EFFECTS OF DRYING METHODS ON POSTHARVEST QUALITY OF HOLY BASIL *OCIMUM TENUIFLORUM L.*

by

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(Under the Direction of David Berle)

ABSTRACT

Holy basil (*Ocimum tenuiflorum L.* 'Kapoor') was grown in Athens, Georgia during 2018 and 2019 to evaluate the effects of drying method on final moisture content, presence of fungal contaminants, and color retention of harvested holy basil. Methods included passive solar convection, room equipped with dehumidifier and fans, commercial food dehydrator, and as a control, a shaded barn. All treatments removed water more effectively than the control. Drying method had no effect on fungal colony count. In a second experiment, in which harvested holy basil was washed or unwashed, washing fresh plant material reduced fungal colony count. Colorimetric analysis detected consistent differences between all methods compared to the control. No differences were found in pixel intensity. Results suggest holy basil growers can affect market quality by changing postharvest handling practices.

INDEX WORDS: *Ocimum tenuiflorum*, holy basil, drying methods, passive solar dryer, medicinal herb dehydration, final moisture content, colorimeter, RGB histogram

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DEDICATION

To the hardest working people I know: my mother, Arlene Begani and my father, Thomas Begani. Their encouragement and constant belief in my ability to pursue my dreams and goals has meant the world to me. To David Berle, for inspiring me and so many others to grow in new directions and to serve the greater community. To Pamela Lewis who graciously welcomed me into the Horticulture family. To Paul Thomas, a dear friend for teaching us to take good care of our plants and of each other.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Holy Basil Biology

Ocimum tenuiflorum L. *(syn. Ocimum sanctum L.*), is commonly referred to as holy basil, or tulsi. It belongs to the *Lamiaceae* family, which includes many commercially important medicinal and culinary herbs, such as basil, rosemary, mint, lavender, and oregano. Holy basil has been genetically traced to north central India, though the plant has naturalized across sub-tropical and tropical regions of North Africa, China, and Southeast Asia (Shasany, 2016). Due to cultivation and human consumption, the species has been introduced and distributed globally across the Middle East, Australia, and South and Central America (Shasany, 2016).

Holy basil is an herbaceous, branching subshrub that typically grows 3 feet in height and 2 feet wide, with mature leaves up to 2 inches long. The plant may grow taller under wild conditions (Joseph and Nair, 2013). Holy basil is perennial in frost-free hardiness zone 10 andwarmer, but grown as an annual in colder climates (Meyers, 2003). Leaf color ranges from green to purple, and flower color may range from white to purple, depending on variety. The flowers are “perfect” and capable of both self and cross pollination. The stems are quadrangular and may become woody if allowed to grow perennially. The leaves are opposite, ovate, and have serrated edges. Both stems and leaves have trichome hairs, which is a characteristic of plants in the *Lamiaceae* family. The glandular trichomes are predominantly on the surface of the leaves and secrete pungent aromatic compounds that contribute to the signature smell and flavor. These

glands serve as a natural defense mechanism for the plant, which may repel or injure some herbivores upon contact, but maintain an attractive aroma for pollinators (Hartmann, 2008).

Holy Basil as Medicine

Humans have relied on the medicinal value of plant materials throughout ancient history, and their use has been recorded for over 5000 years (Bent, 2007). About one third of pharmaceuticals on the market are synthesized from compounds produced in plants, and 75% of the synthetic byproducts are used in ways attributed to their traditional use (Mafimisebi et al., 2013). Medicinal and aromatic plants are still used today as the primary form of healthcare in most developing countries, representing about 80% of the world's population (FAO, 2005). In the United States and Europe, approximately 40-50% of the population uses herbal remedies as a form of medicine (Ramzan, 2015). Modern pharmaceutical practice has rapidly replaced traditional herbal medicine in industrialized nations over the past 200 years, due to major advancements in sanitation, synthetic drugs, and regulations requiring clinical efficacy (Bent, 2007).

American interest in botanical products, including nutraceuticals, cosmeceuticals, functional foods, and natural pet care, has been thriving over the past two decades, and is expected to grow steadily as the benefits of herbal products receive more mainstream attention (Craker, 2007). Sales of natural herbal products in the US have increased on an annual basis since 2000, reaching over $9.6 billion in 2019 (Smith, et al., 2020). Holy basil is in the top 30 most popular herbs sold in natural markets, with $3.1 million dollars in sales in 2019, contributing to the growing popularity of herbal formulations (Smith, et al., 2020). The demand for nutritional herbal products is projected to continue growing, based on trends involving an increasing aging population of consumers with chronic health concerns seeking alternative

therapies (Kantor et al., 2016). An expanding body of research on herbal products is available online, allowing individuals to manage their own health issues using nutritional therapy and creating further growth in this market (Mellentin, 2018).

Holy basil is valuable for its aromatic and flavorful qualities, and is used in teas, extracts, essential oils, cosmetics, and nutritional supplements. It has been documented in sacred texts dating from 200 BC (Pattanayak et al., 2010). The Indian Ayurvedic system of medicine regards the plant as the "incomparable one" and considers it to be the most important plant among hundreds for treating health conditions such as malaria, influenza, diarrhea, asthma, and migraines (Bano et al., 2017, Gupta et al., 2002, Vasudevan et al., 1999). The most significant benefits observed in trial participants are positive results for metabolic activity in diabetic patients, immunomodulation, and neurocognitive function (Jamshidi and Cohen, 2017).

Naturally occurring volatile oil extracts from holy basil include eugenol, methyl eugenol, carvacrol, caryophyllene, and ursolic acid, which have numerous documented adaptogenic, antimicrobial, anticarcinogenic, anti-inflammatory, and antiviral properties (Singh et al., 2012, Gupta et al., 2002, Vasudevan et al., 1999). As consumer interest in alternative medicine grows, there is huge potential for the pharmaceutical industry and the herbal supplement producers to optimize their formulations and increase overall health benefits.

Phytochemicals

Plants synthesize various levels of chemical substances as a response to their immediate environment. These secondary metabolites help defend against various environmental factors as opposed to primary growth and maintenance (Stahl, 1888). Aromatic phenolic compounds and terpenoids found within the chemical profile of holy basil have medicinal, dental, flavoring, food science, cosmetic, and pest control applications (Shasany, 2016). A combination of eugenol,

linalool, and estragole compose the signature scent of holy basil (Rastogi et al., 2015). Eugenol and methyl-eugenol compounds are both aromatic phenols which have a defining role in the profile of holy basil due to their high concentration and commercial marketability for growers (Baseer and Jain, 2016). Although eugenol is historically extracted from clove (*Eugenia caryophyllata*) buds, holy basil has proved to be a more economical source for commercial extraction (Prakash and Gupta, 2005).

Holy basil varieties exhibit differences in chemical profile based on the chemotype of the individual cultivar (Shasany, 2016). Fourteen varieties of holy basil were evaluated at the University of Georgia in Athens, GA to determine the chemotype and essential oil yield of each one under local growing conditions between 2015 and 2016 (Fuller et al., 2018). Total yield and essential oil profile can differ between plants based on genetic predisposition as well as regional microclimates, seasonal temperature variations, rainfall, and time and date of harvest (Vasudevan et al., 1999, Bowes and Zheljazkov, 2004, Selvam et al., 2013, Nadukeri et al., 2018).

Drought, temperature, salinity, and soil pH cause variation in the amount of volatile chemicals released by plants. Because individual species synthesize certain compounds as a result of external stress factors, studies have demonstrated changes in production levels of biochemicals by holy basil in different habitats (Selvam, et al., 2013). In general, holy basil conforms to the hypothesis that stress causes plants to produce greater amounts of defensive substances. It is logical, therefore, to assume post-harvest handling may also affect phytochemicals.

Holy Basil Production Practices

Holy basil and culinary basil (*Ocimum basilicum*) have similar cultural requirements, but the two plants require different approaches to growing, handling, and processing. Both species

are consumed fresh or dried into flavorful herbal products such as tea, spice blends, and body care products. Information is readily available for culinary basil due to its widespread use, however the two *Ocimum* species are genetically and morphologically distinct (Shasany, 2016). There is a lack of holy basil production and harvesting information in the US, however many varieties are known to thrive under climatic conditions in the Southeastern US. Due to the lack of research, growers must depend on a combination of experience, experimentation, and recommendations from other regions. Depending on the local conditions and method of production practiced by commercial growers, the resulting quality and value of the end product may vary greatly.

In India, the most common holy basil varieties cultivated are *O. tenuiflorum* 'Rama', the green-leaved specimen and *O. tenuiflorum* 'Krishna' with dark green to purple leaves (Upadhyay, 2017). *O. tenuiflorum* 'Kapoor' is a popular commercial cultivar, grown for its highly productive yields under temperate North American climatic conditions. The essential oil content of 'Rama' and 'Krishna', and *O. gratissimum* 'Vana' is high, but under typical SE US conditions, 'Kapoor' has a consistently higher overall essential oil yield per hectare (Fuller et al., 2018). Each variety has a unique habit, with some displaying a wide sprawling structure while others form clumped shape with a compact structure which adds to the ease of the harvest.

Commercial, field-grown culinary basil is produced in the US, mainly in southern and southwestern regions where temperatures remain above 4 °C throughout the cropping season. Soil and air temperatures above 15 °C are ideal for optimal growth rates and can directly affect the number of harvests growers can expect to achieve throughout the year (Meyers, 2003). Both holy basil and culinary basil are extremely tender. A light frost will instantly destroy this crop, while cold damage to the foliage is evident at temperatures as low as 4 °C in the field and during

storage (Simon, 1995). Multiple harvests may be performed, depending on the length of the growing season in a particular region. In the Southeastern US, the growing season is approximately 200 days, therefore plants could potentially be harvested 5 times.

Started indoors in soilless potting mix, holy basil seeds germinate in 8-14 days. Small plants can be transplanted into the field in 4-6 weeks. Holy basil prefers moist, well-draining soil. It is typically planted on raised beds 60 to 90 cm wide accommodate double rows 30 cm apart, with plants spaced at least 15 cm apart in the row. Holy basil is adaptive to many soil types, but prefers a pH near 6.4 (Tucker and DeBaggio, 2000).

Fertilizer is typically applied to culinary basil at 150-250 kg/ha nitrogen (Bufalo et al., 2015). Higher rates encourage more foliar growth at the expense of lower essential oil concentration in the leaves and flowers (Rhodes and Chong, 2016, Bufalo et al., 2015). A recent study conducted on holy basil in India, subjecting plants to various fertilization regimes, determined 150 kg/ha N the optimal N rate for higher yield and essential oil production (Nadukeri et al., 2018).

Holy basil is typically harvested at the flowering stage, when essential oil production is highest. Harvesting both leaves and flowers contributes to the diversity of the essential oil profile (Vasudevan et al., 1999). Plants are typically cut 10-15 cm above ground to promote regrowth for subsequent harvests (Bowes and Zheljazkov, 2004). In the Southeastern US, flowers normally develop on the plants six weeks after transplanting into the field, and regrow after harvesting at approximately six week intervals until the final harvest just before first frost.

Postharvest Handling and Drying

Washing is recommended by both the Food and Drug Administration and the American Herbal Products Association to remove debris and contaminants (FDA, 2013, AHPA, 2017).

Depending on field and farm conditions, dried herbs and spices may contain microorganisms and pathogens from wildlife or other vectors. Growers following GAPs and GMPs for herbal products will reduce their risk of exposing the consumer to potentially unsafe levels of contamination (FDA, 2013). To date, few studies have examined how washing of medicinal herbs affects volatiles or overall product quality.

Harvested holy basil may be sold immediately as fresh material, or dried on-site to extend shelf life and storage capabilities, while ensuring year-round product availability. Holy basil is traditionally sold and consumed as a tea, using hot water decoction of fresh or dried aerial plant parts. Herbal supplements and liquid solvent extracts may be obtained from the herb and bottled or encapsulated using its dry powdered form. Essential oil extraction requires a distillation process that may use either fresh or dried material. Marketing of holy basil varies by grower and the ultimate form in which the herb will be used.

Postharvest handling must cater to the specific customer, whether sold wholesale, retail, processed or as a raw product. The market for fresh culinary and medicinal herbs is highly specialized and depends on partnerships between growers, local farmers markets, herbal practitioners, and contracts with larger-scale supplement distributors.

Drying a perishable product is complex and requires crop-specific procedures to maintain safety, quality, and efficiency throughout the process. There are cost considerations involved with selecting a postharvest dehydration strategy. The initial investment in drying equipment, combined with energy use, labor intensiveness, and processing time affects the net value of the crop. The dominant producers of medicinal plants are located in developing countries where access to modern equipment and resources may not be available (FAO, 2005).

Common drying methods for most medicinal herbs range from primitive ancient designs to modern industrial-scale technologies. In many remote rural areas, where a large portion of herbs, spices, and teas are produced, drying technology may be limited to structures that can be built using local materials and do not require fuel or power.

Sun drying is common in rural areas because it does not require an external fuel source and minimizes equipment costs. In climates that typically experience daily temperatures above 30 ˚C, direct sun drying is a practical option (Chua and Chou, 2003). Plants dried in direct sunlight, however, exhibit UV-induced discoloration, as well as degradation of nutrients and essential oils (Rocha et al., 2011). Solar UV radiation does provide some anti-microbial activity in drying leaves, however results from sun drying experiments are inconsistent due to uneven surface exposure throughout (Bordoux et al., 2016). Light-permeable material may protect the herbs from moisture, insect, and animal contamination, but weather and exposure to the outdoor elements inevitably affects end quality.

Worldwide, convective dryers are the most popular type of drying method for food dehydration (Bordoux et al., 2016). They can be constructed out of simple materials and adjusted in scale to fit a small space or an entire room, as required for dry herb production. The mechanism for convective water removal is a source of heated air allowed to pass over the wet material and ventilate diffused water vapor out of the unit. Convective drying is considered an active process if external energy inputs are used to control heat and air movement. A simple unit can be built into an existing structure using a heat source combined with a fan and vent system. Growers can control temperature and airflow using any available energy source, depending on cost and availability.

A passive convective dryer can be made using solar radiation as an energy source to create a thermal air gradient. Solar dryers are built using wood and plastic sheeting. They collect heat in a separate chamber, then expose the drying plant material to the hot air, while protecting it from sunlight. As the warmed air passes through the drying chamber section, water is evaporated and released through a chimney vent. Solar convection drying is highly dependent on availability of the sun and a low relative humidity. Cloudy or humid days can slow the process. An electric fan is often added to increase air movement, and supplemental heating is added to compensate for low overnight temperatures or poor weather. This hybrid design represents an integral type system with both active and passive drying mechanisms. Few studies have been conducted using solar convective drying of fresh herbs.

A heat pump convection system is a more complex technology. Air is circulated from the drying chamber across a condenser that captures and evaporates the moisture from the fresh plant material. The latent heat warms the dry air entering back into the chamber. As a closed system, this technology is independent of existing weather or room air conditions, (Perera and Rahman, 1997). Larger-scale convective systems typically include conveyor belts or fluidized bed equipment to remove water more efficiently. In general, unless thick stems or roots are present, medicinal plants can be dried satisfactorily in thin layers using basic convection drying equipment (Muller and Heindl, 2006).

Medicinal herbs undergo compositional changes in color, texture, flavor, microbiology, and biochemistry throughout the postharvest drying process. As with most medicinal herbs, the goal of holy basil dehydration is to reduce moisture content within the tissues to a level that preserves nutrients and essential oils, while inhibiting microbial activity. The rate of water removal is controlled by three factors: temperature, airflow, and relative humidity (Muller and

Heindl, 2006). Generally, the relative humidity of the air corresponds negatively with air temperature, and therefore drying time is reduced under hotter, dryer conditions. Maintaining steady airflow promotes continuous evaporation and minimizes the time heat-sensitive biochemicals are exposed to temperature elevation.

Temperature studies have been conducted on culinary basil and other herbs to determine optimal ranges for drying which retain desired coloration, minimize damage to sensitive tissues, and conserve volatile oils. It is likely that holy basil drying conditions are similar to the ranges for culinary basil and other medicinal herbs, but to date, specific temperature and humidity guidelines have not been documented for holy basil. At a drying temperature of 60 °C, the effects on individual essential oil compounds vary in culinary basil. (Filho et al., 2006, Muller and Heindl, 2006). One study, completed in the southeastern US, reported significant changes in holy basil essential oil composition between different drying methods, but did not identify changes in total oil yield (Bowes and Zheljazkov, 2004).

Holy Basil Quality

Herbal supplements are regulated in the United States by the Food and Drug Administration (FDA) under different criteria than food products, requiring medicinal plant growers to navigate a multitude of policies related to quality and safety standards. The commercial production of medicinal herbs falls under the food, supplement, or cosmetics category, depending on their intended use. Regulations for quality, safety, and product testing for herbal products increased in 1994 with the Dietary Supplement Health and Education Act (DSHEA) standards implemented by the FDA. These standards require accurate labelling of all ingredients and legally prohibit manufacturers from including adulterated material or falsely representing the contents of their product. This has created a new market for superior quality

medicinal plant material verified by measurable tests for identity, potency, and contamination from sources such as microbes, pesticides, and foreign matter. (Sanzini et al., 2011). Growers and manufacturers are expected to take a proactive approach to minimize safety hazards involved in producing and processing raw plant material at every step of the harvest.

The FDA establishes recommended Good Agricultural Practices (GAPs) and Good Manufacturing Practices (GMPs) in cooperation with the National Sanitation Foundation (NSF) and many industry representatives. The American Herbal Products Association (AHPA) has created and established detailed guidelines for growers and manufacturers to aid in compliance with federal regulations and ensure quality in botanical products not formally addressed by FDA standards (AHPA, 2017). Due to the cooperative efforts from governmental agencies, consumer advocacy, academic research, and industry stakeholders, the increased regulation of herbal products and supplements will continue to expand to meet the high demands of an increasing market with an active interest in traceability, quality, and safety issues. Farmers invested in supplying manufacturers with plant material rely upon these groups to determine the best postharvest methods to use for their specific needs.

Qualitative analyses for herbal teas and supplements must include the overall sensory attributes of the product, including color, appearance, and aroma. The connection between visual cues and our perception of the quality, freshness, and flavor of a food or drink is well established, with food color being the most influential factor (Spence, 2015). The color of any food product is typically the first factor we encounter before purchasing, preparing, or enjoying the item. Herbal products, like holy basil, must appeal to the senses of the consumer or manufacturer to convey freshness and quality of the material. Medicinal herbal products have a wide range of sources and may contain natural pigments from leaf, stem, fruit, and flower parts

which are extracted during hot-water tea preparation. The resulting color of the dried plant material is affected by variety, processing method, and storage conditions and may correspond to measurable levels of bioactive compounds.

Aside from enhancing visual appearance, the color of the finished herbal product can indicate important quality information. An important relationship exists between drying temperature, biochemical potency, and color retention. A temperature of 50° C has been determined as the generally acceptable limit for drying medicinal herbs without negative effects on color or volatile oil compounds, although specific species and compounds must be studied individually (Muller and Heindl, 2006). Based on this principle, changes in color correspond to changes in chemical composition, and may help aid growers and buyers in assessing the quality of their product based on a color gradient. Color scale values have not been published for postharvest fresh or dry holy basil.

Culinary basil was analyzed with respect to different drying methods, including oven-drying, microwave-drying, and freeze-drying (Di Cesare et al., 2003). In the report, color intensity, chlorophyll level, and volatile compound content were directly related, and appeared greener on the color intensity scale under lower temperature microwave- and freeze-drying methods compared to conventional oven-drying at 50° C. Four culinary herbs (mint, thyme, lemon balm, and sage) were dried at 24°C and 40°C, and the lower temperature was associated with higher amounts of total phenols, antioxidants, and flavonoids while preserving color (Rababah, et al., 2015). Ten culinary herbs were used to classify drying treatments (sun drying, freeze drying, and oven drying) based on their impact on visual appearance. While freeze drying had no impact, oven drying had a lower impact, and sun drying had a strong impact on color (Lafeuille, et al., 2014). The appropriate methods for drying holy basil to conserve both color

and medicinal value need to be determined through evaluation of specific drying practices and temperature studies.

The Federal Food, Drug, and Cosmetic Act of 1938 (FD&C) grants the FDA authority over tea processing and manufacturers. Teas are commonly treated as food products although medicinal teas may be classified as supplements. (Richman, 1983) All food products sold in the US marketplace are subject to routine inspection and analysis of their contents. Teas are subject to purity testing typically using USP sampling guidelines as a standard (FDA, 2020). Tea and supplement production facilities are inspected annually, or more often if there are health risks reported to be associated with a specific product (Pirina, 2004).

Medicinal herbs are generally dried to 8-12% of their fresh weight to achieve the appropriate moisture level and limit microbial growth during extended storage (Muller and Heindl, 2006). Microorganisms such as bacteria, yeasts, and fungi are incapable of reproducing if storage conditions are maintained properly at relative humidity level below 60% (Muller and Heindl, 2006). The US Pharmacopoeia (USP) has established specific quality specifications within their monograph of holy basil leaves to be included in dietary supplements. According to USP, holy basil leaves should not contain more than 10% final moisture (USP, 2018). USP standardization also requires certain botanical characteristics, quantitative tests for total ash content, loss on drying, microbial count, pesticide analysis, and foreign matter content. Although USP certification is not required, many herbal supplement manufacturers maintain USP requirements and include their label on the product to assure quality, and therefore each ingredient must meet USP guidelines.

Criteria for the microbiological safety of dried medicinal herbs differs according to the intended use of the product. Herbal teas, culinary herbs, solvent extracts, and encapsulated

supplements each have unique qualitative requirements (AHPA, 2017). The USP recommends not more than 10^3 cfu/g total yeasts and molds for dry holy basil leaves (USP, 2018). The AHPA recommends not more than 10^5 cfu/g for finished dried herbs and 10^3 cfu/g for extracts. (AHPA, 2017). Total fungal counts are measured although tests performed on dry goods are directed at specific pathogenic microorganisms such as *Staphylococcus aureus*, *Bacillus cereus* and *Aspergillus niger* (USP, 2016).

The fungal load of dried herbal products may be reduced using chemical pretreatments such as peracetic acid (PAA) (Suslow, 2000), but must not exceed the threshold for contamination by fungicides or other chemical residues. The United States Pharmacopoeia (USP), World Health Organization (WHO), and the Food and Agriculture Organization (FAO) have limited the amount of acceptable residual pesticides present in herbal products to levels safe for long-term exposure by consumers (USP, 2018, WHO, 2007).

The increasing demand for plant-based ingredients and extracts by the pharmaceutical, cosmetic, and food industries depends on access to reliable sources of commercially acceptable dried plant matter (FAO, 2005). The Food and Agriculture Organization (FAO) predicts long-term demand for essential oils in industrial, culinary, and medicinal use will outpace the ability for tropical growers to produce them. A study in India found 47% of herbs sampled directly from the local retail markets did not meet the World Health Organization (WHO) limits for fungal contamination (Aiko and Mehta, 2016). Medicinal herb farmers in Brazil reported up to 60% of herbal products failing to meet these specifications (Rocha et al., 2011).

To solve microbiological contamination issues, some producers employ irradiation to kill all microorganisms in lieu of correcting unsanitary farm and factory procedures (FAO, 2005). While irradiation can eliminate pathogens, it is not intended as a replacement for good sanitation

and proper storage conditions. A European case study found 73.9% of dry herb and spice samples were found to be suspected of irradiation without proper labelling (EU, 2004). A comprehensive review of contaminants in medicinal herbal products found that field cultivation and harvest time are the critical control points at which contamination occurs, followed by postharvest holding, treatment (washing/drying/packaging), and transportation (Kneifel et al., 2002).

Clearly, there are improvements to be made in the design and implementation of drying processes by herb suppliers worldwide. Research is needed to inform policymakers and create guidelines to help growers achieve quality standards and prevent contaminated product from entering the marketplace. To meet the increased domestic and international quality criteria for medicinally valuable plants such as holy basil, growers and processors require practical guidelines to be developed based on interdisciplinary research between agriculturalists, food scientists, and pharmacologists.

Purpose

The purpose of this study was to develop a postharvest drying protocol for growers in order to produce high quality holy basil for the domestic marketplace, and contribute to the growing body of research on medicinal herbs in the US. The objective was to compare commonly used herb drying techniques of a highly productive holy basil cultivar to determine the effects of each method on the quality of the final product. Each method evaluated represents a unique approach to postharvest handling of fresh holy basil. These methods include zero to low-cost construction materials, standard farm-scale equipment, and a commercial grade dehydrator to examine the potential for each drying method to generate a commercially acceptable medicinal herbal product. The quality factors analyzed include the following: 1) water

removal from fresh whole holy basil plant; 2) final moisture content of dried holy basil leaves; 3) color retention of dried holy basil leaves; 4) total fungal colony forming units on dried holy basil.

CHAPTER 2

EFFECTS OF DRYING METHODS ON POSTHARVEST QUALITY OF HOLY BASIL *OCIMUM TENUIFLORUM L.*[1]

[1] Begani, S.B., D.C. Berle, E. Little, and S. Nambeesan. To be submitted to HortTech.

ABSTRACT

Holy basil (*Ocimum tenuiflorum* L. ‘Kapoor’) was grown and dried at the University of Georgia Athens campus during 2018 and 2019 to evaluate postharvest handling and drying methods. The purpose was to develop a commercially acceptable postharvest protocol for growers and contribute to the growing body of research on medicinal herbs in the US. Drying methods included a passive solar convection dryer, a room equipped with dehumidifier and fans, and a commercial food dehydrator. The control treatment was a shaded barn. Treatments were evaluated using quality factors of dry holy basil, including: water removal, moisture content, color retention, and fungal colony count.

All treatments removed water more effectively than the control. Colorimetric analysis detected consistent differences between all drying methods compared to control. No differences were observed in RGB pixel intensity. Drying method had no effect on fungal colony count. Washing the fresh plant material reduced fungal colony count to the commercially acceptable level below 10^5 cfu/g dry holy basil.

INDEX WORDS: *Ocimum tenuiflorum*, holy basil, drying methods, passive solar dryer, medicinal herb dehydration, final moisture content, colorimeter, RGB histogram

Holy basil (*Ocimum tenuiflorum*) is a popular herb valued for its medicinal, aromatic, and flavorful qualities. Holy basil has been cultivated in India for therapeutic use since 200BC (Pattanayak et al., 2010) and is used in teas, extracts, essential oils, cosmetics, and nutritional supplements. Naturally occurring phytochemical extracts from holy basil include eugenol, methyl eugenol, carvacrol, caryophyllene, and ursolic acid, which have adaptogenic, antimicrobial, anticarcinogenic, anti-inflammatory, and antiviral properties (Singh et al., 2012, Gupta et al., 2002, Vasudevan et al., 1999).

The Food and Agriculture Organization (FAO) predicts long-term demand for essential oils in industrial, culinary, and medicinal use will outpace the ability for tropical growers to produce them (FAO, 2005). The climate in the SE US is ideal for cultivation of holy basil and there is potential for growers in the region to meet the growing demand for this herb, which ranks consistently in the top 30 herbs sold in the natural foods market (Smith, May, Eckl, and Reynolds, 2020). While information is readily available for culinary basil, due to its widespread use, there is much less postharvest handling information for holy basil. The two *Ocimum* species are genetically and morphologically distinct and contain unique essential oil profiles (Shasany, 2016).

Holy basil is dehydrated to reduce moisture content within the tissues to a safe level, without losing essential oils. Most medicinal herbs are dried to 8-12% moisture to limit microbial growth during extended storage (Muller & Heindl, 2006). The US Pharmacopoeia (USP official reference standard for holy basil is set at a maximum of 10% final moisture (USP, 2018).

When dried, holy basil undergoes compositional changes in color, texture, flavor, microbiology, and biochemistry (Bowes and Zheljazkov, 2004, Sims et al., 2014, Singh et al.,

2013). Drying conditions for holy basil may fall within the temperature and humidity ranges specified for culinary basil and other medicinal herbs, but specific guidelines have not been thoroughly researched. One Southeastern US study reported significant changes in holy basil essential oil composition between different drying methods, but did not identify changes in total oil yield (Bowes & Zheljazkov, 2004).

A study of culinary basil in Italy found that, color intensity, chlorophyll level, and volatile compound content were directly related, and dried material appeared greener on the color intensity scale under low temperature freeze-drying and microwave-drying methods, compared to conventional oven-drying at 50 °C (Di Cesare, Forni, Viscardi, & Nani, 2003). At 60 °C, the effects of heat on culinary basil vary depending on the individual compounds analyzed within its oil profile (Filho et al., 2006; Muller & Heindl, 2006).

Four culinary herbs (mint, thyme, lemon balm, and sage) were dried at 24°C and 40°C, with the lower temperature associated with greater amounts of total phenols, antioxidants, and flavonoids, while preserving color (Rababah, et al., 2015). Ten culinary herbs were used to classify various drying treatments (sun-drying, freeze-drying, and oven-drying) based on their impact on visual appearance. While freeze-drying had no impact, oven-drying had a lower impact, and sun-drying had a strong impact on color (Lafeuille, et al., 2014).

Standards for fungal load vary, depending on the agency. The USP recommends not more than 10^3 cfu/g total yeasts and molds for dry holy basil leaves (USP, 2018). The American Herbal Products Association (AHPA) and World Health Organization (WHO) recommend not more than 10^5 cfu/g for finished dry herbs. (AHPA, 2017, WHO, 2007). A study in India found 47% of herbs sampled directly from the local retail markets did not meet these standards (Aiko

and Mehta, 2016). Medicinal herb farmers in Brazil have reported 60% of herbal products failing to meet these specifications (Rocha et al., 2011).

Postharvest washing is recommended by both the Food and Drug Administration (FDA) and the AHPA to remove debris and contaminants (FDA, 2013, AHPA, 2017). Depending on field and farm conditions, dried herbs and spices may contain microorganisms and pathogens from wildlife or other vectors. Growers following Good Agricultural Practices (GAPs) and Good Manufacturing Practices (GMPs) for dried herbal products will reduce the risk of exposing consumers to potentially unsafe levels of contamination (FDA, 2013). To date, few studies have examined how washing of medicinal herbs affects volatile compounds or overall product quality.

Specific criteria for the microbiological safety of dried medicinal herbs differs according to the intended use of the product (Pirina, 2004). Herbal teas, culinary herbs, solvent extracts, and encapsulated supplements each have unique qualitative requirements (AHPA, 2017, Richman, 1983). Both tea and supplement producers are required to conform to GAPs and GMPs standards. Products are routinely screened for accurate botanical identity, mislabeling, adulteration, and contamination from pesticides and pathogens according to the US FD&C Act (FDA, 2020).

The purpose of this study was to compare commonly used herb drying techniques to determine the effects of each method on the quality of the final product, using the following quality factors: water removal, final moisture content, fungal colony forming units, and color retention of the holy basil leaves. An additional goal was to evaluate the effects of washing fresh plant material on these same quality factors. This study intentionally focused on quality factors that could be evaluated with simple economical lab procedures.

MATERIALS AND METHODS

Cultivation

The experiment was conducted during the growing seasons in 2018 and 2019, at the University of Georgia UGArden farm (lat. 33°53′55.5″N; long. 83°22′09.2″W) in Athens, GA. *O. tenuiflorum* 'Kapoor' plants were grown from seeds obtained from Strictly Medicinal Seeds (Williams, OR). The cultivar 'Kapoor' was selected for its yield and oil content, based on a previous study (Fuller et al., 2018).

Plants were seeded in a greenhouse using Sunshine Natural and Organic Professional Growing Mix (SunGro®, Agawam, MA) in 72-cell flats on June 10, 2018 and May 25, 2019. Seedlings were hardened off for 1 week and transplanted into raised beds after 34 days. A weather station (Meter Group, Pullman, WA) was installed onsite to record temperature, relative humidity, and rainfall for each drying period (Table 2.1).

Plants were grown in a Cecil sandy clay loam. Soil test results in 2018 obtained from the University of Georgia Soil Test Lab indicated high levels of P and K (soil test index 257 and 408 lbs/A, respectively) with only N recommended. Nitrogen was applied at the rate of 120 kg/ha N. In both 2018 and 2019, using organic hydrolyzed poultry meal (13-0-0) (Mason City Byproducts, Inc., Mason City, IA). In 2019, the soil test reported inadequate P and K (32 and 149 lbs/A, respectively). P was applied at 110 lbs/A and K at 110 lbs/A. Soil amendments were incorporated prior to planting using a walk-behind tractor (BCS, Abiategrasso (MI), Italy) with power harrow attachment.

Three raised field beds, 1 m wide × 9 m long, were planted, each containing 60 plants with 30 cm spacing. The two outer rows were planted as a buffer for the center row. Plants were mulched with pine straw and drip irrigation tubing provided approximately 2.5 cm water in

weekly applications when there was no rain. Plants were pruned back to 3 nodes after 12 days to encourage branching.

Harvest Protocol

Leaves and flowers were harvested after 6 weeks, as the plants reached full-flower. All plants were harvested from the center row during morning hours. Aerial plant parts were cut 10 cm above ground. Harvested material was gathered and placed in a 40-gallon bin and mixed into one homogenous batch. Each treatment consisted of 1,800 g fresh holy basil, separated into 3 screens, each containing 600 g. Screens were spaced 25 cm apart on metal racks (WinCo®, Boise, ID) within each of the four drying treatments. The plants entered the drying chambers in their raw, unwashed state prior to processing. All equipment was sanitized before and after use.

HOBO® dataloggers (Onset® Computer Corporation, Bourne, MA) were placed on the center screen to monitor temperature and relative humidity for the duration of the drying period (Table 2.2). Plants were examined to determine dryness based on the breaking point of stem tissue, which signified completion of the drying process. Stems were discarded before processing and subsequent analyses. Mean, max, and min daily temperatures and cumulative rainfall were recorded for the drying period for all harvests in 2018 and 2019 (Table 2.1). Drying times differ according to these seasonal fluctuations (Table 2.2).

Five total harvests were made in 2018 and 2019: harvest H1 (Aug 28) and H2 (Oct 2) in 2018, and harvest H3 (Aug 8), H4 (Sep 6), and H5 (Oct 16) in 2019. Plants were allowed to grow back until reaching their full-flower stage before they were harvested again. The first damaging frost occurred at a later date in 2019, which allowed for harvest H5.

Drying Treatments

Solar dryer

The solar dryer (SD) was constructed in 2019 using a design provided by Wheaton Labs (Missoula MT, 2009) (Figure 2.1A). The dryer is passive, allowing incoming air to be heated using solar radiation, and airflow is controlled using only the passive air gradient with no external energy input. Plants are protected from direct exposure to the sun in a separate drying chamber. Screens were placed in the solar dryer on custom wooden slats to provide 25 cm spacing. The solar dryer was aligned south to maximize light exposure and heat production. Daily temperature and RH fluctuations were recorded (Tables 2.1 and 2.2).

Drying room

The drying room (DR) was established in a pre-existing barn structure at the UGArden farm (Figure 2.1B). The walls were cement block with a concrete floor. A standard room dehumidifier (Hisense DH70K1G) was used to remove moisture from the air and a fan (Lasko WTA 2551) was placed in the room to keep air moving. The dehumidifier and fan were used at full power, based on their respective capabilities. Temperature and RH in the room was maintained at approximately 35 ℃ and 35% RH (Table 2.2).

Commercial dehydrator

A food dehydrator (FD) (Nyle Systems 2.5 Brewer, ME) was used, providing customizable temperature, relative humidity, and time control (Figure 2.1C). The drying chamber held one single metal rack. Temperature and RH settings were programmed at 40 °C and 25% RH (Table 2.2). In 2019, the food dehydrator was inoperable and no data was collected during harvests H3 and H4 due to a defective compressor, and was repaired prior to H5.

Control

For the control (CO), a shaded open-air barn was used (Figure 2.1D). This space represents a typical shed or barn space found on most farms. A single rack containing 3 screens

was placed adjacent to a wall and protected from direct sun and rain. This treatment received no additional inputs and was completely dependent on weather conditions. Daily temperature and RH fluctuations were recorded. (Tables 2.1 and 2.2)

Water removal

After drying was determined to be complete for each treatment, plants were removed from screens and weighed with stems intact to determine the total amount of water removed from the whole plants. Water content inside the plant tissue at the time of harvest determined how much must be removed to complete the drying process. To determine the volume of water removed, moisture removal was calculated in grams and converted to ml by:

600 g – g dry plant weight = ml water removed

Final moisture content

Leaves and flowers were separated from the stems and weighed to determine the actual yield of usable herbage from each treatment. Three samples of 10 g holy basil leaves and flowers from each treatment were placed in a lab oven (Napco Inc. Model 630, South Haven, MI) at 80 °C until a constant dry weight was achieved. The final moisture content of the processed plants was determined by:

(10 g – dry weight g @ 80° C) /10g x 100%

Color retention

Three samples from each harvest were analyzed using a Precision Colorimeter (3nh NR110) to determine L*a*b*Ch colorspace values. Forty colorimeter readings per sample were obtained from the surfaces of the leaves. In addition, 5 g holy basil leaves were ground with a mortar and pestle and passed through a sieve (Market Grade #20, Chicago, IL). Individual petri dishes containing 5 g ground holy basil were directly placed on the scanner (Epson Perfection

V600 Photo) producing 48-bit 600 dpi TIF format images analyzed using the RGB Color Histogram plugin (ImageJ Version 1.52a.).

Fungal colony count

Three samples from each treatment in each harvest were analyzed to determine the total fungal colony forming unit count per gram of dry holy basil (cfu/g). The method was based on the World Health Organization guidelines for assessing quality of herbal medicines with reference to contaminants and residues (WHO, 2007). For each sample, 1g dry holy basil was passed through a sieve (Market Grade #10, Chicago, IL) and diluted with 100 ml sterile distilled water. A surfactant (Tween20, Sigma-Aldrich, St. Louis, MO) was added at a rate of 0.01% to facilitate separation of spores from leaf material. Subsequent 10-fold dilutions of the original concentrate were prepared in glass vials up to 10^{-3}.

One hundred µl of each dilution was pipetted in triplicate onto Rose Bengal agar with chloramphenicol (Hardy Diagnostics, Santa Maria, CA) in sterile 100 x 15 mm petri dishes (VWR, Radnor, PA) in a laminar-flow hood. The suspension was immediately spread over the plate surface using a bent glass rod. Plates were incubated at 21 °C for 5 days and the dilutions that contained 10-150 cfu were counted to determine total fungal colonies per gram of dried holy basil for each treatment (Figure 2.2).

Washed vs. unwashed

In a separate study, freshly harvested holy basil samples were placed into unwashed or washed treatments in three additional harvests in 2019. Unwashed plants were placed directly from the field onto screens. Washed plants were submerged into two consecutive municipal water baths before placement onto the drying screens. Municipal water was tested for chlorine content using a Sensafe Free Chlorine Test Kit (Industrial Test Systems, Inc.). Three screens,

each with 600g of harvested material, were placed onto the drying rack and dried to completion in the drying room. Temperature, RH, and drying time were recorded for each harvest (Table 2.3). Wash treatments were repeated 3 times in 2019 for 3 separate harvests, W1 (July 25), W2 (Aug. 28), and W3 (Oct 25).

Statistical analysis

Due to significant effects of year and harvest, data for all treatments is analyzed and presented for each individual harvest (H1-H5). Data from results was analyzed using one-way ANOVA and Tukey's range test ($p \leq 0.05$) by JMP Version 14.1.0 (SAS Institute Inc., Cary, NC, 1989-2019).

RESULTS

Water removal

The solar dryer (SD), drying room (DR), and food dehydrator (FD) treatments removed significantly more water compared to the control (CO) for H2 in 2018, and H3, H4, and H5 in 2019 (Figure 2.3A-B). There were no differences among the SD, DR, and FD treatments, however. For harvest H1 in 2018, there were no differences between treatments and CO (Figure 2.3A).

Final moisture content

Each drying treatment produced variable results depending on individual harvests (Figure 2.4A-B). During harvest H1, only the SD and FD treatments had lower final moisture content (FMC) (7.3% and 7.1%, respectively) than CO (9.0%) (Figure 2.4A). For harvest H2, all treatments were significantly different from one another, as well as the control (Figure 2.4A). The FD had the lowest FMC (6.9%), followed by DR (7.9%), SD (9.8%), and CO (13.5%). Data from harvest H3 showed no differences between any treatment and control. In harvest H4, the

drying room (8.7%) was lower than both SD (13.8%) and the control (13.9%) (Figure 2.4B). In harvest H5, CO (24.5%) had an extremely high FMC due to cooler fall weather conditions. All treatments were lower than CO for harvest H5, and FD (8.3%) was significantly lower than DR (11.1%) (Figure 2.4B).

The target FMC for medicinal herbs is 8-12% (Muller and Heindel, 2006), and not more than 10% for dry holy basil leaves (USP, 2018). The SD met the 8-12% target for harvest H2 and H5, and the USP target for H1, H2, and H3 (Figure 2.4A-B). The drying room met the 8-12% target for H1, H3, H4, and H5, and the USP target for H1, H2, H3, and H4 (Figure 2.4A-B). The food dehydrator met the 8-12% target for H5, and the USP target for H1, H2, and H5 (Figure 2.4A-B). The CO met both targets for H1 and H3 (Figure 2.4A-B).

*Color retention: Colorimeter L*a*b*Ch values*

The colorimeter produces values for lightness (L*), red/green (a*), blue/yellow (b*), chroma (C), and hue (h). The a* value represents the red/green scale and b* is the yellow/blue scale. C is the chroma value and is calculated to match the perception of the human eye. Hue is the angle of each sample around the CIELAB color wheel. Values >90° are closer to green, while <90° falls closer to red.

In 2018 and 2019 for all harvests H1-H5, CO produced significantly higher red/green (a*) values (4.5, 5.5, 4.8, 3.3, 5.3) than all other treatments, indicating more redness (Tables 2.4 and 2.5). DR had greener a* values than all treatments in harvests H2, H3, H4, and H5, (-0.9, -1.0, -0.2, -1.0). For harvest H1 SD had the greenest a* value (0.8), (Tables 2.4 and 2.5).

The lightness (L*) value differed between treatments for harvest H1, H2, H3, and H5 (Tables 2.4 and 2.5). For three of these harvests, DR showed lighter values than the other

treatments, (40.6, 43.1, 45.5) while SD was lighter for harvest H1 (37.6). For harvest H2 and harvest H5, DR was more yellow and less blue than both CO and SD (Tables 2.4 and 2.5).

Hue angles (h) were consistently greater, indicating different shades of green and red in dry herbage for all treatments compared with CO. For harvests H2, H3, H4, and H5, DR had a greener hue angle (92.2°, 92.6°, 90.2°, 92.1°) (Tables 2.4 and 2.5). For harvest H1, SD had the largest hue angle (86.9°). There were no consistent trends among harvest or year on the blue/yellow (b*) scale, or chroma (C), (Tables 2.4 and 2.5).

Color retention: RGB pixel intensity

The RGB histograms represent the intensity of the high-resolution scanned image of the powdered holy basil leaves. In 2018, SD showed a slightly, yet significantly lower red intensities for harvest H1 and H2 (84.25, 89.22) compared to CO (89.33, 99.5). For harvest H1, FD (84.5) was lower than CO (Table 2.6). For harvest H3, DR (104.67) had lower red intensity than CO (112) (Table 2.7). There were no differences in green or blue pixel intensity values, in either year (Tables 2.6 and 2.7). In 2018, there was one case where FD had a higher blue intensity (27.4) than CO (31.05) for harvest H2 (Table 2.6).

Fungal colony count

For harvests H1, H2, H4, and H5, there were no differences between any drying treatment and control (Figure 2.5A-B). In harvest H3, SD had a lower cfu count ($2.4^{x}10^{5}$ cfu/g) than both DR ($4.2^{x}10^{5}$ cfu/g) and CO ($4.5^{x}10^{5}$ cfu/g) (Figure 2.5B).

Washed vs. unwashed

Holy basil dried in the drying room after washing (W) in tap water during all three harvests W1-W3 contained significantly lower cfu counts ($7.3^{x}10^{4}$ cfu/g, $9.8^{x}10^{4}$ cfu/g, $1.3^{x}10^{5}$ cfu/g) compared with the unwashed (UW) plants ($3.7^{x}10^{5}$ cfu/g, $2.2^{x}10^{5}$ cfu/g, $4.7^{x}10^{5}$ cfu/g)

(Figure 2.6). The washing treatment did not affect the final moisture content of the product overall, or in any individual harvest. There were no differences in L*a*b*Ch colorimeter values overall between treatments (Table 2.8). There were no differences in RGB pixel intensity between washing treatments (Table 2.9).

DISCUSSION

Differences between drying methods were observed in both the 2018 and 2019 crop seasons. For all drying treatments, water removal was greater, final moisture lower, and colorimeter measurements were greener than observed in the control.

Dehydration of medicinal herbs to 8-12% moisture is recommended to limit microbial growth while preserving color and medicinal compounds (Muller & Heindl, 2006). According to the USP monograph, holy basil leaves should contain less than 10% final moisture, which is also a standard for pharmaceutical grade products (USP, 2018). All treatments, except CO, were below the 12% FMC target for H1, H2, H3, and H5. SD was above the 12% target for H4. All treatments, except CO, dried the product below 8% FMC value during at least one of the harvests. Over-drying herbal products below 8% may produce a product of lower value due to loss of volatile compounds, as well as using more energy (Muller and Heindl, 2006). DR showed the most consistent FMC, drying below 12% for all harvests, and only one harvest was over dried by 0.1%. Both DR and FD were able to consistently meet the 10% USP target range for all harvests except H5.

All treatments were more green and less red on the a* scale compared to CO. Significantly higher lightness (L*) values and hue angles (h) were observed for all treatments compared to the control for all harvests. Similar differences in L*, a*, and hue were also found in

a study of culinary basil using a variety of drying methods, where loss of chlorophyll and volatile compounds were correlated with color (Di Cesare et al., 2003).

Figure 2.7A-D depicts the dry holy basil material from harvest H2 in 2018. Color differences which may not be apparent to the human eye are detected by the colorimeter. The DR produced the lightest L* and greenest a* and hue values compared to all other treatments during harvest H2, as well as H3, H4, and H5. The SD, DR, and FD treatments were also lower than CO in FMC during H2. Images scanned and analyzed using RGB histograms did not provide enough information to distinguish between drying treatments. Although several individual harvests showed redder values in CO, RGB pixel intensity values did not give a clear picture overall.

Drying treatment had no effect on number of fungal cfus on dried holy basil. Significantly lower fungal cfus were detected in washed plants vs unwashed. The degree and duration of heat applied throughout the process is evidently not lethal to many fungal colony-forming species. The fungal cfu counts were slightly higher in 2018. During the experiment, weather conditions ranged from excessively hot and dry during the summer days, with periods of brief and/or heavy rainfall, as well as decreasing fall temperatures. Rainfall was higher overall in 2018 with record-breaking numbers of spring rain days for Athens, GA. In 2019, temperatures were slightly higher than 2018. Weather variation (wind, rainfall, season) is known to have an impact on naturally occurring fluctuations in the local microbiota (Collier and Ferguson, 1953). The SD experienced higher maximum internal temperatures (Table 2.2) and demonstrated some ability to reduce fungal activity during harvest H3 (Figure 2.6B).

Microbes, such as fungal spores, are present in the atmosphere and do not necessarily pose any health risk to the consumer. The main focus of the FDA safety monitoring approach to herbal products is on specific tests for microorganisms that are toxic or pathogenic to humans,

such as *Staphylococcus aureus*, *Bacillus cereus* and *Aspergillus niger* (USP, 2016). In general, microorganisms such as bacteria, yeasts, and fungi are incapable of reproducing and do not pose any risk if storage conditions are maintained properly at 60% RH (Muller and Heindl, 2006).

This study demonstrated that method of drying could affect moisture, fungal count and color of dried holy basil. The color differences associated with various drying treatments suggests potential for colorimetry to be an efficient quality indicator. Alone, washing harvested holy basil reduced the fungal count more than any drying treatment, highlighting the importance of postharvest washing. Although the commercial food dehydrator allows greater precision in temperature and RH control, these results do not indicate that this form of drying equipment is superior to the simpler low-cost methods.

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Table 2.1. Outdoor temperature and rainfall during drying periods. Mean, minimum, and maximum temperature (°C) and total rainfall (cm) for holy basil harvest H1, H2, H3, H4, and H5, in 2018 and 2019.

		Temp °C			RH %			Rain cm
Year	**Harvest**	**Mean**	**Max**	**Min**	**Mean**	**Max**	**Min**	**Total**
2018	**H1**	26.2	33.3	20.1	74.5	98.7	38.9	0.2
	H2	24.1	33.7	15.8	77.5	98.8	36.2	2.2
2019	**H3**	27.5	35.2	21.6	74.8	98.5	32.3	0.6
	H4	26.6	36.2	17.2	65.2	98.4	20.2	5.6
	H5	15.6	26.2	5.1	71.8	100	24.5	7.3

Table 2.2. Temperature, relative humidity, and drying time per drying treatment and harvest in 2018 and 2019. Mean temperature (°C), RH (%), and duration of drying period (d) in drying chamber. Drying treatments include control (CO), drying room (DR), commercial food dryer (FD), and solar dryer (SD) for each holy basil harvest H1, H2, H3, H4, and H5, in 2018 and 2019.

Year	Harvest	Dryer	Temp (°C)	RH (%)	Drying days (d)
2018	**H1**	**CO**	27.7	71.2	8.5
		DR	32.8	37.2	8.5
		FD	41.6	25.8	2.5
		SD	33.4	54.3	8.5
	H2	**CO**	25.8	71.7	7.5
		DR	32.4	32.1	7.5
		FD	41.9	23.4	3.5
		SD	31.9	25.8	5.5
2019	**H3**	**CO**	29.1	70.89	8.0
		DR	37.6	38.42	7.0
		FD	-	-	-
		SD	34.6	56.9	7.0
	H4	**CO**	27.3	67.21	8.0
		DR	36.5	40.01	8.0
		FD	-	-	-
		SD	34.1	50.76	8.0
	H5	**CO**	16.5	68.36	9.0
		DR	27.5	41.16	8.0
		FD	40.1	29.97	4.0
		SD	19.7	59.95	8.0

Table 2.3. **Temperature, relative humidity, and drying time per wash treatment and harvest in 2019.** Mean temperature (°C), RH (%), and duration of drying period (d) were in chamber for each drying treatment, in 2019. Washing treatments include unwashed holy basil (UW) and holy basil submerged in two municipal water baths (W) for harvest (W1, W2, and W3).

Year	Harvest	Treatment	Temp(°C)	RH(%)	Drying days
2019	**W1**	**UW**	35.5	40.96	8.0
		W	35.8	37.29	8.0
	W2	**UW**	35.9	37.34	8.0
		W	35.7	41.59	8.0
	W3	**UW**	29.7	36.63	8.0
		W	29.7	37.66	8.0

Table 2.4. Colorimeter values per holy basil drying treatment and harvest in 2018. Mean colorimeter values using 40 measurements for each drying treatment. Drying treatments include control (CO), drying room (DR), commercial food dryer (FD, and solar dryer (SD) for each holy basil harvest (H1, H2, H3, H4, and H5) in 2018 and 2019. Values are average of three treatment replications.

Colorimeter values

Year	**2018**						
Harvest		**H1**			**H2**		
Value	**Dryer**		**±SE**			**±SE**	
L [y]	**SD**	37.6	0.03	a[z]	35.4	0.11	c
	DR	34.5	0.12	B	40.6	0.29	a
	FD	33.7	0.94	B	38.1	0.62	b
	CO	35.4	0.5	ab	33.6	0.38	d
				*			*
a [x]	**SD**	0.8	0.27	B	1.9	0.36	b
	DR	3.2	0.76	ab	-0.9	0.31	c
	FD	3.0	1.07	ab	0.1	0.23	c
	CO	4.5	0.09	a	5.5	0.35	a
				*			*
b[w]	**SD**	17.0	0.23	a	15.2	0.38	b
	DR	16.4	0.15	a	18.9	0.86	a
	FD	15.3	0.79	a	17.5	0.41	ab
	CO	17.4	0.45	a	16.1	0.13	b
				NSD			*
C[v]	**SD**	17.1	0.26	a	15.4	0.44	b
	DR	17.2	0.33	a	19.0	0.88	a
	FD	16.1	0.33	a	17.6	0.42	ab
	CO	18.2	0.48	a	17.2	0.2	ab
				NSD			*
h[u]	**SD**	86.9	0.85	a	83.1	1.32	b
	DR	78.3	2.33	ab	92.2	0.88	a
	FD	77.5	4.05	ab	89.2	0.57	a
	CO	74.9	0.27	b	70.9	1.03	c
				*			*

NSD, * not significant, significant at P value ≤ 0.05, respectively.

[z] Means (±SE) followed by the same letter within the same column not different according to Tukey's honestly significant difference test (α = 0.05)

[y] L value represents lightness (0 = black, 100 = white)

[x]a value represents the red+/green- scale (0-60)

[w]b value represents the yellow+/blue- scale (0-60)

[v]C (chroma) is the color intensity (0-60)

[u]h Hue angles (0-360°) >90° are closer to green, while <90° falls closer to red

Table 2.5 Colorimeter values per holy basil drying treatment and harvest in 2019. Mean colorimeter values using 40 measurements for each drying treatment. Drying treatments include control (CO), drying room (DR), commercial food dryer (FD, and solar dryer (SD) for each holy basil harvest H1, H2, H3, H4, and H5, in 2018 and 2019. Values are average of three treatment replications.

Colorimeter values

Year	**2019**									
Harvest		**H3**			**H4**			**H5**		
Value	**Dryer**		**±SE**			**±SE**			**±SE**	
L [y]	**SD**	41.5	0.5	a[z]	43.4	1.09	a	40.7	0.59	bc
	DR	43.1	0.43	a	43.1	1.8	a	45.5	0.51	a
	FD	-			-			41.7	1.27	ab
	CO	37.6	0.7	b	40.5	0.65	A	37.5	0.87	c
				*			NSD			*
a [x]	**SD**	0.0	0.36	b	-0.4	0.75	b	3.3	0.28	b
	DR	-1.0	0.24	c	-0.2	0.62	b	-1.0	0.21	c
	FD	-			-			1.9	0.76	b
	CO	4.8	0.13	a	3.3	0.46	a	5.3	0.22	a
				*			*			*
b[w]	**SD**	18.3	0.47	a	20.2	0.79	a	19.1	0.54	b
	DR	18.7	0.34	a	20.7	0.83	a	22.4	0.21	a
	FD	-			-			20.4	0.82	ab
	CO	17.9	0.72	a	19.5	0.52	a	18.4	0.39	b
				NSD			NSD			*
C[v]	**SD**	18.4	0.48	a	20.3	0.8	a	19.6	0.52	b
	DR	18.8	0.33	a	20.8	0.84	a	22.6	0.81	a
	FD	-			-			20.7	0.81	ab
	CO	18.6	0.75	a	19.9	0.53	a	19.3	0.41	b
				NSD			NSD			*
h[u]	**SD**	90.0	0.67	b	90.5	2.01	a	79.7	1.09	b
	DR	92.6	0.46	a	90.2	1.69	a	92.1	0.63	a
	FD	-			-			84.0	2.11	b
	CO	74.9	0.3	c	80.3	1.06	b	73.7	0.56	c
				*			*			*

NSD, * not significant, significant at P value ≤ 0.05, respectively.

[z] Means (±SE) followed by the same letter within the same column not different according to Tukey's honestly significant difference test (α = 0.05)

[y]L value represents lightness (0 = black, 100 = white)

[x]a value represents the red+/green- scale (0-60)

[w]b value represents the yellow+/blue- scale (0-60)

[v]C (chroma) is the color intensity (0-60)

[u]h Hue angles (0-360°) >90° are closer to green, while <90° falls closer to red

Table 2.6. Red, green, and blue pixel intensity of holy basil leaves per drying treatment and harvest in 2018. Mean red, green, and blue (RGB) intensity values for each drying treatment. Drying treatments include control (CO), drying room (DR), commercial food dryer (FD, and solar dryer (SD) for each holy basil harvest H1, H2, H3, H4, and H5, in 2018 and 2019. Values are average of three treatment replications.

Pixel intensity values

Year	**2018**						
Harvest		**H1**			**H2**		
Value	**Dryer**		**±SE**			**±SE**	
Red	**SD**	84.25	0.45	b[z]	89.22	0.75	b
	DR	86.02	0.7	ab	92.32	1.99	ab
	FD	84.5	1.09	b	90.57	1.59	ab
	CO	89.33	1.09	a	99.5	3.06	a
				*			*
Green	**SD**	90.39	1.57	a	92.22	0.89	a
	DR	94.17	2.21	a	98.48	2.41	a
	FD	94.62	1.54	a	95.31	0.23	a
	CO	89.57	1.69	a	94.39	2.92	a
				NSD			NSD
Blue	**SD**	25.45	1.17	a	30.84	0.82	ab
	DR	24.18	1.24	a	29.38	0.62	ab
	FD	23.88	0.65	a	27.4	0.87	b
	CO	22.62	0.46	a	31.05	0.79	a
				NSD			*

NSD, * not significant, significant at P value ≤ 0.05, respectively.

[z] Means (±SE) followed by the same letter within the same column not different according to Tukey's honestly significant difference test ($\alpha = 0.05$)

Table 2.7 Red, green, and blue pixel intensity of holy basil leaves per drying treatment and harvest in 2019. Mean red, green, and blue (RGB) intensity values for each drying treatment. Drying treatments include control (CO), drying room (DR), commercial food dryer (FD, and solar dryer (SD) for each holy basil harvest H1, H2, H3, H4, H5, in 2018 and 2019. Values are average of three treatment replications.

Pixel intensity values

Year	**2019**									
Harvest		**H3**			**H4**			**H5**		
Value	**Dryer**		**±SE**			**±SE**			**±SE**	
Red	**SD**	105.67	2.4	ab[z]	106.67	2.19	a	126.33	2.33	a
	DR	104.67	1.2	b	115	2.89	a	122.67	2.96	a
	FD							118.67	1.2	a
	CO	112	1	a	113.67	2.03	a	123.33	3.67	a
				*			NSD			NSD
Green	**SD**	99.33	0.33	a	101	3.21	a	107.33	1.45	a
	DR	99.67	3.18	a	104	3.21	a	110	3.79	a
	FD							105	1.73	a
	CO	95	1.53	a	102.67	1.76	a	105.67	2.84	a
				NSD			NSD			NSD
Blue	**SD**	26.33	2.33	a	25.33	1.45	a	25	0.58	a
	DR	25.33	0.33	a	26	1.73	a	30.33	2.03	a
	FD							27	1	a
	CO	23.66	1.21	a	31.67	3.28	a	29	1	a
				NSD			NSD			NSD

NSD, * not significant, significant at P value ≤ 0.05, respectively.

[z] Means (±SE) followed by the same letter within the same column not different according to Tukey's honestly significant difference test (α = 0.05)

Table 2.8: Mean colorimeter values for per wash treatment and harvest in 2019. Mean colorimeter values using 40 measurements for each washing treatment- unwashed holy basil (UW) and holy basil submerged in two municipal water baths (W) for harvest (W1, W2, and W3). Values are average of three treatment replications.

Colorimeter values

Year	**2019**							
Harvest		**W1**			**W2**			**W3[t]**
Value	**Treatment**		**±SE**			**±SE**		
L[y]	**UW**	43.8	1.28	a[z]	45.0	0.77	a	-
	W	42.7	0.73	a	45.6	0.74	a	-
				NSD			NSD	
a[x]	**UW**	-0.6	0.44	a	-1.95	0.37	a	-
	W	-0.0	0.37	a	-2.4	0.32	a	-
				NSD			NSD	
b[w]	**UW**	19.8	0.88	a	21.6	0.8	a	-
	W	19.3	0.57	a	21.5	0.41	a	-
				NSD			NSD	
C[v]	**UW**	19.9	0.89	a	21.72	0.8	a	-
	W	19.3	0.57	a	21.8	0.45	a	-
				NSD			NSD	
h[u]	**UW**	91.0	1.16	a	94.4	0.89	a	-
	W	89.8	1.1	a	95.6	0.67	a	-
				NSD			NSD	

NSD, * not significant, significant at P value ≤ 0.05, respectively.

[z] Means (±SE) followed by the same letter within the same column not different according to Tukey's honestly significant difference test (α = 0.05)

[y]L value represents lightness (0 = black, 100 = white)

[x]a value represents the red+/green- scale (0-60)

[w]b value represents the yellow+/blue- scale (0-60)

[v]C (chroma) is the color intensity (0-60)

[u]h Hue angles (0-360°) >90° are closer to green, while <90° falls closer to red

[t] Colorimeter readings data for W3 samples not available due to file error

Table 2.9: Red, green, and blue pixel intensity per wash treatment and harvest in 2019. Mean red, green, and blue (RGB) intensity values for each washing treatment- unwashed holy basil (UW) and holy basil submerged in two municipal water baths (W) for harvest (W1, W2, and W3). Values are average of three treatment replications.

Pixel intensity value									
Year	**2019**								
Harvest		**W1**			**W2**			**W3**	
Value	**Treatment**		**±SE**			**±SE**			**±SE**	
Red	**UW**	127	4.36	a[z]	116.7	2.18	a	143.3	1.2	a
	W	127	0.7	a	116.3	1.86	a	133.7	4.48	a
				NSD			NSD			NSD
Green	**UW**	115.3	4.33	a	109	2.52	a	129.3	1.76	a
	W	118.7	2.33	a	110.3	2.85	a	121.3	3.84	a
				NSD			NSD			NSD
Blue	**UW**	26.3	1.67	a	26.3	2.33	a	37.6	2.33	a
	W	27.7	2.4	a	25.3	0.33	a	36.3	0.88	a
				NSD			NSD			NSD

NSD, * not significant, significant at P value ≤ 0.05, respectively.

[z] Means followed by the same letter within the same column not different according to Tukey's honestly significant difference test ($\alpha = 0.05$)

A. 

B. 

C. 

D. 

Figure 2.1A-D. Holy basil drying treatments in 2018 and 2019. Drying treatments include solar dryer (A), drying room (B) commercial food dryer (C) and the control in a shaded barn (D).

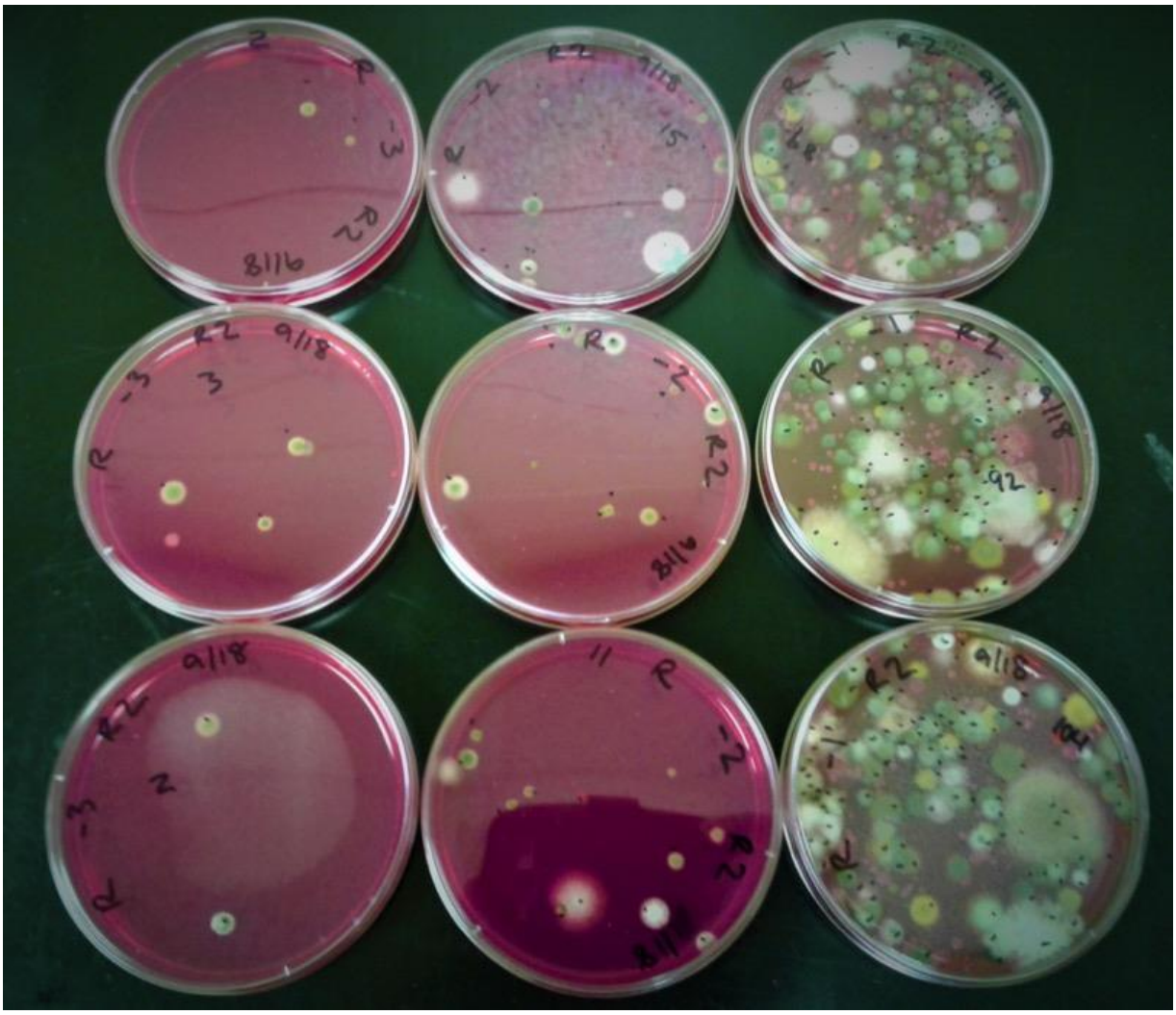

Figure 2.2. Serial dilution plates of holy basil dried in drying room. Harvest H1 in 2018. Prepared using 1g dry holy basil in aqueous suspension with surfactant. Plates pipetted in triplicate up to 10^{-3} concentration using Rose Bengal agar and incubated for 5 days at 21°C. Plates containing 10-150 cultures were counted to determine total fungal cfu count per gram of holy basil.

A.

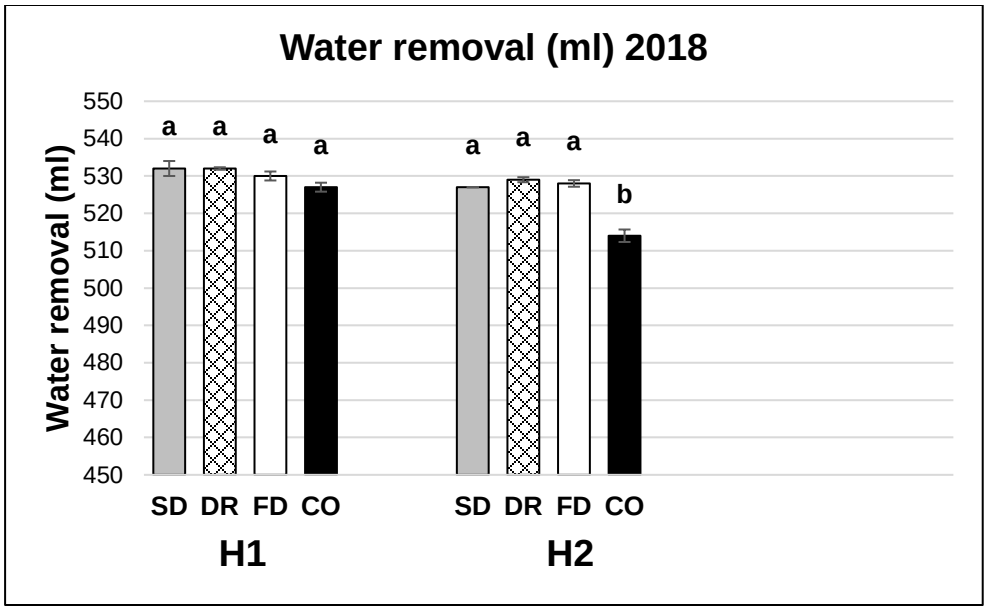

Figure 2.2A. Mean water volume (ml) removed per drying treatment and harvest. 600g fresh holy basil per drying treatment, during each harvest, H1(A), H2(A) Drying treatments include control (CO), drying room (DR), commercial food dryer (FD, and solar dryer (SD) for each harvest in 2018. Values are average of three treatment replications. Similar letters above bars indicate no difference between treatments (P-value ≤ 0.05).

B.

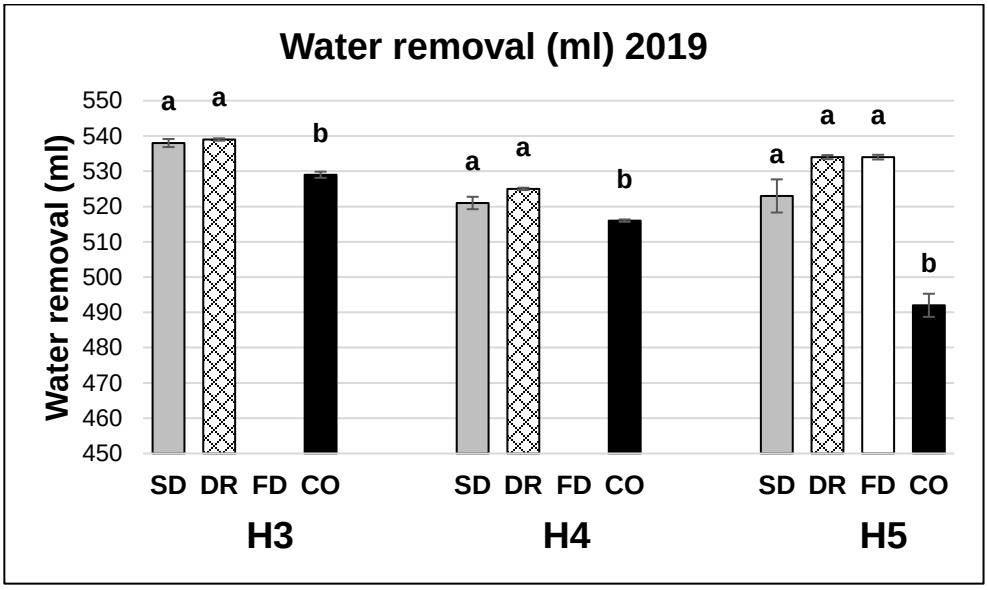

Figure 2.2B. Mean water volume (ml) removed per drying treatment and harvest. 600g fresh holy basil per drying treatment, during each harvest, H3(B), H4(B), and H5(B). Drying treatments include control (CO), drying room (DR), commercial food dryer (FD, and solar dryer (SD) for each harvest in 2019. Values are average of three treatment replications. Similar letters above bars indicate no difference between treatments (P-value ≤ 0.05).

A.

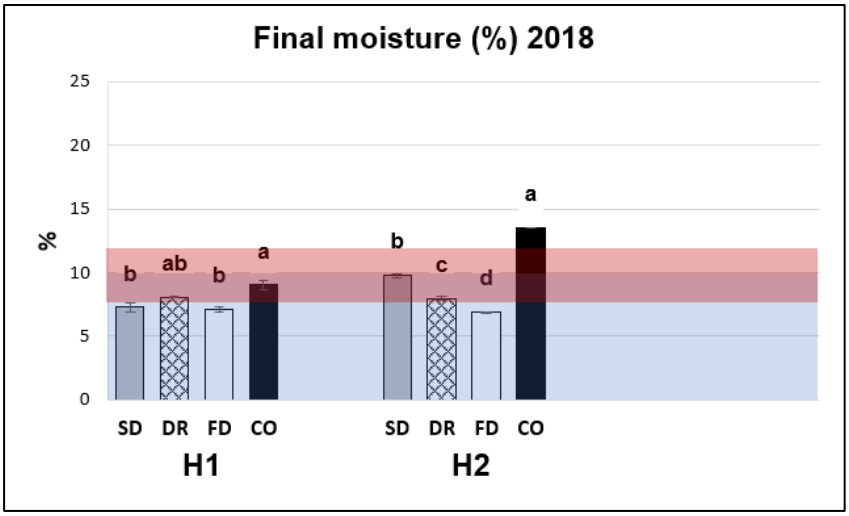

Figure 2.4A. Mean final moisture content per drying treatment and harvest. Drying to constant dry weight at 80°C during harvest H1(A), H2(A). Drying treatments include control (CO), drying room (DR), commercial food dryer (FD), and solar dryer (SD) for each holy basil harvest (H1, H2) in 2018. Values are average of three treatment replications. Similar letters above bars indicate no difference between treatments (P-value ≤ 0.05). Red region represents the 8-12% FMC target range recommended by the AHPA. Blue region represents the >10% target FMC range required by the USP.

B.

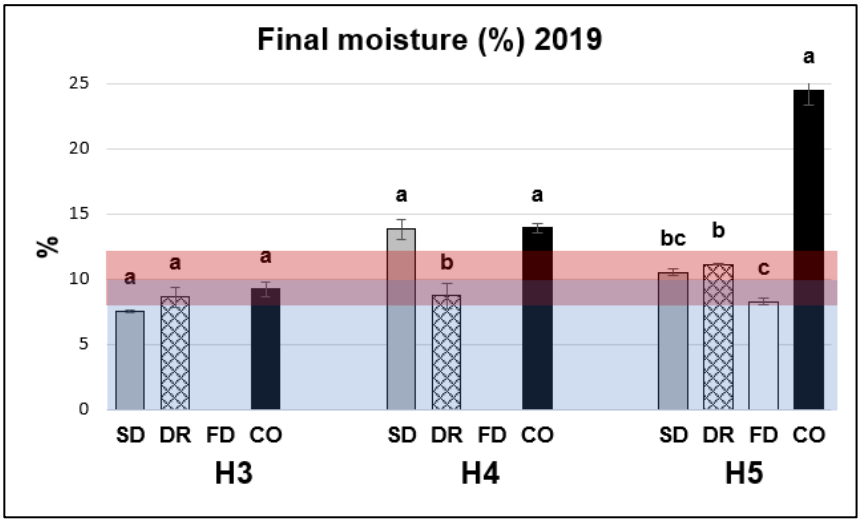

Figure 2.4B. Mean final moisture content per drying treatment and harvest. Drying to constant dry weight at 80°C during harvest H3(B), H4(B), and H5(B). Drying treatments include control (CO), drying room (DR), commercial food dryer (FD), and solar dryer (SD) for each holy basil harvest (H3, H4, and H5) in 2019. Values are average of three treatment replications. Similar letters above bars indicate no difference between treatments (P-value ≤ 0.05). Red region represents the 8-12% FMC target range recommended by the AHPA. Blue region represents the >10% target FMC range required by the USP.

A.

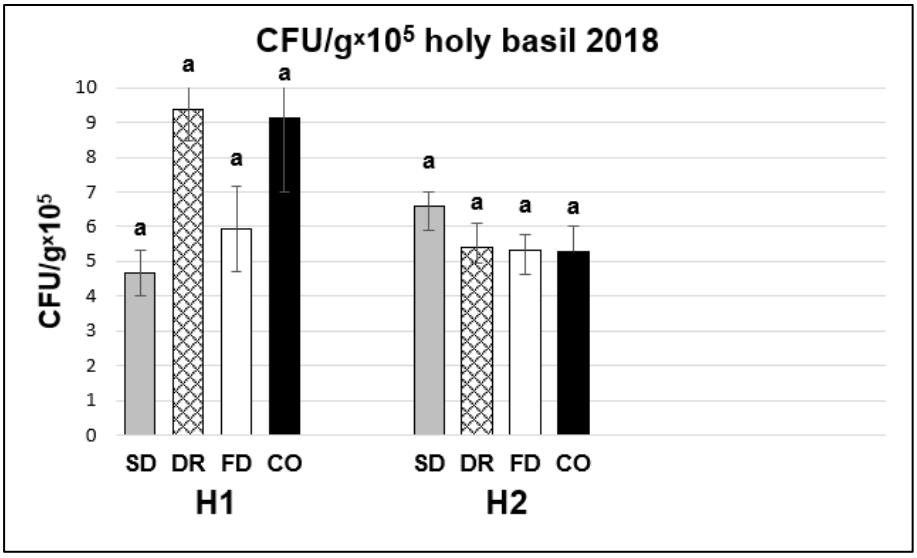

Figure 2.5A. Fungal colony forming units per drying treatment and harvest. Counted per gram of dried holy basil during harvest H1(A), H2(A). Drying treatments include a control (CO), drying room (DR), commercial food dryer (FD), and solar dryer (SD) for each holy basil harvest (H1, H2) in 2018. Values are average of three treatment replications. Similar letters above bars indicate no difference between treatments (P-value ≤ 0.05).

B.

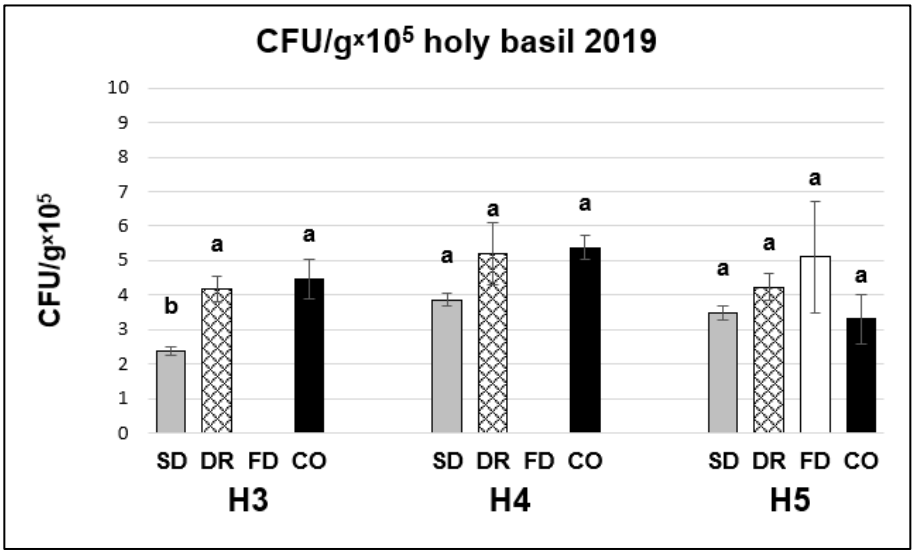

Figure 2.5B. Fungal colony forming units per drying treatment and harvest. Counted per gram of dried holy basil during harvest H3(B), H4(B), and H5(B). Drying treatments include a control (CO), drying room (DR), commercial food dryer (FD), and solar dryer (SD) for each holy basil harvest (H3, H4, and H5) in 2019. Values are average of three treatment replications. Similar letters above bars indicate no difference between treatments (P-value ≤ 0.05).

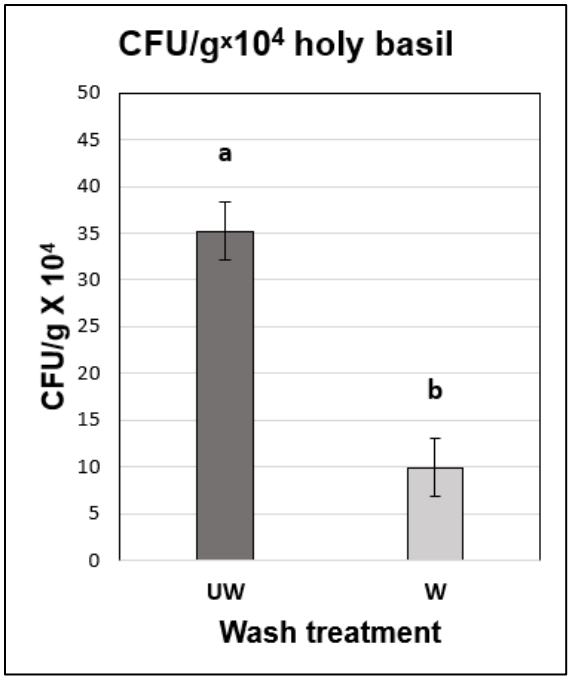

Figure 2.6. Fungal colony forming units per wash treatment. Counted on holy basil for all wash treatments for all harvests, W1, W2, and W3 in 2019. After harvesting, unwashed (UW) and washed (W) holy basil was dried in the drying room. Similar letters above bars indicate no difference between treatments (P-value ≤ 0.05). Values are the average of three treatment replications.

A.  B. 

C.  D. 

Figure 2.7A-D. Holy basil dried using four different methods from harvest H2 in 2018. Samples collected during harvest H2 (Oct 6, 2018) and dried using solar dryer (A), drying room (B), commercial food dryer (C), and the control in a shaded barn (D). Moisture removal, final moisture content, fungal colony counts, colorimeter measurements, and pixel intensity values were analyzed for each sample. During harvest H2, the control removed less moisture, produced a higher final moisture content, and retained less greenness compared to all other drying treatments.

CHAPTER 3

CONCLUSIONS

The quality factors were analyzed to assess the final quality of the holy basil. Color and microbial of dried holy basil are compared with the goal of helping growers choose the most efficient method for herb dehydration. Holy basil dried in the solar dryer, drying room, and commercial dryer differed from the control in terms of final moisture, color, and fungal count. Each harvest represents the performance of each dryer under a unique set of environmental conditions. All of the drying methods evaluated were capable of drying the material to completion, although precise control over the drying environment was not always possible. Seasonal fluctuations in airflow, temperature, and humidity were common. Drying equipment availability, time of year, and dryer capacity must all be considered when selecting a method to dry fresh herbs.

Limitations of this study

. The solar dryer and the barn were directly exposed to external airflow and thus more likely to be influenced by weather. The solar dryer had limited space, but is cheap and could be built to the scale of the growing operation. Passive solar drying was optimal during hot sunny periods, which are common. but unpredictable in the southeastern US during the typical holy basil harvest season. Humid summer air in the barn prevented drying of the leaves and stems and resulted in an unmarketable product.

The drying room and commercial food dehydrator were in controlled environments, however the ability of the drying equipment to remove moisture from the holy basil, while

circulating humid outdoor air is a limiting factor. The drying room was large enough for an entire plot to be harvested at once, but the total drying time was longer than the commercial dryer. The cost of operating the dehumidifier and fan inside the drying room during hot humid summer days is comparable to air-conditioning a small room. The drying room represents the most versatile system in terms of size, affordability, and quality control.

A commercial dehydrator allows optimal drying conditions for quickly drying a relatively small batch of product. This method would not accommodate a large harvest at one time. The commercial unit also requires a large initial investment and specialized training to obtain, operate, and maintain. While the commercial dryer appears to allow the greatest control over the drying process, its size severely limits production capabilities. Unfortunately during a large portion of the study the commercial unit was inoperative due to programming and construction flaws. Repairs were complex and expensive and required multiple specialists to resolve each issue. An additional drawback to this unit was the noise produced by the compressor. In a small farm facility space may be limited and the loud volume of this processing equipment may negatively affect working conditions.

Dry holy basil coloration is based on aesthetic principles, but may also indicate overall quality differences. All drying treatments showed greener coloration than the control, suggesting a colorimeter is a possible tool to distinguish between holy basil dried using one of the treatments and material dried in the barn.

The RGB pixel intensity method relies on free software and simple office equipment, which could benefit growers and producers by providing an easier way to assess color quality. These images could potentially serve as an archive to compare the coloration of a holy basil crop

with years past. However, in this study, the RGB method produced inconsistent results, preventing any conclusions to be drawn.

Surprisingly, the wide range of temperatures, humidity fluctuations, and drying times per treatment did not have an effect on viable spores present on the plants harvested from the field. Greater temperatures may be required to fully inactivate fungal colonies and this may be examined in future studies using precise temperature control. The solar dryer experienced higher maximum internal temperatures, demonstrating some ability to reduce fungal activity and should be examined more closely. Higher temperatures lower overall dried holy basil quality, and may be problematic.

Washing the holy basil in water baths proved effective in reducing fungal cfus and improved the quality of the final dried product. The plate count method using agar media proved to be a successful way to test whether plants have been washed prior to drying and processing. Testing for contamination and impurities is time consuming, whereas a rapid method like this provides instant results. Development of better testing methods which can be performed by farm personnel could aid both growers and manufacturers who work with perishable herbal materials.

Microbes, such as fungal spores, are present in the atmosphere and do not necessarily pose a health risk to the consumer. The main focus of the FDA safety monitoring approach to herbal products is on specific tests for microorganisms that are toxic or pathogenic to humans. All tests are performed during routine inspections and are also available to producers and manufacturers to monitor on their own. In general, by following GMP and GAP guidelines for sanitation and process control, and proper storage, the integrity of the product is protected and there is minimal risk for contamination.

Without directly comparing temperatures and humidity inside each drying chamber at all times, it is difficult to make a clear judgement on which is the "best practice" for drying herbs. Since each dryer is capable of drying the holy basil to completion it may be more advantageous to have multiple drying options available on the farm and choose the most efficient dryer for the job. Microwave-drying and freeze-drying methods were not evaluated in this study, however both of these low-temperature drying methods have great potential, as they have demonstrated the ability to conserve important phytochemical compounds, while saving time.

Next Steps

To improve current holy basil production in the Southeast, more research is needed on field conditions, postharvest, drying, and storage methods and the effect on final quality. Additional medicinal herb species should also be compared using the solar dryer, drying room, and the commercial food dryer to observe patterns and determine if these patterns are species-specific.

Holy basil, and other medicinal herbs, must be dehydrated properly to preserve the biochemical compounds within the plant. The goal is to dry the product quickly yet effectively, maintaining a temperature below the threshold so sensitive compounds are not damaged. These compounds vary widely between species, cultivar, weather, field production practices, and are most certainly affected by drying conditions. Research is critical to determine how the drying process affects the plant biochemical extracts, and exactly how much heat can be applied and for how long.

Direct comparison between holy basil dried at different temperatures and relative humidity (RH) values would give a clearer picture of their effect on herb quality. Using the commercial food dryer or separate chambers, temperature and RH applied consistently

throughout the drying period may help growers choose the appropriate setting for holy basil processing. Higher temperatures may be needed to reduce the fungal cfu count, but should not be set so high as damage valuable phytochemicals. A complete evaluation of essential oil content is necessary to provide a more complete picture of the effect of drying treatment on herb quality.

Complimentary studies are necessary to assess quality by exploring drying kinetics on holy basil as well as individual compounds found within the herb. Water activity (a^w) is the standard measurement used in food safety monitoring programs, but ideal a^w must be determined separately for each species. This is common in a food science setting and requires sophisticated gravimetric equipment to develop a moisture sorption isotherm unique to each product. Instant water activity tools are becoming more common and provide growers a way to determine with more accuracy whether their product is fully dry and ready for long-term storage. Over-drying is common to avoid the potential risks associated with under-drying, and using unsophisticated methodology may result in improper moisture levels during storage. The commercial dryer is most suited to perform tests on herbal materials to determine precisely how temperature, RH, and drying period duration affect the overall quality of the end product.

Storage of medicinal herbal products may also impact holy basil moisture content, as well as fungal count. While herbal teas are typically stored at room temperature, future research could investigate how storage conditions affect chemical compounds and microbial activity found within the herb. A storage study should investigate the effects of dry storage compared with standard refrigeration and freezing in order to form guidelines for processors and manufacturers.

Washing the fresh plant material produced a greater effect on fungal colony count than any of the experimental drying methods. The unwashed holy basil did not meet the minimum recommended requirements for herbal products sold in the US, therefore washing is necessary if

the grower aims to meet these specifications. Herbs grown for supplement manufacturers or pharmaceuticals must contain less than 10^3 fungal cfus/g dry herbage, and may require an additional antimicrobial treatment. Potential studies should examine the effects of common postharvest wash treatments such as organic vinegar, peroxyacetic acid, and up to 200ppm chlorine applied to fresh plants before drying begins. In addition to their effect on microbial activity, these wash treatments would also need to be evaluated based on their effect on the phytochemicals in the herb.

The solar dryer demonstrated the ability to reduce the fungal cfu content at higher temperatures. This drying treatment may have more potential to dry products effectively at certain times of year, and a year-round assessment of the temperature maintained within the drying chamber will help to determine the best practice for drying crops using this method. For some herbs with fewer volatile compounds, the solar dryer could be an inexpensive drying method.

A complete cost-benefit-analysis of each drying method would account for initial cost of equipment as well as estimated and actual maintenance expenses. Dryer capacity and time required to dry product also affect the total processing capabilities for each method. Losses due to equipment failure and weather events may also be factored into the overall value for each dryer. There may be a premium market price associated with herbs processed to meet the standards used for herbal supplements as opposed to teas and extracts.

A simple consumer preference analysis using teas infused with material from each drying method may be an interesting compliment to this research. Since the holy basil produced at the farm is used for herbal teas, the specific compounds that are extracted during traditional brewing methods may differ greatly from the extracts obtained using solvents and essential oil distillation.

Herb growers must adapt their growing practices to the changing market and be prepared to accommodate supplement manufacturers as well as researchers who depend on pharmaceutical grade plant material. Future studies should incorporate the phytochemistry of the herb throughout the postharvest handling, drying, and storage processes.

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