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Effect of Distillation Time on *Mentha canadensis* Essential Oil Yield and Composition

Valtcho D. Zheljazkov[1]
University of Wyoming, Sheridan Research and Extension Center, 663 Wyarno Road, Sheridan, WY 82801

Tess Astatkie
Nova Scotia Agricultural College, Department of Engineering, 50 Pictou Road, P.O. Box 550, Truro, Nova Scotia B2N 5E3, Canada

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Abstract. **Japanese cornmint (*Mentha canadensis* L.) is a major essential oil crop grown in Asia, South America, and to a limited extent in eastern Europe. Japanese cornmint oil is the only commercially viable source for crystalline menthol. We hypothesized that the length of the distillation time (DT) will have an effect on Japanese cornmint essential oil content and composition. Therefore, the objective was to evaluate the effect of eight DTs (1.25, 2.5, 5, 10, 20, 40, 80, and 160 min) on essential oil content and composition. The essential oil content (0.43% to 1.06% range) reached maximum at 10 min DT; further increase in DT did not significantly increase essential oil content. The concentrations of alpha-pinene (0.14% to 0.76% range), beta-pinene (0.23–0.81), 3-octanal (0.19–0.34), limonene (0.69% to 1.53%), eucalyptol (0.06% to 0.12%), isopulegone (0.42% to 0.56%), and isomenthone (4.4% to 5.7%) were highest at 1.25 to 5 min DT and generally decreased to their respective minimums at 160 min DT. The concentration of menthone (4.3% to 6.3%) was highest at 1.25 min DT, decreased at 2.5 min, and was lowest at 10 to 160 min DT. The concentration of piperitone (0.98% to 1.27%) was lowest at 1.25 min DT and higher at 5 to 40 min DT compared with other DTs. The concentration of menthol (74% to 79%) was low at 1.25 min, then increased at 10 to 80 min DT, and reached a maximum at 160 min DT. Generally, the yield of most individual essential oil constituents was lower at 1.25 min DT relative to the other DT and reached maximum at 10 to 20 min DT. The yield of menthol was low at 1.25 to 2.5 min DT and rose at 5 min to 160 min DT. The results suggest that different DTs can be used to maximize recovery of certain constituents. Also, the results demonstrated that there are no oil yield gains after 20 min DT, which is much lower than the usual distillation time of 60 min or more. This study can be used as a reference when comparing reports in which different DTs were used.**

A synonym of *Mentha canadensis* L. is *Mentha arvensis* L. Japanese cornmint and its commercial varieties are natural hybrids and are propagated exclusively vegetatively (Topalov, 1989). Japanese cornmint includes two cytotypes, one with $2n = 72$ (*M. arvensis*) and the other with $2n = 96$ chromosomes (*M. canadensis*) (Gobert et al., 2002). However, the two names have been and continue to be used interchangeably by many authors. Most authors from Asian countries continue to designate Japanese cornmint as *M. arvensis*.

Japanese cornmint is considered a subtropical plant and it is grown widely in India, Vietnam, China, in some south American countries (Brazil), and in a smaller acreage in eastern Europe (Bulgaria, Romania) (Chand et al., 2004; Clark, 1998, Lawrence, 2007; Topalov and Zheljazkov, 1991; Zheljazkov et al., 1996a).

Japanese cornmint essential oil is rich in menthol and the species is currently the only commercial source for the production of natural menthol (Clark, 1998; Lawrence, 2007). Japanese cornmint essential oil, crystalline menthol, and the dementholized oil are some of the most widely used flavor and fragrance agents in beverages and in the food, pharmaceutical, flavor, and fragrance industries (Clark, 1998; Chand et al., 2004; Galeotti et al., 2002; Mint Industry Research Council, 2011; Shrivastava et al., 2002).

The United States has not been and does not currently produce Japanese cornmint despite some incorrect reports (Singh and Saini, 2008). However, the United States is one of the largest importers of crystalline menthol and dementolized oil from Japanese cornmint. As a result of inconsistent supply and quality of these products, the U.S. industry and individual peppermint producers are interested in domestic production of Japanese cornmint. The United States is a major producer of peppermint and spearmint [National Agricultural Statistics Service (NASS), 2011], species closely related to Japanese cornmint. For 2011, the values of peppermint and spearmint oils produced in the United States were $128 million and $45 million, respectively (NASS, 2011). The need for domestic production of Japanese cornmint has triggered some recent studies in Mississippi (Zheljazkov et al., 2010a, 2010b) and in Wyoming (Zheljazkov, 2011, unpublished data). This recent research clearly demonstrated Japanese cornmint could be a viable crop in the United States, from the humid hot environment in Mississippi to the dry, short-season climate in Wyoming.

Japanese cornmint oil content and composition may be affected by a number of factors such as fertilization (Zheljazkov et al., 2010a), methods of propagation (Zheljazkov et al., 1996a) and harvesting stage (Topalov and Zheljazkov, 1991; Zheljazkov et al., 2010b).

We hypothesized that the length of the DT will have an effect on Japanese cornmint essential oil content and composition. Indeed, various authors used different DTs for extraction of Japanese cornmint, ranging from 60 min (Zheljazkov et al., 2010a, 2010b) to 180 min (Phatak and Heble, 2002). The objective of this study was to evaluate the effect of eight different DTs (1.25, 2.5, 5, 10, 20, 40, 80, and 160 min) on essential oil content and composition of Japanese cornmint and develop a regression model that describes their relationship.

Materials and Methods

Steam distillation and distillation times. Japanese cornmint cultivar Arvensis 2 was used in this study. A Japanese cornmint plantation with 'Arvensis 2' was established at the North Mississippi Research and Extension Center in the spring of 2007 using a certified virus-free material, as described in Zheljazkov et al. (2010a), and was maintained as perennial thereafter. The plant material used in this study was harvested in late July 2010 at flowering, when the essential oil is highest (Zheljazkov et al., 2010b). Plants were harvested at 5 or 6 cm above the soil surface, the fresh yield recorded, and the plants were dried at 35 to 40 °C (Zheljazkov et al., 2010b).

Each distillation subsample consisted of 200 g of dried shoots (including stems, leaves, and inflorescences). The essential oil was extracted using the traditional steam distillation process in 2-L steam distillation units (Gawde et al., 2009). Eight different DTs were evaluated, 1.25, 2.5, 5, 10, 20, 40, 80, and 160 min, all in three replicates. The beginning of each DT was measured from the moment when the first drop of essential oil was deposited in the Florentine (a separator vessel). At the end of each DT, the electric power was turned off, and the oil was immediately collected. The oil samples were measured on analytical balance; the oil content (yield) was calculated as grams of oil per 100 g of dried Japanese cornmint

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[1]To whom reprint requests should be addressed; e-mail vjeliazk@uwyo.edu; valtcho.pubs@gmail.com.

shoots. The term "content" is traditionally used to denote the concentration of essential oil in 100 g biomass. The term "concentration" is used to denote the concentration of individual oil constituents as percentage of the total oil. The yield of the various constituents was calculated from the essential oil content (yield) and the concentration of individual constituents in the oil. The oil samples were kept in a freezer at –5 °C until the compositional analyses.

Gas chromatography analysis of essential oil. The essential oil samples of Japanese cornmint (all in three replicates per DT) were analyzed on a Hewlett Packard gas chromatograph 6890 GC with an autosampler [carrier gas helium, 40 cm·sec^{-1}, 11.7 psi (60 °C), 2.5 mL·min^{-1} constant flow rate; injection: split 60:1, 0.5 µL, inlet 220 °C; oven temperature program: 60 °C for 1 min, 10 °C/min to 250 °C]. The column was HP-INNOWAX (crosslinked polyethylene glycol; 30 m × 0.32 mm × 0.5 µm), and the flame ionization detector temperature was 275 °C.

Statistical analysis. The effect of distillation time on essential oil content and the concentration and yield of alpha-pinene, sabinene, beta-pinene, myrcene, 3-octanal, limonene, eucalyptol, isopulegon, menthone, isomenthone, menthol, piperitone, menthyl acetate, and beta bourbonene were determined using a one-way analysis of variance. For each response, the validity of model assumptions, namely normal distribution, constant variance, and independence of the error terms, was verified by examining the residuals. Specifically, 1) by constructing normal probability plot of the residuals and running a test for normality to verify the normal distribution assumption; 2) plotting the residuals vs. the fitted values to verify the constant variance assumption; and 3) independence was assured by randomizing the run orders when conducting the experiment (Montgomery, 2009). Because the effect of DT was significant ($P < 0.05$) on all responses, multiple means comparison was completed 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 SAS (SAS Institute Inc., 2008).

The most appropriate model (Bates and Watts, 2007) that described the relationship between DT and essential oil content was asymptotic regression (Eq. 1), whereas the model that describes the relationship between DT and the concentrations of 3-octanal, menthone, isomenthone, and menthol was the power regression model (Eq. 2). The relationship between the concentration of the other constituents and the yields of all constituents was described by a third-order polynomial (Eq. 3). While the third-order polynomial model is linear, the other two, asymptotic and power models, are nonlinear and required iterative estimation of the parameters (Bates and Watts, 2007) using the NLIN Procedure of SAS (SAS Institute Inc., 2008). The figures as well as the third-order polynomial fits were completed using Minitab 16 software (Minitab, State College, PA).

$$Y = \theta_1 - \theta_2(\exp(-\theta_3 x)) + \varepsilon \quad (1)$$

$$Y = \theta_1 x^{-\theta_2} + \varepsilon \quad (2)$$

$$Y = \beta_0 + \beta_1 x + \beta_2 x^2 + \beta_3 x^3 + \varepsilon \quad (3)$$

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

Results

Effect of the length of the distillation time on the essential oil content and the concentration of individual oil constituents as percentage from total oil. The essential oil content (0.43% to 1.06% range) was low at the shortest DT (1.25 min) and increased significantly with increase in DT to reach maximum at 10 min DT (Table 1; Fig. 1). Further increase in DT beyond 10 min did not significantly increase the essential oil content. The concentrations of alpha-pinene (0.14% to 0.76% range), beta-pinene (0.23–0.81), 3-octanal (0.19–0.34), limonene (0.69% to 1.53%), eucalyptol (0.06% to 0.12%), isopulegone (0.42% to 0.56%), and isomenthone (4.4% to 5.7%) were high at the initial shorter DT (1.25–5 min) and generally decreased to reach their respective minimum concentrations at the longest DT (160 min) (Table 1; Fig. 1). The concentration of sabinene (0.06% to 0.32%) was high at 1.25 to 40 min DT, lower at 80 min relative to the shorter DT, and the lowest at 160 min DT. The concentration of menthone (4.3% to 6.3%) was highest at 1.25 min DT, decreased at 2.5 min, and was lowest at 10 to 160 min DT. The concentration of myrcene (0.23% to 0.45% range) was high at 2.5 to 80 min DT and lower at 160 min DT relative to the other DTs. The concentration of piperitone (0.98% to 1.27% range) was lowest at 1.25 min DT and then increased at 5 to 40 min DT. The concentration of menthol, the major oil constituent, was 74.3% at 1.25 min, then increased to 77.2% to 77.6% at 10 to 80 min DT, and reached a maximum of 79% at 160 min DT (Table 1; Fig. 1).

Effect of distillation time on the yield of essential oil constituents. Generally, the yield of most individual essential oil constituents was low at 1.25 min DT, increased, and reached maximum at 10 min DT and did not change until 80 min DT (Table 2). At 160 min DT, the yields decreased relative to the 80 min DT (Fig. 2). The yield of menthol was low at 1.25 to 2.5 min DT and higher at 5 to 160 min DT relative to the shortest DT (Table 2).

Relationship between distillation time and the concentration and yield of essential oil constituents. The fitted regression model shown in Figure 1 suggests that the relationship between DT and essential oil content can be modeled by the asymptotic regression model (Eq. 1) and that the potentially maximum value of essential oil content is predicted to be 1.03%. The relationships between DT and the concentrations of 3-octanal, menthone, isomenthone,

Table 1. Mean essential oil (EO) content (%) and the concentrations (%) of alpha-pinene, sabinene, beta-pinene, myrcene, 3-octanal, limonene, eucalyptol, isopulegon, menthone, isomenthone, menthol, piperitone, and beta bourbonene obtained from the eight distillation times (DTs).

DT (min)	EO content (%)	Alpha-pinene (%)	Sabinene (%)	Beta-pinene (%)	Myrcene (%)	3-octanal (%)	Limonene (%)	Eucalyptol (%)	Isopulegon (%)	Menthone (%)	Isomenthone (%)	Menthol (%)	Piperitone (%)	Beta bourbonene (%)
1.25	0.43 d[z]	0.76 a	0.32 a	0.81 a	0.32 bc	0.34 a	1.53 a	0.12 a	0.56 a	6.30 a	5.72 a	74.3 d	0.98 d	0.26 a
2.5	0.63 c	0.67 ab	0.31 a	0.73 ab	0.42 ab	0.28 b	1.39 ab	0.10 ab	0.55 ab	5.37 b	5.27 b	75.4 cd	1.12 c	0.24 ab
5	0.83 b	0.59 ab	0.27 ab	0.64 b	0.45 a	0.24 c	1.25 bc	0.08 b	0.53 ab	4.81 bc	4.96 c	76.7 bc	1.25 ab	0.22 bcd
10	1.04 ab	0.55 bc	0.26 ab	0.61 bc	0.44 ab	0.23 c	1.20 bc	0.08 bc	0.51 bc	4.57 c	4.83 cd	77.2 b	1.27 a	0.21 d
20	1.05 a	0.53 bc	0.25 ab	0.60 bc	0.45 a	0.22 cd	1.19 bc	0.08 bc	0.50 cd	4.40 c	4.72 de	77.1 b	1.27 a	0.22 cd
40	1.05 a	0.48 bc	0.23 ab	0.56 bc	0.45 a	0.21 cd	1.16 bc	0.06 c	0.47 de	4.52 c	4.67 de	77.1 b	1.18 abc	0.23 bcd
80	1.06 a	0.37 c	0.19 b	0.46 c	0.38 ab	0.21 cd	1.04 c	0.08 bc	0.45 ef	4.53 c	4.56 ef	77.6 b	1.16 bc	0.24 abc
160	0.93 ab	0.14 d	0.06 c	0.23 d	0.23 c	0.19 d	0.69 d	ND	0.43 f	4.32 c	4.41 f	79.0 a	1.12 c	0.24 ab

[z]Means followed by the same letter are not significantly different at the 5% level of significance using Duncan's multiple range test.
ND = not detected.

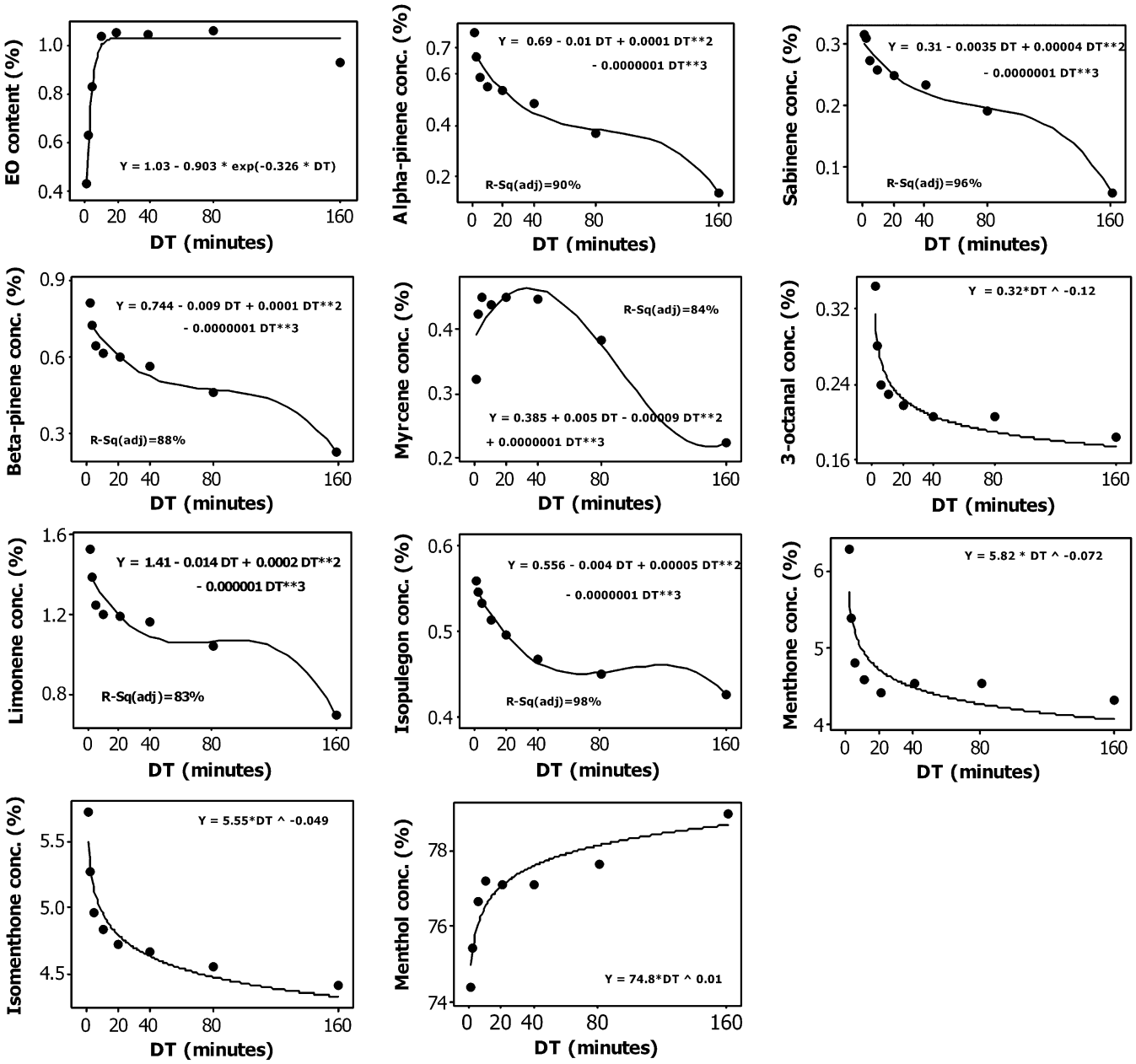

Fig. 1. Plots of essential oil (EO) content and the concentrations of 10 constituents vs. distillation time along with the fitted asymptotic, power, and third-order polynomial regression models. Equations of the fitted models are shown within each plot. Adjusted R^2 values are shown only for the linear regression model.

and menthol (Fig. 1) were adequately described by the power regression model (Eq. 2). The fitted models also indicate that at DT of 1 min, the predicted concentrations of 3-octanal, menthone, isomenthone, and menthol are 0.32%, 5.82%, 5.55%, and 74.8%, respectively. Figures 1 and 2 show that the relationships between DT and the concentration of the other constituents as well as the yields of all constituents can be modeled by the third-order polynomial regression model (Eq. 3). However, because the adjusted R^2 (percentage of the variability in Y explained by the model, adjusted for the number of parameters in the model) values of the third-order polynomial regression models were only moderate, the fitted models are not shown in Figure 2.

Concluding Discussion

Generally, menthol concentration of the oil in this study was higher than that in some previous reports (Murray et al., 1972, data for Michigan; Pandey et al., 2003; Zheljazkov et al., 1996a, 1996b) and similar to other reports (Murray et al., 1972, data for Indiana; Zheljazkov et al., 2010a). For example, Murray et al. (1972) reported that menthol concentration in their study ranged from 48% to 71%, and in very few hybrids, it reached 74%. Pandey et al. (2003) reported menthol concentration of 71%. Zheljazkov et al. (1996a) reported menthol concentration range of 58% to 64% for two cultivars of Japanese cornmint, whereas Zheljazkov et al. (1996b) reported menthol concentration from 58% to 61% for one cultivar of Japanese cornmint. Furthermore, Zheljazkov et al. (2010a) reported that menthol concentration in two Japanese cornmint cultivars was ≈50% in 2007; however, in 2008, the menthol concentration was 67% to 76% in 'Arvensis 2' and 73% to 78% in 'Arvensis 3'. One needs to keep in mind that these reports were based on different cultivars or hybrids of Japanese cornmint grown in various environmental conditions.

Results from this study confirmed the hypothesis that DT affects essential oil content and composition of Japanese cornmint. The results suggest that DT could be used as an economical method for producing oil with dissimilar composition from the same biomass.

Table 2. Mean yields (mg) of alpha-pinene, sabinene, beta-pinene, myrcene, 3-octanal, limonene, isopulegon menthone, isomenthone, menthol, piperitone, menthyl acetate, and beta bourbonene obtained from the eight distillation times (DTs).

DT (min)	Alpha-pinene (mg)	Sabinene (mg)	Beta-pinene (mg)	Myrcene (mg)	3-octanal (mg)	Limonene (mg)	Isopulegon (mg)	Menthone (mg)	Isomenthone (mg)	Menthol (mg)	Piperitone (mg)	Menthyl acetate (mg)	Beta bourbonene (mg)
1.25	6.6 b[z]	2.8 bc	7.1 bc	2.9 d	3.0 b	13.2 b	4.8 c	49.4 c	49.4 c	639 b	8.5 e	49.9 c	2.2 d
2.5	9.5 ab	4.3 ab	10.1 ab	5.5 bc	3.7 ab	18.9 ab	6.9 bc	66.5 bc	66.5 bc	921 b	13.6 de	69.2 bc	3.0 cd
5	9.3 ab	4.4 ab	10.2 ab	7.0 ab	4.0 ab	19.9 a	8.9 ab	83.0 ab	83.0 ab	1272 a	20.1 bc	94.6 ab	3.7 bc
10	12.0 a	5.7 a	13.4 a	9.5 a	5.0 a	25.7 a	10.8 a	101.0 a	101.0 a	1595 a	26.4 ab	112.5 a	4.5 ab
20	11.1 a	5.2 a	12.5 a	9.3 a	4.5 a	24.8 a	10.5 a	100.0 a	100.0 a	1628 a	26.9 a	117.8 a	4.4 ab
40	10.8 a	5.1 a	12.4 a	9.6 a	4.5 a	25.0 a	10.1 a	98.3 a	98.3 a	1614 a	25.2 ab	117.6 a	4.7 ab
80	8.9 ab	4.6 ab	10.8 a	8.8 a	4.5 a	23.5 a	9.8 a	97.4 a	97.4 a	1635 a	25.5 ab	119.1 a	5.0 la
160	2.9 c	1.6 c	4.6 c	4.4 cd	3.2 b	12.9 b	7.1 bc	74.5 b	74.5 b	1325 a	18.5 cd	93.8 ab	4.1abc

[z]Means followed by the same letter are not significantly different at the 5% level of significance using Duncan's multiple range test.

Second, the results demonstrated that there are no oil content (yield) gains after 20 min DT, which is only one-third of the usual distillation time of 60 min (Topalov and Zheljazkov, 1991; Zheljazkov et al., 2010a, 2010b) and one-ninth of that in other reports (Phatak and Heble, 2002). Reducing DT would increase the capacity output and save energy and time at the producer level. Although the maximum menthol concentration in the oil was achieved at 160 min DT, the maximum menthol yield was reached much earlier, at 10 to 20 min DT. These results are relevant to the distillation of dried Japanese cornmint biomass. Usually, when Japanese cornmints, peppermint, or spearmints are harvested, they are left to dry for 3 to 5 d out in the field before oil extraction.

Third, the findings of this study demonstrated that DT must be taken into consideration when comparing reports on Japanese cornmint essential oil content and composition. Some articles do not mention the DT they used for steam distillation of Japanese cornmint. However, our results demonstrated that DT must be reported. This article can be used as a reference point when comparing results from reports in which different DTs were used.

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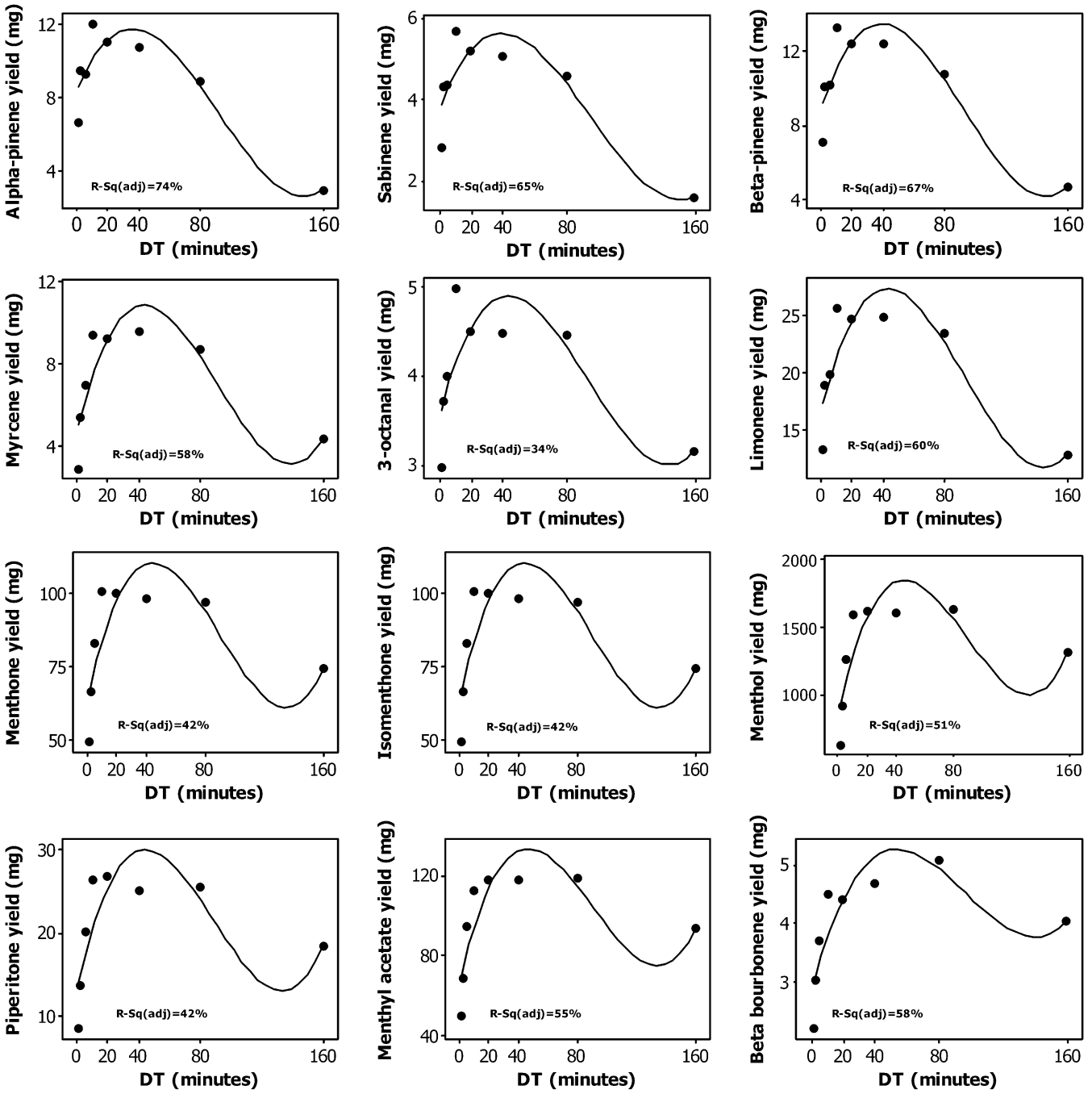

Fig. 2. Plots of the yields of 12 constituents vs. distillation time along with the fitted third-order polynomial regression model. The fitted models are not shown in the plots as a result of the relatively low adjusted R^2 values shown within each plot.