CHEMICAL CONSTITUENTS AND COMBINED RELATIVE ANTIOXIDANT ACTIVITY OF SAGE PLANT PARTS VARYING IN PHYSIOLOGICAL AGE AND EFFECT OF HARVEST METHOD AND HARVEST HEIGHT

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CHAPTER I

INTRODUCTION

In the food industry preservatives are widely used to maintain nutritive value and prevent quality deterioration of foods. The largest class of these substances, known as antioxidants, extends shelf life by preventing oxidation when added at a lower concentration compared to that of the oxidizable substrate. The action of antioxidants occurs by one or more of the following mechanisms: 1) decreasing oxygen concentration, 2) interception of singlet oxygen or 3) scavenging initial radicals. This results in delay in the start of autocatalytic oxidation and effectively lengthens the induction period (Shahidi, 1997). The process of oxidation is related to the free radical chain mechanism following steps of initiation, propagation and termination. Radicals which are produced at the initiation stage, react with polyunsaturated fatty acids (PUFA) in the food at the propagation stage until termination reaction is reached resulting in production byproducts which accumulate to sufficient levels to impart characteristic rancid flavors and odors (Shaidi, 1997).

Hydro-peroxy radicals, formed when an energetic radical species becomes stabilized by reacting with an oxygen molecule, cause oxidation of PUFA. During the oxidative reaction the radical absorbs a hydrogen atom from one of the unsaturations in the fatty acid molecule, leaving it with a carbon centered radical. The PUFA is then broken into its characteristic reaction products. An antioxidant may act to absorb the

radical energy interrupting the chain reaction and preventing reaction with PUFA (Elstner, E.F. 1982; Halliwell, B. 1987).

The most commonly used antioxidants are the synthethic compounds butylated hydroxyl anisole (BHA) and butylated hydroxytolune (BHT). They are volatile and decompose at high temperature. They have also been found to be carcinogens and mutagens. Because of the disadvantages of such synthetic food preservatives and consumers preference for a safe and natural product, herbs have been given attention as a source of natural antioxidants (Wong, et al., 1995; Stephens, 1977). Some of the widely studied herbs for antioxidant production are rosemary (*Rosmarinus officinalis L.*) and sage (*Salvia officinalis L.*). Studies on herb field production have shown that unlike rosemary, which is not adaptable to most parts of Oklahoma due to susceptibility to winter freeze injury, sage is well adapted to most parts of Oklahoma (Maness, 1997).

Traditionally herbs are dried in a variety of ways. Drying is a common processing step to avoid microbial spoilage and stabilize flavor for a longer shelf life, to protect against enzymatic and hydrolytic spoilage, and maintain availability of product throughout the year (Nora and Dawn, 1993; Torrey, 1974). Depending on the availability of facilities and intended end use, forced- air drying, drying in place, drying by hanging, microwave drying and drying on a stationary bed are commonly used methods. The largest producers of sage use forced air drying. Edible sage may also be dried commercially with microwave. Highly dried CO_2 may be used without heat or vacuum for drying (Nora and Dawn, 1993; Torrey, 1974).

Herb quality is effected by stage of development at harvest. The upper leaves for sage and oregano (*Origanum vulgare L.*) gave higher yield of dried leaves/ha with high yield of essential oil as compared with the lower leaves (Putievsky and Basker, 1978).

Herbs that are sources of antioxidants are extracted using different extraction techniques. The activity of natural antioxidants is dependant on the type of extraction technique and the extractants. In a test for measuring antioxidant activity of thyme (*Thymus vulgaris L.*), oregano, lavender (*Lavandula angustifolia*) sage, majoram (*Origanum majorana L.*) and rosemary, hexane extracts showed higher concentration of carnosic acid and carnosol than extracts from methanol and acetone. Beta carotene bleaching test and diffusion method, antioxidant activity assays, using five different methods of extraction ((acetone oleoresins (AO), deodorized acetone oleoresins (DAO), deodorized water extracts (DWE), methanol-water extracts (MWE) and carbon dioxide extracts (CO_2)), on majoram, oregano, hyssop (*Lophantus anisatus*), thyme, sage, lavender, and catnip (*Nepeta cataria L.*) indicated that for sage and thyme, acetone oleoresins had highest antioxidant activity. Antioxidant properties of bleached alcoholic extract of sage using supercritical carbon dioxide extraction showed greater antioxidant activity of Rosmanol-9-ethyl ether than BHT (Chen, Shi, and Ho, 1992; Dapkevicious et al., 1998; Djamarti et al., 1992). Ethyl acetate, hexane and methanol extracts of fresh sage were effective for preservation of alpha tocopherol and its preservation activity was directly related to the amount of sage extract (Beddows, 2000).

Five of the discovered phenolic compounds of sage (carsonol, carnosic acid, rosmarinol, epirosmanol and methyl carnosate) are also found in rosemary. These

compounds, when combined with α-tocopherol, show synergism that increases antioxidant activity. Many components of diterpenes, tri-terpenes like thujone and camphor, α- and β-pinene, limonene, myrcene, cineole, linalool and flavonoids have been found to be major components of sage antioxidant activity (AOA). Rosmarinic acid, luteolin-7-o-β-glucopyranoside, isolaricirenisol-3- α-O-β-D-glucose pyranoside also have high radical scavenging capacity. Antioxidant activity of polyphenols is due to radical scavenging by hydroxyl groups in ortho and Para positions (Fankel and Huang, 1996; Rhyu, 1979; Wang, 1998). The mechanism of protection by phenolic compounds is high at the stage of propagation because they interrupt the formation of hydro-peroxides, essentially blocking their action prior to reaction with PUFA and leading to longer shelf life of the food being preserved (Basaga et al., 1997). It is believed that flavonoids, terpenoids and phenolic acids contribute to the "health" property of sage used as a popular folk medicine for the treatments of various ailments. Rosmarinic acid derivatives are more predominantly responsible for the AOA of the non-diterpenoid component in sage (Karousou, 1998; Yinrong, 2001).

Activity of antioxidants leads to shelf stability of food products. Marketing cost of a product is partially determined by the time the food can remain on the shelf. In order to achieve effective result in the use of natural antioxidants, pH of the food, flavor interaction with additives and type of processing of the food needs to be known. It is necessary to test the thermal stability of antioxidant preparations to be used at a temperature equal to or exceeding that used in the food preparation process, so that the final quality of the product is guaranteed. In essential oil of thyme added to fat containing foods, carvacrol and especially thymol are the phenolic components mainly responsible

for antioxidant activity. Carnosol and carnosic acid were effective phenolic compounds in sage and rosemary (Cuvelier, 1996; Farag et al., 1989).

The optimal substrates for measuring antioxidant activity are the actual food items, though there is a great difference between substances. Spices with high antioxidant activity should provide the product long shelf life. The ability of spice extracts to scavenge hydroxyl radicals measures the antioxidative effect of them. These radicals are also highly reactive with organic compounds other than phenols (Madsen et al., 1996).

Hydrophobic antioxidants tend to have higher antioxidant activity as compared to hydrophilic antioxidants. Comparison of electrochemical oxygen depletion and electron spin resonance (ESR) spin trapping assays on sage, majoram, summer savory (*Origanum onites*), Turkish oregano (*Origanum vulgare L.*), and rosemary show different results. In the ESR assay, phenolic compounds act as scavengers of free radicals active in the early stages of oxidation, while the oxygen consumption method measures potential activity at the stage of propagation during lipid oxidation, when radicals increase substantially in numbers. This led to a suggestion that the use of combined herbs might give better protection of foods from oxidation (Madsen et al., 1996). In measuring antioxidant activity spices need to be evaluated in experiment with foods. Since this is time consuming and antioxidant activity depends on the substrate, which will be unique to each food preparation, screening systems have been used to compare the relative antioxdative characteristics of different spices and spice extracts.

Most methods assessing antioxidant activity vary depending on the generated radical species, reproducibility of the generation process and the end point that is used. The end point could be inhibition of spontaneous oxidation or loss of

chemiluminescence. A decline in chemiluminescence occurs when a luminescent compound reacts with a free radical, degrading the compound into a colorless end product. In the inhibition assays antioxidant action induces a longer lag phase; the antioxidant intercepts free radicals and prevents oxidation of the luminescent indicator until the antioxidants reach an equilibrium concentration with the free radicals acting on the luminescent indicator. After equilibrium is reached, the indicator compounds are oxidized at a constant and linear rate equivalent to the rate of generation of free radicals. This linear decline in luminescent indicator continues until both indicator and antioxidants are depleted, after which no further change in response can be measured. Beta-carotene bleaching test is commonly used, because it is simple and gives visual evidence of results. Absorbance is measured intermittently by a spectrophotometer at a wavelength of 490 nm and antioxidant activity is measured in relative terms usually in comparison to a known antioxidant compound. The total peroxy trapping parameter (TRAP) assay is a widely used assay of the radical absorbing capacity, but doesn't maintain stability. Oxygen radical absorbance capacity (ORAC) assay depends on the detection of chemical damage to phycoerythrin, causing a decrease in its fluorescence emission. Inhibition of reactive species action is a reflection of protection of phycoerythrin from loss, and is a measure of antioxidant capacity against a carefully added reactive species. ORAC measures the time of inhibition of oxidation and the percentage the oxidation inhibition displayed at different times after the lag phase in relation to the standard antioxidant trolox. ORAC overcomes problems related to the quantitation of antioxidant capacity through integration of oxidation inhibition percentage

and the time in which inhibition lasts (Alho 1999; Cao 1999). Typically, the activity of antioxidants is expressed in relation to coincident trolox activity, as trolox equivalents.

Plants grown for leaf production have similar harvesting requirements. In the production of tea, the shoot and the first three leaves known for tea harvest were harvested every one to three weeks depending on the availability of water, nutrient availability, and air temperature (Burgess and Carr, 1992). Hand plucked tea was richer in green tea biochemical precursors and high tea quality constituents than shear plucked tea. The decrease in components important for tea quality in shear plucked leaves was caused by deterioration due to mechanical injury during shear harvesting (Ravichandran, 1998).

During wet warm season harvesting time of alfalfa was shorter than during cool and dry season. Dry yield was found to depend on precipitation received prior to re-growth. Growth rate was determined by optimum day and nighttime temperature, quality of solar radiation, and adequate soil temperature. At temperatures substantially above the optimum for growth, such as during mid summer months, a decline in yield occurs. Following the appropriate time of harvest, the proportion of harvested shoots could be increased by the growth of axillary buds. The number of axillary buds leads to formation of more new shoots, which in turn leads to the production of a higher number of young leaves. In the production of dill (*Aethum graveolus*), harvest at flower initiation stage results in higher dry matter production than at the complete flowering stage (Muleo and Thomas 1977; Singh et al., 1953; Sharatt et al., 1986). Leaf age and time of harvest of sage plant was found to affect the quality of medicine that was obtained. Young leaves have been observed to have highest essential oil content (Tekelova et al., 1994). Higher

amount of 1,8-cineol and much lower total thujone was collected from *Salvia fruticosa* during mid summer harvest. During the same period higher amount of camphor was also collected from oils of *Salvia fruticosa* (Karousou et al., 1998).

In the growing system of perennial crops, maintaining the stand life of a plant can optimize production throughout successive seasons and offset loses of profit due to progressive yearly yield decline. Knowledge of time of harvest, which ends early enough during the season to insure replenishment of photosyntate to enhance winter survival and late enough to accumulate maximum yield, is important for profitable production over several years. According to Ogg (1988) cited by Maness (1997) in the production of alfalfa (*Medicago sativa*) a final harvest cut at time near fall frost was observed to bring injury and death of the plant. In Alabama alfalfa plants need six to eight weeks recovery time between the last harvest and fall frost (Sharratt et al., 1987). The optimum time for last harvest of alfalfa was mid September. Sage followed the same pattern of survival. A final harvest of sage in mid November, following the first killing frost, allowed for harvest of growth which occurred from mid September to frost and gave highest herbal yield with less plant mortality (Motes et al., 1994).

Objectives

The objectives of this study were:

1. To map antioxidant and antioxidant activity production in sage vegetative tissues.
2. Using concentration and content antioxidant maps, to establish cutting heights most likely to result in increased antioxidant yield
3. To compare potential harvesting methods for enhancing antioxidant activity and dry yield production over the harvest season.
4. To evaluate the effects of harvesting frequency on antioxidant activity and dry yield production.

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Results from the study may be of importance in answering, which tissue of a stem contributes maximum AO and AOA, how often should the plant material be harvested at given heights of harvest and what type of harvesting machinery is needed for maximum AO and AOA production.

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CHAPTER II

CHEMICAL COMPOUNDS AND RELATIVE ANTIOXIDANT ACTIVTY OF SAGE TISSUES VARYING IN PHYSIOLOGICAL AGE

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Abstract

Consumer preference and industry demand for natural food preservatives led us to evaluate sage (*Salvia officinalis L.*) as a potential source of extractable antioxidants. The distribution of natural antioxidants in tissues varying in physiological age was investigated to evaluate plant growth stages best suited for antioxidant harvest. Plant material was separated into nine different tissue fractions: terminal unexpanded leaf (apical meristem), fully expanded leaf pairs from terminal growth downwards (leaf one to leaf six), leaf axillary buds and stem. Each was lyophilized, ground, extracted with hexane and injected onto GC for quantitative analysis of antioxidant components (AO; alpha thujone, beta thujone, camphor, alpha pinene, beta pinene, cineole and camphene,). In terms of concentration (mg AO/gm sage), the apical meristem was richest in AO (19 %), leaves 1 through 6 had progressively decreasing AO (16 % to 7 %) followed by axillary buds (8 %) and stem (2%). In terms of content (mg AO/plant part), distribution increased slightly from leaf one (15 %) to leaf 2 (23 %) and then decreased progressively down the stem (leaf 3 to 6 from 20 % to 6%). Axillary buds, stem and terminal buds had

1%, 2% and 4% AO content accordingly. Sage should be harvested at a young growth stage for maximum AO yield. Allowing plants to regrow to the third or forth fully expanded leaf would apparently allow for recovery of from 70 % to 80 % of the total potential AO.

Introduction

Leaf age and time of harvest of sage was found to affect the quality of medicine that was obtained. Young leaves have been observed to have high essential oil content (Tekelova et al., 1994). Ethyl acetate, hexane and methanol extracts of fresh sage was effective for preservation of alpha tocopherol, and the preservation activity was directly related to the concentration of sage extracts (Beddows, 2000). Many components of diterpenes, tri-terpenes like thujone and camphor, α- and β-pinene, limonene, myrcene, cineole, linalool and flavonoids have been found to be major components of sage antioxidant activity (AOA) (Frankel and Huang, 1996; Rhyu, 1979; and Wang, 1998).

The upper leaves for sage and oregano (*Origanum vulgare L.)* gave high dried leaves/ha with higher yield of essential oil than the lower leaves (Putievsky and Basker, 1978). According to Robinson and Maness (1996), in comparing the effect of harvesting method on AOA, flail harvesting of sage stems versus hand harvesting of only expanded leaves as a source of plant material at the same height resulted in a difference in AOA. AOA for hand harvested leaves increasing from 50% of BHT standard for flail harvest to 55% BHT standard.. In comparing hand harvest of only fully expanded leaves versus hand harvest of the whole plant material, there was a further increase in AOA from 55% of BHT standard for hand harvested leaves to 65% of BHT standard for the hand harvested whole plant material. The lower AOA level of hand harvested leaves was

expected to be due to the absence of apical meristem, which may contribute a substantial amount of AOA. To maintain quality of tea, the shoot and the first three leaves known for tea harvest were harvested every one to three weeks depending on the availability of water, nutrients and air temperature (Burgess and Carr, 1992). Studies done on tea show that hand plucked tea leaves were richer in their green tea biochemical precursors than shear plucked tea leaves. The shearing process apparently decreased important chemical constituents due to mechanical injury during shearing (Ravichandran, 1998).

The purpose of this study was:

1. To map antioxidants and antioxidant activity production in sage vegetative tissues.
2. Using concentration and content maps, to establish cutting heights most likely to result in increased antioxidant yield.

Results from this study may be of importance in explaining the distribution of antioxidants and antioxidant activity between sage plant tissues varying in physiological age. The practical application of this study will be to determine an optimum cutting height for enhanced antioxidant yield of sage.

Materials and methods

Plant Material:

Single plant selections from 6 four year old plants were made in a producer field north of Stillwater, OK from transplants propagated from seed of Dalmatian sage. Plants were initially monitored for production of antioxidant compounds (AO) in the two seasons preceeding this study, and three plants differing in antioxidant production were selected for this study. Vegetative plant material was obtained in late summer by hand cutting 35 fully developed current season growth for each of three plants. Stems were placed into a freezer bag and transported on ice to laboratory facilities in Stillwater.

Sage tissue separation:

Harvested stems were inspected, damaged stems were discarded and a set of 25 stems from each of the three plants were trimmed to six fully expanded leaves or less. Tissues were separated into nine fractions as depicted in Figure 2.1. Tissue fractions included terminal unexpanded leaves with apical meristem, fully expanded leaf pairs counted from terminal growth downwards (leaf one to leaf six), the combined leaf axillary buds and the stem. Following separation, tissues were dried in accordance with the following section.

Drying procedure:

Separated plant materials were sealed inside a freezer bag and held in a cold room at 4°C for a maximum of two days and then placed into cheese cloth, securely tied with a string and frozen in liquid N_2. Lyophilization was conducted in a VirTis shelf freeze drier (Consol 12, Vir Tis, Gardner NY 12525) with a shelf temperature maintained at

15°C and condenser temperature at -40°C for a duration of at least 48 hours. The dried sample material was sealed inside a labeled freezer bag and held in a freezer at -20°C until needed for extraction. A day or two before analysis samples were brought to room temperature and ground into fine particles with a UDY mill (UD corporation, Boulder, CO), placed into brown capped bottles and held in a cold room at 4°C to await extraction. Following extraction samples were held at -20°C for use in re-extraction if deemed necessary.

Extraction procedure:

Extraction was carried out in duplicate for each sample using hexane. Two aliquots of 100 mg each were accurately weighed into two-dram vials and a magnetic stir bar was added. Two ml of hexane was added and samples were capped and stirred using a magnetic stirrer for a duration of 20 min. The capped vials were then placed into a SpeedVac (Model S V C-100 H; Savant, Instruments Inc. Farmingdale. NY) and centrifuged for five minutes. The supernatant was decanted into a second two-dram vial and centrifuged for an additional five minutes to remove small amounts of transferred particulates, and then transferred into a 10 ml volumetric flask. Two more extractions were conducted as above for a total of three extractions and the 10 ml volumetric flask was brought to volume with hexane. Preliminary experiments indicated greater than 95% recovery of measured compounds using this extraction procedure. Extracted samples were placed into capped brown bottles and held in the refrigerator for one week or less until analysis was conducted. Preliminary trials indicated that AO was stable under these conditions.

Analysis of AO chemical components:

Samples of extracted material were brought to room temperature and analyzed for the content of chemical constituents of sage using Gas Chromatography. A 1.94 ml sample was added to 0.06 ml linalyl acetate to make 280 nmol linalyl acetate (Sigma Chemical Co. St. Louise, Mo) internal standard. Gas chromatography was conducted with a Varian 360 gas chromatograph (Varian Associates, Walnut Creek, CA), equipped with a septum programmable injector and flame ionization detector at 300°C. The initial injector temperature was 55°C. Samples were injected slowly for cold on column injection, and immediately following injection an injector temperature ramp from 55°C to 290°C was accomplished at a rate of 100°C min^{-1}. Chemical components were separated on a DB 1 column (30 x 0.25 mm, 0.25 μm film thickness; J& W Scientific, Folsom, CA) with helium as carrier gas. The initial column temperature was 40°C with hold for 1 minute. Components were then separated with a linear temperature program from 40°C to 290°C at 5°C/min, and held at 290°C for 10 minute. Chemical component peaks were identified according to co-elution with authentic standards (α-thujone, β-thujone, camphor, α- pinene, β-pinene, cineole and camphene; Sigma Chemical Co., St. Louis, MO) and quantitated relative to linalyl acetate internal standard.

Antioxidant activity:

One ml of the extract was placed into a 100 ml volumetric and brought to volume with hexane. Antioxidant activity was measured by oxygen radical absorbing capacity (ORAC) using a modified version of Cao et al., (1995). The reagents used for analysis were phycoerythrin (Sigma Chemical, St. Louis, MO); 2, 2'-azobis (2-amidinopropane)

dihydrochloride (AAPH); (Wako Chemicals Co., Richmond, VA) 6-hydroxy-2,5,7,8-tetramthylchroman-2 carboxylic acid (trolox); Aldrich Chemical Co., Milw. WI) phosphate buffer and sample.

Reagents were prepared as follows: Phosphate buffer was prepared by mixing 0.75M K_2HPO_4 and 0.75 M NaH_2PO_4 at 61.6:38.9 v/v and pH was adjusted to 7 using potassium hydroxide. A stock solution for phycoerythrin (PE) was prepared by dissolving 1 gm of phycoerythrin in 5.9 ml of phosphate buffer and kept refrigerated for not longer than one month prior to use. A working solution of PE was prepared prior to analysis by adding 300 µl of PE stock to 13.4 ml of phosphate buffer. Trolox stock solution was prepared by dissolving 5 mg of trolox in 200 ml of phosphate buffer to make a 100 µM solution. The working solution of trolox was prepared by 1:10 dilution of the stock in phosphate buffer to make 10uM. AAPH working solution was prepared just prior to use by dissolving 79 mg of AAPH in 5 ml of phosphate buffer.

Reaction mixtures for analysis consisted of 20 µl of sample, 160 µl PE working solution, 20 µl trolox working solution for standard and 20 µl hexane as reagent blank. Reaction was carried out in a 48 well plate (Falcon multi well; Sigma Chemical Co. St. Louis, MO) arranged in 8 columns of six wells each. During each run, column one was hexane reagent blank, column 2 was trolox and columns 3-8 were sample, with each of the samples replicated over each of the six wells per column. A Perkin-Elmer HTS-7000 Microplate Reader was set for ORAC assay at 535nm for excitation and 595nm for emission. Target temperature was 36°C, with a cycle period of two minutes, giving 35 cycles in a 70 minute run. The fluorescence readings were converted into ORAC values,

expressed in terms of trolox equivalent. Micro plates were replaced after 4 uses to prevent excessive background readings caused by hexane reacting with the plastic of the wells.

Statistical design and analysis:

Analysis of variance (ANOVA) for a randomized complete block design was performed to determine quantitative difference in AO chemical constituents and AOA of the different plant tissues using Statistical Analysis System (SAS Institute, Carry, NC). Data was analyzed with multiple range test comparison of protected LSD, to identify differences among means of chemical compound concentration, content and ORAC values at $P<0.05$.

Results

Individual sage plants were found to have variation in concentration of antioxidant compounds. In the preliminary study, 6 sage plants were selected randomly from transplants propagated from Dalmatian seed. Whole stems containing up to six leaves were cut for analysis of concentration of antioxidant compounds. Results show that for all the compounds analyzed, randomly selected plants yielded different concentration and there was year to year variation in AO concentration (Table 2.1). Plant selection for the three sample plants used in this study (plant 6, 37 and 49) was made based on the ratio of concentration of the two major chemical compounds, α-thujone and camphor, and on the total concentration of AO compounds. Plants 6, 37 and 49 had a ratio and total AO concentration falling between the highest and the lowest ratio of α-thujone to camphor of the six plants (Table 2.2). Sage stems from the selected plants were separated into nine plant parts as depicted in Fig. 2.1. Results are presented in terms of concentration or content, for antioxidant compounds (AO) and for antioxidant activity (AOA) in ORAC units. AO and ORAC concentration values were expressed according to the amount of the individual chemical compounds, sum of their total, and ORAC units and dry weight basis. Since sage tissues vary substantially in their weight contribution to the total weight of the sampled stems, content was also described in terms of quantitated amount per tissue to evaluate actual contribution to yield. Chemical compounds analyzed were: α-thujone, β- thujone, camphor, α-pinene, β-pinene, cineole and camphene. Plant tissues were separated into nine fractions: leaf one to six of fully expanded leaves, apical tissues, leaf axillary buds and stem (Fig. 2.1). Concentration

and content of α-thujone, β-thujone, camphor, α-pinene, β-pinene, cineole and camphene were analyzed to determine distribution of antioxidant in these plant tissues (Table 2.3 and 2.4).

Concentration of chemical compounds and oxygen radical absorbing capacity (ORAC) values of sage

Concentration of α-thujone was highest in young growth and tended to decline in tissues of greater physiological age (Table 2.3). Terminal buds had significantly higher concentration of α-thujone than leaves two, three, four, five, six, stem and axillary buds, and were not different from leaf one. Leaves one and two were similar in α-thujone concentration. Concentration of α-thujone between leaves three, four, five and auxiliary buds was also not significantly different, but was higher than the stem and leaf six and lower than leaf one, leaf two and terminal buds. There was no significant difference in concentration of α-thujone between leaf six and stem.

Similar to α-thujone, concentration of β-thujone decreased progressively as tissue physiological age increased (Table 2.3). Terminal buds were highest, followed by leaves one and two, then leaves three, four, five, six, axillary buds and the stem. Axillary buds had a β-thujone concentration lower than leaf two but higher than the stem. Concentration of β-thujone from leaf one was significantly higher than leaves two and three. There was no significant difference between leaves four, five, six and axillary buds.

Camphor concentration was highest in leaves one through four but concentration didn't differ until leaf six, axillary buds and the stem (Table 2.3). There was a higher degree of variability for camphor measurements than for any other chemical compound,

as depicted by the high LSD value. This may have led to difficulty in determination of differences in camphor concentration. There was no significant difference between leaves one, two, three, four, five, axillary and terminal buds in concentration of camphor, and they were all higher than stem.

Terminal buds, leaf one, two and three had highest and similar cineole concentration (Table 2.3). Leaves three, four and five were similar, with no difference between leaf six and axillary buds. The stem and axillary buds contained the lowest cineole concentration.

Leaf two had significantly higher concentration of camphene than terminal buds, leaf six, stem and axillary buds (Table 2.3). Leaves one, three, four, five, six and terminal buds were similar in camphene concentration, but were higher than axillary buds and stem.

Similar to camphene, α-pinene concentration was higher in leaf two, than in terminal buds, leaf six, stem and axillary buds (Table 2.3). Leaves one, three, four, five and six were similar. Leaf six was higher than stem and axillary buds with no difference between stem, axillary and terminal buds.

Concentration of β-pinene followed a pattern more similar to α-thujone than α-pinene and camphene (Table 2.3). Terminal buds had significantly higher concentration of β-pinene than leaves three, four, five, six and axillary buds. All leaves were similar in β-pinene concentration. Leaves three, four, five and six had similar β-pinene concentration to the stem and axillary buds.

Terminal buds had significantly higher concentration of total chemical compounds than leaves three, four, five, six, stem and axillary buds (Table 2.3). Axillary

buds were lower in total concentration than terminal buds and leaves one and two, but higher than the stem. There was no difference between leaves four, five, six and axillary buds. The stem was lowest in total chemical compound concentration. There was no significant difference in concentration of total chemical components between leaves one, two and three. There was no significant difference in ORAC values between the different plant tissues of sage (Table 2.3).

Content of chemical compounds and oxygen radical absorbing capacity values of sage

Separated plant tissues varied in herbal yield (Table 2.4). Fresh and dry herbal yield of the samples show that the highest herbal yield was obtained from leaf two downwards to leaf five. Axillary buds had the lowest contribution of herbal yield followed by terminal buds. Since the purpose of this study was to map the potential antioxidant yield of sage, results were corrected for weight contribution discrepancies by expression of total content per plant part.

Content of α- and β-thujone was significantly higher in leaves two and three than leaves one, four and five, with no significant difference between leaves two and three and also between leaves one, four and five for α-thujone content (Table 2.5). Leaf six, stem, terminal and axillary buds had lower content of α-thujone with no significant difference between them. Similar to α-thujone there was no significant difference in content of β-thujone between leaves one, four and five. However, their content was significantly higher than leaf six, stem, axillary and terminal buds.

Leaves one through four had a higher and similar content of camphor than leaves five, six, axillary and terminal buds and the stem (Table 2.5). There was no significant difference in camphor between leaf six, stem, axillary and terminal buds.

Leaves two, three and four were highest in camphene content (Table 2.5). Leaves one and five were similar, and lower in camphene content than leaves two, three and four but higher than leaf six, axillary and terminal buds and the stem, which were similar. Cineole contents exhibited similar differences to camphene. Leaves two and three were higher than leaves one, five, six, axillary and terminal buds and stem. Leaf one had higher cineole content than leaf six, stem, axillary and terminal buds with no significant difference between them. Leaf four and five also had higher content of cineole than stem, axillary and terminal buds.

Content of α-pinene was highest in leaves two, three, four and five (Table 2.5). There was no significant difference in α-pinene content between stem and axillary and terminal buds (Table 2.5). Content of β-pinene was higher in leaf two than leaves one and five (Table 2.5). There was a difference in β-pinene content between leaves one, three and four, but higher than leaf six, stem, axillary and terminal buds.

Leaves one through four were highest in total chemical compound content, and leaf five was similar in content to leaves one and four (Table 2.5). Axillary buds were lower in total content than leaf six, but similar to stem and terminal buds. Oxygen radical absorbing capacity (ORAC) values of leaves two, three and four were significantly higher than leaves one, five, six, stem, axillary and terminal buds (Table 2.5). There was no significant difference in ORAC values between leaves one, five, six, stem, axillary and terminal buds.

Discussion

In millet, genotypes showed large variability on protein and fat content in grain. Differences in genotype between plants influenced the concentration of essential amino acids (Buerkert et al., 2001). Plant to plant variation has influence in quality of a product. Mapping of antioxidant in plant tissues of sage was conducted to determine distribution of antioxidants in different tissues of a plant to establish harvest height of sage for optimal AO production. Our study confirms what was reported by Tekelova et al., 1994); Higher concentration of the chemical components of sage like alpha and β-thujone, which are the major antioxidant yield contributors of sage, was found from younger tissues, terminal buds followed by leaf one. Further down the stem the older leaves contained progressively decreasing concentration of α and β-thujone. Leaf one and two had the same concentration of α and β-thujone. Axillary buds, while physiologically young, had triple the concentration of α and β-thujone than the stem. Concentration of total chemical components provide similar evidence with the highest concentration from terminal buds, doubling the concentration of leaf five and six and accounting for 19% of the total concentration. Leaves two, three and four had 15%, 12% and 10% concentration coverage, respectively. Stems had only 2% and axillary buds only 8% contribution to the total concentration of the chemical compounds analyzed.

On a content basis, leaves two, three and sometimes four were highest in AO. Leaf two had the highest AO chemical components, with 23% contribution to the total followed by leaves three, four and one having 20, 17 and 15%, respectively. Unlike

antioxidant mapping of concentration, terminal bud AO content was only 4% contribution to the total content. Although the concentration in terminal buds was usually highest (Table 2.3), the low weight contribution to the total weight of stem components (Table 2.4) led to this low total contribution.

Putting the results of AO concentration and content mapping together, we conclude that, younger tissues contain a higher amount of antioxidant. Based on the overall results, harvest up to 4th leaf provides 73% of concentration and 79% of content and harvest up to 3rd leaf gives 62% of concentration and 62% of content of antioxidant chemical components. Regrowth to a shorter height would lead to more frequent harvests with high AO content.

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Table 2.1 Comparison of sage plants in antioxidant chemical compound concentration.

Plant No.	2		6		18	
Year	1999	2000	1999	2000	1999	2000
Chemical compounds	Concentration mg/gm					
α-thujone	4.590	3.959	2.402	2.751	2.745	4.636
β-thujone	0.790	1.047	0.785	2.055	0.392	0.652
Camphor	3.008	2.089	2.897	3.407	3.109	2.907
α-pinene	1.304	1.182	1.072	1.126	0.935	0.931
β-pinene	0.279	0.264	0.247	0.275	0.293	0.418
Cineole	1.544	1.358	1.310	1.800	1.376	1.926
Camphene	0.499	0.515	0.497	0.671	0.564	0.805
Total	6.217	10.413	9.210	12.084	9.413	12.275

Plant No.	23		37		49	
Year	1999	2000	1999	2000	1999	2000
Component	Concentration mg/gm					
α-thujone	1.811	3.888	1.741	3.383	1.980	2.802
β-thujone	0.814	1.374	0.866	1.866	1.074	1.443
Camphor	1.463	1.610	3.379	1.219	2.349	1.978
α-pinene	1.684	1.993	0.882	2.266	1.093	7.983
β-pinene	0.171	0.154	0.294	0.235	0.207	0.153
Cineole	0.402	0.539	1.340	1.835	0.915	0.851
Camphene	0.231	0.280	0.590	0.309	0.365	0.348
Total	6.576	9.838	9.092	11.112	7.983	8.493

Table 2.2 Comparison of sage plants in terms of ratio of α-thujone to camphor concentration.

	α-thujone/camphor	
Year	1999	2000
Plant No.		
2	1.5	1.9
6	0.8	0.8
14	0.9	1.4
18	0.9	1.6
23	1.2	2.4
37	0.5	2.8
42	0.8	1.0
49	0.8	1.4

Table 2.3 Concentration of chemical compounds (mg/gm dry weight) and ORAC units/gm dry weight of sage plant parts.

	Concentration (mg/gm)								
Plant parts	a-thujone	b-thujone	Camphor	a-pinene	b-pinene	Cineole	Camphene	Total[y]	ORAC[z]
Leaf one	3.62	2.19	3.36	0.82	0.21	1.60	0.40	12.20	112.62
Leaf two	3.30	1.86	2.50	1.26	0.21	1.62	0.53	11.27	112.51
Leaf three	2.74	1.41	2.32	1.08	0.15	1.29	0.44	9.42	131.61
Leaf four	2.22	1.16	2.32	1.06	0.13	1.17	0.44	8.50	98.08
Leaf five	1.78	1.07	1.88	1.03	0.12	1.13	0.41	7.41	46.97
Leaf six	1.33	0.84	1.51	0.72	0.10	0.90	0.28	5.68	121.97
Axillary bud	1.79	1.31	1.78	0.24	0.07	0.66	0.09	5.95	129.70
Stem	0.54	0.27	0.20	0.05	0.08	0.08	0.06	1.29	85.73
Terminal bud	4.35	2.73	2.07	0.47	0.28	1.85	0.28	14.22	108.91
$LSD_{0.05}$	0.85	0.54	1.51	0.41	0.11	0.59	0.19	3.03	95.29

[y] Total: all chemical compounds quantitated in sage.

[z] ORAC: oxygen radical absorbing capacity(trolox equivalent/gm dry weight).

Table 2.4 Herbal yield of plant parts for analysis of chemical compounds and content of antioxidants of sage.

	Plant selections					
Plant No.	6		37		49	
Plant parts	Fresh weight	Dry weight	Fresh weight	Dry weight	Fresh weight	Dry weight
Leaf one	5.371	1.846	3.955	1.366	6.065	2.318
Leaf two	9.862	3.329	6.952	2.379	9.888	3.499
Leaf three	10.956	3.519	7.764	2.572	10.626	3.425
Leaf four	11.543	3.398	8.098	2.522	10.120	2.860
Leaf five	11.952	3.256	5.190	1.537	9.935	2.504
Leaf six	10.789	2.847	2.168	0.619	3.557	0.976
Axillary buds	0.716	0.235	1.333	0.447	0.495	0.170
Stem	8.602	2.580	6.454	2.130	9.099	2.838
Terminal buds	1.292	0.406	0.968	0.311	1.834	0.638

[z]Weight is expressed in gram from 25 stems.

Table 2.5 Comparison of chemical compounds (mg/plant part) content and ORAC (ORAC units/plant part) of sage.

	Content (gm/plant part)								
Plant parts	a-thujone	b-thujone	Camphor	a-pinene	b-pinene	Cineole	Camphene	Total [y]	ORAC[z]
Leaf one	6.82	4.12	6.25	1.38	0.35	2.79	0.71	22.42	$2.2*10^5$
Leaf two	10.25	5.78	7.86	3.70	0.61	4.84	1.60	34.64	$3.5*10^5$
Leaf three	8.77	4.54	7.47	3.34	0.48	4.04	1.35	30.01	$4.0*10^5$
Leaf four	6.51	3.45	6.89	3.02	0.39	3.41	1.27	24.94	$2.8*10^5$
Leaf five	4.45	2.70	4.83	2.44	0.30	2.78	1.04	18.54	$1.2*10^5$
Leaf six	2.25	1.49	2.61	1.21	0.18	1.64	0.53	9.91	$1.4*10^5$
Axillary bud	0.52	0.37	0.45	0.09	0.02	0.21	0.03	1.69	$3.4*10^4$
Stem	1.32	0.67	0.50	0.12	0.18	0.19	0.13	3.11	$2.2*10^5$
Terminal bud	1.93	1.20	1.03	0.19	0.11	0.75	0.12	6.23	$5.4*10^4$
$LSD_{0.05}$	2.85	1.48	2.52	0.97	0.25	1.14	0.46	7.44	$2.1*10^4$

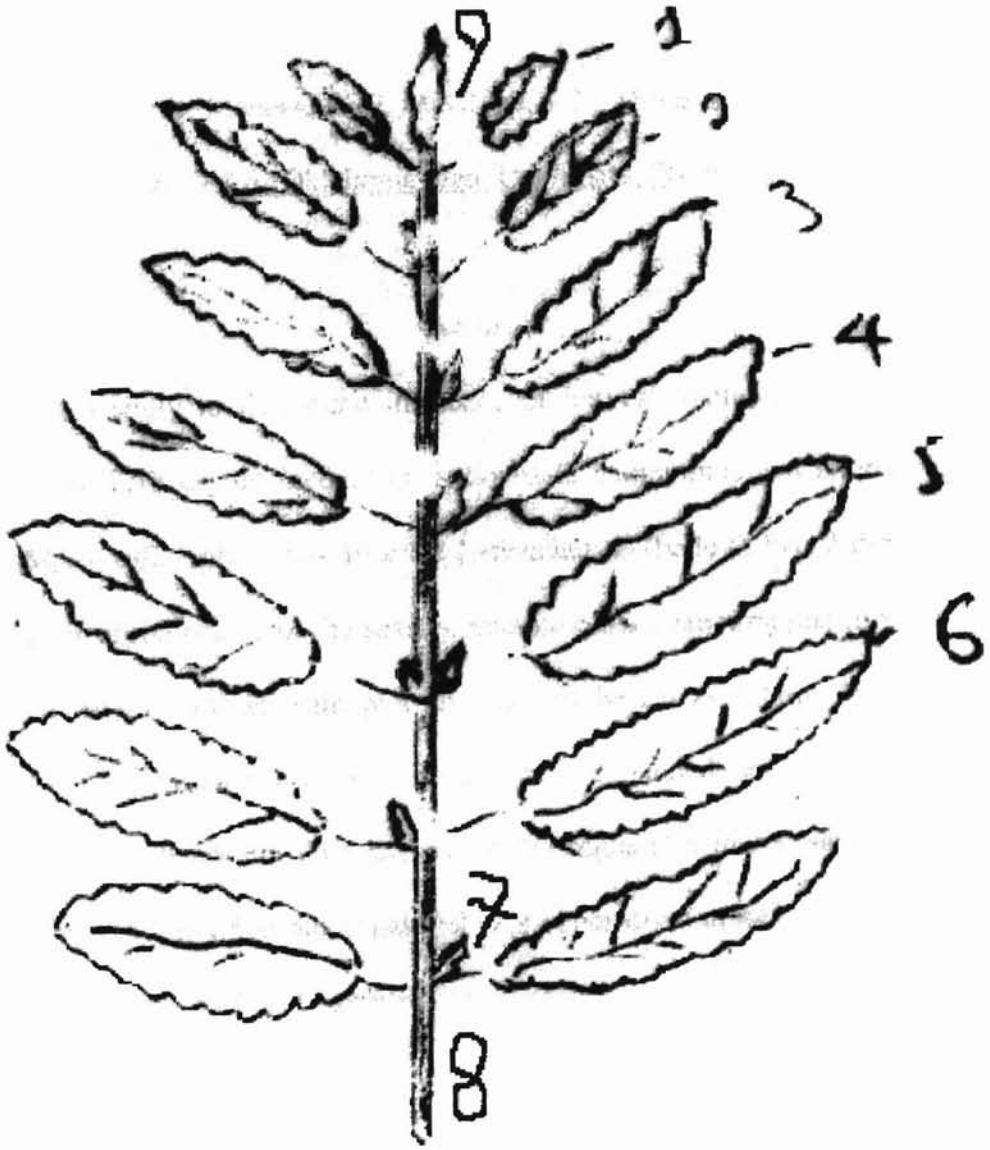
[y] Total: all chemical components quantitated in sage.

[z] ORAC: oxygen radical absorbing capacity (trolox equivalent/plant part dry weight).

Figure 2.1

Diagram of sage with paired leaves, meristematic growth, axillary buds and stem.

Leaves numbered from 1-6 are expanded leaves. Number 7 denotes axillary buds, 8 the stem and 9 terminal meristematic growth.



CHAPTER III

INFLUENCE OF HARVEST METHOD, HARVEST HEIGHT, TIME OF HARVEST AND METHOD OF DRYING ON ANTIOXIDANT YIELD AND ANTIOXIDANT ACTIVITY OF SAGE

(*Salvia Officinalis*)

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Abstract

In a study to determine influence of harvest method, harvest height, time of harvest and herb drying method on antioxidant compounds and antioxidant activity of sage (*Salvia officinalis*), two different harvesting methods at two different heights were imposed over the course of the season. Sample plots were arranged in a split plot design with harvest method as main plot and harvest height as subplot. Field samples were collected after regrowth to the two and five fully expanded leaf stage by first harvesting a sub sample by hand and then harvesting the remainder of the same plot by flail type harvester. The harvested plant material was either dried at 44°C or was frozen in liquid N_2 and lyophilized. Dried samples were ground and then extracted using hexane. To determine the effect of harvesting time, the multiple harvests in a season were grouped into three (early, middle and late) harvests. The two leaf stage had 6 seasonal harvests

and the five leaf stage had 4 seasonal harvests. Extracts were injected onto GC for quantitative analysis of antioxidant components (α-thujone, β-thujone, camphor, α-pinene, β-pinene, cineole and camphene) and the same extracts were diluted appropriately and utilized for antioxidant activity (AOA) analysis, using oxygen radical absorbing capacity assay (ORAC). Results demonstrate that harvest method and harvest height significantly effected AO and AOA yield. Early harvest had lowest antioxidant yield followed by middle and late harvests. Younger tissues (harvesting at the two leaf stage) had 43% more yield of antioxidant chemical components than harvesting at the five leaf stage. Harvesting sage by hand at 5 leaf height for AO and AOA production gave 55% more yield of antioxidant as compared to the flail harvest. When sage was harvested at the two leaf stage by hand, antioxidant yield was increased by 73% as compared to harvest by flail type harvester at the five leaf stage.

Introduction

Sage is a hardy perennial plant in the family Labiatae. It originates from the Mediterranean region, but it is widely adapted to most parts of Europe and North America. The largest producers in the world are Yugoslavia and Albania. Other than for its antioxidant effect, sage is widely known for its pharmaceutical use against aging and for spices used for seasoning poultry, sausages and hamburger (Vandemark and Splinsttoeter, 1979; Putievsky and Dudai, 1986). Sage is quite diverse, with more than 500 species, of which the most widely known is *Salvia officinalis*. The plant may be propagated vegetatively from stems. It may also be propagated from seeds. It grows well as a perennial in soil with slightly alkaline pH, and is tolerant to water stress once the

plant is established (Maness, 1997). The leaves are arranged parallel and have distinctive color, which is used for identification of variety. The top one half to one third of the plant is harvested, dried and extracted for antioxidant production. Vegetative parts include the youngest meristematic growth at the apical portion, followed down the stem by four to six paired leaves and abundant axillary leaf buds along the stem. Plants of sage cultivated under different climatic conditions showed similar essential oil yield, (Putievsky et al., 1992). According to Ogg (1988) cited by (Maness, N 1997) in the production of alfalfa (*Medicago sativa*) a cut at time near fall frost was observed to bring injury and death of the plant. In Alabama alfalfa plants need six to eight weeks recovery time between the last harvest and fall frost. The optimum time for last harvest of alfalfa was mid September. Sage followed the same pattern of survival. A final harvest of sage in mid November, following the first killing frost, gave highest herbal yield with less plant mortality (Motes et al., 1994).

Studies on tea show that care during harvest to prevent excessive damage is essential to maintain high quality. Hand harvested tea leaves were richer in their green tea biochemical precursors and quality constituents than shear harvested tea, due to deterioration from mechanical injury caused with shear harvesting (Ravichandran, 1998). Robinson and Maness (1996) compared the effect of harvesting method, using flail and sickle type mechanical harvesters with hand harvesting with only fully expanded leaves as a control. Plant material was harvested at the same height. The sickle type harvester cut at one location on the stem, the flail type harvester chopped the plant material into small particles and hand harvesting included fully expanded leaves only. AOA level was found to be 50% and 65% of Butylated hydroxyl tolune (BHT) standard for flail and

sickle type harvested sage, accordingly. The hand harvested sage had intermediate AOA, 55% of Butylated hydroxyl tolune (BHT) standard. The difference in AOA between the sickle and hand harvested leaf material was thought to be due to the absence of apical meristem in the hand harvested leaves, which may contribute a substantial amount of AOA.

This study will address the effect of harvest method with the inclusion of unexpanded leaves, apical meristem and axillary leaf buds by the use of flail and hand harvest (simulating mechanical sickle harvest) methods. In addition, the effect of harvesting height and method of drying will be addressed for AO and AOA.

The objectives of this study were:

- To compare the potential harvesting methods after plant regrowth to two heights and method of drying for enhancing antioxidant and antioxidant activity and dry yield over the harvesting season.
- To evaluate the effect of harvesting frequency on antioxidant yield and antioxidant activity and dry yield.

Results from this study may be of importance in determining how often sage should be harvested based on height of harvest and what type of harvesting machinery is needed for maximum AO and AOA production.

Materials and methods

Harvesting procedures for improving sage dry yield for production of antioxidants and AOA:

Samples were harvested from plots at the Oklahoma Vegetable Research Station at Bixby in the year 2000. The sage planting was established in 1998 by transplanting Dalmatian sage in rows 1m apart and 0.45m between plant spacing. Sage plots were 10 m long and arranged in a split plot design with 5 replications; one 5m section was harvested after regrowth to two fully expanded leaves and the other 5m section was harvested after regrowth at 5 fully expanded leaves. A 1 m tilled alley was maintained between the subplots to allow for harvester starting and stopping. The method of harvest (flail versus hand harvest) was the main plot and harvest height (harvest after regrowth to two leaves versus harvest after regrowth to five leaves) was the sub plot. At time of harvest, 50 stems in each sub-plot were obtained by hand, weighed and placed on ice inside freezer bags. The same plots were then flail harvested at the proper height with a Lawn Genie flail harvester (Mathews, Co., Crystal Lake IL.). The harvested material was weighed for fresh yield and a sub sample of flail-harvested material was weighed and sealed into a freezer bag, placed on ice and then both hand and flail harvest samples were transported to laboratory facilities in Stillwater. Total plot yield was obtained by adding the 50-stem sample weight to the total flail harvested weight. Plants were harvested as soon as specified height of harvest requirements were met, leading to a different frequency of harvest for the two harvest heights under study.

Upon arrival at laboratory facilities, the 50-stem hand harvested sample was separated into two 25-stem sample, and along with an equivalent weight of the flail harvested samples, placed into one of the two drying treatments. The first sets were put in a paper bag and samples were dried in forced draft ovens for a maximum duration of 44 hours at 50° C. The drying process in the forced draft oven continued until samples reached equilibrium weight. The remaining 25-stem hand harvested sample and corresponding weight of the flail harvest sample were placed into labeled freezer bags and frozen at –20°C for 24 hours. They were then lyophilized in a VirTis shelf freeze drier (Consol 12 VirTis, Gardner NY) with shelf temperature maintained at 15°C and condenser temperature at –40°C for a duration of at least 48 hours. Dry weights for both drying treatments were recorded and utilized to convert fresh yield into dry yield. Freeze and forced air dried samples were sealed in freezer bags and stored at –20°C to await grinding just prior to extraction.

Extraction:

Samples were ground to a fine powder with a UDY mill (UD Corporation, Boulder, CO). Duplicate 100 mg aliquots for each sample were accurately weighed into separate two dram vials containing a magnetic stir bar. Two ml of hexane was added, vials were capped and continuously stirred with magnetic stirrer for a duration of 20 minutes. Capped vials were then placed into a SpeedVac (model SVC-100 H Savant, Instruments Inc. Farmingdale. NY) and centrifuged for five minutes. Supernatants were decanted into a second two-dram vial which was subsequently placed into the SpeedVac and centrifuged for another five minutes to remove any remaining particulates and the supernatant was transferred into a labeled 10 ml volumetric flask. Two more extractions

were conducted as above for a total of three extractions. The 10 ml volumetric flask was brought to volume with hexane. Extracted samples were placed into a brown bottle, securely capped and held for one week or less at 4°C to await analysis. Preliminary trials indicate greater than 95% recovery for all measured compounds using this procedure.

Analysis of AO chemical components:

Samples of extracted material were brought to room temperature and analyzed for the content of chemical components using Gas Chromatography. A 1.94 ml sample was added to 0.06 ml linalyl acetate to make 280 nmol linalyl acetate (Sigma Chemical Co. St. Louis, MO) internal standard. Gas chromatography was conducted with a Varian 3600 gas chromatograph (Varian Associates, Walnut Creek, CA), equipped with a septum programmable injector and flame ionization detector at 300°C. The initial injector temperature was 55°C. Samples were injected slowly for cold on column injection, and immediately following injection an injector temperature ramp from 55°C to 290°C was accomplished at a rate of 100 °C min^{-1}. Chemical components were separated on a DB 1 column (30 x 0.25 mm, 0.25 um film thickness; J& W Scientific, Folsom, CA) with helium as carrier gas. The initial column temperature was 40°C with hold for 1 minute. Components were then separated with a linear temperature program from 40°C to 290°C at 5°C/min, and held at 290°C for 10 min. Chemical component peaks were identified according to co-elution with authentic standards (α-thujone, β-thujone, camphor, α-pinene, β-pinene, cineole and camphene (Sigma Chemical Co., St. Louis, MO) and quantitated relative to linalyl acetate internal standard.

Antioxidant activity:

One ml of the extract was placed into a 100 ml volumetric and brought to volume with hexane. Antioxidant activity was measured by oxygen radical absorbing capacity (ORAC) using a modified version of Cao et al.(1995). The reagents used for analysis were phycoerythrin (Sigma Chemical, St. Louis, MO); 2, 2'-azobis (2-amidinopropane) dihydrochloride (AAPH; Wako Chemical Co. Richmond, VA), 6-hydroxy-2,5,7,8-tetramthylchroman-2 carboxylic acid (trolox; Aldrich Chemicals, Inc. Milw. WI), phosphate buffer and sample.

Reagents were prepared as follows: Phosphate buffer was prepared by mixing 0.75M K_2HPO_4 and 0.75 M NaH_2PO_4 at 61.6:38.9 v/v and pH was adjusted to 7 using potassium hydroxide. The stock solution for phycoerythrin (PE) was prepared by dissolving 1 gm of phycoerythrin in 5.9 ml of phosphate buffer and kept refrigerated for not longer than a month prior to use. A working solution of PE was prepared prior to analysis, by adding 300 µl of PE stock to 13.4 ml of phosphate buffer. Trolox stock solution was prepared by dissolving 5 mg of trolox in 200 ml of phosphate buffer to make a 100 µM solution. . The working solution of trolox was prepared by 1:10 dilution of the stock in phosphate buffer to make 10µM. AAPH working solution was prepared just prior to use by dissolving 79 mg of AAPH in 5 ml of phosphate buffer.

Reaction mixtures for analysis consisted of 20 µl of sample, 160 µl PE working solution 20 µl trolox working solution for standard and 20 µl hexane as reagent blank. Reaction was carried out in a 48 well plate (Falcon multi well; Sigma Chemical Co., St. Louis, MO) arranged in 8 columns of six samples each. During each run, column one was

hexane reagent blank , column 2 was trolox and columns 3-8 were samples, with each sample replicated over each of the six wells per column. A Perkin-Elmer HTS-7000 Microplate Reader was set for ORAC assay at 535nm for excitation and 595nm for emission. Target temperature was 36°C, with a cycle period of two minutes, giving 35 cycles in a 70 min. run. The fluorescence readings were converted into ORAC values and were expressed in terms of trolox equivalent. Micro plates were replaced after 4 uses to prevent excessive background readings caused by hexane reacting with the plastic of the wells.

Statistical design and analysis:

In order to be able to compare unequal harvests for sage harvested at the two leaf stage versus sage harvested at the five leaf stage, the effect of harvest time on the basis of concentration and content of chemical components and ORAC values were grouped into early harvest (from late April to late May), middle harvest (mid June to early August), and late harvest (covering a period from mid August to mid September). Analysis of variance (ANOVA) for a split plot design was conducted with method of harvest as main plot and harvest height (regrowth to) two fully expanded leaves versus regrowth to five fully expanded leaves) as subplot with 5 replications using Statistical Analysis System (SAS Institute, Carry, NC). Data was analyzed with multiple range test comparison of protected LSD, to determine significance differences among treatment means at $P<0.05$.

Results

The effect of the different treatments on antioxidant yield was analyzed in terms of concentration and content. Concentration of chemical compounds was expressed according to the amount of the different chemical compounds and total sum of the chemical compounds analyzed per unit dry weight of sage. Content of the different chemical compounds and the total sum of all analyzed chemical compounds, as well as ORAC equivalents, were quantitated from sage per unit of planted area. Chemical compounds analyzed were α-thujone, β-thujone camphor, α-pinene, β-pinene, cineole, and camphene. Samples for this study were harvested in the 2000 growing season during a period from late April to early September and overall results are presented in appendix B.

Since harvesting frequency, and eventual total number of harvests was different for plants harvested after regrowth of only two leaves versus plants harvested after regrowth of five leaves, the multiple harvests obtained during the period of late April to early September were divided into three groups: early harvest (late April to late May), middle harvest (mid June to early August), and late harvest (mid August to early September). Results of chemical concentration will be presented first, followed by content and ORAC.

Interaction of harvest height and harvest time on concentration of chemical compounds of sage

The interaction of harvest height and harvest time was significant at $P<0.05$ for α-thujone, camphor, cineole, β-pinene and the sum of all detected compounds, but not for β-thujone, α- pinene and camphene (Table 3.1). Alpha thujone was the most prominent chemical constituent identified in hexane extracts of sage and differences in its concentration was mirrored by camphor. Concentration of α-thujone and camphor were significantly higher in middle and late harvests than the early harvest for both harvest heights. When harvested at the two leaf stage, α-thujone and camphor were significantly higher at the middle harvest than the late harvest. At the five leaf stage there was no difference in α-thujone and camphor concentration between the middle and late harvests. Early harvest at the five leaf stage resulted in a lower concentration of α-thujone and camphor than harvest during the same time period at the two leaf stage.

Cineole and β- pinene concentrations didn't exhibit the same interaction with harvest height and harvest time as α-thujone and camphor. Concentration of cineole was generally five to six times higher than β-pinene, but differences within the harvest height and harvest time interaction were the same. The sum of concentrations for all chemical compounds (total) for the interaction of harvest height and harvest time ranged from almost 5 mg/gm to over 9 mg/gm depending on treatment. Like α-thujone and camphor the total concentration was lowest at the early harvest time and five leaf stage was lower than the two leaf height stage. Unlike α-thujone and camphor the middle and late

harvests at the two leaf height didn't differ in total concentration, while the same harvests at five leaf height did differ.

Interaction of harvest method and harvest time on concentration of chemical compounds of sage

The interaction of harvest method and harvest time was significant at $P< 0.05$ for α-pinene, β-thujone, cinole and the total detected chemical compound concentration, but not for α-thujone, camphor, β-pinene, and camphene (Table 3.2) Concentration of α-pinene and β-thujone harvested by both flail and hand was lowest in early harvested sage, and flail harvested was lower than hand harvested at this stage. Late harvested sage contained a higher concentration of α-pinene and β-thujone than the middle harvest for the flail treatment, but there was no difference when harvested by hand. Cineole concentration progressively decreased with harvest time for the hand harvest method, but increased from the early harvest to the late harvest for the flail harvest method. The interaction of harvest method with harvest time exerted a substantial influence on total concentration of chemical compounds which varied from less than 4 mg/gm to over 10 mg/gm depending on treatment. Similarly to α-pinene and β-thujone concentration of total chemical compounds from sage was lowest for flail harvest at early harvest time. There was no difference between the middle and late harvest times for flail harvested sage, but for hand harvest the middle time was greater than the late harvest, which in turn was greater than the early harvest.

Influence of the main effect of harvest method on concentration of camphene

Concentration of camphene from hand harvested sage was significantly higher than that of flail harvested sage (Table 3.3).

Interaction of harvest height and drying method on concentration of chemical compounds of sage

The interaction of harvest height and drying method on chemical concentrations was only significant for α-thujone, cineole and the total chemical compounds (Table 3.4). Differences in concentration of α-thujone and total chemical compounds in this interaction were similar, with freeze dried sage at the two leaf height consistently higher than five leaf height within either drying method. Alpha thujone concentration differed from the total concentration in that there was no difference between harvest heights for the oven drying treatment, whereas there was a difference in terms of total concentration. While the freeze drying treatment caused greater concentration than the oven drying treatment of cineole for the two leaf height, there was no difference between drying treatments at the five leaf height. Like α-thujone there was no difference in cineole concentration between harvest heights in the oven dried treatment.

Interaction of harvest method and harvest height on concentration of α-thujone and the total chemical compounds of sage

The interaction of harvest method and harvest height was only significant for α-thujone and total chemical compound concentrations (Table 3.5). Alpha thujone and total chemical compound concentrations were highest for hand harvest at two leaf height followed by hand harvest at five leaf height. There was no difference in concentration of

α-thujone for flail harvest between harvest heights but flail harvest at two leaf height resulted in greater concentration of total chemical compounds than at the five leaf height.

Interaction of harvest method, harvest time and drying method on concentration of camphor and total chemical compounds of sage

The three way interaction of harvest method, harvest time and drying method was only significant for camphor and the total chemical compounds in sage (Table 3.6). Within harvest methods, concentration of both α-thujone and total chemical compounds was least at the early harvest, regardless of drying method. Oven drying did result in less camphor concentration in late harvested sage as opposed to the middle harvest, but there was no difference in camphor concentration between these harvest times for freeze dried sage. The middle and late harvests were not different in terms of total concentration for flail harvest and freeze dried treatments, but differences existed between other treatments within harvest method. For flail harvested sage, oven drying of the late harvest time resulted in higher total chemical compounds concentration than the middle harvest. For hand harvested sage in either freeze or oven drying treatments, total chemical concentration was greater in the middle time period than in the late time period.

Interaction of harvest method, harvest height, harvest time, and drying method on concentration of chemical compounds of sage

The interaction of harvest method, harvest height, harvest time and drying method had significant difference in concentration of α-thujone, camphor, β-pinene, cineole and total chemical compounds but not β-thujone, α-pinene and camphene (Table3.7). Concentration of α-thujone harvested by hand from the middle harvest at two leaf stage

and dried with either oven or freeze drying was significantly higher than the early harvest followed by the late harvest. Concentration of α-thujone from the middle harvest of freeze dried sage with the same type of harvesting method was higher than oven dried sage. The same trend was observed in camphor concentration, except for the flail harvested at five leaf stage and oven dried, where the late harvest had higher concentration of camphor followed by the middle harvested sage. Early harvested sage from either two or five leaf stage, by flail or hand, but dried with oven had significantly higher concentration of β-pinene than middle harvest followed by late harvest with the exception of hand harvested at five leaf stage. As opposed to camphor, concentration of β-pinene from the middle harvest either at two or five leaf stage and freeze dried was lower than early and late harvest. In comparing the different drying methods within the same harvest method, harvest height and time of harvest, freeze drying for most combinations gave higher concentration of β-pinene than oven drying. Early harvested sage at two leaf stage and freeze dried was significantly higher than sage harvested at the same time of harvest, harvest height and drying method, but harvested at the five leaf stage. Highest concentration of total chemical compounds from the four way interaction within the same harvest method and harvest height but freeze dried was obtained from the middle harvest followed by late harvest. The same combination of treatments with oven drying gave lower concentration of total chemical compounds.

Content of individual and total chemical compounds of sage

Expression of results in terms of content (kg/ha) as opposed to the preceding results for concentration (mg/gm dry weight) describes the amount of chemical compounds produced by sage, in terms most applicable to describe actual production. Although the main effect of harvest height didn't significantly affect sage herb yield, the two leaf height did result in greater than a 500 kg dry sage ha $^{-1}$ yield increase over harvest at the five leaf stage (Table 3.8). Since the concentration of various compounds did change in relation to harvest height, content of these compounds would be expected to be significantly influenced by this herb yield discrepancy. Where appropriate ORAC results are also presented in ORAC units ha $^{-1}$.

Main effect of harvest height on the individual compounds and the total content and oxygen radical absorbing capacity of sage

The main effect at harvest height on chemical content was significant at $P<0.05$ for all compounds except α-pinene (Table 3.9). In all cases harvest at the two leaf height resulted in greater content and ORAC yield than harvest at the five leaf height.

Main effect of harvest method on individual compounds, the total content and oxygen radical absorbing capacity of sage

The main effect of harvest method was significant for all compounds except α- and β-pinene (Table 3.10). In all cases hand harvest resulted in greater content and ORAC yield than flail harvest. Chemical compounds ranged from 1/3 to 1/2 greater content in hand as compared to flail harvested sage.

Interaction of harvest height and harvest method on total chemical compound content of sage

The interaction of harvest height and harvest method for chemical content was only significant at $P<0.05$ for total chemical compounds (Table 3.11). For each harvest method sage harvested at the two leaf height had a higher total content of chemical compounds than sage harvested at the five leaf height. Hand harvested sage at two leaf stage had up to double the total content of chemical compounds than the other combinations. The only treatment that was not different was flail vs hand harvested sage at the five leaf height.

Interaction of harvest height and drying method on individual and total chemical compound content of sage

The interaction of harvest height and drying method was significant at $P<0.05$ for β-pinene, cineole and total chemical compound content but not for α and -β-thujone, camphor, α-pinene and camphene (Table 3.12). In all cases, highest content was obtained by freeze drying at the two leaf height. Within the two leaf height, oven drying resulted in less content of all interacting compounds, but no difference between drying methods was observed at the five leaf height.

Interaction of harvest height and harvest time on individual and total chemical compound content and oxygen radical absorbing capacity of sage

The interaction of harvest height and harvest time was significant at $P< 0.05$ for all chemical compound contents and ORAC units except α-pinene and camphene

(Table 3.13). Within harvest heights, differences between chemical compound content mirrored those of ORAC values. For harvest at the two leaf height, highest values were obtained at the early harvest period, with no difference between the middle and late harvests. At the five leaf height highest values were observed for the middle harvest, and the total chemical contents were greater at the late harvest than at the early harvest whereas ORAC values were greater at the early harvest versus the late harvest. The same differences at the two leaf height were observed for α-thujone and β-pinene. At the five leaf height the middle harvest was also highest for α-thujone but not for β-pinene. For either two or five leaf heights, camphor was lowest at the early harvest and either not different between middle and late harvest at the two leaf height or higher at the middle versus the late harvest at the five leaf height. Across harvest heights and harvest times, β-thujone content was highest at the middle harvest of five leaf height, followed by the early harvest at two leaf height, but no other differences were obtained.

Interaction of harvest method and harvest time on the individual and total chemical compound content and oxygen radical absorbing capacity of sage

The interaction of harvest method with harvest time was significant at $P< 0.05$ for all chemical compound contents, and for the total chemical compound content and ORAC values except for β-pinene (Table 3.14). Differences in cineole mirrored that of ORAC units within harvest methods. For flail harvest method, the early harvest was highest, with no difference between middle and late harvests, whereas in hand harvest method, early harvest was higher than the late harvest. For hand harvested sage, α-thujone content also mirrored cineole content and ORAC units, yielding progressively

less α-thujone with harvest time, but unlike cineole and ORAC units, α-thujone content didn't differ with time of harvest for the flail harvest method. Contents of camphor, β-thujone, α-pinene and camphene for flail harvested sage changed equivalently- early harvest was lowest with no difference between the middle and late harvest. For hand harvested sage, contents of α-thujone and camphene didn't change as harvest time progressed, camphor content was highest at the middle harvest with no difference between the early and late harvests, and β-thujone content was lowest at the late harvest, with no difference between the early and late harvests. Total chemical compound content for flail harvested sage was higher at the middle harvest than the early harvest, but didn't differ between early and late harvests or between middle and late harvests. Similar to differences in β-thujone content for hand harvested sage, the total chemical compound content was lowest at the late harvest but didn't differ between early and middle harvests.

Interaction of harvest method, harvest height and harvest time on α-thujone, camphor and total chemical compound content of sage

The three way interaction of harvest method, harvest height and harvest time was significant at $P< 0.05$ only for contents of α-thujone, camphor and total chemical compounds (Table 3.15). Changes in α-thujone content and camphor content for five leaf stage only, mirrored that of total chemical compound content. Within flail harvested samples there was no difference between harvest times for the two leaf height, but for the five leaf height sage harvested at the middle time period had highest content, followed by late harvest and then early harvest. Within the hand harvest method, α-thujone and total contents at the two leaf height were highest at the early harvest but not

different at the middle and late harvests, and at the five leaf height the middle harvest time was highest, with no difference between the early and late harvests. Camphor content for the flail harvest method at the two leaf height was lowest at the early harvest, and not different between middle and late harvests. There was no difference in camphor content at two leaf height between harvest times.

Discussion

This research compared the effect of harvest height, harvest method, time of harvest and type of drying on the individual and combined total amount of chemical compounds, previously reported as antioxidant constituents of sage (Tekelova et al., 1994). Since prior studies for sage and other crops (Ravichandran, 1998) have established that younger tissues have higher antioxidant concentration, we hypothesized that more frequent harvest of younger sage would result in greater antioxidant production. Results from this study shows that sage harvested at young physiological age gives higher content of almost all measured chemical compounds which are the major contributors of antioxidant and antioxidant activity of sage (table 3.9). This result extends the observation made by Tekelova et al., (1994) that young sage leaves yield higher essential oil than older ones by establishing that antioxidant compounds in the essential oils are also produced in higher quantity in younger tissues.

Hand harvesting was conducted to stimulate action of a sickle type mechanical harvester with one cut of the stem to obtain leaves and merismatic growth with minimal plant damage. Thirty percent of the total chemical composition of sage was composed of α-thujone. In a similar study with Dalmatian sage, hand harvest also gave higher antioxidant activity than flail harvest, although individual chemical compounds were not evaluated (Robinson, 1996). Our results reflect a higher yield of antioxidants and antioxidant activity in hand harvested sage for most compounds analyzed and for ORAC units than flail harvested sage (Table 3.10). This effect was likely due to a substantial

reduction in plant maceration and damage during harvest by hand as opposed to harvest by flail.

Our flail harvesting procedure highly macerated the plant material and exposed the many cut surfaces to oxidation. Harvesting at a younger physiological age, using hand harvest gave highest total yield of all the major chemical compounds, with an approximate doubling in production of antioxidants when hand harvest at the two leaf stage was compared to flail harvest at the five leaf stage (Table 3.11).

Herbal yield of sage comparison for the full season from the two leaf versus five leaf stage didn't show any significant difference, although the two leaf harvest resulted in greater than 500 kg/ha increase in dry yield (Table 3.8). We believe the increase in antioxidant content for two leaf heights versus five leaf heights was due to higher concentration interacting with the slight, yet insignificant difference in herbal yield.

In order to see the effect of harvest time, which is a range including the multiple harvests during the growing season of sage for antioxidant production, the multiple harvests were divided into three groups of early , middle and late season. Harvesting at the five leaf stage had four harvests when the two leaf stage had six harvests for the full season. In comparing the effect of harvest time in relation to harvest height, on antioxidant and antioxidant activity, results indicate that yield of the different chemical compounds which are known to be the major contributors of AO and AOA was highest for most measured chemical compounds of sage harvested during early period of the season, at the two leaf height, but highest during the middle part of the season when harvested at the five leaf height (Table 3.13). Highest AOA can be expected from sage during the first two thirds of the harvest season, irrespective of harvest height. In

comparing freeze and oven drying methods at different harvest height, antioxidant and antioxidant activity of most of the chemical compounds and the total of all the compounds analyzed in this study, we see that freeze drying of sage harvested at young physiological age had higher AO and AOA than oven dried sage. For other treatments no difference was observed between the different drying methods.

Recommendations

In our study we have seen that harvesting young tissues (two leaf stage) of sage for antioxidant production had higher yield than older tissues (five leaf stage). Harvesting younger tissues allowed more frequent harvesting, than harvesting old tissues and the overall seasonal yield AO and AOA increased for hand harvest of younger tissues. We recommend that sage for antioxidant production needs to be harvested at young stage. Further, there is a need to develop a harvesting mechanism of sage which is similar to hand harvest with a single cut without macerating and exposing the tissues to oxidation. Since our dry herb yield was consistently higher for two leaf stage versus five leaf height stage, yet barely missed a statistically significant difference, we would also recommend repetition of this study over several years to firmly establish this important yield comparison.

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Table 3.1 Interaction of harvest height and harvest time on concentration of chemical compounds of sage.

		concentration(mg/gm dry weight)				
Harvest height [x]	Harvest time [y]	α–thujone	Camphor	β–pinene	Cineole	Total [z]
2	Early	2.48	1.48	0.27	1.54	6.66
2	Middle	3.26	3.21	0.18	1.23	9.45
2	Late	2.78	2.76	0.20	1.14	8.62
5	Early	1.78	1.02	0.20	1.00	4.72
5	Middle	3.01	2.68	0.15	0.99	8.24
5	Late	3.23	2.87	0.20	1.16	9.25
$LSD_{0.05}$						
Overall		0.44	0.45	0.05	0.26	1.15
Harvest time for the same harvest height		0.37	0.29	0.05	0.17	0.95

[x]Harvest height: 2- harvesting at two leaf stage, 5-harvesting at 5 leaf stage.

[y] Harvest time: early harvest is before May 31, medium harvest is between June 16 and August 1 and late harvest is after August 9.

[z] Total: all the chemical compounds quantitated in sage.

Table 3.2 Interaction of harvest method and harvest time on concentration of chemical compounds of sage.

Harvest method [x]	Harvest time [y]	Concentration(mg/gm dry weight)			
		β-thujone	α-pinene	Cineole	Total [z]
Flail	Early	0.18	0.21	0.94	3.78
	Middle	0.41	0.61	0.98	7.85
	Late	0.52	0.80	1.12	8.73
Hand	Early	0.45	0.37	1.59	7.60
	Middle	0.58	0.63	1.36	10.46
	Late	0.50	0.74	1.16	9.14
$LSD_{0.05}$					
Overall		0.13	0.15	0.26	1.15
Harvest time for the same harvest method		0.08	0.14	0.17	0.95

[x] Harvest method: flail is flail type harvester and hand is hand harvest.

[y] Harvest time: early harvest was before May 31, middle harvest was between June 16 and August 1 and late harvest was after August 9.

[z] Total: all the chemical compounds quantitated in sage.

Table 3.3 Influence of the main effect of harvest method on concentration of camphene of sage.

Harvest method [z]	Concentration(mg/gm dry weight)
	camphene
Flail	0.61
Hand	0.74
$LSD_{0.05}$	0.08

[z] Harvest method: flail is flail type harvester and hand is hand harvest.

Table 3.4 Interaction of harvest height and drying method on the concentration of chemical compounds of sage.

Harvest height [y]	Drying method	Concentration (mg/gm dry weight)		
		α–thujone	Cineole	Total [z]
2	Freeze	3.32	1.44	9.05
5	Freeze	2.62	1.03	6.92
2	Oven	2.55	1.19	7.72
5	Oven	2.28	1.04	6.55
$LSD_{0.05}$				
Overall		0.43	0.28	1.01
Drying methods for the same harvest height		0.30	0.14	0.80

[y]Harvest height: 2-harvesting at two leaf stage, 5- harvesting at five leaf stage.

[z]Total :all the chemical compounds quantitated in sage.

Table 3.5 Interaction of harvest method and harvest height on concentration of α- thujone and total chemical compounds of sage.

Harvest method [x]	Harvest height [y]	concentration (mg/gm dry weight)	
		α–thujone	Total [z]
Flail	2	2.31	6.75
	5	2.12	5.86
Hand	2	3.57	10.02
	5	2.78	7.61
$LSD_{0.05}$		0.30	0.78

[x] Harvest method: flail is flail type harvester and hand is hand harvest.

[y] Harvest height: 2-harvesting at two leaf stage, 5- harvesting at five leaf stage.

[z] Total: all the chemical compounds quantitated in sage.

Table 3.6 Interaction of harvest method,drying method and harvest time on concentration of camphor and the total chemical compounds of sage.

Harvest method [x]	Drying method	Harvest time [y]	Concentration(mg/gm)	
			Camphor	Total [z]
Flail	Freeze	Early	0.75	4.09
		Middle	3.00	9.05
		Late	2.58	8.88
	Oven	Early	0.64	3.47
		Middle	2.15	6.64
		Late	2.76	8.57
Hand	Freeze	Early	1.76	7.99
		Middle	3.54	10.70
		Late	2.95	9.40
	Oven	Early	1.86	7.22
		Middle	3.61	10.22
		Late	2.95	8.89
$LSD_{0.05}$				
Overall			0.68	1.92
Same or different harvest time and drying method within harvest method			0.59	0.79

[x] Harvest method: flail is flail type harvester and hand is hand harvest.

[y] Harvest time: early harvest is before May 31, middle harvest is between June 16 and August 1 and late harvest is after August 9.

[z] Total: all the chemical compounds quantitated in sage.

Table 3.7 Interaction of harvest method, harvest height, harvest time, and drying method on concentration (mg/gm dry weight) of chemical compounds of sage.

Harvest method [w]	Harvest height [x]	Harvest time [y]	Drying method	Concentration (mg/gm)				
				α -thujone	Camphor	β–pinene	Cineole	Total[z]
Flail	2	Early	Freeze	1.82	0.81	0.33	1.17	4.58
	2	Middle		3.32	3.16	0.18	1.18	9.29
	2	Late		2.90	2.27	0.21	1.17	8.44
	5	Early		1.41	0.68	0.23	0.68	3.59
	5	Middle		3.20	2.53	0.17	0.98	8.32
	5	Late		3.49	2.89	0.20	1.14	9.33
	2	Early	Oven	1.21	0.71	0.17	0.87	3.49
	2	Middle		2.13	2.21	0.15	0.84	6.67
	2	Late		2.66	2.86	0.19	1.12	8.56
	5	Early		1.16	0.57	0.23	0.89	3.44
	5	Middle		2.26	1.98	0.14	0.77	6.55
	5	Late		2.91	2.66	0.17	1.07	8.58
Hand	2	Early	Freeze	3.93	2.17	0.33	2.25	10.06
	2	Middle		4.30	3.74	0.22	1.56	11.55
	2	Late		2.85	2.74	0.19	1.10	8.29
	5	Early		2.41	1.36	0.17	1.08	5.92
	5	Middle		3.06	2.95	0.14	0.93	8.14
	5	Late		3.67	3.17	0.26	1.37	10.52
	2	Early	Oven	3.09	2.25	0.22	1.85	8.51
	2	Middle		3.38	3.72	0.18	1.34	10.30
	2	Late		2.86	3.15	0.21	1.17	9.21
	5	Early		2.11	1.48	0.17	1.19	5.92
	5	Middle		3.53	3.28	0.17	1.27	9.96
	5	Late		2.95	2.75	0.17	1.06	8.56
$LSD_{0.05}$								
Overall				0.73	0.68	0.09	0.39	1.91
Same or different harvest method, harvest date, drying method, and harvest height.				0.39	0.59	0.09	0.34	1.93

[w] Harvest method: flail is flail harvest and hand is hand harvest.

[x] Harvest height: 2-harvesting at two leaf stage, 5- harvesting at five leaf stage.

[y] Harvest time: early harvest is before May 31, middle harvest is between June 16 and August 1 and late harvest is after August 9.

[z] Total :all chemical compounds quantitated in sage.

Table 3.8 Influence of harvest height on fresh and dry herbal yield (kg/ha) of sage.

Harvest height [z]	Weight (kg/ha)	
	Fresh	Dry
2	23694	5575
5	21175	4917
$LSD_{0.05}$	NS	NS

[z] Harvest height: 2 is harvest at two leaf stage and 5 is harvest at five leaf stage.

Table 3.9 Main effect of harvest height on individual and total chemical content (kg/ha) and oxygen radical absorbing capacity of sage.

Harvest height [x]	Content (kg/ha)							
	α–thujone	β–thujone	Camphor	β-pinene	Cineole	Camphene	Total [y]	ORAC [z]
2	29.47	4.34	22.11	2.71	15.47	3.46	82.38	$15.3*10^{9}$
5	21.48	3.06	15.18	1.84	9.82	2.36	57.67	$12.9*10^{9}$
$LSD_{0.05}$	6.28	0.84	5.35	0.50	3.40	0.51	15.09	$0.4*10^{9}$

[x] Harvest height 2 is harvest at two leaf stage and 5 is harvest at five leaf stage.

[y] Total: all chemical comopounds quantitated in sage.

[z] ORAC: oxygen radical absorbing capacity(Trolox equivalent units/ ha).

Table 3.10 Main effect of harvest method on individual chemical compounds content(kg/ha) and oxygen radical absorbing capacity of sage.

	Content (kg/ha)						
Harvest method [x]	α-thujone	β-thujone	Camphor	Cineole	Camphene	Total [y]	ORAC [z]
Flail	19.45	2.75	14.10	9.78	2.52	54.91	$1.1*10^{9}$
Hand	31.40	4.64	23.10	15.40	3.29	85.13	$1.5*10^{9}$
$LSD_{0.05}$	6.28	0.84	5.35	3.40	0.51	15.09	$0.4*10^{9}$

[x] Harvest method: flail is flail type harvester and hand is hand harvest.

[y] Total: all chemical compounds quantitated in sage.

[z] ORAC: oxygen radical absorbing capacity(trolox equivalent units /ha).

Table 3.11 Interaction of harvest height and harvest method on total chemical compound content(kg/ha) of sage.

Harvest height [x]	Harvest method [y]	Content (kg/ha) Total [z]
2	Flail	60.37
5	Flail	49.45
2	Hand	104.37
5	Hand	65.88
$LSD_{0.05}$		10.68

[x] Harvest height : 2 is harvest at two leaf stage and 5 is harvest at five leaf stage.

[y] Harvest method: flail is flail type harvester and hand is hand harvest.

[z] Total: all chemical compounds quantitated in sage.

Table 3.12 Interaction of harvest height and drying method on chemical compounds content of sage.

Harvest height [x]	Drying method [y]	Content (kg/ha)		
		β-pinene	Cineole	Total [z]
2	Oven	2.07	13.80	74.7
5	Oven	1.81	10.25	57.09
2	Freeze	3.35	17.11	90.04
5	Freeze	1.85	9.38	58.24
$LSD_{0.05}$				
Overall		0.61	2.75	12.97
Drying method for the same harvest height		0.59	1.38	6.09

[x] Harvest height: 2 is harvest at two leaf stage and 5 is harvest at

[y] Drying method: oven is drying by heat and freeze is freeze drying.

[z] Total: all chemical comopounds quantitated in sage.

Table 3.13 Interaction of harvest height and harvest time on individual and total chemical compounds content (kg/ha) and oxygen radical absorbing capacity (ORAC units/ha) of sage.

Harvest height [w]	Time of harvest [x]	Content (kg/ha)						
		α–thujone	β–thujone	β–pinene	Camphor	Cineole	Total [y]	ORAC [z]
2	Early	3.36	0.44	0.45	1.54	0.24	8.87	$3.8*10^8$
2	Middle	2.04	0.32	0.11	2.03	0.75	5.89	$1.9*10^8$
2	Late	1.88	0.34	0.13	1.90	0.75	5.80	$1.7*10^8$
5	Early	2.20	0.30	0.28	0.94	0.14	5.57	$3.1*10^8$
5	Middle	4.24	0.60	0.22	3.80	0.14	11.61	$4.2*10^8$
5	Late	2.16	0.33	0.13	1.91	0.76	6.08	$2.0*10^8$
$LSD_{0.05}$								
Overall		0.80	0.10	0.24	0.50	0.40	1.70	$0.8*10^8$
Harvest time for the same harvest height		0.53	0.10	0.14	0.37	0.29	0.13	$0.7*10^8$

[w] Harvest height: 2 is harvest at the two leaf stage and 5 is harvest at five leaf stage.

[x] Harvest time: early is harvest before May 31, middle is between June 16 and August 1 and late is after August 9.

[y] Total: All chemical compounds quantitated in sage

[z] ORAC :Oxygen Radical Absorbing Capacity (trolox equivalent /ha)

Table 3.14 Interaction of harvest method and harvest time on individual and total amount of chemical compounds content (kg/ha) and oxygen radical absorbing capacity (ORAC units/ha) of sage.

Harvest method [w]	Harvest time [x]	Content (kg/ha)							
		α–thujone	β–thujone	Camphor	α–pinene	Cineole	Camphene	Total [y]	ORAC [z]
Flail	Early	1.74	0.20	0.62	0.24	1.34	0.17	4.71	$2.9*10^9$
	Middle	2.15	0.32	2.01	0.49	0.74	0.31	6.13	$2.0*10^9$
	Late	1.95	0.34	1.79	0.51	0.74	0.31	5.77	$1.9*10^9$
Hand	Early	3.82	0.52	1.85	0.45	2.40	0.32	9.73	$4.0*10^9$
	Middle	3.03	0.46	2.93	0.51	1.08	0.34	8.50	$2.9* 10^9$
	Late	2.05	0.33	2.01	0.48	0.77	0.33	6.11	$1.8* 10^9$
$LSD_{0.05}$									
Overall		0.85	0.12	0.58	0.24	0.41	0.15	1.76	$0.8*10^9$
Harvest time for same harvest method		0.53	0.09	0.37	0.14	0.29	0.09	1.29	$0.7*10^9$

[w] Harvest method: flail is flail type harvester and hand is hand harvest.

[x] Harvest time: early is harvest before May 31, middle is between June 16 and August 1 and late is after August 9.

[y] Total: all chemical comopounds quantitated in sage.

[z] ORAC: oxygen radical absorbing capacity(trolox quivalent/ha).

Table 3.15 Interaction of harvest method, harvest height and harvest time on α-thujone, camphor and total chemical compounds content (kg/ha) of sage.

Harvest method [w]	Harvest height [x]	Harvest time [y]	Content (kg/ha)		
			α–thujone	camphor	Total [z]
Flail	2	Early	1.90	0.68	5.20
	2	Middle	1.60	1.62	4.71
	2	Late	1.85	1.75	5.68
	5	Early	1.59	0.56	4.23
	5	Middle	3.78	3.17	10.41
	5	Late	2.06	1.83	5.85
Hand	2	Early	4.83	2.39	12.54
	2	Middle	2.49	2.43	7.07
	2	Late	1.92	2.05	5.92
	5	Early	2.82	1.32	6.91
	5	Middle	4.65	4.43	12.81
	5	Late	2.17	1.98	6.31
$LSD_{0.05}$					
overall			0.88	0.67	2.03
Same or different harvest time within the same harvest method and harvest height.			0.75	0.53	1.29

[w] Harvest method: flail is flail type harvester and hand is hand harvested.

[x] Harvest height: 2 is harvest at two leaf stage and 5 is harvest at five leaf stage.

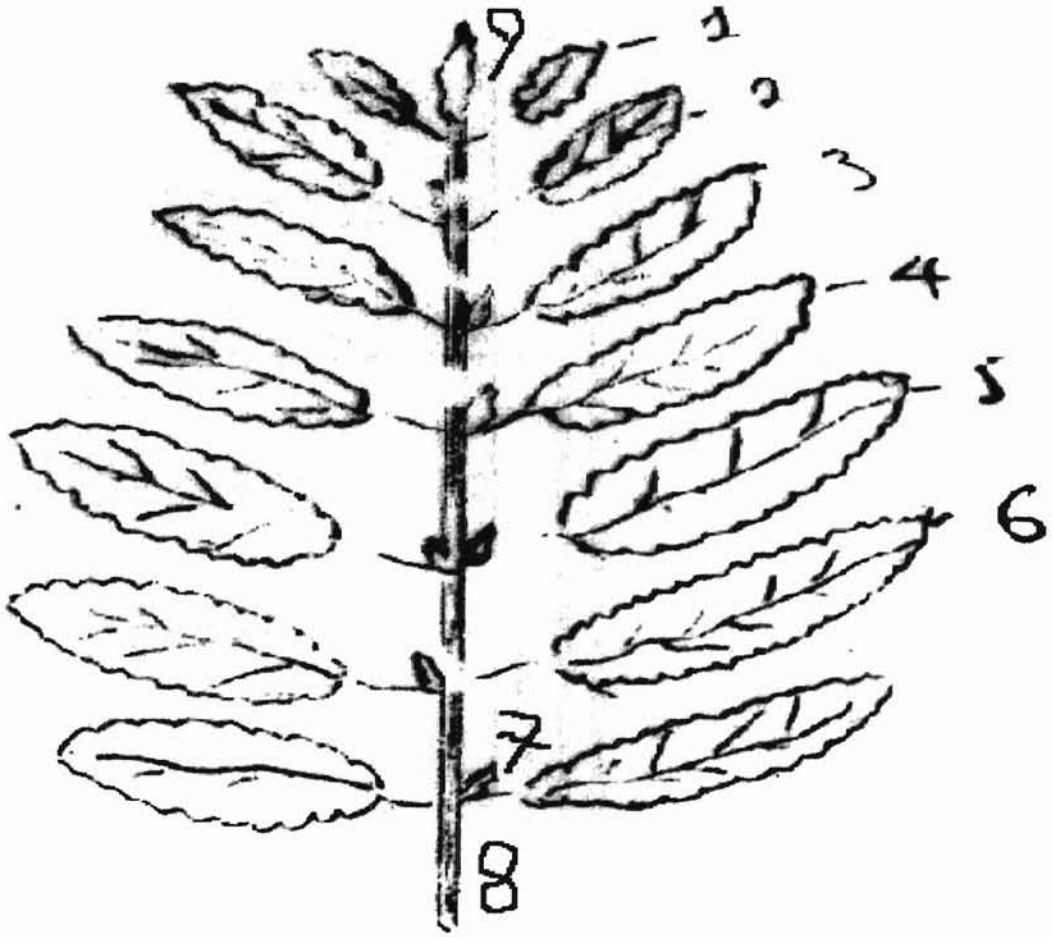
[y] Harvest time: early is harvest before May 31, middle is between June 16, and August 1 and late is after August 9.

[z] Total: all chemical compounds quantitated in sage.

Figure 3.1

Diagram for harvest height determination of sage.

Leaves numbered from 1-6 are fully expanded laves. Number 7denotes axillary buds, 8 the stem and 9 meristematic growth. Harvesting at two leaf stage follows regrowth the second pair of leaves and five leaf stage the fifth fully expanded leaves. In both cases the harvesting material includes the pair of leaves above the cutting point, the stem, meristematic growth and axilllary buds.



Appendix A

Distribution of chemical compounds and ORAC values among plant parts of sage.

	Percentage value								
Plant parts	a-thujone	b-thujone	Camphore	a-pinene	b-pinene	Cineole	Camphene	Total [x]	ORAC[y]
Leaf one	15.8	17.0	16.3	8.7	12.7	13.5	9.6	14.8	12.0
Leaf two	24.0	24.0	20.6	24.0	23.5	23.9	24.2	23.2	18.7
Leaf three	20.6	18.8	20.5	21.7	18.4	19.8	20.9	20.2	23.2
Leaf four	15.2	14.3	18.7	19.7	15.3	16.9	19.8	16.8	15.8
Leaf five	9.9	10.6	12.1	15.9	12.2	13.6	15.1	11.8	6.3
Leaf six	5.1	5.4	5.1	7.4	6.0	6.7	6.3	5.8	8.1
Axillary buds	1.5	1.8	1.3	0.5	0.8	1.0	0.4	1.2	2.1
Stem	3.4	3.0	1.5	0.7	6.5	0.1	2.1	2.3	11.0
Terminal buds	4.5	5.0	3.1	1.2	4.5	3.5	1.6	4.1	2.8
$LSD_{0.05}$									
Plant parts within a plant material	4.3	4.5	4.5	6.7	9.1	5.3	6.6	4.4	11.19

[x] Total :all chemical components quantitated in sage.

[y] ORAC: oxygen radical absorbing values (trolox equivalent/pant part).

* Percentage value is based on harvest up to six expanded leaf of a plant material.

Appendix B

Interaction of harvest method, harvest height, harvest time and drying method on concentration (mg/gm dry weight) of chemical compounds of sage.

Harvest method [w]	Harvest height [x]	Harvest time[y]	Drying method	Concentration (mg/gm)							
				α-Thujone	β-Thujone	Camphor	α -Pinene	β–pinene	Cineole	Camphene	Total[z]
Flail	2	Early	Freeze	1.82	0.22	0.81	0.21	0.33	1.17	0.18	4.58
	2	Middle		3.32	0.48	3.16	0.58	0.18	1.18	0.39	9.29
	2	Late		2.90	0.58	2.27	0.85	0.21	1.17	0.45	8.44
	5	Early		1.41	0.15	0.68	0.18	0.23	0.68	0.11	3.59
	5	Middle		3.20	0.40	2.53	0.69	0.17	0.98	0.36	8.32
	5	Late		3.49	0.53	2.89	0.76	0.20	1.14	0.41	9.33
	2	Early	Oven	1.21	0.17	0.71	0.18	0.17	0.87	0.17	3.49
	2	Middle		2.13	0.33	2.21	0.60	0.15	0.84	0.40	6.67
	2	Late		2.66	0.48	2.86	0.76	0.19	1.12	0.49	8.56
	5	Early		1.16	0.16	0.57	0.27	0.23	0.89	0.15	3.44
	5	Middle		2.26	0.40	1.98	0.64	0.14	0.77	0.36	6.55
	5	Late		2.91	0.49	2.66	0.82	0.17	1.07	0.46	8.58
Hand	2	Early	Freeze	3.93	0.60	2.17	0.45	0.33	2.25	0.33	10.06
	2	Middle		4.30	0.64	3.74	0.65	0.22	1.56	0.65	11.55
	2	Late		2.85	0.46	2.74	0.54	0.19	1.10	0.41	8.29
	5	Early		2.41	0.37	1.36	0.30	0.17	1.08	0.30	5.92
	5	Middle		3.06	0.40	2.95	0.40	0.14	0.93	0.40	8.14
	5	Late		3.67	0.54	3.17	0.93	0.26	1.37	0.93	10.52
	2	Early	Oven	3.09	0.48	2.25	0.36	0.22	1.85	0.36	8.51
	2	Middle		3.38	0.59	3.72	0.64	0.18	1.34	0.64	10.30
	2	Late		2.86	0.56	3.15	0.73	0.21	1.17	0.73	9.21
	5	Early		2.11	0.34	1.48	0.38	0.17	1.19	0.38	5.92
	5	Middle		3.53	0.55	3.28	0.74	0.17	1.27	0.74	9.96
	5	Late		2.95	0.43	2.75	0.76	0.17	1.06	0.76	8.56
$LSD_{0.05}$											
First				0.73	NS	0.68	NS	0.09	0.39	NS	1.91
Same or different harvest method, harvest date, drying method, and harvest height.				0.39	NS	0.59	NS	0.09	0.34	NS	1.93

[w] Harvest method: flail is flail harvest and hand is hand harvest.

[x] Harvest height: 2-harvesting at two leaf stage, 5- harvesting at five leaf stage.

[y] Harvest time: early harvest is before May 31, middle harvest is between June 16 and August 1 and late harvest is after August 9.

[z] Total : all chemical compods quantitated in sage.

VITA

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Master of Science

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