HUNGARIAN UNIVERSITY OF AGRICULTURE AND LIFE SCIENCES

EFFECT OF DIFFERENT PRESERVATION METHODS ON THE ACTIVE SUBSTANCES AND ORGANOLEPTIC PROPERTIES OF SELECTED SPICES

Doctoral Thesis

URBASHI HAZARIKA

Supervisor: BEÁTA GOSZTOLA (Ph.D., Assoc. Prof.)

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Budapest, Hungary

The Doctoral School of Horticultural Sciences

Name: **Urbashi Hazarika**

Department: Medicinal and Aromatic Plants

Head: **Zámboriné dr. Németh Éva**
Professor, DSc
MATE, Institute of Horticultural Sciences

Supervisor: **Beáta Gosztola**
Associate professor, PhD
MATE, Institute of Horticultural Sciences
Department of Medicinal and Aromatic Plants

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Approval of the Head of Doctoral School Approval of the Supervisor(s)

1. Introduction

Spices have been identified as sources of various phytochemicals, many of which possess powerful antioxidant activity and various pharmacological attributes. The demand for spices is persistent throughout the year although the fresh plant materials are available only for a short period of time. The plants must be well preserved after harvesting to prevent their biological deterioration which will help to elongate the shelf life and to secure the active substances present in them. The time of harvest, the phenological stage of harvesting and post-harvest processes (e.g. cleaning, drying, shredding, chopping, etc.) can significantly influence the quantity and quality of final plant product. Therefore, selection of the adequate preservation methods is highly important. Several drying methods (natural and artificial) are in use commercially for preserving spices. These can reduce the moisture content of plants thus inhibiting the enzymatic degradation processes. Natural drying (drying in the sun or in shade) has been in use since ancient times. Among artificial drying methods, freeze-drying or lyophilization is one of the most recent, non-convective technique that is used for drying spices. Another non-convective method that has been employed in recent years for drying spices is microwave drying. However, its effect on the active substances is still not well known. Freezing is another preservation method that is widely used to enhance the shelf life of fruits and vegetables, but it is a less known technique in case of spices. Surprisingly, there are no research results in connection with freezing and its effect on the active substance content in spices.

2. Objectives

The main objectives of this research was to investigate the effect of conservation: freezing and drying (with different convective and non-convective techniques) on the active compounds (EO content and composition, total phenolic and rosmarinic acid content, total antioxidant capacity) and organoleptic properties (colour and taste) of selected spice species. The experiment was conducted on some important spice species, which

- accumulate the EO primarily or exclusively in glandular hairs or trichomes: *Melissa officinalis* L., *Mentha x piperita* L., *Mentha spicata* L. var. *crispa*, *Ocimum basilicum* L., *Origanum majorana* L., *Origanum vulgare* L. subsp. *hirtum* (Link) Ietswaart, *Origanum vulgare* L. subsp. *vulgare*, *Salvia rosmarinus* L., *Salvia officinalis* L., *Satureja hortensis* L., *Thymus vulgaris* L., *Helichrysum italicum* L., *Lavandula angustifolia* Mill., *Lavandula x intermedia* Emeric; and

- accumulate the EO primarily or exclusively in secretory ducts: *Anethum graveolens* L., *Artemisia dracunculus* L., *Levisticum officinale* Koch., *Petroselinum crispum* (Mill) Nym. var. *neapolitanum*.

Examining the effects of preservation methods, we aimed

a. to observe and compare the effects of the relatively well known convective drying methods (drying in the sun, in shade, at 40 and 60°C in oven) with the literature data and the relatively newer preservation methods (lyophilization, microwave drying and freezing).

b. to explore the applicability of non-conventional drying methods like lyophilization and microwave drying (at 250 and 700 W) in the preservation of selected species.

c. to study the effectiveness of freezing methods (slow and fast) in preserving the quality of the final product.

d. to describe the correlations between preservation methods and changing of active substances and organoleptic properties of processed raw material of selected important spices. Additionally, to investigate whether there is any correlation between the location of EO accumulation and the effect of certain preservation methods. Furthermore, to explore, whether the trends observed are general or species-specific.

e. to investigate how chopping before preservation affects the examined characteristics (in case of those species where it is relevant).

3. Materials and Methods

3.1. Time and place of the experiment

The research was carried out in the Department of Medicinal and Aromatic Plants, at the Institute of Horticultural Sciences of Hungarian University of Agriculture and Life Sciences (MATE), between 2020 and 2022. The plant material of the research was produced in the Soroksár Experimental and Research Farm from March to September 2020-2022.

3.2. Examined plant material and applied preservation methods

Eighteen well-known, medicinally also important spices were included in the examinations, of which fourteen species synthesize the EO primarily or exclusively in glandular hairs or trichomes, and four species mainly in internal secretory ducts. Information about the plant materials examined in this research are given in Table 1.

Table 1. The common and scientific name, variety, plant family, examined plant parts and harvest time of species involved in the study

Studied plant species	Common name	Scientific name	'Variety' or accession	Plant family	Examined plant parts	Place of the cultivation	Year of the experiment	Harvest time
Species accumulating the EO primarily or exclusively in glandular hairs or trichomes	Italian strawflower	*Helichrysum italicum* L.	Commercial sample from Jelitto	*Asteraceae*	flowering leafy shoots	Soroksár	2020	July
	True lavender	*Lavandula angustifolia* Mill.	'Budakalászi 80'	*Lamiaceae*	flowers	Soroksár	2022	June
	Hybrid lavender	*Lavandula* x *intermedia* Emeric	'Judit'	*Lamiaceae*	flowers	Soroksár	2022	June
	Lemonbalm	*Melissa officinalis* L.	'Lemona'	*Lamiaceae*	leaves	Soroksár	2021	August
	Peppermint	*Mentha* x *piperita* L.	'Mexián', Gene bank accession Nr. LAMIMENTA18	*Lamiaceae*	leaves	Soroksár	2021	June
	Spearmint	*Mentha spicata* L.var. *crispa*	Gene bank accession LAMIMENTA5	*Lamiaceae*	leaves	Soroksár	2020	August
	Sweet basil	*Ocimum basilicum* L.	'Genovese', 'Ohře'	*Lamiaceae*	leaves and flowers	Soroksár	2020-2021	June-July
	Marjoram	*Origanum majorana* L.	'Egyptian', 'Magyar'	*Lamiaceae*	flowering leafy shoots	Soroksár	2020-2021	June-July
	Greek oregano	*Origanum vulgare* L. subsp. *hirtum* (Link) Ietswaart	Commercial sample from Jelitto	*Lamiaceae*	flowering leafy shoots	Soroksár	2021	August
	Common oregano	*Origanum vulgare* L. subsp. *vulgare*	Commercial sample from Jelitto	*Lamiaceae*	flowering leafy shoots	Soroksár	2020	July
	Rosemary	*Salvia rosmarinus* L.	'Harmat'	*Lamiaceae*	leaves	Soroksár	2021	September
	Garden sage	*Salvia officinalis* L.	'Regula', 'Extrakta'	*Lamiaceae*	leaves	Soroksár	2021	August
	Summer savory	*Satureja hortensis* L.	Gene bank accession Nr. LAMISATU22, 'Saturn'	*Lamiaceae*	flowering leafy shoots	Soroksár	2020	July
	Garden thyme	*Thymus vulgaris* L.	'French Summer', 'Deutscher Winter'	*Lamiaceae*	leafy shoots	Soroksár	2021	June
Species accumulating the EO primarily or exclusively in secretory ducts	Dill	*Anethum graveolens* L.	Commercial sample from Hungary	*Apiaceae*	leafy shoots	Fajsz	2021	August
	French tarragon	*Artemisia dracunculus* L.	'Zöldzamat', 'Artemis'	*Asteraceae*	leaves	Soroksár	2020	August
	Lovage	*Levisticum officinale* Koch.	'Mittelgroblättriger', Gene bank accession Nr. ASTLEVI44	*Apiaceae*	leaves	Soroksár	2021	June
	Flat-leaf parsley	*Petroselinum crispum* (Mill) Nym. var. *neapolitanum*	Commercial sample from Hungary	*Apiaceae*	leaves	Fajsz	2021	August

A total of approximately 4-5 kg of fresh plant material were harvested from each plant species according to the proper phenophase period of the plants, from June to September. After harvest, the samples were sorted, leaving only the valuable plant parts (Table 1) and were thoroughly mixed to attain a homogeneous mixture. In case of parsley and dill, half of the harvested plant materials were chopped into 0.5 cm pieces. Then plant materials of each plant species were divided into ten parts for the applied treatments: *fresh (control), sun drying, shade drying, oven drying at 40°C and 60°C, lyophilization, microwave drying at 250 W and 700 W and freezing (slow and fast)*.

During **sun drying**, the fresh plant materials were placed outside in a densely latticed, top open compartment. The temperature as recorded by the RHT Datalogger changed between 19-72°C during the measurements. The average duration of drying was 2-8 days depending on the plant species and the actual temperature. For **Shade drying**, the plant materials were kept in a sun-protected, dark but well-ventilated room. Here the RHT 10 Datalogger recorded 18-30°C during the day and 17-25°C at night in relation to every plant species. The average duration of drying was 5-13 days depending upon the plant species.

Oven drying was carried out in a convection oven at **40** and **60°C**. The duration of drying ranged between 3-8 hrs for oven drying at 60ºC and 15-35 hrs for oven drying at 40ºC. Microwave drying was carried out in a domestic microwave oven at **250 W** and **700 W** for 12-21 mins and 4-8 mins, ventilated every 3 and 1 min, respectively. In case of all drying treatments, the drying process was carried out until the mass consistency became constant (up to 4-9% moisture content, depending on species). For **lyophilization**, the fresh samples were quick frozen at -80ºC and then freeze-dried in a lyophilizer for 48 hours at -109ºC. The ready samples (with 1-5% moisture content) were packed in polyethylene bags and stored in a domestic refrigerator at 4ºC until processing.

Slow freezing was accomplished in a 230 l domestic freezer in which fresh plant materials packaged in polyethylene bags were kept at -18°C. For **fast freezing**, the fresh samples were quick frozen at -80°C and then stored at -18°C in a domestic freezer until use for a maximum of 2 months.

3.3. Measurement and evaluation methods

3.3.1. Chemical analyses

Essential oil (EO) extraction

From the examined plant species, 50 g of fresh and 20 g of dried plant materials were subjected to hydro-distillation in a Clevenger-type apparatus using 1000 ml of water for 2 hours

(except of peppermint and lavender species where the distillation time was only 1 hour) according to the methods recommended by the 7th Hungarian Pharmacopoeia. Measurements were recorded in three replications for each treatment, and data were referenced to the dry matter content of the samples.

Determination of essential oil (EO) composition

The EO composition was carried out in three replications per treatment using Gas Chromatography-Mass Spectrometry (GC-MS). The percentage composition of the EO was evaluated from the GC peak areas and were expressed as total area percentages.

Total phenolic content (TPC) and total antioxidant capacity (TAC)

The TPC and TAC were determined from aqueous extracts using modified method of Singleton and Rossi (1965) for TPC and modified FRAP method of Benzie and Strain (1996) for TAC in 3 biological replications from three parallel measurements. Data were expressed as mg GAE/g (d.w.) for TPC and mg AAE/g (d.w.) for TAC.

Rosmarinic acid (RA) determination

RA content was determined by an HPLC method in three replications according to the Monograph *Melissae folium* of Ph. Eur. 6.5.

Total hydroxycinnamic acid (THA) determination

The content of THA was determined by using a spectrophotometer according to the monograph *Melissae folium* of Ph. Hg. VIII. The percentage content of THA was expressed as rosmarinic acid. The results were always calculated with reference to the dry matter content of samples.

3.3.2. Sensory evaluation

E-tongue analysis

The e-tongue assessments were conducted with an Alpha Astree electronic tongue (Alpha M.O.S., Toulouse, France). These evaluations took place within the Department of Food Measurement and Process Control at the Institute of Food Science and Technology of MATE. Each plant sample was measured in separate days using nine consecutive runs, which resulted in nine replicates and in total 90 observations per sample set.

Colour measurements

For measuring colour, L* (lightness/darkness), a* (red/green coordinate) and b* (yellow/blue coordinate) values were recorded using a colorimeter and the quotient a*/b* was

calculated as a*/b* value is considered as a criterion for assessing the colour quality of dried products. Measurements were taken in six replications per treatment.

3.3.3. Scanning electron microscopic (SEM) observations

The SEM investigation was performed at Bay Zoltan Nonprofit Ltd. for Applied Research in Miskolc, Hungary using a JSM-IT700HR-LA (JEOL, Japan) scanning electron microscope. The examinations were conducted using plant materials from peppermint 'Mexián', lovage 'Mittelgroblättriger', lemonbalm 'Lemona' and rosemary 'Harmat'.

3.4. Statistical analysis

A one-way analysis of variance (ANOVA) accompanied by Tukey's or Games-Howell's test was performed to the data at 5% significance level using IBM SPSS 29. Homogeneity of variances was checked using Levene's test ($p < 0.05$). Linear discriminant analysis (LDA) was carried out for analysing the e-tongue data.

The measurements carried out by species are shown in Table 2.

4. Results and discussion

4.1. Effect of different preservation methods on the essential oil (EO) content

The effect of different preservation methods on the EO content and composition of plants was investigated for 20 taxa of 14 plant species. Based on our results, it was found that the applied preservation methods affected the *EO content* of the examined plant species, but not in the similar manner and range. Freezing (both slow and fast) and the gentle methods, like drying in shade and in oven at 40°C (which operated at low temperatures) proved to be the best methods; these caused only 2-28% EO content reduction. Whereas oven drying at 60°C (drying at higher temperature) reduced the EO content significantly (in case of *Artemisia dracunculus*, *Ocimum basilicum*, *Origanum vulgare* subsp. *hirtum* and *Thymus vulgaris* by 74-93%; for *Origanum majorana*, *Salvia rosmarinus* and *Salvia officinalis* by 5-26%). According to SEM pictures, the high drying temperature damaged the glandular hairs and tissue structure, leading to a major loss of volatiles (Figure 1). Sun drying also caused higher decrease, depending on the actual drying temperature.

Lyophilization was found to be a rather good preservation method for species, which contain the EO in external glandular hairs. In case of some plants (e.g. *Melissa officinalis*, *Thymus vulgaris, Origanum majorana, Salvia officinalis* or *Salvia rosmarinus*) it proved to be as good as oven drying at 40°C in preserving volatiles, and for some species, like *Mentha* x *piperita*, *Ocimum basilicum*, *Satureja hortensis* or *Lavandula spp.,* it was somewhat less

Table 2. Plant species examined in chemical and sensory evaluations

Examined plant species		'Variety' or accession		Evaluations						
				EO content and composition	TPC	TAC	RA	THA	Colour	Taste
Species accumulating the EO primarily or exclusively in glandular hairs or trichomes	*Helichrysum italicum* L.	Commercial sample from Jelitto			x	x				
	Lavandula angustifolia Mill.	'Budakalászi 80'		x						
	Lavandula x *intermedia* Emeric	'Judit'		x						
	Melissa officinalis L.	'Lemona'		x	x	x		x	x	
	Mentha x *piperita* L.	'Mexián'		x	x	x			x	
		Gene bank accession Nr. LAMIMENTA18								x
	Mentha spicata L.var. *crispa*	Gene bank accession Nr. LAMIMENTA5			x	x				
	Ocimum basilicum L.	'Genovese'		x	x	x				x
		'Ohré'		x					x	
	Origanum majorana L.	'Egyptian'		x						x
		'Magyar'		x					x	
	Origanum vulgare L. subsp. *hirtum* (Link) Ietswaart	Commercial sample from Jelitto		x						
	Origanum vulgare L. subsp. *vulgare*	Commercial sample from Jelitto								x
	Salvia rosmarinus L.	'Harmat'		x	x	x	x			
	Salvia officinalis L.	'Regula'		x	x	x			x	
		'Extrakta'								
	Satureja hortensis L.	Gene bank accession Nr. LAMISATU22		x						x
		'Saturn'		x						
	Thymus vulgaris L.	'French Summer'		x	x	x				
		'Deutscher Winter'		x			x			
Species accumulating the EO primarily or exclusively in secretory ducts	*Anethum graveolens* L.	Commercial sample	Whole						x	
			Chopped						x	
	Artemisia dracunculus L.	'Zöldzamat'		x						
		'Artemis'		x	x	x				x
	Levisticum officinale Koch.	'Mittelgroblättriger'		x	x	x			x	x
		Gene bank accession Nr. ASTLEVI44		x						
	Petroselinum crispum (Mill) Nym. var. *neapolitanum*	Commercial sample	Whole	x					x	
			Chopped	x					x	

Note: EO = Essential oil; TPC = Total phenolic content; TAC = Total antioxidant capacity; RA = Rosmarinic acid content; THC = Total hydroxycinnamic acid content

effective, but still better compared to drying at 60°C. However, for those species, which contain the volatiles primarily in endogenous secretory ducts, lyophilization was found to be the worst preservation technique, causing 79-97% EO loss. According to SEM pictures, in this case the structural expansion of pores due to water converting into ice indicated the diffusion of volatiles (Figure 2).

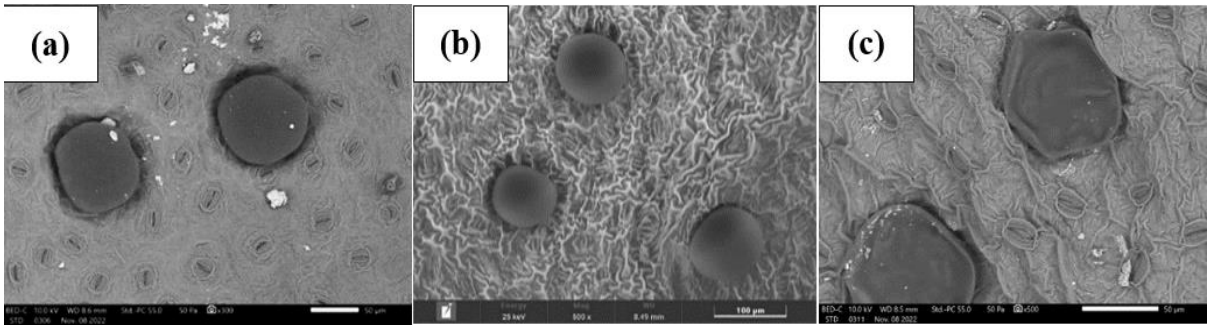

Figure 1. Peltate glands on the lower epidermis of *Mentha* x *piperita* leaves preserved by lyophilization (a), oven drying at 40ºC (b), and at 60ºC (c)

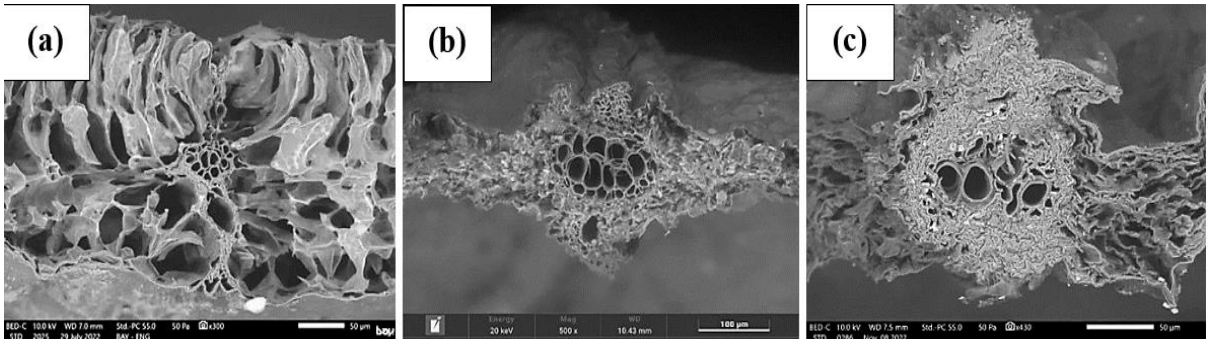

Figure 2. Cross-section of a *Levisticum officinale* leaf preserved by lyophilization (a), oven drying at 40ºC (b) and oven drying at 60°C (c)

Microwave dryings (both 250 W and 700 W) proved to be the worst methods for conserve species with external glandular hairs (e.g. members of the *Lamiaceae* plant family), as these techniques caused a huge, 72-97% EO loss (Figure 1). Based on SEM photos (Figure 1), microwaves destroyed the glandular hairs completely. Nevertheless, in relation to species with internal secretory ducts (e.g. members of the *Apiaceae* plant family), microwave drying, especially at higher power (700 W) was found to be quite effective (it caused only 13-59% decrease in volatiles). Drying at 250 W resulted in a higher, 13-73% EO loss.

According to these data, it can be concluded that there is a strong relationship between the type of secretory structures in plants and the effects of lyophilization and microwave drying methods.

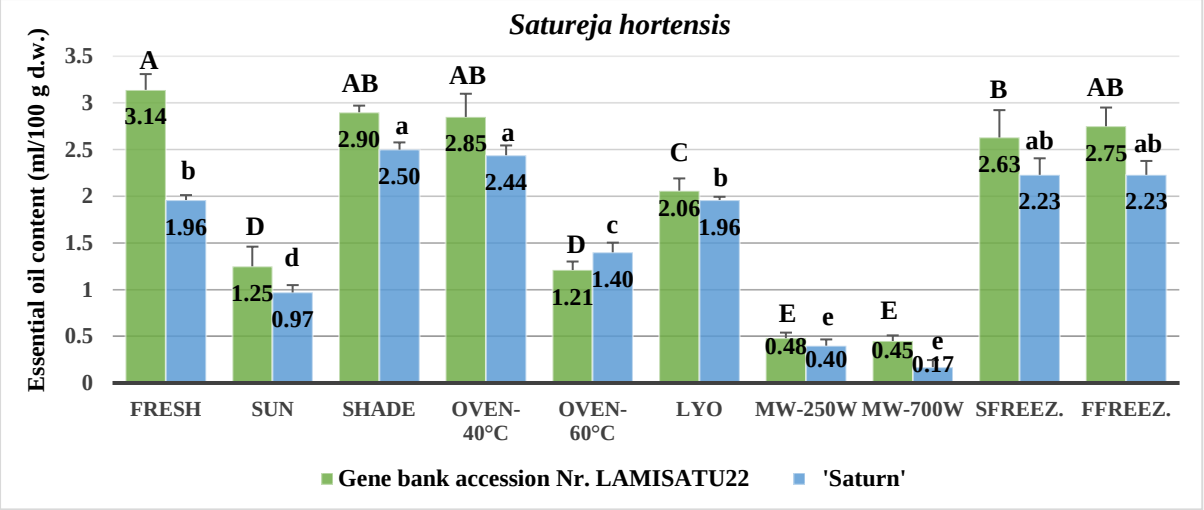

Figure 3. Essential oil content (ml/100 g d.w.) of fresh and preserved leafy shoots of *Satureja hortensis* L. gene bank accession Nr. LAMISATU22 and 'Saturn'

†Different letters indicate significant differences between means at $p < 0.05$. †Fresh = Fresh leaves; Sun = Sun drying; Shade = Shade drying; Oven-40°C = Oven drying at 40°C; Oven-60°C = Oven drying at 60°C; Lyo = Lyophilization; MW-250W = Microwave drying at 250 W; MW-700W = Microwave drying at 700 W; SFreez. = Slow freezing; FFreez. = Fast freezing

Chopping of parsley leaves before preservation reduced the EO content of fresh leaves by 41%, and that of preserved leaves by 31-51%, depending on the preservation method. The applied treatments, however, affected the EO content of chopped leaves in exactly the same way as for the whole leaves (Figure 4).

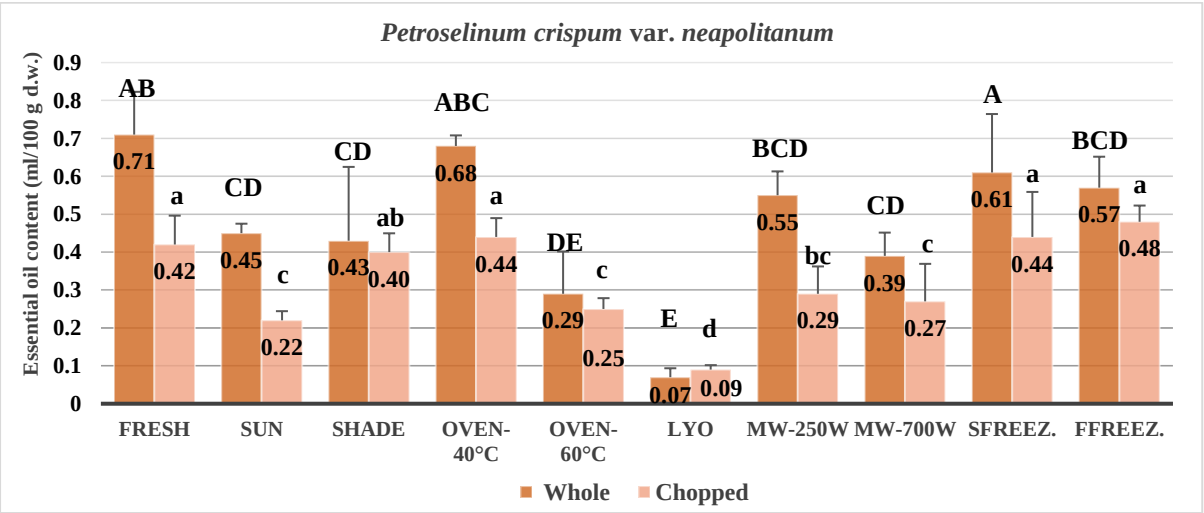

Figure 4. Essential oil content (ml/100 g d.w.) of fresh and preserved, whole and chopped leaves of *Petroselinum crispum* var. *neapolitanum*

†Different letters indicate significant differences between means at $p < 0.05$. †Fresh = Fresh leaves; Sun = Sun drying; Shade = Shade drying; Oven-40°C = Oven drying at 40°C; Oven-60°C = Oven drying at 60°C; Lyo = Lyophilization; MW-250W = Microwave drying at 250 W; MW-700W = Microwave drying at 700 W; SFreez. = Slow freezing; FFreez. = Fast freezing

4.2. Effect of different preservation methods on the essential oil (EO) composition

In our experiment, the preservation methods also affected the *EO composition* of plant species, but not equally and not to the same extent. In particular, those treatments influenced the composition significantly, which caused a huge, more than 70% of EO loss. These processes were lyophilization in connection with species containing the EO in internal secretory ducts, and microwave drying for species accumulating the volatiles in external glandular hairs (Table 3).

Table 3. EO composition of fresh and preserved leaves of *Salvia officinalis* L. 'Regula'

Compounds	LRI[a]	Total GC area percentages (%)									
		Fresh	Sun	Shade	Oven-40°C	Oven-60°C	Lyo	MW-250W	MW-700W	SFreez.	FFreez.
α-Pinene	938	1.61	1.72	1.40	1.71	1.18	1.22	0.26	0.23	1.70	1.05
Camphene	952	3.78	3.64	3.19	3.45	2.44	2.95	0.52	0.41	3.73	2.55
β-Pinene	981	1.70	1.70	1.49	1.60	1.06	1.54	0.21	0.18	1.79	1.46
Limonene	1029	1.85	2.10	1.97	1.92	1.38	1.92	0.39	0.25	2.10	1.89
1,8-Cineol	1034	**7.57**	**8.84**	**9.46**	**9.38**	**8.60**	**9.40**	**1.88**	**1.86**	**7.17**	**7.03**
α-Thujone	1105	**34.53**	**26.52**	**29.74**	**27.32**	**26.55**	**28.45**	**11.49**	**8.37**	**27.05**	**28.79**
β-Thujone	1113	**7.07**	**8.40**	**7.58**	**8.34**	**6.81**	**6.66**	**2.52**	**1.78**	**7.15**	**7.75**
Camphor	1144	**18.25**	**15.70**	**17.58**	**17.22**	**18.39**	**17.52**	**8.98**	**7.03**	**15.03**	**15.88**
Borneol	1165	2.03	2.96	2.69	2.07	2.63	2.56	1.88	2.08	2.30	2.31
Bornyl acetate	1284	0.75	1.16	0.84	0.94	1.29	1.57	1.61	1.86	1.14	1.18
E-Caryophyllene	1420	3.23	3.99	3.66	5.27	6.08	4.82	**11.11**	**9.85**	4.17	4.00
α-Humulene	1454	3.64	3.96	3.89	4.42	4.86	4.72	**9.34**	**8.91**	4.27	4.48
Caryophyllene oxide	1590	0.57	0.90	0.74	0.80	0.94	0.77	1.97	2.20	1.00	0.88
Viridiflorol	1598	**5.22**	**6.15**	**6.10**	**6.02**	**7.22**	**6.16**	**18.71**	**21.40**	**7.42**	**7.76**
Humulene epoxide II	1614	0.73	1.24	1.12	0.93	1.02	0.96	2.32	2.78	1.15	1.11
Manool	2060	**3.87**	**4.45**	**3.48**	**3.62**	**5.10**	**3.92**	**18.94**	**22.52**	**7.75**	**7.09**

Note: Major compounds are shown in bold. [a] Linear retention indices (LRI) calculated relative to the elution ranking of n-alkanes on HP-5 column. Fresh = Fresh sample; Sun = Sun drying; Shade = Shade drying; Oven-40°C = Oven drying at 40°C; Oven-60°C = Oven drying at 60°C; Lyo = Lyophilization; MW-250W = Microwave drying at 250 W; MW-700W = Microwave drying at 700 W; SFreez = Slow freezing; FFreez = Fast freezing.

Microwave treatment at 700 W exhibited a slightly more pronounced effect compared to treatment at 250 W. Furthermore, for oven drying at 60°C, when it resulted in a spectacular reduction in the EO content, compositional changes were also observed. Examining the changes in composition, it was established that the ratio of compounds with lower molecular weight notably decreased (these were presumed to have evaporated more readily), leading to an increase in the proportion of larger molecules. This alteration was consistent across all species studied. Additionally, during lyophilization new components (such as trans-anethole, estragole, α-terpinyl acetate, etc.) appeared in the EO primarily for those species, which contained the volatiles in endogenous secretory cavities (e.g. *Levisticum officinale*, *Petroselinum crispum* var. *neapolitanum* or *Artemisia dracunculus*).

In connection with parsley leaves, chopping before preservation further reduced the proportion of monoterpenes in the EO compared to whole leaves and increased the ratio of larger molecules (apiole and myristicin). This change was more spectacular, where the EO loss caused by different preservation methods proved to be higher.

4.3. Effect of preservation methods on the phenolic compounds (total phenolic content (TPC), total antioxidant capacity (TAC), rosmarinic acid (RA) content and total hydroxycinnamic acid (THA) content)

In connection with *phenolic compounds* (TPC, RA and THA content) furthermore TAC, eleven taxa of ten plant species were investigated. The different preservation techniques had very characteristic, consistent effects on the plant species examined. According to our observations, shade drying, oven drying at 40°C, lyophilization and microwave drying at 700 W were the most suitable methods for preserving the mentioned characteristics. These methods caused 1-55% loss in TPC, 1-46% loss in TAC and 1-24% reduction in RA and THA content, depending on plant species. The notable effectiveness of microwave drying at 700 W is likely due to its remarkably short drying duration, typically ranging from 4 to 8 minutes. Sun drying also proved to be a suitable method in preserving the TPC, TAC, RA and THA content quite well (e.g. for *Thymus vulgaris*, *Levisticum officinale*, *Helichrysum italicum* and *Melissa officinalis*), depending on the drying temperature (Figure 5).

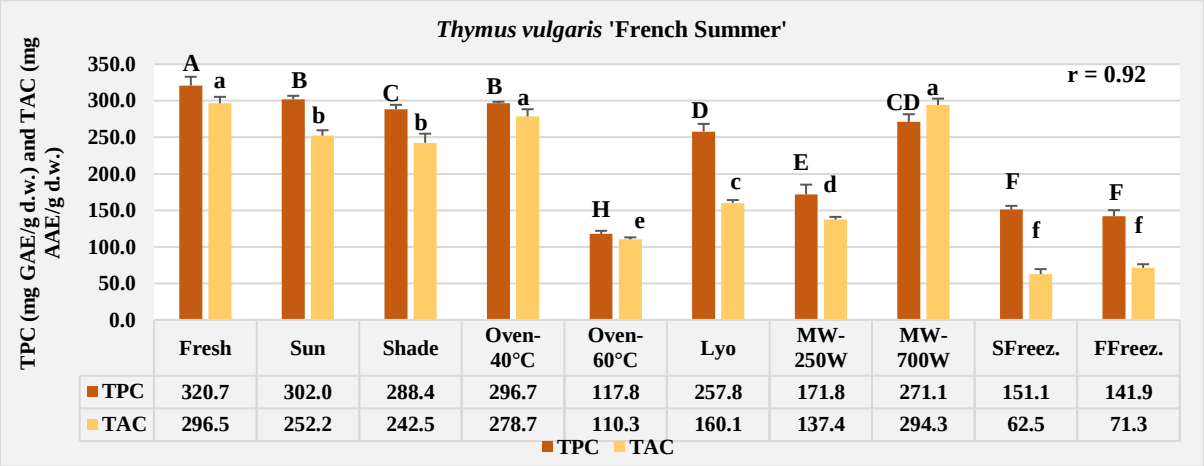

Figure 5. Total phenolic content (TPC) and total antioxidant capacity (TAC) of fresh and preserved leafy shoots of *Thymus vulgaris* L. 'French Summer'

†Different letters indicate significant differences between means at $p < 0.05$. †Fresh = Fresh leaves; Sun = Sun drying; Shade = Shade drying; Oven-40°C = Oven drying at 40°C; Oven-60°C = Oven drying at 60°C; Lyo = Lyophilization; MW-250W = Microwave drying at 250 W; MW-700W = Microwave drying at 700 W; SFreez. = Slow freezing; FFreez. = Fast freezing

The worst methods for preserving phenolic compounds and other molecules with antioxidant activity were oven drying at 60°C, microwave drying at 250 W and slow and fast

freezing methods. These techniques reduced the TPC and TAC by 30-87%, the RA content by 47-92% and the THA content by 75-81%. Accordingly, these processes cannot be recommended for the preservation of phenolic compounds and TAC of herbs and spices (Figure 6).

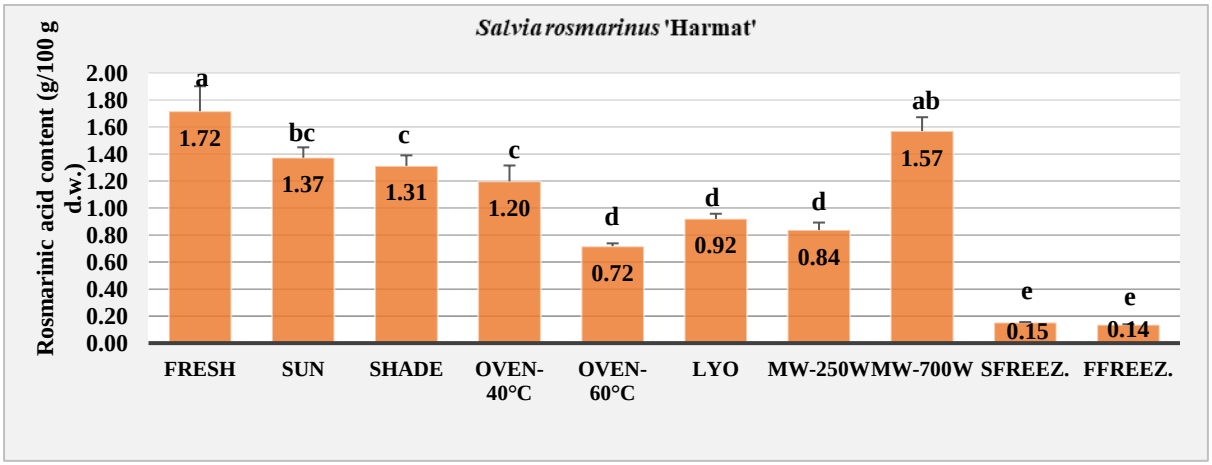

Figure 6. Rosmarinic acid content (g/100 g d.w.) of fresh and preserved leaves of *Salvia rosmarinus* L. 'Harmat'

†Different letters indicate significant differences between means at $p < 0.05$. †Fresh = Fresh leaves; Sun = Sun drying; Shade = Shade drying; Oven-40°C = Oven drying at 40°C; Oven-60°C = Oven drying at 60°C; Lyo = Lyophilization; MW-250W = Microwave drying at 250 W; MW-700W = Microwave drying at 700 W; SFreez. = Slow freezing; FFreez. = Fast freezing

Based on our data, a very strong relationship was detected between the TPC and TAC of aqueous extracts prepared from the examined, fresh and treated plant samples of species, suggesting that the antioxidant activity of the extracts was mainly due to the phenolic compounds contained in them.

4.4. Effect of preservation methods on the organoleptic characteristics

4.4.1. Colour

The preservation techniques also significantly affected the *colour characteristics* (the lightness (L*), greenness (-a*), yellowness (b*)) of the studied 10 plant species. Freezing (both slow and fast) was found to be an excellent method for maintaining the vibrant colour of herbs and spices. Lyophilization exhibited similar effectiveness in preserving the natural colour of fresh plant materials. Although the lyophilized samples appeared slightly lighter, their original hues remained perfectly intact. Microwave drying at 700 W and oven drying at 40°C also emerged as preferred techniques, showcasing good preservation of the original colour of raw samples. However, the other treatments, especially drying at 60°C resulted in significant colour degradation. At this elevated temperature, the chlorophyll content suffered damage, resulting

in a noticeable reduction in green hue. Consequently, the samples exhibited a darker, greyer appearance (Figure 7).

Figure 7. **Colour characteristics of fresh and preserved leaves of *Mentha x piperita* 'Mexián'**

4.4.2. Taste

For the first time, the *taste* of extracts made from seven herbs and spices was evaluated using electronic tongue (e-tongue) instrument. The results obtained showcased the applicability of e-tongue in distinguishing between various preservation methods. Linear discriminant analysis (LDA) models generated from e-tongue data for classifying different treatments yielded clear and distinct results. In our experiment, we observed a pronounced contrast in the taste profiles of alcoholic extracts derived from plant samples. The e-tongue results unambiguously distinguished between the tastes of fresh samples and those subjected to different treatments, each treatment imparting a distinct flavour profile. Generally, fresh samples exhibited tastes most closely resembling those of frozen and lyophilized samples. Both microwave treatments (at 250 W and 700 W) resulted in extracts with remarkably similar flavours, as did the two different freezing methods (slow and fast) (Figure 8). Our findings suggest a strong correlation between the flavour of samples and the content and composition of EOs in plant materials. However, other compounds also influence the flavour besides volatiles.

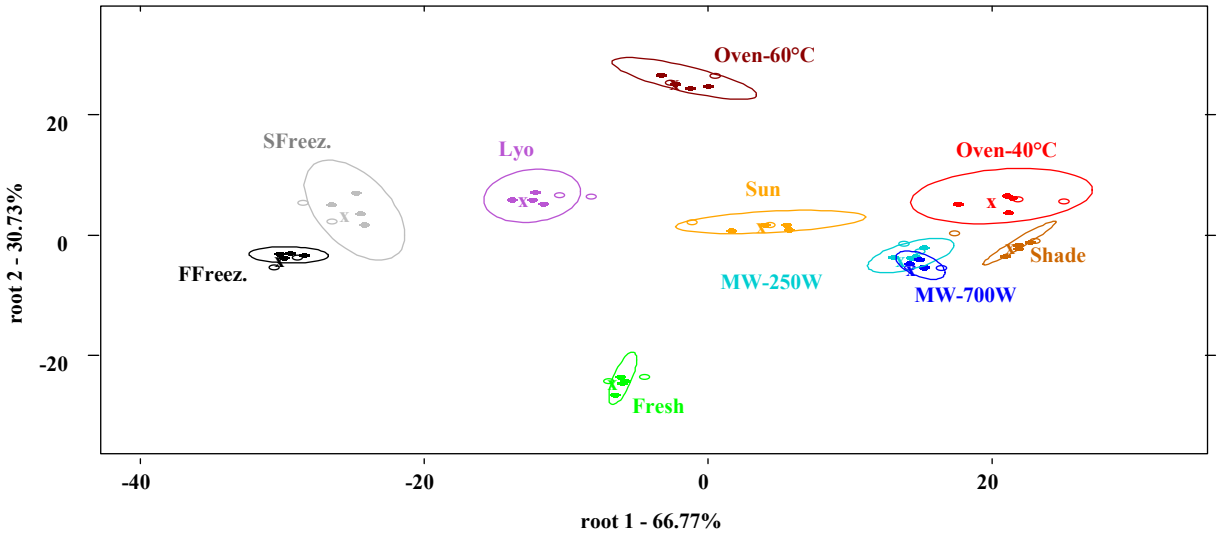

Figure 8. Linear discriminant analysis classification model for the grouping of preservation methods based on electronic tongue results applied on *Origanum vulgare* L. subsp. *vulgare*

†Fresh = Fresh leaves; Sun = Sun drying; Shade = Shade drying; Oven-40°C = Oven drying at 40°C; Oven-60°C = Oven drying at 60°C; Lyo = Lyophilization; MW-250W = Microwave drying at 250 W; MW-700W = Microwave drying at 700 W; SFreez. = Slow freezing; FFreez. = Fast freezing

5. CONCLUSIONS AND RECOMMENDATIONS

The findings of this research demonstrated the effects of examined preservation methods on the quality of medicinally important spices. Based on our results, we have found that in case of EO content and composition, the plant material had a great importance in terms of whether it accumulates the volatiles externally in glandular hairs or internally in secretory ducts, since certain preservation techniques had different effects on the different types of plant species. However, in connection with other properties (e.g. TPC, TAC, colour or taste), the preservation processes had the same effect on the examined plants, so in this case those had a greater impact on the quality of the final product than the used plant material.

Drying herbs and spices naturally (in the sun or in shade) is one of the oldest and most cost-effective preservation method used for a variety of spices. According to our data, shade drying proved to be a more advantageous method compared to sun drying. Although drying in shade is considered a very lengthy process in contrast to sun drying, this method was very efficient in maintaining the amount of volatiles and the original EO composition of plant species, presumably due to the low drying temperature. This method also preserved the phenolic compounds and the samples' antioxidant capacity very well. Although it did not always preserve the colour of the samples perfectly, overall we found it to be a very effective preservation method. In contrast, drying in the sun proved to be a more unpredictable process.

Depending on the actual drying temperature, sometimes it's effect was more similar to shade drying, but other times it's effect was closer to oven drying at 60°C.

Convective drying at 40°C - as expected - effectively retained the volatiles, the phenolic compounds and the organoleptic attributes of the examined spices. Conversely, oven drying at 60°C significantly decreased the content of active ingredients and other quality characteristics. Hence, convective drying at low temperature is recommended in order to get the final product of the optimal quality.

Freezing emerged as one of the most efficient preservation method in maintaining the original colour, volatile content and composition of plants, independently of the place of EO accumulation. These favourable outcomes are likely attributable to the immobilization of water in the form of ice, which slows down the degradation rate during the freezing process compared to preservation methods with higher temperatures. In our research there were no spectacular differences between the effects of slow and fast freezing methods, even the taste of their samples was also similar. However, freezing techniques significantly decreased the amount of phenolic compounds and the antioxidant capacity proving it as a least advisable method when the goal is to preserve such active substances.

Microwave drying is a lesser-known technique for the preservation of spices. Currently, there are limited researches on its applicability. Within this study, microwave drying exhibited a significant impact on the quality of studied plants. For species that contain the EO in external glandular hairs (e.g. the members of *Lamiaceae* plant family), microwave drying was not a suitable preservation method. It damaged the glandular hairs, causing a huge loss of volatiles. In the minimal remaining EO, mainly the larger compounds could be detected. However, for species that accumulate the EO in internal secretory ducts (e.g. the *Apiaceae* family), the caused EO loss was not so significant, mainly at higher power. Drying at 700 W proved to be much better compared to drying at 250 W. 700 W was able to retain not only the EO, the phenolic compounds and antioxidant capacity, but the original colour of samples as well, in contrast to drying at 250 W, which caused a lot of damage and loss. The increased microwave power substantially reduces the drying time, thereby safeguarding bioactive compounds from evaporation and enhancing colour retention. According to this, drying at 700 W was found to be a suitable method for the preservation of the above-mentioned active substances. Otherwise, the two microwave drying methods resulted in a relatively similar taste.

Lyophilization is an innovative technology that is still not widely used for preserving spices. Based on our findings, lyophilization retained the quality and quantity of volatiles in relation to species where EO is accumulated in external glandular hairs. Hence, it can be

recommended as an effective technique for preserving volatiles for these species. However, it caused a huge loss of EO in species that accumulate volatiles primarily or exclusively in endogenous secretory ducts. For these species, it modified the EO composition too: the ratio of larger molecules increased, and in some plant species, new aroma compounds appeared. Lyophilization also preserved the phenolic compounds and antioxidant capacity of studied plant species in a high amount, as well as the original colour of fresh plant materials.

Improving the quality characteristics of the medicinally important spices is a constant demand. When selecting a preservation method, it is recommended to consider the end-use of spices and optimize the preserving conditions based on the specific requirements of each plant species. Every method has its advantages and disadvantages, and the optimal choice may depend on the specific spice, its intended use and the availability of resources. Further research is also recommended to develop novel technologies and optimize the existing ones to preserve spices of the superior quality while becoming more environmentally and economically sustainable.

6. NEW SCIENTIFIC FINDINGS

It was the first, really comprehensive study on the preservation of spices, in which the effects of 9 preservation methods (lyophilization, fast and slow freezing, drying in the sun, in shade, in oven at 40 and 60°C, by microwave at 250 W and 700 W) were investigated and evaluated in connection with many characteristics (EO content and composition, TPC, TAC, RA and THA content of aqueous extracts, colour, flavour) for 18 plant species. The following scientific results have been identified for the first time during the research:

1. There is a strong correlation between the location of EO accumulation (the type of secretory structures) and the effects of certain preservation methods (such as lyophilization and microwave drying):
   - For species that contain the EO in external glandular hairs (e.g. members of the *Lamiaceae* plant family), lyophilization is a suitable preservation method, whereas microwave drying damages the epidermal glands, causing a huge loss of volatiles.
   - However, in species that contain the EO mainly in endogenous secretory ducts (e.g. members of the *Apiaceae* plant family), microwave drying is an appropriate method (especially at higher power), but lyophilization is not. It causes a very porous tissue

structure, due to which volatiles can evaporate from the internal cavities very easily, resulting in huge EO loss.

2. Preservation methods that result in substantial volatile loss (exceeding 70%), also cause significant alterations in the EO composition. However, those methods that result in lower volatile loss (less than 70%), do not modify the characteristic composition significantly.

3. The applicability of *freezing* in the preservation of spices has been evaluated complexly for the first time. It has been established, that freezing is obviously suitable for preserving the EO content and the original composition of plants, independently of the place of EO accumulation. It can perfectly preserve the original colour of plant materials as well. Conversely, it drastically reduces the TPC, TAC, RA and THA content of samples. Thus, it is not suitable for the preservation of phenolic compounds at all.

4. *Microwave drying at 700 W* is an effective method for preserving the EO content and original composition of those species, which accumulate the volatiles primarily in endogenous secretory ducts. This technique also conserves the TPC, TAC, RA and THA content of samples very well. Moreover, it is suitable for retaining the original colour of raw plant materials too. These statements are already not valid for microwave drying at 250 W.

5. The applicability of *lyophilization* in the preservation of spices has also been clarified. For species that accumulate the volatiles in external glandular hairs, it is a good technique to conserve the EO content and the original composition at a high level. It also retains the TPC, TAC, RA and THA content of samples very well, moreover, their original colour too. Although lyophilized samples become slightly paler, but the natural hues are preserved.

6. Chopping before preservation reduces the EO content of *Petroselinum crispum* var. *neapolitanum* leaves by 30-50%, and increases the ratio of larger molecules (apiole and myristicin) within the EO. However, it has no effect on colour characteristics.

7. The electronic tongue instrument was used for the first time to evaluate the taste of extracts made from spices. The results showed that this method was perfectly suitable to distinguish the flavour of samples preserved in different ways. The applied treatments were successfully classified using LDA models on e-tongue data, where fresh samples clearly separated from the others according to their taste, for each species.

Publications

Journal articles

1. Gosztola, B., **Hazarika, U.** (2020): The effect of different preservation methods on the colour and active substance content of chives leaves. *Kergazdaság*, **52**(2), 68-81.
2. **Hazarika, U.**, Gosztola, B. (2020): Lyophilization and its effects on the essential oil content and composition of herbs and spices - A review. *Acta Sci. Pol. Technol. Aliment.* **19**(4), 467-473. https://doi.org/10.17306/J.AFS.2020.0853 (IF: 1.5)
3. Gosztola, B., Radácsi, P., **Hazarika, U.** (2022): The effect of different preservation methods on the colour and active substance content of garden sage leaves. *Kergazdaság*, **54**(4), 34-50.
4. **Hazarika, U.**, Kovács, Z., Bodor, Z., Gosztola, B. (2022): Phytochemicals and organoleptic properties of French tarragon (*Artemisia dracunculus* L.) influenced by different preservation methods. LWT, **169**, 114006. https://doi.org/10.1016/j.lwt.2022.114006 (IF: 6)

Conference paper (Abstract)

1. **Hazarika, U.**, Gosztola, B. (2021): Effect of different preservation methods on the active compounds and organoleptic properties of Garden Sage (*Salvia officinalis* L.) leaves. 51st International Symposium on Essential Oils (ISEO), November 12-14, 2021 (Online). Book of Abstract, Pp.22.
2. Balkis, A., **Hazarika, U**., Gosztola, B., Kovács, Z. (2023): Drying-induced alterations of the phytochemicals content and the water spectral pattern of selected herbs. 3rd Aquaphotomics European Conference, September 02-04, 2023, Rome, Italy, Book of Abstracts, Pp.14.
3. Gosztola, B., Kovács, Z., Bodor, Z, **Hazarika, U**. (2023): Effects of chopping and different preservation methods on the volatiles and organoleptic properties of Parsley (*Petroselinum crispum* (Mill) Nym. var. *neapolitanum*) leaves. 53rd International Symposium on Essential Oils (ISEO), September 13-16, 2023. Book of Abstract, Pp.1.

Short Communication

1. **Hazarika U.**, Gosztola, B. (2020): The effect of different preservation methods on the colour and active substance content of Summer Savory (*Satureja hortensis* L.) leafy shoots. Macedonian Pharmaceutical Bulletin, 66 (Suppl. 2) 31-32. DOI: 10.33320/maced.pharm.bull.2020.66.04.015
2. **Hazarika U.**, Gosztola, B. (2022): Active substances and colour characteristics of Peppermint (*Mentha x piperita* L.) leaves influenced by different preservation methods. Macedonian Pharmaceutical Bulletin, 68 (Suppl. 2) 207-208. DOI: 10.33320/maced.pharm.bull.2022.68.04.095