, Cisneros-Zevallos, & Jacobo-Velazquez, 2018). Currently, phenolic compounds (along with compounds that participate in Maillard reactions) are known to be important aroma precursors in coffee, although they may not correlate with coffee quality in terms of differentiating between commodity and specialty coffee (L.W. Lee, 2015). Phenolic compounds from green coffee extracts are also used to preserve other products, such as cookies or protein hydrolysates (Budryn, Zaczyńska, & Rachwał-Rosiak, 2017; Budryn & Nebesny, 2013).

One standard assay used to quantify total phenolic content is the Folin-Ciocalteu (F-C) assay. The F-C assay, developed in 1927 to measure tyrosine, reacts with phenols and produces a blue color change in the presence of phenolic compounds (absorbance at 765 nm). This method relies on a standard, usually gallic acid, and data are reported in mg Gallic Acid Equivalent (GAE) per gram on a dry basis (Singleton, Orthofer, & Lamuela-Raventós, 1999). The F-C reagent has been found to be reactive towards other compounds and it has been suggested that the F-C assay is closer to a measure of total antioxidant capacity instead of phenolic content (Everette, et al., 2010). Although problems exist with the F-C method, it is more accessible than other methods that rely on mass spectrometry for total phenol quantification. Method for preparing green coffee for the F-C assay include: (1) extracting 1 g of ground coffee (< 0.5 mm) in 40 mL of 50% methanol and HCl (to a pH = 2) by shaking the mixture for 60 minutes at 25°C, then removing the supernatant and adding 40 mL of 70% acetone to the precipitate and repeating the shaking step, then combining both extracts (Tripetch & Borompichaichartkul, 2019); (2) sonicating 2 g of ground coffee with 20 mL DI water for 20 minutes and then stirring for 20 minutes at 35°C, then centrifuging and removing the supernatant (Priftis, et al., 2015); (3)

stirring 35 g ground green coffee with 700 mL DI water at 80°C for 30 minutes in the dark (Siva & Noor-Azlin, 2016); and (4) boiling ground green coffee (particle size from 480-680 µm) with water in a pressure vessel at 110°C for 10 minutes, cooling to 40°C for 20 min, then filtering under a vacuum and repeating these steps three times before freeze drying (Budryn G. , et al., 2014). This final method increased CGA recovery but decreased caffeine recovery, so it would not be useful if both caffeine and CGA were examined.

The total phenolic content of green coffee determined using F-C is between 40.14 +/- 1.11 mg GAE/g (db) (Tripetch & Borompichaichartkul, 2019) or between 3.2% and 5.2% by mass (Priftis, et al., 2015). High-performance liquid chromatography-diode array (HPLC-DAD) analysis identified a phenolic content between 6-7% (w/w) in green coffee extracts (Baeza, Sarriá, Bravo, & Mateos, 2016). HPLC may also be used for total phenolic quantification. Instead of measuring total polyphenol content, some researchers chose to measure antioxidant activity (instead of in tandem). One common assay for estimating antioxidant capacity is the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. Another method is the 2,2'-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS*) assay.

The polyphenolic and antioxidant content of roasted coffee beverages are well studied, but the behavior of these compounds during green coffee storage is not. One study found that, during a 15-month storage study, total phenolic content of green coffee was stable, especially when the beans were stored in hermetic packaging (Tripetch & Borompichaichartkul, 2019). Another study stored green Robusta coffee at 9°C, 26°C, and 35°C for five days and determined a higher phenolic content in coffee stored at 9°C (Siva & Noor-Azlin, 2016). More research on the changes in phenolic content during specialty coffee storage, and if these effects are correlated

to quality losses, may be beneficial for understanding quality degradation and other problems faced by the specialty coffee industry.

1.8 Conclusion & Research Objectives

Coffee is an agricultural commodity produced in tropical climates around the globe. Specialty coffee can be differentiated from commodity coffee as coffee of high quality and with distinct, desirable attributes. High-quality coffee can be produced by manipulating pre-harvest variables, such as climate, variety, and shade amount. Harvesting methods can also be leveraged to improve the final quality of coffee, along with post-harvest steps such as sorting. Packing choices, storage conditions, and shipping methods are other variables that must be controlled to maintain not only the quality but the safety of green coffee. If green coffee is allowed to rehydrate to 12.5% MC or above, usually by storage in > 75% RH environments, mold growth and mycotoxin production may result. Mold and mycotoxins will compromise the safety and quality of the coffee. Storing all green coffee between 9 – 11% MC (wb) (< 75% RH) and 10 and 15°C is generally agreed upon in the literature for maintaining quality, primarily by ensuring green coffee bean viability is maintained, and prolonging shelf life.

Based on the current literature, it is apparent that there is limited data on specialty green coffee behavior during storage. There are only general pre- and post-harvest guidelines to maintain the shelf life and quality of specialty coffee. More research is needed on changes that occur in specialty green coffee during storage and how these changes are correlated with quality loss. The most common quality control variable used for green coffee is MC. MSIs are valuable tools for determining the MC of a product at a known a_w/ERH. Adsorption and desorption MSIs have been created for green coffee, but there is limited data on green coffee “as is.” Working

MSIs can be used to fill this research gap. MSI data can then be used to determine m_o and H_s, both of which may be practically applied to the coffee industry as a measure of product stability.

Working MSIs may be useful tools for specialty coffee importers and roasters, but more research is also needed on the physical and chemical changes that occur in specialty grade green coffee during roasting. Some chemical compounds of interest include caffeine, CGAs, and total phenolics. Physical data, such as MC and a_w, and any other values that are commonly recorded in the coffee industry can also be leveraged. The objectives of this thesis are as follows:

(1) to use the equilibrium method to create working MSIs for specialty *Coffea arabica* green coffee at applicable temperatures (20, 30, and 40 °C) and over two production years (2019 and 2020) for the first time, to the author's knowledge,

(2) to assess some thermodynamic properties of green coffee, namely net isosteric heat of sorption (H_s) and monolayer moisture content (m_o), and,

(3) to correlate physical green coffee data, which is rapidly obtainable, with MSI, quality, and chemical data quality changes that may be useful to specialty coffee producers.

The overall hypotheses of this research are that specialty green coffee will follow a type II isotherm pattern, that MSIs can aid in the prediction of the end of specialty coffee status, and that rapidly obtainable physical data can be correlated to quality losses.

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Chapter 2. Creation of a Working Moisture Sorption Isotherm for "as is" Colombian *Coffea arabica* Specialty Green Coffee

Abstract

Understanding the moisture sorption characteristics of stored specialty green coffee may be useful for maintaining specialty coffee quality and estimating specialty coffee shelf life. Working moisture sorption isotherms (MSIs) were created using "as is" specialty Colombian *Coffea arabica* green coffee at 20, 30, and 40°C, over a water activity (a_w) range of 0.11 to 0.98, for two consecutive production years (2019 and 2020). The equilibrium isotherm method was used. The Guggenheim-Anderson-de Boer model was used to fit isotherm data and estimate monolayer moisture content (m_o). Net isosteric heat of sorption (H_s) was calculated from isotherm data. Isotherms from both production years revealed specialty green coffee follows the Type II isotherm classification typical of amorphous materials. The isotherms from the 2019 and 2020 production years were significantly different, where equilibrium moisture content (EMC) values from the 2020 production year were significantly greater than EMC values from the 2019 production year. The m_o was estimated to be 6.17±0.18% (db), which roughly correlated to 0.33 a_w. This indicated green coffee is most stable when held close to 0.33 a_w; however, this a_w value is not practical for green coffee storage. H_s decreased as moisture content increased and increased rapidly for MC values below the monolayer moisture content. Evidence suggests that specialty green coffee should be stored in a narrower MC range, closer to 9% MC, to preserve quality and extend shelf life.

2.1 Introduction

Specialty grade coffee, primarily produced from *Coffea arabica*, can be defined as coffee with superior flavor attributes and low defect counts. A functional definition published by the

Specialty Coffee Association (SCA) in 2021 states that “specialty coffee is a coffee or coffee experience recognized for its distinctive attributes, and because of these attributes, has significant extra value in the marketplace” (SCA, 2021). To be deemed ‘specialty,’ the beans must be graded based on physical and organoleptic attributes following strict procedures set by the Coffee Quality Institute (CQI) to standards set by the SCA. For the last five years (2017-2021), approximately two-thirds of coffee consumed in the US has been specialty coffee (National Coffee Association (NCA) of USA, 2021).

Green coffee, the seed of the coffee tree, is an agricultural commodity that has been described as hygroscopic, meaning it readily takes up moisture from its environment. Therefore, water is an essential variable for the storage life and quality of specialty green coffee. Moisture content is a standard quality control variable, where specialty coffee should have a MC between 9 and 11%, with 12% being the upper recommendation for most green coffee (Palacios-Cabrera et al., 2007). The SCA has also recommended 0.70 water activity (a_w) for specialty-grade green coffee (SCA, 2018). However, implementing a_w measurement as a standard quality measure throughout the specialty coffee industry may be expensive, and some may argue it is more difficult to practically apply to the coffee industry (Fretheim, 2019). Furthermore, these standards were created to prevent mold growth and toxin formation, not necessarily to preserve the quality of the product. A white paper published by Ian Fretheim of Café Imports commented on the current use of a_w in the specialty coffee industry. It provides an analysis of an extensive a_w dataset for specialty green coffee (2019). This research identified the mean observed a_w for green coffee samples to be 0.554±0.057, which is lower than the SCA recommendation, and ideal storage conditions should be 60% RH and 65-70°F (Fretheim, 2019). This research also suggested that more work is needed to correlate a_w to quality losses (Fretheim, 2019). Because a_w

is a measure of product stability, it can be used to estimate product shelf life and quality; however, more research is needed to achieve this goal. One tool that may be useful is the moisture sorption isotherm (MSI).

MSIs are product-specific plots that depict MC as a function of a_w at a specified temperature. MSIs are commonly produced using the equilibrium (or static) method. The equilibrium method relies on sample equilibration to a known a_w environment, created using saturated salt slurries, in a closed system at a specified temperature (Schmidt, 2012; Greenspan, 1976). In a closed system at equilibrium, the a_w of a sample is equal to the %RH of the air in the system; thus, a_w and %RH are often used interchangeably, where a_w is equal to %RH/100. MSIs are modeled using multi-parameter equations based on MC and a_w data. Many studies have shown the GAB equation to be the best at fitting isotherm data for green coffee (Rocculi et al., 2011; Goneli et al., 2013; Pittia et al., 2007), although hundreds of models for isotherms exist (Labuza and Altunakar, 2007). Current literature on green coffee MSIs dry the coffee bean close to 0% MC and track rehydration, following the adsorption isotherm, or model how the bean dries during post-harvest treatment, following the desorption isotherm (Corrêa et al., 2010; Goneli et al., 2013, Ramírez-Martínez et al., 2013, Pittia 2007; Oliveira et al., 2017; Nilnont, 2012; Rocculi et al., 2011; Palacios-Cabrera et al., 2004; Iaccheri et al., 2015; Iaccheri et al., 2019). To the author's knowledge, only one study examined green coffee in an "as is" state, using the working isotherm method, where "as is" means the standard green coffee product how it would be stored in a warehouse, just prior to roasting (Bucheli et al., 1998). The working isotherm may be especially useful to members of the specialty coffee industry because it explores moisture sorption behavior of green coffee from the "as is" state.

The GAB equation may be used to fit isotherm data, but it also estimates isotherm parameters such as the monolayer moisture content (m_o). The m_o is defined as the MC where all available binding sites on a product have one water molecule associated with them, forming a monolayer, and is the MC at which the product is the most stable. Estimating the m_o of green coffee allows for identifying a_w and MC ranges that may optimize the stability and shelf life of green coffee. Another value that can be estimated from isotherm data is net isosteric heat of sorption (H_s). H_s, or differential enthalpy, indicates how strongly associated water is to molecules in a product and can be used to estimate product stability (Corrêa et al., 2010; Aguerre et al., 1988; Iglesias et al., 1989). H_s can be calculated from the Clausius-Clapeyron equation, where the resulting heat of sorption is temperature-independent. Then, at any combination of known temperature and a_w values, the sorption behavior of a product can be estimated.

Most members of the specialty coffee industry have methods for determining MC that are rapid and accurate. If there was a robust a_w-MC dataset, a_w could be extrapolated from MC data, and coffee producers could utilize estimated a_w values (without having to measure a_w directly) to gain insight into the stability of their product. In addition, establishing standard m_o and H_s values for specialty green coffee may also provide helpful information for maintaining product quality. Moisture sorption isotherms (MSIs) are a particularly useful tool in this regard. Thus, the objective of this research was to create working MSIs for “as is” specialty *C. arabica* green coffee at three temperatures from two production years. A secondary objective was to use MSI data, m_o, and H_s, to identify storage conditions that may prolong specialty green coffee shelf life and quality.

2.2 Methods

2.2.1 Coffee Sourcing

Washed *C. arabica* green coffee beans of specialty grade were provided by Counter Culture Coffee Roasters (Durham, NC), sourced from La Golondrina, Colombia. Two harvest years were used for isotherm development. The first replicate used green coffee harvested between May and July of 2019, and the second was between May and July of 2020. A representative sample was removed from the original, previously unopened bags, and vacuum-sealed and stored in the dark at 20°C±1°C until analysis. The initial moisture content of the green coffee was determined when the initial samples were prepared for the isotherm following AOAC 968.11 (105°C for 12 hours).

2.2.2 Working Isotherm Preparation

The following method was repeated for both the 2019 and 2020 production year green coffee. The equilibrium method for MSI development was used on "as is" green coffee samples to create a working MSI. Six saturated salt solutions, or salt slurries, were created in 1 L batches by mixing an excess amount of salt with deionized water, heating to 50°C±5°C for two hours, and then cooling to 20°C, stirring frequently. The following salts were used: lithium chloride (LiCl), magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$), magnesium nitrate hexahydrate ($Mg(NO_3)_2 \cdot 6H_2O$), sodium chloride (NaCl), potassium chloride (KCl), and potassium sulfate (K_2SO_4) (Fischer Scientific, Hampton, NH). This method was adapted from Yu et al. (2008). The salt slurries provide a_w ranges from 0.11 to 0.97 (ERH values from 11% to 98%), depending on storage temperature. 30 mL of each salt slurry was added to a clean, dry 4-ounce Ball® mason jar. Plastic pizza savers were trimmed and placed into the mason jars to act as a stand to keep the sample and sample tray from contacting the salt slurry. Four 9±1 g replicates were placed onto

perforated aluminum sample trays and placed on inert plastic pizza trays (Amazon, Seattle, WA). Chambers were stored at 20°C, 30°C, and 40°C in incubators (MyTempMini Incubators, Benchmark, NJ) in the dark until sample equilibrium was reached, indicated by a change of less than 0.0010 g after three weeks of consecutive weighing.

2.2.3 Isotherm Data Collection

Once equilibrium was reached, samples were removed from incubators, and the final a_w of the sample was determined using an Aqualab 4TE water a_w meter as a validation step (Decagon Devices, Philadelphia, PA). The a_w meter was turned on 15 minutes prior to sample analysis. For samples stored at 30°C and 40°C, the a_w meter was placed into an insulated plastic box with heating pads to create an ambient environment equal to the temperature of the incubation chamber. This reduces the risk of condensation forming on the bean and reduces equilibration time within the a_w meter. The a_w meter was calibrated before each use using at least two standard salt solutions (Decagon, Philadelphia, PA). Green coffee samples were left to equilibrate in the sample chamber on continuous mode for two hours (see Appendix B), at which point the a_w on the instrument screen was recorded and the sample was removed.

2.2.4 UV-C Sample Treatment for Mold Prevention

Mold growth on the green coffee samples was identified on samples stored with KCl and K_2SO_4 during preliminary experiments. These salts correspond to a_w values between 0.823 and 0.851 for KCl and 0.964 and 0.976 for K_2SO_4, depending on temperature. Currently, there are no standard methods for mold growth prevention during green coffee isotherm development. Green coffee samples were exposed to UV-C radiation for 90 minutes, turning every 30 minutes, to reduce or eliminate mold growth during salt slurry equilibration. Samples were treated and placed in incubation jars in a biosafety hood to reduce the risk of contamination.

2.2.5 Isotherm Modeling

Green coffee MC after equilibration was calculated following Equation 2:

Equation 6

$$m_{db} = \frac{(w_f - w_i) + (w_i) * \frac{\%H_2O}{100}}{w_i * \frac{100 - (\%H_2O)}{100}}$$

where w_f is the weight of the green coffee after equilibration, w_i is the initial weight of the green coffee, % H_2O is the wet weight (wb) MC of the initial green coffee, calculated using the AOAC oven method, and m is moisture content (db). M_{db} is equivalent to the equilibrium moisture content (EMC) of green coffee on a dry basis and is also expressed in g/ g green coffee. M_{db} was be multiplied by 100 to get % MC or g water per 100 g dry green coffee. Data obtained during isotherm production was used to fit the GAB model (equation 3):

Equation 7

$$m_{eq} = \frac{Ckm_o a_w}{(1 - ka_w)(1 - ka_w + Cka_w)}$$

where m_{eq} is the equilibrium moisture content (g/100 g dry solids), C and k are dimensionless constants related to water binding and partitioning, a_w is the water activity, and m_o is the monolayer moisture content. Nonlinear regression (quadratic modeling) was used to estimate the C, k, and m_o for each production year and temperature. This was accomplished by transforming a_w and EMC into a_w/EMC and plotting against a_w. This plot can then be fit to a quadratic model and analyzed for fit (JMP 15.0, SAS, Morrisville, NC). C, k, and m_o were solved for using the method described below (Blahovec & Yanniotis, 2007). The quadratic equation was expressed as:

Equation 8

$$ak^2 + bk + c = 0$$

where a was the intercept of the quadratic model, b was the slope of the quadratic model, and c was the quadratic. Equation 8 can then be solved for k, and then k can be used to solve the following equations:

Equation 9

$$C = \frac{b}{ak} + 2$$

and,

Equation 10

$$m_o = \frac{1}{b + 2ka}$$

for C and m_o. The model adequacy was analyzed based on mean relative percent deviation (P) (equation 11) and determination coefficient (R^2). In Equation 11, N is the number of experimental data, m_i is the experimental data, and m_{pi} is the predicted value from the model.

Equation 11

$$P = \frac{100}{N} \sum_{i=1}^{N} \left(\frac{|m_i - m_{pi}|}{m_i}\right)$$

A second method for fitting MSI data to the GAB equation utilized the Excel Solver add-in to Excel (Windows) to find the best combination of C, K, and m_o variables to minimize the sum square error (SSE) between the experimental and predicted MSI values. Both data were reported. For clarity, the first method will be referred to as the quadratic fit method and the second method will be referred to as the Excel Solver method.

2.2.6 Net Isosteric Heat of Sorption

Net Isosteric Heat of sorption (H_s) from the Clausius-Clapeyron equation (equation 12) can be determined if MSIs have been constructed for more than one temperature. The Clausius-Clapeyron equation is as follows:

Equation 12

$$ln\frac{a_2}{a_1} = \frac{H_s}{R}(\frac{1}{T_1} - \frac{1}{T_2})$$

where a_1 is the a_w at T_1 (Kelvin, K), a_2 is the a_w at T_2 (K), R is the gas constant (1.987 cal/mol*K), and H_s is the heat of sorption (cal/mol). Ln(a_w) vs. 1/T (in Kelvin) at a constant moisture content was plotted, and the slope (equal to H_s/R), was used to solve for H_s. H_s at a given temperature and moisture content could then be used to determine a_w. A_w at a constant MC was calculated using equation 13 below (Quirijns et al., 2005).

Equation 13

$$a_w = \frac{\left[2 + \left(\frac{x_m}{x} - 1\right)C_g - \left\{\left(2 + \left(\frac{x_m}{x} - 1\right)C_g\right)^2 - 4\left(1 - C_g\right)\right\}^{\frac{1}{2}}\right]}{\left[2K\left(1 - C_g\right)\right]}$$

2.2.7 Statistics

For each production year and temperature, four replicates of each saturated salt slurry were created. Salt slurry chambers were prepared and placed in the incubators in a randomized manner. The mean, standard deviation (SD), sum square error (SSE), and coefficient of variance (CV) for each treatment were reported. Data were checked for normality using the Wilkes-Shapiro Test. If the data were normally distributed, a full factorial analysis of temperature and production year with salt slurry as a grouping variable was conducted in JMP by fitting the data to the Standard Least Squares model (SAS, Cary, NC). Then, the fit was further analyzed using Tukey's HSD test to check for significant differences between production year and temperature.

2.3 Results and Discussion

The initial MC (db) of specialty green coffee samples from La Golondrina, Colombia, was 9.2% in 2019 and 12.1% in 2020. The average EMC (db) for green coffee for each

temperature and production year post-incubation is shown in Table 2.1. Green coffee stored in chambers at 0.54, 0.51, and 0.48 a_w ($Mg(NO_3)_2$ treatment depending on temperature) had EMC values closest to the initial MC but still decreased slightly. Green coffee stored in chambers with lower a_w environments (LiCl and $MgCl_2$ at roughly 0.30 and 0.11 a_w, respectively, depending on temperature) lost moisture, and coffee stored in chambers with higher a_w environments (NaCl, KCl, and K_2SO_4 at roughly 0.75, 0.83, and 0.97, respectively, depending on temperature) gained moisture.

Table 2.1 Average A_w, Equilibrium Moisture Content (EMC), Standard Deviation, and Coefficient of Variation (CV) Determined of Specialty Colombian Green Coffee by Temperature and Production Year.

2019										
Salt	20°C				30°C				40°C	
	a_w	EMC	CV		a_w	EMC	CV		a_w	EMC	CV
LiCl	0.11	3.88±0.04	1.10		0.11	3.08±0.06	1.90		0.11	2.54±0.06	2.09
$MgCl_2$	0.33	7.31±0.05	0.64		0.32	6.32±0.18	2.92		0.32	5.81±0.09	1.48
$Mg(NO_3)_2$	0.54	10.47±0.07	0.63		0.51	9.4±0.13	1.42		0.48	8.38±0.1	1.15
NaCl	0.76	15.73±0.1	0.61		0.75	14.43±0.33	2.25		0.75	14.1±0.23	1.60
KCl	0.85	22.24±0.31	1.39		0.84	20.44±0.7	3.41		0.82	17.14±1.44	8.37
K_2SO_4	0.98	62.06±1.17	1.88		0.97	60.28±14.49	24.04		0.96	52.7±9.47	17.96
2020										
Salt	20°C				30°C				40°C	
	a_w	EMC	CV		a_w	EMC	CV		a_w	EMC	CV
LiCl	0.11	5.95±0.04	0.69		0.11	5.24±0.02	0.32		0.11	4.81±0.04	0.80
$MgCl_2$	0.33	9.17±0.06	0.67		0.32	8.65±0.21	2.48		0.32	8.01±0.24	2.96
$Mg(NO_3)_2$	0.54	12.52±0.04	0.31		0.51	11.42±0.06	0.54		0.48	10.39±0.13	1.27
NaCl	0.76	17.7±0.09	0.53		0.75	16.77±0.17	0.98		0.75	16.43±0.23	1.38
KCl	0.85	38.6±5.02	13.00		0.84	33.3±8.94	26.84		0.82	28.35±3.32	11.69
K_2SO_4	0.98	78.38±2.48	3.17		0.97	64.17±19.54	30.45		0.96	63.38±32.33	51.01

*EMC on a db, average of 4 data points; a_w values from Greenspan (1976)

Specialty green coffee EMC values for a given salt treatment were compared between production years in Table 2.2. Average EMC for each production year at a given temperature was significantly different for LiCl, MgCl, and $Mg(NO_3)_2$ ($p < 0.05$). There was no significant difference between average EMC values by production year for either KCl or K_2SO_4, likely because the standard deviations were large due to physical changes in the green coffee beans at high temperatures at MC values. The data shown in Table 2.2 indicated a significant difference in EMC values of specialty green coffee between production years. As an agricultural commodity, green coffee may be highly variable from year to year, so it may be challenging to extrapolate isotherm data from one production year to another. This research should be repeated with other origins or production years to validate further if there is a significant difference between production years. If there is enough evidence to refute this, then data can be extrapolated indefinitely, providing valuable tools for shelf life, a_w, and EMC prediction to the coffee industry.

Table 2.2 Average EMC for each RH treatment by production year and temperature.

	LiCl			$MgCl_2$		
	20°C a_w = 0.11	30°C a_w = 0.11	40°C a_w = 0.11	20°C a_w = 0.33	30°C a_w = 0.32	40°C a_w = 0.32
2019	3.88±0.04a	3.08±0.06b	2.54±0.06c	7.31±0.05a	6.32±0.18b	5.81±0.09c
2020	5.95±0.04d	5.24±0.02e	4.81±0.04f	9.17±0.06d	8.65±0.21e	8.01±0.24f

	$Mg(NO_3)_2$			NaCl		
	20°C a_w = 0.54	30°C a_w = 0.51	40°C a_w = 0.48	20°C a_w = 0.76	30°C a_w = 0.75	40°C a_w = 0.75
2019	10.47±0.07a	9.4±0.13b	8.38±0.1c	15.73±0.1a	14.43±0.33b	14.1±0.23b
2020	12.52±0.04d	11.42±0.06e	10.39±0.13a	17.7±0.09c	16.77±0.17d	16.43±0.23d

	KCl			K_2SO_4		
	20°C a_w = 0.85	30°C a_w = 0.84	40°C a_w = 0.82	20°C a_w = 0.98	30°C a_w = 0.97	40°C a_w = 0.96
2019	22.24±0.31a	20.44±0.7a	17.14±1.44a	62.06±1.17a	60.28±14.49a	52.7±9.47a
2020	38.6±5.02a	33.3±8.94a	28.35±3.32a	78.38±2.48a	64.17±19.54a	63.38±32.33a

*$p < 0.05$

**each salt treatment was analyzed separately; letters between salt treatments are not connected

***a_w from Greenspan (1976)

The plotted experimental (observed) EMC and a_w data for the 2019 and 2020 production years at each temperature (20, 30, and 40°C) are shown in Figures 2.1 and 2.2. Both figures also include MSI datapoints predicted using the GAB equation. For all MSIs, the GAB equation model did not fit the final points at 0.96, 0.97, or 0.98 a_w (depending on temperature) as the GAB model fails above 0.95 a_w.

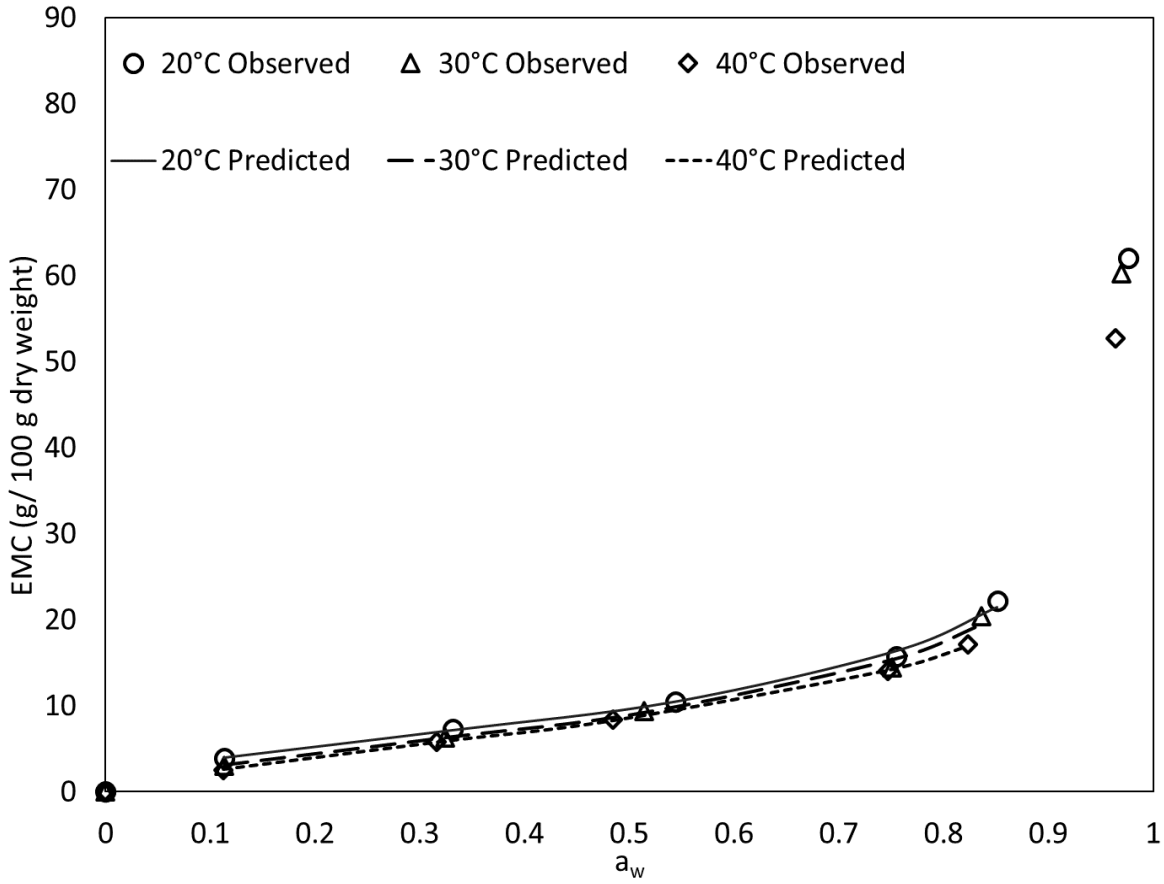

Figure 2.1 Working MSI for Specialty *C. arabica* Green Coffee from the 2019 Production Year at 20°C, 30°C, and 40°C with Experimental Equilibrium Moisture Content (EMC) Values (indicated by ○, □, ◇ points) and Predicted EMC Values from the GAB Equation (indicated by ─, ┄, ···).

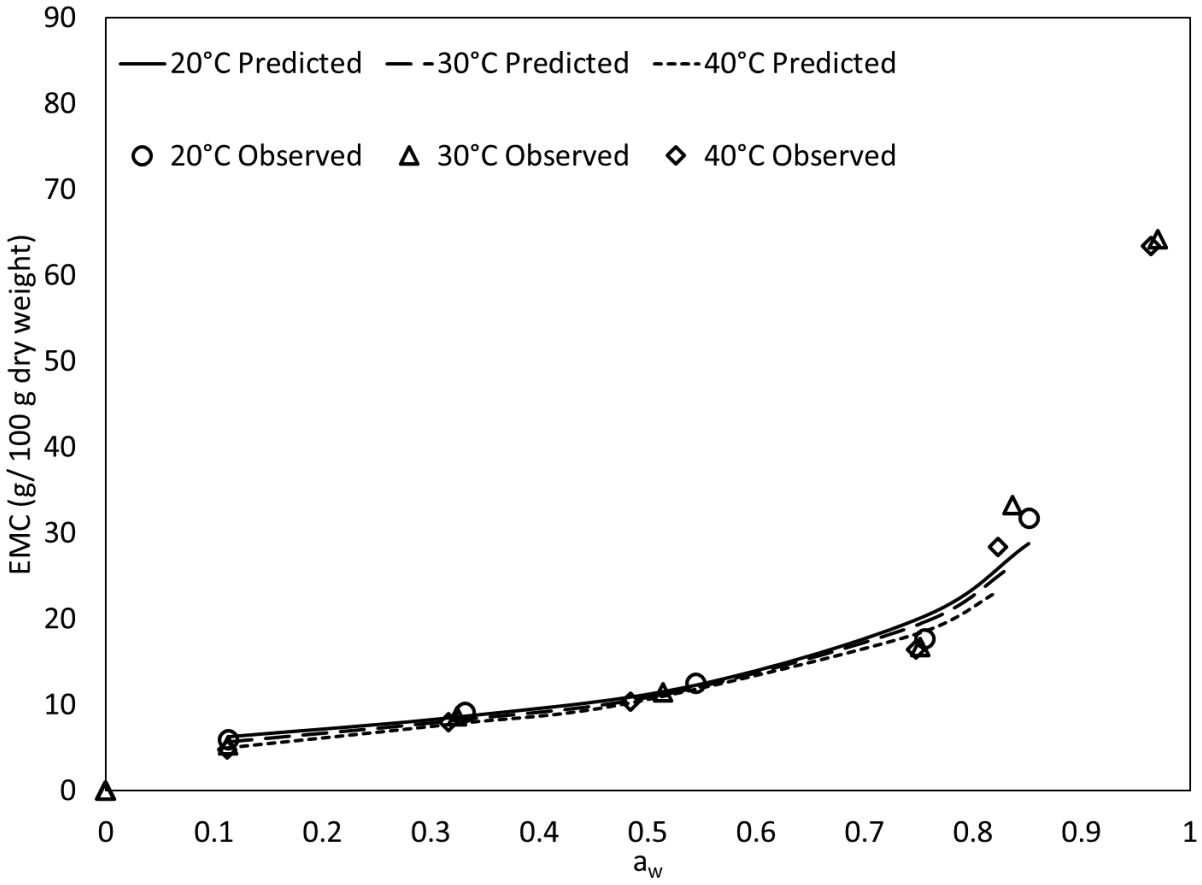

Figure 2.2 Working MSI for Specialty *C. arabica* Green Coffee from the 2020 Production Year at 20°C, 30°C, and 40°C with Experimental Equilibrium Moisture Content (EMC) Values (indicated by ○, □, ◇ points) and Predicted EMC Values from the GAB Equation (indicated by —, ---, ···).

For both production years and all temperatures, green coffee followed a type II isotherm pattern. This pattern is typical of amorphous solids, protein, seeds, and other heterogeneous products, which agrees with previous research on adsorption and desorption isotherms for green coffee (Pittia, Nicoli, & Sacchetti, 2007; Nilnont, et al., 2012; Iaccheri, et al., 2015; Corrêa, Goneli, Júnior, Oliveira, & Valente, 2010). Some literature has noted that green coffee follows the type III isotherm classification (Iaccheri, et al., 2019) or avoided categorizing the isotherm shape at all (Rocculi, et al., 2011). Some reported MSIs used a narrow range of a_w values and may have missed vital inflection points typical of type II isotherms around 0.2 a_w and 0.8 a_w. For products that follow the type II isotherm shape, if a_w is constant, the product will have a lower

EMC at higher temperatures (Labuza & Altunakar, 2007). It is difficult to say if all specialty green coffee data follows this trend because the a_w of the salt slurry decreases as temperature increases (Greenspan, 1977), thus comparing the EMC values between $Mg(NO_3)_2$ at 20°C and 40°C must take into consideration that the a_w of $Mg(NO_3)_2$ at both temperatures is not the same. A_w values for salt slurries at each temperature are shown in Table 2.1. However, EMC did decrease as temperature increased for salt slurries with approximately the same a_w across the range of temperatures. For example, for both the 2019 and 2020 production years, the EMC of green coffee stored at 0.11 a_w (LiCl) significantly decreased as temperature increased. The EMC of green coffee stored at 0.75 a_w (NaCl) at 30°C or 40°C also decreased, but not significantly. In general, specialty green coffee followed a type II isotherm pattern, and sorption behavior was significantly different between production years at the same temperature for a_w environments below 0.80.

Estimated GAB parameters from the quadratic fit method are shown in Table 2.3. GAB parameters include C, k, and m_o. Recall that m_o is the monolayer moisture content, where the product is the most stable. M_o is generally considered to be temperature independent (Quirijns et al., 2005; Staudt et al., 2013; Iglesias et al., 1989). C and k are dimensionless parameters that can also be used to understand sorption properties better. C is a measure of the binding strength of water in the monolayer; k is a ratio of the partition of water in the bulk phase to the partition of water sorbed in the multilayer (right above the monolayer) (Quirijns et al., 2005; van den Berg, 1981). A larger C value indicates water is strongly bound in the monolayer and a greater k indicates multilayer water behaves more like bulk water (Quirijns et al., 2005). For the quadratic fit method, the 2019 data fit the GAB equation better than the 2020 data. R^2 values were greater in 2019 than 2020 (> 0.91 in 2019 vs $0.77 < R^2 < 0.88$ for 2020). For both the 2019 and 2020

production years, C and k values generally decreased as temperature increased. C and k values estimated from the 2019 production year were similar to other reported values from adsorption and desorption isotherms (Goneli, 2013). 2020 Production year data had C values much greater than expected (usually C < 20). A different method for fitting the 2020 production year data may be necessary to get more realistic estimated parameters. To further understand variances in C and k, more research should be conducted across production years to see if fluctuations can be attributed to processing differences. Ideally, robust C, k, and m_o values for specialty green coffee could be identified for easier isotherm prediction in the future.

Table 2.3 GAB Estimated Parameters, Sum Square Error (SSE), Determination Coefficient (R^2), and Mean Relative Percent Deviation (P) Determined using the Quadratic Fit Method for Specialty Green Coffee Over Two Production Years.

Temp (°C)	k	C	m_o	SSE of Fit	R^2	P (%)
2019 Production Year						
20	0.85	13.04	6.15	3.05E-05	0.98	0.59
30	0.84	8.20	6.06	8.45E-05	0.92	0.74
40	0.79	6.17	6.54	5.33E-05	0.91	0.13
2020 Production Year						
20	0.92	89.62	6.18	2.60E-04	0.86	1.86
30	0.92	44.75	6.03	3.56E-04	0.77	3.10
40	0.90	24.81	6.03	1.83E-04	0.88	2.38

Table 2.4 GAB Estimated Parameters and SSE Determined using the Excel Solver Method for Specialty Green Coffee Over Two Production Years.

Temp (°C)	k	C	m_o	SSE
2019 Production Year				
20	0.88	16.89	5.66	0.66
30	0.94	16.71	4.65	2.78
40	0.80	6.48	6.35	0.02
2020 Production Year				
20	0.96	20.00	5.84	22.49
30	0.99	72.56	5.36	22.86
40	0.99	89.97	5.00	11.05

Estimated GAB parameters calculated using the Excel Solver method are shown in Table 2.4. The quadratic fit method produced lower SSE than the Excel Solver method; thus, fitting GAB data using parameters estimated from the quadratic fit method was likely more appropriate. Overall, although R^2 values are relatively low, a mean relative percentage deviation below 10% is considered acceptable in terms of fitting quality. Thus, the quadratic fit GAB model was deemed acceptable at fitting isotherm data and parameter estimations from this model were referenced.

Table 2.3 shows m_o values, estimated from the GAB equation using the quadratic fit method, ranged from 6.17±0.18% (db), which roughly correlated to 0.33 a_w. This agrees with ranges provided in the literature. Previous researchers reported the following green coffee m_o values: 5.28±0.26% (Rocculi, et al., 2011), 7.62±0.13% (Iaccheri, et al., 2015), 5.30±0.01% (Iaccheri, et al., 2019), 5.67-7.92% depending on temperature (Goneli et al., 2013), and 4.34% (Pittia et al., 2007). There was no trend in m_o related to temperature, but as previously stated, m_o is temperature independent. Differences in m_o between temperatures may be due to differences in the model fit, not the actual m_o changing. It should be noted previously reported m_o values were estimated from adsorption data or desorption data, not working isotherm data. M_o is the MC where a product is the most stable because at the m_o, there are decreased rates of chemical reactions influencing product stability (Taoukis & Richardson, 2007). However, storing specialty green coffee near 6% MC (db) (roughly 5.66% wb) is impractical. It would be difficult for members of the specialty coffee industry to achieve MC values that low, and the roasting behavior of low MC beans is unwieldy and generally avoided. Getting MC as close to m_o as possible by maintaining MC around 9% (wb) may be desirable.

Net isosteric heat of sorption (H_s) was calculated for the following MC values (db): 3, 9, 10, 11, 12, and 14%. 3% MC depicts H_s below the estimated m_o, 9-12% are reasonable MC values for green coffee, and 14% gives insight into the heat of sorption for a green coffee with too high of a MC. The MC for each temperature was mathematically extrapolated from isotherm data. An expected trend was noticed in the isotherm data: a_w increased as temperature increased at constant EMC values. H_s values were calculated by conducting a natural log transformation of a_w (y-axis), plotting the inverse of temperature (in Kelvin) on the x-axis, and fitting points with a linear equation. Each linear equation is composed of three a_w: temperature combinations at a constant MC. Equations are shown in Table 2.5. The slope of the line is equal to $-H_s$ divided by the gas constant, so the slope can be used to solve for H_s. H_s values are shown in Table 2.5.

Table 2.5 Net Isosteric Heat of Sorption (H_s) of Specialty Green Coffee at a Constant Moisture Content.

EMC (% db)	H_s Linear Equation	R^2	H_s (cal/mol)
3	y = -3382.1x + 8.4365	0.91	6720.23
9	y = -624.88x + 1.4203	0.98	1241.65
10	y = -505.37x + 1.1185	0.99	1004.17
11	y = -421.34x + 0.9127	0.99	837.20
12	y = -359.49x + 0.7653	0.99	714.31
14	y = -275.09x + 0.5711	0.99	546.60

Net isosteric heat of sorption was estimated to be significantly higher for green coffee at 3% MC than beans stored at 9%+ MC. This trend follows thermodynamic principles for amorphous materials because, at low MC, a_w is also low, and 3% is below the m_o, indicating the

water is tightly bound and would take a lot of vaporization energy to remove from the product. The relationship between water content and the energy it takes to remove water is non-linear, indicated by the almost doubled H_s between 9% and 12% when ΔMC is only 3%. Other coffee literature has found similar trends for H_s (Correa et al., 2010; Hayakawa et al., 1978).

2.5 Conclusion

MSI data is useful for determining product stability at a known a_w or MC. To the author's knowledge, this research successfully created the first working MSIs for "as is" specialty green coffee. For both production years, specialty *C. arabica* green coffee followed a type II isotherm and had an estimated m_o of 6.17±0.18% (db), which loosely correlated to 0.33 a_w. However, it is impractical to hold specialty green coffee at the m_o. Instead, holding specialty green coffee at the low end of the recommended 9-12% MC range may be desirable, as even small changes in MC influence product stability, indicated by H_s.

Green coffee samples take a long time to equilibrate following the equilibrium isotherm method. Determining MSIs for every origin, production year, or variety of green coffee would be inefficient and unrealistic. Ideally, a few robust, well-constructed MSIs for specialty green coffee could provide relevant data to the coffee industry. There was a significant difference between MSIs based on production year, indicating it may not be possible to create robust MSIs for specialty coffee for a_w and MC prediction. To determine how MSI data can be related to specialty green coffee quality and shelf life, a look into the physical and chemical changes occurring during storage may be necessary.

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Chapter 3. Physical and Chemical Analysis of Specialty Green *C. arabica* Coffee over a Three-Month Controlled Storage Period

Abstract

Rapid and accessible indicators of specialty coffee quality may be desirable by the industry. Physical and chemical variables, along with cup scores, were quantified for specialty green coffee stored at known optimal and sub-optimal storage conditions. Green coffee beans were stored in three relative humidity environments, 33%, 54%, and 75%, for 3, 6, 9, and 12 weeks at approximately 20°C and physical variables (weight change, bulk density, moisture content, a_w, color) as well as chemical variables (caffeine, 3-CQA, total phenols, ochratoxin, aflatoxin) were quantified for samples at each time and humidity combination. Cupping data was also obtained. Specialty green coffee stored at 75% RH changed the most during the storage period and experienced bean swelling, indicated by decreased bulk density by storage week 12, % MC (wb) increase, a_w increase, and color changes (primarily L*). Specialty green coffee stored at 33% humidity experienced weight and moisture loss, a_w decrease, and some color changes. Green coffee stored at 54% RH experienced the least change over the three-month storage period and was identified as the most stable storage humidity for green coffee at 20°C. Additionally, if a significant difference was noted between treatment times for a given humidity environment, most changes occurred within the first 3-weeks of storage. Significant quality losses were not achieved during this study.

3.1 Introduction

Coffee is an agricultural commodity that comes from the fruit of trees of the Rubiaceae family, genus *Coffea*. *Coffea arabica*, colloquially referred to as arabica coffee, is one of many species of the *Coffea* genus and is also one of the most traded coffees, taking up approximately

70% of the coffee market (ICO). Coffee, originally from Ethiopia, is produced in tropical environments, typically in the Torrid Zone between the Tropic of Capricorn and the Tropic of Cancer. Some coffee producing countries today include, but are not limited to, Brazil, Ethiopia, Colombia, El Salvador, and Indonesia. Specialty coffee is a classification of coffee, usually arabica coffee, that was functionally defined by the Specialty Coffee Association (SCA) as "coffee…recognized for its distinctive attributes, and because of these attributes, has significant extra value in the marketplace" (Giuliano et al., 2021). The market demand for specialty coffee is increasing (Ramírez-Correa et al., 2020).

To be classified as specialty, green coffee must be graded as a raw commodity, fall below the set guideline for defect allowances, and then be roasted and tasted following a procedure called cupping, by certified coffee graders. Production of specialty coffee requires great care, both in pre- and post-harvest treatment, to maximize the differentiating attributes and prevent quality losses. There are many steps in the processing of the coffee fruit to the tradeable raw commodity referred to as green coffee. These steps are summarized in Figure 3.1.

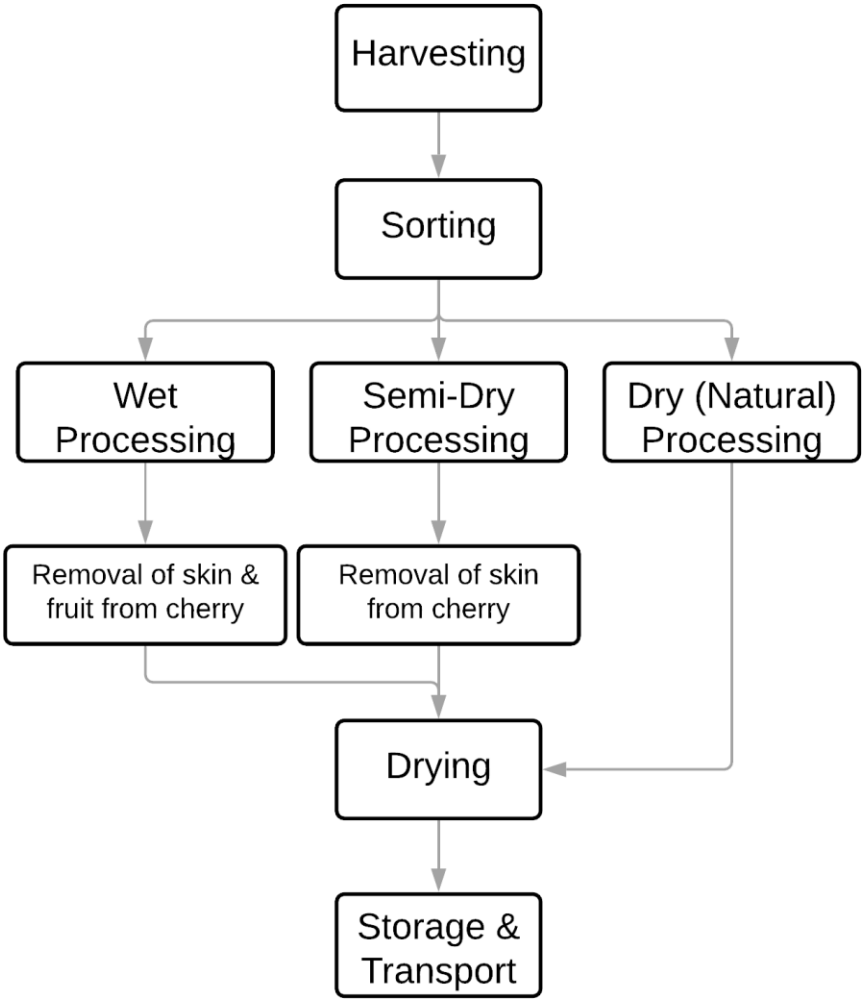

Figure 3.1 A Brief Overview of the Main Steps in Coffee Processing.

A few key drivers of specialty green coffee quality are the sorting step, the time interval for drying the harvested coffee, the end moisture content of the drying step, and the storage conditions of the green coffee post-harvest and processing. Green coffee can spend months to years in storage (Scheidig & Schieberle, 2006), where commodity coffee is often stored longer than specialty coffee. For both types of coffee, maintaining proper storage conditions is essential to preserve safety and quality.

Literature on green coffee quality losses during storage have been correlated to lipid oxidation (Rendón et al., 2014; Cong et al., 2020), coffee seed viability losses (Selmar et al., 2008; Sivetz & Foote, 1963), nonenzymatic browning reactions (Pokorný et al., 1975), free amino acid content (Pokorný et al., 1975). There is currently limited research on specialty green coffee changes during storage and their relation to cup quality. However, one study found that alternating temperatures contributed the most to moisture content gain during green coffee storage, primarily from condensation formation (Palacios-Cabrera et al., 2004). Packaging and other storage practices can be leveraged to reduce potential quality losses from green coffee rewetting and fungal growth (Poltronieri & Rossi, 2016; Palacios-Cabrera et al., 2004), but even small changes in the environment may influence coffee quality.

Quantifying quality changes in green coffee relies on analytical techniques that require some expertise, whether the technique is cupping, sample extraction for analysis on a High-Performance Liquid Chromatography (HPLC) machine or utilizing Raman spectroscopy for quality estimation (Abreu et al., 2019). Unfortunately, many members of the specialty coffee industry – notably smaller specialty coffee roasters – do not have access to complicated laboratory techniques for quality estimation and may not be certified to cup and grade specialty coffee. Thus, it is desirable to identify rapid, accessible predictors of quality (such as moisture content or color) and correlate them to cupping scores and other data obtained from chemical analysis.

According to anecdotal information, specialty coffee roasters hold green coffee in-house for an average of three months. The goal of this research is to explore how different physical and chemical attributes of a single-origin specialty green coffee change during a three-month controlled storage period at three different relative humidity environments (33% RH, 54% RH,

and 75% RH) at ambient temperature (20°C±1°C). Physical attributes, including moisture content (MC, % wet weight basis), water activity (a_w), bulk density (g/mL), color (L*a*b*) were measured, along with cupping score, fade rating, ochratoxin (OT) content (ppb), total aflatoxin (AF) content (ppb), caffeine content, chlorogenic acid content (3-caffeoylquinic acid, g/ 100 g dry weight green coffee), and total phenolic content (g/ 100 g dry weight), were quantified at 0, 3, 6, 9, and 12 weeks in an attempt to identify easy-to-measure variables that may be predictors of quality changes. This data also expanded on three treatment conditions utilized for specialty Colombian *C. arabica* green coffee moisture sorption isotherm (MSI) creation (see Chapter 2) as green coffee from the same production year (2020) was used for both experiments.

3.2 Methods

3.2.1 Coffee Sourcing

Washed *C. arabica* green coffee beans of specialty grade were provided by Counter Culture Coffee Roasters, Durham, NC, sourced from La Golondrina, Colombia. Green coffee was from the 2020 harvest year, harvested between May and July 2020. Coffee seeds were depulped the same day, dry fermented overnight, washed with water, dried for approximately seven days, transported to a new location for dry milling, and then transported to Buenaventura for shipping to the port in Charleston, SC. A representative sample was removed from the original, previously unopened bags, and vacuum sealed and stored in the dark at 20°C +/- 1°C until analysis. The initial moisture content of the green coffee was determined when the initial samples were prepared for the isotherm following AOAC 968.11 (105°C for 12 hours).

3.2.2 Chamber Preparation

Three different saturated salt slurries ($MgCl_2 \cdot 6H_2O$, $Mg(NO_3)_2 \cdot 6H_2O$, NaCl) were prepared in 1 L batches following methods by Yu et al (2008). This involved mixing an excess

amount of salt with deionized water, heating to 50±5°C for two hours, and then cooling to 20°C, stirring frequently. The salts were then placed into borosilicate glass containers with airtight lids, either 9x13 (Ello Duraglass, from Amazon.com) or 30 oz, 8x6 (M Micro, from Amazon.com). Three replicates of each salt were placed into individual 30 oz containers and four replicates of each salt were placed into individual 9x13 containers. Six pizza savers were placed in each container. Trays for holding green coffee in each chamber were constructed from plastic mesh embroidery sheets (Buygo, from Amazon.com).

3.2.3 Non-Destructive Analysis

100±1 g of green coffee were added to the 30 oz containers. The samples were stored in the dark at 20±1°C for 12 weeks and sampling with replacement was conducted every three weeks. Initial data on bulk density, moisture content, L*a*b* values, and water activity were collected using a representative sample from the original population of green coffee. At each three-week time point, samples were removed from the air-tight container, a picture was taken, and the beans were weighed (Sartorius Quintix 224 -1S, Goettingen, Germany) to track weight change over time. Bulk density was obtained by weighing the amount of green coffee necessary to fill a graduated cylinder to 40 mL. Then moisture content on a wet weight basis (wb) was obtained in triplicate using an Agratronix Portable Coffee Moisture Tester (Agratronix, Streetsboro, Ohio) method validated using oven method AOAC 968.11. L*a*b* values were collected in triplicate, where each triplicate was an average of three readings, using a HunterLab ColorFlex EZ colorimeter (HunterLab, Reston, Virginia). Two a_w sample cups were then filled halfway with green coffee and the remaining green coffee was returned to the sample chamber and covered until a_w analysis was complete. The a_w sample was allowed to equilibrate on continuous mode in a Waters 4TE a_w meter for two hours, at which point the sample a_w was

recorded and the sample was returned to the chamber. The chamber was placed back in a dark cabinet at room temperature (20±3°C). After 12 weeks, the green coffee was nitrogen flushed, vacuum sealed, and placed into the freezer.

3.2.4 Destructive Analysis

Chamber Preparation. 250±10 g of green coffee was placed into 12 9x13 air-tight containers and stored in the dark at room temperature (20°C±3°C). An initial 100g sample was nitrogen flushed, vacuum sealed, and stored in a -50°C freezer to act as a control. After 3, 6, 9, and 12 weeks, the samples were removed from the chamber.

Roasting and Cupping Data. 150 g from each time point was nitrogen flushed, vacuum-sealed, labeled with a random letter code, and sent to Counter Culture Coffee Roasters (Durham, NC) to be roasted and cupped following SCAA standards. Cupping scores along with the degree of fade were reported.

Grinding Procedure. Samples were ground using a Waring Power Grinder from frozen in 30-second increments to prevent heat buildup (Waring Spice Grinder Model WSG60, Torrington, CT). Ground particles were placed into a series of sieves on a sieve shaker (Model SS-15, Gilson) and shaken for 2 minutes. Particles that passed through a 710 µm mesh sieve were collected and particles larger than the 710 µm mesh size were added to a vacuum freezer bag, vacuum sealed, and placed into the freezer for 10 minutes to cool the sample. This procedure was repeated until 80 g of green coffee grounds was collected. All materials were cleaned and dried thoroughly between samples to prevent cross-contamination. Grounds were stored in a -50°C freezer until extraction.

Ochratoxin and Aflatoxin ELISA. Three 10 g samples were obtained from the 80 g of ground coffee and analyzed for Ochratoxin (OT) content using a Veratox® for Ochratoxin Grani

ELISA kit following kit procedures (Neogen, Lansing, Michigan). Three more 10 g samples were obtained from the remaining ground green coffee and analyzed for total aflatoxin (AF) using a Veratox® MAX for Total Aflatoxin kit following kit procedures (Neogen, Lansing, Michigan). Samples were read on an EnSpire 2300 Multilabel plate reader at 650 nm (PerkinElmer, Waltham, Massachusetts). Absorbance data was converted into micrograms/kg for reporting.

HPLC Analysis of Caffeine and Chlorogenic Acid. Green coffee extract was prepared by combining 0.100±0.02 g of each ground green coffee sample in triplicate with 10 mL methanol: water: acetic acid solution (30:67.5:2.5 vol/vol). The mixture was placed in conical centrifuge tubes, which were then submerged in an agitating water bath at 30°C for 60 minutes for extraction. See Appendix E for validation data. The extract was removed from the tube and diluted 1:1 with HPLC-grade water, and the diluted extract was filtered into labeled HPLC vials for analysis. The HPLC vials were randomly arranged in the autosampler tray, with HPLC-grade methanol rinse vials prepared every four tubes. Six vials of 0.5 mM caffeine: CGA mix were also prepared to check retention time to ensure the column is in working order and two retention time vials were run every replicate. At the end of every replicate run (13 samples plus rinses and retention time vials), a water rinse vial was used to clean the column.

Six standard solutions were created and used to develop a standard curve using caffeine and Chlorogenic acid (specifically 3-caffeoylquinic acid, or 3-CQA) powders (Sigma-Aldrich, St. Louis, MO). Serial dilutions of a 10 mM mix of caffeine and 3-CQA were done in methanol to achieve 2.5 mM, 1.25 mM, 0.625 mM, .3125 mM, .15625 mM, and .078 mM caffeine and 3-CQA mixes.

HPLC-grade methanol, HPLC-grade water, and citric acid monohydrate used for HPLC sample preparation and analysis were purchased from Fischer Scientific (Hampton, NH). The stationary phase was a Waters X-Bridge C-18, 2.5 micrometer column (100 mm x 4.6 mm) (Waters Corporation, Milford, MA). Two mobile phases were used and run at a gradient: Mobile phase A (90% 20 mM citric acid monohydrate and 10% methanol) and mobile phase B (10% methanol). The HPLC was run at room temperature, 20±3°C and at a flow rate of 0.7 mL/min. Injection volume was 0.1 microliters. Rinses and standard curve solutions had two injections per vial. Waters software, Breeze, was used to run the HPLC and autosampler and collect absorbance and retention time data (Waters Corporation, Milford, MA). Data was exported and transformed to determine caffeine and chlorogenic acid concentration in each sample.

Folin-Ciocalteu (F-C). Green coffee extract was prepared by following the same extraction protocol used for caffeine and 3-CQA analysis. Serial dilutions of a stock gallic acid phenol solution (0.5 g gallic acid into 100mL of a 10:90 mL ethanol: DI water solution) were made to obtain the absorbance of 0, 50, 100, 150, 250, 500, and 700 milliequivalents (meq) of gallic acid for standard curve creation (gallic acid source). Absorbance was determined at 765 nm and concentration and absorbance data were used for standard curve creation. 0.1 mL of green coffee extract (diluted 1:1 with DI water) was mixed with water and reagents, left to incubate in the dark, and measured on a spectrophotometer. All absorbance data was obtained using an XLS UV-Vis Spectrophotometer (Perkin Elmer, Waltham, MA).

3.2.5 Statistics

Data was checked for overall normality and normality within treatment groups and time points by checking goodness of fit to the normal distribution. If the normality assumption was upheld, a full factorial analysis of the % RH treatment and time point on each dependent variable

was conducted in JMP by fitting the data to the Standard Least Squares model (SAS, Cary, NC). Least Squares Means Difference Tukey's HSD was then run to which variables had a significant effect on the dependent variable. Levels not connected by the same level were significantly different from each other. Each measurement was plotted over time to visualize changes over time with standard deviation bars shown.

3.3 Results and Discussion

*3.3.1 Weight, Bulk Density, Moisture Content, A_w, and L*a*b* Changes During Storage*

Weight change during storage was significantly different between humidity treatments ($p < 0.01$). Within humidity treatments, there was no significant difference between weekly weight changes after the initial time point. This indicates that most changes occur within the first three weeks of storage. Table 3.1 below shows grouping letters for weight change. Changes over time are graphically represented in Figure 3.2.

Table 3.1 Average Weight Change (n = 3) of Specialty Green Coffee Stored over Time at 33%, 54%, and 75% RH and 20°C (averages connected by the same letter are not significantly different from one another, $p < 0.05$).

Week	Average Weight Change		
	33%	54%	75%
0	0b	0b	0b
3	-2.77c	0.04b	5.91a
6	-2.92c	-0.02b	6.01a
9	-2.97c	-0.02b	5.61a
12	-3.00c	-0.13b	5.64a

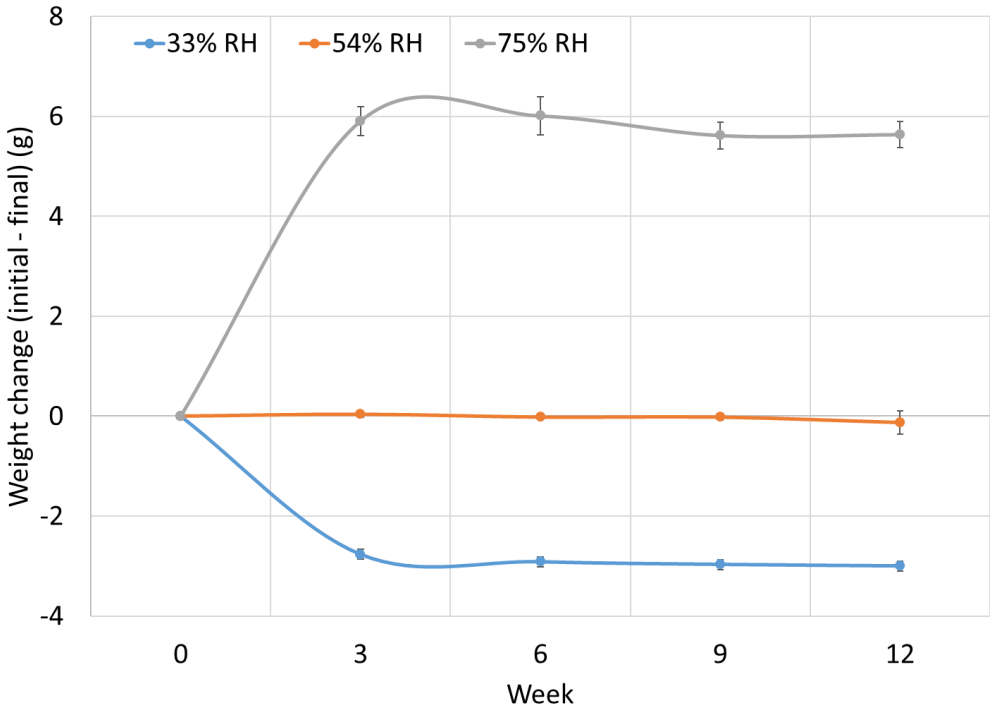

Figure 3.2 Average Weight Change (n = 3) of Specialty Green Coffee Stored Over Time at 33%, 54%, and 75% RH and 20°C.

Bulk density (g/mL) during storage did not significantly change ($p < 0.05$) over the three-month storage period for green coffee stored at 33% RH. Bulk density for green coffee stored at 54% RH was significantly decreased at week 3, but the remaining time points (week = 6, 9, and 12) were not significantly different from the initial bulk density. Green coffee stored at 75% RH displayed significant decreases in bulk density at week 3, 6, 9, and 12. Bulk density at week 12 was significantly less than week 3. At the end of the three-month storage period, green coffee stored at 75% RH had a significantly smaller bulk density than green coffee stored at 33% and 54% RH. This indicates that green coffee increased in size over time when stored at 75% RH, as a smaller bulk density means a substance takes up less mass per unit volume. Table 3.2 below shows average bulk density for each treatment and significant differences between treatments. Figure 3.3 graphically represents bulk density changes over time.

Table 3.2 Average Bulk Density (g/ mL) (n = 3) of Specialty Green Coffee Stored Over Time at 33%, 54%, and 75% RH and 20°C (averages connected by the same letter are not significantly different from one another, $p < 0.05$).

Week	Bulk Density (g/mL)		
	33%	54%	75%
0	0.70ab	0.69ab	0.70a
3	0.68abc	0.66cde	0.66de
6	0.70a	0.67bcd	0.66def
9	0.69ab	0.69ab	0.65ef
12	0.70a	0.69ab	0.63f

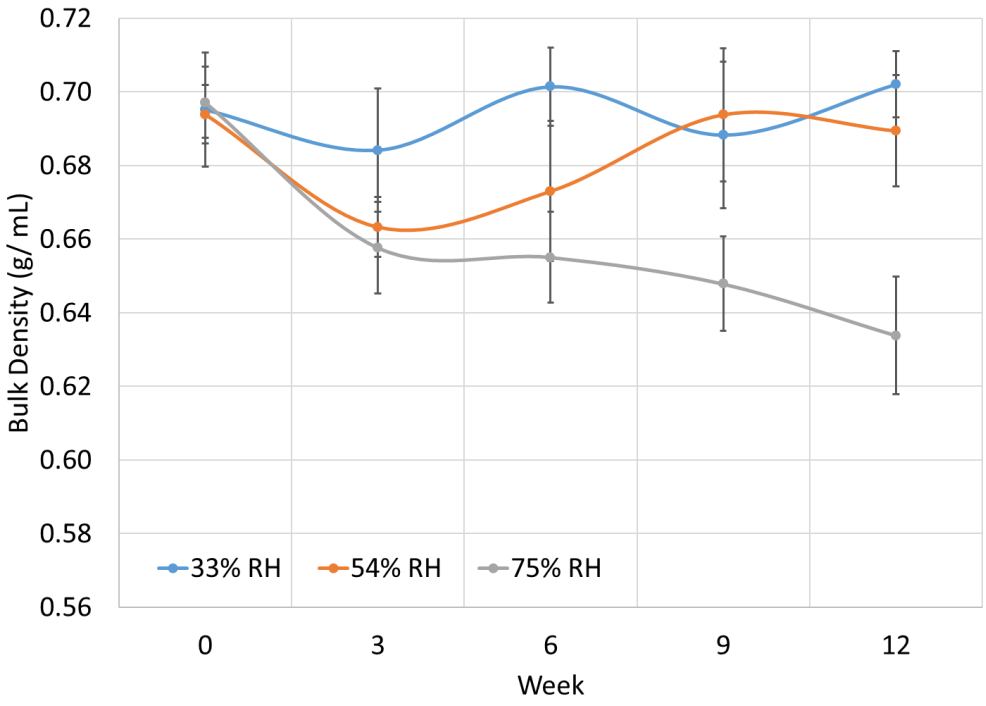

Figure 3.3 Average Bulk Density (g/ mL) (n = 3) of Specialty Green Coffee Stored Over Time at 33%, 54%, and 75% RH and 20°C.

Average MC (% wb) between RH treatments was significantly different after the initial time point ($p < 0.01$). The initial MC for green coffee stored at 75% RH was significantly lower than the initial MC for 33% and 54% RH. This suggests some initial sample variation. The MC of green coffee stored at 33% RH (for week = 3, 6, 9, 12) was significantly lower than all other

treatments. The MC of green coffee stored at 75% RH (for week = 3, 6, 9, 12) was significantly greater than all other treatments. This trend is expected, based on weight change data from Table 3.1 and Figure 3.2 above, indicating that weight change was influenced primarily by MC. Table 3.3 below shows grouping letters, representative of significant differences, for MC. These data are graphically represented in Figure 3.4.

Table 3.3 Average Moisture Content (% MC wet weight basis) (n = 3) of Specialty Green Coffee Stored Over Time at 33%, 54%, and 75% RH and 20°C (averages connected by the same letter are not significantly different from one another, $p < 0.05$).

Week	% MC (wet weight basis)		
	33%	54%	75%
0	10.92d	10.86d	10.50e
3	9.46f	10.61de	14.64a
6	9.70f	10.89d	14.56ab
9	9.53f	10.76de	13.94c
12	9.51f	10.69de	14.22bc

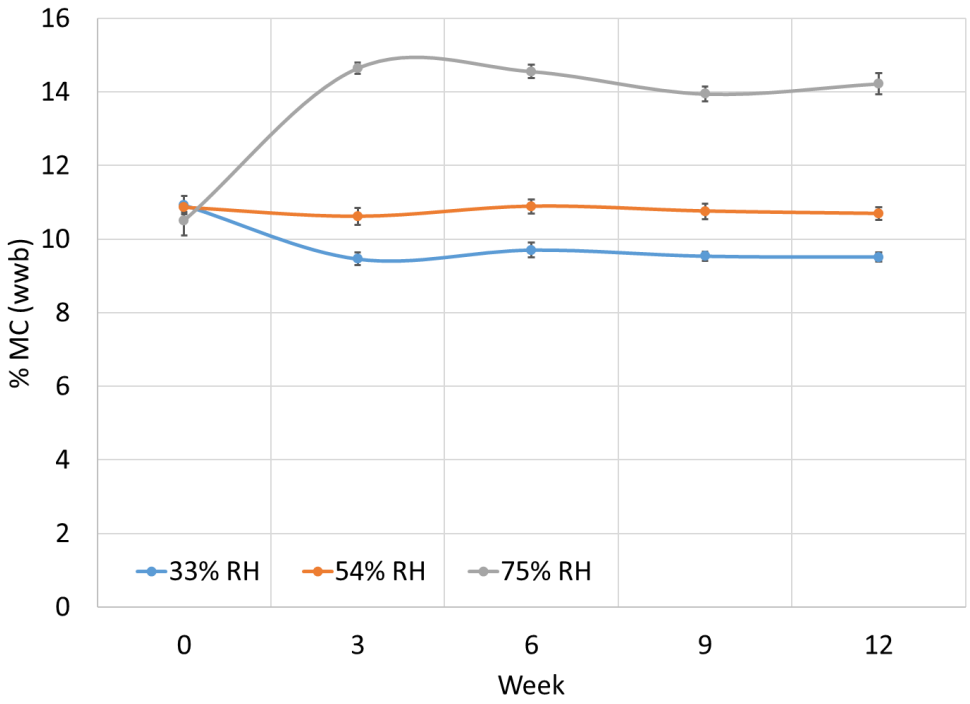

Figure 3.4 Average Moisture Content (% MC wet weight basis) (n = 3) of Specialty Green Coffee Stored Over Time at 33%, 54%, and 75% RH and 20°C.

Between treatments, a_w was significantly different after the initial time point ($p < 0.01$). Green coffee stored at 54% RH showed no change over time within treatment. Green coffee stored at 33% RH also had no significant differences between time points after the initial measurement. There were slight fluctuations in a_w over time (i.e., 0.32 a_w at week 9, but 0.34 a_w at week 6 and 12) and, although not significantly different, reveal some slight variation in a_w due to ambient conditions during sample equilibration in the a_w meter or other variables may be present. Green coffee stored at 75% RH experienced the same trend. Table 3.4 below shows grouping letters for significant differences in a_w. Average a_w values over time are shown in Figure 3.5. Note that the general trend shown in Figure 3.5 matches the trend for MC and weight change, which is to be expected as all three dependent variables are influenced by water content. Some research has indicated that specialty coffee, when stored at ideal conditions for quality

maintenance (i.e. 60% RH and 65-70°F), will equilibrate to approximately 0.554 a_w (Fretheim, 2019). As shown in Table 3.4, green coffee stored at 54% elicited a_w values in line with these recommendations. It should be noted that the SCA recommendation for specialty green coffee a_w is 0.70, but based on the % MC (wb), a_w, and bulk density data explored thus far, that a_w recommendation may be too broad to be useful.

Table 3.4 A_w (n = 3) of Specialty Green Coffee Stored Over Time at 33%, 54%, and 75% RH and 20°C (averages connected by the same letter are not significantly different from one another, $p < 0.05$).

Week	a_w		
	33%	54%	75%
0	0.55b	0.54b	0.53b
3	0.33c	0.54b	0.75a
6	0.34c	0.54b	0.76a
9	0.32c	0.54b	0.75a
12	0.34c	0.54b	0.76a

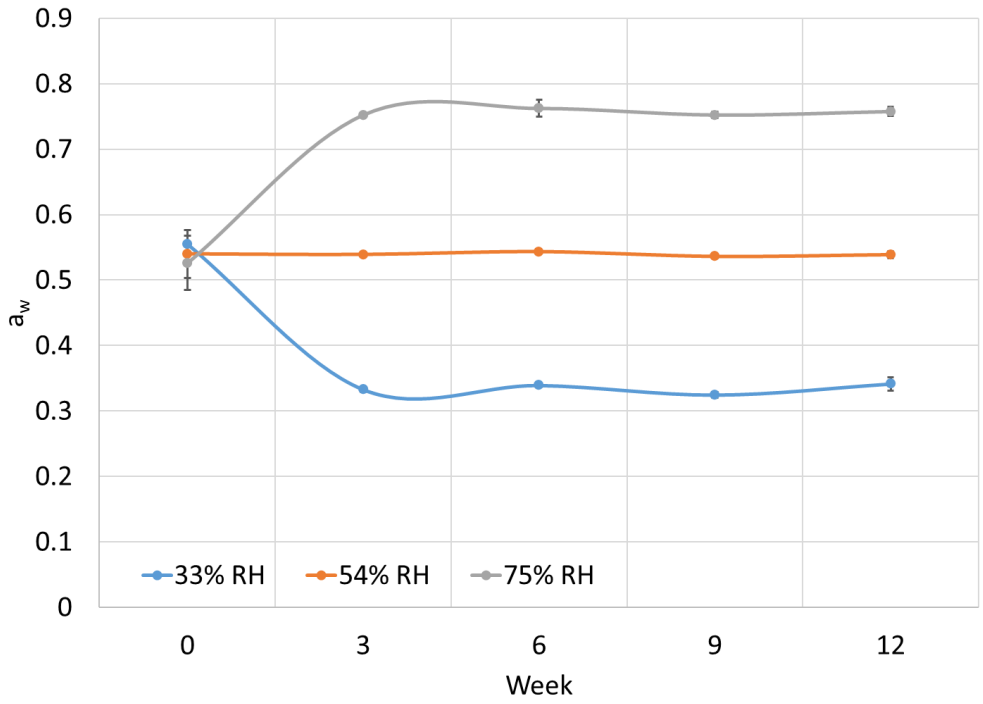

Figure 3.5 A_w (n = 3) of Specialty Green Coffee Stored Over Time at 33%, 54%, and 75% RH and 20°C.

Green coffee color was measured in the L*a*b* color space. Lightness, greenness, and blueness of green coffee are sometimes used as coffee quality variables (Rojas, 2009). However, there is limited literature data on how these colors change in specialty green coffee over time. Each color value was analyzed independently. Lightness was indicated by L*, where larger L* values indicated a lighter sample. Lightness values were significantly different between all treatments at week 3 and 12 ($p < 0.01$). Green coffee stored for 12 weeks in the 75% RH environment was significantly lighter than all other sample treatment combinations. Green coffee stored for 9 and 12 weeks at 33% RH was significantly lighter than coffee stored at 54% RH, although still significantly darker than beans stored at 9 and 12 weeks at 75% RH. RH environments that dehydrated (33%) or rehydrated (75%) the green coffee may have lightened the coffee over time. Average L* data and significant differences, represented by grouping

letters, are shown in Table 3.5. Average L* change over time is shown in Figure 3.6. Green coffee L* values stored between 60% and 80% RH experienced L* increases (bean lightening) over time (Rendón et al., 2014). L* data shown in Table 3.5 indicates that specialty green coffee stored around 54% RH should not experience L* changes, and RH should be kept below 60% to mitigate potential color changes.

Table 3.5 L*a*b* (n = 3) of Specialty Green Coffee Stored Over Time at 33%, 54%, and 75% RH and 20°C (averages connected by the same letter are not significantly different from one another, $p < 0.05$).

Week	L*			a*			b*		
	33%	54%	75%	33%	54%	75%	33%	54%	75%
0	46.37defg	46.36defg	46.17efg	1.18a	1.16a	1.05a	16.56bc	16.39bc	16.38bc
3	47.17cd	45.83g	46.07df	1.24a	1.14a	1.07a	17.51a	16.44bc	15.12d
6	46.99cdef	46.28defg	47.68bc	1.24a	1.18a	1.22a	17.53a	16.72b	16.18c
9	47.11cde	46.16efg	48.61b	1.22a	1.24a	1.08a	17.64a	16.71b	16.20c
12	47.45c	46.03fg	49.71a	1.24a	1.13a	1.19a	17.48a	16.31bc	16.75b

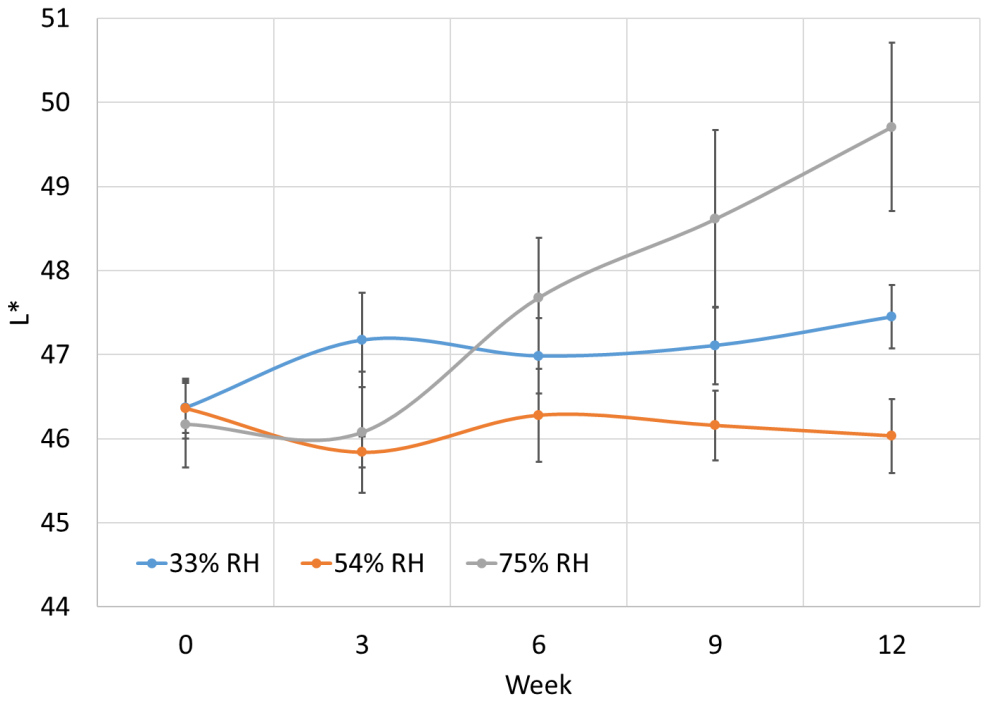

Figure 3.6 L* (n = 3) of Specialty Green Coffee Stored Over Time at 33%, 54%, and 75% RH and 20°C.

Redness or greenness of the green coffee can be quantified using a* values. Lower a* values indicated a greener sample and higher a* values indicated a redder sample. There was no significant difference in any treatment or time point. Based on this data, it can be assumed that time was not an effect for a* values; thus, a* values can be averaged by RH and the overall means can be compared. When averaged by RH, beans stored at 75% RH were significantly more green (lower a*) than beans stored at 33% RH (higher a*) (Table 3.6). Although statistically significant ($p < 0.05$), the difference may be too small to be observed by the human eye. Thus, it may be concluded that a* was not significantly affected by storage time or RH. Other literature has shown that a* significantly increased over a 15-month storage period, but when measured at three months, no significant difference was present (Rendón et al., 2014).

Average a* data between treatments is shown in Table 3.5, along with significant differences.

Average a* changes over time is shown in Figure 3.7.

Table 3.6 Average a* Values from Specialty Green Coffee Stored at Different RH Environments Where Storage Time is Not an Effect (averages connected by the same letter are not significantly different from one another, $p < 0.05$).

% RH	Average a*
33%	1.22a
54%	1.17ab
75%	1.12b

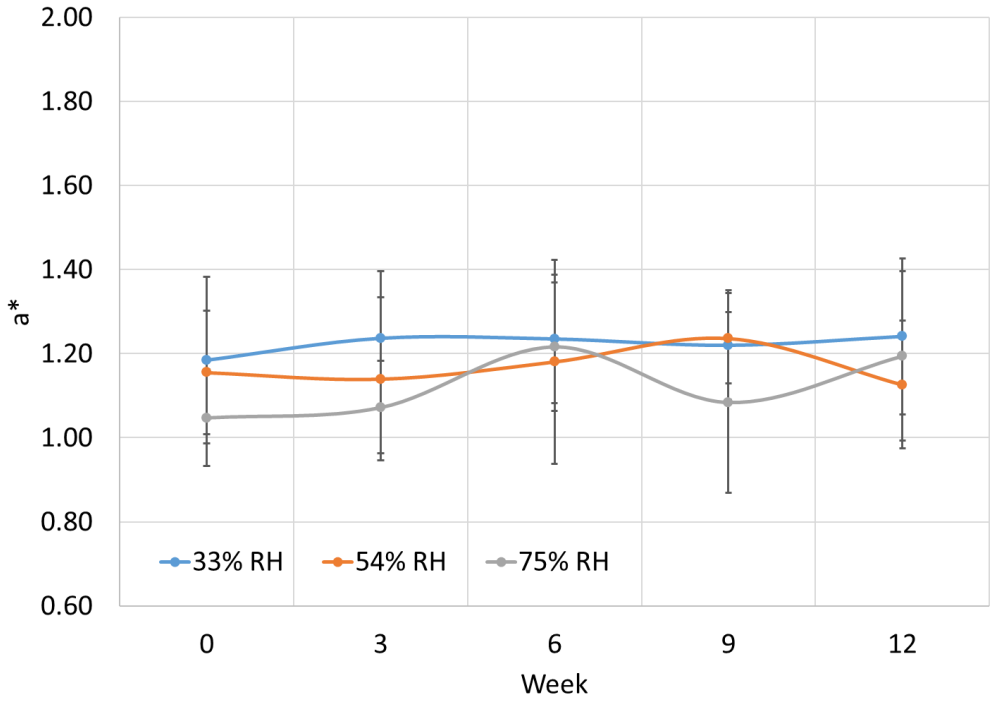

Figure 3.7 a* (n = 3) of Specialty Green Coffee Stored Over Time at 33%, 54%, and 75% RH and 20°C.

Yellowness or blueness of the green coffee can be quantified using b* values. Higher b* indicated yellower color and lower blue indicated a bluer color. Green coffee stored at 54% RH experienced no significant change over time. Green coffee stored at 33% RH was significantly

yellower (larger b*) after week three and for each remaining time point ($p < 0.05$). Green coffee stored at 75% RH was significantly bluer (smaller b*) than all other RH/time treatments, but this trend was not seen in the following weeks. B* data showed no overall trend, meaning it is likely that storage at the tested conditions has no significant effect on the yellow-blue color space. Existing literature on b* changes over time during coffee storage indicated no changes occurred; however, only one RH environment was tested (Rendón et al., 2014). Thus, more research is needed to draw a general conclusion. Average b* data between treatments is shown in Table 3.5, along with significant differences. Average b* changes over time are shown in Figure 3.8.

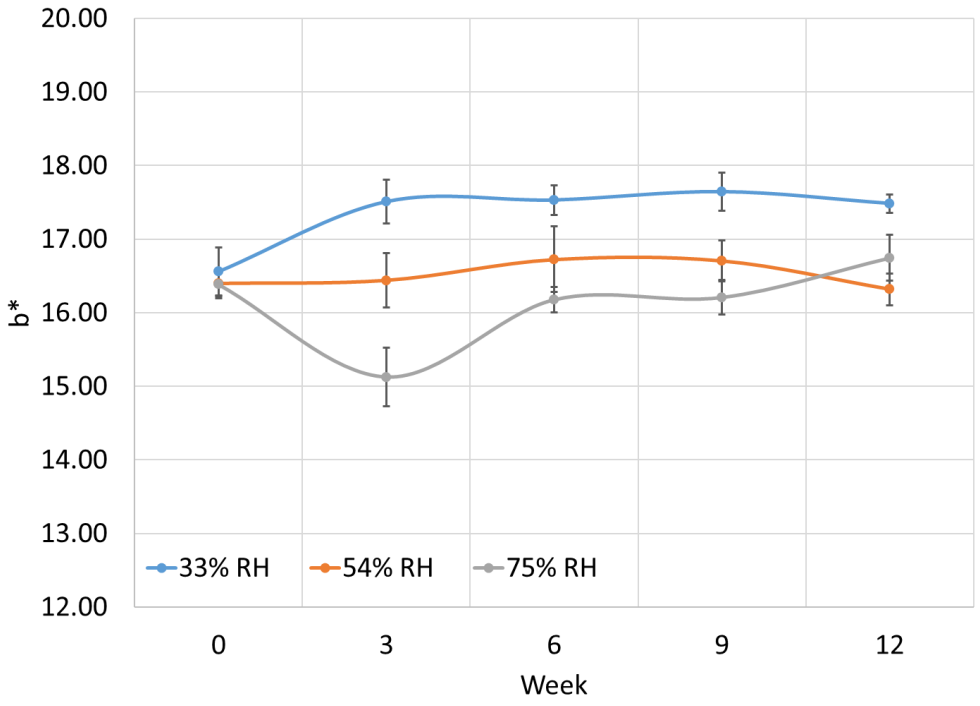

Figure 3.8 b* (n = 3) of Specialty Green Coffee Stored Over Time at 33%, 54%, and 75% RH and 20°C.

Considering the results for L*a*b*, it was likely that any color change captured by color analysis was caused by bean lightening, indicated by significant differences in L* between groups. The lightest samples were the green coffee samples stored at 75% RH for 12 weeks.

These samples also had the smallest bulk density, largest MC, largest weight increase, and highest a_w. Thus, it was likely that bean swelling due to moisture gain had a significant effect on the color of the green coffee bean. Overall, green coffee beans stored at 75% RH experienced the most changes over time. Green coffee stored at 50% RH experienced no weight, moisture content, bulk density, water activity, L*, a*, or b* changes over time. Green coffee stored at 33% RH lost weight and moisture, decreased in a_w, had a slight increase in L* over 12 weeks, and a yellower bean (higher b*). For significant physical changes (i.e., moisture content, a_w, and weight change), most of the effect took place by the first time point, equivalent to three weeks. This indicated that detrimental effects from improper storage need time to affect the green coffee, assuming the coffee seed does not come into direct contact with water.

3.3.2 Cupping and Fade

Cupping data, reported in cup score, obtained from Counter Culture Coffee Roasters (Durham, NC) was reported along with fade rating. Green coffee must be cupped by certified individuals to be classified as specialty. Another measure of quality occasionally reported in tandem with cup score is fade rating. Fade rating has been used in the specialty coffee industry to describe coffee that has flattened in flavor and has a noticeable increase in off-flavor not initially present in the green coffee (i.e., not ferment, potato defect, or other common quality defect). Fade rating is a subjective and nonstandard analysis but provides an extra data point for a given cup quality score (i.e., two coffees can score an 84 for cupping, but one may have no fade while one may have some fade present). Fade rating was reported on a scale of 0-10, where 0 is no fade and 10 is significantly faded. At the time of initial import by Counter Culture, the green coffee was scored at 86 on the SCAA scale with no fade. All cup scores measured from the treatments were less than the initial cup score. There was no significant difference between cup scores or

fade ratings between storage times and treatments after the initial time point. Cup scores are shown in Figure 3.9 and fade ratings are shown in Figure 3.10. Cup score and fade rating data are listed in Table 3.7. Although not statistically significant, the average cup score (average of scores from each time point) for beans stored at 75% RH was lower than beans stored at 50% and 33% RH. If considering cup score alone, all samples cupped were scored at above 80, meaning the green coffee could still be considered specialty grade. However, all samples had some degree of fade, meaning it is likely the quality degraded during storage for all samples. Green coffee was stored in N_2 flushed and vacuum-sealed packages until all samples were roasted and cupped, but there was a time delay between the point at which the samples were removed from the incubator and when they were roasted and cupped due to logistical problems. In the future, time delays should be avoided. Overall, it is difficult to draw any conclusions about quality changes during storage. There were no significant differences between treatments and there was no replication due to logistical constraints. More research is needed to connect specialty green coffee quality changes to storage conditions.

Table 3.7 Cup Score and Fade Rating of Specialty Green Coffee Stored Over Time at 33%, 54%, and 75% RH and 20°C.

Week	Cup Score				Fade Rating		
	33%	54%	75%		33%	54%	75%
3	83.5	86	83		2	2.5	2
6	82.5	83.25±0.35	82.25±0.35		3	2.5±0.71	3.25±0.35
9	83.5	82.5	83		2	3	2.5
12	82.5	84	82.5		3	1.5	2.5

*Initial cup score was 86, only had duplicates for 54% and 75% RH treatments at week 6

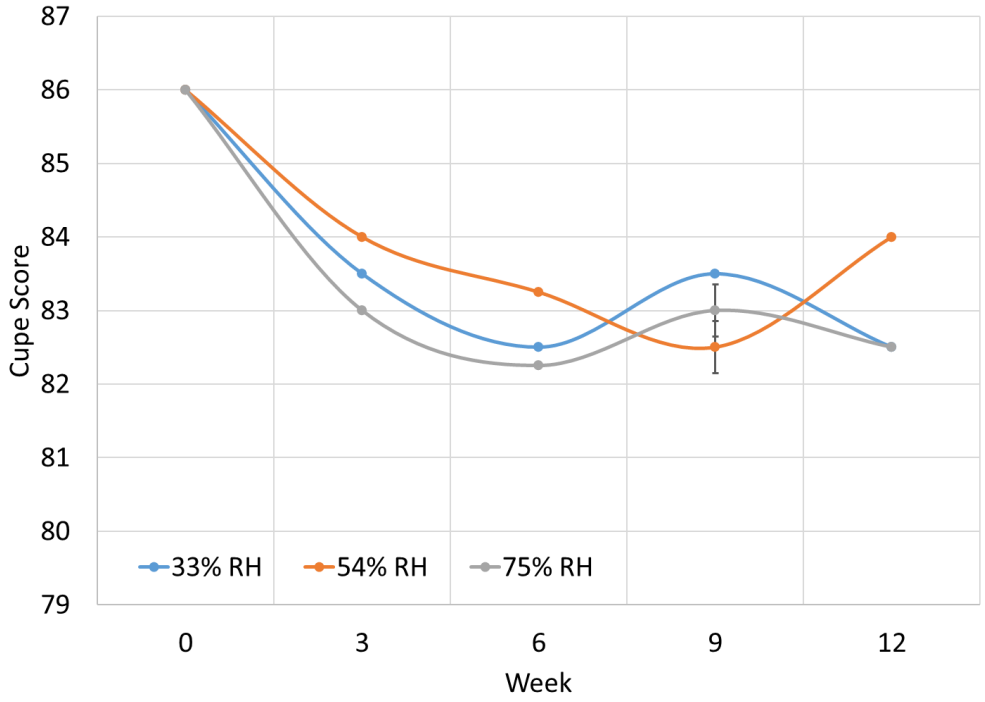

Figure 3.9 Cup Score of Specialty Green Coffee Stored Over Time at 33%, 54%, and 75% RH and 20°C.

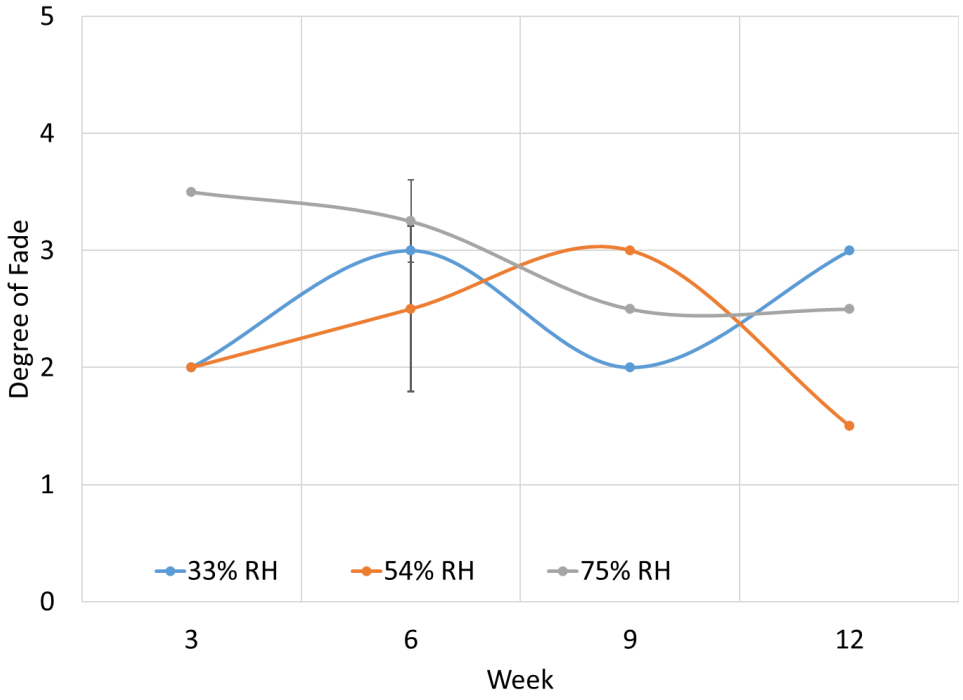

Figure 3.10 Fade Rating of Specialty Green Coffee Stored Over Time at 33%, 54%, and 75% RH and 20°C.

3.3.3 Ochratoxin and Aflatoxin

In addition to quality losses, improper storage can cause fungal growth and mycotoxin production, impacting the safety of green coffee. Two mycotoxins of concern are Ochratoxin (OT) and Total Aflatoxin (AF), produced from different species of *Aspergillus* and *Penicillium*. Both OT and AT are carcinogenic Contamination levels indicated in the literature vary between 0.2 and 360 ppb for OT in green coffee, consistent with the reported data (Poltronieri & Rossi, 2016; Mutua, 2000). Allowed levels of OT, specifically Ochratoxin A (OTA), in coffee vary by country but fall between 5-20 ppb (Mutua, 2000). AF limits in food products also fall between 4-20 ppb (Al-Ghouti et al., 2020); AFs have been identified in green coffee at concentrations ranging from 4.28 to 17.45 ppb (Soliman, 2002; Al-Ghouti et al., 2020; Jeszka-Skowron et al., 2017). OT and AF may be produced in green coffee between 10°C and 35°C and 0.80 and 0.99

a_w (Suárez-Quiroz, et al., 2004; Palacios-Cabrera et al., 2004; Gil-Serna et al., 2014; Pardo et al., 2005).

OT content of specialty green coffee followed a normal distribution. There was no significant difference between RH/storage time treatments. AF content of specialty green coffee was not normally distributed because all measurements for total aflatoxin were above quantifiable limits of 50 ppb; thus, the ppb values are unreliable. See Appendix K for standard curves used to calculate OT and AF concentrations. All samples had both OT and AF present. It is difficult to draw conclusions about the exact AF contamination levels because of the high concentrations, but there were no significant differences between treatment times or RH conditions. For more accurate AF estimation, the ELISA could be redone at a higher dilution. It was surprising and concerning that all samples were contaminated with OT and AF. These findings imply that even specialty green coffee may have been exposed to unfavorable storage conditions. More replication is necessary to determine average contamination levels of specialty coffee by mycotoxins. Research should also be conducted to validate claims that OT and AF are degraded to a safe degree during roasting. OT and AF content are shown in Table 3.8, along with significant differences between treatments, indicated by letter assignment. Figures 3.11 and 3.12 graphically represent AF and OT data.

Table 3.8 Average Ochratoxin (OT, ppb) and Total Aflatoxin (AF, ppb) Content (n = 3) of Specialty Green Coffee Stored Over Time at 33%, 54%, and 75% RH and 20°C (averages connected by the same letter are not significantly different from one another, $p < 0.05$).

Week	OT (ppb)				AF (ppb)		
	33%	54%	75%		33%	54%	75%
0	20.51a*	36.21a*	45.87a*		251.83a*	262.66a*	270.16a*
3	31.44±14.79a	46.87±13.79a	53.77±21.14a		253.78±5.96a	253.00±2.16a	210.84±76.71a
6	39.10±35.47a	43.02±13.33a	79.40±30.76a		232.96±26.70a	255.21±4.41a	252.24±5.17a
9	40.80±15.97a	38.59±7.57a	56.22±15.87a		236.81±20.53a	220.44±39.03a	250.74±5.03a
12	54.63±8.94a	59.48±3.89a	37.54±24.43a		254.29±5.76a	257.91±15.36a	259.45±8.82a

*No replication for initial treatments so no standard deviation values

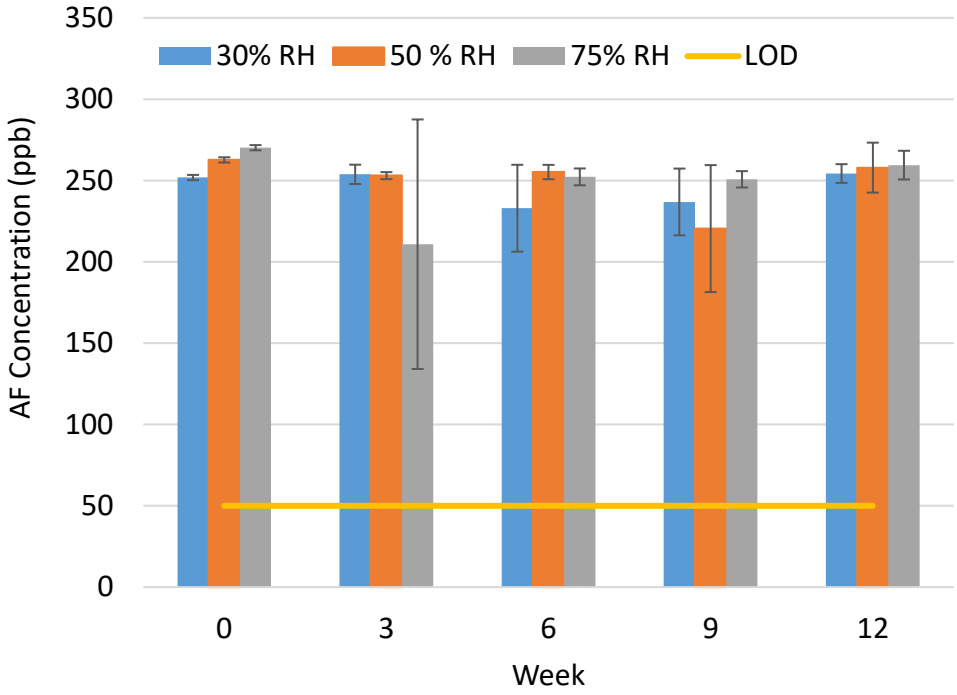

Figure 3.11 Average Total Aflatoxin (AF, ppb) Content (n = 3) of Specialty Green Coffee Stored Over Time at 33%, 54%, and 75% RH and 20°C.

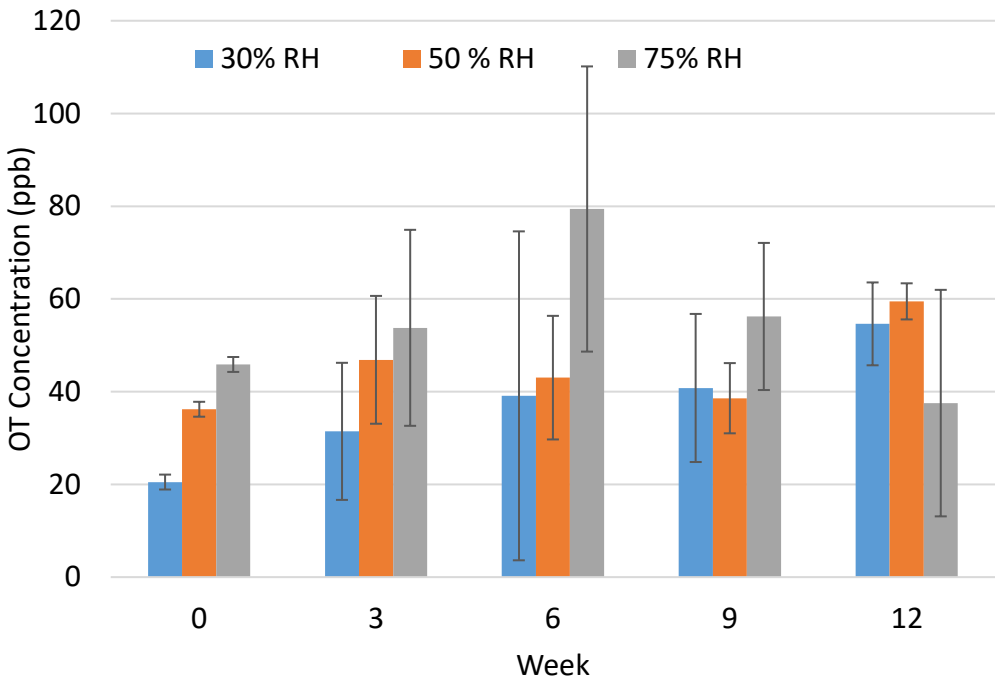

Figure 3.12 Average Ochratoxin (OT, ppb) Content (n = 3) of Specialty Green Coffee Stored Over Time at 33%, 54%, and 75% RH and 20°C.

3.3.4 Total Phenolic Content

Polyphenolic compounds are present in relatively large quantities in coffee and act as anti-inflammatories and metabolic regulators (Fukagawa et al., 2017). They are also believed to be essential aroma precursors in coffee, although they may not directly correlate with coffee quality differentiation (Lee et al., 2015). Total phenolic content was determined using the FC method, which utilized a standard curve of a known phenol stock solution (r^2 = 0.9988) to convert absorbance to a g total phenols per 100 g green coffee on a dry basis (Singleton et al., 1999). See Appendix K for the standard curve used to calculate total phenolic content. There was no significant difference in the concentration of total phenolic compounds in green coffee for any treatment combination. This is expected because even as total phenolic compounds degrade, new phenolic compounds are being formed, a phenomenon often described in coffee roasting (Farah

et al., 2005; Tfouni et al., 2012). Due to the nature of the FC analysis, which relies on spectrophotometry and sample dilution, large standard deviations are possible. Experimental total phenolic content of specialty green coffee was estimated to be between 22 and 37 g/ 100 g dry green coffee. Previous literature has identified the total phenolic content of green coffee to be around 40 mg GAE/g (it is not possible to convert this to reported units due to limited data), or between 3.2% and 5.2% by mass (Tripetch et al., 2019; Priftis et al., 2015). Average total phenolic content of specialty green coffee for each treatment is shown in Table 3.9 and graphically represented in Figure 3.13.

Table 3.9 Average Total Phenolic Content (g/ 100 g dry green coffee) (n = 3) of Specialty Green Coffee Stored Over Time at 33%, 54%, and 75% RH and 20°C (averages connected by the same letter are not significantly different from one another, $p < 0.05$).

Week	Total Phenolic Content (g/ 100 g dry green coffee)		
	33%	54%	75%
0	34.02a*	31.18a*	30.44a*
3	36.88±3.37a	35.56±1.47a	33.07±2.27a
6	23.95±11.73a	22.32±4.85a	30.21±2.26a
9	24.66±14.68a	28.91±13.85a	21.91±8.98a
12	37.23±4.29a	22.46±6.30a	32.69±4.69a

*No replication for initial treatments so no standard deviation values

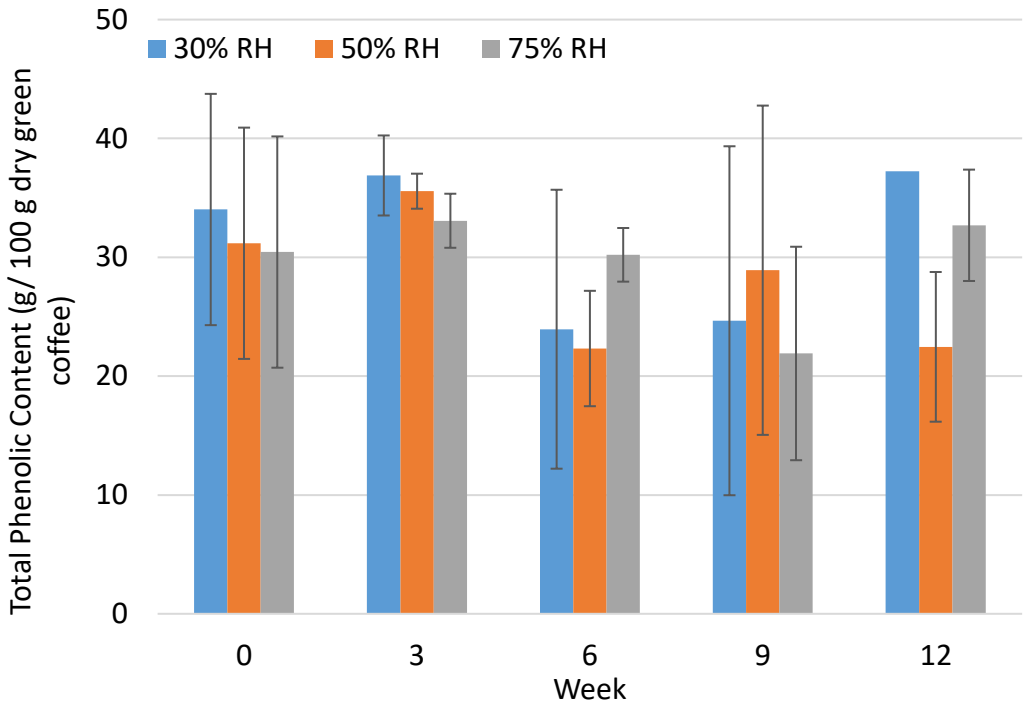

Figure 3.13 Average Total Phenolic Content (g/ 100 g dry green coffee) (n = 3) of Specialty Green Coffee Stored Over Time at 33%, 54%, and 75% RH and 20°C.

3.3.5 Caffeine and CGA

Caffeine and chlorogenic acids (CGA) are perhaps the most cited components of coffee. Caffeine is a psychoactive compound in *C. arabica* at about 1.2 g per 100 g (Mazzafera et al., 2010; Ayu, 2020). CGAs are polyphenolic and antioxidant compounds present at about 5-12% by weight in *C. arabica* (Farah et al., 2006; Farah et al., 2008). Although not the dominant CGA in green coffee, 3-caffeoylquinic (3-CQA) was used as an indicator of CGA content due to standard availability and other experimental constraints. Caffeine and 3-CQA concentrations were determined using a standard curve of each compound (r^2 = 1, r^2 = 0.9983 respectively) in tandem with area unit data from Breeze software and converted to a g per 100 g green coffee basis. See Appendix K for the standard curves used to calculate caffeine and 3-CQA concentration. Caffeine was significantly different between treatments for beans stored at 75%

RH for 6 weeks and beans stored at 33% RH for 3 weeks ($p < 0.05$), where the beans stored at 75% RH for 6 weeks had the lowest caffeine content. All other treatments were not significantly different from each other. To the author's knowledge, no data on caffeine content changes during green coffee storage exist. However, one study examined caffeine in organic roasted coffee and reported a significant increase during storage (Król et al., 2020). Caffeine change over time for different RH environments is depicted in Figure 3.14, and average caffeine values are shown in Table 3.10.

Table 3.10 Average Caffeine (g/ 100 g green coffee) and 3-CQA (g/ 100 g green coffee) Content (n = 3) of Specialty Green Coffee Stored Over Time at 33%, 54%, and 75% RH and 20°C (averages connected by the same letter are not significantly different from one another, $p < 0.05$).

Week	Caffeine				Chlorogenic Acid (3-CQA)		
	33%	54%	75%		33%	54%	75%
0	0.63ab	0.59ab	0.62ab		6.35a	5.79a	6.09a
3	0.67±0.02a	0.59±0.05ab	0.60±0.04ab		6.59±0.12a	5.89±0.56a	5.90±0.26a
6	0.65±0.02ab	0.62±0.04b	0.56±0.05ab		6.42±0.19a	6.05±0.23a	5.75±0.51a
9	0.62±0.03ab	0.63±0.03ab	0.59±0.02ab		6.30±0.25a	6.15±0.38a	5.98±0.24a
12	0.63±0.03ab	0.59±0.01ab	0.63±0.02ab		6.23±0.33a	6.10±0.02a	6.34±0.26a

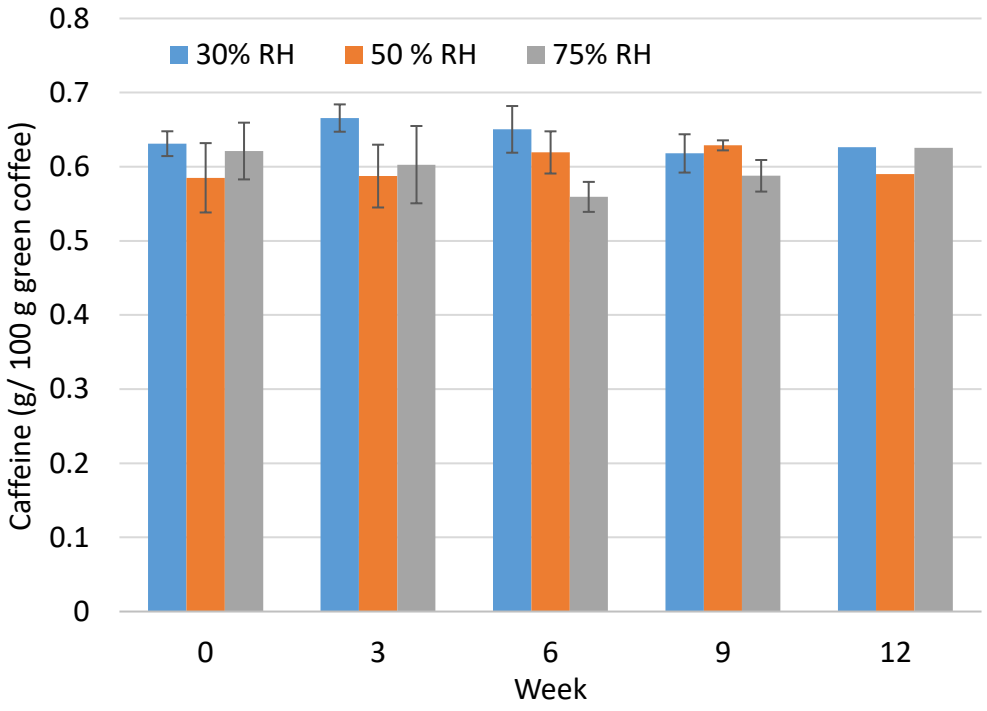

Figure 3.14 Average Caffeine Content (g/ 100 g dry green coffee) (n = 3) of Specialty Green Coffee Stored Over Time at 33%, 54%, and 75% RH and 20°C.

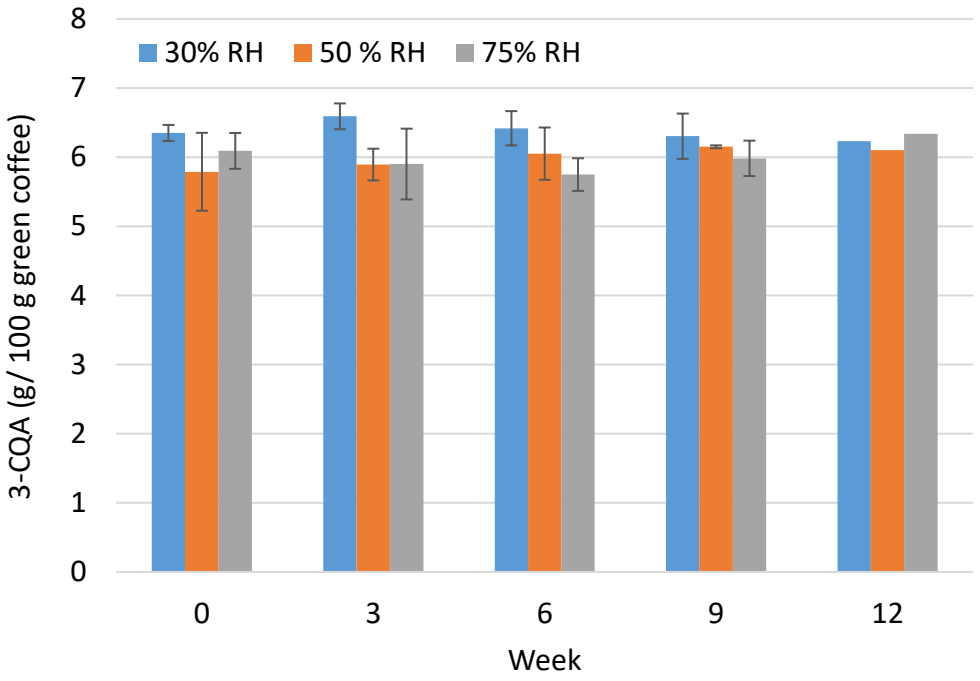

Figure 3.15 Average 3-CQA Content (g/ 100 g dry green coffee) (n = 3) of Specialty Green Coffee Stored Over Time at 33%, 54%, and 75% RH and 20°C.

The same trend was found for 3-CQA content (6.378 g/100 g for 33% RH storage), where no other combination of variables showed significant differences. 3-CQA change over time is shown in Figure 3.15; average 3-CQA values are shown in Table 3.10. Since 3-CQA is not the most dominant CGA in green coffee, another CGA may have been better to use, but 3-CQA was the most cost-effective for this exploratory analysis of CGA change over time. Other research using 5-CQA as an indicator of coffee quality found that 5-CQA decreased during storage, but no claims were made about the statistical significance of the decrease (Rendón et al., 2014). Data for both caffeine and 3-CQA fell within expected ranges.

3.5 Conclusion

The research discussed above sought to explore how physical and chemical attributes of specialty Colombian *C. arabica* green coffee changed at 3-, 6-, 9-, and 12-week time intervals

and 33%, 54%, and 75% RH storage conditions at approximately 20°C. Physical changes in green coffee beans during storage at 75% humidity can be described by an increase in weight, MC, a_w, bean size (decreased bulk density), and lightness (L*). No physical changes of green coffee beans during storage at 50% humidity were observed. Physical changes in green coffee beans during storage at 30% humidity can be described by a decrease in weight, MC, water activity, a slight increase in L* over a long time (12 weeks), and a more yellow-colored bean (higher b*). Most significant changes in physical variables occurred in the first three weeks of the storage experiment. One unexpected outcome of this research was the lack of significant differences between cup scores for each treatment. Insignificant results may have been impacted by challenges faced while obtaining cupping data—primarily the lack of replication and storage time before being roasted and cupped. These challenges could have hindered the identification of any significant differences in cup quality correlated with storage time and RH condition. More research is needed to correlate physical and chemical changes to specialty coffee quality data to be able to identify rapid, accessible methods for estimating specialty coffee quality.

Chemical analysis showed that all samples of specialty green coffee were contaminated with OT and AF, and there were no significant changes or trends in caffeine, 3-CQA, or total phenolic content for different treatments over the storage period. In the future, more specific chemical indicators of quality may be explored to successfully correlate chemical changes with cup quality losses and rapid physical analyses.

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Chapter 4. Conclusions & Future Work

The aim of this thesis was threefold: (1) to use the equilibrium method to create working MSIs on "as is" specialty *Coffea arabica* green coffee from Colombia over two production years (2019 and 2020); (2) to assess some thermodynamic properties of green coffee, namely net isosteric heat of sorption (H_s) and monolayer moisture content (m_o), and correlate physical and chemical characteristics of specialty green coffee with MSI and thermodynamic data; and, (3) to identify rapid, accessible predictors of quality changes that may be useful to the specialty coffee industry from a three-month controlled humidity storage experiment. Green coffee followed a Type II isotherm pattern for both production years. However, EMC values were significantly different between production years. This difference could be attributed to differences in the treatment of the coffee prior to the MSI experiment, as both green coffees did not start at the same MC. M_o values estimated from working isotherm were 6.17±0.18% (dry weight basis). H_s was found to be significantly greater below the m_o, as expected. The m_o is much too low to be a practical MC for green coffee storage due to roasting behavior and storage environment limitations. However, maintaining green coffee MC close to the m_o, at the lower end of the 9-12% recommended MC range, may be advantageous for quality preservation.

The MSI experiments were the precursor to the second phase of research. Intermediate a_w values from MSI construction (0.33, 0.54, and 0.75 a_w) corresponded to suboptimal (0.33 and 0.75 a_w) and optimal (0.54 a_w) % MC for green coffee and were further explored over a three-month storage period. Green coffee beans stored at 75% humidity increased % MC (to 14%), a_w, L*, and weight (presumably from water) over 12 weeks. Bulk density decreased, indicating an increase in bean size. For the suboptimal RH conditions (33% and 75% RH), weight gain, a_w change, and %MC change happened by week 3. Physical data showed maintaining an a_w of 0.54

produced the most stable product (no changes in weight, MC, a_w, color). Chemical analysis of caffeine, 3-CQA, and total phenolic content showed no significant difference for any time or RH treatment combination. OT and AF content also were not significantly different between storage and RH treatments. All samples, even the control, contained both mycotoxins. The lack of statistical significance could be due to the high standard deviation for the rapid ELISA method, so a more sensitive method may be desirable if the goal is to achieve accurate mycotoxin counts instead of only confirming the presence of the mycotoxins. Interestingly, cup scores and fade rating of the treated specialty green coffee followed no trend and seemed to be independent of any variables explored. This may be due to a few of the study limitations, including cupping and experiment timing. Specialty coffee quality is a multi-dimensional variable that may not be able to be correlated to any rapidly obtainable variable without extensive research and replication. In general, specialty coffee quality may be more robust than previously thought but maintaining a_w < 0.60 (< 60% RH environment) at room temperature conditions seems to prevent any physical changes from occurring over three months. A cost-effective way to maintain this a_w must be identified to optimize the quality and shelf life of specialty green coffee. Furthermore, most changes occur within three weeks of exposure to suboptimal conditions, suggesting there is time to react and prevent damage if the coffee has not been allowed to rehydrate from direct contact with water. MSI and physical data suggest the Specialty Coffee Association a_w limit of 0.70 for green coffee safety is not low enough to ensure green coffee quality is maintained—an a_w recommendation closer to 0.60 would be more robust.

This research attempted to understand specialty coffee quality through the lens of both an academic and a specialty coffee roaster. The overarching goal was to try and provide the industry with relevant and accessible results to improve coffee knowledge in the industry. More work is

necessary to accomplish this goal. Some suggested future projects include the following: (1) since green coffee moisture sorption is time-dependent, different Dynamic Dewpoint Isotherm methods may need to be considered for rapid isotherms that are closer in EMC to the equilibrium method; (2) explore the behavior of specialty green coffee that has adsorbed/ absorbed/ desorbed water multiple times; (3) estimate MSI construction using the knowledge that green coffee equilibrates in about three weeks (even if weight change for the equilibrium isotherm method is not below the mg threshold); (4) redo the storage experiments with smaller intervals for the first three weeks and force quality losses to ensure the end of specialty shelf life is found (cupping score > 80); (5) explore if there is a rapid method for quantifying green coffee viability and correlate to quality as there is growing research relating viability to quality; (6) use more specific indicators of quality (some have been identified in recent research) that may indicate the very beginning of degradation and track their change during storage to estimate quality without relying on roasting and cupping. This thesis emphasizes the need for practical methods the specialty coffee industry can utilize to preserve green coffee and further basic research to refine these practical applications.

APPENDICES

Appendix A: MSI Preliminary Experiments

The objectives of the preliminary MSI experiments were to estimate equilibration time and ensure the salt solutions and temperatures selected would suffice. Green coffee was initially stored at 20°C, 35°C, and 50°C. The salt solutions worked well, but the 50°C treatment was identified as too hot to be practical for specialty green coffee storage application. Thus, the temperatures were modified to 20°C, 30°C, and 40°C. During the incubation, mold was identified on green coffee stored at RH > 80%. This was problematic and needed a solution, see appendix C and the figure below from the final defense.

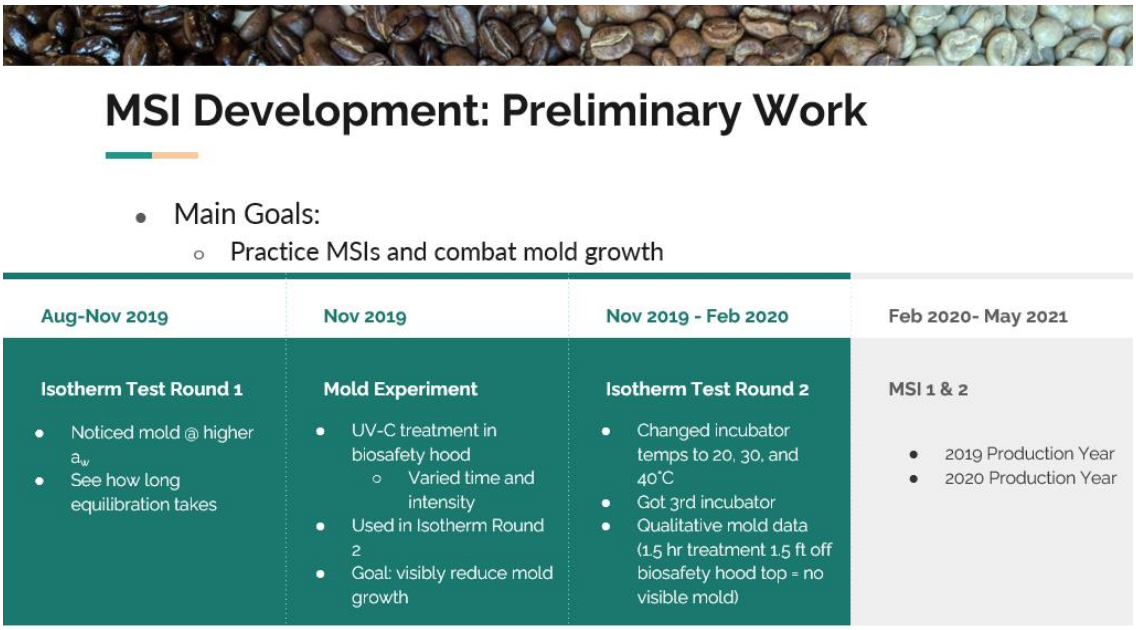


Figure A.1 Overview of Preliminary Research for Moisture Sorption Isotherm Construction.

Appendix B: A_w 2-hr time determination

Green coffee was placed in a calibrated a_w meter on continuous mode and a_w was tracked every 0:30 seconds for 1 minute, then every minute until the time had reached 20 minutes, then every 5 minutes until the time had reached an hour, and then every 10 minutes until the time had reached two hours. When there was less than 0.005 change in the a_w, the time was recorded. It was noted that as the a_w got further from the ambient room RH (about 50%), the equilibration time took longer.

Table B.1 A_w Change Over Time for Two Hours.

Time Range (minutes)	a_w change test 1	a_w change test 2
0-2:00	0.00	0.00
2:00-10:00	0.02 (0.2841 @ 2 min, 0.2992 @ 10 min)	0.09 (0.6393 @ 2 min, 0.7306 @ 10 min)
10:00-20:00	0.01 (0.2992 @ 10 min, 0.3063 @ 20)	0.01 (0.7306 @ 10 min, 0.7433 @ 20 min)
20:00-60:00	0.007 (0.3036 @ 20 min, 0.3130 @ 60 min)	0.01 (0.7433 @ 20 min, 0.7529 @ 60 min)
60:00-90:00	0.005 (0.3130 @ 60 min, 0.3135 @ 90 min)	0.001 (0.7529 @ 60 min, 0.7540 @ 90 min)
90:00-120:00	0.002 (0.3135 @ 90 min, 0.3156 @ 120 min)	0.001 (0.7540 @ 90 min, 0.7554 @ 120 min)

Appendix C: MSI Sterilization Experiments

Green coffee was found to mold when stored at > 80% RH during the MSI preliminary experiments. To combat mold growth, UV exposure was tested. A biosafety hood was cleaned with 70% ethanol following proper cell culture cleaning procedure. The mason jars were also cleaned with ethanol solution. Beans were placed on a metal baking dish on the bottom level of the biosafety hood and tested for 30-minute intervals for either one or two hours. Then, beans were put into the jars inside the biosafety hood to reduce the risk of contamination. This was also repeated with the beans elevated about one foot off the biosafety hood on a makeshift stand. The elevated position plus one-hour UV treatment successfully reduced visible mold growth. See below for some pictures. Moisture content and color values were taken on beans independent of this experiment as a quick way to see if the UV treatment dried out the coffee or changed the color, which would both serve as indicators for the treatment significantly altering the coffee. No significant difference was found.

Figure C.1 Mold Growth After First Preliminary Isotherm Experiment.

Figure C.2 No Visible Mold After UV-C Treatment and Incubation.

However, after opening a few times, some mold would grow – on the left is after opening for the first time after incubation, on the right is opening the third time (hard to tell, but there is more mold visible).

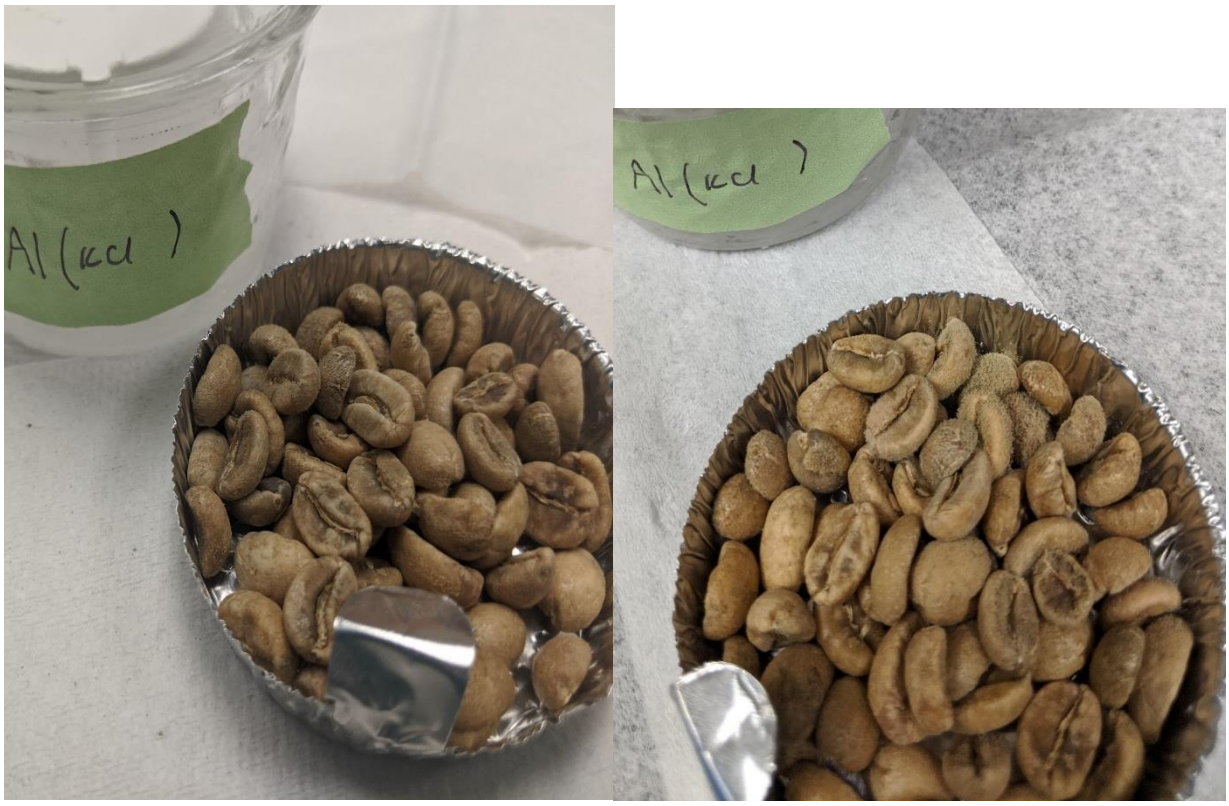


Figure C.3 Green Coffee Beans Opened for the First Time After Incubation (left) Vs. Green Coffee Beans Opened and Closed Three Times (right).

Appendix D: H_s Calculation Curve

MSI data was transformed and a_w at a fixed MC was calculated to estimate H_s from linear models. The linear fits for each MC are shown below.

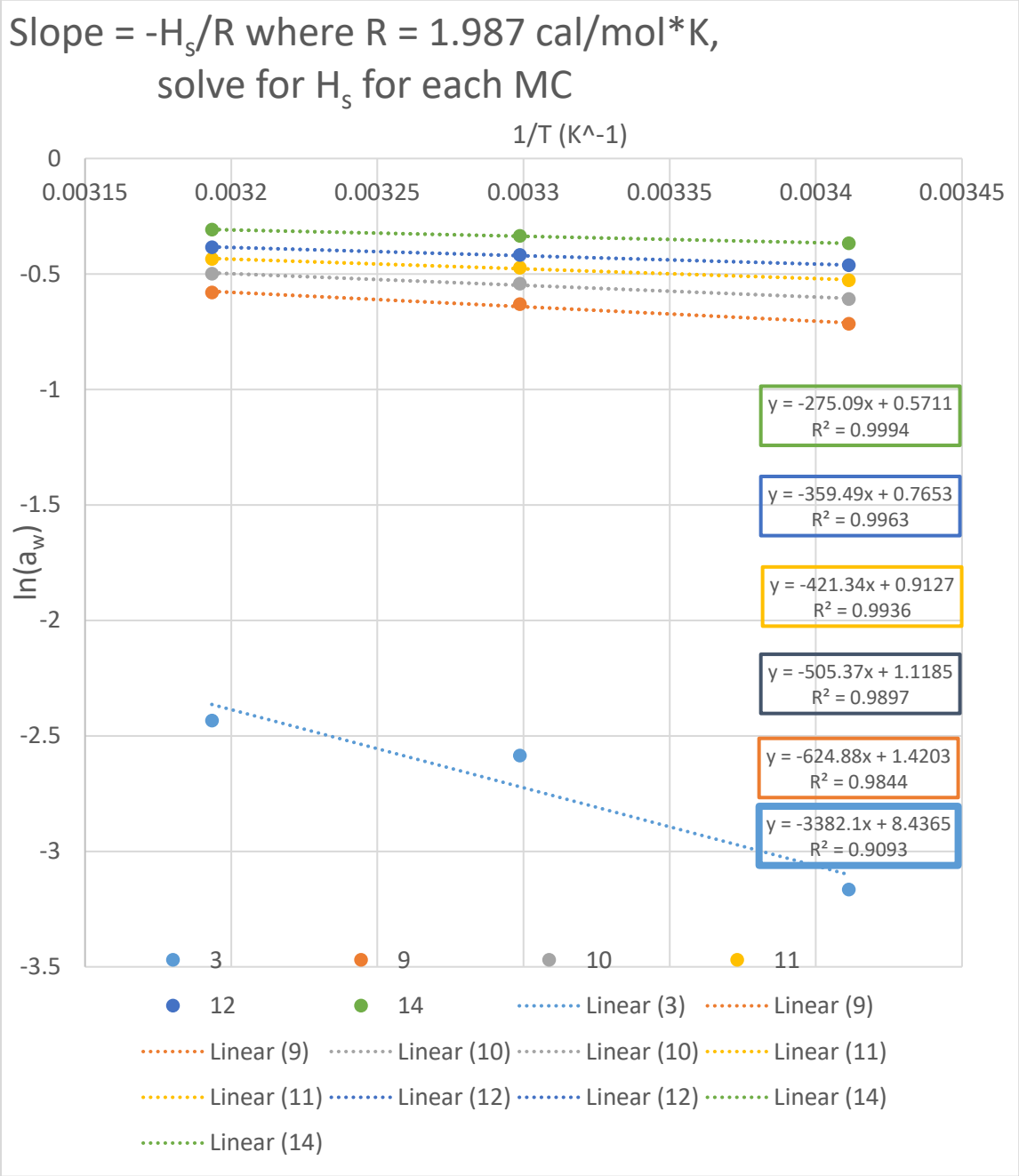


Figure D.1 Transformed A_w Vs. Temperature (in Kelvin) Data at a Constant Moisture Content (wet weight basis) with Linear Fit Included for Net Isosteric Heat of Sorption (in cal/mol) Estimation.

Appendix E: Sample Preparation for Extraction

Many methods exist for extracting caffeine, 3-CQA, and phenolic compounds for green coffee. Validation of existing methods was the goal of this experiment. Methods were selected from the literature for their simplicity while still expecting good compound recovery. The following methods were tested using 0.1 g ground green coffee where M: W: A is methanol: water: acetic acid at a 30: 67.5: 2.5 ratio and M: W is methanol: water at a 70:30 ratio:

Table E.1 Treatment Scheme for Green Coffee Extraction Method Selection.

Time (minutes)	Treatment	Solution
30	Sonic bath	M: W: A
30	Shaking water bath	M: W: A
60	Sonic bath	M: W: A
60	Shaking water bath	M: W: A
30	Sonic bath	M: W
30	Shaking water bath	M: W
60	Sonic bath	M: W
60	Shaking water bath	M: W
10	Beaker + 0.1 g ground green coffee	Boiling water
10	Beaker + 0.2 g ground green coffee	Boiling water

Samples were run through the HPLC, and the max absorbance unit was examined, along with peak differentiation. The boiling water samples had the highest AU, suggesting high

recovery, but there were many other peaks present, so there was too much noise. The best treatment overall was the 60-minute shaking hot water bath treatment. The methanol: water samples made the supernatant very cloudy, and a lipid-like layer formed in the conical centrifuge tube. Tubes and the water bath are shown below.

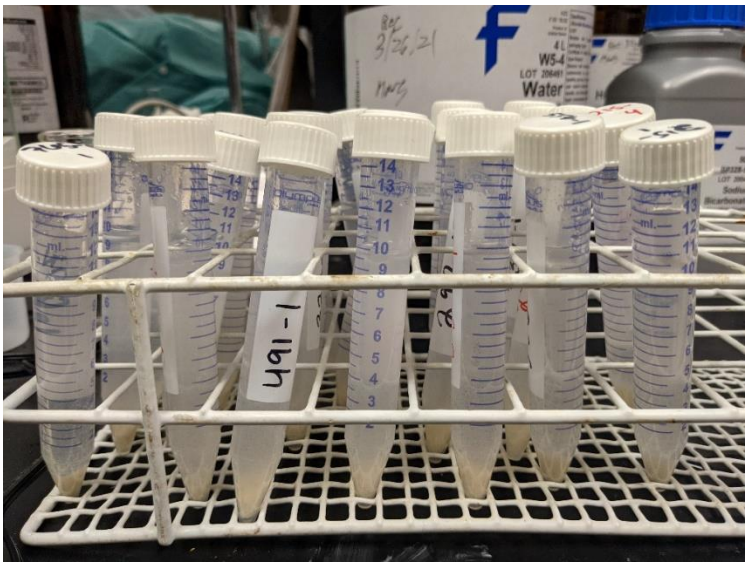


Figure E.1 Green Coffee Precipitate and Supernatant Post-Extraction.

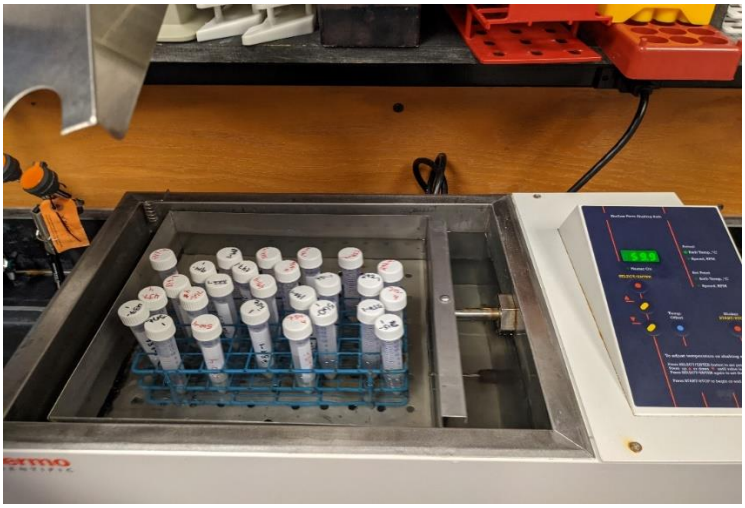


Figure E.2 Water Bath and Extraction Tube Set-Up.

Appendix F: Grinding and Grinder Cleaning Method

Grinding green coffee is difficult because of the rubbery nature of the beans. One thing to be cautious of is generating heat and roasting the coffee powder during grinding. The following method was used to prevent this:

1. Green coffee was removed from the incubator change, N_2 flushed, vacuum-sealed, labeled, and frozen in a -40°C freezer until ready for grinding and extraction.
2. Green coffee was removed from the freezer, placed into the grinder, and then ground at 30-second intervals until the beans no longer felt cold (about 3-4 rounds).

Figure F.1 Grinder (left), Frozen Green Coffee Beans (middle), and Green Coffee Beans After Grinding for One 30-Second Interval (right).

3. The grounds were sieved using the Gilson sieve shaker, fines were saved for extraction, but anything that was too large in particle size was put back into the vacuum bag and placed back in the freezer for 10 minutes to keep the beans cold.
4. After 10 minutes had elapsed, the procedure was repeated. Until > 90% of the green coffee was ground down. This usually took 7-12 repetitions. After the third repetition,

a smaller grinder was used because the volume of coffee beans had decreased.

Figure F.2 Final Ground Green Coffee Samples.

Some more pictures are shown on the next page.

Figure F.3 Ground Green Coffee Separated by Particle Size Using a Sieve Shaker.

Appendix G: Texture Analysis

Thirty green coffee beans were randomly sampled from the remaining 100 g of green coffee and were analyzed using a TA.XT Plus Texture Analyzer (Texture Technologies Corp., Scarsdale, New York) equipped with a 50 kg load cell. The cell was calibrated with a 5 kg weight. All data were obtained at 20°C for the three salt treatments. The length, width, and height of each bean were measured with calipers and then subjected to uniaxial compression using a flat metal plate until the probe had moved 2.7 mm through the green coffee bean, by which time the bean had split, at a rate of 0.83 mm/second. The flat side of the bean was facing up. Trigger force was set to 0.5 N. The peak force at the time of initial fracture was recorded. If no fracture event was recorded, peak force through 2.7 mm was recorded. After texture analysis was complete, the green coffee sample was nitrogen flushed, vacuum sealed, and placed into a -50°C freezer until they could be ground for further chemical analysis.

Texture data was obtained from TA.XT software using a macro to extract fracture data. Uniaxial compression data obtained by the TA.XT can generally be defined in two categories: beans that had a detectable fracture event and beans that did not have a detectable fracture event. Examples of TA.XT graphs are shown in Figure X below. In general, almost no beans stored at 75% RH had detectable fracture events. Instead, the beans split like baked beans. Conversely, almost all beans at 33% RH had detectable fracture events. Beans stored at 33% humidity had significantly higher instances of fracture events when subjected to uniaxial compression.

Appendix H: Hyperspectral Imaging

From chapter 3: 100 g +/- 1 g of green coffee was placed into 12, 30 oz containers (three for each salt slurry). A 100 g sample was nitrogen flushed, vacuum sealed, and stored in the dark as an initial control sample. At 3, 6, 9, and 12 weeks, the samples were removed from the chamber, nitrogen flushed, vacuum sealed, assigned a random three-digit number code, and placed into the dark until all samples were collected. All samples were shipped to RingoAI (California) for HSI using a SPECIM contracted by CoffeeSeed. HSI data was obtained, but time constraints made it difficult to analyze this data. An example plot from the experiment is shown below. It is hard to say if there was a significant difference between peaks in the figure below.

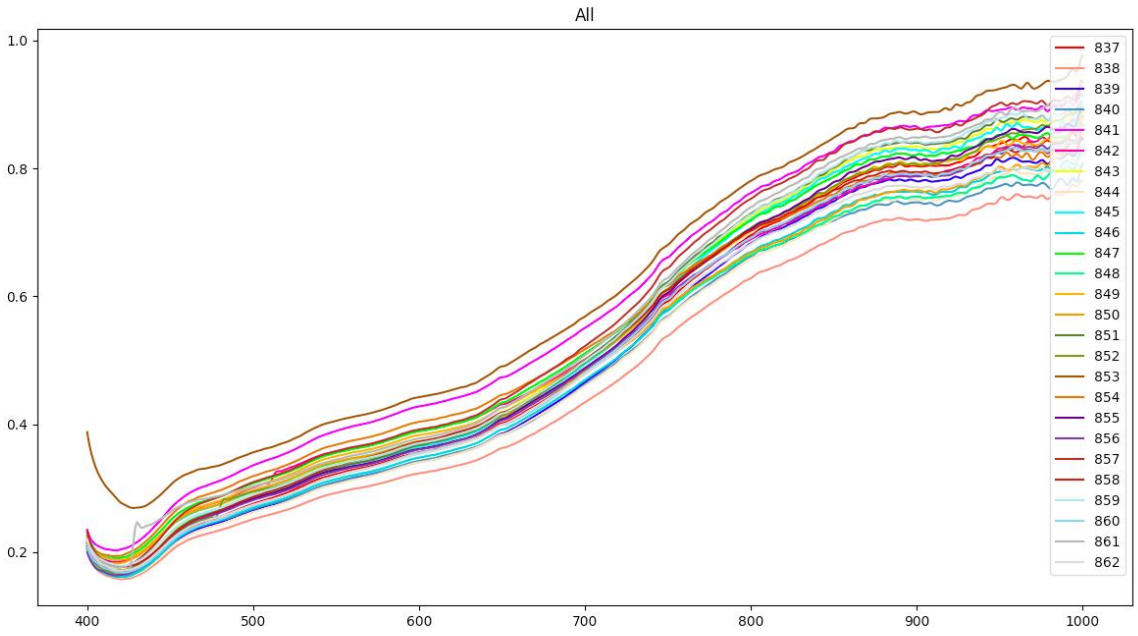


Figure H.1 An Example Plot of Hyperspectral Imaging Data for Each Green Coffee Bean.

Appendix I: Germination, Viability, & Sterilization Preliminary Experiments

In addition to the research discussed in this paper, we also ran an experiment to attempt to germinate specialty green coffee seeds. Below are some pictures and a brief discussion of what worked and what did not work.

Brief Background Information

Coffee beans are alive and are respiring after harvesting and processing and during early stages of storage. This is an important consideration for storage as respiration is an exothermic process, thus coffee beans will produce heat as they respire during storage. Aside from heat production, respiration during storage may also cause quality losses. Oxygen availability, moisture content, and ambient and bean temperatures are all factors that regulate respiration and germination. The amount of reducing sugars present in green coffee is also an indicator of respiration (Ribeiro 2011). Rice, a comparable grain to coffee beans, undergoes a sharp increase in respiration rates when it has a moisture content above 14%.

In addition to being a driving factor of quality degradation, respiration is a necessary process for seed germination. Germination can be prevented by processing the seeds to a state of dormancy, but coffee beans do not need to be dormant to germinate like some other plant seeds. Germination can be triggered by high humidity, air temperatures of 30-35°C, or soil temperatures between 28 and 30°C. Germination rates are lower in beans that were dried at high drying temperatures.

It has been argued that final cup quality losses are linked to coffee bean viability losses. It has been suggested that beans dried or stored in a way that maintains seed viability (i.e., beans can germinate) will produce a better final cup flavor and color than beans that have lost their ability to germinate. Robusta coffee loses viability at ambient conditions after two months, while

arabica coffee loses viability after six months; however, beans stored at 10-15°C and 10-11% MC may be dormant for two years (Wintgens, 2012). Arabica beans stored at 63% RH and 22 C for two years lost 100% of their viability by the end of the first year of storage. This was true for hulled dry, semi-dry, and wet-processed coffee (Selmar, 2007). Green coffee stored with parchment has a higher final cup quality. Parchment coffee also remains viable for longer. Furthermore, removal of parchment may cause damage to the seed, resulting in viability losses. Accumulation of free fatty acids is also associated with loss of seed viability (Rendon). In general, low humidity environments and low temperatures lengthen seed viability. Freezing coffee beans has conflicting data on whether viability is damaged.

Trying to germinate coffee seeds can be difficult because of endogenous microbes. Chlorine solutions have been used for sterilizing (Suárez-Quiroz, et al., 2004). Also, some researchers have suggested that the blue-green color development of reimbibed seeds (a post-mortem reaction, see Figure I.11) may indicate that the beans are relatively fresh/ have not had any other quality losses (Selmar, 2007).

Purpose

To determine whether viability or germination rates of green coffee beans would be effective for estimating quality or predicting the end of shelf life for specialty coffee (specialty coffee score dropping below 80 on SCAA score).

Part 1

1. 6 replicates of 200 specialty green coffee beans were counted out and separated by size. Counts of each size were recorded.

Figure I.1 Green Coffee Beans Separated by Size Before Germination.

2. Three replicates were placed on soaked germination paper, no more than 50 per paper, separated by size. The paper was wrapped up and then wrapped in a plastic bag to keep moisture in and then placed in an incubator at 30°C for 15 days, checked after 5 days and 8 days.

Figure I.2 Green Coffee Beans on Germination Paper (left), Wrapped Up (middle), and Wrapped in Plastic Bags to Retain Moisture During Incubation (right).

3. The other three replicates were placed in water for 24 hours and then were wrapped up following the method in step 2. After 24 hours, some radicels were observed. Beans were placed on germination paper grouped by size and whether they had radicels or not.

Figure I.3 Green Coffee in Water Before 24-Hour Soak Pre-Germination Trial.

Figure I.4 Green Coffee in Water After 24-Hour Soak Pre-Germination Trial.

Figure I.5 Example of Green Coffee with Radicels After a 24-Hour Water Soak.

4. Beans from the lot were also sorted by damage and photographed up close, then germinated individually.
5. All beans molded over time, so none were successfully germinated. Instead, final counts of radicel development were noted.
6. Then, the beans were cut in half and soaked in tetrazolium to check for embryo viability. Although it was realized later the beans were cut the wrong way and the embryo was not always showing when cut in half lengthwise.

Figure I.6 Green Coffee Cross-Section After Tetrazolium Soak.

7. Beans were also float tested to check for damage (beans that float are less dense than water and are usually low quality – method adapted from cotton/corn, not used for coffee to the author's knowledge).
8. Molded beans were sent to the micro lab to get an idea of the species present on the beans. Results mentioned the following: *Aspergillus fumigatus, A. flavus, Aspergillus sp. Penicillium, Paecilomyces*.

Experimental questions were: (1) Is there a difference between soaked vs. unsoaked beans in germination rates; (2) Is there a difference between soaked vs. unsoaked beans in viability - if they did not germinate were they still viable; and (3) Does size affect germination rate? When germination failed, research questions became (1) How does mold affect viability; (2) Can we prevent molding during attempted germination; and (3) Does radicel growth after a soak indicate bean quality? One note is that the mold smelled terrible. We recommend double masking and wearing gloves to prevent exposure to mold spores on top of the normal PPE.

Part 2

Takeaways from part 1 were as follows: (1) beans had a heavy micro load so needed some treatment to prevent mold growth during incubation; (2) radicels should be counted after

soaking as they might be a rapid indicator of the quality of bean; (3) tetrazolium soak needs to be done on the bean cut in half from top to bottom, exposing the embryo.

1. Steps from Part 1 were repeated with some modifications. Beans were soaked in hydrogen peroxide for 30 or 60 minutes (peroxide soak for 30 or 60 min, followed by either no water soak or a 24-hour water soak, or just a water soak with no peroxide soak). We thought about testing acetone, but acetone is not a food-safe treatment option that the coffee industry could use.

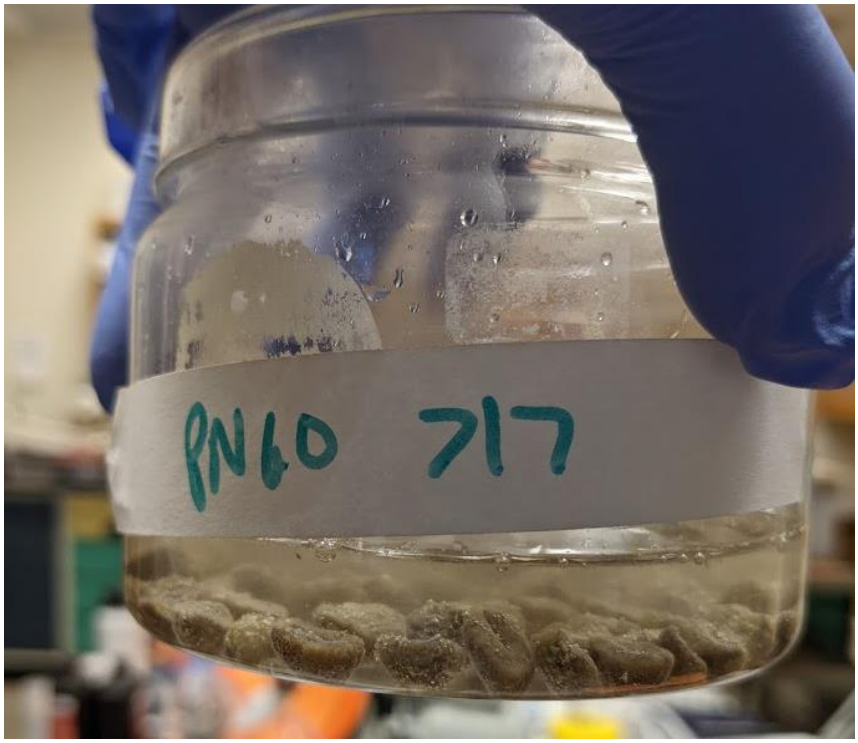


Figure I.7 Green Coffee After 60-Minute Soak in Hydrogen Peroxide.

2. Beans still molded, but beans soaked in peroxide, then water, had higher rates of germination.
3. Bean embryos were tested for viability.

a. Picture of a non-viable embryo

Figure I.8 Non-Viable Green Coffee Embryos Post-Germination Attempt.

b. Picture of a viable embryo

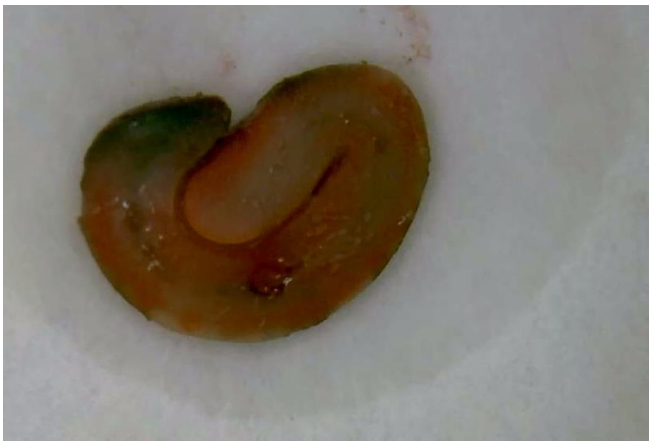


Figure I.9 Viable Green Coffee Embryo Post-Germination Attempt.

4. Pictures were taken of all beans separated by treatment, size group, and radicel growth.

Experimental questions to answer in future experiments are as follows: (1) Does acetone, chlorine, hydrogen peroxide reduce the incidence of mold; (2) Does acetone, chlorine, or hydrogen peroxide increase germination rates (namely by decreasing mold); (3) What is the percentage of beans that show radical growth after a 24-hour soak; (4)

Does seed density (float test) correlate to quality; and (5) Can we quantify damage to see if damage is correlated with mold growth?

Next Steps

1. Explore radicel counting method in relation to quality
2. Maybe find method that works for germination
3. Identify if bean size affects viability
4. Test UV treatment method used on MSI experiment for germination

Figure I.10 Close-Up Images of Molded Green Coffee Post-Germination.

Figure I.11 An Image of Blue-Green Post-Mortem Reactions**.**

Appendix J: Pictures of Storage Containers from Chapter 3

Pictures of large storage chambers used for destructive analysis (roasting, caffeine, 3-CQA, total phenol, AF, OT) are shown below. Note that the same lighting should have been used but was not.

Table J.1 Pictures of Storage Containers from Chapter 3 Organized by Treatment and Time.

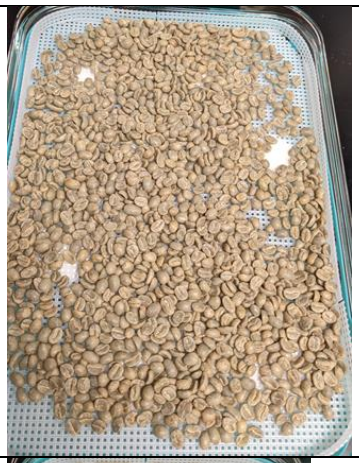
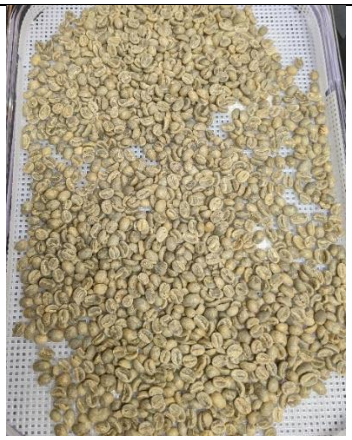
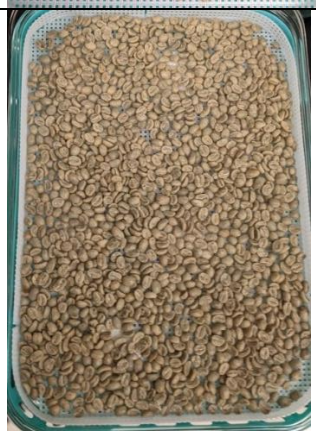
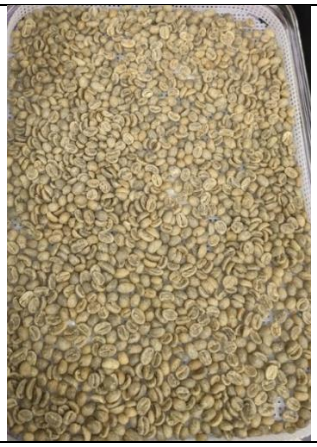
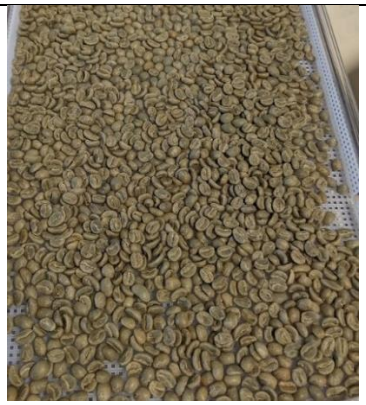
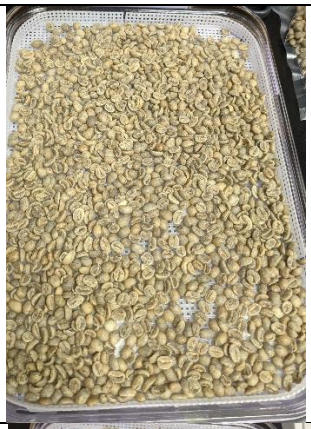
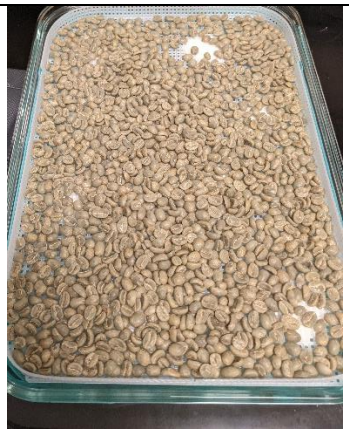
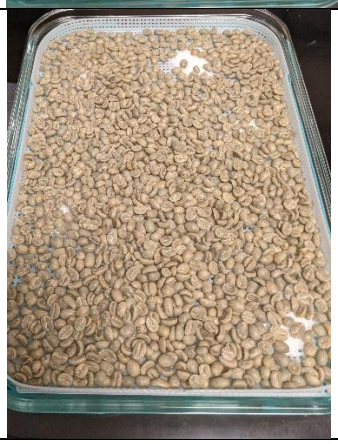
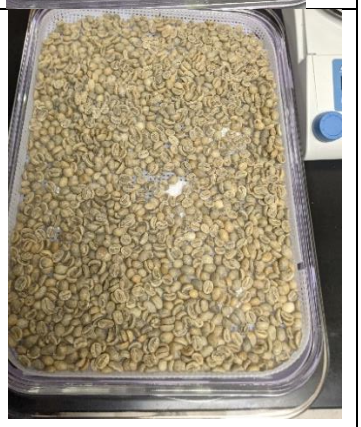
Week	33% RH	54% RH	75% RH
3			
6			

Table J.1 (continued)

Week	33% RH	54% RH	75% RH
9			
12			

Appendix K: Standard Curves for F-C, Caffeine, 3-CQA, AF, and OT Determination

The following standard curves are relevant to Chapter 3.

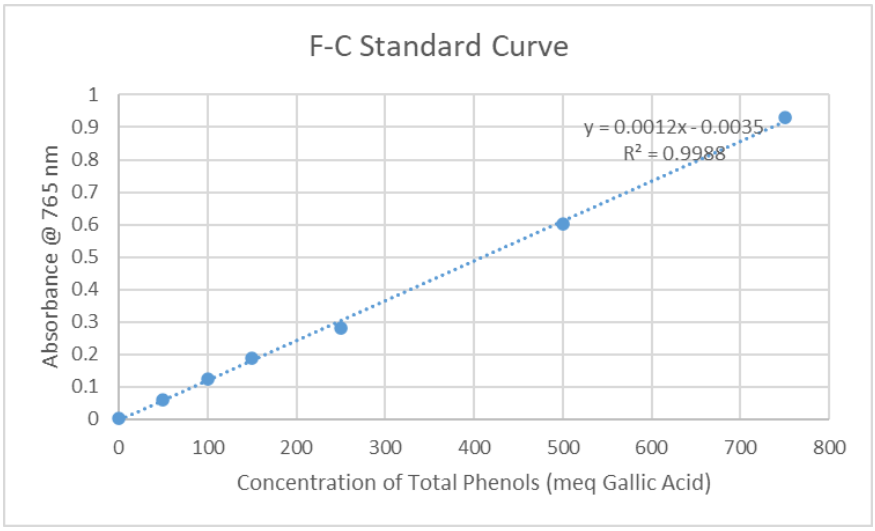


Figure K.1 Standard Curve for Green Coffee Total Phenolic Content Quantification.

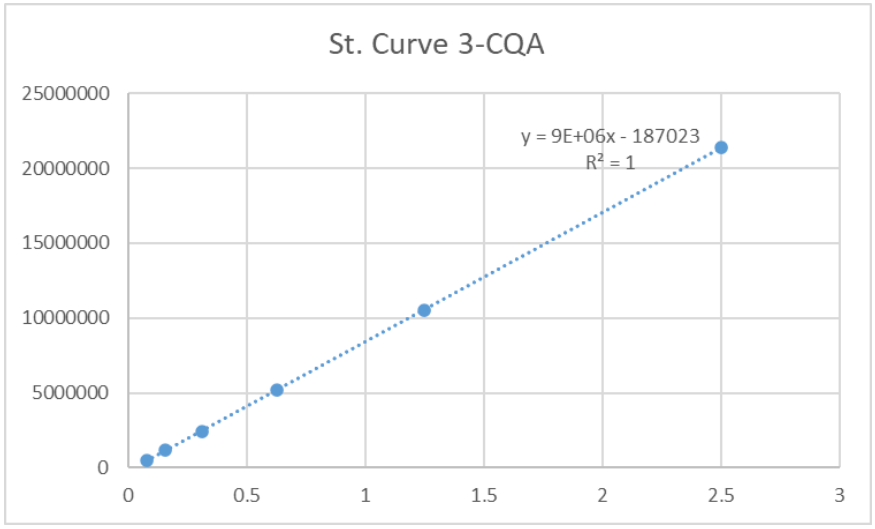


Figure K.2 Standard Curve for Green Coffee 3-Caffeoylquinic Acid Quantification.

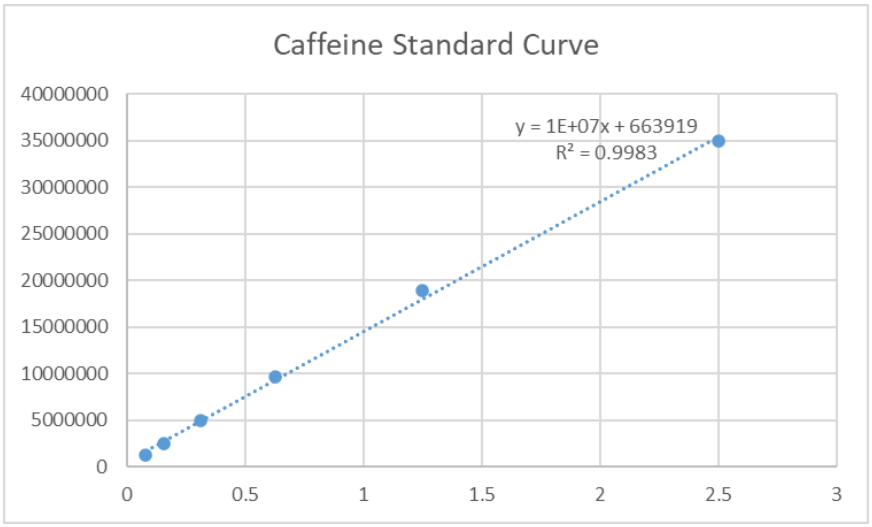


Figure K.3 Standard Curve for Green Coffee Caffeine Quantification.

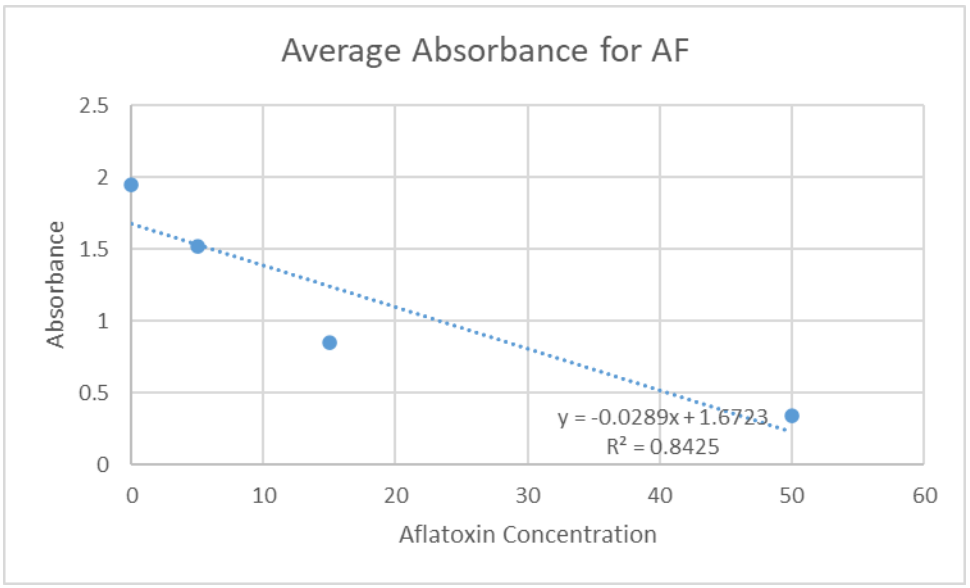


Figure K.4 Standard Curve for Green Coffee Total Aflatoxin Quantification.

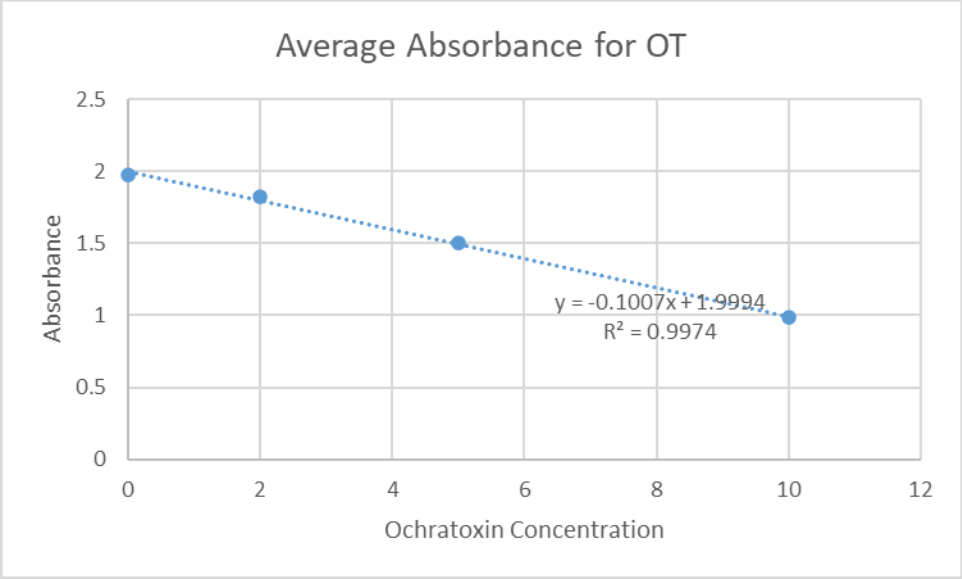


Figure K.5 Standard Curve for Green Coffee Ochratoxin Quantification.

Appendix L: Key Terms

Carcinogenic – has the potential to cause cancer

Cup score – score out of 100 of SCA scale on cupped coffee (following SCA cupping standards)

Equilibrium moisture content (EMC) – Moisture content at which a sample is at equilibrium with a known a_w/ RH environment

Coffee fade – Fade, on a scale of 0 to 10, is when coffee has flattened in flavor (loss of attributes) and has a noticeable increase in off-flavor not initially present in the green coffee (i.e., not ferment, potato defect, or other common quality defects)

Immunotoxic – toxic to the immune system

Monolayer moisture content (m_o) – the MC where all available binding sites on a product have one water molecule bound to them, forming a monolayer, and is the MC at which the product is the most stable

Nephrotoxic – toxic to kidneys

Net isosteric heat of sorption (H_s) – indicates how strongly bound water is to particles in a product and can be used to estimate product stability

Teratogenic – causes, or relates to, developmental malformations/ abnormalities

Working moisture sorption isotherm – Working MSIs start with the product "as is," usually at an intermediate a_w, and data on adsorption and desorption is collected