INFLUENCE OF HARVESTING TIME ON THE YIELD AND CHEMICAL COMPOSITION OF SAGE (*Salvia officinalis* L.)

Renata Baranauskiene[1*], Edita Dambrauskiene[2], Petras Rimantas Venskutonis[1], Pranas Viskelis[2]

*[1]Kaunas University of Technology, Department of Food Technology, Radvilenu pl. 19, Kaunas, LT 50254, Lithuania *e-mail: renata.baranauskiene@ktu.lt*
[2]Lithuanian Research Centre for Agriculture and Forestry, Institute of Horticulture, Babtai, Kaunas distr., LT 54333, Lithuania

Abstract
Sage (*Salvia officinalis* L.) was harvested at different periods from May 23 to July 11. The yield of crop depended on plant growing phase and was 3.0–10.0 of fresh and 0.5–2.7 t ha^{-1} of dried herb. Total essential oil (EO) content increased from May 23 to June 20 and was 0.1–0.2 in fresh and 0.4–1.0 cm^3 100 g^{-1} in dried herb. Seventy compounds were identified in sage EOs by GC and GC-MS. It is evident that *S. officinalis* grown in Lithuania depends to α-thujone and camphor chemotype: the content of α-thujone in EO varied in the range of 15.2–39.7%; that of β-thujone 5.3–7.9%. Other important components were 1,8-cineole, camphor, borneol, α-humulene, viridiflorol and manool. In addition, the amounts of dry soluble solids, ascorbic acid, carotenes, nitrates and total sugars were determined in fresh raw material at different growth stages.
Key words: Sage, *Salvia officinalis*, seasonal variation, essential oil, chemical composition, crop yield.

Introduction
Sage (*Salvia officinalis* L., Lamiaceae) and its products, such as EOs and oleoresins have been widely used as food flavourings and health promoting agents (Perry et al., 1999; Perry et al., 2003; Farhat, et al., 2009). In Lithuania aerial parts of sage are used in herbal teas and in the mixtures of medicinal plants for healing digestion and circulation disturbances, bronchitis, angina, skin and other diseases. Sage is also a natural source of flavonoids and polyphenolic compounds possessing strong antioxidant, radical-scavenging and antibacterial activities (Dapkevičius et al., 1998, Delamare et al., 2007).
The studies of sage EO indicate that its composition may be variable (Farhat et al., 2009; Delamare et al., 2007; Marić, Maksimović, Miloš 2006; Mockutė et al., 2003; Mirjalili et al., 2006). ISO 9909 for medicinal uses regulates the amounts of the following constituents in sage EO: *cis*-thujone (18.0–43.0%), camphor (4.5–24.5%), 1,8-cineole (5.5–13.0%), *trans*-thujone (3.0–8.5%), α-humulene (≤12.0%), α-pinene (1.0–6.5%), camphene (1.5–7.0%), limonene (0.5–3.0%), bornyl acetate (≤2.5%) and linalool + linalyl acetate (≤1.0%) (Santos-Gomes, Fernandes-Ferreira, 2001; Bernotienė et al., 2007). EO composition of *Salvia* species depends on many factors and sometimes does not match the profile defined by the ISO 9909 (Farhat et al., 2009). Therefore, the present study was aimed at evaluating and comparing the biological and chemical properties of *S. officinalis* grown in Lithuania at different growth stages. The results presented in this study expand our knowledge on sage and may be practically applied in the developments of commercial cultivation of this valuable herb.

Materials and Methods
Sage (*Salvia officinalis* L.) was cultivated in the experimental fields of the Lithuanian Institute of Horticulture. The plants were harvested at various vegetation phases: May 23 – regrowth; June 7 – intensive vegetative growth; June 20 – butonization (just before flowering, formation of inflorescences); June 27 – full flowering; and July 11 – after flowering (in fruiting – seeds ripening). Harvested herbs were dried at 40 ºC in the dark.
The yield of EO was determined by hydrodistillation of 100 g herb in a Clevenger-type apparatus during 3 hr. Soluble solids were determined by refractometer method using an Abbe refractometer (AOAC, 1990a). Ascorbic acid was determined by titrimetric method using

2,6-dichloroindophenol (AOAC, 1990b). Sucrose was determined by reducing sugars before and after inversion; sugars (reducing) were determined by inversion method (AOAC, 1990c). Nitrates were determined on a potentiometer pH-150 with an ion selective electrode EM-020604 (Methodology directions, 1990). Carotenes were determined by spectrophotometric method measuring extinction at 450 nm in hexane (Scott, 2001).
The EOs diluted in pentane (10 µl in 1 ml) were analyzed on a *Fisons 8000* GC equipped with a flame ionization detector and a DB-5 fused silica capillary column (50 m×0.32 mm i.d.×0.25 µm). GC–MS analyses were performed using a Perkin Elmer *Clarus 500* GC coupled to a Perkin Elmer *Clarus 500* series mass selective detector in the electron impact ionization mode at 70eV, the mass range was *m/z* 29–550 using an Elite–5MS capillary column (30 m×0.25 mm i.d. ×0.25 µm) (Baranauskiene, 2007).
The components were identified by comparing their Kováts retention indices (KI) relative to C_8-$C_{30,32}$ *n*-alkanes, obtained on nonpolar DB-5 GC column with those provided by Adams (2001) and by comparing their mass spectra with the data present in NIST (vers.1.7), NBS 75K/WILEY 275 and EPA/NIH mass spectral libraries.
All analyses were replicated four times. Data were statistically handled by one-way analysis of variance (ANOVA). Duncan's multiple-range test was applied for the calculation of the significant differences among the harvesting time treatments of plant biochemical composition and the amounts of individual EO components ($P=0.05$). The effect of different growing time on the yield of sage was described by regression analysis.

Results and discussion
Sage crop yield and EO content. The crop and the quality of plant material are the most important characteristics in commercial cultivation of spices and aromatic herbs.

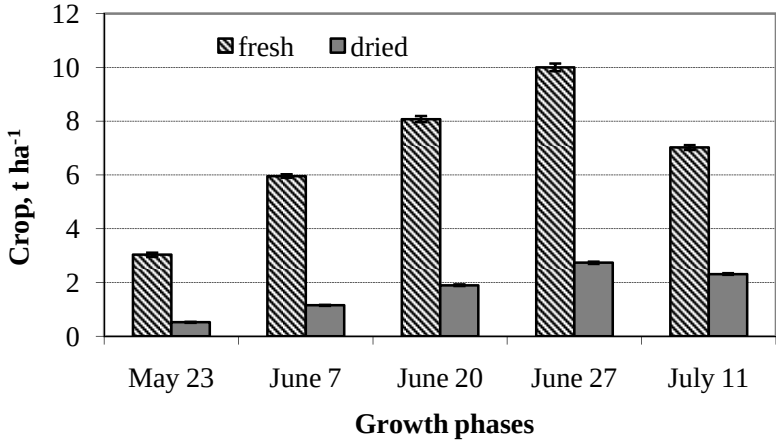

Figure 1. The yield of crop (t ha^{-1}) of sage raw material at different growth phases

It is obvious that the crop yield of fresh harvested sage continuously increased from May 23 till June 27 from 3.0 to 10.0 t ha^{-1} (Figure 1). The maximum peak was reached at full flowering stage and afterwards remarkably decreased after flowering at seeds ripening (June 11) to 7.0 t ha^{-1}. The effect of different growing time on the yield of fresh sage herb can be described by the third-order polynomial regression equation $y=-0.3417x^3+2.2176x^2-1.7174x+2.9473$ with a determination coefficient $R^2 = 0.985$. Sage herb may be used in its dried form or for further processing, e.g. isolation of EO, extraction. Therefore it is important to assess the yield of dried raw materials. After drying, the mass of sage decreased ~3–6 times. The similar tendency at different vegetative phases was obtained with crop yield of dried plant material $0.5\rightarrow2.7\rightarrow2.3$ t ha^{-1} compared to that of a fresh one (Figure 1). The output of dry sage may be described by the third-order polynomial regression equation: $y=-0.1144x^3+0.8857x^2-1.3203x+1.0869$, $R^2=0.992$.

EO content at different harvesting time was in the range of 0.1–0.3 cm^3 100 g^{-1} (fresh) and 0.4–1.0 cm^3 100 g^{-1} (dried) (Figure 2). In another study the yield of EO (w/w %) based on the dry weight of sage from Iran was in the following order: floral budding (0.9%)>vegetative (0.7%)>flowering (0.5%)>immature fruit (0.4%)>ripen fruit (0.2%) (Mirjalili et.al., 2006). Generally, the amount of EO is at its highest level at the floral budding stage when the oil is intensively biosynthesized, and decreased gradually at the fruiting phase, as observed in other plant species.

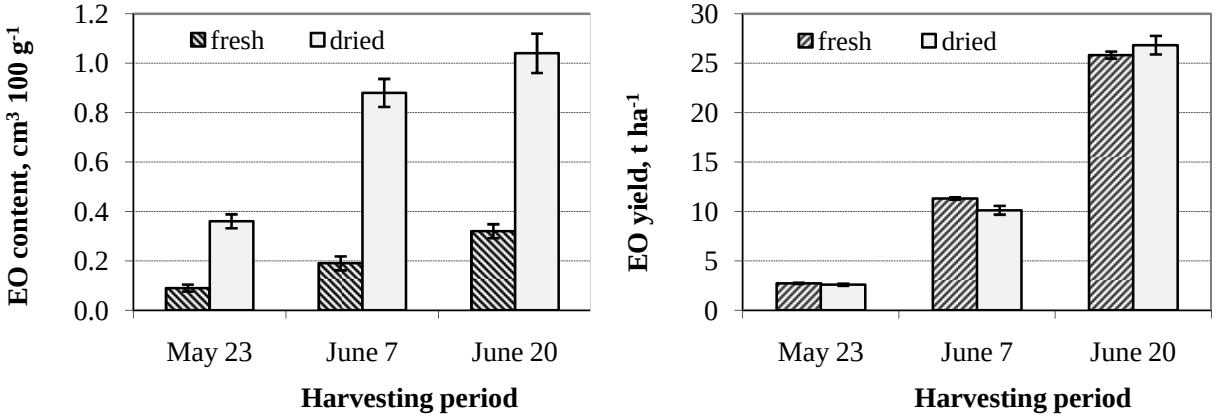

Figure 2. The content of EO (cm^3 100 g^{-1}) and the yield of EO (t ha^{-1}) in sage raw material at different growing phases

The total productivity of oil in fresh sage varied from 2.7 to 25.8 dm^3 ha^{-1}. After drying EO content slightly reduced in May 23 – June 7, and was from 2.6 to 26.8 dm^3 ha^{-1}; that indicates that the losses of volatiles during drying were not significant. The total productivity of oil from dried herb on June 20 was determined in higher amount compared to that of fresh sage and constituted 25.8 and 26.8 dm^3 ha^{-1} in fresh and dried herb, respectively (Figure 2).

EO composition. The composition of sage components exceeding 1 % in EO is presented in Table 1. The major components were α-thujone, camphor, α-humulene, manool, 1,8-cineole, viridiflorol, borneol and β-thujone, however, their content at different vegetative phases varied in a rather wide range. For instance, the percentage of α-thujone from May 23 to June 20 steadily increased from 29.4 to 39.7% (fresh herb). The content of viridiflorol also incresed at the same period, while the changes of camphor, α-humulene and manool were not so consistent. For example, from May 23 to June 7 the content of camphor significantly decreased from 12.8 to 5.3% and afterwards on June 20 again increased up to 13.7 (fresh herb). After drying it varied between 8.3% (June 7) and 17.7% (June 20). In contrary, the percentages of α-humulene and manool significantly increased from May 23 to June 7 and afterwards on June 20 decreased more than twice. The percentages of 1,8-cineole, borneol, β-pinene were continously decreasing during the whole vegetative period, while the content of β-thujone was almost stable (5.8–6.1%). Drying of fresh sage resulted in the changes in the composition of EO, most likely due to losses of the most volatile constituents. For example, the percentage of α-thujone significantly decreased after drying and was from 24.7 to 30.1%.

In general, it could be concluded that amounts of toxic thujones and other regulated compounds, such as camphor, 1,8-cineole, α-humulene, camphene, α-pinene, limonene and bornyl acetate met the requirements of ISO9909 standard.

Table 1

Variation in chemical composition of EOs of sage at different growth stages, GC peak area percentage[1]

Compound	KI	Identi-fication	May 23		June 7		June 20	
			fresh	dried	fresh	dried	fresh	dried
α-Pinene	939	KI, MS	1.93b	2.13bc	1.68a	2.31c	1.64a	4.92d
Camphene	954	KI, MS	2.51c	1.67b	0.95a	1.49b	3.20d	5.08e
β-Pinene	981	KI, MS	5.70d	2.04b	4.24c	1.43a	2.36b	1.88ab
1,8-Cineole	1049	KI, MS	8.91e	6.58d	5.66b	5.16c	4.18a	5.92b
α-Thujone	1117	KI, MS	29.35b	24.67a	30.26b	25.24a	39.72c	30.05b
β-Thujone	1126	KI, MS	6.05b	6.50c	5.83b	7.89d	6.12bc	5.26a
Camphor	1156	KI, MS	12.81d	9.58c	5.25a	8.28b	13.72e	17.67f
Borneol	1175	KI, MS	7.45e	5.39d	2.74b	2.99b	2.23a	4.88c
Isobornyl acetate	1291	KI, MS	0.81c	0.94d	0.55b	0.19a	0.57b	2.64e
Bornyl acetate	1298	KI, MS	0.09a	1.47d	0.32b	5.23e	0.11a	1.03c
Carvacrol	1311	KI, MS	tr[2] a	2.74c	0.52b	3.34d	nd[3]	tr a
β-Caryophyllene	1434	KI, MS	2.35bc	2.78c	3.70d	3.50d	1.20a	1.99b
α-Humulene	1471	KI, MS	7.90b	12.93d	13.79e	9.39c	6.02a	5.65a
Viridiflorol	1613	KI, MS	4.37a	5.74b	7.04c	8.43d	7.62c	5.15ab
Humulene epoxide II	1627	KI, MS	0.25a	1.08d	0.31a	0.59b	0.75c	0.58b
Manool	2084	KI, MS	3.81a	7.85b	10.39d	7.08b	4.77c	2.82a
Total identified, %			99.94	99.13	98.95	99.30	99.10	99.60
RSD[4], %			8.81	7.48	6.64	5.25	11.20	8.75

[1]Average GC peak area percentage of four replicates. [2]tr, peak area percent ≤0.04%. [3]nd, not determined.
[4] %RSD, average coefficient of variance of individual compounds. a-f, Values within rows followed by the same letter do not differ statistically at *p=0.05*.

The concentration of the main sage EO components was also expressed in absolute units, mg kg^{-1} (Figure 3). Significant increase in the concentration of α- and β-thujones was observed during the studied period of vegetation. In general, the amounts of α-thujone, β-thujone, α-pinene, 1,8-cineole, and viridiflorol were increasing with the increase of the total EO content. The highest amounts of α-humulene, β-caryophyllene and manool were observed on June 7 during intensive vegetative growth; while on June 20 it again decreased.

After drying some losses of volatiles were observed. For example, the amount of α-thujone was from 264.2 to 1271.0 mg kg^{-1} in fresh herb; while after drying it decreased 1.3–1.8 times and was 148.0–721.3 mg kg^{-1}. The same changes were observed for β-thujone, exception for herb harvested on June 7, when its amount in dried herb (134.1 mg kg^{-1}) was higher than that in fresh herb (110.7 mg kg^{-1}). Also, the amounts of some other components during butonization (June 20) were higher in EO from dried herb compared to the fresh one (Figure 3).

Biochemical composition of sage. The amounts of dry soluble solids, vitamin C, carotenes, nitrates and total sugars are presented in Table 2. It was observed that carotenes were biosynthesized more intensively at the second half of vegetation, while in the period from June 20 to June 27 their concentration was similar (*P=0.05*). The content of dry soluble substances and total sugars was different at various growth phases (*P=0.05*) and varied in the range of 6.3 and 11.3 % and 1.46–3.47 %, respectively. The concentration of vitamin C was very low (9.6 mg 100 g^{-1}) at the beginning of plant vegetation, however it increased up to 17.6 mg 100 g^{-1} during intensive flowering phase (Table 2). The amounts of nitrates were quite high during all plants vegetation period and varied from 720 to 980 mg kg^{-1}; it continuously decreased during plant vegetation from May 23 to July 11.

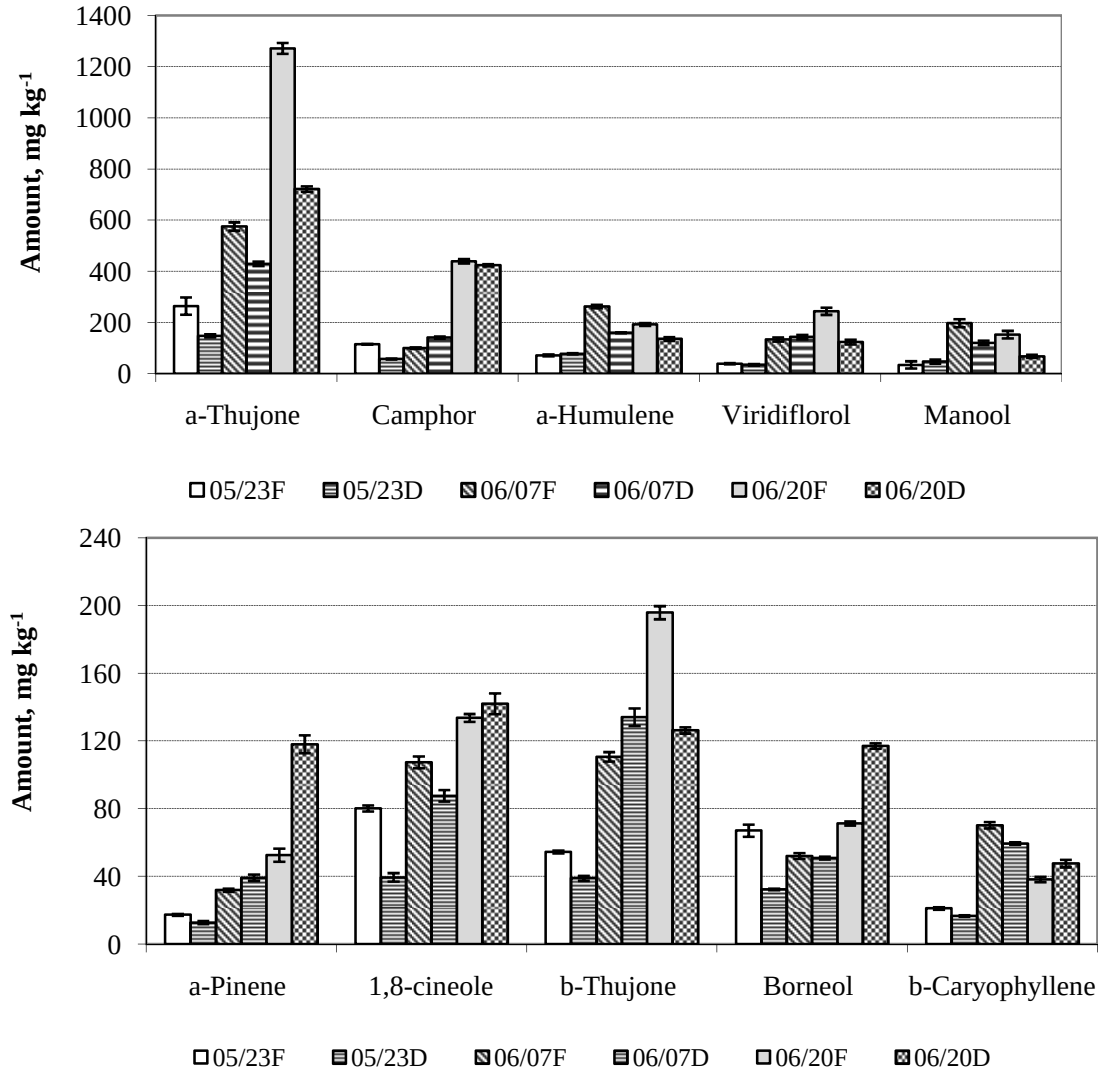

Figure 3. The concentrations of individual constituents in sage at different harvesting period: F=fresh herb; D=dried herb

Table 2

Chemical characteristics of sage (fresh herb) at different growing phases

Growing time	Dry soluble solids, %	Vitamin C, mg %	Carotenes, mg %	Nitrates, mg kg^{-1}	Total sugars, %
May 23	6.3a	9.6a	5.5a	980e	1.46a
June 7	9.2b	15.0c	9.2c	880d	1.68b
June 20	9.9c	17.6d	11.2b	830c	2.68c
June 27	10.5d	17.0b	11.6b	760b	3.06d
July 11	11.3e	16.8b	12.8d	720a	3.47e

a-e, Values within columns followed by the same letter do not differ statistically at *p=0.05*.

It could be concluded that biochemical composition of *S. officinalis* considerably depends on plant growth phase. In the beginning of plant vegetation after winter the amounts of dry soluble solids, total sugars, vitamin C and carotenes were low, and continuously increased until the intensive flowering phase or after flowering in seed ripening. The content of nitrates was continuously decreasing during all vegetation period.

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