Distillation Time Modifies Essential Oil Yield, Composition, and Antioxidant Capacity of Fennel (*Foeniculum vulgare* Mill)

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Abstract: Fennel (*Foeniculum vulgare* Mill) is an essential oil crop grown worldwide for production of essential oil, as medicinal or as culinary herb. The essential oil is extracted via steam distillation either from the whole aboveground biomass (herb) or from fennel fruits (seed). The hypothesis of this study was that distillation time (DT) can modify fennel oil yield, composition, and antioxidant capacity of the oil. Therefore, the objective of this study was to evaluate the effect of eight DT (1.25, 2.5, 5, 10, 20, 40, 80, and 160 min) on fennel herb essential oil. Fennel essential oil yield (content) reached a maximum of 0.68% at 160 min DT. The concentration of trans-anethole (32.6–59.4% range in the oil) was low at 1.25 min DT, and increased with an increase of the DT. Alpha-phellandrene (0.9–10.5% range) was the lowest at 1.25 min DT and higher at 10, 80, and 160 min DT. Alpha-pinene (7.1–12.4% range) and beta-pinene (0.95–1.64% range) were higher in the shortest DT and the lowest at 80 min DT. Myrcene (0.93–1.95% range), delta-3-carene (2.1–3.7% range), cis-ocimene (0–0.23% range), and gamma-terpinene (0.22–2.67% range) were the lowest at 1.25 min DT and the highest at 160 min DT. In contrast, the concentrations of paracymene (0.68–5.97% range), fenchone (9.8–22.7% range), camphor (0.21–0.51% range), and cis-anethole (0.14–4.66% range) were highest at shorter DT (1.25–5 min DT) and the lowest at the longer DT (80–160 min DT). Fennel oils from the 20 and 160 min DT had higher antioxidant capacity than the fennel oil obtained at 1.25 min DT. DT can be used to obtain fennel essential oil with differential composition. DT must be reported when reporting essential oil content and composition of fennel essential oil. The results from this study may be used to compare reports in which different DT to extract essential oil from fennel biomass were used.

Key words: Aromatic plants, steam distillation, essential oil yield, trans-anethole, alpha-phellandrene, fenchone, alpha-pinene, paracymene

1 INTRODUCTION

Fennel (*Foeniculum vulgare* Mill) is a major essential oil crop grown for production of essential oil or as herb throughout the world; it is one of the principal essential oil crops in the temperate region¹. Current production of fennel essential oil is concentrated in Europe (Bulgaria, France, Germany, Italy, Romania, Hungary, Turkey, Ukraine, Russia, Yugoslavia), in some Asian countries (India, China), in South America (Argentina) and in the United States. Fennel is used as spice (seeds), edible herb (aboveground plant parts or the roots), and as medicinal herb. Fennel has been used as medicinal herb since ancient

times, especially in medical systems of various cultures around the Mediterranean region². For example, Bulgarian folk medicine has been utilizing fennel as baby colic remedy, for treating stomach pain, indigestion, as anesthetic, antispasmodic, carminative, for increasing milk supply in nursing mothers². Fennel oil is also used as an aromatic ingredient in the food, canning, and beverage industries. Indeed, fennel extract has shown some antioxidant properties^{3,4}, and anti-inflammatory properties⁵.

Commercially, fennel essential oil is obtained via steam distillation of the fresh herbage (all aboveground plant parts harvested and distilled at flowering stage), or from

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fennel seed (fruits). The chemical profile of fennel herb oil and fennel seed oil are different; most of the commercially available fennel oil is produced from fennel herbage.

The length of the distillation time (DT) was found to affect both essential oil yield and oil profile of some other aromatic plants such as Japanese cornmint (*Mentha canadensis* L.)⁶⁾, oregano (*Origanum vulgare* L.)⁷⁾, pine (*Pinus ponderosa* Dougl. ex Laws)⁸⁾, (*Mentha piperita* L.), lemongrass (*Cymbopogon flexuosus* Steud.) and palmarosa (*Cymbopogon martinii* Roxb.)⁹⁾, of female and of male Rocky Mountain juniper (*Juniperus scopulorum* Sarg.)^{10, 11)}, but DT effect for fennel was not reported. We hypothesized that DT can modify fennel oil yield, composition, and antioxidant capacity. If the hypothesis is correct, then perhaps it would be possible to obtain fennel oil with specific composition by modifying the DT. The objective of this study was to evaluate the effect of eight DT (1.25, 2.5, 5, 10, 20, 40, 80, and 160 min) on fennel herb essential oil, and to develop regression models that describe the relationship between DT and essential oil content, and the concentration and yield of its constituents.

2 MATERIALS AND METHODS

The fennel used in this study was grown in 2010 at the North Mississippi Research and Extension Center at Verona, MS (34° 43' 22" N and -88° 43' 22" W). Certified seeds from Bulgarian fennel cultivar 'Shumen' were used in this study. This cultivar is commercially grown for essential oil production in Bulgaria¹²⁾. Transplants were started in a greenhouse in January and transplanted out in the field in May 2010. Fennel transplants were transplanted at previously prepared raised beds, with drip tape irrigation tubing at 5-6 cm deep, running in the middle of each bed. Plants were spaced 45 cm apart and grown under irrigation. Phosphorus and potassium were provided prior to planting based on soil test reports. Nitrogen was applied at 120 kg/ha. Plants were harvested at flowering, by cutting with a hedge trimmer at 10-12 cm above the soil surface, and processed immediately for essential oil extraction.

2.1 Steam distillation and distillation times (DT)

Fresh fennel samples (400 g of above ground herbage, including stems, leaves, umbels with immature seeds), in 3 replicates, were extracted via steam distillation in 2-L steam distillation units as described previously⁹⁾. Briefly, the apparatus includes a 2 L pear shaped flask filled with water on a hotplate and a 2-L bioflask, which is positioned above. The bioflask contains the fennel herbage. The still-head is attached to the top of bioflask, and directs the steam to the condenser, which allows for the co-distilled steam and oil to simultaneously collect and separate in a collector, analogous to the Florentine vessel in large com-

mercial installations.

The DT evaluated in this experiment included eight different times: 1.25, 2.5, 5, 10, 20, 40, 80, and 160 min. All these DT were measured from the beginning of the distillation (the moment the first drop of essential oil was deposited). At the end of the DT, the power was turned off, the still head was removed, and the oil was separated from the water. The resulting essential oils were measured on an analytical scale, and kept at -5°C until GC/MS analyses were performed. The essential oil content (yield) was calculated as grams of oil per weight of fresh fennel herbage.

2.2 Gas chromatography (GC) analysis of the fennel essential oil

All fennel essential oil samples (in three replicates) were analyzed on a Hewlett Packard gas chromatograph 6890 GC with an auto sampler (carrier gas helium, 40 cm/sec, 11.7 psi (60°C), 2.5 ml/min constant flow rate; injection: split 60:1, 0.5 µL, injector temperature of 220°C; oven temperature program: 60°C for 1 min, 10°C/min to 250°C). The column was HP-INNOWAX (cross-linked PEG; 30 m × 0.32 mm × 0.5 µm), and the flame ionization detector (FID) temperature was 275°C.

2.3 The antioxidative capacity of the essential oil extract

The antioxidative capacity of the oil extracts was determined by the oxygen radical absorbance capacity (ORAC) method as described previously^{13, 14)}. Briefly, samples of extracted oil were prepared for antioxidant capacity tests by mixing 10 ± 1 mg oil with 1 mL of water, acetone (1:1) with 7% methyl-β-cyclodextrins (w:v). The test was prepared in a 96 well plate by first transferring 25 µL of 74 mM phosphate buffer saline (pH 7.4) to each well. The test sample (25 µL) or Trolox (25 µL) (which served as the standard) was added to different wells at concentration of 0.2, 0.4, 3.3, 6.5, 10, 13, 25, 50 µg/ml followed by 150 µL of fluorescein (8.16 × 10⁻⁵ mM). The samples were incubated at 37 °C for 10 min, with 3 min intermittent shaking (linear, at medium speed). After incubation, the reaction was activated by adding 153 mM 2, 2'-azobis(2-amidinopropane) hydrochloride (25 µL), i.e., the radical initiator, to each well. All samples/standards were prepared in 96 well plates and monitored with a BMG Labtech FLUOstar Optima microplate reader (Durham, NC). Fluorescence was measured every 1.5 min at an excitation and emission wavelength of 485 nm and 520 nm, respectively, until the decreasing fluorescence values plateaued. From these data, the area under the decay curve was calculated and the results are shown as µmole Trolox equivalents/g of extract. Each sample was tested in triplicate.

2.4 Statistical analyses

In total, 29 essential oil constituents were identified; of these, 12 constituents with the highest concentration were

selected for statistical analyses. The effect of distillation time on essential oil content (%), and the concentrations (%) and yield (mg) of alpha-phellendrene, trans-anethole, alpha-pinene, beta-pinene, myrcene, paracymene, delta-3-carene, cis-ocimene, gamma-terpinene, fenchone, camphor, and cis-anthole was determined using a one-way analysis of variance. For each response, the validity of model assumptions was verified by examining the residuals as described previously¹⁵. The effect of distillation time was significant (p-value < 0.05) on essential oil content, all concentrations, and all yields except paracymene and cis-anthole. Subsequently, multiple means comparison was completed for all responses significantly affected by distillation time using Duncan's multiple range test at the 5% level of significance, and letter groupings were generated. The analysis was completed using the GLM Procedure of SAS¹⁶.

Regression analysis to determine the relationship between distillation time and the concentration of each of the constituents suggested that seven of them (essential oil content, and the concentration of paracymene, delta-3-carene, gamma-terpinene, fenchone, camphor, and cis-anthole) can be adequately modeled using the Power (Eq. 1) model, and three of them (the concentration of trans-anethole, alpha-pinene, and beta-pinene) can be adequately modeled using the second order polynomial (Eq. 2) model. There was no clear relationship between distillation time and the concentration of myrcene and cis-ocimene. The relationship between distillation time and the yields of all ten constituents (alpha-phellendrene, trans-anethole, alpha-pinene, beta-pinene, myrcene, delta-3-carene, cis-ocimene, gamma-terpinene, fenchone, and camphor) were adequately modeled by the Power (Eq. 1) model. While the Power model is nonlinear, the second order polynomial model is linear. The parameters of the nonlinear model were estimated iteratively using the NLIN Procedure of SAS¹⁶.

$$Y = \theta_1 x^{\theta_2} + \varepsilon \quad (\text{Eq. 1})$$

$$Y = \beta_0 + \beta_1 x + \beta_2 x^2 + \varepsilon \quad (\text{Eq. 2})$$

Where Y is the dependent (response) variable, x is the independent (distillation time) variable, and the error term ε is assumed to have normal distribution with constant variance.

3 RESULTS

The following constituents were identified in fennel oil: alpha-thujene, alpha-pinene, camphene, sabinene, beta-pinene, myrcene, alpha-phellendrene, alpha-terpinene, paracymene, delta-3-carene, cis-ocimene, gamma-terpinene, fenchone, linalool, trans-pinene hydrate, camphor, trans-verbenol, 4-terpineol, estragole, fenchyl acetate, cis-anethole, anisaldehyde-para, dimethyl acetal, trans-anethole, para-anisyl methyl Ketone, germacrene-D, trans-methylisoeugenol, delta-cadinene, and 1-(4-methoxy phenyl)-1-propanone. Of these, only the constituents with concentrations greater than 1% of the oil were statistically analyzed.

Fennel essential oil content increased with increase of DT and reached a maximum of 0.68% at 160 min DT (Table 1, Fig. 1). The concentration of alpha-phellandrene (0.9-10.5% concentration range in the oil) was the lowest at 1.25 min DT and higher at 10, 80, and 160 min DT. The concentration of the main constituent trans-anethole (32.6 - 59.4% range) was low at 1.25 min DT, generally increased with an increase of the DT at 40-160 min DT. The concentrations of alpha-pinene (7.1-12.4% of the oil) and beta pinene (0.95-1.64% range) were higher in the shortest DT and the lowest at 80 min DT. However, the concentrations of both constituents at 160 min DT were not different from their respective concentrations at 1.25 or 2.5 min DT. The

Table 1 Mean essential oil (EO) content (%), and the concentrations (%) of alpha-phellendrene, trans-anethole, alpha-pinene, beta-pinene, myrcene, and paracymene obtained from the 8 distillation times (DT).

DT (min)	EO content	Alpha-phellendrene	Trans-anethole	alpha-pinene	beta-pinene	myrcene	paracymene
1.25	0.06 f	0.90 b	32.58 d	11.8 ab	1.50 ab	0.93 c	5.97 a
2.5	0.11 ef	7.58 ab	39.25 cd	12.4 a	1.64 a	1.65 ab	4.67 ab
5	0.14 e	5.01 ab	42.15 cd	10.3 abc	1.37 abc	1.31 bc	3.27 abc
10	0.25 d	10.10 a	46.51 bc	8.7 abc	1.17 bc	1.44 b	1.80 bc
20	0.29 d	4.71 ab	50.30 abc	8.4 bc	1.19 abc	1.21 bc	3.42 abc
40	0.40 c	7.70 ab	55.03 ab	8.2 bc	1.17 bc	1.38 b	2.20 bc
80	0.52 b	10.49 a	59.39 a	7.1 c	0.95 c	1.57 ab	1.02 c
160	0.68 a	10.03 a	52.69 ab	11.9 ab	1.42 ab	1.95 a	0.68 c

Within each column, means sharing the same letter are not significantly different at the 5% level.

Fig. 1 Plot of Distillation time (DT) vs. Essential oil content, and the concentration of 9 constituents along with the fitted Power and second-order polynomial regression models. Equations of the fitted models are shown within each plot.

Table 2 Mean concentrations (%) of delta-3-carene, cis-ocimene, gamma-terpinene, fenchone, camphor, and cis-anthole obtained from the 8 distillation times (DT).

DT (min)	delta-3-carene	cis-ocimene	gamma-terpinene	fenchone	camphor	cis-anthole
1.25	2.08 c	*	0.22 c	22.7 a	0.51 a	4.66 a
2.5	2.71 bc	0.14 b	0.75 c	20.8 ab	0.45 ab	1.46 ab
5	2.60 bc	0.12 b	0.53 c	22.0 ab	0.46 ab	0.69 ab
10	2.99 ab	0.13 b	1.03 bc	20.3 ab	0.44 ab	0.24 b
20	2.72 bc	0.09 b	0.64 c	16.6 bc	0.36 bc	0.83 ab
40	2.85 bc	0.13 b	1.02 c	12.9 cd	0.28 cd	0.43 ab
80	3.23 ab	0.15 b	1.89 ab	9.8 d	0.21 d	0.17 b
160	3.72 a	0.23 a	2.67 a	10.6 d	0.23 d	0.14 b

Within each column, means sharing the same letter are not significantly different at the 5% level.

Table 3 Mean yield (mg) of alpha-phellendrene, trans-anethole, alpha-pinene, beta-pinene, myrcene, delta-3-carene, cis-ocimene, gamma-terpinene, fenchone, and camphor obtained from the 8 distillation times (DT). The effect of DT on the yields of paracymene and cis-anthole was not significant (p-values 0.227 and 0.771 respectively), and their overall means were 5.87 mg and 2.19 mg respectively.

DT (min)	Alpha-phellendrene	Trans-anethole	alpha-pinene	beta-pinene	myrcene	delta-3-carene	cis-ocimene	Gamma-terpinene	fenchone	camphor
1.25	0.7 c	15.62 e	7.3 d	0.93 d	0.6 f	1.3 e	NA	0.17 d	14.1 c	0.32 c
2.5	9.2 c	35.01 de	13.7 cd	1.81 cd	1.4 ef	3.0 de	0.16 c	0.85 d	22.9 c	0.50 bc
5	6.9 c	60.10 d	14.7 cd	1.96 cd	1.9 de	3.7 d	0.16 c	0.63 d	31.5 bc	0.45 c
10	25.5 c	117.40 c	21.9 bc	2.93 bc	3.6 c	7.6 c	0.33 c	2.60 cd	51.2 ab	1.10 ab
20	13.5 c	146.37 c	24.2 bc	3.45 bc	3.5 cd	7.9 c	0.27 c	1.76 cd	48.4 ab	1.06 ab
40	29.6 bc	218.49 b	31.9 b	4.59 b	5.5 bc	11.3 c	0.50 bc	3.89 c	51.3 ab	1.11 a
80	54.5 ab	306.01 a	36.1 b	4.83 b	8.1 b	16.7 b	0.75 b	9.73 b	50.5 ab	1.09 ab
160	68.6 a	325.34 a	80.8 a	9.61 a	13.2 a	25.2 a	1.58 a	17.99 a	70.8 a	1.56 a

Within each column, means sharing the same letter are not significantly different at the 5% level.

concentrations of myrcene (0.93-1.95% range), delta-3-carene (2.1-3.7% range), cis-ocimene (0-0.23% range), and gamma-terpinene (0.22-2.67% range) were the lowest at 1.25 min DT and the highest at 160 min DT.

In contrast, the concentrations of paracymene (0.68-5.97% range), fenchone (9.8-22.7% range), camphor (0.21-0.51% range), and cis-anethole (0.14-4.66% range) were highest at shorter DT (1.25-5 min DT) and the lowest at the longest (80-160 min) DT (Table 1, Table 2, Fig. 1).

However, the yield of individual constituents (that was calculated from the essential oil yield and the concentration of the respective constituent in the oil) increased with an increase of DT and reached maximum at 160 min DT (alpha-pinene, beta-pinene, myrcene, delta-3-carene, cis-ocimene, gamma-terpinene) or at 10-160 min DT (fenchone and camphor) (Table 3, Fig. 2).

The Power and second order polynomial regression models shown in Fig. 1 and Fig. 2 describe the relationship between DT and the corresponding response variable very well; and the fitted models shown within each plot can be

used to predict the values of the response variable at any given DT. While the magnitude and signs of the coefficients for the polynomial model are not physically meaningful, the sign of the second parameter of the Power model (e.g., 0.416 for essential oil content, Fig. 1) indicate whether it is increasing (positive) or decreasing (negative); and its magnitude indicate the rate of increase or decrease.

DT also had an effect on antioxidant capacity of the fennel oil; fennel oils from the 20 and 160 min DT had higher antioxidant capacity than the fennel oil obtained at 1.25 min DT (Table 4).

4 DISCUSSION

The results from this study confirmed the hypothesis that DT has significant effect on fennel essential oil content, composition and antioxidant capacity. Essential oil composition of fennel in this study was similar to previous reports¹⁷⁻²⁴. In particular¹⁷, and²³ used the same cul-

Fig. 2 Plot of Distillation time (DT) vs. the yield (mg) of 10 constituents along with the fitted Power regression model. Equations of the fitted models are shown within each plot.

Table 4 Antioxidant capacity of fennel oils from 1.25 min, 20 min, and 160 min distillation times (DT).

DT (min)	Antioxidative capacity (μM Trolox/g)
1.25	131 b
20	164 a
160	161 a

Means followed by the same letter are not significantly different at the 5% level.

tivar of fennel as in this study, and reported similar essential oil composition to the one from this study. For example, the main oil constituents in this study were trans-anethole, fenchone, alpha-phellandrene, alpha-pinene, whereas the main constituents in fennel oil in literature report¹⁷⁾ were anethole, fenchone, methyl chavicol, alpha-phellandrene, alpha-pinene. Also, anethole concentration in this study varied from 33 to 53% of the oil, while in literature report¹⁷⁾ anethole concentration varied between 40 and 80% of the oil. On the other hand, literature report²¹⁾ found 35-82% anethole concentration range in fennel seed oils obtained from five different countries. However, generally the fennel seed and fennel herb oil may have dissimilar composition.

Fennel essential oil composition depends on various factors among which are genotype^{18, 19, 24)}, cultivar and harvest stage^{17, 25)}, nitrogen application¹⁸⁾, origin²¹⁾, type of extraction²³⁾ to name a few.

According to²⁶⁾, methyl-chavicol in fennel oil should be less than 10% of the oil and fenchone should be less than 7.5%. The concentration of methyl-chavicol (estragole) in this study was 1.98-2.5% (data not shown), whereas the concentration of fenchone was 9.8-23% (Table 1). Longer DT significantly reduced the concentration of fenchone in the oil.

The antioxidative capacity obtained at 20 min DT was significantly higher (164 μM Trolox/g) than that obtained at 1.25 min DT (131 μM Trolox/g). This could be due in part to the substantially lower levels of compounds obtained from the 1.25 min DT (0.06) compared to that from the 20 min DT (0.29) and 160 min DT (0.68). Although the total amounts of essential oil contents were different for the 20 min and 160 min DT, the antioxidative responses from these distillation times were not significantly different (161-164 μM Trolox/g). The similar levels of alpha-pinene, and beta-pinene may be acting together in combination, with the main contributor being trans-anethole as this compound is relatively high in both the 20 and 160 DT.

This study demonstrated that DT can significantly alter fennel essential oil composition. Therefore, DT must be taken into consideration when comparing literature report where different distillation times have been used.

5 CONCLUSIONS

For high essential oil content, fennel biomass needs to be distilled for 160 min. For high trans-anethole oil, fennel biomass should be distilled for 80 min. For low concentration of fenchone, fennel biomass needs to be distilled for 80-160 min.

DT can be used to obtain fennel essential oil with differential composition. DT can also change the antioxidant capacity of fennel oil. DT must be reported when reporting essential oil content and composition of fennel essential oil. The results from this study will help when comparing reports in which different DT were used to extract essential oil from fennel biomass.

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