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INFLUENCE OF PRESERVATION METHOD ON PEPPERMINT, BASIL AND ROSEMARY PLANT EXTRACTS CHEMICAL COMPOSITION

Denisa CIOTEA, Mona Elena POPA
University of Agronomic Sciences and Veterinary Medicine of Bucharest,
59 Mărăşti Blvd, District 1, Bucharest, Romania

Corresponding author email: denisa.ciotea@gmail.com

Abstract

The aim of this paper is to present the most used preservation methods and their impact on the content and composition of following plant materials: Mentha piperita, Ocimum basilicum and Rosmarinus officinalis. The preservation methods used in this work were natural drying, freezing storage and gamma rays irradiation. Steam distillation method was used to extract the essential oils from the preserved samples. Essential oil content varied according to the preservation method tested on the plant materials. Fresh plant distillation yield for peppermint and rosemary were 0.35% and for basil was 0.88%. Dried plant and gamma rays irradiated plant distillation yield for rosemary was 1.94%, for peppermint was 0.55%, and for basil was 0.29%. Frozen plant distillation yield for rosemary was 0.50%, for peppermint was 0.15%, and for basil was 0.05%. Chemical composition of studied essential oils are analyzed by Gas Chromatography coupled with Mass Spectrometry Detector (GC-MSD). The primary constituent detected in peppermint oil was menthan-ol, in rosemary oil was eucalyptol and in basil essential oil was estragole. As the main conclusion is the preservation methods clearly influence the content and the chemical composition of essential oils obtained from plant material.

Key words*: drying, essential oils, freezing, gamma rays irradiation, plant material preservation.*

INTRODUCTION

For centuries, plant materials have been used for a wide variety of purposes in different industries like perfumery, cosmetics, medicines and food industry. In food industry, aromatic plants are used to season the meat products, beverages and sweets. Lelieveld (2015) found that below a certain dose of food (vegetables, spices), there is no effect to humans, so it can be used safely. Plants degrade easily and lose their volatile compounds quickly, so they need to be preserved immediately after the harvesting process. The preservation methods used in this study are natural drying, freezing and gamma irradiation.

Drying is one of the oldest techniques for plant storage. Drying is a thermodynamic process involving heat and mass transfer, like diffusion, where the water is evaporated into the environment (Sultanova et al., 2020). Medicinal plants are composed of more water than dry substances. The moisture content in plant is approximately 10-12% (Safarov J., 2017). Natural drying process influences the color of the leaves, the weight of herbs, the volume of herbs and their volatile content (Qiaoxian, 2019; Chakraborty & Dey, 2016).

Freezing is also a thermodynamic process which involves cooling the herbs to their freezing point. Freezing is a preservation technique, where plant cells and membranes are breaking down at low temperature and the water is crystallizing in the form of ice. The metabolic process slows down and the microbiological growth is stopped. Harvesting factors also contribute to the freezing performance (Celli et al., 2016; Neri et al., 2020).

Gamma irradiation is a preservation method because it is used to extend the shelf-life of plant materials. Gamma irradiation is also used in decontamination of dried plants (Pereira et al., 2018). Plant irradiation using ionizing radiation is known to be a safe method of treatment (Gerolis et al., 2017).

In recent years, research has focused on different methods to extract essential oils from plant materials like hydrodistillation, steam distillation, solvent extraction, microwave extraction and extraction with supercritical CO_2. The most used method to extract the essential oils is steam distillation, because it has a great efficiency and the oil quality is higher than other extractive methods. Steam distillation method uses heat from water steam to extract the oil through vaporization. The oil is then separated from the water (Radwan et al., 2020; Salamon et al., 2019).

The purpose of this study was to determine how preservation method influence the chemical composition of *Mentha piperita, Ocimum basilicum* and *Rosmarinus officinalis.*

MATERIALS AND METHODS

This study was performed to evaluate the effect of preservation methods like drying, freezing and gamma irradiation on the chemical composition of peppermint (*Mentha piperita*), basil (*Ocimum basilicum*) and rosemary (*Rosmarinus officinalis*) essential oil.

The studied plants were cultivated by established growers in Constanta area, in spring of 2020. Immediately after harvest two quarters of the plant material was stored in a drying room at 21ºC, a quarter of the plant material was freezing at -21ºC in a Samsung (RB31FERNDSA) freezer and a quarter remained fresh. Half of the dried plant material was gamma irradiated in a Gamma Irradiator GC5000 with a ^{60}Cobalt source at a dose rate of 10Gy. The 12 samples of essential oils were extracted by steam distillation. The distillation process was made in lab. To proceed to steam distillation, the plant material was placed in a recipient. This recipient was connected to water and a condenser. The water vapour crosses the plant material and then it goes to the condenser equipment. The essential oil is easily separated by decantation (Radwan et al., 2020). After the distillation proccess, the essential oils were kept in a refrigerator until the analysis.

GC-MS analysis was performed on GC 7890A chromatograph using Zebron capillary. The parameters are:

- Inlet temperature: 250ºC;
- Helium flow rate: 0.8 ml/min;
- 50:1 split ratio;
- Column temperature: 50ºC for 1 min;
- Rate of expansion: 8ºC/min up to 100ºC, 2 min;
- Maintain at 110°C for 2 min;
- Rate of expansion: 5°C/min up to 185ºC;
- Maintain at 280°C for 10 min.

The Mass Spectrometry (MS) detection was performed on 7000A TQMSA detector and Zebron capillary (Deleanu et al., 2018).

The Electron Ionization used was 70 eV (electron Volt). The source temperature was set at 230°C, the transfer temperature was set at 280°C and the quadrupole temperature was set at 150°C (Deleanu et al., 2018).

The antioxidant activity of these oils is evaluated using DPPH method. The DPPH ethanol solution was mixed with each sample of oil (Deleanu et al., 2018).

The control used at this analysis is DPPH - ethanol solution. The plate with the oil samples and the control sample were incubated. Incubate condition were: a dark room and maintained for 30 minutes. The plates were read at 518 nm.

The anti-radical activity (XY) of the oil samples were determined using the formula:

$$XY\% = 100 - \left(\frac{(B_{OS} - B_{OSD})\ x\ 100}{B_C}\right)$$

Where:
XY = Anti-radical Activity;
B_{OS} = oil sample absorbance;
B_{OSD} = oil sample absorbance without DPPH;
B_C = control sample absorbance (Deleanu et al., 2018).

RESULTS AND DISCUSSIONS

The chemical composition of fresh (FrP), dried (DrP), irradiated (IrP) and frozen (FroP) extracted peppermint essential oils are presented in Table 1.

Terpenes alcohols (Menthan-ol) and terpenes (Limonene) were found in high concentrations in Fresh peppermint oil, where Menthan-ol is 40.52% and Limonene is 14.6%, in Dried peppermint oil Menthan-ol is 44.6% and Limonene is 16.5%, in Irradiated peppermint oil Menthan-ol is 44.1% and Limonene is 16.3%, in Frozen peppermint oil Menthan-ol is 43.7% and Limonene is 15.86%.

Other terpenes found in low concentrations in peppermint oil were Cymene, Eucalyptol, L-β-Pinene, Terpinene, α-Pinene, Terpinolene, 3-Carene and ketones like Menthone.

The results obtained for Menthan-ol found in *M. piperita* extracted oil are in agreement with those reported by Bishr & Salama (2017) - 32.24%; Salamon et al. (2019) - 39%; Mattazi et al. (2015) - 41.23%.

The chemical composition of fresh (FrB), dried (DrB), irradiated (IrB) and frozen (FroB) extracted basil essential oils are presented in Table 2.

Table 1. Chemical composition of fresh (FrP), dried (DrP), irradiated (IrP) and frozen (FroP) extracted peppermint essential oils

No	Detected Compound	Retention Time (min)	FrP (%)	DrP (%)	IrP (%)	FroP (%)
1	Menthan-ol	14.7	40.52	44.6	44.1	43.7
2	Limonene	9.8	14.6	16.5	16.3	15.86
3	Cymene	8.7	8.2	7.2	6.75	6.7
4	L-β-Pinene	8.4	4.8	4.8	4.6	4.54
5	Eucalyptol	9.7	4.8	3.5	4.6	4.56
6	Terpinene	9.8	3.9	2.9	3.85	3.8
7	α-Pinene	6.8	2.9	3.15	3.1	2.9
8	Menthone	15.4	2.6	1.7	2.51	2.5
9	Terpinolene	12.1	2.5	1.6	2.25	2.24
10	3-Carene	8.7	2.1	2.1	2.1	2
11	D-Menthone	15.3	1.6	1.65	0.52	0.5
12	β-Pinene	9.2	1.47	1.5	1.7	1.68
13	Octanol	8.7	1.1	1.1	1.01	1
14	Isomenthol	16.4	1	1	-	0.96
15	Caryophyllene	24.5	1	1	1.1	1
16	O-Menthone	13.2	0.9	0.95	0.9	0.88
17	Neo-menthol	13.4	0.9	0.8	0.9	0.88
18	β-Phellandrene	7.6	0.77	0.9	0.9	0.88
19	α-Terpinene	8.5	0.7	0.86	0.85	0.72
20	2-Menthene	8.9	0.52	0.63	0.62	0.6
21	Camphene	7.5	0.35	0.42	0.41	0.4
22	Isopulegol	14.6	0.35	0.2	0.33	0.32
23	Fenchone	10.4	0.25	0.26	0.25	0.24
24	Germa-crene	26.2	0.09	0.1	0.07	0.07
25	Caryophyllene oxide	28.4	0.09	0.1	0.02	0.1
26	Pulegone	18.4	-	-	-	-
27	Isomenthol acetate	18.7	-	-	-	-
Total			**98.01**	**99.52**	**99.74**	**99.03**

Table 2. Chemical composition of fresh (FrB), dried (DrB), irradiated (IrB) and frozen (FroB) extracted basil essential oils

No	Detected Compound	Retention Time (min)	FrB (%)	DrB (%)	IrB (%)	FroB (%)
1	Estragole	15.8	75.5	78.3	76.5	77.2
2	β-Linalool	11.5	17.8	16.3	18.1	17.3
3	1599Tetramethy1.4.7-cycl-cat.	27.3	1.6	1.2	1.1	1.2
4	Humulene	23.4	0.7	0.2	0.19	0.24
5	β-Citral	18.5	0.6	0.2	0.31	0.24
6	Bergamotene	24.6	0.59	0.6	0.55	0.5
7	Caryo-phyllene	21.4	0.51	0.56	0.48	0.42
8	Isomenthol	14.8	0.36	0.3	0.28	0.24
9	Farnesene	22.7	0.3	0.1	0.2	0.23
10	β-Cubebene	26.8	0.3	0.4	0.2	0.25
11	3-Carene	9.7	0.16	0.17	0.16	0.16
12	β-Pinene	8.5	0.15	0.15	0.15	0.15
13	Limonene	11.3	0.14	0.16	0.14	0.14
14	Eucalyptol	11.6	0.14	0.16	0.14	0.14
15	Menthone	13.6	0.12	0.13	0.12	0.12
16	Methoxycinnamald.	28.3	0.11	-	-	-
17	α-Pinene	8.3	0.09	0.08	0.09	0.09
18	Bisabolene	26.7	0.03	0.05	0.04	0.03
19	Ethylhexanol	8.7	-	-	-	-
20	Cymene	8.6	-	-	-	-
21	Dimethyl-octadienol	16.5	-	-	-	-
22	Citral	15.3	-	-	-	-
23	Caryophyllene oxide	25.3	-	-	-	-
Total			**99.2**	**99**	**98.7**	**98.65**

Phenolics derivatives (Estragole) and terpene alcohols (Linalool) were found in high concentrations in Fresh basil oil, where Estragole is 75.5% and Linalool is 17.8%, in Dried basil oil Estragole is 78.3% and Linalool is 16.3%, in Irradiated basil oil Estragole is 76.5% and Linalool is 18.1%, in Frozen basil oil Estragole is 77.2% and Linalool is 17.3%.

Other study obtained as principal constituent, Linalool: Abou El-Soud et al. (2015) - 48.4%; Rezzoug et al. (2019) -52.1%; Stanojevic et al. (2017) - 31.6%.

The chemical composition of fresh (FrR), dried (DrR), irradiated (IrR) and frozen (FroR) extracted rosemary essential oils are presented in Table 3.

Table 3. Chemical composition of fresh (FR), dried (DR), irradiated (IR) and frozen (FRR) extracted rosemary essential oils

No	Detected Compound	Retention Time (min)	FrR (%)	DrR (%)	IrR (%)	FroR (%)
1	Eucalyptol	11.2	40.5	40.2	39.5	37.3
2	Camphor	12.6	18.4	17.9	16.7	16.3
3	α-Pinene	6.8	11.3	9.51	8.9	7.7
4	Safrole	20.1	4.87	5.23	4.23	4.5
5	Phellandrene	9.2	4.8	4	4.2	3.9
6	Limonene	11.2	3.27	3.5	3.21	3.33
7	Camphene	7.5	2.7	2.1	1.8	1.9
8	Linalool	11.6	2.6	2.8	2.44	2.34
9	β-Pinene	7.9	2.5	2	1.7	1.65
10	L-β-Pinene	9.2	2.4	2.6	2.1	2.3
11	Terpineol	16.5	2.3	5.01	4.5	4.3
12	Terpinen-4-ol	16.3	1.23	1.5	1.32	1.43
13	Isoborneol	13.5	0.98	1.21	0.74	0.68
14	Cymene	8.7	0.68	0.6	0.56	0.51
15	Terpinene	8.6	0.65	0.7	0.6	0.57
16	Tricyclene	6.9	0.4	0.6	0.5	0.3
17	α-Phellandrene	10.4	0.4	0.5	0.35	0.33
Total			**99.98**	**99.96**	**93.35**	**89.34**

Terpenes (Eucalyptol and α-Pinene) and terpenoids (d-Camphor) were found in high concentrations in Fresh rosemary oil, where Eucalyptol is 40.5%, d-Camphor is 18.4% and α-Pinene is 11.3%, in Dried rosemary oil Eucalyptol is 40.2%, d-Camphor is 17.9% and α-Pinene is 9.51%, in Irradiated rosemary oil Eucalyptol is 39.5%, d-Camphor is 16.7% and α-Pinene is 8.9%, in Frozen rosemary oil Eucalyptol is 37.3%, d-Camphor is 16.3% and α-Pinene is 7.7%.

Terpene alcohols are also found in low concentrations in rosemary oil like α-Terpineol, Linalool, Terpinen-4-ol and terpenes like β-Phellandrene, Limonene, Camphene and β-Pinene.

Salamon et al. (2019), Ojeda-Sana et al. (2012) and Mattazi et al. (2015) found that the major constituents of rosemary oil were Cineole, Camphor and α-Pinene.
Essential oils antioxidant activity is expressed as the essential oil concentration needed to inhibit the formation of radicals by 50%.
The antioxidant activities of Fresh, Dried, Irradiated and Frozen Peppermint essential oils are presented in Figure 1. Dried peppermint oil (IC_{50}DrP = 40.27 mg/ml) has higher antioxidant potential than irradiated peppermint oil (IC_{50}IrP = 47.56 mg/ml), frozen peppermint oil (IC_{50}FroP = 51.75 mg/ml) and fresh peppermint oil (IC_{50}FrP = 53.43 mg/ml).

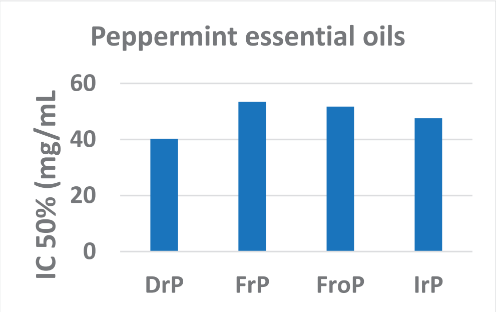

Figure 1. Inhibitory Concentration of Peppermint oil (FrP-fresh peppermint oil; DrP- dried peppermint oil; IrP- irradiated peppermint oil; FroP- frozen peppermint oil)

The antioxidant activities of Fresh, Dried, Irradiated and Frozen Basil essential oils are presented in Figure 2. Dried basil oil (IC_{50}DrB = 45.3 mg/ml) has higher antioxidant potential than frozen basil oil (IC_{50}FroB = 47.6 mg/ml), irradiated basil oil (IC_{50}IrB = 51.7 mg/ml) and fresh basil oil (IC_{50}FrB = 53.5 mg/ml).

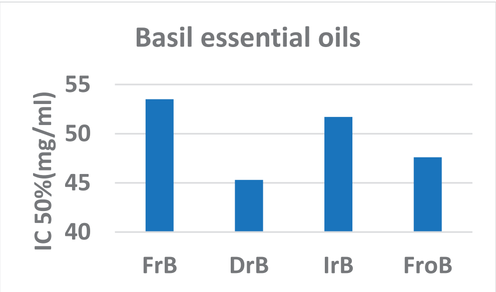

Figure 2. Inhibitory Concentration of Basil oil (FrB-fresh basil oil; DrB- dried basil oil; IrB- irradiated basil oil; FroB- frozen basil oil)

The antioxidant activities of Fresh, Dried, Irradiated and Frozen Rosemary essential oils are presented in Figure 3. Dried rosemary oil (IC_{50}DrR = 14.3 mg/ml) has higher antioxidant potential than fresh rosemary oil (IC_{50}FrR = 18.2 mg/ml), irradiated rosemary oil (IC_{50}IrR = 19.4 mg/ml) and frozen rosemary oil (IC_{50}FroR = 21.8 mg/ml).

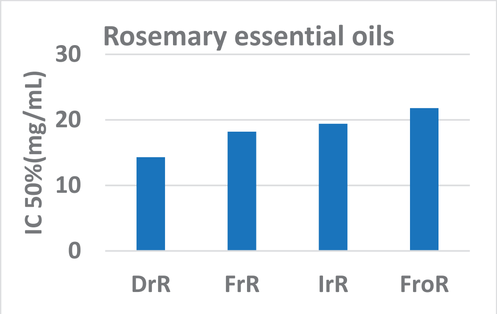

Figure 3. Inhibitory Concentration of Rosemary oil (FrR-fresh rosemary oil; DrR- dried rosemary oil; IrR- irradiated rosemary oil; FroR- frozen rosemary oil)

In their research, Hossain et al. (2010) obtained that dried rosemary and basil samples achieved higher antioxidant activity while fresh samples has loss of antioxidants.

CONCLUSIONS

In conclusion, the principal compounds obtained by Gas-cromatography for peppermint essential oil (98.01-99.74%) were: Menthan-ol, Limonene, Cymene, L-β-Pinene, Eucalyptol, α-Pinene and Menthone; for basil essential oil (98.65-99.2%) were: Estragole, Linalool and 1.5.9.9-Tetramethyl-1.4.7-cycloundecatriene; for rosemary essential oil (93.35-99.98%) were: Eucalyptol, Camphor, α-Pinene, Safrole, β-Phellandrene, Limonene, Camphene, Linalool and β-Pinene.
The preservation method influence the composition of the essential oil. Fresh plants components are very unstable and easily evaporate, while dried plants components are stable and concentrated. Irradiated plants show no differences in composition compared with the dried ones. Frozen plants components are easily disintegrated because of the lower temperature.

The dried samples of studied aromatic plants (peppermint, basil and rosemary) has the highest antioxidant activity.

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