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

Doctoral (Ph.D.) Dissertation

By

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DECLARATION

I declare that the thesis titled “Effect of different preservation methods on the active substances and organoleptic properties of selected spices” is my work and that it has not been submitted before for any degree or examination in any other University.

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List of abbreviations

AAE	Ascorbic acid equivalent
ANOVA	Analysis of variance
CPD-VMFD	Convective pre-drying and vacuum-microwave finish-drying
d.w.	dry weight
DMAPP	Dimethylallyl pyrophosphate
DNA	Deoxyribonucleic acid
DOXP	deoxy-D-xyloluse
EMA	European Medicines Agency
EO	Essential oil
FIR	Far-infrared drying
FRAP	Ferric reducing antioxidant power
G3P	D-glyceraldehyde 3-phosphate
GAE	Gallic acid equivalent
GC-MS	Gas Chromatography-Mass Spectrometry
HPLC	High-performance liquid chromatography
IFSET	Ion selective field effect transistor
IPP	Isopentenyl pyrophosphate
ISO	International Organization for Standardization
LDA	Linear discriminant analysis
LRI	Linear retention indices
MAP	Medicinal and aromatic plants
MATE	Magyar Agrár-és Élettudományi Egyetem (Hungarian University of Agriculture and Life Sciences)
PAL	Phenylalanine ammonia lyase
Ph. Eur.	European Pharmacopoeia
Ph.Hg.	Hungarian Pharmacopoeia
RA	Rosmarinic acid
SEM	Scanning electron microscopic
TAA	Total antioxidant activity
TAC	Total antioxidant capacity
TFC	Total Flavonoid Content
THA	Total hydroxycinnamic acid
TIC	Total ion current
TPC	Total phenolic content
UV	Ultraviolet

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1. INTRODUCTION

The quality of food is playing an important role in our daily life and the use of culinary spices, which have biologically active ingredients, is an integral part of this. Spices have been identified as sources of various phytochemicals, many of which possess powerful antioxidant activity and various pharmacological attributes (Velioglu et al., 1998; Dragland et al., 2003; Newman and Cragg, 2012). In general, spices contain secondary metabolites such as volatile oils, which are a complex mixture of hydrocarbons and their oxygenated derivatives that determine the organoleptic properties and consumer value of them to a significant extent. Volatile oils are synthesized by different plant organs, externally and internally. Those species which contain the volatile oils externally in glandular hairs (e.g. garden sage, sweet basil, marjoram, etc.) on their surface are more sensitive to external influences compared to those species which accumulate the volatiles within their organs, in secretory cells or in intercellular secretory ducts (e.g. lovage, dill, etc.). Furthermore, spices also contain phenylpropanoids, phenolic compounds, tannins, coumarins and many other important organic molecules, which also contribute to the taste, aroma and health 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 quality of final plant product. The different preservation methods greatly influence the essential oil (EO) content and composition, the content and quality of phenolic compounds, the antioxidative properties of the product, as well as the organoleptic features (taste, colour and smell). 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 (Jin et al., 2018). Natural drying (drying in the sun or in shade) has been in use since ancient times. This is a traditional way of preserving spices, but these are time consuming, hardly adjustable and degrades the quality in many cases. For this reason, artificial methods of drying involving new techniques are more commonly used in present times, such

as hot air oven drying, microwave drying, vacuum drying, lyophilization, infrared drying, etc., where the main physical parameters of the drying process can be controlled (Janjai et al., 2008).

Among artificial drying methods, freeze-drying or lyophilization is one of the most recent, non-convective technique that is used for drying spices. This drying technology requires low temperature and shorter processing time leading to promising results, but its effect on active compounds is still not well known (Kamath, 2006).

Another non-convective method that has been employed in recent years for drying spices is microwave drying. This technique uses microwave energy to generate heat directly within the plant materials, which significantly speeds up the drying process compared to other conventional methods. 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.

Most of the previous researches in the literature were limited to evaluation of only a few preservation methods, in various combinations, which makes it difficult to compare and evaluate them. Moreover, only 1-2 plant species were examined simultaneously, so it is challenging to generalize about plant species as a whole. In addition, preservation methods affect the qualitative and quantitative characteristics of the final product, but in most of the previous researches only few of these aspects were studied. The situation is further complicated by the fact that the results obtained were also found to be rather contradictory.

Therefore, the aim of this research was to carry out a large, comprehensive study involving numerous spices, in which the effects of the most commonly used or potentially beneficial preservation methods on the quality of these plant species were investigated. Further aim was to draw general conclusions that could help the companies to produce spices with the highest quality.

2. OBJECTIVES TO ACHIEVE

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. LITERATURE OVERVIEW

3.1. Introduction of medicinally important spices

Medicinal plants have been used since ages for different applications in every part of the world. These plants can be utilized for medical purposes, either directly or indirectly, due to their content of active ingredients. The active ingredients are synthesized biologically and accumulated in the plant in very small concentrations, sometimes less than 1% of the dry material content of the plant. Generally not the entire plant is used but only those specific parts such as leaves, fruits, roots etc. which contain the desired active compounds (Hornok, 1992).

Before the arrival of modern medicine, MAPs served as primary healthcare remedies, a tradition persisting globally, while also being esteemed for their role in manufacturing various industrial products. Although many MAPs are used traditionally, but most of these plant species are still not registered. Approximately 35,000 to 75,000 plants are utilized for medicinal purposes, but only a very small percentage of these species are consistently grown through cultivation, while the rest are collected in their natural habitats (Öztekin and Martinov, 2014).

Certain types of medicinal plants are utilized not only for their medicinal properties but also for enhancing the flavour, enjoyment of foods and beverages, as seasonings. These MAPs are called as spices. Spices are not only used for seasoning, flavouring, but many of them are suitable for colouring foods as well. Spices are also used in medicine, cosmetics or perfume production (Hornok, 1992).

Spices have long history of aromatic use, cultural significance, culinary importance, and the growing body of scientific evidence supporting their health benefits. These play a diverse role in enhancing culinary experiences, offering a spectrum of flavours, hues, scents, and aiding in food preservation (Douglas et al., 2005). They originate from various plant parts, including bark, buds, flowers, fruits, leaves, rhizomes, roots, seeds, stigmas and styles, or even the entire plant tops. The term “herbs and spices” refer to those spice species, of which we use only or primarily the green leafy parts. Majority of the well-known spices originate from Europe, Africa and Asian countries (Öztekin and Martinov, 2014).

Spices play a vital role in pharmacology, featuring prominently in numerous drugs and dietary supplements. Each species has a different physiological effect depending on its active ingredients. For example, notable spices utilized in pharmacology encompass *Lavandula spp.* for antifungal, antibacterial, anti-inflammatory and sedative properties (Cavanagh and Wilkinson, 2002), *Thymus vulgaris* for strong antibacterial, antifungal, antiviral, antiparasitic,

spasmolytic and antioxidant activities (Pirbalouti et al., 2011), *Helichrysum italicum* L. for antimicrobial and anti-inflammatory properties (Viegas et al., 2014), *Melissa officinalis* L. for various medicinal uses such as sedative, antimicrobial, antispasmodic, antiviral (Allahverdiyev et al., 2004; Schnitzler et al., 2008), *Ocimum basilicum* for antibacterial, antioxidant and digestive properties (Juliani and Simon, 2002; Hussain et al., 2008; Jayasinghe et al., 2003; Lee and Scagel, 2009; Kwee and Niemeyer, 2011), *Rosmarinus officinalis* for mainly antimicrobial and anti-inflammatory applications (Arranz et al., 2015; Haloui et al., 2000; Rožman and Jeršek, 2009; Teixeira et al., 2013), *Levisticum officinale* for diuretic, carminative, antimicrobial and anti-inflammatory activities (Boligon et al., 2013). Although the bioactive compounds present in spices have many promising industrial and medical applications, it should be noted that certain substances may exhibit toxic effects too.

Spices can also be used as natural food preservatives due to their high antioxidant activity (Hinneburg et al., 2006). These boast abundant antioxidants, comprising flavonoids, volatiles, lignans, polyphenolics, carotenoids, coumarins, plant sterols, etc. The phenolic substances, notably containing at least two hydroxylic groups in the ortho or para positions, such as caffeic acid, stand out as the most potent antioxidants present in spices (Embuscado, 2015).

In addition, spice extracts possess a range of other beneficial properties, including e.g. sunscreen, anti-aging, anti-inflammatory, moisturizing, antioxidant and anti-cellulite properties, due to which the cosmetics industry also prefers to use them. Natural skincare products, unlike synthetic cosmetics, are gentle, biodegradable and exhibit a low toxicity profile (Chanchal and Swarnlata, 2008).

Spices, with their extensive culinary history, not only enhance flavour but also offer functional benefits such as salt, fat and sugar reduction, vitamin enrichment and the provision of essential micro- and macro elements in food products (Leja and Czaczyk, 2016).

3.2. Most important active substances of spices

Active constituents found in spices are secondary metabolites with significant variability in distribution across the plant kingdom. Their precise role in plant life remains largely unknown in many cases. The most important groups of active substances for our topic are essential oils and phenolics.

Volatiles and essential oils (EOs)

Volatiles are those secondary metabolites, which can be synthesized by different plant organs including leaves, rhizomes, roots, bark, wood, flowers, seeds, peel, fruits, etc. both

internally and externally. Internally, volatiles are present in secretory cells (e.g. *Laurus nobilis*) or in intercellular secretory ducts (e.g. *Levisticum officinale*, *Anethum graveolens*, etc.), and externally in glandular hairs (e.g. *Ocimum basilicum*, *Origanum majorana*, etc.).

Spices deploy a diverse array of glandular hairs, each uniquely shaped and sized to serve specific purposes. These glands serve primarily to safeguard the plant's various organs and to entice pollinators. Glandular trichomes can vary based on the shape of their secretory heads and the morphology and composition of the substances they secrete. They fall into two main categories: peltate and capitate hairs (Figure 1). Capitate glands typically have 1-4 rounded secretory cells positioned horizontally, along with a stem comprising one or several elongated cells, and a basal cell (Hazzoumi et al., 2020). In some species, such as certain *Salvia* species, the head cell may be notably large (Werker et al., 1985). On the other hand, peltate hairs have heads composed of 4-18 flatter cells arranged on a horizontal plane, along with a stem cell and a basal cell (Hazzoumi et al., 2020). Werker et al. (1994) have further classified these glands into short-term and long-term ones. Short-term glands swiftly secrete to shield young organs, while long-term glands gradually accumulate secretory substances in the subcuticular space, contributing to the safeguarding of mature organs like flowers and aiding in pollination. They suggested that capitate hairs function as short-term glands, while peltate hairs serve as long-term glands. This classification hinges on distinctions in structure, secretion mechanisms and secretion timing between the two gland types. The glandular trichome consists of multiple cells that carry out distinct functions: secretory cells synthesize terpenes, which then move into a subcuticular space at the top of the trichome for storage, while basal cells are responsible for anchoring the structure to the epidermis. These glands exhibit variations in morphology, biochemistry and secretion patterns (Hazzoumi et al., 2020).

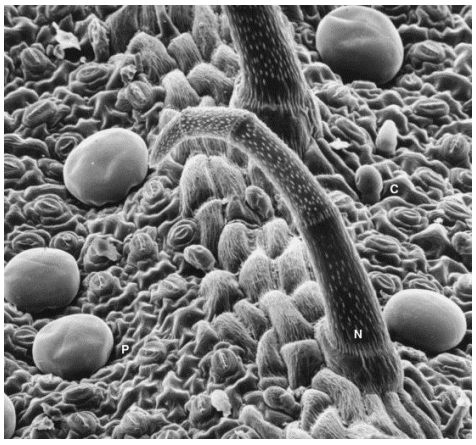

Figure 1. Scanning electron micrograph of a peppermint leaf (abaxial surface) showing peltate (P) and capitate (C) glandular trichomes and non-glandular trichomes (N); Scale bar = 50 µm (https://www.cell.com/trends/plant-science/abstract/S1360-1385(01)02056-8)

Essential oil as defined by the International Organization for Standardization (ISO) is a product obtained from a natural raw material of plant origin, by steam distillation, by mechanical processes from the epicarp of citrus species, or by dry distillation, after separation of the aqueous phase, if any, by physical processes (Başer and Buchbauer, 2009).

EOs are very complex mixtures, often with hundreds of constituents, specific to different plant species or taxonomic units. However, composition exhibits considerable variability, influenced by factors such as plant species (Sarac and Ugur, 2008), geographic origin and environmental conditions (Sarac and Ugur, 2008; Mechergui et al., 2010), seasonal variations in harvesting (Hussain et al., 2008; Figueiredo et al., 2008), methods of drying (Di Cesare et al., 2003) or techniques employed in extraction (Burt, 2004; Karakaya et al., 2011). EOs are particularly susceptible to degradation when exposed to heat, moisture and oxygen. Aging processes often result in the formation of oxygenated terpenes, chemical transformations or polymerization, leading to a decline in quality. Consequently, it is imperative for producers, traders and essential oil manufacturers to rigorously monitor the quality control processes (Turek and Stintzing, 2013).

EOs are derived from various parts of plants, primarily through processes like steam distillation or water distillation (Hyldgaard et al., 2012). Typically, EOs are associated with distillation, where volatile compounds are expected to be extracted. The final composition varies when plant material is exposed to high temperatures in the presence of water over an extended period, compared to when water vapor swiftly passes through the plant material in a shorter duration (Chamorro et al., 2012). Supercritical CO_2 extraction stands out as an alternative to distillation by preserving the integrity of the final product without introducing chemical alterations. This method retains delicate compounds and a diverse array of other components in their original state (Herzi et al., 2013).

EOs exhibit a diverse range of bioactivities and serve as valuable natural reservoirs of antimicrobial and antioxidant agents (Bassolé and Juliani, 2012; Iqbal et al., 2013; Victoria et al., 2012). Wei and Shibamoto (2010) identified specific terpenes and terpenoids within essential oils that contribute to antioxidant activity. These include menthone and isomenthone in peppermint. Similarly, thymol and carvacrol, the primary compounds found in thyme oil, demonstrate robust antioxidant properties (Burdock, 2005).

EOs demonstrate versatile effectiveness in inhibiting a wide range of bacterial pathogens too (Teixeira et al., 2013). The EO of *Origanum vulgare* and *Lavandula officinalis* is found to be effective against *Escherichia coli* and *Staphylococcus aureus* (Martucci et al., 2015); *Satureja montana* against *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, *Streptococcus*

mutans, *Streptococcus sanguis*, *Streptococcus salivarius*, *Enterecoccus faecalis*, *Lactobacillus acidophilus* (Nikolić et al., 2014); *Ocimum basilicum*, *Salvia rosmarinus*, *Origanum majorana*, *Mentha piperita*, *Thymus vulgaris* against *Clostridium perfringens* (Radaelli et al., 2016); *Ocimum gratissimum* against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Hyldgaard et al., 2012), just to mention a few research results.

EOs are also reported to have antifungal activity. Bouchra et al. (2003) discovered that the EOs from *Origanum compactum* and *Thymus glandulosus*, contained potent compounds such as carvacrol, linalyl acetate and thymol. These compounds exhibited remarkable antifungal properties, completely inhibiting the growth of *Botrytis cinerea* mycelium at just 100 ppm concentration. Similarly, Serrano et al. (2005) effectively reduced the growth of yeasts and molds on stored cherries by incorporating active packaging materials infused with eugenol, menthol, thymol and eucalyptol. Moreover, citral, citronellol and geranyl acetate demonstrated significant cell cycle inhibitory effects against *Candida albicans* (Zore et al., 2011).

Some EOs also seem to be effective in cancer therapy (EO of *Origanum vulgare*, *Thymus vulgaris*). Key components of them exhibit promising potential by demonstrating both cytotoxic effects and the ability to inhibit tumour growth. When integrated with conventional cancer therapies, EOs can effectively mitigate the adverse effects of medication (Hadfield, 2001). Their cytotoxic properties stem from their ability to disrupt cellular integrity, resulting in processes such as necrosis, apoptosis and cell cycle arrest, as well as impairing key organelle functions (Bhardwaj et al., 2013; Russo et al., 2015). However, it is crucial to thoroughly assess both the anticancer efficacy EOs and their safety on normal cell lines (Sieniawska et al., 2016).

In recent clinical applications, EOs have emerged as promising treatments for inflammatory conditions (e.g. rheumatism, arthritis, allergies, eczema or other inflammations in the body). For instance, the EO of *Melaleuca alternifolia* has demonstrated notable anti-inflammatory properties, primarily attributed to its key compound, α-terpineol (Koh et al., 2002; Caldefie-Chézet et al., 2006; Hart et al., 2000). Similarly, *Pelargonium* x *asperum* EO contains geraniol and β-citronellol, both of which exhibit anti-inflammatory effects (Maruyama et al., 2005). Additionally, 1,8-cineole, found in various EOs, has been identified to possess anti-inflammatory properties (Yoon et al., 2000). These findings underscore the potential of volatiles in mitigating inflammation through diverse molecular mechanisms.

Volatiles are sensitive to sunlight, high temperature, moisture and oxygen. Hence, processing and preserving plant parts containing volatiles requires a great deal of expertise. During preservation, it is important to avoid high temperatures, as EOs evaporate at relatively high temperatures (Öztekin and Martinov, 2014).

Terpenes

The constituents of volatiles are terpene molecules, primarily monoterpenes and sesquiterpenes, which could be terpene hydrocarbons or oxygenated compounds too (Mohamed et al., 2010). Terpenes are synthesized within the cytoplasm of plant cells via the mevalonate pathway, which is also known as isoprenoid pathway, and via the DOXP (deoxy-D-xyloluse) pathway (Hyldgaard et al., 2012) (Figure 2).

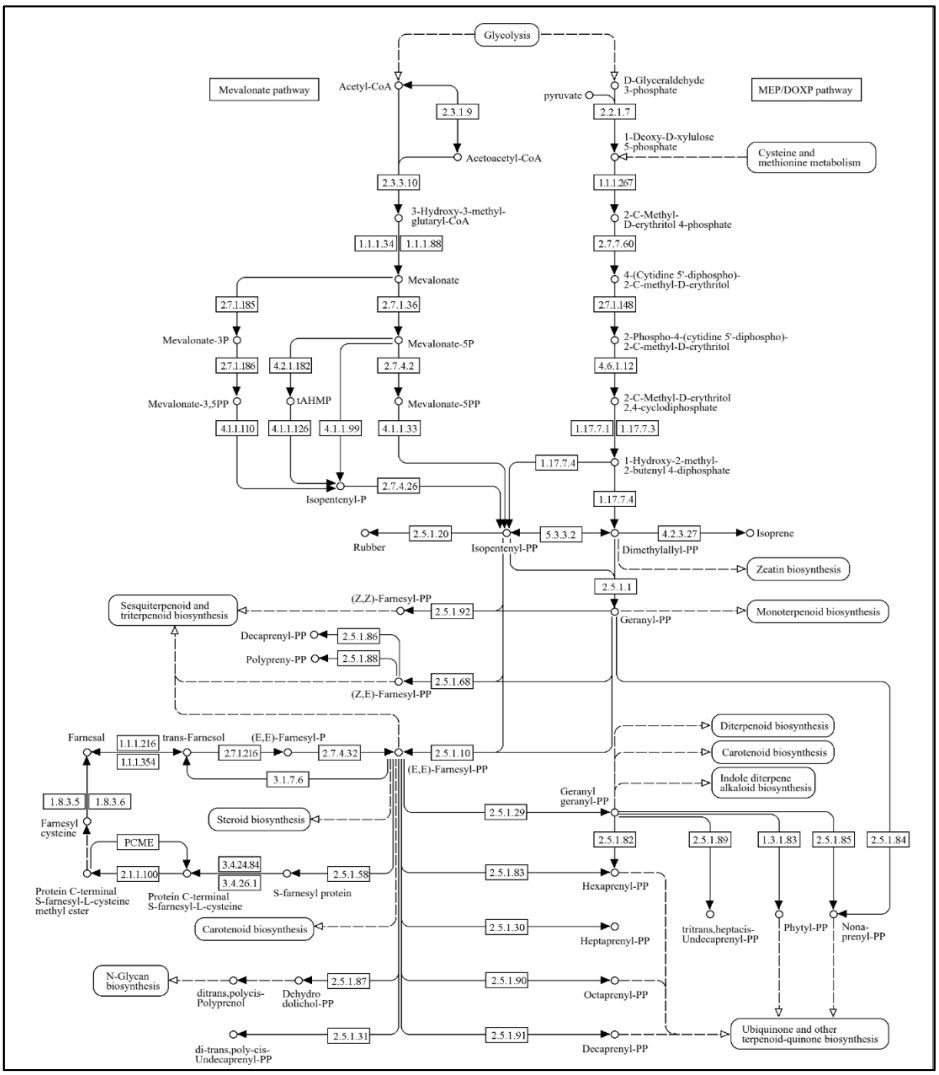

Figure 2. Biosynthesis of terpenes via mevalonate and DOXP (deoxy-D-xyloluse) pathways (https://www.genome.jp/pathway/map00900)

The mevalonate pathway accounts for conversion of acetyl-CoA and ends with the production of two five-carbon building blocks called isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP) (Miziorko, 2011). Whereas, in the DOXP pathway, pyruvate and D-glyceraldehyde 3-phosphate (G3P) convert to IPP and DMAPP by seven enzymatic reaction steps (Wiesner and Jomaa, 2013).

Terpenes consist of isoprene (C_5) units. They can be classified based on the number of isoprene units they contain. This classification includes monoterpenes (C_{10}), sesquiterpenes (C_{15}), diterpenes (C_{20}), etc. Volatile components come from these three groups primarily.

Monoterpenes with a chemical formula $C_{10}H_{16}$ have two isoprene units attached together. Monoterpenes are classified as acyclic (e.g. ocimene (Figure 3), linalool, linalyl acetate, etc.), monocyclic (e.g. limonene, carvone, etc.), bicyclic (e.g. pinene, camphene, sabinene, etc.) or aromatic ones (e.g. thymol, carvacrol, etc.) (George et al., 2015). Due to their rapid reaction to heat sources and air these compounds oxidize easily (Hunter, 2009).

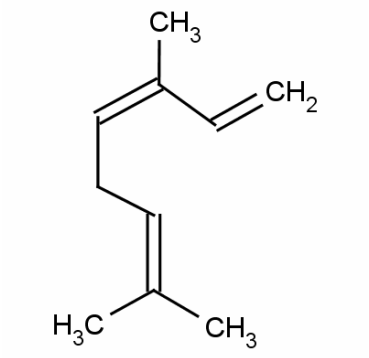

Figure 3. Skeleton of monoterpene (Z)-beta-Ocimene
(https://www.chemnet.com/cas/en/3338-55-4/OCIMENE.html)

Sesquiterpenes (e.g. caryophyllene, germacrene D, caryophyllene oxide, etc.), with a chemical formula of $C_{15}H_{24}$ (Figure 4), are the next class following monoterpenes. These are already larger molecules with higher molecular weight and higher boiling point. They are composed of three isoprene units combined together (Croteau et al., 2000). These compounds are grouped into linear, branched or cyclic sesquiterpenes.

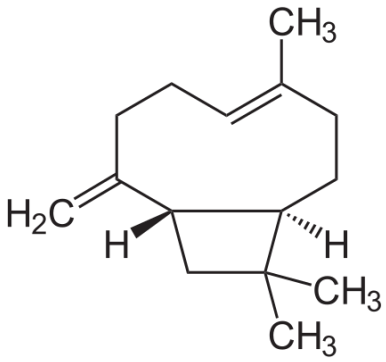

Figure 4. Skeleton of sesquiterpene Caryophyllene
(https://en.wikipedia.org/wiki/Caryophyllene)

Diterpenes (e.g. sclareol, manool, phytol, etc.), triterpenes (saponins, ursolic acid, etc.) and tetraterpenes (e.g. carotenes) are typically found in essential oils at very low concentrations

(Mohamed et al., 2010; Bakkali et al., 2008). The recovery of these compounds can be enhanced by prolonging steam distillation times and their presence in essential oils can also be influenced by the specific extraction method employed (Başer and Demirci, 2007).

Oxygenated terpenoids (e.g. linalool, menthol, geraniol) are known for their strong fragrance (Darjazi, 2011). Terpenoids can be further categorized into aldehydes, ethers, alcohols, esters, ketones, phenols and epoxides (Hyldgaard et al., 2012).

Phenolics

Besides volatiles, spices also contain the largest group of secondary natural metabolites 'phenolics', which are derived primarily via the shikimic acid or phenylpropanoid pathways (Dhifi et al., 2016; Cheynier et al., 2013). Phenolic compounds are classified into different groups like flavonoids, anthocyanins, tannins, coumarines, lignins, lignans, stilbenes, etc.

Flavonoids are the largest and most diverse group of phenoloids, comprising over 8000 different molecules. They can be categorized into six main sub-groups: flavonols, flavones, flavonones, flavanols, isoflavonoids and anthocyanins. They have many different pharmacological effects, such as antioxidant, anti-inflammatory, antispasmodic, liver protective effect, vascular wall protective effect, etc. (Durazzo et al., 2019; Mark et al., 2019).

Tannins are complex phenolic polymers that serve as the primary type of phenolic compounds present in plant tissues. They are classified into two main subclasses: hydrolysable tannins and condensed tannins. Both groups have strong antioxidant effects. Although tannins have not been extensively researched for their bioactive potential, likely due to their structural complexity, proanthocyanidins, a type of condensed tannins, have gained attention in recent years for their associated health benefits (anti-inflammatory, anti-diabetic, anti-carcinogenic, etc.) (Mark et al., 2019).

Phenolic acids, another important phenoloid group, are cinnamic acid derivatives. Their fundamental structure consists of a phenolic ring and a carboxylic acid function. Vanillic acid, caffeic acid, rosmarinic acid are some of the most common phenolic acids found in MAPs.

Rosmarinic acid (RA) stands as a prevalent phenolic compound found in various plant species, notably within the *Lamiaceae* and *Boraginaceae* families. It is formed by the esterification of caffeic acid and 3,4-dihydroxyphenyllactic acid (Figure 5), with its biosynthetic pathway and physiological functions extensively studied (Marchev et al., 2021). Additionally, owing to its capacity to hinder lipid peroxidation and bacterial proliferation, RA is approved for use as a natural antioxidant or preservative within the food industry (Marchev et al., 2021). The growing need for RA highlights the importance of scaling up its production.

However, its concentration in plants typically remains below 1% of the dry weight and is influenced by various factors such as plant physiology, growth stages, harvest techniques and environmental conditions (Petersen, 2013).

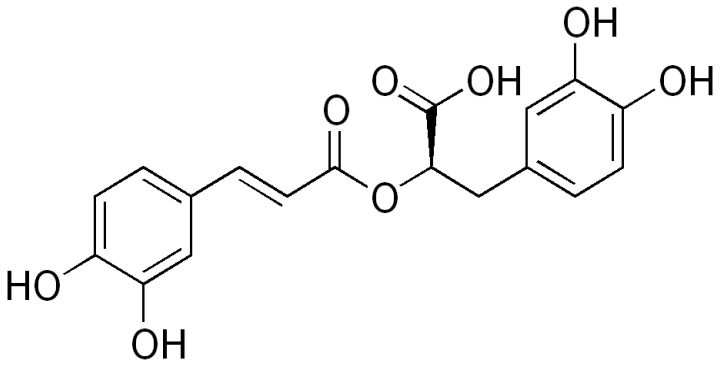

Figure 5. Structure of rosmarinic acid (https://en.wikipedia.org/wiki/Rosmarinic_acid)

Phenolic compounds not only regulate the plant's colour, the pigmentation or astringency but also serve as natural shields against UV radiation. Additionally, they play a crucial role in safeguarding plants from parasites and insects (Pratyusha, 2022).

These compounds are so bioactive substances that are typically linked to a protective role in maintaining overall human health when included in a regular diet. Research has shown that these compounds exhibit inhibitory effects against the progression of various serious diseases, including cancer, Alzheimer's and diabetes (Caleja et al., 2017; Durazzo et al., 2019). These desirable effects are primarily attributed to the antioxidant and radical scavenging activities of phenolic compounds, which can help to postpone or prevent the oxidation of DNA, proteins and lipids. The antioxidant and antimicrobial properties of phenolic compounds present in plant extracts have played a crucial role in utilizing these molecules as preservatives. This application has been instrumental in extending the shelf life of various food products (Mark et al., 2019).

3.3. Presentation of the important spice species selected for our investigation

There are many spices that are utilized in culinary practices and for their therapeutic properties around the world. Information about the medicinally important spice species that were selected for this study are given in Table 1(a) and 1(b). Species were classified according to whether they accumulate the volatiles in external glandular hairs or mainly in endogenous secretory ducts.

Table 1(a). Important spice species accumulating the EO primarily or exclusively in glandular hairs or trichomes

Latin name (english name)	Life form	Official drugs	Important active substances	Pharmacological effects
***Helichrysum italicum* L.** (italian strawflower)	Nanophane-rophyte (N)	*Helichrysi flos* (Ph. Eur.)	0.2-0.4% of EO comprising mainly sesquiterpenes (Bianchini et al., 2001; Paolini et al., 2006; Morone-Fortunato et al., 2010)	Used in digestive disorders, choleretic and antispasmodic (EMA, 2016)
***Lavandula angustifolia* Mill.** (true lavender)	Nanophane-rophyte (N)	*Lavandulae flos, Lavandulae aetheroleum* (Ph. Eur.)	2-3% of EO mainly comprising of linalool and linalyl acetate; flavonoids and cinnamic acid derivatives (Batiha et al., 2023)	Anxiolytic and sedative (EMA, 2012)
***Lavandula x intermedia* Emeric** (hybrid lavender)	Nanophane-rophyte (N)	*Lavandulae aetheroleum* (Ph. Eur.)	6-10% of EO mainly comprising of linalool and linalyl acetate; flavonoids and cinnamic acid derivatives (Denys et al., 2002)	Anxiolytic and sedative (EMA, 2012)
***Melissa officinalis* L.** (lemonbalm)	Hemi-cryptophyte (H)	*Melissae folium, Melissae folii extractum siccum* (Ph. Eur.) *Melissae aetheroleum*	0.2-0.5% EO comprising of acyclic monoterpenes like citral, citronellal, geraniol, as well as flavonoids and cinnamic acid derivatives such as rosmarinic acid and caffeic acid (Tóth, 2005).	Carminative, anxiolytic and sedative (EMA, 2013)
***Mentha* x *piperita* L.** (peppermint)	Hemi-cryptophyte (H)	*Menthae piperitae aetheroleum, Menthae piperitae folii extractum siccum, Menthae piperitae folium* (Ph. Eur.)	2-4% EO, predominantly composed of monocyclic monoterpenes like menthol, menthone and piperitone, as well as flavonoids and rosmarinic acid (Tóth, 2005).	Antiemetic, carminative (EMA, 2020)
Mentha spicata* L.var. *crispa (spearmint)	Hemi-cryptophyte (H)	*Menthae crispae folium, Menthae crispae aetheroleum*	0.1-2.1% EO, containing monoterpenes (e.g. carvone, limonene), sesquiterpenes (e.g., β-caryophyllene, germacrene D), phenolic acids, flavonoids and lignans (Bardaweel et al., 2018).	Antibacterial, antiseptic, digestive, carminative, anti-inflammatory (Mahendran et al., 2021)
***Ocimum basilicum* L.** (sweet basil)	Therophyte (Th)	*Basilici herba, Basilici aetheroleum*	0.5-2.5% EO with acyclic (e.g. linalool), bicyclic (e.g., camphor) and aromatic monoterpenes (e.g., methyl chavicol); flavonoids, rosmarinic acid (Tóth, 2005).	Antiseptic, digestive, carminative, anti-inflammatory (Hornok, 1992)
***Origanum majorana* L.** (marjoram)	Therophyte (Th)	*Majoranae herba, Majoranae aetheroleum*	1.5-2.5% EO with mainly monocyclic (e.g. terpinene-4-ol, gamma-terpinene) and bicyclic monoterpenes (e.g. sabinene, sabinene hydrate); flavonoids, rosmarinic acid (Tóth, 2005).	Carminative, for the relief of skin irritation (EMA, 2023)
***Origanum vulgare* L. subsp. *hirtum* (Link) Ietswaart** (greek oregano)	Hemi-cryptophyte (H)	*Origani herba* (Ph. Eur.), *Origani aetheroleum*	About 5% of EO containing monoterpenes specifically carvacrol and thymol, as well as flavonoids, phenolic acids and rosmarinic acid (Węglarz et al., 2020)	Antiseptic, digestive, carminative, anti-inflammatory (Hornok, 1992)
Origanum vulgare* L. subsp. *vulgare (common oregano)	Hemi-cryptophyte (H)	*Origani herba, Origani aetheroleum*	Up to 1% of EO comprising of monoterpenes like carvacrol, cis/trans sabinene hydrate; sesquiterpenes like germacrene D, β-caryophyllene and caryophyllene oxide as well as flavonoids, phenolic acids and rosmarinic acid (Węglarz et al., 2020)	Antiseptic, digestive, carminative, anti-inflammatory (Hornok, 1992)
***Salvia rosmarinus* L.** (rosemary)	Nanophane-rophyte (N)	*Rosmarini aetheroleum, Rosmarini folium* (Ph. Eur.)	0.5%-3.5% EO (Teshale et al., 2022) Spanish type comprising of camphene, 1,8-cineole and camphor; in Moroccan type: mainly 1,8-cineole (Ph. Eur.)	Antispasmodic, gastrointestinal relaxant, anti-dyspeptic (EMA, 2022a); analgesic, peripheral vasodilator (EMA, 2022b)
***Salvia officinalis* L.** (garden sage)	Nanophane-rophyte (N)	*Salviae officinalis folium* (Ph. Eur.), *Salviae officinalis aetheroleum*	1-2.5% EO, primarily comprising α- and β-thujone, borneol, camphor and pinenes, as well as flavonoids, cinnamic acid derivatives such as rosmarinic acid and caffeic acid (Tóth, 2005)	Anti-dyspeptic, anti-inflammatory (EMA, 2017)
***Satureja hortensis* L.** (summer savory)	Therophyte (Th)	*Saturejae herba, Saturejae aetheroleum*	Upto 3% of EO comprising of terpenes like carvacrol, thymol, β-pinene, p-cymene, limonene, camphene as well as flavonoids and rosmarinic acid (Ejaz et al., 2023)	Antiseptic, digestive, carminative, anti-inflammatory (Hornok, 1992)
***Thymus vulgaris* L.** (garden thyme)	Chamae-phyte (Ch)	*Thymi herba* (Ph. Eur.), *Thymi aetheroleum* (EMA)	2-4.5% EO, primarily composed of aromatic monoterpenes such as thymol, carvacrol and p-cymene, along with flavonoids and cinnamic acid derivatives like rosmarinic acid and caffeic acid (Tóth, 2005).	Expectorant (EMA, 2020); antiseptic, antibacterial, antifungal (Hornok, 1992)

Table 1(b). Important spice species accumulating the EO primarily or exclusively in secretory ducts

Latin name (english name)	Life form	Official drugs	Important active substances	Pharmacological effects
***Anethum graveolens* L.** (dill)	Therophyte (Th)	*Anethi herba, Anethi fructus, Anethi aetheroleum*	0.5-2.5% of EO comprised of carvone, limonene and α-phellandrene (Hornok, 1992)	Sedative, antiseptic (Hornok, 1992)
***Artemisia dracunculus* L.** (French tarragon)	Hemi-cryptophyte (H)	*Dracunculi herba, Dracunculi aetheroleum*	0.5-2.5% of EO with phenylpropane derivatives, such as estragole, and bicyclic monoterpenes like pinenes and camphene (Tóth, 2005)	digestive, appetizer, blood pressure reducer (Hornok, 1992)
***Levisticum officinale* Koch.** (lovage)	Hemi-cryptophyte (H)	*Levistici radix*(Ph.Eur.), *Levistici folium, Levistici fructus, Levistici aetheroleum*	0.2-1.5% EO with mainly ftalid sesquiterpenes, like Z-ligustilide (Tóth, 2005)	Diuretic, carminative, digestive (Hornok, 1992)
Petroselinum crispum* (Mill) Nym. var. *neapolitanum (flat-leaf parsley)	Hemithero-phyte (TH)	*Petroselini folium, Petroselini fructus, Petroselini aetheroleum*	0.05-0.5% of EO comprising of apiole, 1,3,8-p-menthatriene, β-phellandrene, myristicin and myrcene (Simon and Quinn, 1988) as well as phenolic compounds like flavonoids	Diuretic, antispasmodic, antiseptic, antidiabetic, uterus contractor (Agyare et al., 2017)

3.4. Preservation of spices

The demand for spices is increasing day-to-day, but the fresh plant materials are available only for a short period of time. Hence, preservation is utmost necessary in order to prolong their shelf life, for easy transportation and storage. Preservation techniques not only influence the quantity but also the quality of the active ingredients in the final product.

The physical and chemical properties of spices after harvest are determined by the amount of moisture present in them. Fresh plant materials usually contain more than 75% of water, which leads to microbial growth and tissue degradation during the post-harvest process; thus, this amount needs to be lowered below 10% according to the European Pharmacopoeia.

Naturally, the time of harvest and the stage of harvesting of herbs and spices are very crucial in terms of active ingredient content, but the different preservation methods also greatly influence the EO content and composition, the content and quality of the phenolic and other compounds and their antioxidant activity in the final product, as well as the organoleptic properties (the taste and colour), which are very important for a spice product.

3.4.1. Different preservation methods and their effects on the quality of spices

There are various preservation methods for spices to maintain their flavour, aroma and quality. Drying is one of the most important post-harvest operations, which lowers the plants' moisture content, due to this inhibits enzymatic and microbial activities and, as a result, increases the shelf life of the final products, enabling them to be used for a prolonged period (Rocha et al., 2011). Drying is that process, during which the moisture content is reduced to the level of storability, usually with the help of some technical equipment's controlled use. At

the end of it the moisture content of dried plant material cannot be more than 5-15% depending on plant species and plant parts. The moisture content of plant parts is categorized based on how water is bound, as outlined below (Hornok, 1992):

Chemically bound water has a high binding energy, making its removal challenging without destroying the material itself. Consequently, conventional drying methods are ineffective in this case.

Physicochemically bound water presents itself in two distinct forms based on binding strength: adsorbed water and osmotically bound water. The binding strength of adsorbed water is high; therefore it is not removed by drying. Meanwhile, besides adsorbed water lies osmotically bound water inside the cells, and it can be removed by drying.

Mechanically bound water is located in the microcapillaries and on the surfaces. This moisture can be evaporated the easiest way.

During drying the movement and pace of water extraction are governed by both the concentration gradient and temperature gradient. These two factors affect the drying jointly and simultaneously. Due to variances in concentration, water naturally moves from areas of higher concentration to those of lower concentration (Hornok, 1992).

The main aims of drying MAPs are as follows (Hornok, 1992):

- To obtain a final product without any change or with the desired change in the amount and composition of active substances present.
- To ensure no negative changes in the appearance characteristics.
- To allow the dried plant material to be preserved for a long period of time without significant losses in its quality.
- To use a drying method that is simple, economical and less time-consuming.

The process of moisture removal also results in a reduction in the weight and volume of plant materials, with a positive outcome for transport and storage (Calixto, 2000).

Numerous convective and non-convective drying methods are used for preserving herbs and spices, including solar drying (Özcan et al., 2005), hot-air drying (Demiray and Tulek, 2014), freeze-drying (Gümüşay et al., 2015), microwave drying (Arslan et al., 2010), etc. However, every technique has both advantages and disadvantages. Drawbacks could be, for example the extended drying time, the high energy consumption, the high drying temperature, resulting in dehydrated products of suboptimal quality (Moses et al., 2014; Karam et al., 2016). Given that medicinal plants and their active substances are sensitive to heat, there is a strong preference for processing them under gentle drying conditions.

3.4.1.1. Convective drying methods

Convective drying is a common method used to remove moisture from plant materials, by using hot air or gas. The process typically involves two stages as described by Chandramohan (2019). In the first stage, energy (in the form of heat) is transferred from the ambient air to the damp product. This can occur through convection, conduction, radiation or a combination of these mechanisms. In the second stage, the moisture content from within the material is transferred to its outer surface. Generally, heat energy is first transferred to the product's surface and then penetrates inward. The first stage is influenced by environmental conditions such as air temperature, humidity, flow conditions, product surface area and pressure. The second stage is dependent on the movement of internal moisture within the solid, which is a function of the product's physical structure, temperature and moisture distribution. The factors influencing these two stages serve as constraints that determine the drying rate and total drying time.

The common convective drying methods used to preserve spices are sun drying and shade drying (natural and cheap methods) furthermore oven drying (artificial and more expensive, but shorter drying method).

Sun drying

Sun or solar drying, recognized as the most ancient drying method, is prevalently employed in tropical or subtropical regions for drying a variety of agricultural products like cocoa beans, tropical spices (cinnamon, cloves, nutmeg, black pepper, etc.), including medicinal plants and aromatic herbs (Orphanides et al., 2016). This technique involves laying fresh herbs on ventilated drying racks for direct sunlight exposure (Janjai and Bala, 2012). Sun drying is often a very fast technique but can result in high drying temperatures (up to 50-70°C).

Due to this, sun drying might not always be the optimal method for certain spices, as it can significantly degrade their colour and aroma. For instance, in *Chamaemelum nobile*, key volatile components like isobutyl isobutyrate, 3-methylbutyl isobutyrate and propyl tiglate were found in lower concentrations in sun-dried samples compared to those dried using hot air at 40°C (Omidbaigi et al., 2004). Similarly, sun-dried *Cymbopogon citratus* showed a reduction in total EO content when compared with hot-air dried samples (Hanaa et al., 2012). In the case of *Ocimum basilicum* L., sun drying led to a more significant loss of EO content than shade drying or hot-air drying at 40°C (Hassanpouraghdam et al., 2010). Additionally, sun drying was observed to cause more damage to the epidermal surface, shrinkage of glandular trichomes

and a greater reduction in mineral content in *Vernonia amygdalina* leaves compared to shade drying (Alara et al., 2018).

Shade drying

Shade drying is a method of drying spices that leverages solar energy as a heat source, similar to sun drying, but with some differences. The key distinction lies in the placement of spices under shade in a well-ventilated, low humidity environment, away from direct sunlight, creating conditions ideal for drying spices. During this process the air, heated by solar energy, passes through the spices aiding in their drying (Sharma et al., 2009). Shade drying offers several advantages over sun drying. It is particularly beneficial for preserving light-sensitive substances and minimizing light-induced chemical reactions, such as oxidation. However, it is important to note that the drying time for shade drying is much longer compared to sun drying, due to the lower drying temperature (Pirbalouti et al., 2013a).

Researches have shown that shade drying is very effective in preserving the quality characteristics of plant products. Omidbaigi et al. (2004) found that the oil content of the shade-dried *Chamaemelum nobile* L. flowers was the highest (1.9% w/w) compared to sun-drying (0.4% w/w) and oven-drying at 40°C (0.9% w/w). In another study, Khorshidi et al. (2009) proved that the maximum EO percentage in *Salvia rosmarinus* was obtained from shade dried leaves (1.8%) than oven dried at 45°C (1.5%). Sárosi et al. (2013) found that shade dried leaves retained the highest amount of EO (1.73 ml/100 g d.w.) in comparison with those samples that were lyophilized (1.04 ml/100 g d.w.) or dried at 50°C (0.69 ml/100 g d.w.). In relation to *Ocimum basilicum* L., Hassanpouraghdam et al. (2010) found that shade dried leaves contained the highest EO content (0.90%) than sun dried (0.50%) or oven dried at 60°C (0.40%). Shade drying was a better drying method for keeping the higher amounts of total phenolics, antioxidant activity and flavonoids in *Mentha* x *piperita* L., *Melissa officinalis* L. and *Salvia officinalis* L. when compared to oven dried method as investigated by Rababah et al. (2015). Furthermore, shade drying treatment also preserved the colour characteristics better than oven drying at 40°C.

In the process of shade drying, the lack of direct solar exposure and the minimal convective heat flow are beneficial for preserving volatiles in plants, especially those that are sensitive to heat. The gentle heat involved in shade drying helps in maintaining the integrity of trichomes on plant materials, which are crucial for storing EOs. A study by Mokhtarikhah et al. (2020) found that spearmint (*Mentha spicata* L.) leaves retained their glandular trichomes more effectively when shade dried, as opposed to using artificial methods like oven drying,

vacuum drying or infrared drying. Additionally, the trichomes on the surface of *Lippia citriodora* leaves experienced less damage from shade drying compared to other drying treatments (Ebadi et al., 2015).

While shade drying has its benefits, such as preserving the colour and aroma of plant materials, it also has its drawbacks. One of the main disadvantages is that shade drying is a slower process compared to other drying methods. It typically takes around 7-10 days or even longer for the plant materials to dry completely, which may not be suitable for situations requiring quicker processing or preservation of freshness. For some spices, significant losses in functional properties have been observed. For example, the total antioxidant activity (TAA) of *Mentha* x *piperita* L. and *Melissa officinalis* L. significantly decreased after shade drying, furthermore losses of ascorbic acid and carotenoids were noted (Capecka et al., 2005). In this experiment, TAA was expressed as a percentage of inhibition of linoleic acid peroxidation by herb extracts in comparison to the oxidation level in the control.

Aroma compounds in some shade-dried spices were also found to be lower in content compared to those dried by other methods, as seen in *Thymus vulgaris* L. (Rahimmalek and Goli, 2013). It might be due to the longer drying time that caused degradation of active ingredients. Despite these limitations, shade drying remains popular in rural areas and small businesses due to its low cost and ability to produce high-quality dried products, if it is properly applied (Janjai and Bala, 2012).

Oven drying (artificial drying)

In the manufacturing industry, particularly in non-tropical countries where sun drying and shade drying are less effective, hot-air drying, often referred to as oven drying, is a widely utilized method (Orphanides et al., 2016). This technique, which employs convection for heat transfer, is preferred in these regions due to its efficiency and reliability. A key advantage of hot-air drying is the precise control it offers over critical parameters such as drying temperature, air velocity and drying time. This level of control is not typically achievable with traditional drying methods like sun or shade drying. Consequently, extensive research and optimization of various aspects of hot-air drying process have been conducted (Orphanides et al., 2016).

The drying process of spices significantly affects their aroma and flavour profiles. Shaw et al. (2006) suggest that hot-air drying at temperatures between 40 to 60°C is effective for drying spices. However, this range of temperatures can lead to undesirable changes in the aroma of dried culinary spices, according to Antal et al. (2011). Specifically, increasing the drying temperature from 40 to 60°C can result in a decrease in total volatile content and

alterations in flavour, including less fresh-like aroma and an increase in spiciness, hay-like, sweet, earthy and woody flavours. This phenomenon was observed in dried *Ocimum basilicum* L. leaves as per the study by Calín-Sánchez et al. (2012). Similar, EO content reducing effects have been reported in other spices as well. Increasing drying temperatures from 30 to 70°C in *Mentha* x *piperita* led to similar changes (Rohloff et al., 2005). Furthermore, *Achillea fragrantissima*, when dried at temperatures increasing from 35 to 45°C, exhibited analogous alterations (Abaas et al., 2013). *Salvia officinalis* L. also demonstrated similar trends when the drying temperature was raised from 30 to 60°C (Venskutonis, 1997).

The temperature used in hot-air drying significantly influences the quality and characteristics of dried products. For instance, Prothon et al. (2003) observed that higher drying temperatures can cause undesirable changes such as tissue collapse. Similarly, Tambunan and Yudistira (2001) found that increased drying temperatures led to a loss of bioactive compounds, and Calín-Sanchez et al. (2013) reported an increase in colour alteration at higher temperatures. Drying the leaves at 60°C caused significant damage to the epidermal surfaces and cell walls, more so than at 40 and 50°C (Alara et al., 2018).

Conversely, some studies report benefits of higher drying temperatures. Shahhoseini et al. (2013) found that *Aloysia citrodora* dried at 50°C had a higher concentration of volatiles than when dried at lower temperatures. Piga et al. (2007) observed a similar trend in *Thymus vulgaris* L. leaves. These data show that the literature on the subject is often contradictory.

Regarding the antioxidant capacity in herbs, Yi and Wetzstein (2011) demonstrated that drying temperatures affected the antioxidant capacity in *Salvia rosmarinus* L. and *Mentha* x *piperita* L., with lower temperatures preserving more antioxidants. Harbourne et al. (2009) observed similar results for *Filipendula ulmaria* and *Salix alba*, where lower temperatures preserved more total phenols, salicylates and quercetin.

3.4.1.2. Non-convective drying methods

Non-convective drying methods refer to techniques of removing moisture from materials without the use of forced air or convection currents. In this case, drying is not carried out by external heat transfer. These methods are often employed when the material being dried is sensitive to high temperatures or airflow (Thamkaew et al., 2021).

Microwave drying

Microwave drying has been studied in the spice processing industry for its effectiveness in speeding up water evaporation from materials, thereby offering shorter drying times than traditional methods (Chi et al., 2003). In relation to this technique, electromagnetic radiation is

used for drying. In convective heating the caloric energy transmits from the surface to the interior, while microwaves heats the inner part at first, pushing the moisture to the lower pressure space on the cool surface, since the surrounding air is not affected by microwaves (Figure 6). This mechanism of water pump considerably accelerates and improves removing the moisture. Due to it, this method is noted for its lower energy consumption (Di Cesare et al., 2003). Compared to hot-air drying, microwave drying results in less shrinkage and better colour and rehydration capacity of the dried products (Kathirvel et al., 2006). The quality of microwave-dried products depends on various factors including the microwave power, drying time, initial moisture content and the material's dielectric properties (Moses et al., 2014).

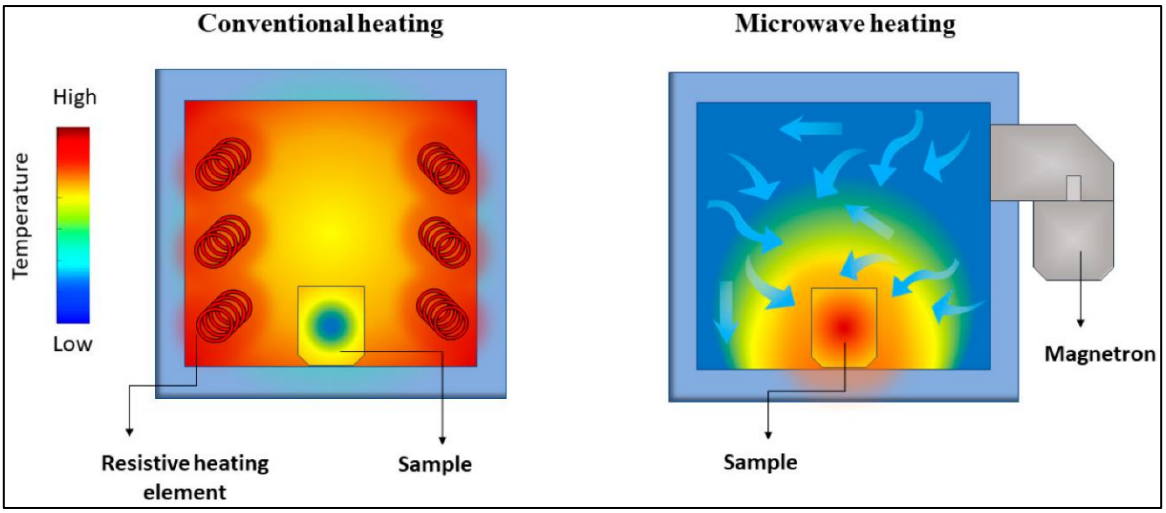

Figure 6. Representation of the temperature gradient in conventional (left) and microwave-assisted (right) heating of a solid sample (https://www.mdpi.com/1420-3049/26/10/2882)

Research on *Petroselinum crispum* L. demonstrated that increasing microwave power from 360 to 900 W reduced the drying time by 64%, with the dried parsley maintaining a good colour, only slightly darker than its fresh state (Soysal, 2004). A similar trend was observed in coriander, where an increase in microwave power from 180 to 360 W led to a higher diffusivity but a decrease in rehydration capacity of dried leaves (Sarimeseli, 2011). In studies comparing various drying methods, it was found that spices dried using microwaves generally had superior quality. Specifically, *Ocimum basilicum* L. leaves dried with microwaves retained more volatiles than those dried by oven drying at 50°C or freeze-drying. Additionally, microwave-dried basil leaves exhibited less colour change than convectively dried ones, potentially due to the shorter drying time associated with microwave drying (Di Cesare et al., 2003).

In another study, Arslan and Özcan (2008) compared microwave drying (using 700 W), sun drying and hot-air drying (at 50°C) for *Salvia rosmarinus* L. leaves. They found that the colour of the microwave-dried rosemary leaves was better preserved than that of the hot-air-

dried ones. Similarly, coriander foliage dried with a microwave power of 295 W showed better colour retention compared to convective drying at 50°C. In contrast, microwave drying method has been observed to cause a substantial reduction in the aromatic compounds of certain spices like *Origanum majorana* (Raghavan et al., 1997) and *Salvia rosmarinus* L. (Rao et al., 1998), more than other drying techniques such as convective shade and sun drying.

In some cases, microwave drying has been shown to effectively preserve bioactive compounds in dried products. Specifically, using a microwave power of 850 W for drying *Coriandrum sativum* leaves resulted in a higher preservation of trans-β-carotene compared to conventional convective drying at 45°C (Divya et al., 2012). This method was also found to be beneficial for drying *Salvia officinalis* L. leaves, where the microwave-dried product at 850 W exhibited superior retention of total phenolic compounds, flavonoid content and antioxidant activity relative to those dried using convective drying at 45°C (Hamrouni-Sellami et al., 2013).

Microwave drying can be effectively integrated with traditional drying methods like hot-air drying, often serving as a preliminary step to decrease initial moisture or as a final drying phase (Orphanides et al., 2016). A significant limitation of microwave drying, however, is its tendency to heat materials unevenly. This can cause temperature gradients, particularly in larger products, leading to inconsistent dehydration, potential overheating and a decline in product quality (Ozkan et al., 2007). While microwave drying is notably quicker compared to conventional methods, further research is necessary for spices to refine microwave drying parameters in order to enhance the quality of final product (Moses et al., 2014).

Microwave-vacuum drying

In recent years, there has been growing interest in utilizing a synergistic approach combining microwave and vacuum drying techniques (Orphanides et al., 2016). This innovative method involves employing microwave irradiation for heating within a sub-atmospheric pressure drying chamber. The vacuum environment serves as the catalyst for water evaporation, leading to significantly accelerated drying rates compared to traditional convective or microwave drying methods (Soysal, 2004). Notably, when compared to conventional hot-air drying, microwave-vacuum drying demonstrates remarkable efficiency, reducing drying times by an impressive 70–90% while also yielding superior product quality (Giri and Prasad, 2007). For instance, in a study focusing on *Lippia berlandieri*, the thymol content in vacuum-microwave dried samples was observed to be 1.3 times higher than those dried using hot air drying (Yousif et al., 2000).

Microwave-vacuum drying has emerged as a compelling alternative to traditional hot-air drying methods, showcasing notable advantages in certain contexts. For instance, in the case of *Mentha cordifolia* leaves, this technique exceled in preserving colour and texture, because according to SEM pictures, it revealed enhanced porosity and reduced collapse compared to conventional hot-air drying (Therdthai and Zhou, 2009). Yet, the efficacy of vacuum drying is constrained by the limitations of the vacuum pump, particularly when confronted with high initial moisture loads from food materials. Notably, while microwave-vacuum drying demonstrates efficacy for some spices like *Mentha cordifolia*, its suitability varies across different plant species. For instance, *Salvia rosmarinus* exhibited low quality when subjected to microwave-vacuum drying, experiencing heightened loss of volatiles compared to hot-air drying methods. Furthermore, the sensory quality of microwave-vacuum-dried rosemary was notably inferior to that of dried using hot-air at 60°C (Szumny et al., 2010).

In summary, microwave-vacuum drying represents a promising method in drying technology, but further optimization and exploration is necessary to unlock its full potential.

Infrared drying

This drying process boasts several notable advantages: its adaptability, simplicity, rapid heating rate and expeditious drying rate have been underscored (Ashtiani et al., 2017). Throughout this method, electromagnetic energy in the form of infrared wavelength radiation permeates the material, instigating internal heat generation via molecular vibrational state alterations (Krishnamurthy et al., 2008). Notably, infrared drying exhibits superior energy efficiency when combined with hot-air drying methodologies. Despite its potential, there has been a lack of recent research into spice drying utilizing infrared techniques. In a comparative study involving *Mentha* x *piperita* leaves, infrared drying showed higher energy efficiency and accelerated drying rates in contrast to convective drying methods, even when subjected to varying temperatures (30, 40 and 50°C) (Ashtiani et al., 2017).

Infrared irradiation, well-suited for thin-layer drying, owes its efficacy to its minimal penetration depth within materials and its reliance on material-specific contact areas. Notably, it outpaces traditional hot-air drying methods in terms of speed (Ashtiani et al., 2017; Torki-Harchegani et al., 2017) and maintains a high drying rate even at lower moisture levels (Pääkkönen et al., 1999). However, further studies of this drying technique in culinary spices are needed to test the effect of the process on the overall quality and quantity of the final product.

Lyophilization

Lyophilization, commonly known as freeze-drying, is a dehydration technique employed to prolong the shelf life of products and optimize their suitability for transportation (Kasper and Friess, 2011; Oyinloye and Yoon, 2020). This advanced drying method utilizes low temperature and shorter processing time, making it a distinct approach (Barresi et al., 2009).

Lyophilization consists of three primary stages: 1. freezing (to convert most of the water into ice); 2. primary drying (to sublime the ice); 3. secondary drying (to remove unfrozen water through desorption) (Figure 7). Occasionally, an optional step called 'annealing' is employed to crystallize certain formulation components (Nireesha et al., 2013).

This process is particularly suitable for preserve compounds that are thermolabile or unstable in aqueous solutions but remain stable in a dry state. The unique aspect of lyophilization, that it is conducted at low temperatures and pressures, which prevents most degradation and microbiological reactions by eliminating the liquid water, resulting in a high-quality final product (Ratti, 2001).

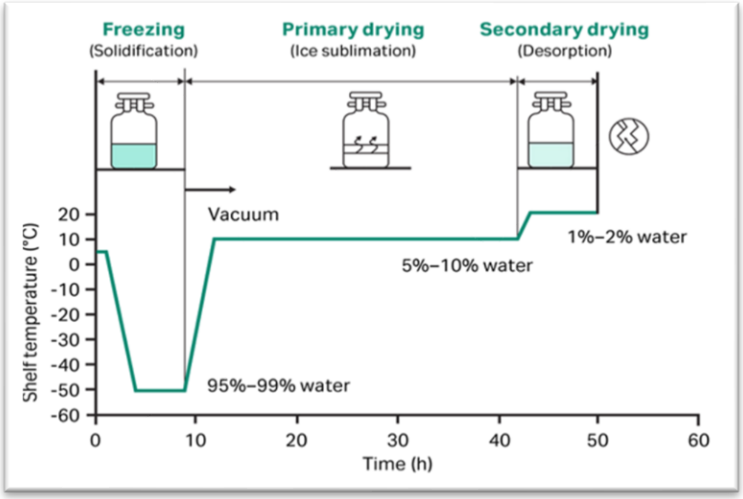

Figure 7. Process of lyophilization
(https://www.cytivalifesciences.com/en/us/solutions/genomics/knowledge-center/advantages-of-lyophilization)

The history of lyophilization dates back to prehistoric times when Eskimos used it to preserve fish (Pržić et al., 2004). In 1935, Flosdof was the first to employ lyophilization for preserving biological materials, and during World War II, it gained prominence as an innovative technique for preserving blood plasma and reactivating it for soldiers (Bode et al., 2007). Today, freeze-drying finds numerous applications, particularly in the pharmaceutical and food industries.

According to Thamkaew et al. (2021), lyophilization helps in better preservation of the EO content in many spice species compared with other drying and preservation techniques. However, the effect of freeze-drying on the active compounds of plants is not entirely unanimous, and data in the literature are often contradictory.

In *Ocimum basilicum* L. 'Green' and 'Purple' landraces, the lyophilized samples recorded higher EO yield compared with other drying techniques. Here, presumably the low temperature maintained during freeze-drying reduced the negative effects caused by the destructive forces of high heat on oil glands (Pirbalouti et al., 2013a). On the other hand, another report concluded that the total EO content of *Ocimum basilicum* and *Laurus nobilis* leaves was considerably decreased during freeze-drying (Díaz-Maroto et al., 2002; Díaz-Maroto et al., 2004). The oil loss could be associated with the operating pressure and processing time during the lyophilization process (Antal et al., 2011). In another study, it was reported that lyophilized samples of *Ocimum basilicum* had lower retention of volatiles compared to microwave dried leaves (Di Cesare et al., 2004).

In relation to *Coriandrum sativum* L., the highest EO yield was obtained from lyophilized aerial parts, and no significant differences were observed among the oil yields distilled from fresh plant materials and those dried by lyophilization (Pirbalouti et al., 2017). Likewise, in case of aerial parts of *Satureja bachtiarica*, freeze-drying recorded a higher percentage of EO yield than oven drying at 65ºC, sun-drying or shade-drying (Pirbalouti et al., 2013b). However, in case of *Perilla frutescens* leaves, the lowest EO yield was observed for lyophilization compared to oven-drying, shade-drying, sun-drying or mid-infrared-drying.

The amount of isolated EO was greatly influenced by the chamber pressure and the freezing temperature in the research of Antal et al. (2014). A decrease in drying chamber pressure reduced the freeze-drying time but increased the release of volatile compounds. Freeze-dried *Melissa officinalis* leaves at high pressure (250–300 Pa for 14 hours) exhibited a higher content of EO (0.252 v/w%) compared with those freeze-dried at low (50–80 Pa for 12 hours) pressure (0.191 v/w%). According to the researchers, it was due to the fact that volatiles of lemonbalm were found in peltate glandular trichomes on the surface of leaves, and low pressure in the freeze-dryer chamber strongly affected them, which resulted in significant loss of volatiles. In connection with EO composition, the ratio of citral, citronellal, geraniol and limonene was significantly higher at high pressure than at low pressure. In another research of Antal et al. (2011), the higher chamber pressure also extended the drying time but preserved the major volatile compounds (carvone and citronellol) of *Mentha spicata* L. better. The quality

of the freeze-dried product was assessed as being lower than the raw plant material, but higher compared to the conventionally dried ones.

Several studies investigated the effect of lyophilization on the EO composition too. Cálin-Sánchez et al. (2013) noted that lyophilization could also cause a significant changing in the aroma compounds of dried herbs and spices. The ratio of thymol, which is present in the leaves of *Lippia berlandieri* and *Thymus vulgaris*, was relatively higher in the lyophilized samples, than in samples dried using convective techniques (Venskutonis et al., 1996; Yousif et al., 2000). The thymol content was also reported to be higher in freeze-dried thyme leaves compared with oven-dried (30–50ºC) and shade-dried ones (Sárosi et al., 2013). In comparison to the fresh, sun-dried and oven-dried samples of *Satureja bachtiarica*, the carvacrol content was the highest in freeze-dried sample (Pirbalouti et al., 2013b). Lyophilization preserved a larger amount of citral (64.7%) than oven-drying at 40ºC (55.4%) in the case of *Lippia citriodora* (Ebadi et al., 2015).

In another research, the statistical analysis showed a significant reduction in thymol concentration in air-dried samples, while its concentration remained unchanged in lyophilized plant materials (Yousif et al., 2000). The study of Gardeli et al. (2010) confirmed that lyophilized *Foeniculum vulgare* herb maintained its volatile composition much better than conventionally dried samples. Similarly, the retention of the major compounds (α-phellandrene and 3,6-dimethyl-2,3,3a,4,5,7a-hexahydrobenzofuran) of fresh *Anethum graveolens* herb was better in the freeze-dried sample compared to the hot-air dried samples. However, in the process of freeze-drying, high amount of neophytadiene was also produced, and the formation of this compound during processing altered the flavour of the final product (Houpalathi et al., 1985).

In relation to *Ocimum basilicum* L. landraces 'Green' and 'Purple', the main compound methyl chavicol was found to be in the highest ratio in freeze-dried samples (Pirbalouti et al., 2013a). In the study of Bowes and Zheljazkov (2004), the ratio of eugenol (the main compound) was proved to be the highest in the freeze-dried samples of *Ocimum basilicum* 'Mesten' and *Ocimum sanctum* 'Local'. In *Laurus nobilis* leaf, freeze-drying led to an increase in the concentration of eugenol compared to shade drying and oven drying at 45ºC (Díaz-Maroto et al., 2002).

Freeze-drying resulted in a significant change in the volatile composition of *Anthriscus sylvestris* (Bos et al., 2002) and *Petroselinum crispum* (Díaz-Maroto et al., 2003) compared to the fresh plant materials. In a study by Díaz-Maroto et al. (2002), also a significant change was observed in the EO composition of lyophilized *Petroselinum crispum* L. leaves compared with the air-dried sample: the ratio of p-mentha-1,3,8-triene and apiole, which are responsible for

the characteristic odour of parsley, decreased, while the proportion of myristicin, another active substance found abundantly in parsley, increased (Díaz-Maroto et al., 2002).

According to Hossain et al. (2010), in certain herbs, such as *Salvia rosmarinus*, *Origanum vulgare*, *Origanum majorana*, *Salvia officinalis*, *Ocimum basilicum* and *Thymus vulgaris*, the freeze-dried samples had lower total phenolic and rosmarinic acid content, furthermore antioxidant capacity compared to convective drying methods.

3.4.1.3. Freezing

Freezing has been a staple preservation technique in the food industry for generations. Surprisingly, it was not until a few decades ago that researchers delved deeply into the mathematical principles behind freezing as a preservation method. This preservation method leverages the advantages of low temperatures, where microorganisms are unable to thrive, the chemical reactions are minimized, and cellular metabolic processes are slowed down (Delgado and Sun, 2001).

Freezing preservation is effective in maintaining the quality of agricultural products during extended storage periods. This technique is often considered more effective than canning and dehydration, particularly in terms of retaining their sensory attributes and nutritional properties (Fennema, 1977). The safety and nutritional quality of frozen products are further enhanced when they are produced using high-quality raw materials, processed under good manufacturing practices and stored at the recommended temperatures. Freezing has been widely used for long-term food preservation, significantly extending shelf life by reducing the temperature to -18°C or below. In general, slow freezing methods tend to result in the formation of ice crystals predominantly in extracellular spaces, whereas rapid freezing rates lead to the generation of small ice crystals that are evenly distributed throughout the tissue (Delgado and Sun, 2001).

Freezing effectively slows microbial growth and chemical changes that could spoil the food or degrade its quality (George, 1993). According to Arthey (1993), compared to emerging minimal processing technologies, industrial freezing remains the most effective method for maintaining quality over extended storage periods. In terms of processing time, energy use and cost, freezing stands out. It requires the shortest processing time compared to other conventional methods like drying. Although these methods may use less energy than freezing and its associated storage, freezing can be as cost-efficient as other preservation techniques when considering overall expenses (Harris and Kramer, 1975).

Scientists and researchers have persistently engaged in efforts to achieve success in commercial freezing trials across various food commodities. However, there is still almost no research available on the effects of freezing on the quality of spices, with a few exceptions. For example, in the research of Tomsone and Kruma (2014), freezing was identified as one of the most effective methods for preserving the phenolic compounds and antioxidant activity in horseradish leaves and lovage leafy shoots, particularly in relation to maintaining their total phenolic and flavonoid content.

There is indeed a lack of comprehensive scientific research specifically addressing the impact of freezing on preserving the volatiles, phenolic compounds and organoleptic characteristics of spices. This gap in research highlights an area where further studies could be beneficial to understand the full scope of freezing.

3.4.1.4. Summary of research results on the examined species

Data of previous literature findings related to the investigated species of this thesis are summarized in Table 2 (a) and (b) for easier overview, focusing on the impact of various preservation methods.

Table 2 (a). Literature data on the effects of preservation methods connected to species accumulating the EO primarily or exclusively in glandular hairs or trichomes

Latin name (English name)	Preservation method(s)	Analysed parameter	Results		Reference
			Best method(s)	Worst method(s)	
***Helichrysum italicum* L.** (italian strawflower)	Shade drying, lyophilization	TPC, TFC	Shade drying	Lyophilization	Nebrigić et al. (2023)
***Lavandula angustifolia* Mill.** (true lavender)	Sun drying, shade drying, oven drying at 40°C, microwave drying at 900 W	EO content and composition	Shade drying	Microwave drying at 900 W	Mirjalili et al. (2019)
***Lavandula x intermedia* Emeric** (hybrid lavender)	Oven drying at 30, 40, 50 and 60°C	EO content and composition	Oven drying at 30°C	Oven drying at 60°C	Erbaş and Baydar (2008)
***Melissa officinalis* L.** (lemonbalm)	Sun drying, shade drying, oven drying at 40°C	EO content and composition	Shade drying	Sun drying	Khalid et al. (2008)
	Shade drying, oven drying at 35ºC and 55ºC	EO content and composition	Oven drying at 35ºC	Oven drying at 55ºC	Mirahmadi et al. (2017)
	Shade drying, microwave drying at 500 and 1000 W, oven drying at 40°C, lyophilization	EO content and composition	Oven drying at 40°C	Microwave drying at 500 W	Ghasemi et al. (2013)
	Shade drying, oven drying at 40°C	TPC, antioxidant activity, TFC, colour	Shade drying	Oven drying at 40°C	Rababah et al. (2015)
	Convective drying 30, 45, 60, 75 and 90°C	EO content and composition, SEM of leaf structure	Convective drying 30 and 45°C	Convective drying 60°C	Argyropoulos and Müller (2014)
***Mentha* x *piperita* L.** (peppermint)	Chamber drying at 30°C, 50°C, 70°C	EO content and composition	Drying at 30°C	Drying at 50°C and 70°C	Rohloff et al., (2005)

	Shade drying, hot air drying at 50°C, 60°C and 70°C, microwave drying at 200, 400 and 800 W	EO yield	Shade drying, hot air drying at 50°C, 60°C and 70°C	Microwave drying at 200, 400 and 800 W	Beigi et al. (2018)
	Sun drying, oven drying at 40°C, 70°C	TPC, TAC	Sun drying, oven drying at 40°C	Oven drying at 70°C	Yi and Wetzstein, (2011)
Mentha spicata* L.var. *crispa (spearmint)	Hot air drying at 43°C, lyophilization	EO content and composition	Lyophilization	Hot air drying at 43°C	Antal et al. (2011)
***Ocimum basilicum* L.** (sweet basil)	Sun drying, shade drying, oven drying at 40°C and 60°C	EO content and composition	Shade drying	Sun drying, oven drying at 60°C	Hassanpouraghdam et al. (2010)
	Sun drying, shade drying, microwave drying at 500 W, oven drying at 40 and 60°C, lyophilization	EO content and composition, colour	All parameters: shade drying, Oven drying at 40ºC, freeze drying	All parameters: Microwave drying at 500 W, sun drying, oven drying at 60°C	Pirbalouti et al. (2013a)
	Forced air drying at 27°C, lyophilization	EO content and composition	Forced air drying at 27°C	Lyophilization	Bowes and Zheljazkov (2004)
	Hot air drying at 48°C, vacuum-microwave drying	EO composition, colour, SEM of leaf structure	Vacuum-microwave drying	Hot air drying at 48°C	Yousif et al. (1999)
***Origanum majorana* L.** (marjoram)	Shade drying, lyophilization, vacuum oven at 70°C	TPC, TAC, RA	Shade drying	Lyophilization	Hossain et al. (2010)
	Convective drying at 45±2°C, microwave drying at 175 W, 385 W and 595 W	EO content and composition	Convection drying at 45°C	Microwave drying at 175 W	Raghavan et al. (1997)
***Origanum vulgare* L. subsp. *hirtum* (Link) Ietswaart** (Greek oregano)	Sun drying, shade drying, oven drying at 45°C	EO yield and composition	Oven drying at 45°C	Sun drying and shade drying	Özer et al. (2018)
Origanum vulgare* L. subsp. *vulgare (common oregano)	Lyophilization, convective drying at 50, 60, 70°C, vacuum-microwave drying at 240, 360 and 480 W	TPC, TAC	Lyophilization	Convective drying at 60 and 70°C	Jałoszyński et al. (2008)
***Salvia rosmarinus* L.** (rosemary)	Shade drying, lyophilization, vacuum oven at 70°C	TPC, TAC, RA	Shade drying	Lyophilization	Hossain et al. (2010)
	Convective drying at 60°C, vacuum microwave drying at 480 W, combined drying with convective pre-drying (CPD) followed by VM finish-drying (VMFD) at 360 or 480 W	EO composition, sensory analysis	Combined drying with convective pre-drying (CPD) followed by VM finish-drying (VMFD)	Vacuum microwave drying at 480 W	Szumny et al. (2010)
	Sun drying, oven drying at 40°C, 70°C	TPC, TAC	Sun drying, oven drying at 40°C	Oven drying at 70°C	Yi and Wetzstein (2011)
	Microwave drying at 700 W, sun drying and hot air drying at 50°C	Colour	Microwave drying at 700 W	Hot air drying at 50°C	Arslan and Ozcan (2008)
***Salvia officinalis* L.** (garden sage)	Shade drying, lyophilization, vacuum oven at 70°C	TPC, TAC, RA	Shade drying	Lyophilization	Hossain et al. (2010)
	Microwave drying at 600 W and 800 W, Far-infrared (FIR) at 45°C	TPC	Microwave drying at 800 W	Far-infrared (FIR) at 45°C	Hamrouni-Sellami et al. (2013)
	Drying at 45, 50, 55, 60, 65°C	TPC, antioxidant activity, colour	Drying at 45°C	Drying at 55, 60 and 65°C	Doymaz and Karasu (2018)
***Satureja hortensis* L.** (summer savory)	Sun drying, shade drying, oven drying at 45°C	EO content and composition	Oven drying at 45°C	Sun drying	Sefidkon et al. (2006)
***Thymus vulgaris* L.** (garden thyme)	Oven drying at 40-100°C, microwave drying at 650 W	EO content	Oven drying at 40-60°C	Oven drying at 70-100°C, microwave drying at 650 W	Deans et al. (1991)
	Shade drying, oven drying at 30-50°C, lyophilization	EO content and composition, sensory analysis	Lyophilization, oven drying at 40°C, shade drying	Oven drying at 30°C and 50°C	Sárosi et al. (2013)
	Shade drying, lyophilization, vacuum oven at 70°C	TPC, TAC, RA	Shade drying	Lyophilization	Hossain et al. (2010)

Table 2 (b). Literature data on the effects of preservation methods connected to species accumulating the EO primarily or exclusively in secretory ducts

Latin name (English name)	Preservation method	Analysed parameter	Results		Reference
			Best method(s)	Worst method(s)	
***Anethum graveolens* L.** (dill)	Hot air drying at 25, 40, 50°C, lyophilization	EO composition	Lyophilization	Hot air drying 25, 40, 50°C	Houpalahti et al. (1985)
***Artemisia dracunculus* L.** (French tarragon)	Hot air drying at 45, 60, 90°C	EO content and composition	Hot air drying at 45 and 90°C	Hot air drying at 60°C	Arabhosseini et al. (2006)
***Levisticum officinale* Koch.** (lovage)	Shade drying, oven drying at 40°C, microwave drying at 360 W, lyophilization	EO yield and composition	Oven drying at 40°C	Lyophilization	Złotek et al. (2023)
	Freezing, shade drying	TPC, TFC, antioxidant activity	Lyophilization	Shade drying	Tomsone and Kruma (2014)
Petroselinum crispum* (Mill) Nym. var. *neapolitanum (flat-leaf parsley)	Shade drying, oven drying at 45°C, lyophilization	EO composition	Shade drying	Oven drying at 45°C, lyophilization	Díaz-Maroto et al. (2002)
	Microwave drying at 360, 450, 540, 630, 720, 810 and 900 W	Colour	Microwave drying at 900 W	Microwave drying at 360 W	Soysal (2004)
	Hot air drying at 75°C, vacuum-microwave drying	Colour, aroma	Vacuum-microwave drying	Hot air drying at 75°C	Böhm et al. (2002)

4. MATERIALS AND METHODS

4.1. 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 (47°23′49″ N, 19°09′10″ E, 120 m a.s.l.) located in Soroksár (Pest county, Hungary) from March to September 2020-2022 (Figure 8). Here the soil is sandy-loam with a pH of 7.8 and the humus content is low.

Figure 8. The Experimental and Research Farm located in Soroksár, Hungary (Photo: Hazarika, 2020)

4.2. Examined plant material

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 3.

Most of the examined raw materials of plant species were produced in the experimental field in Soroksár (Figure 9), except parsley and dill. The propagation materials came from commercial trade or from the gene bank of the Department of Medicinal and Aromatic Plants of MATE (Table 3).

Table 3. 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 cultiva-tion	Year of the experi-ment	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

Our aim was to produce so plant materials that are as homogeneous as possible; hence we mainly used varieties. The fresh plant materials of parsley and dill were purchased from a firm named Házi Piros Paprika Kft. located in Fajsz in county Bács-Kiskun (Hungary). The processing of parsley and dill leaves started within 3 hours after the harvest.

Every year the propagation of annual plants (e.g. sweet basil, marjoram, summer savory, etc.) was carried out by producing seedlings in March in greenhouse for 2 months. The seedlings that had developed 5-6 leaves were transplanted into plots in the open field in June. In case of perennial plants (Italian strawflower, lemonbalm, Greek oregano, common oregano, garden thyme, French tarragon, lovage), the 1-3-years-old plant stands were harvested. Meanwhile, the plant stands of peppermint, spearmint, rosemary, garden sage and lavenders ranged from 3 to 10 years in age at the time of harvesting. During the vegetation period regular irrigation and mechanical weed control were carried out.

(a) **(b)** **(c)**

Figure 9. Plant stands in the experimental farm in Soroksár:
(a) Lovage 'Mittelgroblättriger', (b) Lemonbalm 'Lemona', (c) Garden thyme 'French Summer'
(Photo: Hazarika, 2021)

Samples of species were collected in the appropriate phenophase suggested by the scientific literature, from June to September. A total of approximately 4-5 kg of fresh plant material were harvested from each plant species. After harvest the samples were sorted, leaving only the valuable plant parts (Table 3) and were thoroughly mixed to attain a homogeneous mixture (Figure 10). In case of parsley and dill, the homogenised plant material was divided into two parts: one part remained in whole, while the other was chopped into pieces of about 0.5 cm immediately after harvest. Then plant materials of each plant species were divided into ten parts for the applied treatments.

(a) **(b)**

(c)

Figure 10. Homogenized fresh plant materials after harvesting: (a) Rosemary 'Harmat' leaves, b) Garden sage 'Regula' leaves, c) Lovage 'Mittelgroblättriger' leaves (Photo: Hazarika, 2021)

4.3. Applied preservation methods

In this research, plant samples were preserved by the treatments listed below. So, treatments were chosen for the experiment that could be interesting and useful for the practice:

1) Sun drying
2) Shade drying
3) Oven drying at 40°C
4) Oven drying at 60°C
5) Lyophilization/ freeze drying
6) Microwave drying at 250 W
7) Microwave drying at 700 W
8) Slow freezing
9) Fast freezing

The plant materials treated by the above preservation methods were compared to the freshly harvested plant material (control). Fresh samples of each plant species were examined within three hours after harvest, until then those were kept under refrigerated conditions.

During **sun drying**, the fresh plant materials were placed outside in a densely latticed, top open compartment to receive full sunlight during daytime (Figure 11) but placed in a sheltered protected room at night. During drying the ambient air temperature was measured with a datalogger (RHT 10, Extech Instruments, Nashua, USA), which detected wide range of temperature during the day. The measured data can be seen in Appendix 2.

Figure 11. Sun drying of sweet basil 'Genovese' leaves and flowers with the datalogger (Photo: Hazarika, 2020)

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 duration of natural drying methods (sun and shade) is presented in Table 4.

Oven drying at 40ºC and **60ºC** were carried out in a convection oven (Memmert UF 260, Memmert GmbH, Büchenbach, Germany), in which fresh plant materials were spread in thin layer and dried. The duration of drying ranged between 3-8 hrs for oven drying at 60ºC and 15-35 hrs for oven drying at 40ºC (Table 4). The dried plant materials were packed in paper bags and kept at room temperature in a dry, protected place until laboratory analyses.

For **microwave drying**, a 20 l domestic microwave oven (SMW 1917WH, Sencor, Opava, Czech Republic) was used in which at once 50 g fresh plant parts were dried at **250 W** and **700 W** for 12-21 mins and 4-8 mins (Table 4), ventilated every 3 and 1 min, respectively. The ready dried samples were stored at room temperature in paper bags protected against sunlight, heat and moisture for a maximum of 2 months until laboratory analyses.

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).

Table 4. Duration of natural (sun and shade), oven and microwave drying for the examined plant species

Examined plant species		'Variety' or accession		Duration of drying					
				Sun (no. of days)	Shade (no. of days)	Oven-40°C (hrs)	Oven-60°C (hrs)	MW-250W (mins)	MW-700W (mins)
Species accumulating the EO primarily or exclusively in glandular hairs or trichomes	Italian strawflower	Commercial sample from Jelitto		5	10	35	7	30	8
	True lavender	'Budakalászi 80'		3	5	21	6	21	7
	Hybrid lavender	'Judit'		3	6	21	6	20	6
	Lemonbalm	'Lemona'		3	6	15	4	12	4
	Peppermint	'Mexián'		3	8	30	5	15	6
		Gene bank accession Nr. LAMIMENTA18		3	8	30	5	15	6
	Spearmint	Gene bank accession Nr. LAMIMENTA5		3	8	30	5	15	6
	Sweet basil	'Genovese'		4	7	30	6	20	7
		'Ohře'		3	6	30	6	20	7
	Marjoram	'Egyptian'		5	9	20	5	15	6
		'Magyar'		5	8	20	5	15	6
	Greek oregano	Commercial sample from Jelitto		3	4	15	3	16	6
	Common oregano	Commercial sample from Jelitto		4	7	22	4	16	6
	Rosemary	'Harmat'		2	5	21	6	19	7
	Garden sage	'Regula'		8	13	15	4	12	4
		'Extrakta'		8	13	15	4	12	4
	Summer savory	Gene bank accession Nr. LAMISATU22		2	12	20	6	15	6
		'Saturn'		2	12	20	6	15	6
	Garden thyme	'French Summer'		5	8	21	5	21	7
		'Deutscher Winter'		5	8	21	5	20	7
Species accumulating the EO primarily or exclusively in secretory ducts	Dill	Commercial sample from Hungary	Whole	4	9	26	12	21	7
			Chopped	3	6	20	8	19	6
	French tarragon	'Zöldzamat'		3	10	32	6	18	6
		'Artemis'		3	10	32	6	18	6
	Lovage	'Mittelgroblättriger'		3	8	32	4	18	7
		Gene bank accession Nr. ASTLEVI44		3	7	32	4	18	7
	Flat-leaf parsley	Commercial sample from Hungary	Whole	5	11	28	8	19	6
			Chopped	3	5	23	6	16	5

Note: Sun = Sun drying; Shade = Shade drying; Oven-40°C = Oven drying at 40°C; Oven-60°C = Oven drying at 60°C; MW-250W = Microwave drying at 250 W; MW-700W = Microwave drying at 700 W

For **lyophilization**, the fresh samples were quick frozen at -80ºC in a Blizzard Ultra Low Temperature Freezer (NU-99828J, NuAire, Inc., Plymouth, USA) and then freeze-dried in a lyophilizer (Scan Vac Cool Safe, LaboGene, Lillerød, Denmark) (Figure 12) for 48 hours at -109ºC. The lyophilization process was conducted within the Department of Food Chemistry and Analysis at the Institute of Food Science and Technology, located on the Buda campus of MATE. The ready samples (with 1-5% moisture content) were packed in polyethylene bags and stored in a domestic refrigerator at 4ºC until processing.

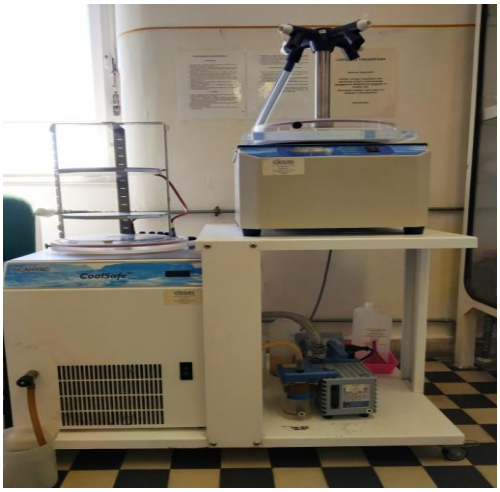

Figure 12. Scan Vac Cool Safe lyophilizer (Photo: Hazarika, 2021)

Slow freezing was accomplished in a 230 l domestic freezer (ZRAN230FW, Zanussi-Electrolux, Stockholm, Sweden) 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 in a Blizzard Ultra Low Temperature Freezer (NU-99828J, NuAire, Inc., Plymouth, USA), and then stored at -18°C in a domestic freezer until use for a maximum of 2 months.

4.4. Measurement and evaluation methods

4.4.1. Chemical analyses

4.4.1.1. 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. The essential oils were stored in airtight vials in a refrigerator at 4ºC for 1-3 months prior to GC-MS analysis.

Measurements were recorded in three replications for each treatment, and data were referenced to the dry matter content of the samples given in Appendix 2. In the thesis, the terms “volatiles” and “essential oil (EO)” are used as synonyms for the simplicity.

4.4.1.2. Determination of essential oil (EO) composition

The EO composition was carried out in three replications per treatment using an Agilent Technologies 6890N instrument equipped with an HP-5MS (6890N, Agilent Technologies International Sàrl, Rolle, Switzerland) capillary column (5% phenyl, 95% dimethyl polysiloxane, length: 30 m, film thickness: 0.25 µm, id. 0.25 mm) and an Agilent Technologies MS 5975 inert mass selective detector for the identification of components with the following temperature program: initial temperature 60°C, heating at a rate of 3°C/min up to 240°C, the final temperature was kept for 5 min. Carrier gas was helium (constant flow rate: 1 $mLmin^{-1}$) with a split ratio of 30:1 and injection volume: 0.2 µL (1% in n-hexane). Both the injector and detector temperatures were 250°C. Ionization energy was 70 eV. The MS was recorded in full scan mode that revealed the total ion current (TIC) chromatograms. A mixture of aliphatic hydrocarbons (C9-C23) in n-hexane (2.1, Sigma-Aldrich, Steinheim, Germany) was injected to calculate the linear retention indices. The mass spectra and linear retention indices (LRI) were contrasted with those in mass spectral library references (NIST MS Search 2.0 library, Wiley 275, Gaithersburg, USA) and home-made library mass spectra gathered from data obtained from standard pure compounds (2.2, Sigma-Aldrich, Steinheim, Germany). The percentage composition of the EO was evaluated from the GC peak areas and were expressed as total area percentages.

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

Measurements were carried out from aqueous extracts. For the extraction, 100 ml of boiling distilled water was added to 1 g of powdered plant material and was left to soak for 24 h. For fresh or frozen plant materials pulverization was not possible therefore in this case plant parts were finely chopped. After filtration, extracts were stored in the freezer until analyses.

The TPC of the aqueous extracts was determined by the modified method of Singleton and Rossi (1965). During TPC determination, the absorbance was measured at 760 nm in a Thermo Evolution spectrophotometer (201, Labomed Inc., Los Angeles, USA) after 5 min incubation period in hot water (50°C). The standard curve was prepared using 0.3 mol/l gallic acid. The straight line equation of calibration was $y = 0.1243x-0.0216$ and the $R^2 = 0.9998$. The TPC of the sample was expressed as mg of gallic acid equivalent (GAE) per g of dry weight

(d.w.) of the extract. Three parallel measurements were performed from the three biological replications for each treatments.

The TAC of aqueous extracts was determined by FRAP (ferric reducing antioxidant power) method according to the modified procedure of Benzie and Strain (1996). The absorbance was measured at 596 nm in a Thermo Evolution 201 spectrophotometer. The straight line equation of calibration was y = 0.2527x+0.0166 and the R^2 = 0.9996. The antioxidant capacity of samples was expressed as mg of ascorbic acid equivalent (AAE) per g of dry weight (d.w.) of the extract. Triplicate measurements were carried out from the three biological replicates for each treatments.

4.4.1.4. Rosmarinic acid (RA) determination

Rosmarinic acid content was determined by an HPLC method in three replications according to the Monograph *Melissae folium* of Ph. Eur. 6.5 by Corvinus-Fitolabor Kft. in Budapest. The HPLC system (Water Corporation, UK) consisted of a 1525 binary pump with a 717plus autosampler, a Jetstream column thermostat and a 2998 PDA detector, controlled by an Empower2 software. A Kinetex C-18 column was used, with 100 mm L4.6 mm i.d. and 2.6 µm particle size. All solvents were HPLC grade. 0.5 g powdered dry plant material was suspended in 45 ml methanol. The suspension was heated for 30 mins in water bath, after that it was cooled and filtered (by 45 µm filter) into a 100 ml flask. The filtrate was completed by methanol to 50 ml volume. 1:19:80 phosphoric acid:acetonitrile:water (mobile phase A) and 1:40:59 phosphoric acid:methanol:acetonitrile (mobile phase B) were used as solvents at a flow rate of 1 ml/min for the elution. The gradient program started at 100% A and after solvent B was increased linearly and attained 35% in 10 mins, then 100% in 2 mins. Eventually, 100% A was reached at 2 mins. The post-time for the equilibration of the initial solvent composition was set for 8 mins. The column temperature was maintained at 35°C and the injection volume of 5µl was used in all experiments. Data were referenced to the dry matter content of samples.

4.4.1.5. 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. 0.2 g of powdered drug was used for preparing the stock solution in 190 ml of alcohol (50% V/V). The solution was boiled in a water-bath under a reflex condenser for 30 mins. Then it was cooled and filtered. The filter was rinsed with 10 ml of alcohol (50% V/V). The filtrate was diluted to 200 ml with alcohol (50% V/V).

Test solution: to 1 ml of the stock solution in a test-tube 2 ml of 0.5 M hydrochloric acid, 2 ml of a solution prepared by dissolving 10 g of sodium nitrite and 10 g of sodium molybdate in 100 ml of water were added. Then 2 ml of dilute sodium hydroxide solution was added and was diluted to 10 ml with water and then it was mixed. *Compensation solution:* in a test tube 1.0 ml of the stock solution, 2 ml of 0.5 M hydrochloric acid, 2 ml of dilute sodium hydroxide solution were placed and diluted to 10.0 ml with water. Absorption values of the test solutions were measured at wavelength of 505 nm and compared with that of the compensation solution. The percentage content of THA was expressed as rosmarinic acid. The results referring to the THA content were calculated with reference to the dry matter content of samples.

4.4.2. Sensory evaluation

4.4.2.1. 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 (Figure 13).

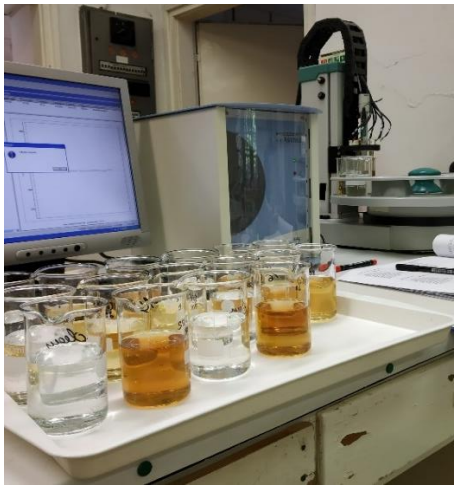

Figure 13. Alpha Astree electronic tongue (Photo: Hazarika, 2020)

The instrument is equipped with a 16-position autosampler. The sensor head consists of Ag/AgCl reference electrode, a sensor array of seven ion selective field effect transistor (IFSET) based on potentiometric chemical sensors (ZZ, BA, BB, CA, GA, HA, JB) and a stirrer. Before the measurements, the instrument was setup according to the manufacturer's instructions (Alpha and Alpha, 2003). This included a pre-conditioning with 0.01 mol/l hydrochloric acid solution then a conditioning and a calibration step. For the last two steps, a mixture of all the used samples was used. The e-tongue was controlled by the software

AlphaSoftsoft.ver. 12.3. Sample preparation for the electronic tongue measurements was done by 10-times dilution of the 25% ethanolic herb extract. Sensors were cleaned with distilled water between subsequent measurements until stable potential was obtained. 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.

4.4.2.2. Colour measurements

The colour of plant samples was determined using a Konica Minolta tristimulus colorimeter (CR-410, Konica Minolta Inc., Osaka, Japan). A white calibration tile standard prepared by the manufacturer was used to calibrate the instrument. L* (lightness/darkness), a* (red/green coordinate) and b* (yellow/blue coordinate) values were recorded 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.

4.4.3. 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 (Figure 14). The examinations were conducted using plant materials from peppermint 'Mexián', lovage 'Mittelgrobblättriger', lemonbalm 'Lemona' and rosemary 'Harmat'. The samples were mounted on stubs using double sided carbon tape. The surfaces of samples were not coated with conductive material (low vacuum was used). Back scattered electron images were taken with the parameters as follows: accelerating voltage: 10 kV; probe current – Std. PC 55.0; working distance: 7.0–9.3 mm; low vacuum mode (50 Pa).

Figure 14. Scanning Electron Microscope (Photo: Szabó, 2022)

The measurements carried out in the species are shown in Table 5.

Table 5. 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
		'Ohře'		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; THA = Total hydroxycinnamic acid content

4.5. Statistical analysis

IBM SPSS version 29 software (International Business Machines Corporation, North Castle, USA) was used to analyse the data. 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. Normality of the residuals was proven by the absolute values of skewness and kurtosis of them. Homogeneity of variances was checked using Levene's test ($p < 0.05$). The relationship between TCP and TAC was analysed using Pearson's correlation coefficient (r). The values between 0.7 and 1.0 indicated a strong positive linear relationship through a firm linear rule (Ratner, 2009).

Linear discriminant analysis (LDA) was used for the multi-class classification of different plant samples treated by different preservation methods in the context of e-tongue analysis. LDA classification models were developed for the grouping of different preservation methods. To assess the reliability of each LDA model in identifying the preservation methods, a cross-validation strategy was implemented. This involved partitioning the data into two segments: a training set, comprising two-thirds of the dataset, and a validation set. Specifically, the training set included sensor readings from the first two repetitions of each plant sample at various concentrations, while the validation set consisted of readings from the third repetition. The data splitting was done three times to ensure that each sample was used at least once in the training and validation set.

5. RESULTS AND DISCUSSION

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

In our experiment, the applied preservation methods had influence on the EO content of the studied plant species, but not to the same way and extent.

5.1.1. Species accumulating the EO primarily or exclusively in glandular hairs or trichomes

***Mentha x piperita* L. 'Mexián'**

The fresh peppermint leaves contained 3.58 ml/100 g d.w. of EO. Examining the preservation methods, the highest EO content (3.10-3.35 ml/100 g d.w.) was obtained in oven drying at 40°C and freezing methods (Figure 15). Sun drying, shade drying and lyophilization also resulted in rather higher values (3.06, 2.90 and 2.69 ml/100 g d.w., respectively). However, in oven dried sample at 60°C the EO content significantly decreased (1.82 ml/100 g d.w.), and the volatiles were almost completely lost during microwave drying (at 250 W: 0.14 ml/100 g d.w., at 700 W: 0.09 ml/100 g d.w.).

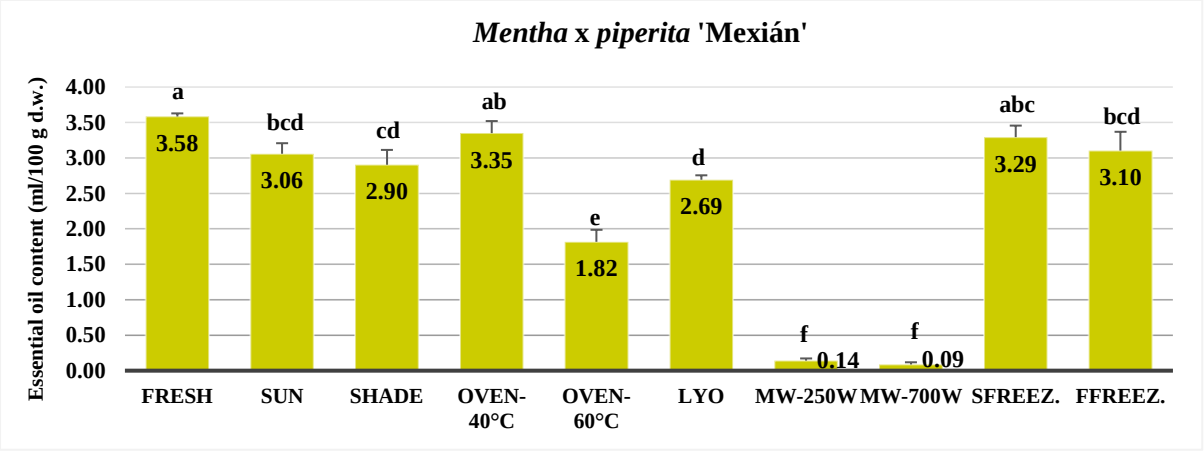

Figure 15. Essential oil content (ml/100 g d.w.) of fresh and preserved leaves of *Mentha* x *piperita* L. 'Mexián'

†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

According to the microphotographs of glandular hairs captured using SEM (Figure 16), lyophilization did not damage the external glandular hairs on the lower epidermis of *Mentha* x *piperita* leaves. The peltate glands remained plump, and no ruptures could be seen on the cuticle cover of leaves dried at lower temperature (40°C) as well. On the contrary, peppermint leaves dried at 60°C and 250 W caused severe shrinkage of the leaf structure and cuticle layer

of the glandular trichomes, suggesting loss of volatiles. Because if the cuticle layer is damaged, cracks and splits open, that eventually causes loss of volatiles through evaporation.

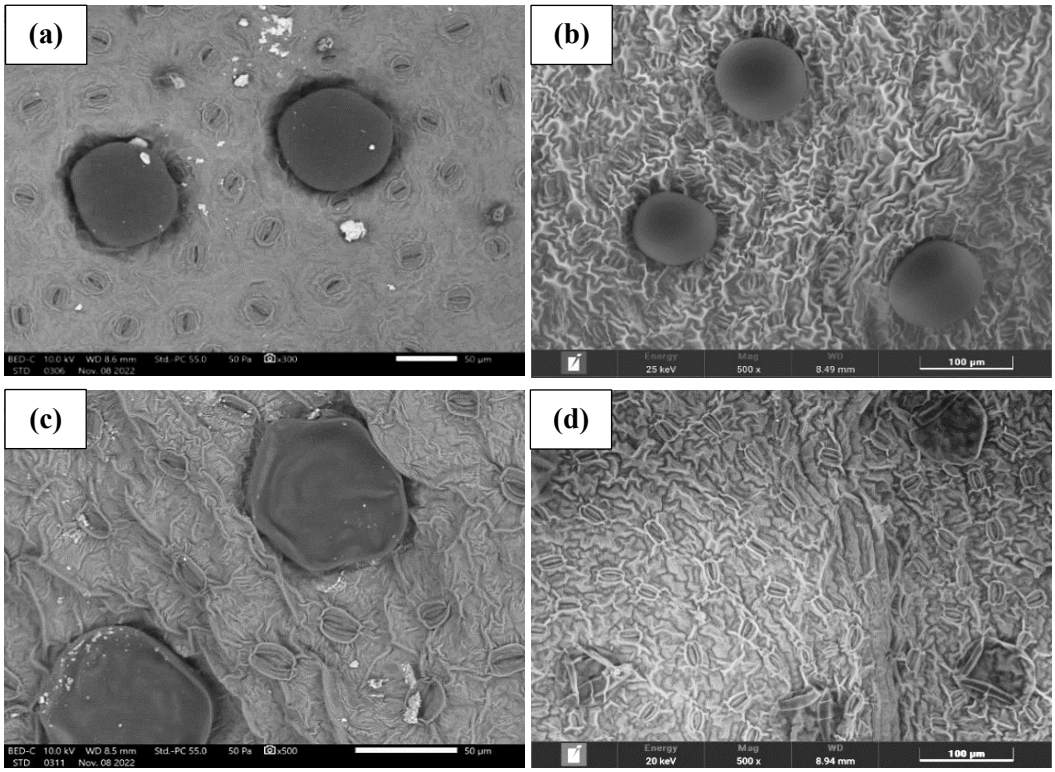

Figure 16. Peltate glands on the lower epidermis of *Mentha* x *piperita* leaves preserved by lyophilization (a), oven drying at 40ºC (b), at 60ºC (c), and microwave drying at 250 W (d)

***Melissa officinalis* L. 'Lemona'**

In fresh lemonbalm leaves, 0.39 ml/100 g d.w. EO content was found. When preservation techniques were applied, a rise in the EO content ranging between 13-36% was observed, depending on the specific method utilized (Figure 17). This is presumably a result of sampling error: the freshly harvested and instantly distilled leaves might have contained more dirt and sand, which could lower the EO content referred to the dry matter content.

Lyophilization resulted in the highest essential oil accumulation (0.53 ml/100 g d.w.), but this value did not differ significantly from that of the samples dried in the oven at 40°C, frozen (slow and fast) or dried naturally (sun and shade) (0.44-0.52 ml/100 g d.w.). In this case, drying in sun and shade were carried out at very similar temperatures (Appendix2/2). The lowest EO content was detected in oven dried at 60°C and microwave dried (250-700 W) samples. These preservation methods caused 72-95% loss in the volatile content.

The microphotographs of *Melissa officinalis* L. glandular hairs captured using SEM revealed the presence of EO on the surface of the leaves in peltate glandular hairs (Figure 18).

According to the SEM images, oven drying at 40°C did not damage the external glandular hairs and these remained plump. It also appeared that the protective hairs on the leaves surrounding the glandular trichomes were also not damaged. In contrast, leaves dried at 60°C and microwaved resulted in split open of the glandular trichomes, indicative of oil loss, with most showing signs of damage. The SEM images support our research findings.

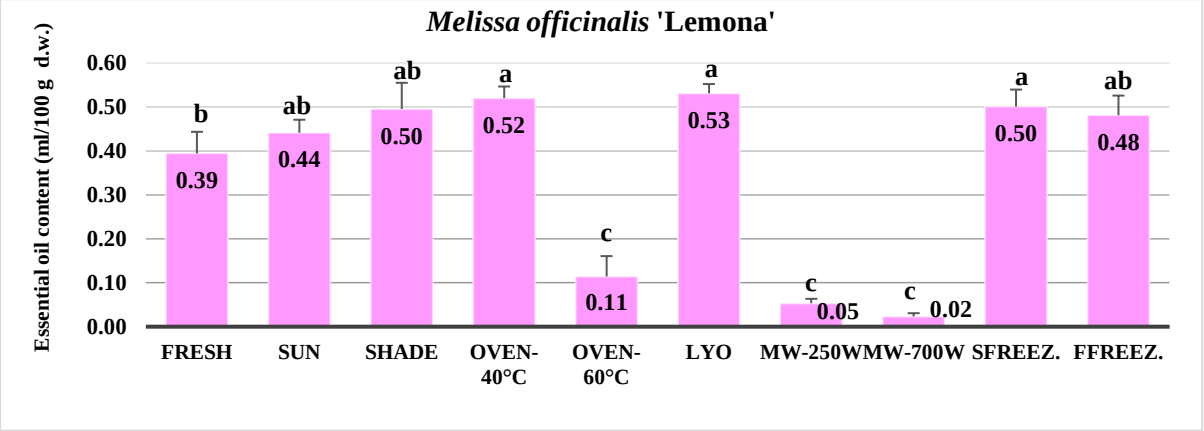

Figure 17. Essential oil content (ml/100 g d.w.) of fresh and preserved leaves of *Melissa officinalis* L. 'Lemona'

†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

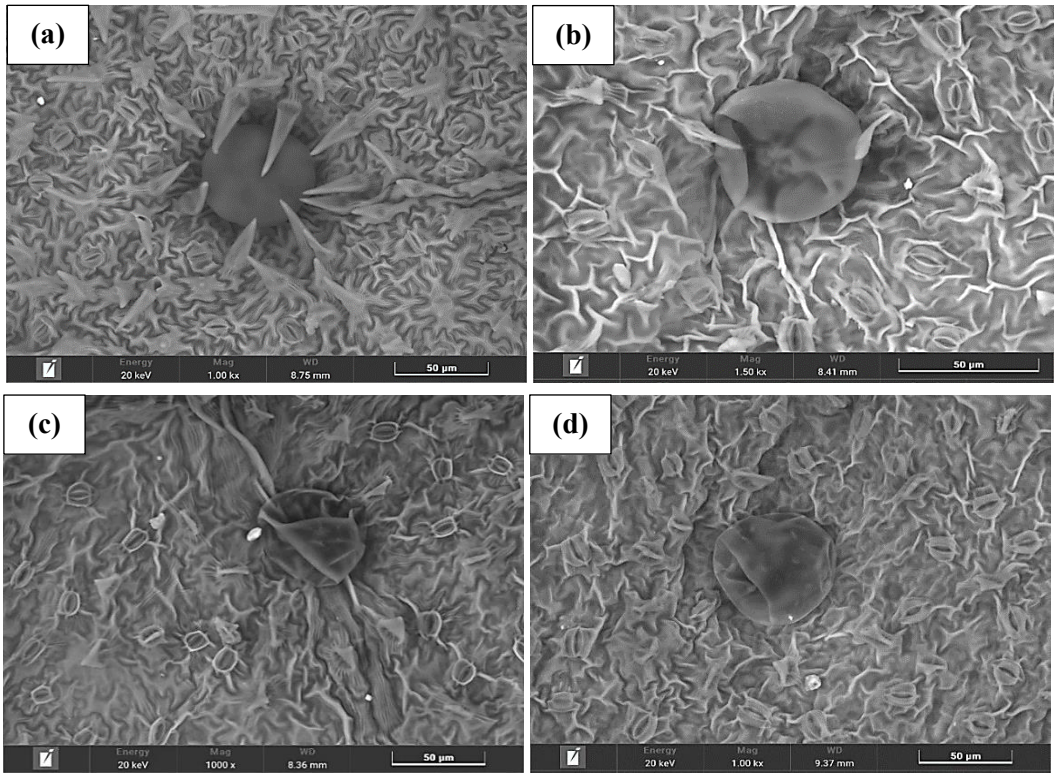

Figure 18. Peltate glands on the lower epidermis of *Melissa officinalis* L. leaves preserved by oven drying at 40ºC (a), at 60ºC (b), microwave drying at 250 W (c) and at 700 W (d)

***Ocimum basilicum* L. 'Genovese' and 'Ohře '**

For sweet basil two varieties were examined, but in different years. The EO content of variety 'Genovese' was a little higher than that of 'Ohře'. In fresh leaves of 'Genovese', a volatile content of 0.85 ml/100 g d.w. was measured. Preservation methods like shade drying, oven drying at 40°C, freezing (slow, fast) and sun drying were effective in retaining the highest amount of EO (0.82-0.92 ml/100 g d.w.). Lyophilization also preserved the EO moderately well (0.63 ml/100 g d.w.). However, the least suitable methods were found to be oven drying at 60°C and microwave drying (250 W, 700 W). These preservation methods reduced the EO content by 64-92% (Figure 19).

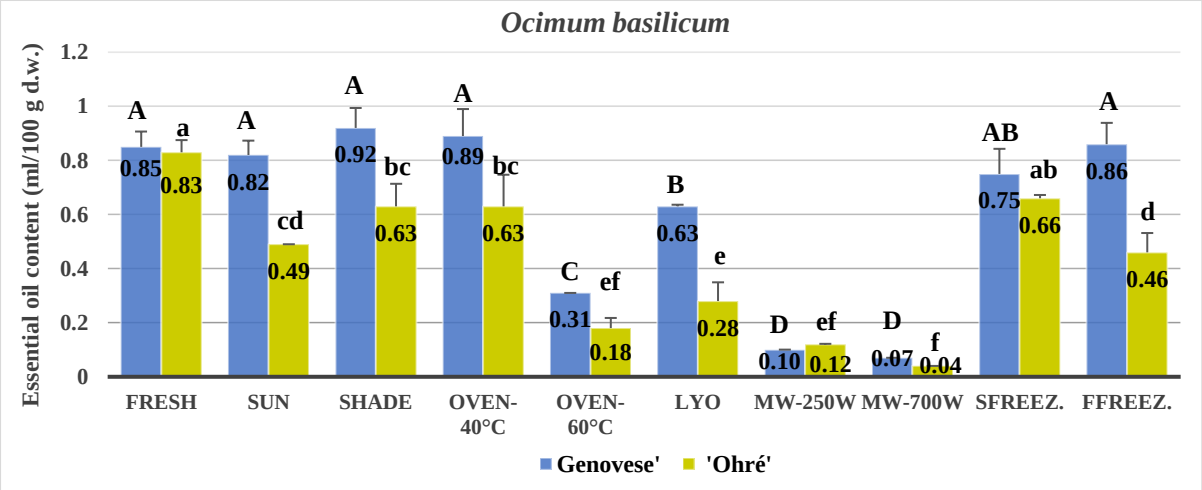

Figure 19. Essential oil content (ml/100 g d.w.) of fresh and preserved leaves of *Ocimum basilicum* L. 'Genovese' and ' Ohře'

†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

Slow freezing, shade drying and oven drying at 40°C preserved the highest amount of EO (0.63-0.66 ml/100 g d.w.) in case of variety ' Ohře' as well. On the other hand, fast freezing and sun drying (with sometimes 50-60°C drying temperature) significantly (by 41-45%) reduced the volatile content compared to that of the fresh leaves, which recorded as 0.83 ml/100 g d.w. (Figure 15). The reduction in EO content for fast-frozen samples could be attributed to errors in sample handling, likely due to partial melting during preparation for distillation. For this variety lyophilization also caused a significant loss (66%) in the EO content. The least suitable methods were oven drying at 60°C and microwave drying (with 78-95% EO loss).

***Origanum majorana* L. 'Magyar' and 'Egyptian'**

Many of the applied preservation methods had significant effect ($p < 0.05$) on the EO content of flowering leafy shoots for both examined varieties of marjoram (Figure 20). Variety

'Magyar' had a slightly higher EO content compared to 'Egyptian'. For variety 'Magyar' in some cases we measured higher EO content in the preserved samples than in the fresh plant material (1.49 ml/100 g d.w.), that is again certainly the outcome of a sampling error similarly to lemonbalm. The highest EO content was found in oven dried at 40°C, shade dried and frozen samples (1.53-1.73 ml/100 g d.w.). Lyophilization and sun drying also preserved the EO content relatively well (1.39 ml/100 g d.w.). On the other hand, in oven dried sample at 60°C the EO content significantly decreased (by 24%) and the volatiles were almost completely lost during microwave drying.

In fresh flowering shoots of 'Egyptian', 1.29 ml/100 g d.w. of EO content was obtained. Oven drying at 40°C could preserve the highest EO content (1.54 ml/100 g d.w.). No significant differences were found in the EO content of natural drying methods (sun and shade drying), freezing (slow, fast), oven drying at 60°C and lyophilization (1.00-1.19 ml/100 g d.w.). However, the least suitable preservation method was found to be microwave drying again (with 91-95% EO loss) (Figure 20).

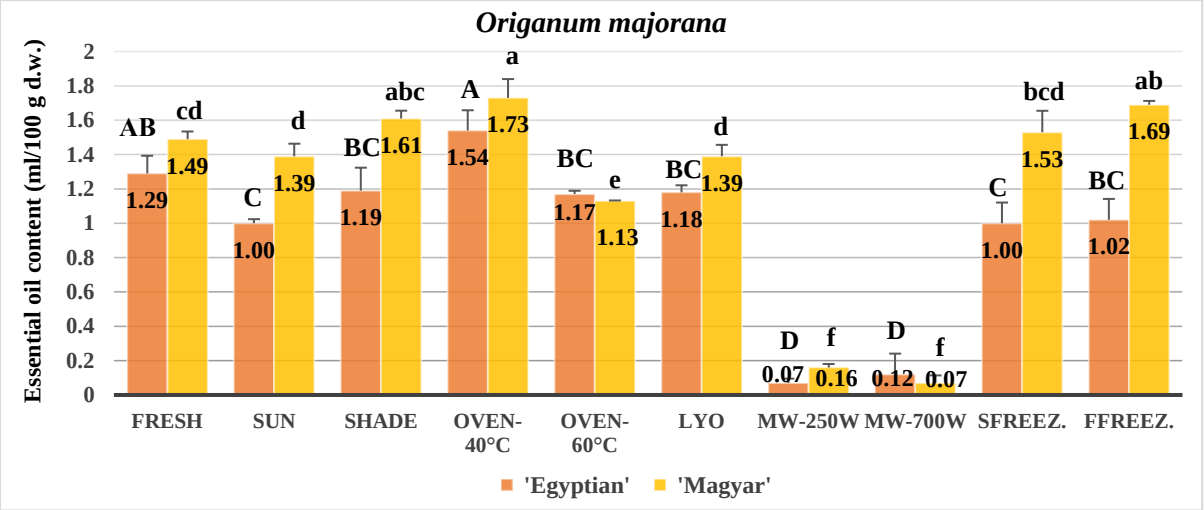

Figure 20. Essential oil content (ml/100 g d.w.) of fresh and preserved leafy shoots of *Origanum majorana* L. 'Egyptian' and 'Magyar'

†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

Thymus vulgaris L. 'Deutscher Winter' and 'French Summer'

According to our data, the variety 'French Summer' had a slightly higher EO content compared to 'Deutscher Winter'. The leafy shoots of variety ‘French Summer’ that were sun dried and oven dried at 40°C (2.01 and 2.03 ml/100 g d.w., respectively) preserved the highest amount of volatiles (Figure 21). The amount of it did not differ significantly from the accumulation of fresh sample (1.91 ml/100 g d.w.). No significant differences were found

between shade drying, lyophilization and freezing (slow and fast) either. These methods could preserve the EO content considerably higher in this variety. The worst preservation methods were found to be oven drying at 60°C and microwave drying treatments. These methods caused a 76-80% loss of volatiles.

For 'Deutscher Winter' the highest amount of EO was preserved by shade drying and oven drying at 40°C (1.92 and 1.90 ml/100 g d.w., respectively), but rest of the preservation methods also proved to be very effective. Only oven drying at 60°C and microwave drying methods reduced the EO content drastically. Similar to 'French Summer', these preservation methods resulted in a significant loss of volatiles (67-73%) in this case too (Figure 21).

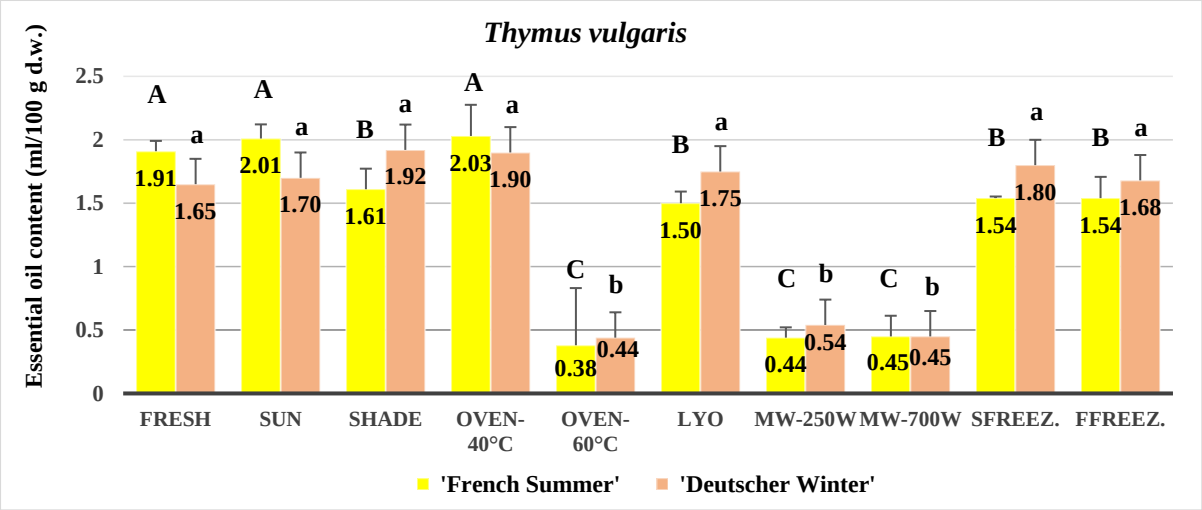

Figure 21. Essential oil content (ml/100 g d.w.) of fresh and preserved leafy shoots of *Thymus vulgaris* 'French Summer' and 'Deutscher Winter'

†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

Satureja hortensis L. LAMISATU22 and 'Saturn'

In relation to *Satureja hortensis* L., the gene bank accession Nr. LAMISATU22 had higher EO content than variety 'Saturn' (Figure 22). Fresh leafy shoots of LAMISATU22 yielded 3.14 ml/100 g d.w. of EO. The highest volatile content was obtained from shade drying, oven drying at 40°C and freezing methods (2.63-2.90 ml/100 g d.w.), but lyophilization also preserved the EO content (2.06 ml/100 g d.w.) moderately well. In case of sun drying (where sometimes the temperature reached the 60-70°C) and oven drying at 60°C, the volatile content significantly decreased, by almost 61%. But microwave drying at 250 W and 700 W were identified as the least effective methods (with 0.45-0.48 ml/100 g d.w. EO content).

The fresh leafy shoots of 'Saturn' contained a lower EO content (1.96 ml/100 g d.w.) than few of the samples preserved by the applied methods did, which is probably due to a handling

mistake during sample preparation. In line with the findings of LAMISATU22, the highest EO content was measured in samples dried in shade, oven dried at 40°C and slow and fast frozen (2.23-2.50 ml/100 g d.w.). Lyophilization resulted in a moderate preservation of EO content as well, with a measured amount of 1.96 ml/100 g d.w. Sun drying and oven drying at 60°C caused a 29-51% loss of volatiles. However, the least suitable methods were microwave drying techniques, where the EO almost completely evaporated (Figure 22).

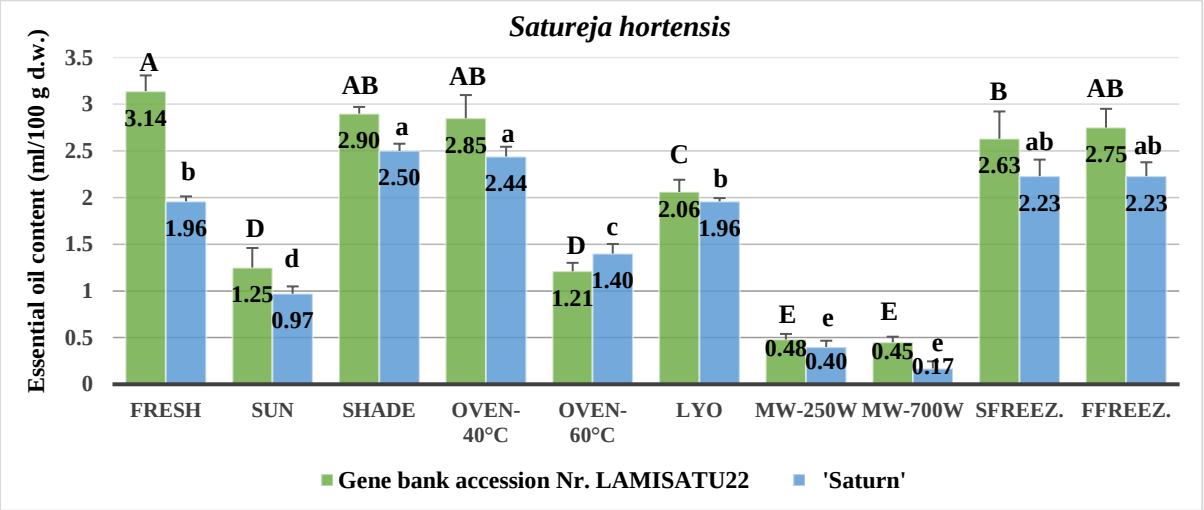

Figure 22. Essential oil content (ml/100 g d.w.) of fresh and preserved leafy shoots of *Satureja hortensis* L. gene bank accession Nr. LAMISATU22 and 'Saturn'

†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

Lavandula x *intermedia* 'Judit' and *Lavandula angustifolia* 'Budakalászi 80'

Among the studied lavender species, *Lavandula angustifolia* 'Budakalászi 80' had lower volatile content than *Lavandula* x *intermedia* 'Judit'. For hybrid lavender 'Judit', fast and slow freezing techniques retained the highest amount of EO (12.52-12.53 ml/100 g d.w.). This results didn't differ significantly from the accumulation of fresh sample (12.66 ml/100 g d.w.). Shade drying, oven drying at 40°C and sun drying (where the temperature was around 30°C) also preserved the EO content relatively well (10.60-11.85 ml/100 g d.w.). However, oven drying at 60°C and lyophilization resulted in 29-32% loss of volatiles, but the lowest EO content was observed in case of microwave drying, similarly to the previous species (Figure 23).

The EO content of fresh true lavender 'Budakalászi 80' flowers was measured at 5.27 ml/100 g d.w. Slow and fast freezing techniques preserved the highest levels of EO in this species as well (5.45 and 4.96 ml/100 g d.w., respectively). The other methods also retained the EO content (4.22-4.73 ml/100 g d.w.) quite well, except microwave drying. This method, when applied at 250 and 700 W resulted in a 72-83% loss of volatiles (Figure 23).

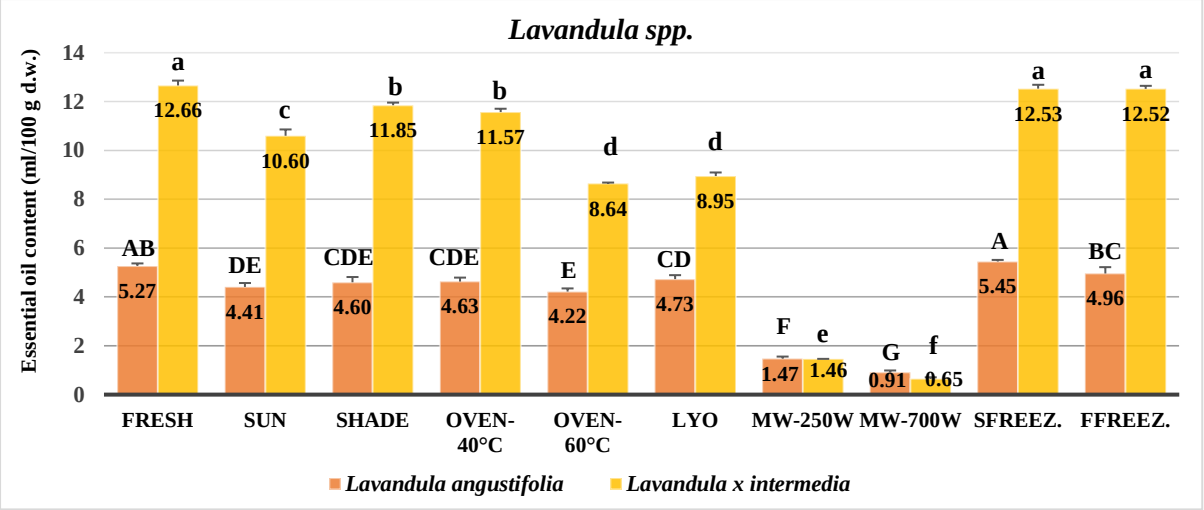

Figure 23. Essential oil content (ml/100 g d.w.) of fresh and preserved flowers of *Lavandula angustifolia* 'Budakalászi 80' and *Lavandula* x *intermedia* 'Judit'

†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

Origanum vulgare subsp. *hirtum* commercial plant material

The EO content of Greek oregano was significantly influenced by the preservation methods ($p < 0.05$). The highest EO content was obtained by slow freezing method (2.76 ml/100 g d.w.), which did not differ significantly from the volatile content of fresh flowering leafy shoots (2.84 ml/100 g d.w.). Fast freezing also resulted in high values (2.24 ml/100 g d.w.). However, the other preservation methods caused significant loss (75-95%) of volatiles, oven drying at 60°C and microwave drying treatments the most (Figure 24). According to the results, it seems that glandular hairs of oregano are very sensitive to heat.

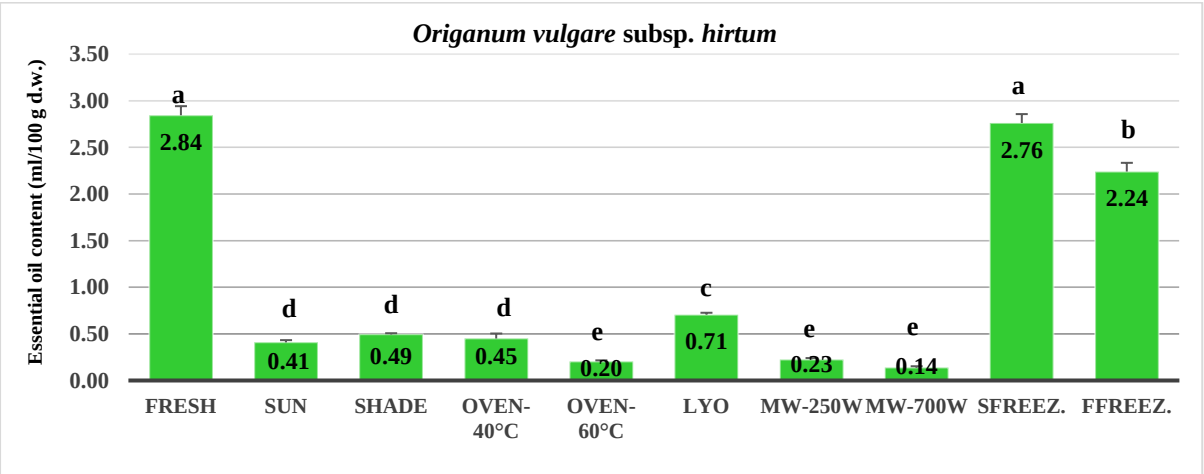

Figure 24. Essential oil content (ml/100 g d.w.) of fresh and preserved leafy shoots of *Origanum vulgare subsp. hirtum* commercial sample

†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

Salvia officinalis L. 'Regula'

Fresh garden sage 'Regula' leaves contained 1.38 ml/100 g d.w. average EO content (Figure 25), which was not significantly ($p > 0.05$) modified by most of the applied preservation techniques (sun and shade drying, lyophilization and oven drying at 40°C). Both slow and fast freezing methods preserved the EO content relatively well (1.12 ml/100 g d.w.), and in this case oven drying at 60°C also did not reduce the amount of volatiles spectacularly. This might be due to the dense layer of protective hairs on the leaves, which likely protect the glands during high-temperature processes. However, these hairs could not provide protection against microwave drying, which caused 81-90% EO loss in this case too.

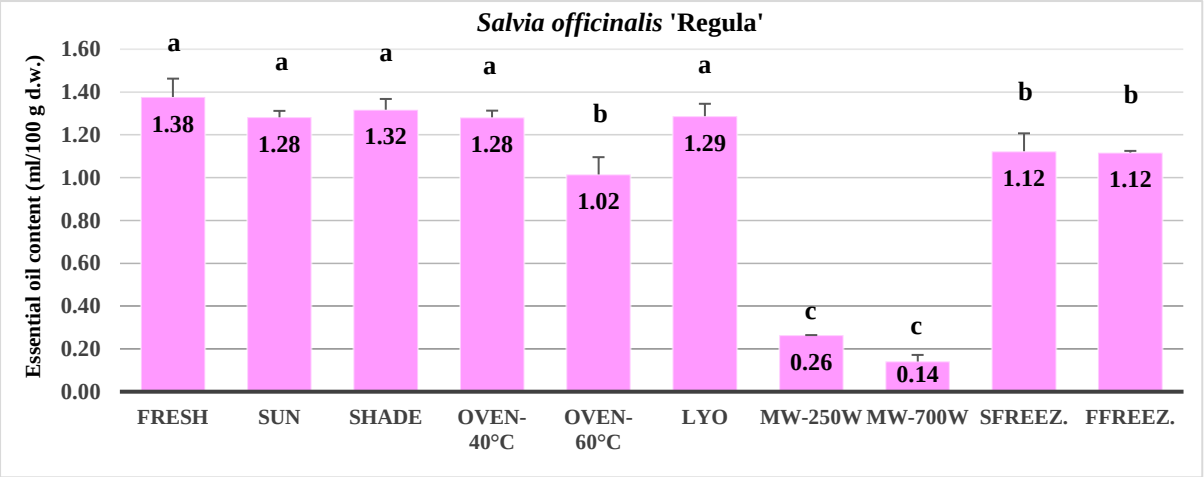

Figure 25. Essential oil content (ml/100 g d.w.) of fresh and preserved leaves of *Salvia officinalis* 'Regula'

†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

Salvia rosmarinus L. 'Harmat'

In fresh rosemary 'Harmat' leaves, 1.98 ml/100 g d.w. EO content was found. The applied preservation methods did not cause significant changes ($p > 0.05$) in the volatile content, except microwave drying methods. Similarly to garden sage, this could result from the leaves' dense protective hair layer, likely safeguarding the glands. Nevertheless, microwave drying at 250 W and 700 W reduced the amount of volatiles by 87-92% (Figure 26).

The scanning electron microscope (SEM) images reveal that the external glandular hairs of rosemary leaves preserving by oven drying at 40°C, remained intact and plump (Figure 27). Additionally, the dense protective hairs that cover the glandular hairs on the lower epidermis of leaves also can be seen. These appears undamaged too.

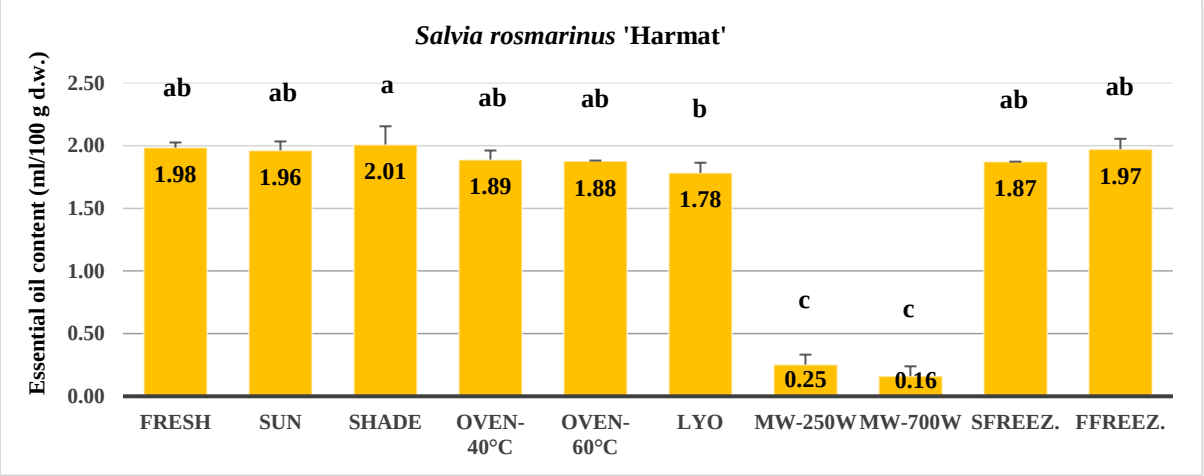

Figure 26. Essential oil content (ml/100 g d.w.) of fresh and preserved leaves of *Salvia rosmarinus* L. 'Harmat'

†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

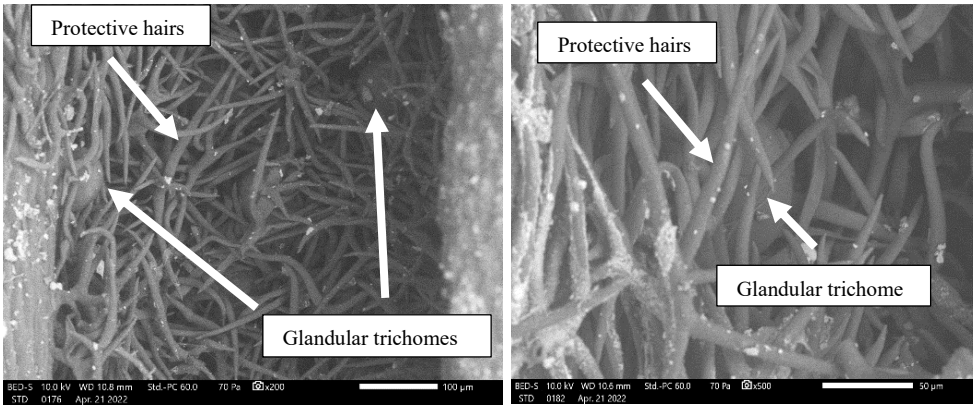

Figure 27. Scanning electron microscope (SEM) observations of the lower epidermis of *Salvia rosmarinus* L. 'Harmat' leaves oven dried at 40°C, showing the protective hairs over glandular trichomes

Conclusions

From our observations, it is evident that among the used preservation methods *freezing* and the gentle, low-temperature drying techniques (*drying in shade and at 40°C*) proved to be the most effective treatments in case of those species, which accumulate the EO externally in glandular hairs. These methods did not reduce the amount of original volatiles of fresh samples considerably. The reason for this is that low temperature spares the heat-sensitive glandular hairs, so these remain intact and can retain the volatiles.

However, in our experiment *drying at 60°C* significantly reduced the EO content of plant materials, although not to the same extent. According to Khangholil and Rezaeinodehi (2008) and Sellami et al., (2011) the differences in the influence of higher drying temperatures on

different plant species can be attributed to the nature of secretory tissues and the differences in their localization. In case of those species, where glandular hairs are mechanically more protected (e.g. by protective hairs), the rate of EO loss will be lower.

The benefits of lower drying temperatures and the disadvantages of higher temperatures have been confirmed in a number of studies, which are in line with our findings. For example, Argyropoulos and Müller (2014) reported that the EO loss increased parallel with the increase of drying temperature. Blanco et al., (2002) also found that the EO content decreased with the increase of drying temperature (40-80°C). According to Shaw et al., (2006) and Alara et al., (2019), the most recommended low convective drying temperature is the 40°C in relation to EO containing plants. Khalid et al., (2008) observed that shade-dried *Melissa officinalis* L. leaves contained the highest volatile content. Mirahmadi et al., (2017) stated that oven drying at lower temperature (35°C) proved to be the best method for drying *Melissa officinalis* L. leaves than drying at higher temperatures (55°C). Mirjalili et al., (2019) also found that shade drying yielded the highest EO content in the flowers of *Lavandula angustifolia*.

The 60°C drying temperature is considered already too high for the preservation of EO containing species according to many scientific findings (Venskutonis, 1997; Rohloff et al., 2005; Pirbalouti et al., 2013a; Calín-Sánchez et al., 2012; Figiel et al., 2010). Other researchers (Thamkaew et al., 2020; Alara et al., 2019) also reported that higher drying temperature could harm the glandular trichomes and secretory cells, resulting in the loss of their volatiles.

Sun drying variably preserved the EO content in glandular hair-containing plant species, with the outcome being dependent on the actual temperature: if the temperature was lower, the EO content was better preserved; if it was higher, the volatile loss was more significant. According to our observations, temperature was the most important factor here too. Similar to our findings, Hassanpouraghdam et al., (2010) found, that in *Ocimum basilicum* the EO content was significantly decreased by sun drying. Sefidkon et al., (2006) also described that in *Satureja hortensis* the EO content was least preserved by sun drying (at high temperature). Similar results were obtained by Pirbalouti et al., (2013b) too in case of *Satureja bachtiarica*.

Lyophilization proved to be moderately good in preserving volatiles in our research. Sometimes, it could preserve the EO content as good as convective drying at low temperature (40°C). Several studies also reported the capability of lyophilization in preserving volatiles in plant species belonging to the *Lamiaceae* plant family, such as *Mentha spicata* (Antal et al., 2011), *Mentha viridis* (Orphanides et al., 2013), *Dracocephalum moldovica* (Morshedloo et al., 2020), *Thymus daenensis subsp. daenensis* (Rahimmalek and Goli, 2013) or *Salvia lavandulifolia* (Hazrati et al., 2021). Due to the applied lower temperature during freeze drying,

plant parts exhibited less shrinkage compared to convective drying methods, which in turn helped in retaining the EO (Tummanichanont et al., 2017). According to Rahimmalek and Goli (2013), the higher EO yield can be obtained through techniques that use low temperatures and shorter drying times, and freeze drying is just such a method.

In our experiment *microwave drying* proved to be the least suitable method for preserving spice plant species with external glandular hairs, since it damaged the trichomes, resulting in an almost complete loss of volatiles. According to Ozkan et al., (2007) other problem with this method that it could lead to a non-uniformity in heating, which results in the development of temperature gradients in the product causing sometimes overheating.

Several other studies in the literature have come to similar conclusions to ours on the effects of microwave drying. Pirbalouti et al., (2013a) for example found that microwave drying proved to be the least suitable method for preserving *Ocimum basilicum* leaves. Yousif et al., (1999) concluded that microwave drying method led to significant shrinkage in the cuticle layer and structure of *Ocimum basilicum* leaves resulting in loss of volatiles. Szumny et al., (2010) observed that microwave drying of rosemary leaves resulted the highest loss of EO in comparison with convective drying. Raghavan et al., (1997) also stated that microwave drying was a less suitable preservation method in connection with *Origanum majorana* when compared with many other preservation methods, including oven drying, shade and sun drying.

5.1.2. Species accumulating the EO primarily or exclusively in secretory ducts

Levisticum officinale Koch. 'Mittelgroblättriger' and ASTLEVI44

The EO content of leaves for lovage variety 'Mittelgroblättriger' was higher than that of the gene bank accession Nr. ASTLEVI44 (Figure 28). In relation to 'Mittelgroblättriger', the highest EO content was preserved by sun drying and freezing methods (1.61-1.87 ml/100 g d.w.), but shade drying, microwave drying at 250 W and oven drying at 40°C also retained the volatile content relatively well (1.25-1.46 ml/100 g d.w.). However, oven drying at 60°C and microwave drying at 700 W resulted in 39-42% loss, and the lowest EO content was found in the sample preserved by lyophilization (0.22 ml/100 g d.w.).

The preserved leaves of ASTLEVI44 showed a higher EO content compared to the fresh sample (1.20 ml/100 g d.w.), but this result was likely due to measurement inaccuracies. The highest EO accumulation was detected in slow frozen and shade dried samples (1.85 and 1.71 ml/100 g d.w., respectively). No significant differences were found between samples preserved by fast freezing, microwave drying (at 250 and 700 W), oven drying at 40°C and sun drying

(1.23-1.38 ml/100 g d.w.). These methods preserved the volatile content comparatively well. Oven drying at 60°C, however, decreased the EO content significantly (by 28%), and similarly to 'Mittelgroblättriger', lyophilisation almost completely eliminated the volatiles from the leaves in this case too (only 0.15 ml/100 g d.w. could be measured) (Figure 28).

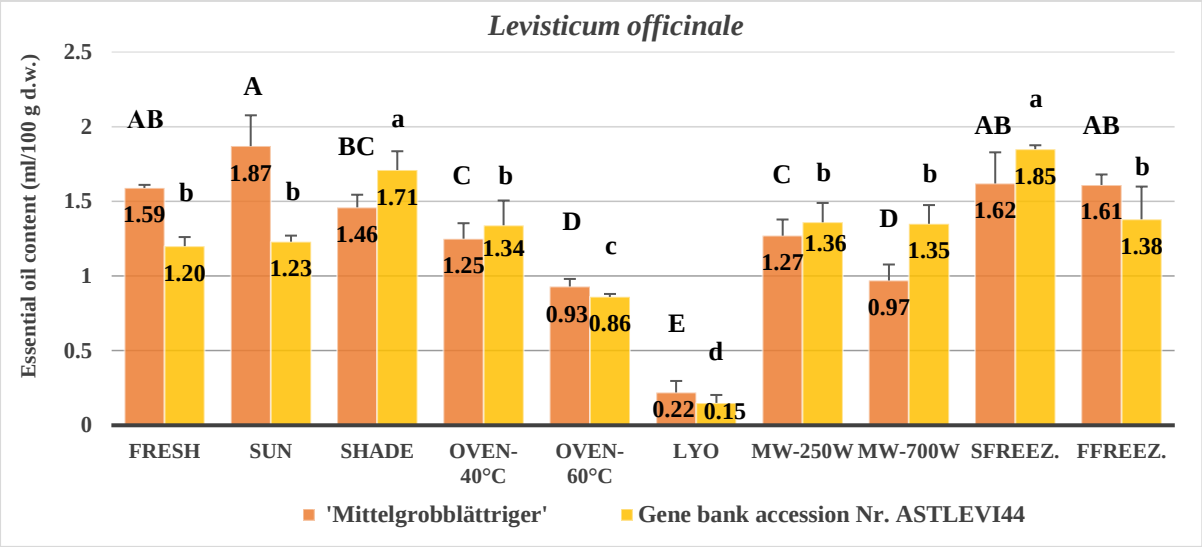

Figure 28. Essential oil content (ml/100 g d.w.) of fresh and preserved leaves of *Levisticum officinale* Koch. 'Mittelgroblättriger' and gene bank accession Nr. ASTLEVI44

†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

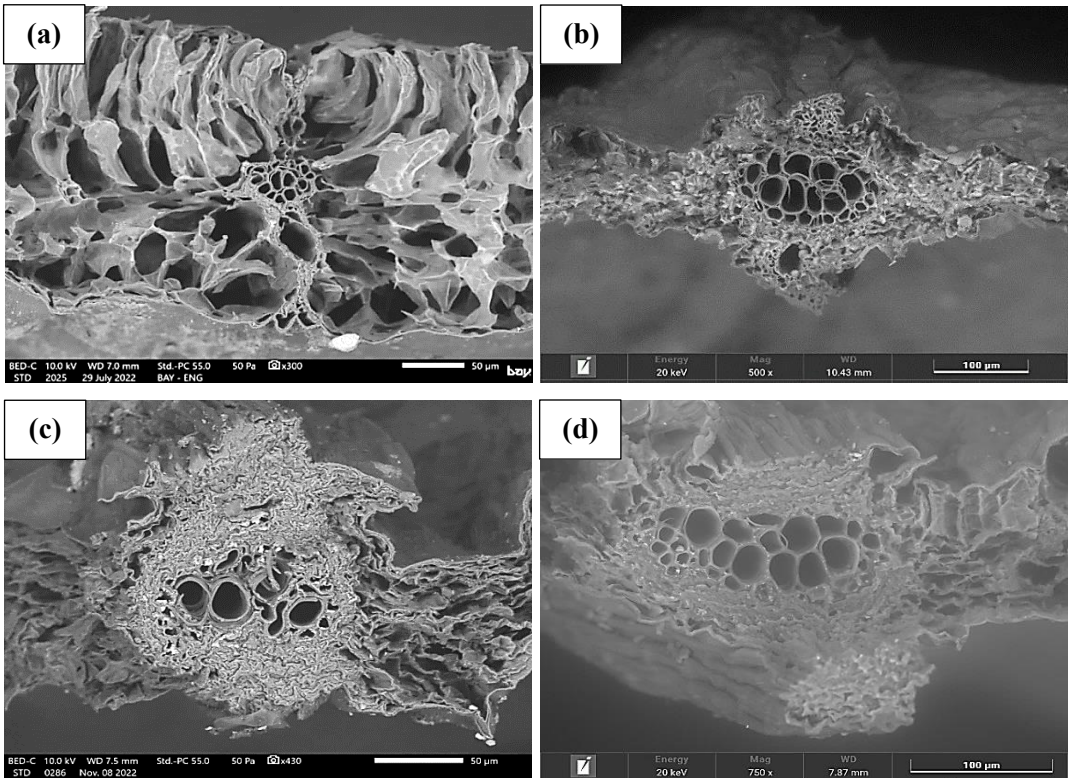

Figure 29. Cross-section of a *Levisticum officinale* leaf preserved by lyophilization (a), oven drying at 40°C (b), oven drying at 60°C (c) and microwave drying at 250 W (d)

According to the SEM cross-sectional images of *Levisticum officinale* leaves (Figure 29), lyophilization led to structural expansion of the pores due to water turning to ice, which could indicate the diffusion of volatiles. However, drying at 40°C, 60°C and 250 W did not change the structure of lovage leaf tissue notably. Presumably, this is the reason why drying at these temperatures resulted in lower EO loss compared to lyophilization.

Artemisia dracunculus L. 'Artemis' and 'Zöldzamat'

The french tarragon variety 'Artemis' had a slightly higher EO content compared to 'Zöldzamat' (Figure 30). Slow and fast freezing treatments ('Zöldzamat': 3.82-3.89 ml/100 g d.w., 'Artemis': 4.11-4.41 ml/100 g d.w.) preserved the EO content the most. The amount of it did not differ significantly from the accumulation of fresh samples. Shade drying also proved to be an effective method, it resulted in a relatively small loss (14–31%) of volatiles. However, the other preservation techniques significantly decreased the EO content of tarragon leaves, especially in the samples of variety 'Zöldzamat'.

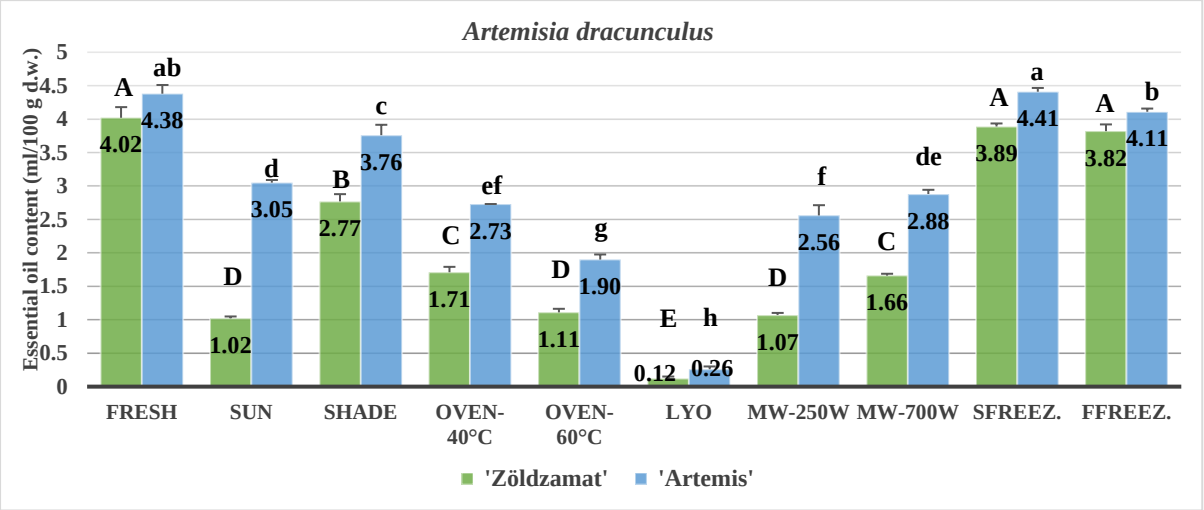

Figure 30. Essential oil content (ml/100 g d.w.) of fresh and preserved leaves of *Artemisia dracunculus* L. 'Zöldzamat' and 'Artemis'

†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

Oven drying at 40°C caused a reduction of 38% and 57% in the volatiles of 'Artemis' and 'Zöldzamat', respectively. Microwave drying also yielded similar values, but dehydration at higher power (700 W) could preserve the EO content significantly better than drying at 250 W, presumably due to the shorter drying time. Sun drying and convective drying at 60°C also caused significant EO loss (72-75%) in tarragon leaves, especially for variety 'Zöldzamat'. However, lyophilization proved to be the least suitable preservation technique for both cultivars

in terms of EO content, since tarragon leaves almost completely lost their volatiles after freeze-drying (in 'Zöldzamat': 0.12 ml/100 g d.w., in 'Artemis': 0.26 ml/100 g d.w. EO could be found).

Petroselinum crispum (Mill) Nym. var. *neapolitanum* whole and chopped leaves

The volatile content of whole, fresh parsley leaves (0.71 ml/100 g d.w.) was not significantly reduced by freezing methods, oven drying at 40°C or microwave drying at 250 W (0.55-0.68 ml/100 g d.w.) (Figure 31). In contrast, natural drying (sun and shade) and microwave drying at 700 W resulted in a significant decrease in the amount of volatiles (20-45%), and in case of drying at 60°C further decrease was detected (59%). But the lowest accumulation level was measured in the lyophilized sample (0.07 ml/100 g d.w.).

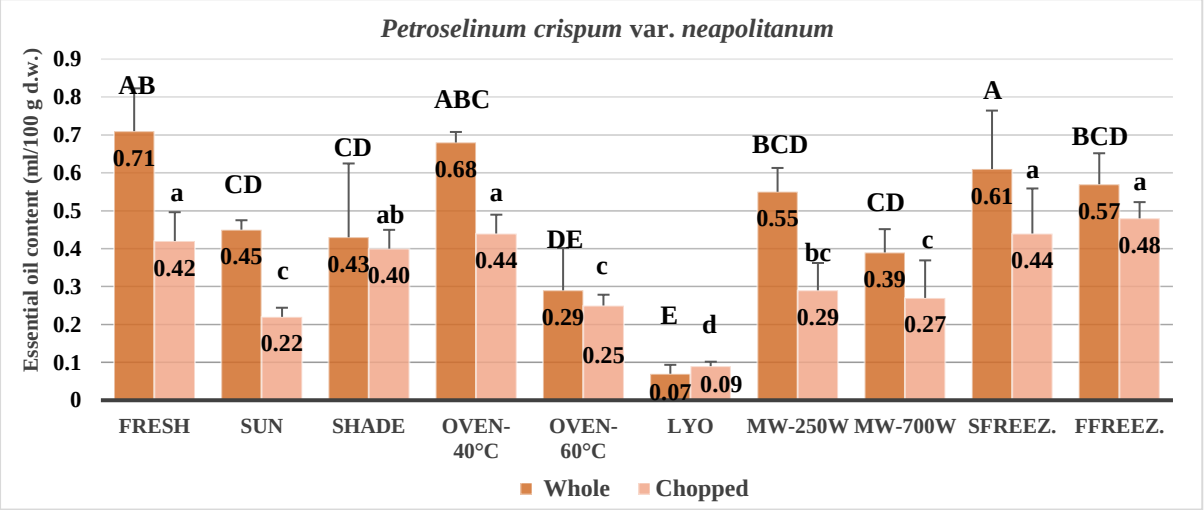

Figure 31. Essential oil content (ml/100 g d.w.) of fresh and preserved, whole and chopped leaves of *Petroselinum crispum* var. *neapolitanum*

†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

In our experiment, chopping the leaves before drying reduced the EO content of fresh leaves by 41%, and that of preserved leaves by 31-51%, depending on the preservation method. The preservation processes, however, affected the EO content of chopped leaves in exactly the same way as for the whole leaves. The lowest EO content was observed in the lyophilized leaves in both cases. Here the volatile content practically disappeared (Figure 31).

Conclusions

Based on our results, it can be established that among the applied preservation methods again *freezing* and the gentle, low-temperature drying techniques (*drying in shade and at 40°C*) proved to be the most effective ones in relation to species accumulating the volatiles in

intercellular secretory ducts. These methods preserved the EO content of samples very well. However, *drying at 60°C* did not spare the volatiles of these species either, causing a loss of 30-70%, depending on species.

In case of internal duct containing plants only a few scientific data are available, but in the research finding of Arabhosseini et al., (2006) the lower drying temperature also resulted in higher EO content for *Artemisia dracunculus* leaves. Rudy et al., (2011) also confirmed that a rise in the drying air temperature from 30 to 70°C caused a decrease in the EO yield from dried *Petroselinum crispum* leaves. Böhm et al., (2002) revealed that at a higher drying temperature of 75°C, 70% of the volatiles of *Petroselinum crispum* were lost, which aligns with our findings.

Microwave drying proved to be moderately good in preserving volatiles in case of species, which accumulate the EO primarily or exclusively in secretory ducts. In our research, it could preserve the EO content as good as convective drying at 40°C. Rezvani Moghaddam et al., (2013) also reported that in addition to shade-drying, microwave drying at 900 W could retain the volatiles the best in relation to *Artemisia dracunculus* leaves.

Nevertheless, summarising our results *lyophilization* was found to be the worst preservation method among the examined treatments, because due to it practically all the volatiles were lost. In the research of Díaz-Maroto et al., (2002) lyophilization also significantly reduced the EO content of *Petroselinum crispum* leaves. Xing et al., (2017) reported that the expansion of porous structures in freeze-dried leaves resulted in loss of volatiles. Another finding by Yousif et al., (2000) confirmed that lyophilization developed a larger porosity in leaves of *Lycium berlandieri*. These findings support our SEM observations of *Levisticum officinale* leaves.

Overall, the preservation methods studied had very similar effects on the EO content of species that synthesised and accumulated the volatiles in the same way. Microwave drying, for example, was an effective preservation method in relation to species with internal secretory ducts, but in case of species with external glandular hairs, it almost completely eliminated the samples' volatiles. In contrast, lyophilisation could retain the EO of plants with external glandular trichomes rather well but proved to be the worst method in the preservation of species with intercellular secretory cavities.

Freezing and low-temperature drying (in shade or at 40°C) proved to be very effective for both groups of plants, but high-temperature drying (in hot sun or at 60°C) had a very negative effect on the EO content of species. For these preservation methods, the form and way of the EO accumulation (externally or internally) were not relevant.

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

In the EO composition analysis, only those main components were presented, the proportion of which in the EO reached the minimum 1-2% in at least one sample of the examined plant species.

5.2.1. Species accumulating the EO primarily or exclusively in glandular hairs or trichomes

***Mentha x piperita* L. 'Mexián'**

The major compounds identified in fresh peppermint leaves were menthone (43.52%), menthol (30.52%), a combination of menthofuran and isomenthone (6.11%) (these two compounds cannot be separated with the applied GC-MS method), 1,8-cineol (5.82%) and limonene (5.74%) (Table 6). Additionally, menthyl acetate was also found in smaller proportion (2.63%).

Table 6. Essential oil composition of fresh and preserved leaves of *Mentha* x *piperita* L. 'Mexián'

Compounds	LRI[a]	Total GC area percentages (%)									
		Fresh	Sun	Shade	Oven-40°C	Oven-60°C	Lyo	MW-250W	MW-700W	SFreez	FFreez
Limonene	**1029**	**5.74**	**4.71**	**4.23**	**4.91**	**2.74**	**4.51**	**0.27**	**0.12**	**7.59**	**5.99**
1,8-Cineole	**1034**	**5.82**	**5.29**	**4.46**	**5.29**	**4.68**	**5.04**	**0.26**	**0.22**	**6.72**	**5.36**
Menthone	**1158**	**43.52**	**38.81**	**39.53**	**41.31**	**38.39**	**36.46**	**14.93**	**9.74**	**35.52**	**38.79**
Menthofuran + Isomenthone	**1168**	**6.11**	**6.42**	**6.43**	**6.52**	**5.92**	**6.56**	**3.91**	**4.08**	**6.49**	**6.68**
Menthol	**1171**	**30.52**	**32.30**	**33.46**	**30.07**	**35.08**	**34.94**	**49.19**	**40.15**	**30.20**	**30.65**
Piperitone	1249	1.41	1.65	1.68	1.57	1.69	1.56	1.94	2.83	1.50	1.51
Menthyl acetate	**1291**	**2.63**	**3.66**	**3.63**	**3.21**	**3.54**	**3.65**	**9.89**	**8.24**	**3.51**	**3.28**
Germacrene D	1482	0.51	1.25	1.38	1.29	1.67	1.20	3.80	6.57	1.06	1.25
Bicyclogermacrene	1497	0.15	0.40	0.45	0.43	0.61	0.43	1.56	2.62	0.41	0.48
Viridiflorol	1598	0.02	0.27	0.24	0.23	0.52	0.29	4.20	5.74	0.27	0.27
unidentified	1702	nd	0.06	0.05	0.05	0.17	0.08	1.92	3.22	0.07	0.07

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. / nd = not detected

The EO composition of peppermint 'Mexián' leaves was largely consistent across the various preservation methods, with the exception of leaves dried using microwaves at 250 and 700 W (Table 6). In the microwave-dried samples, significant changes were observed. The proportions of limonene, 1,8-Cineole, menthone and menthofuran+isomenthone significantly decreased, while there was a marked increase in the ratios of menthol (by 10-19%), menthyl acetate (by 6-7%) and certain sesquiterpenes (germacrene D, bicyclogermacrene, and

viridiflorol) within the EO. It appears that compounds with lower molecular weight evaporated during drying leading to an increase in the ratio of higher molecular weight compounds.

The quality of peppermint EO is characterized by high concentrations of menthol and low levels of menthone (Rohloff et al., 2005). Beigi et al., (2018) also found, that microwave drying (at power levels of 200, 400 and 800 W) reduced the concentrations of menthone (by 6-11%), menthofuran (by 9%), and 1,8-cineole (by 4-6%) compared to fresh peppermint leaves and increased the compositions of menthol (by 2-3%), menthyl acetate (by 5-11%) and several other sesquiterpenes. These findings support our research results, although there are differences in the range of concentrations observed.

Melissa officinalis L. 'Lemona'

In the fresh leaves of lemonbalm variety 'Lemona' the major compounds identified were geranial (40.66%), neral (29.60%), citronellal (6.31%), geraniol (5.59%) and citronellol (4.60%) (Table 7).

Examining the effect of preservation methods, we found that every applied treatments significantly reduced the ratio of citronellol and geraniol (in the preserved samples we found only 0.24-1.05% and 0.05-0.66% proportions, respectively), and increased the ratio of citronellal by 1-5%, except microwave and oven drying at 60°C. In the study of Ghasemi et al., (2013) oven drying at 40°C preserved the original EO composition of lemonbalm leaves very well, but in our research every drying treatment slightly modified the composition.

Table 7. Essential oil composition of fresh and preserved leaves of *Melissa officinalis* L. 'Lemona'

Compounds	LRI[a]	Total GC area percentages (%)									
		Fresh	Sun	Shade	Oven-40°C	Oven-60°C	Lyo	MW-250W	MW-700W	SFreez	FFreez
Citronellal	1155	**6.31**	**9.74**	**10.12**	**10.91**	**5.53**	**8.38**	**3.18**	**2.07**	**10.21**	**8.58**
E-Isocitral	1178	1.21	1.31	1.10	1.56	1.02	1.50	1.05	0.68	1.26	1.16
Citronellol	1223	**4.60**	**0.39**	**0.24**	**0.80**	**1.05**	**0.49**	**0.39**	**0.35**	**0.44**	**0.36**
Neral	1238	**29.60**	**33.43**	**33.26**	**33.11**	**32.37**	**34.41**	**28.18**	**26.60**	**32.07**	**33.16**
Geraniol	1252	**5.59**	**0.24**	**0.05**	**0.60**	**0.66**	**0.38**	**0.40**	**0.32**	**0.19**	**0.22**
Geranial	1268	**40.66**	**45.55**	**46.11**	**43.33**	**46.73**	**43.75**	**40.20**	**40.44**	**45.24**	**47.21**
8-Hydroxyneomenthol	1324	2.08	1.43	1.25	1.51	1.19	1.13	1.34	0.74	1.66	1.30
Citronellyl acetate	1354	1.29	0.82	0.77	0.80	0.59	0.61	0.89	0.11	0.94	0.82
Geranyl acetate	1388	1.01	0.65	0.65	0.79	1.43	0.79	1.48	1.33	0.88	0.91
e-Caryophyllene	1420	1.64	1.29	1.30	1.02	1.32	1.40	**12.98**	**15.72**	1.20	1.27
Caryophyllene oxide	1590	1.09	0.79	0.81	0.57	2.28	0.44	**3.82**	**5.42**	0.76	0.86

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.

According to the data, microwave drying methods caused the most spectacular changes in the volatile composition of lemonbalm leaves. Both the applied wattages (250 and 700 W) significantly reduced the concentration of citronellol (by 4%), citronellal (by 3-4%) and geraniol (by 5%), but the ratio of geranial and neral was found to be similar to the fresh sample, no significant differences were found between them. Furthermore, microwave drying led to a significant increase in the ratio of sesquiterpenes (e-caryophyllene, caryophyllene oxide) too.

***Ocimum basilicum* L. 'Genovese' and 'Ohře'**

The major compounds identified in the extracted EO from the fresh leaves of sweet basil variety 'Genovese' were linalool (52.60%), tau-cadinol (10.24%), eugenol (9.23%), α-trans-bergamotene (5.40%) and germacrene D (4.47%). Most of the applied preservation methods did not cause spectacular changes in the composition (Table 8), only minor variations could be observed. Freezing preserved the original composition the best. The higher drying temperatures increased the ratio of estragole in the EO (e.g. oven drying at 60°C by 7%) and reduced the eugenol's proportion (oven drying at 60°C by 7%). In case of lyophilization, the ratio of linalool decreased a little (by 7%), and the ratio of bornyl acetate increased from 0.53 to 4.40%. But microwave drying treatments completely altered the composition. During this process, the ratio of compounds with lower molecular weight decreased (e.g. the linalool by 46-51%), these likely evaporated. In parallel with this, the proportion of higher molecular weight sesquiterpenes increased (e.g. the tau-cadinol by 12-17%).

Linalool (46.35%), geraniol (9.53%), eugenol (3.10%), germacrene D (3.91%) and tau-cadinol (12.41%) were the predominant compounds in the EO extracted from the fresh leaves of variety 'Ohře'. Similarly to 'Genovese', most of the applied treatments did not affect the composition significantly, except oven drying at 60°C and microwave drying methods. Oven drying at 60°C decreased the ratio of linalool by 16% and increased the ratio of tau-cadinol by 10%. But the biggest difference was observed for microwave dryings, where – similarly to 'Genovese' - the ratio of linalool (by 40-43%), geraniol and eugenol decreased, and the ratio of tau-cadinol (by 16%) and germacrene D increased. For lyophilization, although the EO content significantly changed, the composition was found to be similar to the fresh leaves.

A study conducted by Pirbalouti et al., (2013a) observed a notable reduction in the linalool content for basil landraces oven-dried at 60°C. This finding supports our research results. Bowes and Zheljazkov (2004) observed in their studies, that lyophilization notably decreased the linalool content in flowering shoots of *Ocimum basilicum* 'Mesten', from 51% to 36%. However, this finding contrasts with ours.

Table 8. Essential oil composition of fresh and preserved leaves of *Ocimum basilicum* L. 'Genovese' and 'Ohře'

Compounds	LRI[a]	Total GC area percentages (%)																			
		Fresh		Sun		Shade		Oven-40°C		Oven-60°C		Lyo		MW-250W		MW-700W		SFreez.		FFreez.	
		BaG	BaO	BaG	BaO	BaG	BaO	BaG	BaO	BaG	BaO	BaG	BaO	BaG	BaO	BaG	BaO	BaG	BaO	BaG	BaO
1,8-Cineol	1034	2.55	1.86	6.58	1.83	6.28	1.16	6.34	2.06	5.50	0.50	3.03	1.10	0.33	0.07	0.06	0.01	3.28	1.46	2.83	1.26
Linalool	1097	**52.60**	**46.35**	**42.05**	**47.09**	**50.24**	**47.72**	**44.99**	**44.61**	**49.21**	**30.33**	**46.11**	**40.48**	**6.58**	**6.51**	**1.80**	**3.73**	**48.69**	**44.53**	**46.36**	**44.01**
Estragole	1196	nd	nd	2.17	nd	0.14	nd	1.69	nd	6.96	nd	1.78	nd	6.81	nd	5.76	nd	0.19	nd	0.26	nd
Geraniol	1252	nd	**9.53**	nd	**8.06**	nd	**10.47**	nd	**9.18**	nd	**8.79**	nd	**11.05**	nd	**5.34**	nd	**4.34**	nd	**9.23**	nd	**9.02**
Bornyl acetate	1284	0.53	nd	1.46	nd	1.52	nd	1.23	nd	1.66	nd	4.40	nd	0.79	nd	0.51	nd	0.71	nd	0.59	nd
Eugenol	1361	**9.23**	**3.10**	**5.62**	**1.56**	**2.75**	**1.96**	**7.53**	**2.21**	**2.38**	**0.83**	**10.09**	**6.08**	**5.54**	**1.78**	**7.29**	**1.39**	**7.78**	**2.75**	**10.12**	**3.44**
β-Elemene	1391	0.65	3.23	0.87	2.73	0.85	2.77	0.82	3.20	0.74	3.88	0.48	2.41	1.73	6.52	1.42	6.41	0.83	3.43	0.89	3.33
E-Caryophyllene	1420	nd	1.40	nd	1.38	nd	1.28	nd	1.48	nd	1.90	nd	1.12	nd	3.08	nd	2.96	nd	1.43	nd	1.39
α-trans-Bergamotene	1437	**5.40**	nd	**4.78**	nd	**4.51**	nd	**5.61**	nd	**4.17**	nd	**3.23**	nd	**6.00**	nd	**6.52**	nd	**4.85**	nd	**4.92**	nd
α-Guaiene	1439	0.71	1.01	0.83	0.96	0.84	0.90	0.68	1.06	0.65	1.42	0.35	0.69	1.48	2.47	1.18	2.57	0.59	0.93	0.63	0.93
Germacrene D	1482	**4.47**	**3.91**	**3.06**	**3.12**	**3.34**	**3.26**	**3.09**	**3.83**	**2.57**	**4.76**	**2.19**	**2.65**	**6.57**	**6.85**	**4.89**	**6.92**	**3.54**	**3.67**	**3.53**	**3.74**
β-Selinene	1486	0.51	nd	0.53	nd	0.54	nd	0.59	nd	0.21	nd	0.31	nd	1.66	nd	2.07	nd	0.57	nd	0.71	nd
Bicyclogermacrene	1497	0.98	0.36	0.93	0.28	0.85	0.31	0.86	0.39	0.69	0.60	0.62	0.41	2.44	1.03	1.87	1.07	0.87	0.42	0.89	0.42
α-Bulnesene	1506	3.78	3.32	2.83	2.87	2.93	2.89	2.49	3.35	2.43	4.32	1.77	2.23	5.62	6.11	4.03	6.70	2.86	2.96	2.83	3.17
γ-cadinene	1524	2.78	3.21	2.98	3.47	2.79	3.67	2.84	3.88	2.84	4.96	1.50	2.92	4.83	6.86	4.77	6.88	2.44	3.63	2.47	3.49
Maaliol	1570	nd	0.81	nd	0.97	nd	0.68	nd	0.76	nd	1.49	nd	1.01	nd	2.34	nd	2.19	nd	0.96	nd	1.02
1,10-di-epi-Cubenol	1621	0.95	1.29	1.25	1.62	1.20	1.45	1.10	1.48	1.14	2.44	1.16	1.74	2.77	3.80	3.13	4.15	1.04	1.61	1.13	1.71
tau-Cadinol	1644	**10.24**	**12.41**	**8.94**	**14.36**	**9.06**	**13.74**	**8.71**	**13.27**	**13.68**	**22.92**	**10.89**	**14.37**	**22.47**	**28.41**	**27.32**	**28.72**	**10.80**	**13.45**	**12.33**	**13.62**
α-Cadinol	1658	0.25	0.39	0.52	0.55	0.56	0.48	0.44	0.51	0.31	1.03	0.44	0.75	1.39	2.02	1.76	2.22	0.30	0.52	0.05	0.55
unknown		nd	nd	0.03	nd	0.03	nd	0.04	nd	nd	nd	0.01	nd	2.49	nd	1.39	nd	nd	nd	0.03	nd

Note: Major compounds are shown in bold. / nd = not detected

[a]Linear retention indices (LRI) calculated relative to the elution ranking of n-alkanes on HP-5 column.

BaG = Basil 'Genovese'; BaO = Basil 'Ohře'

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.

***Origanum majorana* L. 'Egyptian' and 'Magyar'**

The fresh sample of marjoram variety 'Egyptian' contained major compounds like cis-sabinene hydrate + linalool (39.48%) (these two compounds cannot be separated with the applied GC-MS method), terpinen-4-ol (18.60%), trans-sabinene hydrate (7.32%), γ-terpinene (6.78%) and α-terpineol (4.02%). The applied preservation techniques did not modify the volatile composition significantly (only a 1-6% fluctuations could be observed in relation to cis-sabinene hydrate + linalool and terpinen-4-ol), but oven drying at 60°C and microwave drying methods resulted in more spectacular changes. Both treatments reduced the ratio of cis-sabinene hydrate + linalool by 7-17% and increased the ratio of terpinen-4-ol by 7-11%. For microwave drying, further changes were found: the ratio of smaller molecules (e.g. sabinene, β-phellandrene) decreased, while the ratio of larger molecules (e.g. β-caryophyllene, carvacrol, bicyclogermacrene) increased (Table 9).

The predominant compounds in fresh leafy shoots of variety 'Magyar' were found to be cis-sabinene hydrate + linalool (32.10%), terpinen-4-ol (22.59%), trans-sabinene hydrate (7.00%), γ-terpinene (7.79%) and α-terpineol (4.74%). The effect of preservation methods showed similar trends with the variety 'Egyptian', but with slight variations in the rate of change. Oven drying at 60°C and microwave drying resulted in a 9-16% decrease in the cis-sabinene hydrate + linalool ratio and 1-3% increase in the terpinen-4-ol proportion. Furthermore, microwave drying methods here also reduced the proportion of compounds with smaller molecular weight and increased the ratio of sesquiterpenes (Table 9).

Our data are in agreement with the findings of Raghavan et al., (1997). They found that microwave drying resulted in a more significant reduction of aroma compounds in marjoram compared to several other drying methods, such as convective drying, shade drying and sun drying. The proportions of the components have also been modified in a similar way to ours.

***Thymus vulgaris* L. 'Deutscher Winter' and 'French Summer'**

The major compounds found in both the thyme varieties were thymol (51.69-55.10%), γ-terpinene (15.55-18.01%), p-cymene (11.46-12.14%) and carvacrol (3.07-3.27%) (Table 10).

Drying in the sun, shade, in oven at 40°C and freezing methods did not influence the characteristic EO composition found in fresh leaves. However, oven drying at 60°C and especially microwave drying altered the EO composition significantly. Oven drying at 60°C considerably reduced the proportion of γ-terpinene by 6-11% and increased the ratio of p-cymene by 3-6%.

Table 9. Essential oil composition of fresh and preserved leafy shoots of *Origanum majorana* L. 'Egyptian' and 'Magyar'

Compounds	LRI[a]	Total GC area percentages (%)																			
		Fresh		Sun		Shade		Oven-40°C		Oven-60°C		Lyo		MW-250W		MW-700W		SFreez.		FFreez.	
		MaE	MaM	MaE	MaM	MaE	MaM	MaE	MaM	MaE	MaM	MaE	MaM	MaE	MaM	MaE	MaM	MaE	MaM	MaE	MaM
Sabinene	976	5.07	4.62	4.51	4.84	2.78	6.19	4.81	5.97	1.46	3.91	1.29	5.13	0.43	0.55	0.03	0.35	5.59	5.66	3.04	5.70
α-Terpinene	1018	3.24	3.76	3.19	4.67	2.55	5.14	3.87	5.11	2.69	4.80	2.07	4.79	2.45	3.40	0.44	2.27	2.83	4.10	2.15	4.20
ß-Phellandrene	1029	2.27	2.20	2.04	2.36	1.60	2.69	2.16	2.68	0.95	1.90	0.97	2.34	0.59	0.56	0.03	0.46	2.35	2.50	1.75	2.52
γ-Terpinene	1056	**6.78**	**7.79**	**6.79**	**8.85**	**6.08**	**9.04**	**7.99**	**9.15**	**6.90**	**9.34**	**5.30**	**8.81**	**7.49**	**8.84**	**1.34**	**7.28**	**5.97**	**7.65**	**5.50**	**7.69**
trans-Sabinene hydrate	1070	**7.32**	**7.00**	**9.19**	**6.87**	**9.56**	**6.96**	**8.78**	**6.75**	**8.89**	**6.26**	**8.43**	**6.72**	**8.51**	**5.30**	**6.78**	**7.62**	**7.63**	**7.43**	**8.71**	**7.57**
α-Terpinolene	1085	1.38	1.49	1.39	1.71	1.28	1.72	1.65	1.76	1.39	1.87	1.13	1.75	1.90	2.11	0.42	1.96	1.19	1.53	1.16	1.56
cis-Sabinene hydrate + linalool	1096	**39.48**	**32.10**	**37.12**	**27.17**	**39.17**	**27.74**	**33.18**	**27.88**	**32.21**	**23.46**	**37.22**	**28.20**	**25.22**	**16.26**	**22.89**	**22.31**	**43.23**	**34.18**	**41.74**	**33.20**
Terpinen-4-ol	1175	**18.60**	**22.59**	**19.00**	**20.04**	**20.27**	**18.45**	**20.22**	**19.09**	**26.74**	**22.50**	**23.85**	**20.65**	**25.89**	**25.31**	**29.14**	**23.95**	**17.25**	**19.13**	**19.49**	**19.26**
α-Terpineol	1189	**4.02**	**4.74**	**4.29**	**4.25**	**4.61**	**4.13**	**3.83**	**4.13**	**4.61**	**4.32**	**5.32**	**4.67**	**4.12**	**4.99**	**4.38**	**3.76**	**3.92**	**4.74**	**4.48**	**4.78**
trans-Sabinene hydrate acetate	1247	0.06	0.29	0.55	4.21	0.34	3.59	0.63	3.17	0.61	5.08	0.34	1.67	0.04	0.76	nd	2.91	0.05	0.41	0.05	0.63
Linalyl acetate	1250	1.29	1.84	1.42	2.45	1.58	2.16	1.41	2.10	1.91	2.75	1.43	1.80	2.12	2.38	2.00	2.43	1.13	1.75	1.43	1.85
Carvacrol	1300	0.44	0.21	0.34	0.42	0.32	0.37	0.35	0.37	0.64	0.58	2.90	0.40	2.25	0.99	3.79	2.42	0.17	0.22	0.28	0.23
β-Caryophyllene	1420	2.23	2.72	2.51	2.92	2.65	2.65	2.66	2.69	3.04	3.42	2.60	2.69	**6.05**	**8.98**	**8.65**	**6.09**	1.77	2.20	2.95	2.21
Bicyclogermacrene	1497	1.87	2.36	1.83	2.52	2.15	2.18	1.94	2.37	2.55	2.66	2.15	2.32	**5.00**	**6.05**	**8.46**	**5.30**	1.24	1.96	2.02	1.96
Spathulenol	1584	0.06	0.13	0.08	0.16	0.08	0.13	0.10	0.13	0.12	0.21	0.07	0.13	0.65	1.65	1.76	1.22	0.04	0.10	0.07	0.09
Caryophyllene oxide	1590	0.08	0.13	0.09	0.12	0.10	0.11	0.10	0.09	0.13	0.13	0.09	0.12	0.59	1.18	1.42	0.87	0.07	0.15	0.12	0.12

Note: Major compounds are shown in bold. / nd = not detected

[a]Linear retention indices (LRI) calculated relative to the elution ranking of n-alkanes on HP-5 column.

MaE = Marjoram 'Egyptian'; MaM = Marjoram 'Magyar'.

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 drying methods increased the ratio of thymol (by 1.4-17.6%) and carvacrol but reduced the proportion of γ-terpinene (by 7.7-13.2%) and p-cymene in general. 700W had stronger effects, and changes were more spectacular for variety 'French Summer' (Table 10). In summary, it can be concluded that microwave and convective drying at higher temperature led to the increase of thymol and carvacrol content, and this correlation has also been described in the research of Rahimmalek and Goli (2013) in connection with *T. daenensis* subsp. *daenensis,* where also thymol and carvacrol were the main EO constituents.

Lyophilization did not influence the characteristic EO composition. In line with our findings, Venskutonis et al., (1996) and Sárosi et al., (2013) also observed that freeze-drying did not significantly alter the EO composition of *Thymus vulgaris*. This observation was mirrored in the study by Rahimmalek and Goli (2013) concerning *Thymus daenensis* subsp. *daenensis* too, where lyophilization had a similar non-significant impact on the EO composition.

***Satureja hortensis* L. LAMISATU22 and 'Saturn'**

The predominant compounds identified in the EO extracted from fresh leafy shoots of summer savory gene bank accession Nr. LAMISATU22 and 'Saturn' were carvacrol (70.39-73.12%) and γ-terpinene (20.61-21.39%). The EO composition of these 2 taxa were very similar (Table 11), only two minor compounds showed differences: carvacryl acetate and β-bisabolene could be detected exclusively in the EO of the variety 'Saturn'.

The EO composition of samples dried in the sun, in shade, in oven at 40 and 60ºC, furthermore by freezing (slow and fast) was found to be very similar to that of fresh flowering leafy shoots. In contrast, samples dried using microwaves exhibited significant changes: the levels of γ-terpinene drastically decreased (at 250 W: by 17-20%, at 700 W: by 19%), likely due to evaporation during drying; while the proportion of carvacrol markedly increased (at 250 W: by 15-23%, at 700 W: by 16-18%) in the EO of both taxa.

Freeze-drying preserved the original EO composition quite well, but it resulted in the appearance of a new compound, trans-anethole (0.81-1.00%), which was not detectable in the fresh plant material, or samples preserved by other techniques (or only in trace) (Table 11). In the research conducted by Pirbalouti et al. (2013b) it was found that freeze-drying (lyophilization) did not significantly alter the EO composition of aerial parts of *Satureja bachtiarica*. Here the primary compounds identified were carvacrol, γ-terpinene, p-cymene and thymol. Similarly, in a study by Yousif et al. (2000) on *Lippia berlandieri*, lyophilization also resulted in a very minor change in the ratio of γ-terpinene in the volatiles of plant shoots.

Table 10. Essential oil composition of fresh and preserved leafy shoots of *Thymus vulgaris* L. 'French Summer' and 'Deutscher Winter'

Compounds	LRI[a]	Total GC area percentages (%)																			
		Fresh		Sun		Shade		Oven-40°C		Oven-60°C		Lyo		MW-250W		MW-700W		SFreez.		FFreez.	
		GtFS	GtDW	GtFS	GtDW	GtFS	GtDW	GtFS	GtDW	GtFS	GtDW	GtFS	GtDW	GtFS	GtDW	GtFS	GtDW	GtFS	GtDW	GtFS	GtDW
α-Thujene	928	1.29	0.67	0.86	1.17	0.75	0.91	0.96	0.84	0.30	0.73	0.82	1.02	0.13	1.10	0.03	0.27	1.22	1.13	0.60	0.93
β-Myrcene	995	1.30	0.98	1.24	1.17	1.22	1.10	1.26	1.12	0.59	0.92	1.09	1.16	0.42	1.01	0.23	0.48	1.36	1.19	1.05	1.09
α-Terpinene	1018	1.92	1.30	1.86	2.37	1.76	1.82	1.86	1.62	0.70	0.94	1.57	1.55	0.55	1.07	0.32	0.50	1.91	1.67	1.57	1.51
p-Cymene	1026	**11.46**	**12.14**	**13.88**	**16.44**	**14.29**	**14.36**	**14.27**	**15.28**	**14.77**	**18.15**	**12.70**	**14.61**	**9.82**	**16.65**	**6.74**	**11.22**	**13.53**	**14.13**	**11.89**	**14.12**
1,8-Cineol	1034	0.41	0.53	0.57	0.57	0.74	0.79	0.51	0.82	1.33	0.99	0.55	0.67	0.89	0.49	0.62	0.53	0.57	0.64	0.57	0.69
γ-Terpinene	1056	**18.01**	**15.55**	**16.16**	**15.86**	**15.29**	**15.24**	**16.57**	**15.07**	**7.04**	**9.25**	**15.35**	**15.26**	**7.16**	**10.28**	**4.81**	**5.69**	**17.48**	**18.37**	**16.26**	**17.46**
Linalool	1097	1.83	1.89	1.93	1.72	2.01	1.87	2.02	1.84	2.85	1.91	1.95	2.10	2.14	1.95	1.81	1.82	1.98	1.44	2.03	1.87
Borneol	1165	0.77	1.29	0.82	0.74	1.02	0.86	0.79	0.95	1.35	1.07	0.87	1.32	1.10	0.81	0.94	0.78	0.96	0.83	0.97	0.85
Thymol	1290	**51.69**	**55.10**	**51.59**	**48.43**	**51.39**	**51.59**	**50.16**	**51.16**	**54.97**	**53.19**	**53.46**	**49.16**	**63.49**	**53.07**	**69.32**	**62.74**	**48.75**	**50.73**	**53.04**	**50.49**
Carvacrol	1300	**3.07**	**3.27**	**3.22**	**3.17**	**3.44**	**3.03**	**3.10**	**2.88**	**3.76**	**3.59**	**3.25**	**3.19**	**4.05**	**3.63**	**4.64**	**4.22**	**2.89**	**2.41**	**3.21**	**2.56**
β-Caryophyllene	1420	1.36	1.13	1.30	1.22	1.22	1.26	1.30	1.20	1.93	1.50	1.41	1.11	2.15	1.41	2.60	2.31	1.46	1.27	1.58	1.29

Note: **GtFS = Garden thyme 'French Summer'; GtDW = Garden thyme 'Deutscher Winter'**

Table 11. Essential oil composition of fresh and preserved leafy shoots of *Satureja hortensis* L. gene bank accession Nr. LAMISATU22 and 'Saturn'

Compounds	LRI[a]	Total GC area percentages (%)																			
		Fresh		Sun		Shade		Oven-40°C		Oven-60°C		Lyo		MW-250W		MW-700W		SFreez.		FFreez.	
		SsL	SsS	SsL	SsS	SsL	SsS	SsL	SsS	SsL	SsS	SsL	SsS	SsL	SsS	SsL	SsS	SsL	SsS	SsL	SsS
β-Myrcene	995	0.62	0.72	0.85	0.69	0.64	1.04	0.92	1.08	0.59	0.69	0.55	0.78	nd	0.07	0.02	0.04	0.53	1.00	1.02	1.00
α-Terpinene	1018	1.66	1.65	2.19	1.71	1.75	2.41	2.26	2.49	1.62	1.74	1.42	1.75	0.03	0.21	0.07	0.12	1.51	2.12	2.37	2.12
p-Cymene	1026	2.01	3.50	2.80	4.78	2.80	4.10	3.03	4.71	3.03	5.11	2.29	4.21	0.63	1.13	0.43	0.73	2.68	4.89	3.09	4.71
γ-Terpinene	1056	**20.61**	**21.39**	**22.35**	**18.75**	**18.96**	**22.34**	**20.99**	**22.01**	**16.76**	**17.09**	**15.57**	**17.59**	**0.60**	**4.06**	**1.29**	**2.28**	**19.42**	**20.65**	**21.85**	**19.63**
trans-Anethole	1283	nd	nd	nd	nd	0.03	nd	nd	nd	nd	nd	**1.00**	**0.81**	nd	nd	0.01	nd	nd	0.01	nd	nd
Carvacrol	1300	**73.12**	**70.39**	**69.38**	**67.77**	**72.75**	**63.15**	**69.61**	**64.49**	**74.08**	**69.06**	**75.56**	**67.78**	**96.48**	**85.31**	**91.26**	**86.41**	**73.56**	**66.09**	**67.23**	**64.11**
Carvacryl acetate	1377	nd	0.49	nd	0.69	nd	0.18	nd	0.57	nd	0.95	nd	0.63	nd	1.01	nd	1.03	nd	0.49	nd	0.56
β-Caryophyllene	1420	0.51	0.49	0.53	1.24	0.89	0.88	0.69	0.95	1.00	1.15	0.60	0.90	0.80	2.61	1.13	2.79	0.58	0.96	0.77	0.91
β-Bisabolene	1508	nd	0.35	nd	0.75	nd	0.67	nd	0.69	nd	0.76	nd	0.69	nd	1.63	nd	1.78	nd	0.78	nd	0.76

Note: Major compounds are shown in bold. / nd = not detected

[a]Linear retention indices (LRI) calculated relative to the elution ranking of n-alkanes on HP-5 column.

SsL = Summer savory gene bank accession Nr. LAMISATU22, SsS = Summer savory 'Saturn'

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.

***Lavandula angustifolia* 'Budakalászi 80' and *Lavandula* x *intermedia* 'Judit'**

In the EO extracted from the fresh flowers of true lavender variety 'Budakalászi 80', the major compounds identified were linalyl acetate (26.02%), linalool (22.88%), lavandulyl acetate (11.13%) and terpinen-4-ol (6.02%). The effect of preservation methods did not cause significant differences in this composition, except microwave drying treatments (Table 12). Microwave drying at 250 W and 700 W significantly decreased the ratio of linalool (by 11-12%, from 22.9% to 11.0-12.3%) and terpinene-4-ol (by 3%, from 6.0% to 2.8-3.2%) while the ratio of linalyl acetate (by 7-8%) and sesquiterpene molecules (e.g. E-caryophyllene, (Z)-beta-farnesene, tau-cadinol) increased. Higher drying temperature (60°C) caused a little change, similar to microwave drying, but the change was not remarkable.

The major EO compounds found in hybrid lavender 'Judit' were linalyl acetate (17.37%), linalool (25.74%), 1,8-cineol (11.58%) and camphor (10.61%). Here also, the applied preservation methods did not affect the original EO composition, except microwave drying treatments. Microwave drying led to a decrease in the ratio of linalool (by 9-15%), 1,8-cineol (by 7-10%) and camphor (by 3-5%), while in parallel with this the ratio of linalyl acetate (by 3-4%) and sesquiterpene compounds increased (Table 12).

Similar to our findings, Mirjalili et al., (2019) found that different preservation processes (shade drying, sun drying, oven drying at 40°C) did not notably affect the ratios of the primary components (linalool, linalyl acetate, lavandulyl acetate, terpinen-4-ol, and α-terpineol) in true lavender. However, in their results microwave drying at 900 W also did not cause any significant changes in the EO composition, which is contrary to our findings.

***Origanum vulgare* subsp. *hirtum* commercial plant material**

The predominant compound in *Origanum vulgare* subsp. *hirtum* fresh leafy shoots was found to be carvacrol (79.27%). Other important compounds present were cis-sabinene hydrate (4.59%), p-cymene (4.07%) and γ-terpinene (3.59%). The applied preservation methods did not change the volatile profile, except 60°C and microwave drying methods (Table 13). Oven drying at 60°C led to a minimal increase in the ratio of cis-sabinene hydrate and carvacrol (by 3%), while decreased the ratio of γ-terpinene (by 3%). The two microwave drying methods significantly increased the ratio of carvacrol by 12-13%, however the cis-sabinene hydrate, p-cymene and γ-terpinene ratio decreased. These compounds presumably evaporated during the drying process. Nhu-Trang et al., (2006) stated that the biosynthesis of carvacrol is strongly associated with its precursors (p-cymene and γ-terpinene), the level of these compounds changes parallel with each other.

Table 12. Essential oil composition of fresh and preserved flowers of *Lavandula angustifolia* 'Budakalászi 80' and *Lavandula* x *intermedia* 'Judit'

Compounds	LRI[a]	Total GC area percentages (%)																			
		Fresh		Sun		Shade		Oven-40°C		Oven-60°C		Lyo		MW-250W		MW-700W		SFreez.		FFreez.	
		LaB	LaJ	LaB	LaJ	LaB	LaJ	LaB	LaJ	LaB	LaJ	LaB	LaJ	LaB	LaJ	LaB	LaJ	LaB	LaJ	LaB	LaJ
3-Octanone	987	1.85	nd	1.14	nd	1.15	nd	1.20	nd	0.74	nd	1.32	nd	0.24	nd	0.14	nd	1.52	nd	1.47	nd
Limonene	1029	0.44	1.48	0.25	1.07	0.27	1.16	0.29	1.18	0.19	1.01	0.32	1.32	0.11	0.37	0.10	0.19	0.38	1.48	0.40	1.47
1,8-Cineol	1034	nd	**11.58**	nd	**11.18**	nd	**11.60**	nd	**12.29**	nd	**11.70**	nd	**11.51**	nd	**4.17**	nd	**1.83**	nd	**11.48**	nd	**11.60**
(Z)-beta-Ocimene	1037	3.36	4.62	3.14	3.02	3.12	3.67	3.21	3.70	2.40	2.77	3.03	4.22	1.12	0.83	0.86	0.26	3.65	4.38	3.49	4.48
(E)-beta-Ocimene	1046	3.71	nd	3.45	nd	3.44	nd	3.12	nd	2.67	nd	3.36	nd	1.21	nd	0.90	nd	3.98	nd	3.95	nd
Linalool	1097	**22.88**	**25.74**	**20.79**	**25.93**	**21.47**	**26.05**	**20.40**	**25.88**	**19.17**	**25.67**	**22.92**	**24.64**	**12.26**	**16.43**	**11.00**	**10.51**	**22.36**	**24.48**	**23.57**	**24.80**
Camphor	1144	0.31	**10.61**	0.36	**11.35**	0.34	**11.12**	0.33	**11.23**	0.32	**11.47**	0.32	**10.65**	0.10	**7.41**	0.09	**5.38**	0.30	**10.43**	0.31	**10.60**
Borneol	1162	1.21	3.42	1.35	3.74	1.37	3.53	1.44	3.69	1.22	3.82	1.40	3.76	0.90	4.00	1.04	3.96	1.34	3.67	1.42	3.65
Lavandulol	1166	1.47	0.92	1.37	0.81	1.36	0.81	1.24	0.80	1.42	0.81	1.51	0.86	0.88	0.79	0.80	0.76	1.40	0.89	1.50	0.87
Terpinen-4-ol	1175	**6.02**	0.53	**5.50**	0.52	**5.66**	0.51	**5.45**	0.48	**5.20**	0.56	**6.27**	0.54	**3.22**	0.36	**2.79**	0.26	**5.83**	0.48	**6.07**	0.47
α-Terpineol	1189	4.64	3.97	2.78	3.33	3.01	3.43	3.08	3.55	2.45	3.19	3.43	3.10	1.46	2.26	1.45	1.91	3.59	3.38	4.07	3.55
Linalyl acetate	1250	**26.02**	**17.37**	**28.85**	**19.94**	**27.92**	**18.90**	**29.15**	**18.48**	**31.72**	**20.12**	**27.85**	**19.82**	**34.16**	**20.49**	**33.02**	**20.93**	**27.09**	**18.38**	**25.70**	**18.07**
Lavandulyl acetate	1285	**11.13**	3.42	**12.34**	3.58	**11.87**	3.52	**11.08**	3.22	**12.71**	3.46	**11.20**	3.34	**12.09**	3.69	**11.62**	3.32	**11.19**	3.43	**11.59**	3.41
E-Caryophyllene	1420	2.47	2.78	3.88	2.93	3.66	2.97	3.83	2.68	4.67	2.92	3.06	3.26	**7.38**	**5.26**	**7.31**	**5.00**	2.76	3.14	2.22	3.02
(Z)-beta-Farnesene	1459	1.82	0.46	3.17	0.49	2.91	0.50	3.10	0.46	3.75	0.49	2.41	0.59	**6.21**	**1.50**	**6.06**	**1.67**	2.08	0.57	1.53	0.52
Germacrene D	1482	0.70	0.72	1.31	0.67	1.21	0.71	1.31	0.70	1.59	0.64	0.97	0.87	2.56	1.57	2.40	1.29	0.92	0.88	0.69	0.85
tau-Cadinol	1644	1.52	1.64	1.45	1.03	1.59	1.02	1.55	0.98	1.61	0.98	1.39	1.43	**5.47**	**9.76**	**7.68**	**17.27**	1.71	1.96	1.80	1.96

Note: Major compounds are shown in bold. / nd = not detected

[a]Linear retention indices (LRI) calculated relative to the elution ranking of n-alkanes on HP-5 column.

LaB = Lavender 'Budakalászi 80'; LaJ = Lavender 'Judit'.

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.

Table 13. Essential oil composition of fresh and preserved leafy shoots of *Origanum vulgare* subsp. *hirtum* commercial sample

Compounds	LRI[a]	Total GC area percentages (%)									
		Fresh	Sun	Shade	Oven-40°C	Oven-60°C	Lyo	MW-250W	MW-700W	SFreez	FFreez
β-Myrcene	995	1.20	0.67	0.77	0.71	0.24	0.53	0.09	0.03	0.85	0.75
p-Cymene	1026	**4.07**	**3.44**	**3.98**	**3.83**	**3.60**	**3.12**	**0.92**	**0.53**	**3.61**	**3.43**
γ-Terpinene	1056	**3.59**	**2.26**	**2.50**	**2.65**	**0.60**	**2.09**	**0.53**	**0.33**	**3.54**	**2.89**
cis-Sabinene hydrate	1096	**4.59**	**5.77**	**4.33**	**5.20**	**7.17**	**3.78**	**2.10**	**1.69**	**4.11**	**4.95**
Terpinen-4-ol	1175	0.59	0.82	0.69	0.76	0.88	0.81	0.78	0.87	0.64	0.59
Carvacrol	1300	**79.27**	**80.28**	**81.54**	**80.32**	**82.39**	**84.36**	**91.47**	**92.30**	**80.27**	**81.09**
β-Caryophyllene	1420	1.80	1.69	1.42	1.55	1.39	1.16	1.89	1.89	1.66	1.52

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.

Salvia officinalis L. 'Regula'

The major compounds identified in the EO extracted from the fresh leaves of garden sage variety 'Regula' were α-thujone (34.53%), camphor (18.25%), 1,8-cineol (7.57%), β-thujone (7.07%), viridiflorol (5.22%) and manool (3.87%) (Table 14). After evaluating the effects of preservation methods, it was found that every treatments reduced the ratio of α-thujone a little (by 4-8%), but otherwise the characteristic EO composition was preserved in most cases.

Table 14. 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.

Only microwave drying methods led to a drastic changes in the composition, resulting in a significant reduction of monoterpene molecules (e.g. camphene, limonene, 1,8-cineol, α- and β-thujone, camphor) in the EO, presumably due to evaporation. However, the proportion of larger molecular-sized compounds, such as sesquiterpene viridiflorol, E-caryophyllene, α-humulene and the diterpene manool increased significantly. Microwave drying at 700 W resulted in more pronounced changes than drying at 250 W (Table 14). Hamrouni-Sellami et al., (2013) also found that microwave drying at 500 W significantly affected the EO composition of *Salvia officinalis* leaves. In their experiment, monoterpenes with smaller molecular sizes, such as β-pinene and camphene, disappeared, while the level of viridiflorol increased.

Salvia rosmarinus L. 'Harmat'

The identified major compounds in the EO of fresh rosemary 'Harmat' leaves were camphor (21.22%), α-pinene (16.43%), 1,8-cineol (15.59%), camphene (5.46%) and limonene (5.39%) (Table 15).

Table 15. EO composition of fresh and preserved leaves of *Salvia rosmarinus* L. 'Harmat'

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	**16.43**	**15.73**	**15.42**	**16.45**	**16.06**	**16.62**	**6.91**	**2.86**	**16.17**	**16.52**
Camphene	952	**5.46**	**4.88**	**5.21**	**5.03**	**4.93**	**5.01**	**1.64**	**0.80**	**5.13**	**4.91**
β-Pinene	981	2.63	1.64	1.79	1.80	1.48	2.19	0.38	0.30	2.54	2.41
β-Myrcene	990	3.95	3.92	4.08	3.81	3.53	3.66	1.73	1.86	3.64	3.70
3-Octanone	995	4.77	5.05	5.03	4.46	4.66	4.91	0.65	0.60	4.63	5.05
α-Phellandrene	1008	1.49	1.61	1.67	1.55	1.47	1.50	0.85	0.79	1.52	1.54
Limonene	1029	**5.39**	**5.74**	**5.81**	**5.64**	**5.18**	**5.37**	**3.48**	**3.97**	**5.53**	**5.68**
1,8-Cineol	1034	**15.59**	**15.46**	**14.92**	**15.52**	**15.90**	**16.19**	**11.36**	**10.95**	**15.50**	**15.88**
Linalool	1097	1.91	1.98	2.01	1.99	1.88	2.00	1.83	1.88	1.96	1.95
Camphor	1144	**21.22**	**22.23**	**21.74**	**21.82**	**22.44**	**22.18**	**27.23**	**24.69**	**21.91**	**21.88**
Borneol	1165	4.11	4.13	4.29	4.12	4.15	4.19	8.29	7.93	3.95	3.96
α-Terpineol	1189	2.03	2.16	2.14	2.03	2.13	2.18	3.89	3.45	2.07	2.05
Verbenone	1209	3.32	3.20	3.15	2.86	2.87	3.44	4.07	3.12	3.22	3.01
Bornyl acetate	1284	1.77	1.50	1.48	2.29	1.56	1.29	2.59	3.16	1.69	1.32
β-Caryophyllene	1420	0.93	0.99	1.13	1.16	1.20	0.76	4.36	6.19	1.16	0.98
α-Humulene	1454	0.31	0.34	0.38	0.39	0.40	0.24	1.55	2.17	0.38	0.33
Caryophyllene oxide	1590	0.46	0.26	0.29	0.30	0.26	0.30	1.60	2.12	0.48	0.46
Cadin-4-en-7-ol	1656	0.04	0.06	0.08	0.07	0.08	0.03	0.71	1.06	0.04	0.04
14-Hydroxy-9-epi-Caryophyllene	1668	0.22	0.38	0.41	0.41	0.51	0.21	2.80	4.07	0.28	0.31

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.

The used preservation methods did not alter the volatile composition of rosemary leaves considerably (except microwave drying), as these treatments did not affect the EO content either. Hence, the EO composition found in preserved leaves remained identical to that of the fresh leaves. Microwave drying techniques, however, led to significant changes in the EO composition. The ratio of α-pinene, camphene and 1,8-cineol (the smaller molecules) decreased (at 250 W: by 4-10%, at 700 W: by 5-14%), while the ratio of camphor (by 3-6%), borneol and many other sesquiterpene compounds increased overall (Table 15).

Conclusions

Based on our observations it can be established that the applied treatments did not influence the EO composition of species, which contain the volatiles externally in glandular hairs, except in some cases oven drying at 60°C, and primarily microwave drying methods. These treatments significantly reduced the EO content of samples as well (especially microwave drying techniques), and the proportion of compounds in the very few remaining EO also changed: the ratio of compounds with lower molecular weight decreased (these are assumed to evaporated), and due to this the ratio of larger molecules increased (these were more difficult to escape). This change was observed in case of all the species studied. Microwave treatment at 700 W resulted in a slightly more drastic effect than at 250 W.

5.2.2. Species accumulating the EO primarily or exclusively in secretory ducts

Levisticum officinale Koch. 'Mittelgroblättriger' and ASTLEVI44

In the EO of fresh leaves of lovage 'Mittelgroblättriger' and gene bank accession ASTLEVI44, the monoterpenes α-terpinyl acetate (41.57-42.89%), β-phellandrene (16.17-18.95%), and the phthalide (Z)-ligustilide (33.90-34.03%) were present in the highest proportions (Table 16). β-myrcene (2.01-2.62%), another monoterpene was also present, but only in a smaller ratio. It can be concluded that both taxa had a very similar EO composition.

Natural drying (in sun and shade), convective drying (at 40 and 60°C), microwave drying (at 250 and 700 W) furthermore freezing methods (slow and fast) did not cause significant changes in the original EO composition. Although there were minor fluctuations in the ratios of major compounds (e.g. in β-phellandrene: 1-10%, in α-terpinyl acetate: 1-12%, in (Z)-ligustilide: 0.2-8.0%), these variations were not so considerable to change the volatile profile.

However, lyophilization substantially altered the composition, notably reducing the presence of smaller molecules (e.g. in case of β-phellandrene and α-terpinyl acetate a 15-18%

and a 31-37% loss could be observed, respectively), presumably due to evaporation. Conversely, there was a remarkable, 36-38% increase in the proportion of larger molecule (Z)-ligustilide in the remaining very little EO after lyophilization. This process led to the appearance of some new components (such as trans-anethole, estragole, carvacrol, methyl eugenol) too, that were not present in the volatile of fresh sample (Table 16). Similarly, in Złotek et al., (2023) experiment, freeze-drying significantly lowered the EO yield of lovage leaves, and in the EO that remained, the quantity of (Z)-ligustilide substantially rose, while other compounds diminished.

***Artemisia dracunculus* L. 'Artemis' and 'Zöldzamat'**

For both french tarragon varieties 'Artemis' and 'Zöldzamat' the phenylpropanes dominated in the EO, namely estragole (77.63-78.02%) and methyl eugenol (4.57-5.41%). Additionally, several monoterpenes such as sabinene (3.19-3.32%), limonene (2%), (Z)-beta-ocimene (4.62-4.89%) and (E)-beta-ocimene (4.8%) were detected in higher ratios. The composition of EOs in both tarragon varieties showed a high degree of similarity (Table 17).

Among the examined preservation methods, freezing and shade drying retained the original ratio of volatiles found in fresh tarragon leaves the most. There were no significant differences between their estragole, methyl eugenol and ocimene content compared to the composition of fresh samples. Oven drying at 40°C and microwave drying at 700 W also did not cause considerable changes. Although the proportion of estragole decreased a little, and parallel with it the ratio of methyl eugenol increased in the samples of each variety, these changes did not prove to be significant.

Convective drying technique with higher temperature (at 60°C and drying in the sun), furthermore microwave drying at 250 W (where the drying time was longer compared to drying at 700 W) further reduced the concentration of estragole within the EO, while the ratio of methyl eugenol increased. In case of both varieties a totally similar trend could be observed.

However, lyophilization was the only preservation technique, which resulted in a really exceptional change in the EO composition. During this process most of the molecules with lower weight (sabinene, limonene, (Z)- and (E)-beta-ocimene) presumably evaporated, the concentration of estragole also reduced, but the ratio of larger methyl eugenol significantly increased (by 3-6%). In lyophilized tarragon leaves other new aroma compounds were also found, such as trans-anethole, α-terpinyl acetate, carvacrol, menthol, l-menthone and linalool. In the sample of 'Artemis' l-carvone also appeared. Trans-anethole, which is a phenylpropane derivative and the isomer of estragole, was detected in a quite high proportion (7.67-8.51%).

Table 16. EO composition of fresh and preserved leaves of *Levisticum officinale* Koch. 'Mittelgroblättriger' and gene bank accession ASTLEVI44

Compounds	LRI[a]	Total GC area percentages (%)																			
		Fresh		Sun		Shade		Oven-40°C		Oven-60°C		Lyo		MW-250W		MW-700W		SFreez.		FFreez.	
		LoM	LoGA	LoM	LoGA	LoM	LoGA	LoM	LoGA	LoM	LoGA	LoM	LoGA	LoM	LoGA	LoM	LoGA	LoM	LoGA	LoM	LoGA
β-Myrcene	994.7	**2.01**	**2.62**	**2.22**	**0.92**	**2.53**	**3.11**	**1.79**	**2.10**	**1.37**	**1.42**	**0.02**	**0.02**	**1.61**	**2.46**	**0.75**	**2.38**	**2.19**	**2.97**	**1.63**	**2.29**
β-Phellandrene	1029	**18.95**	**16.17**	**17.21**	**11.07**	**19.52**	**21.93**	**16.55**	**18.57**	**14.48**	**12.65**	**0.66**	**0.69**	**15.42**	**20.98**	**18.48**	**18.57**	**15.47**	**18.36**	**14.19**	**15.29**
Estragole	1196	nd	nd	nd	0.14	nd	nd	nd	0.85	0.04	0.12	**1.60**	**1.73**	nd	nd	0.04	0.16	nd	nd	nd	0.20
Trans-Anethole	1283	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	**3.37**	**3.10**	nd	nd	nd	nd	nd	nd	nd	nd
Carvacrol	1300	nd	nd	0.05	nd	0.04	nd	0.33	nd	0.52	0.43	**5.73**	**7.32**	0.31	0.12	0.42	0.24	0.05	nd	nd	nd
α-Terpinyl acetate	1349	**41.57**	**42.89**	**39.80**	**54.81**	**42.46**	**39.47**	**41.37**	**39.96**	**43.33**	**40.29**	**10.33**	**6.23**	**39.08**	**40.48**	**42.45**	**34.28**	**42.79**	**37.17**	**46.48**	**44.02**
Methyl eugenol	1411	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	**0.05**	**0.98**	nd	nd	nd	nd	nd	nd	nd	nd
(Z)-3-Butylidene phthalide	1677	0.24	0.36	0.32	0.64	0.25	0.35	0.33	0.53	0.49	0.99	1.22	1.65	0.39	0.28	0.76	0.44	0.46	0.39	0.40	0.59
(E)-3-Butylidene phthalide	1722	0.07	0.09	0.34	0.48	0.46	0.21	0.50	0.30	0.69	0.56	0.38	0.62	0.44	0.10	0.53	0.19	0.19	0.11	0.14	0.14
Z-ligustilide	1742	**33.90**	**34.03**	**34.19**	**28.05**	**27.61**	**26.65**	**31.72**	**33.25**	**31.43**	**39.58**	**69.61**	**72.39**	**37.47**	**33.69**	**41.70**	**40.49**	**33.39**	**37.12**	**32.85**	**34.22**
E-ligustilide	1806	0.75	0.75	2.13	1.97	2.67	0.50	2.93	0.71	3.35	0.84	2.50	1.58	1.97	0.36	1.90	0.69	1.27	0.71	1.12	0.62

Note: **LoM = Lovage 'Mittelgrobblättriger'; LoGA = Lovage gene bank accession ASTLEVI44**

Table 17. Essential oil composition of fresh and preserved leaves of *Artemisia dracunculus* L. 'Zöldzamat' and 'Artemis'

Compounds	LRI[a]	Total GC area percentages (%)																			
		Fresh		Sun		Shade		Oven-40°C		Oven-60°C		Lyo		MW-250W		MW-700W		SFreez.		FFreez.	
		FtZ	FtA	FtZ	FtA	FtZ	FtA	FtZ	FtA	FtZ	FtA	FtZ	FtA	FtZ	FtA	FtZ	FtA	FtZ	FtA	FtZ	FtA
Sabinene	976	**3.32**	**3.19**	**3.87**	**3.59**	**4.18**	**3.52**	**3.81**	**3.79**	**3.53**	**4.58**	**0.25**	**1.02**	**3.03**	**4.24**	**2.85**	**3.46**	**3.42**	**3.10**	**3.84**	**3.50**
Limonene	1029	**2.01**	**2.00**	**2.24**	**2.37**	**2.47**	**2.40**	**2.40**	**2.35**	**2.26**	**2.93**	**0.44**	**0.84**	**1.95**	**2.69**	**1.75**	**2.09**	**2.08**	**2.01**	**2.21**	**2.16**
(Z)-beta-Ocimene	1037	**4.89**	**4.62**	**2.57**	**4.23**	**3.19**	**4.54**	**3.80**	**4.26**	**3.91**	**5.16**	**0.96**	**1.19**	**3.50**	**5.18**	**3.53**	**4.35**	**4.72**	**4.55**	**4.76**	**4.75**
(E)-beta-Ocimene	1046	**4.81**	**4.77**	**2.06**	**4.22**	**2.61**	**4.48**	**3.18**	**4.04**	**3.35**	**5.28**	**0.79**	**0.96**	**2.88**	**5.04**	**3.06**	**4.52**	**4.50**	**4.73**	**4.26**	**4.75**
Linalool	1097	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	**1.41**	**0.32**	nd	nd	nd	nd	nd	nd	nd	nd
L-Menthone	1158	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	**0.55**	**0.64**	nd	nd	nd	nd	nd	nd	nd	nd
Menthol	1171	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	**0.64**	**0.67**	nd	nd	nd	nd	nd	nd	nd	nd
Estragole	1196	**78.02**	**77.63**	**71.33**	**74.17**	**78.22**	**75.89**	**73.46**	**72.49**	**69.75**	**66.19**	**40.03**	**42.60**	**69.86**	**67.41**	**75.67**	**72.56**	**77.44**	**77.01**	**76.08**	**75.67**
L-Carvone	1241	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	**nd**	**2.55**	nd	nd	nd	nd	nd	nd	nd	nd
Trans-Anethole	1283	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	**8.51**	**7.67**	nd	nd	nd	nd	nd	nd	nd	nd
Carvacrol	1300	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	**0.77**	**2.48**	0.05	nd	nd	nd	nd	nd	nd	nd
α-Terpinyl acetate	1349	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	**1.46**	**2.51**	nd	nd	nd	nd	nd	nd	nd	nd
Geranyl acetate	1388	1.11	1.01	2.95	1.70	1.62	1.29	2.32	1.79	3.49	3.03	2.20	2.24	3.54	2.39	2.48	2.11	1.29	1.15	1.39	1.16
Methyl eugenol	1411	**4.57**	**5.41**	**11.01**	**7.58**	**5.61**	**6.19**	**8.06**	**8.44**	**10.22**	**9.11**	**31.82**	**27.21**	**10.03**	**8.71**	**7.58**	**7.96**	**5.01**	**5.97**	**5.17**	**6.12**
Germacrene D	1482	nd	0.03	0.07	0.08	nd	0.05	0.10	0.15	0.29	0.22	1.55	0.61	0.78	0.46	0.53	0.35	0.05	0.05	0.14	0.08
Bicyclogermacrene	1497	nd	0.04	nd	0.06	nd	0.04	0.04	0.10	0.21	0.19	1.24	0.51	0.57	0.42	0.41	0.33	0.08	0.07	0.17	0.11

Note: Major compounds are shown in bold. / nd = not detected / [a]Linear retention indices (LRI) calculated relative to the elution ranking of n-alkanes on HP-5 column.
FtZ = French tarragon 'Zöldzamat'; FtA = French tarragon 'Artemis'. 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.

***Petroselinum crispum* (Mill) Nym. var. *neapolitanum* whole and chopped leaves**

Apiole (39.78%), 1,3,8-p-menthatriene (25.20%), p-cymenene (7.75%), β-phellandrene (7.85%), myristicin (6.90%) and myrcene (4.24%) were identified as the main compounds in the EO of fresh, whole parsley leaves.

The preservation techniques resulted in similar constituents in comparable proportions, with the exception of lyophilization. The EO remaining after it showed a significant decrease in the proportion of smaller components like myrcene (by 4%), β-phellandrene (by 7%), p-cymene (by 7%) and 1,3,8-p-menthatriene (by 24%). Conversely, the ratio of larger molecular size compounds like myristicin and apiole, which are phenylpropene derivatives, nearly doubled as detailed in Table 18. Additionally, some new components, like trans-anethole, estragole and α-terpinyl acetate appeared in the volatiles of lyophilized parsley leaves, which were not present or only in trace in the EO of fresh or other way preserved samples.

A study on parsley conducted by Díaz-Maroto et al., (2002) indicated that lyophilization not only substantially reduced the EO content, but the concentration of each components within the EO as well. Huopalahti et al., (1985) also observed that freeze-drying not only notably reduced the EO content of *Anethum graveolens* L., a related herb by about 25%, but dramatically changed the composition of the EO too: the concentration of monoterpenes was significantly diminished, while the level of neophytadiene diterpene saw a marked increase. These findings align with our results.

In all chopped parsley samples, the ratio of larger compounds such as apiole and myristicin were higher in the EO than in whole leaves, specifically in case of those preservation methods, which caused higher volatile loss (e.g. drying in the sun, at 60°C, microwave drying methods and mainly lyophilization). Based on our results, it could be assumed that the loss of EO and the change in composition during preservation was mainly due to the higher evaporation of monoterpenes, which was more significant for chopped leaves (Table 18).

Conclusions

According to our observations, the treatments applied had a minimal impact on the EO composition of species, which contain the volatiles in internal secretory ducts, except lyophilization. Freeze-drying resulted in the highest EO loss in case of this species by far. Presumably, during this treatment the compounds with smaller molecular weight evaporated to a greater extent compared to larger molecules, thus their proportion decreased in the very little remaining EO. Further interesting phenomenon, that new components also appeared in the EO due to lyophilization, mainly phenylpropene derivatives (e.g. trans-anethole, estragole, etc.).

Table 18. Essential oil composition of fresh and preserved, whole and chopped leaves of *Petroselinum crispum* (Mill) Nym. var. *neapolitanum*

Compounds	LRI[a]	Total GC area percentages (%)																			
		Fresh		Sun		Shade		Oven-40°C		Oven-60°C		Lyo		MW-250W		MW-700W		SFreez.		FFreez.	
		P(W)	P(C)	P(W)	P(C)	P(W)	P(C)	P(W)	P(C)	P(W)	P(C)	P(W)	P(C)	P(W)	P(C)	P(W)	P(C)	P(W)	P(C)	P(W)	P(C)
α-Pinene	938	1.67	0.34	6.79	1.69	1.52	1.27	1.55	1.89	1.46	0.36	0.05	0.02	2.07	0.73	0.68	0.15	2.53	0.76	1.12	0.39
β-Pinene	981	1.31	0.45	3.51	1.45	1.26	1.09	1.16	1.56	1.19	0.37	0.05	0.02	1.39	0.71	0.62	0.18	1.83	0.75	1.08	0.56
Myrcene	995	**4.24**	**1.90**	**5.32**	**2.31**	**3.55**	**2.45**	**2.95**	**3.27**	**2.95**	**1.01**	**0.15**	**0.09**	**3.23**	**2.35**	**2.25**	**0.74**	**4.52**	**2.44**	**3.02**	**2.02**
β-Phellandrene	1029	**7.85**	**4.39**	**10.88**	**5.14**	**7.08**	**5.87**	**6.59**	**6.52**	**7.22**	**2.47**	**0.48**	**0.34**	**6.60**	**4.64**	**4.78**	**1.85**	**8.65**	**5.12**	**6.29**	**4.60**
p-Cymenene	1085	**7.75**	**7.30**	**9.35**	**7.04**	**8.81**	**7.37**	**7.54**	**7.77**	**7.91**	**3.62**	**1.03**	**2.14**	**7.01**	**5.61**	**5.84**	**3.75**	**8.06**	**6.71**	**7.82**	**6.54**
1,3,8-p-Menthatriene	1113	**25.20**	**18.55**	**21.47**	**3.50**	**31.63**	**27.18**	**30.66**	**23.77**	**25.85**	**7.88**	**1.18**	**4.86**	**18.93**	**9.59**	**15.97**	**8.86**	**20.56**	**22.64**	**16.15**	**19.81**
Estragole	1196	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	**0.87**	**1.48**	nd	0.02	nd	nd	0.05	0.06	0.09	0.07
trans-Anethole	1283	nd	0.02	nd	nd	nd	nd	nd	nd	nd	nd	**3.84**	**4.41**	nd	0.03	nd	nd	0.13	0.02	0.11	0.08
α-Terpinyl acetate	1349	nd	0.03	nd	nd	0.02	0.01	0.01	0.01	0.01	0.01	**3.65**	**3.45**	0.02	0.03	0.02	0.02	0.11	0.02	0.02	0.02
β-Caryophyllene	1420	0.84	1.25	0.68	0.72	0.82	0.47	0.71	0.51	1.68	0.81	0.36	1.55	1.63	1.28	2.42	1.55	0.84	0.71	1.18	0.65
Myristicin	1529	**6.90**	**11.76**	**5.32**	**7.33**	**5.89**	**6.73**	**7.40**	**8.82**	**8.90**	**12.43**	**12.37**	**33.83**	**6.71**	**8.86**	**7.56**	**8.97**	**8.78**	**12.08**	**10.62**	**13.10**
Elemicin	1565	0.18	0.49	0.16	0.32	0.20	0.33	0.29	0.40	0.27	0.50	0.43	2.68	0.27	0.39	0.25	0.47	0.30	0.51	0.48	0.44
Apiole	1668	**39.78**	**45.95**	**32.77**	**67.46**	**34.97**	**43.98**	**37.20**	**40.82**	**37.63**	**68.00**	**70.25**	**35.26**	**44.89**	**60.66**	**52.33**	**69.30**	**36.34**	**43.96**	**44.89**	**46.98**

Note: Major compounds are shown in bold. / nd = not detected

[a]Linear retention indices (LRI) calculated relative to the elution ranking of n-alkanes on HP-5 column.

P(W)= Parsley (Whole leaves); P(C) = Parsley (Chopped leaves)

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.

5.3. Effect of preservation methods on the total phenolic content (TPC) and total antioxidant capacity (TAC)

The different preservation methods unambiguously, significantly affected the TPC and TAC of aqueous extracts made from the used plant parts of examined herbs and spice species.

***Thymus vulgaris* L. 'French Summer'**

Fresh leafy shoots of garden thyme 'French Summer' contained 320.7 mg GAE/g d.w. of TPC. Among the studied preservation techniques, drying in the sun, in shade, at 40°C, at 700 W and lyophilization could preserve the highest amount of phenolic compounds (257.8-302 mg GAE/g d.w.) (Figure 32). Microwave drying at 250 W and freezing methods (141.9-171.8 mg GAE/g d.w.) decreased the TPC significantly. However, the lowest TPC was recorded in samples dried at 60°C. This method reduced the amount of phenolic compounds by 63%.

In case of TAC a trend similar to TPC could be observed. The fresh plant material had 296.5 mg AAE/g d.w. of TAC (Figure 32), but samples microwave dried at 700 W, oven dried at 40°C or dried in the sun and in shade also could produce this antioxidant effect (242.5-294.3 mg AAE/g d.w.). Lyophilization preserved the antioxidant capacity quite well (160.1 mg AAE/g d.w.), but microwave drying at 250 W and oven drying at 60°C decreased the TAC significantly (by 54-63%). Nevertheless, freezing methods proved to be the least suitable ones among the examined techniques with 62.6-71.3 mg AAE/g d.w. of TAC. According to data, TPC and TAC had a strong positive linear relationship (r = 0.92) with each other.

***Mentha* x *piperita* L. 'Mexián'**

Fresh 'Mexián' leaves contained 271 mg GAE/g d.w. of TPC. The highest values were measured in oven dried at 40°C, shade dried, lyophilized and at 700 W microwave dried samples (133.9-151.7 mg GAE/g d.w.). Sun drying and freezing methods preserved the TPC moderately well (102.2-123.1 mg GAE/g d.w.). The lowest TPC was recorded in samples dried in the oven at 60°C and microwaved at 250 W (68.7-71.1 mg GAE/g d.w.) (Figure 33).

We observed similar tendencies in TAC of peppermint leaves. 263.2 mg AAE/g d.w. of TAC was measured in the fresh sample. The highest data were found in case of lyophilization, drying at 40°C, at 700 W and in relation to natural drying methods (189-231.7 mg AAE/g d.w.). Freezing also maintained the TAC quite well (149.8-154.4 mg AAE/g d.w.) (Figure 33). However, the lowest data were observed in samples that underwent drying at 60°C and at 250 W. These drying methods led to a 57-64% reduction in the antioxidant capacity. The TPC and TAC exhibited a robust positive linear relationship, with a correlation coefficient of 0.88.

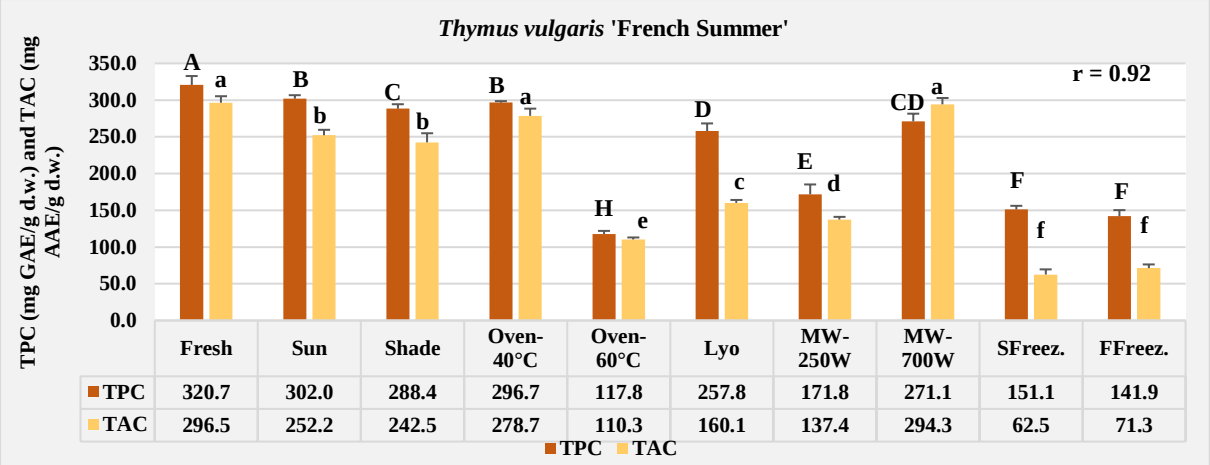

Figure 32. Total phenolic content (TPC) and total antioxidant capacity (TAC) of fresh and preserved leafy shoots of *Thymus vulgaris* L. 'French Summer'

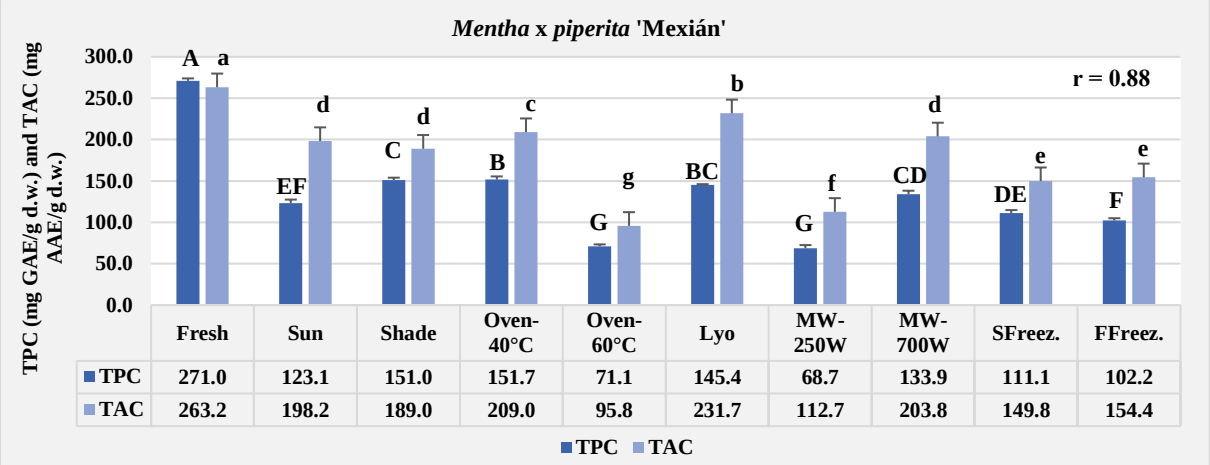

Figure 33. Total phenolic content (TPC) and total antioxidant capacity (TAC) of fresh and preserved leaves of *Mentha* x *piperita* 'Mexián'

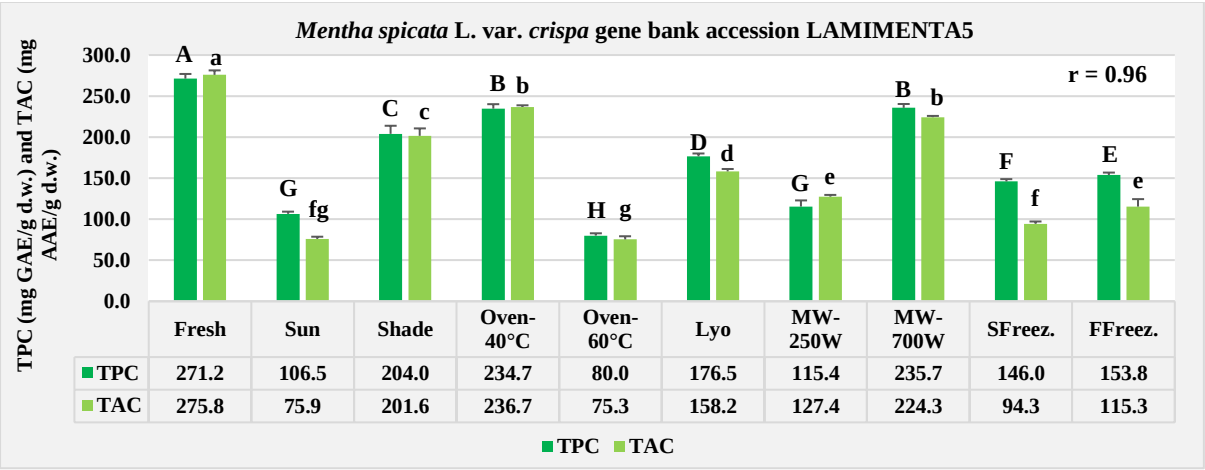

Figure 34. Total phenolic content (TPC) and total antioxidant capacity (TAC) of fresh and preserved leaves of *Mentha spicata* L. var. *crispa* gene bank accession LAMIMENTA5

†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

***Mentha spicata* L. var. *crispa* gene bank accession Nr. LAMIMENTA5**

Fresh spearmint leaves contained 271.2 mg GAE/g d.w. of TPC and had 275.9 mg AAE/g d.w. of TAC. The highest concentrations of TPC and the highest TAC were observed in samples treated with microwave drying at 700 W, shade drying and oven drying at 40°C. These methods yielded TPC ranging from 204.0-235.7 mg GAE/g d.w. and TAC ranging from 201.6-236.8 mg AAE/g d.w. (Figure 34). Lyophilization and freezing methods resulted in moderate data (TPC: 146.0-176.5 mg GAE/g d.w.; TAC: 94.3-158.2 mg AAE/g d.w.).

Conversely, microwave drying at 250 W and primarily oven drying at 60°C appeared to be the least effective methods for preserving phenolic compounds, resulting in substantially (by 54-73%) lower TPC and TAC. In this case again there was a strong, positive correlation between the two characteristics (r = 0.96).

***Artemisia dracunculus* L. 'Artemis'**

Fresh French tarragon 'Artemis' leaves contained 118.1 mg GAE/g d.w. of TPC and the TAC of the aqueous extract made from it was 143.9 mg AAE/g d.w. (Figure 35). The highest TPC and TAC were found in the microwave dried at 700 W sample, furthermore in the lyophilized, oven dried at 40°C and shade dried leaves (TPC: 102.5-118.0 mg GAE/g d.w., TAC: 119.0-142.2 mg AAE/g d.w.). However, in relation to this plant species, freezing, sun-drying and microwave drying at 250 W did not cause spectacular changes either.

On the other hand, oven drying at 60°C proved to be the worst method in preserving the phenolic compounds (70.3 mg GAE/g d.w.) and the antioxidant capacity (64.6 mg AAE/g d.w.). Here again there was a tight positive connection between the samples' TPC and TAC (r = 0.89).

***Levisticum officinale* Koch. 'Mittelgroblättriger'**

The average TPC of fresh lovage 'Mittelgroblättriger' leaves was 112.1 mg GAE/g d.w. (Figure 36). Among preserved samples, the significantly highest values were measured in the naturally dried (sun, shade), at 700 W microwave dried and oven dried at 40°C ones (91.7-106 mg GAE/g d.w.). Lyophilization gave intermediate values, and the lowest TPC data were found in frozen samples and leaves dried at 60°C in oven (56.6-57.7 mg GAE/g d.w.).

The TAC of fresh lovage leaves was measured to be 65.0 mg AAE/g d.w. Microwave drying at 700 W, lyophilization and sun drying proved to be the best methods in preserving the TAC (42.1-49.4 mg AAE/g d.w.). In contrast, oven drying at 60°C was the least suitable method, because it reduced the original antioxidant capacity by 62%. A moderate linear, positive relationship (r = 0.58) was observed between TPC and TAC of lovage samples.

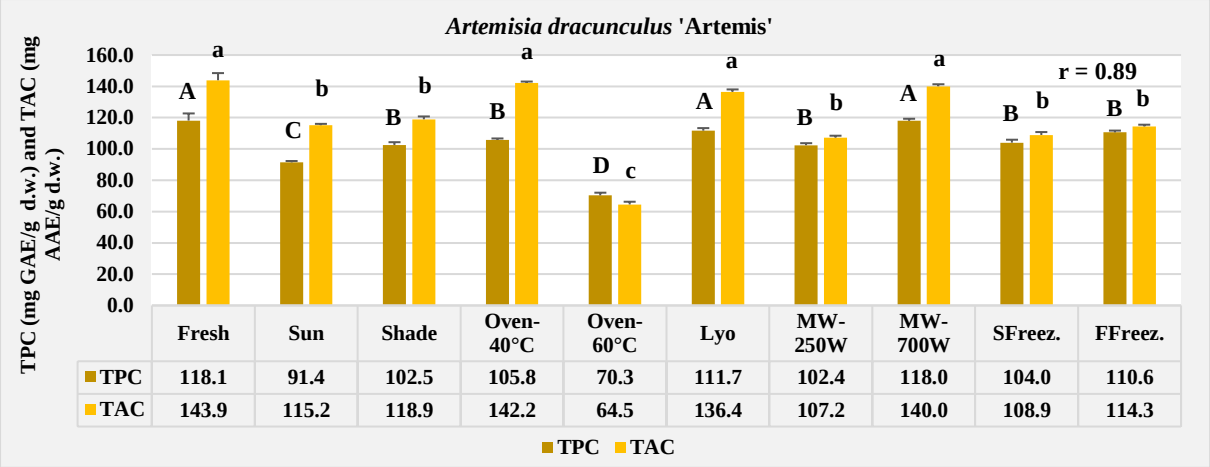

Figure 35. Total phenolic content (TPC) and total antioxidant capacity (TAC) of fresh and preserved leaves of *Artemisia dracunculus* L. 'Artemis'

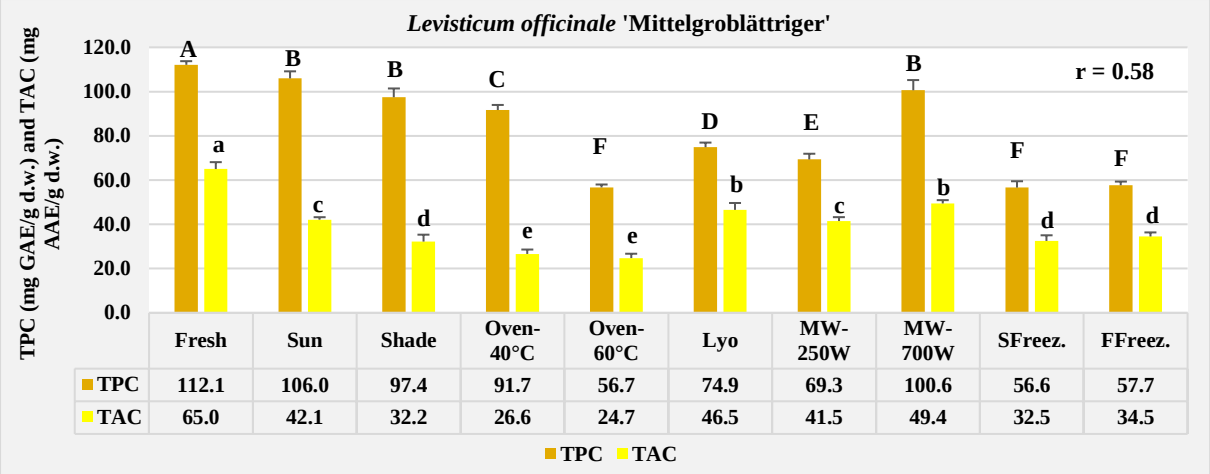

Figure 36. Total phenolic content (TPC) and total antioxidant capacity (TAC) of fresh and preserved leaves of *Levisticum officinale* Koch. 'Mittelgroblättriger'

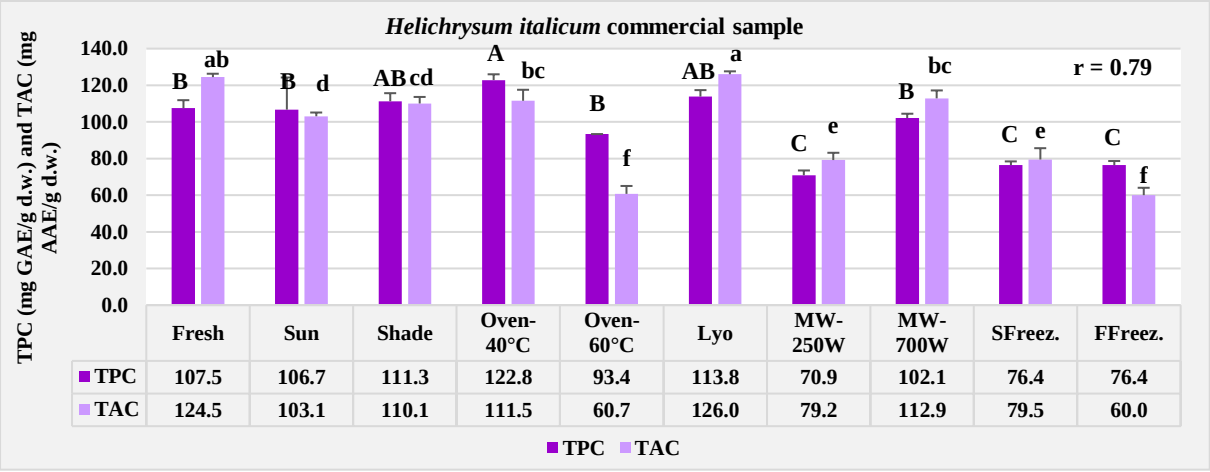

Figure 37. Total phenolic content (TPC) and total antioxidant capacity (TAC) of fresh and preserved flowering leafy shoots of *Helichrysum italicum* L.

†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

***Helichrysum italicum* L. commercial sample**

The aqueous extract prepared from the fresh flowering leafy shoots of Italian strawflower had 107.6 mg GAE/g d.w. of TPC and 124.5 mg AAE/g d.w. of TAC (Figure 37). The highest TPC and TAC were observed for microwave dried sample at 700 W, furthermore in case of lyophilized, oven-dried at 40°C and shade dried flowering leafy shoots (TPC: 102.1-113.8 mg GAE/g d.w.; TAC: 110.1-126.0 mg AAE/g d.w.). However, oven drying at 60°C, microwave drying at 250 W and freezing reduced the phenolic content (70.9-93.4 mg GAE/g d.w.) and the antioxidant capacity (60.0-79.5 mg AAE/g d.w.) the most. The TPC and TAC of Italian strawflower showed a strong, positive linear relationship (r = 0.79).

***Salvia officinalis* L. 'Regula'**

The TPC of fresh garden sage leaves was measured to be 262.5 mg GAE/g d.w. (Figure 38). The highest TPC was attained in samples dried by microwave at 700 W, lyophilized, dried in shade and in oven at 40°C (207.7-252.1 mg GAE/g d.w.). Sun drying and freezing methods already a little reduced the TPC of aqueous extracts (182.1-199.9 mg GAE/g d.w.), but the lowest values were recorded in relation to oven drying at 60°C and microwave drying at 250 W. These methods led to a 34-39% reduction in the amount of phenolic compounds.

The fresh garden sage 'Regula' leaves had 284.4 mg AAE/g d.w. average TAC (Figure 38). Lyophilization, microwave drying at 700 W, oven drying at 40°C and shade drying could preserve the highest TAC (255.2-297.0 mg AAE/g d.w.) measured in the aqueous extracts, while the lowest capacity was recorded in case of freezing, microwave drying at 250 W and oven drying at 60°C (152.2-164.1 mg AAE/g d.w.). According to data, there was a strong positive linear relationship (r = 0.91) between the TPC and TAC of garden sage leaves.

***Ocimum basilicum* L. 'Genovese'**

In the aqueous extract made from the fresh leaves and flowers of sweet basil variety 'Genovese', the average TPC was 182.4 mg GAE/g d.w., while the average TAC was measured to be 146.0 mg AAE/g d.w. (Figure 39). Similar to our previous observations, the highest TPC (144.2-180.0 mg GAE/g d.w.) and TAC (124.6-180.6 mg AAE/g d.w.) were observed in case of microwave drying at 700 W, lyophilization, oven drying at 40°C and shade drying. However, freezing, microwave drying at 250 W and mainly oven drying at 60°C reduced both the TPC (63.7-98.6 mg GAE/g d.w.) and the TAC (47.1-88.3 mg AAE/g d.w.) significantly. The TPC and TAC appeared to be strongly correlated, with a coefficient of 0.96, suggesting a strong interrelationship between the two characteristics.

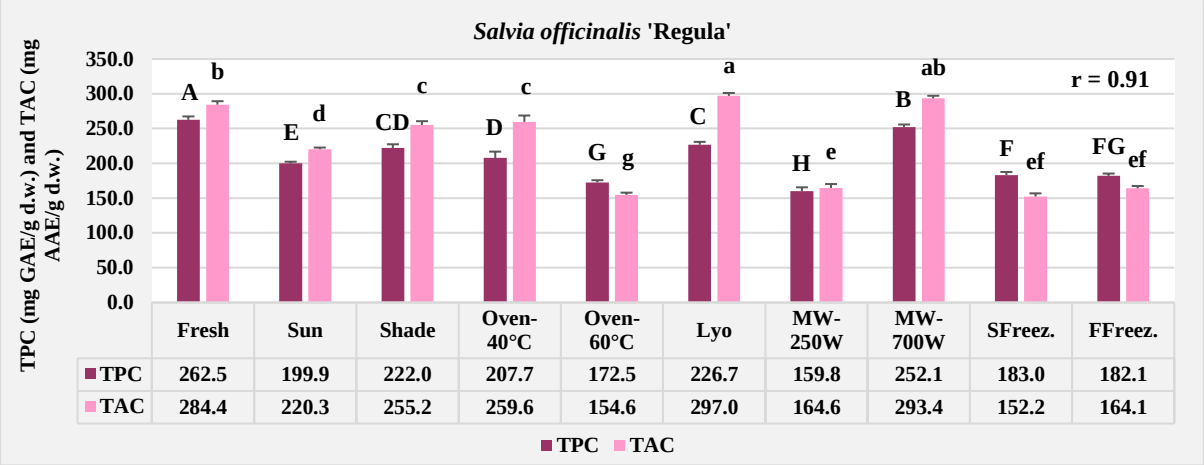

Figure 38. Total phenolic content (TPC) and total antioxidant capacity (TAC) of fresh and preserved leaves of *Salvia officinalis* L. 'Regula'

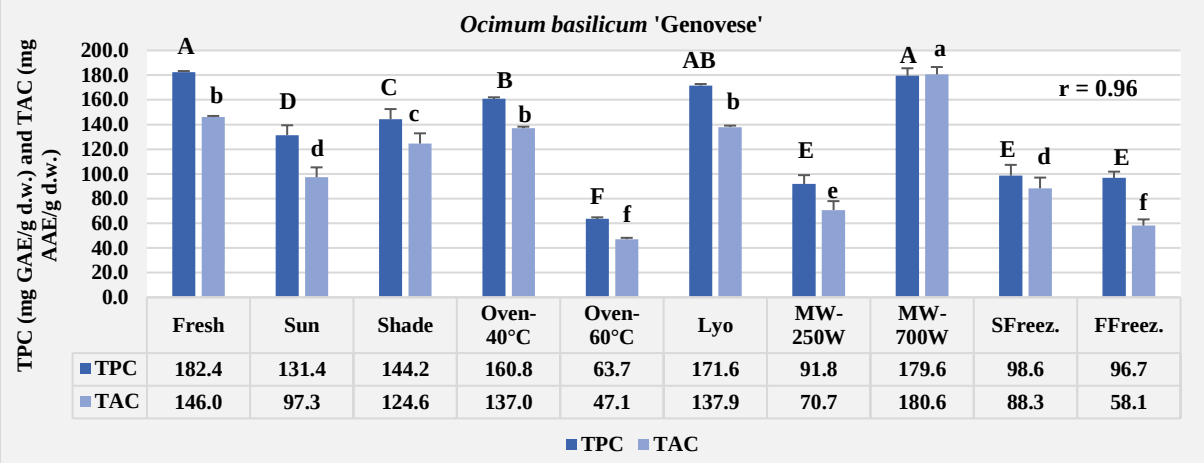

Figure 39. Total phenolic content (TPC) and total antioxidant capacity (TAC) of fresh and preserved leaves and flowers of *Ocimum basilicum* L. 'Genovese'

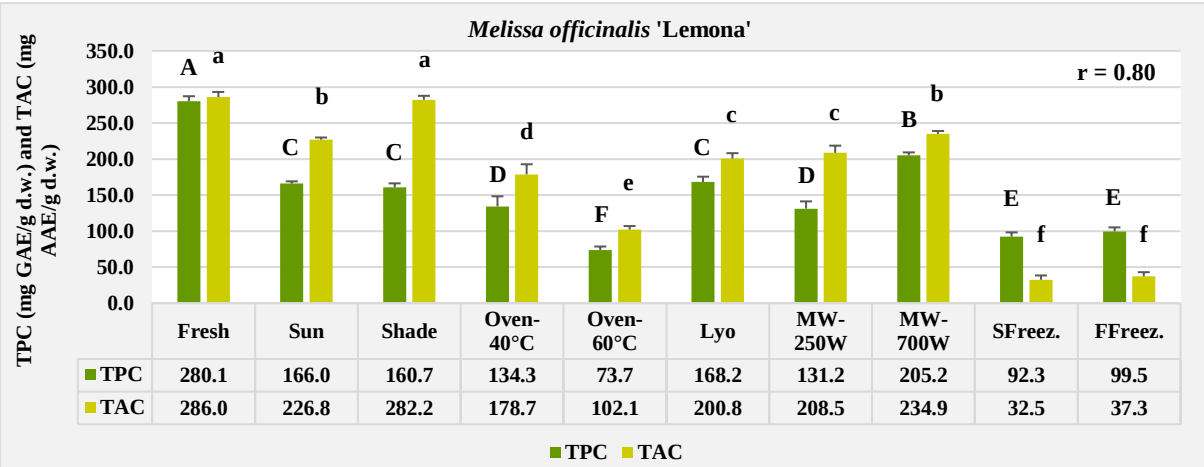

Figure 40. Total phenolic content (TPC) and total antioxidant capacity (TAC) of fresh and preserved leaves of *Melissa officinalis* L. 'Lemona'

†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

***Melissa officinalis* L. 'Lemona'**

The TPC in fresh leaves of lemonbalm variety 'Lemona' was measured to be 280.1 mg GAE/g d.w. (Figure 40). Microwave drying at 700 W, lyophilization and natural drying (in sun and in shade) proved to the best methods in preserving the phenolic compounds (160.7-205.2 mg GAE/g d.w.) of lemonbalm leaves. Nevertheless, the worst methods were microwave drying at 250 W, oven drying at 60°C and freezing, which resulted in 53-74% loss in the TPC.

The TAC of aqueous extract made from fresh lemonbalm leaves was 286.0 mg AAE/g d.w. (Figure 40). In this case shade drying, microwave dryings (700 W, 250 W), sun drying and lyophilization (200.8-282.2 mg AAE/g d.w.) proved to be the best preservation techniques, whereas oven drying at 60°C, furthermore freezing methods reduced the samples' TAC the most (by 64-89%). A strong linear relationship (r = 0.80) was observed between the TPC and TAC of lemonbalm variety 'Lemona'.

***Salvia rosmarinus* L. 'Harmat'**

Fresh rosemary 'Harmat' leaves possessed 142.4 mg GAE/g d.w. of TPC (Figure 41). The highest TPC was obtained by microwave drying at 700 W and oven drying at 40°C (134.5-140.3 mg GAE/g d.w.). However, due to lyophilization and oven drying at 60°C, the TPC decreased, but primarily freezing techniques reduced the amount of phenolic compounds (by 71-74%). In this way, these treatments proved to be the least suitable ones for the preservation of phenols in rosemary leaves.

The TAC in fresh leaves was measured to be 310.6 mg AAE/g d.w. (Figure 41). Drying

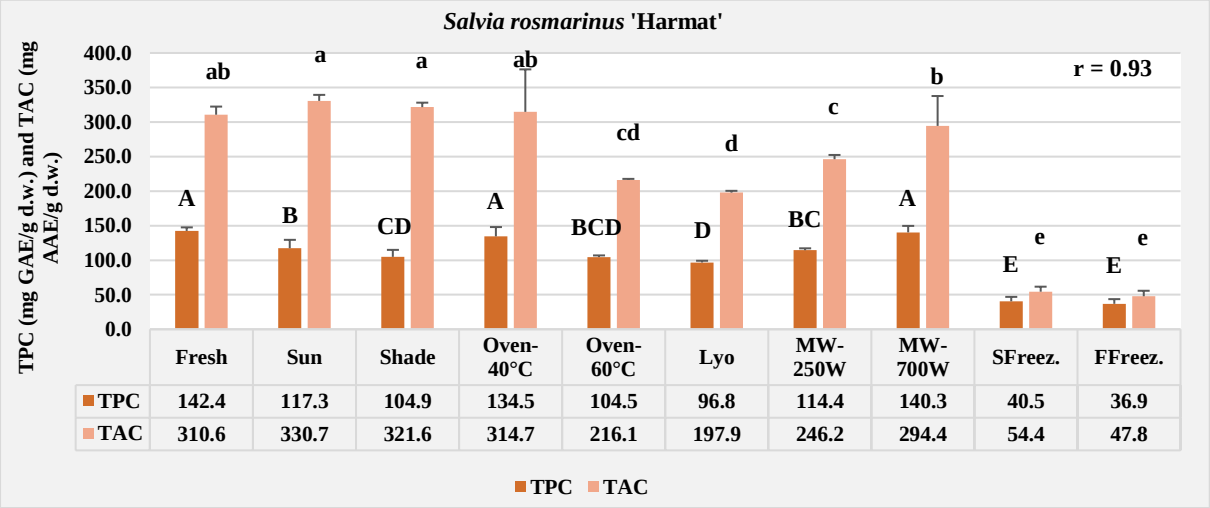

Figure 41. Total phenolic content (TPC) and total antioxidant capacity (TAC) of fresh and preserved leaves of *Salvia rosmarinus* L. 'Harmat'

†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

in the sun, in shade, at 40°C and at 700 W preserved the highest amount of TAC (294.4-314.7 mg AAE/g d.w.). The lowest TAC was found again in frozen samples (47.8-54.4 mg AAE/g d.w., which was an 82-85% decrease). We observed a strong positive, linear relationship (r = 0.93) between the TPC and TAC of aqueous extracts prepared from rosemary leaves.

Conclusions

Based on our findings, it can be concluded that the gentle drying methods with low temperature (drying in shade and at 40°C in oven), furthermore lyophilization and microwave drying at 700 W are the most suitable methods for preserving the TPC and TAC of herbs and spice species in general. The efficiency of microwave drying at 700 W is presumably due to the very short drying time (4-8 minutes). Sun drying also could preserve the TPC and TAC quite well in some cases, depending on the drying temperature. However, oven drying at 60°C, microwave drying at 250 W and freezing (both slow and fast) could be considered as the least suitable methods for preserving the phenolic compounds and other molecules with antioxidant effect. In contrast to our findings, Tomsone and Kruma (2014) found that freezing was one of the most effective techniques for maintaining the TPC and total flavonoid content (TFC), as well as the antioxidant activity in both the leaves and stems of *Levisticum officinale* L. However, we did not experience this.

But there are many research results in the literature, that support our observations. For example, *Thymus vulgaris* samples that were air-dried (in shade) exhibited the highest levels of TPC and TAC compared to oven drying at 70°C (Hossain et al., 2010). In another research study, Arslan et al., (2010) observed that drying peppermint leaves with microwaves at 700 W increased the TPC by 45%. This enhancement in microwave drying was attributed to the tissue structure being disrupted by heat waves, which enhanced the release of polyphenolic compounds into the solvent. In contrast, convective drying at 50°C reduced the phenol content by 53%, likely due to polyphenol instability or enzymatic breakdown.

Higher drying temperatures also diminished the antioxidant capacity in *Mentha piperita* and *Salvia rosmarinus* (Yi and Wetzstein, 2011). In another study, Jałoszyński et al., (2008) found that convection drying of oregano leaves at temperatures of 60 or 70°C led to a significant reduction in polyphenols in extracts, with losses exceeding the 80%. Conversely, microwave drying proved to be more efficient in preserving phenolic compounds, with the extent of preservation improving as the microwave power increased. For instance, increasing the power from 240 W to 480 W reduced the loss of phenolic compounds by about 24%, attributed to the shortened duration of the drying process. Similarly, Rezvani Moghaddam et

al., (2013) observed comparable results with tarragon leaves when microwaved at a higher power of 900 W. Djamila et al., (2021) reached similar conclusions regarding the effectiveness of high-power microwave drying at 600 W for *Mentha aquatica* L., aligning with our findings. According to Divya et al., (2012) and Soysal (2004), the increasing microwave power significantly reduces the drying time, thereby preserving bioactive compounds from evaporation. Hamrouni-Sellami et al., (2013) also found that microwave drying at higher power (800 W) resulted in the highest polyphenol content in *Salvia officinalis* leaves. They found that this method was suitable for preserving compounds with antioxidant effects too.

In contrast with our findings, Rababah et al., (2015) stated that sun-drying (with higher temperature) preserved greater quantities of total phenolics, and antioxidant activity compared to oven drying at 40°C for *Melissa officinalis*. Hossain et al., (2010) observed, that freeze drying led to a greater loss of bioactive compounds, such as phenoloids in *Lamiaceae* herbs like rosemary, oregano, marjoram, sage, basil and thyme. However, according to Nebrigić et al., (2023), the freeze drying process when applied to woody plants like *Helichrysum italicum*, causes less damage to plant tissue. This reduced damage could lead to a lower release of phenolic compounds from the plant, ultimately resulting in a decreased content of these compounds in the extracts.

In our research there was a very strong relationship between the TPC and TAC of aqueous extracts prepared from the examined species, suggesting that the antioxidant activity of the extracts was mainly due to the phenolic compounds contained in them.

5.4. Effect of preservation methods on the content of other phenolic compounds (rosmarinic acid and total hydroxycinnamic acid)

***Salvia rosmarinus* L. 'Harmat'**

As shown in Figure 42, the rosmarinic acid (RA) content of *Salvia rosmarinus* L. 'Harmat' was significantly influenced by the preservation methods. The leaves preserved by microwave drying at 700 W contained the highest amount of RA (1.57 g/100 g d.w.). The amount of it did not differ statistically verifiable from the results found in fresh leaves (1.72 g/100 g d.w.). Leaves drying in the sun, in shade and in oven at 40°C also contained relatively higher amount of RA (1.20-1.37 g/100 g d.w.). However, oven drying at 60°C, lyophilization and microwave drying at 250 W significantly decreased the RA content, by 47-58%. Among the examined preservation techniques, freezing methods proved to be the least efficient ones in maintaining the RA content, because in case of them only 0.14-0.15 g/100 g d.w. could be detected. There was no difference between the effects of fast and slow freezing methods.

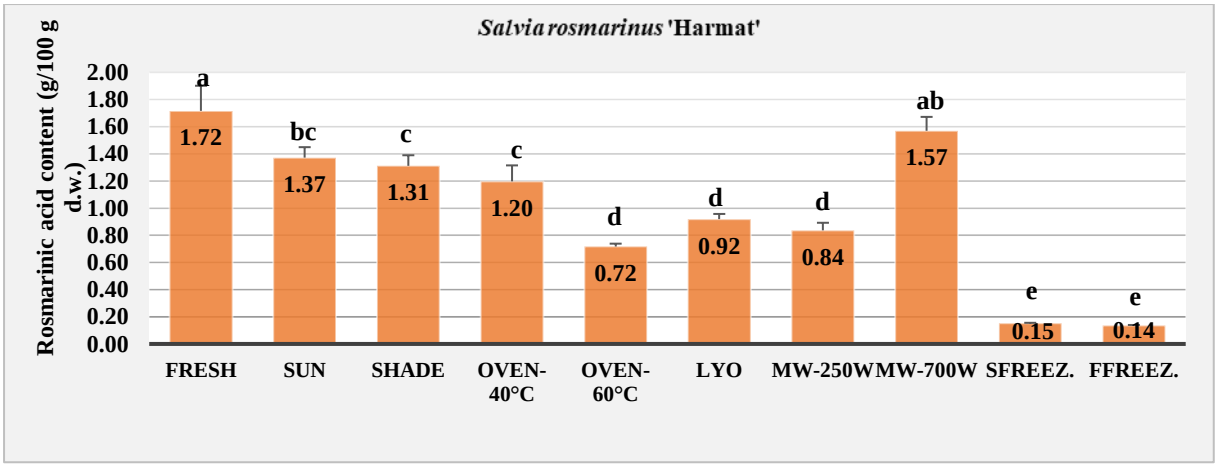

Figure 42. Rosmarinic acid content (g/100 g d.w.) of fresh and preserved leaves of *Salvia rosmarinus* L. 'Harmat'

†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

Thymus vulgaris L. 'Deutscher Winter'

The RA content of garden thyme 'Deutscher Winter' was also significantly affected by the preservation methods (Figure 43). The highest RA content was found in lyophilized sample (2.92 g/100 g d.w.), but it's amount did not differ significantly from the fresh plant material or samples dried naturally (in sun and shade), at 40°C and microwaved at 700 W (2.37-2.53 g/100 g d.w.). However, drying at 60°C and at 250 W caused significant reduction in the RA content by 53% and 35%, respectively. Freezing methods proved to be the least efficient method in preserving the RA content in this case as well (0.05 g/100 g d.w.).

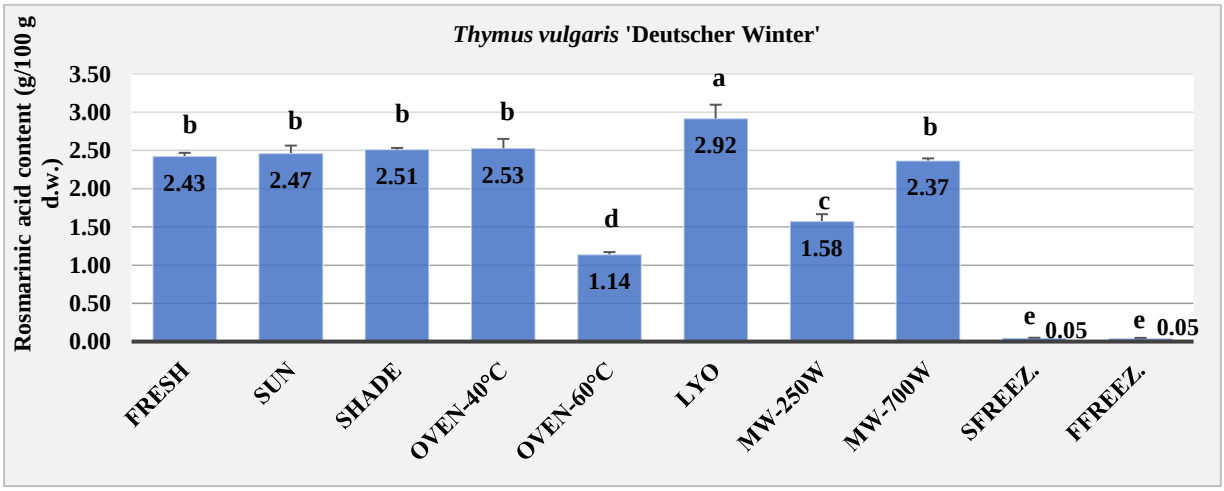

Figure 43. Rosmarinic acid content (g/100 g d.w.) of fresh and preserved leafy shoots of *Thymus vulgaris* L. 'Deutscher Winter'

†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

***Melissa officinalis* L. 'Lemona'**

In the fresh leaves of lemonbalm 'Lemona', 4.62% total hydroxycinnamic acid (THA) content was measured referred to the dry weight. The highest THA content was found in sun-dried leaves (5.05%), but shade drying, oven drying at 40°C, lyophilization and microwave drying at 700 W also preserved the THA content relatively well (3.52-4.61%) (Figure 44). The worst treatments were microwave drying at 250 W, furthermore oven drying at 60°C and frozen treatments, because these resulted in a significant, 75-81% loss in the THA content.

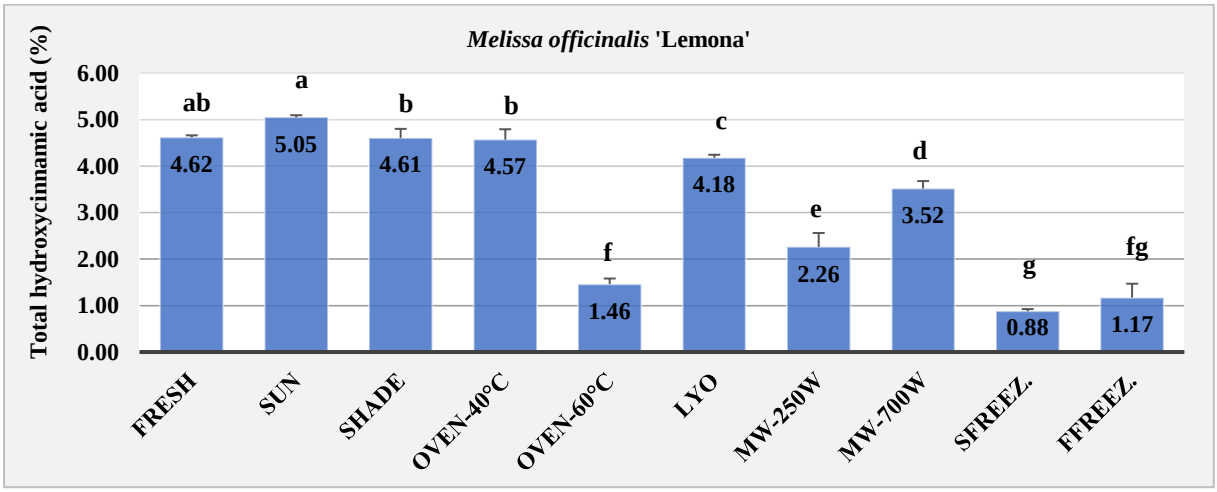

Figure 44. Total hydroxycinnamic acid content (%) of fresh and preserved leaves of *Melissa officinalis* L. 'Lemona'

†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

From our observations it can be concluded that microwave drying at 700 W, oven drying at 40°C and natural drying methods proved to be the most suitable ones for preserving the RA and THA content, while microwave drying at 250 W, oven drying at 60°C and in particular freezing were the worst techniques, causing the highest loss. These results are consistent with our findings on TPC and TAC. Although freezing slows down the process, it does not stop the physical and biochemical reactions responsible for deterioration (George, 1993). According to Mulinacci et al., (2011), it is easy to observe a rapid and noticeable browning of the leaf's surface during thawing. Despite handling the frozen sample within five minutes prior to adding the extractive solution, some degree of browning persists, likely resulting from endogenous enzymes oxidizing phenolic compounds during and after thawing. This phenomenon must be taken into account when quantifying phenolic compounds.

In our research lyophilization was also effective in preserving phenolic compounds, although its efficacy was not always the same. Hossain et al., (2010) found lower RA content

in freeze dried rosemary and thyme samples. While our experimental findings align with this result in the case of rosemary, we observed contrasting results for thyme. The lyophilization processes involve very low temperatures, but these may not completely deactivate the degrading enzymes.

5.5. Effect of preservation methods on the organoleptic characteristics

5.5.1. Colour

The colour characteristics of fresh and processed plant materials of the examined herbs and spices are illustrated in Table 19-20 and Appendix 3/1- Appendix 3/10(Appendix 3).

***Salvia officinalis* L. 'Regula'**

Among the studied preservation methods, lyophilization and microwave drying at 700 W led to a significant increment in L* values of garden sage variety 'Regula' leaves (Table 19). Oven drying at 40 and 60°C resulted in L* values the closest to fresh leaves (L*=35.3).

Examining the green tint (a*), it was found that lyophilized and frozen sage leaves retained their green colour (a*=-3.3 to -3.7) during preservation the best, their a* values did not differ significantly from the fresh sample (a*=-3.2). Microwave drying at 700 W could also slightly retain the green colour. However, in case of the other methods the leaves turned greyish (Appendix 3/1).

Based on the yellow coordinate (b*) values, we found that lyophilized and at 700 W dried samples were significantly more yellow (b*=9.9-10.1) than the fresh sample (b*=8.6). The frozen leaves were the closest to the fresh sage leaves in relation to the b* value. The yellowish hue of leaves significantly decreased due to the other treatments.

The calculated a*/b* ratio shows the proportion of the two coordinates. The larger and more negative the ratio, the more intense is the green colour of the sample. In our experiment, lyophilization and freezing (a*/b*=-0.3 and -0.5, respectively) resulted in a leaf colour most similar to that of fresh sage leaves (a*/b*=-0.4). The other preservation methods significantly affected the external appearance of samples. Sage leaves should be preserved at low temperature to prevent colour change according to Doymaz and Karasu (2018), but in our experiment shade drying and oven drying at 40°C also caused significant changes.

***Ocimum basilicum* L. 'Ohře'**

In case of sweet basil variety 'Ohře', lyophilization resulted in the highest increase in lightness (L*=46.4), significantly higher compared to the fresh sample (L*=36.2). Oven drying at 40°C (L*=36.1) maintained the original shade. However, oven drying at 60°C, freezing

methods (both slow and fast) and microwave drying at 250 and 700 W resulted in the lowest lightness values (L*=28.3-31.1), indicating a darker appearance (Appendix 3/2).

The fresh leaves had a green hue (a*) of -11.1. Lyophilized leaves retained the highest green colour (a*=-7.1), and both freezing methods could also preserve the green tint relatively well. The other preservation methods showed a significant increase in a* value (Table 19).

The b* values showed the highest yellow hue in the fresh sample (b*=21.8), while the significantly lowest data obtained in oven drying at 60°C (b*=8.5). Lyophilization retained a higher yellow hue (b*=16.4), while other methods resulted in 9.1-12.9 of b* values.

According to the a*/b* quotient, lyophilization and freezing (slow, fast) were the most effective in maintaining the original colour of fresh basil leaves (a*/b*=-0.5). The samples, which were preserved by oven drying at 40°C and microwave drying at 700 W, also leaned towards greenness with the a*/b* values of -0.2 and -0.1, respectively. However, the other preservation methods modified the colour, slight browning occurred.

Similarly to our results, Pirbalouti et al. (2013a) also found that lyophilization and oven drying at 40°C as the most suitable preservation techniques for colour retention in basil leaves. These techniques proved to be better than drying at 60°C and at 500 W.

***Mentha* x *piperita* L. 'Mexián'**

In case of peppermint variety 'Mexián', lyophilized leaves had the highest L* value (43.4), suggesting a lighter appearance (Table 19). Oven drying at 40 and 60°C, microwave drying at 250 W and particularly freezing methods significantly reduced the lightness, resulting in a darker product. Natural drying (sun and shade) and microwave drying at 700 W resulted in L* values close to the fresh leaves (Appendix 3/3).

Preservation methods generally decreased the greenness (a*). Sun drying and microwave drying at 250 W caused a moderate decrease, but oven drying at 60°C significantly reduced it (a*=0.6). Lyophilization (a*=-7) preserved the green colour of fresh peppermint sample (a*=-7.3) the most effectively. Rubinskienė et al., (2015) found that peppermint leaves dried by microwave at 500 W had a reddish tint, but we did not experience it. In our research, microwave drying at 700 W (a*=-3.9) and shade drying (a*=-3.4) could preserve the greenness rather well.

The fresh sample had a b* value of 11.0. Lyophilization (b*=11.8) and microwave drying at 700 W (b*=10.3) slightly enhanced the yellow colour of peppermint leaves. Natural drying (sun and shade), oven drying at 40 and 60°C, furthermore microwave drying at 250 W generally decreased the yellowness compared to the fresh product, but freezing methods showed the most substantial reduction in yellow hue with 3.2-4.0 values.

According to the a*/b* data, lyophilization (-0.6) preserved the original colour (-0.7) of peppermint leaves the best. Microwave drying at 700 W, natural drying methods and slow freezing also proved to be effective (a*/b*=-0.3 to -0.4). Sample dried at 60°C (0.1), however, could be characterized by the most different colour that was markedly distinct from that of fresh leaves.

***Levisticum officinale* Koch. 'Mittelgroblättriger'**

In connection with L* value, lyophilization (41.8) and sun drying (38.3) greatly increased the lightness of lovage leaves, but freezing methods significantly led to a darker hue (Appendix 3/4). Other preservation methods resulted in moderate increase in lightness.

The a* values revealed that drying methods decreased the greenish hue of lovage leaves in general (oven drying at 60°C resulting in the least greenness), except lyophilization (a*=-8.9). It could retain the green colour of the fresh sample (a*=-9.4) very well (Table 19).

In case of b* values, sun drying (18.8) significantly increased the yellow hue of leaves, whereas lyophilization (b*=17.1) also enhanced the yellow tint but to a lesser extent. In contrast, freezing methods resulted in a significant decrease. The other methods either slightly increased or maintained the yellow colouration compared to fresh leaves (b*=14.3).

The a*/b* ratio analysis suggested that drying in the sun, shade, at 40°C and 60°C were not the best methods for preserving the original colour of lovage leaves. These caused colour degradation, leaves turned yellow. However, lyophilization (a*/b*=-0.5) preserved the colour of fresh leaves (a*/b*=-0.7) very well, and freezing (slow and fast), furthermore microwave drying at 250 and 700 W also proved to be effective.

Chlorophyll is responsible for the intensity of green colour in plants. When chlorophyll degrades, it leads to a decline in greenness. According to Sledz and Witrowa-Rajchert (2012), the retention levels of chlorophyll during the drying process are influenced by the plant species too. They found that plants of the *Apiaceae* family, like lovage can maintain higher levels of chlorophylls compared to herbs of the *Lamiaceae* family, such as basil, mint or oregano.

***Melissa officinalis* L. 'Lemona'**

As given in Table 19, lyophilization resulted in the highest L* value (37.5) for lemonbalm variety 'Lemona', indicating that lyophilized lemonbalm leaves became lighter in appearance than in fresh state (L*=33.4). Oven drying at 60°C and freezing methods (L*=26.6-27.6) had

Table 19. Colour characteristics (L*, a*, b*, a*/b* values) of fresh and preserved samples of examined species

***Salvia officinalis* 'Regula'**										
Colour characteristics	**Fresh**	**Sun**	**Shade**	**Oven-40°C**	**Oven-60°C**	**Lyo**	**MW-250W**	**MW-700W**	**S.Freez.**	**F.Freez.**
L* (lightness)	35.3^{c}	39.0^{bc}	38.4^{bc}	36.2^{c}	36.9^{c}	43.3^{a}	38.3^{bc}	41.5^{ab}	37.8^{bc}	37.4^{c}
a* (red/green)	-3.2^{a}	0.4^{c}	0.6^{c}	0.4^{c}	0.8^{c}	-3.3^{a}	0.4^{c}	-0.8^{b}	-3.7^{a}	-3.5^{a}
b* (yellow/blue)	8.6^{b}	6.3^{d}	6.8^{cd}	6.0^{d}	6.1^{d}	9.9^{a}	6.3^{d}	10.1^{a}	8.00^{bc}	7.8^{bc}
a*/b*	-0.4^{b}	0.1^{de}	0.1^{de}	0.1^{de}	0.1^{e}	-0.3^{b}	0.1^{d}	-0.1^{c}	-0.5^{a}	-0.5^{a}
***Ocimum basilicum* 'Ohře'**										
Colour characteristics	**Fresh**	**Sun**	**Shade**	**Oven-40°C**	**Oven-60°C**	**Lyo**	**MW-250W**	**MW-700W**	**S.Freez.**	**F.Freez.**
L* (lightness)	36.2^{b}	33.6^{bc}	30.3^{d}	36.1^{b}	28.9^{d}	46.4^{a}	28.3^{d}	29.0^{d}	28.8^{d}	31.1^{cd}
a* (red/green)	-11.1^{a}	1.2^{e}	1.1^{e}	-2.3^{d}	1.5^{e}	-7.1^{b}	1.9^{e}	-1.3^{d}	-4.9^{c}	-5.6^{c}
b* (yellow/blue)	21.8^{a}	10.8^{cde}	9.5^{ef}	12.3^{cd}	8.5^{f}	16.4^{b}	9.1^{ef}	10.5^{def}	11.7^{cd}	12.9^{c}
a*/b*	-0.5^{a}	0.1^{c}	0.1^{c}	-0.2^{b}	0.2^{c}	-0.4^{a}	0.2^{c}	-0.1^{b}	-0.4^{a}	-0.4^{a}
***Mentha* x *piperita* 'Mexián'**										
Colour characteristics	**Fresh**	**Sun**	**Shade**	**Oven-40°C**	**Oven-60°C**	**Lyo**	**MW-250W**	**MW-700W**	**S.Freez.**	**F.Freez.**
L* (lightness)	31.2^{b}	31.0^{b}	32.9^{b}	27.8^{c}	27.8^{c}	43.4^{a}	27.2^{c}	30.2^{bc}	22.8^{d}	21.6^{d}
a* (red/green)	-7.3^{a}	-2.1^{c}	-3.4^{b}	-0.4^{de}	0.6^{e}	-7.0^{a}	-1.8^{c}	-3.9^{b}	-1.2^{cd}	-0.3^{de}
b* (yellow/blue)	11.0^{ab}	8.0^{de}	9.5^{bcd}	7.3^{e}	7.9^{de}	11.8^{a}	8.8^{cde}	10.3^{abc}	4.0^{f}	3.2^{f}
a*/b*	-0.7^{a}	-0.3^{bc}	-0.4^{b}	-0.1^{de}	0.1^{e}	-0.6^{a}	-0.2^{c}	-0.4^{b}	-0.3^{bc}	-0.1^{d}
***Levisticum officinale* 'Mittelgroblättriger'**										
Colour characteristics	**Fresh**	**Sun**	**Shade**	**Oven-40°C**	**Oven-60°C**	**Lyo**	**MW-250W**	**MW-700W**	**S.Freez.**	**F.Freez.**
L* (lightness)	31.6^{d}	38.4^{b}	33.8^{cd}	34.1^{cd}	34.5^{c}	41.8^{a}	32.7^{cd}	34.7^{c}	21.3^{e}	21.6^{e}
a* (red/green)	-9.4^{a}	-4.4^{bc}	-1.8^{de}	-3.0^{cd}	-0.9^{e}	-8.9^{a}	-4.0^{bc}	-4.5^{b}	-1.7^{de}	-1.7^{de}
b* (yellow/blue)	14.3^{c}	18.8^{a}	14.5^{c}	14.4^{c}	14.0^{c}	17.1^{ab}	13.5^{c}	15.3^{bc}	4.5^{d}	4.5^{d}
a*/b*	-0.7^{a}	-0.2^{d}	-0.1^{ef}	-0.2^{de}	-0.1^{f}	-0.5^{b}	-0.3^{cd}	-0.3^{cd}	-0.4^{c}	-0.4^{c}
***Melissa officinalis* 'Lemona'**										
Colour characteristics	**Fresh**	**Sun**	**Shade**	**Oven-40°C**	**Oven-60°C**	**Lyo**	**MW-250W**	**MW-700W**	**S.Freez.**	**F.Freez.**
L* (lightness)	33.4^{b}	30.0^{bcd}	28.5^{cd}	29.6^{cd}	27.6^{d}	37.5^{a}	28.7^{cd}	31.9^{bc}	26.6^{d}	27.5^{d}
a* (red/green)	-7.8^{a}	0.5^{e}	0.3^{e}	-0.1^{e}	2.0^{f}	-4.5^{bc}	0.3^{e}	-1.5^{d}	-3.6^{c}	-5.2^{b}
b* (yellow/blue)	15.0^{a}	8.2^{cd}	8.0^{d}	7.7^{d}	7.6^{d}	11.6^{b}	9.5^{bcd}	11.4^{b}	9.2^{cd}	10.3^{bc}
a*/b*	-0.5^{a}	0.1^{d}	0.1^{d}	-0.1^{cd}	0.3^{e}	-0.4^{b}	0.1^{d}	-0.1^{c}	-0.4^{b}	-0.5^{ab}

Note: 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.
†Different letters indicate significant differences between means at $p < 0.05$ within characteristics (within rows).

the lowest L* values, suggesting darker hues. Other preservation techniques showed intermediate data, indicating varying degrees of colour retention (Appendix 3/5).

Observing the a* coordinate we found that freezing and lyophilization (a*=-3.6 to -4.5) were the most appropriate treatments in preserving the green hue close to that of fresh sample. At the same time, oven drying at 60°C (a*=2.0) caused a spectacular chlorophyll degradation.

Fresh lemonbalm leaves had yellow hue (b*) at 15.0. Among the preservation methods, lyophilization and microwave drying at 700 W recorded the highest data (b*=11.4-11.6). Natural drying (in sun and in shade) and oven drying at 40 and 60°C significantly reduced the yellow intensity. Microwave drying at 250 W and freezing methods resulted in intermediate b* values, indicating a partial preservation of yellow colour compared to the fresh sample.

The a*/b* data of applied methods showed, that lyophilization (-0.4), slow (-0.4) and fast (-0.5) freezing techniques maintained the original hue (-0.5) the most. In our experiment, oven drying at 40°C, furthermore microwave drying at 700 W also proved to be good at preserving the natural colour. In contrast, drying at 60°C (0.3) resulted in the highest a*/b* value, causing the biggest colour degradation.

***Origanum majorana* L. 'Magyar'**

Table 20 shows that lyophilized sample of marjoram variety 'Magyar' became a little lighter in colour (L*=40.8) compared to the fresh sample (L*=39.3). In contrast, freezing (L*=24.7-24.8) resulted in the lowest L* values, indicating a darker appearance. Natural drying, microwave and oven drying methods also led to significant reductions in lightness.

Fresh marjoram sample proved to be the greenest with the lowest negative a* value of -6.0. Freezing methods (a*=-4.8) and lyophilization (a*=-4.4) preserved the greenness of marjoram leafy shoots rather well. However, the other preservation methods reduced it indicating that these techniques caused a spectacular chlorophyll degradation (Appendix 3/6).

The b* value of fresh leafy shoots was found to be 17.1. Lyophilization (b*=14.1) caused the highest yellow hue while the other methods significantly reduced it (b*=8.7-10.9).

The a*/b* quotient of marjoram leafy shoots indicated that lyophilization and freezing (a*/b*=-0.3) closely matched the fresh sample's green hue (a*/b*=-0.4). The other applied preservation methods could not preserve the original colour of fresh sample. Verma et al., (2016) found that shade drying resulted in the best retention of the original colour of fresh herb, but in our research shade drying significantly reduced the intensity of green colour, although the worst method was not this, but drying at 60°C, at 250 W and drying in the sun.

Anethum graveolens L. commercial sample

Whole leaves

The L* value of fresh dill leaves was found to be 22.9. Lyophilized, whole dill leaves resulted in the highest L* value (31.1) demonstrating that these became lighter in appearance. The other techniques caused L* values (21.2-25.5) closer to the fresh leaves (Table 20).

The lowest a* value (-7.0) was observed in case of lyophilized whole leaves indicating that lyophilization process preserved the greenness of fresh leaves (-5.6) very well. Oven drying at 40°C, microwave drying at 700 W and freezing methods also preserved the green colour of whole dill leaves relatively well (a*=-3.1 to -4.9). In contrast, the highest a* data were recorded in samples dried in the sun, in shade, at 60°C and at 250 W (a*=-1.3 to -2.3). These resulted in the highest colour degradation (Appendix 3/7).

The b* coordinate of raw leaves was found to be 8.8. Observing the preservation methods, we found that lyophilized sample had the highest b* value (10.4), while the lowest data were measured in samples drying in the sun, at 60°C, at 250 W and 700 W (b*=4.4-5.8).

The a*/b* quotient showed that every treatments maintained (-0.5 to -0.7) the original hue (-0.6) very well, only oven drying at 60°C, sun and shade drying reduced the green colour a little (a*/b*=-0.2 to -0.4). During sun-drying, the temperature was relatively low, so the drying time was prolonged, which could help the colour degradation.

Chopped leaves

Pre-chopping of dill leaves did not affect the colour changes during the applied preservation techniques. We got very similar results in case of whole and chopped samples. In relation to chopped leaves also lyophilization proved to be the lightest according to its L* value (32.1). The L* data of other methods were lower (21.5-26.1) (Table 20).

Lyophilization (a*=-6.9) retained the greenness of fresh leaves the most in this case too. The highest a* values were found in samples dried in the sun, in shade, at 60°C and at 250 W (a*=-1.8 to -2.3) indicating that these methods significantly reduced the greenish hue.

In connection with b* coordinate, lyophilization resulted in the highest data (b*=11.2), while microwave drying at 250 W and 700 W produced the lowest b* values (4.7-5.7).

According to the a*/b* quotient, oven drying at 40°C (-0.6), lyophilization (-0.6), microwave drying at 700 W (-0.6) and freezing methods (a*/b*=-0.6 to -0.7) preserved the original hue the best for chopped leaves too, similarly to whole leaves. In their case, the colour was almost identical to that measured in the fresh sample. However, drying at 60°C, in the sun and in shade could retain the original hue the least (Appendix 3/8).

Table 20. Colour characteristics (L*, a*, b*, a*/b* values) of fresh and preserved samples of examined species

***Origanum majorana* 'Magyar'**										
Colour characteristics	**Fresh**	**Sun**	**Shade**	**Oven-40°C**	**Oven-60°C**	**Lyo**	**MW-250W**	**MW-700W**	**S.Freez.**	**F.Freez.**
L* (lightness)	39.3^{a}	32.2^{c}	34.6^{b}	34.6^{b}	33.1^{bc}	40.8^{a}	32.0^{c}	34.1^{bc}	24.7^{d}	24.8^{d}
a* (red/green)	-6.0^{a}	1.9^{e}	-0.2^{cd}	-0.3^{c}	1.7^{e}	-4.4^{b}	1.4^{e}	0.3^{d}	-4.8^{b}	-4.8^{b}
b* (yellow/blue)	17.1^{a}	8.7^{e}	10.1^{d}	10.5^{d}	9.7^{de}	14.1^{b}	9.7^{de}	11.8^{c}	10.9^{d}	10.8^{d}
a*/b*	-0.4^{a}	0.2^{d}	-0.0^{b}	-0.0^{b}	0.2^{cd}	-0.3^{a}	0.2^{c}	0.0^{b}	-0.3^{a}	-0.3^{a}
***Anethum graveolens* L. commercial sample, whole leaves**										
Colour characteristics	**Fresh**	**Sun**	**Shade**	**Oven-40°C**	**Oven-60°C**	**Lyo**	**MW-250W**	**MW-700W**	**S.Freez.**	**F.Freez.**
L* (lightness)	22.9^{cd}	24.5^{bc}	25.5^{b}	25.4^{b}	23.9^{bc}	31.1^{a}	21.2^{d}	21.9^{d}	22.8^{cd}	22.8^{cd}
a* (red/green)	-5.6^{b}	-1.3^{e}	-2.1^{de}	-3.7^{bc}	-2.3^{de}	-7^{a}	-2.2^{de}	-3.1^{cd}	-4.9^{b}	-4.9^{b}
b* (yellow/blue)	8.8^{b}	5.6^{bcd}	7.4^{bc}	7.0^{bc}	5.8^{bcd}	10.4^{a}	4.4^{d}	5.3^{cd}	6.8^{bc}	7.1^{bc}
a*/b*	-0.6^{ab}	-0.2^{c}	-0.3^{bc}	-0.5^{abc}	-0.4^{abc}	-0.7^{a}	-0.5^{abc}	-0.6^{abc}	-0.5^{abc}	-0.5^{abc}
***Anethum graveolens* L. commercial sample, chopped leaves**										
Colour characteristics	**Fresh**	**Sun**	**Shade**	**Oven-40°C**	**Oven-60°C**	**Lyo**	**MW-250W**	**MW-700W**	**S.Freez.**	**F.Freez.**
L* (lightness)	23.5^{cd}	24.7^{bc}	24.7^{bc}	26.1^{b}	24.8^{bc}	32.1^{a}	21.5^{e}	22.5^{de}	23.3^{cd}	23.8^{c}
a* (red/green)	-5.6^{b}	-2.1^{de}	-1.8^{e}	-4.5^{b}	-3.1^{cd}	-6.9^{a}	-2.3^{de}	-3.4^{bc}	-4.3^{b}	-4.3^{b}
b* (yellow/blue)	8.8^{b}	6.6^{bc}	6.6^{bc}	8.2^{b}	6.7^{bc}	11.2^{a}	4.7^{d}	5.7^{cd}	6.9^{b}	6.3^{bc}
a*/b*	-0.6^{ab}	-0.3^{c}	-0.3^{c}	-0.6^{ab}	-0.5^{b}	-0.6^{ab}	-0.5^{b}	-0.6^{ab}	-0.6^{ab}	-0.7^{a}
***Petroselinum crispum* (Mill) Nym. var. *neapolitanum* commercial sample, whole leaves**										
Colour characteristics	**Fresh**	**Sun**	**Shade**	**Oven-40°C**	**Oven-60°C**	**Lyo**	**MW-250W**	**MW-700W**	**S.Freez.**	**F.Freez.**
L* (lightness)	30.9^{b}	29^{bc}	29.7^{bc}	28.8^{bcd}	28.6^{bcd}	33.8^{a}	26.2^{d}	27.6^{cd}	23^{e}	22.9^{e}
a* (red/green)	-10.6^{a}	-4^{de}	-3.2^{f}	-5.9^{bcde}	-3.5^{f}	-7.1^{b}	-4.6^{e}	-5.6^{cde}	-6.5^{bc}	-6.1^{bcd}
b* (yellow/blue)	15.6^{a}	10.8^{bcd}	12.5^{b}	11.9^{b}	11.4^{bc}	12.5^{b}	9^{cde}	10.6^{bcde}	8.7^{de}	8.3^{e}
a*/b*	-0.7^{a}	-0.4^{d}	-0.3^{f}	-0.5^{c}	-0.3^{e}	-0.6^{b}	-0.5^{c}	-0.5^{c}	-0.7^{a}	-0.7^{a}
***Petroselinum crispum* (Mill) Nym. var. *neapolitanum* commercial sample, chopped leaves**										
Colour characteristics	**Fresh**	**Sun**	**Shade**	**Oven-40°C**	**Oven-60°C**	**Lyo**	**MW-250W**	**MW-700W**	**S.Freez.**	**F.Freez.**
L* (lightness)	30.9^{b}	32.1^{b}	34.8^{a}	30.8^{b}	28.7^{c}	35.6^{a}	26.5^{de}	27.8^{cd}	24.9^{e}	25.4^{e}
a* (red/green)	-10.6^{a}	-5.6^{bc}	-1.9^{f}	-6.6^{bc}	-3.7^{de}	-3.3^{e}	-4^{d}	-5.6^{c}	-6.9^{b}	-6.8^{b}
b* (yellow/blue)	15.6^{ab}	13.6^{bc}	17.3^{a}	13.1^{cd}	11.4^{de}	14.1^{bc}	9.5^{ef}	10.9^{ef}	9.2^{ef}	9.5^{ef}
a*/b*	-0.7^{b}	-0.4^{d}	-0.1^{f}	-0.5^{c}	-0.3^{d}	-0.2^{e}	-0.2^{e}	-0.5^{c}	-0.8^{a}	-0.8^{a}

Note: 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.
†Different letters indicate significant differences between means at $p < 0.05$ within characteristics (within rows).

***Petroselinum crispum* (Mill) Nym. var. *neapolitanum* commercial sample**

Whole leaves

The L* value of fresh, whole parsley leaves was 30.9. Among the applied preservation methods, lyophilization slightly enhanced the lightness (L*=33.8), whereas the other preservation methods resulted in some decrease (L*=22.9-29.7) (Table 20).

Lyophilization, freezing methods, oven drying at 40°C and microwave drying at 700 W (a*=-5.6 to -7.1) could preserve the greenish hue of fresh leaves (a*=-10.6) the most. In parallel the highest a* data were observed in samples dried in the sun, in shade and at 60°C (a*=-3.2 to -4.0) (Appendix 3/9).

Every treatments reduced the b*values compared to the fresh sample (15.6). The lowest b* coordinates were observed in frozen samples (b*=8.3-8.7), while shade dried and lyophilized whole leaves had the highest ones (12.5).

Evaluating the a*/b* values, freezing could preserve the fresh sample's original colour the most (-0.7), no significant difference was found between them. Leaves that were lyophilized, oven dried at 40°C and microwave dried at 250 W and 700 W also had very similar appearance properties (a*/b*=-0.5 to -0.6). However, drying in the sun, in shade and at 60°C could not preserve the original colour of whole parsley leaves so well (a*/b*=-0.3 to -0.4). In the research of Soysal (2004) microwave drying methods (from 360 W to 900 W) retained the parsley leaves' colour well, exhibiting only a marginally darker hue compared to its fresh state.

Chopped leaves

Chopping parsley leaves before preservation did not affect the colour changes significantly. Similarly to whole parsley leaves, lyophilization resulted in the highest L* value (35.6) in this case as well, indicating that lyophilized leaves became lighter in appearance than fresh leaves (Table 20). Other preservation methods showed intermediate values.

In relation to the a* coordinate, oven drying at 40°C and freezing methods (a*=-6.6 to -6.9) retained the greenish hue of fresh leaves the most for chopped leaves too, whereas shade drying, oven drying at 60°C and lyophilization reduced it the most (-1.9).

The fresh sample's b* value (15.6) was produced by shade drying and lyophilization primarily (14.1-17.3), whereas frozen samples had the lowest data (9.2-9.5).

According to the a*/b* quotient, frozen samples could be characterised by the colour most similar (a*/b*=-0.8) to the fresh sample (-0.7). However, shade drying and oven drying at 60°C resulted in the highest colour degradation (a*/b*=-0.1 to -0.3), just like for the whole parsley leaves (Appendix 3/10).

Conclusions

Based on the above findings, it can be concluded that freezing is a highly suitable method for preserving the colour characteristics of herbs and spices. There was no significant difference between slow and fast freezing; both methods effectively preserved the natural colour of fresh plant materials. Lyophilization also emerged as a preferable method, as it maintained the original colour of raw samples very well. The lyophilized samples became a little lighter, but the original hues were perfectly preserved. Di Cesare et al., (2003) demonstrated that lyophilization also resulted in lighter hues in *Ocimum basilicum* leaves. Freeze drying *Origanum vulgare* and *Thymus daenensis* showed better colour retention in comparison with other drying methods (Yousif et al., 2000; Rahimmalek and Goli, 2013). Oven drying at lower temperatures (at 40°C) and microwave drying at high power (at 700 W) also proved to be appropriate methods. A comparison study of microwave drying (with the microwave power of 700 W), sun drying, and hot-air drying (at 50°C) of *Salvia rosmarinus* leaves showed that the colour of microwave-dried rosemary was better than that of hot-air dried products (Arslan and Ozcan 2008). Ali et al., (2014) found that drying *Moringa oleifera* leaves at 40°C better preserved their colour compared to leaves dried at higher temperatures of 50°C and 60°C.

Natural drying techniques (drying in the sun and in shade), furthermore microwave drying at 250 W were less effective preservation methods from this point of view, the green colour of samples decreased, the hues slightly changed. This may be attributed to the prolonged duration of shade drying, the exposure to high temperatures and UV light during sun drying and the very intense but relatively longer drying time of microwave drying at 250 W. In the contrary, studies have demonstrated that shade drying preserved the colour of dried products across various herbs like *Salvia rosmarinus* (Khorshidi et al., 2009), *Tanacetum parthenium* (Omidbaigi et al., 2007), *Thymus vulgaris* (Sárosi et al., 2013), *Ocimum basilicum* (Hassanpouraghdam et al., 2010), *Mentha spicata*, *Melissa officinalis*, and *Salvia officinalis* (Rababah et al., 2015).

Our observations revealed that drying at 60°C was the least effective preservation technique for retaining the original colour. In this case the high temperature damaged the chlorophyll content, causing a reduction in green colour and due to this samples became darker and greyer. Mahayothee (2020) examined *Zingiber montanum* samples dried through convection at 50°C and 60°C visually, revealing a slight colour reduction compared to alternative drying techniques.

5.5.2. Taste

The e-tongue results revealed this measurement method's capability for the classification of different preservation methods applied on the studied herbs and spice species. LDA models of e-tongue data built for the classification of different treatments showed distinct results in many herbs and spices. Appendix 4/1-Appendix 4/7 (Appendix 4) contains the confusion tables for the classification of different preservation methods.

***Artemisia dracunculus* L. 'Artemis'**

In relation to French tarragon 'Artemis', the average recognition and prediction abilities were 99.00% and 91.98%, respectively (Appendix 4/1). Here a misclassification was observed, because the fast frozen sample belonged to the slow frozen sample in 9.91% and 40.12% during the training and cross-validation. Thus, the two types of freezing could not be discriminated perfectly, but all the other treatments were completely differentiable. The group of fresh sample was close to the group of lyophilized and groups of frozen samples (Figure 45). Based on this, it can be supposed that the taste of these treatments was very similar.

***Mentha* x *piperita* L. LAMIMENTA18**

The classification accuracy reached 100% during both the training and cross validation of peppermint gene bank accession Nr. LAMIMENTA18 (Appendix 4/2). The fresh sample group was located separately from the other samples (Figure 46). The sample groups of lyophilized and oven dried at 60°C were also very distant from the other treatments. However, the clusters of 250 W and 700 W microwave drying methods were very close to each other, similarly to the slow and fast freezing sample groups. Shade dried, oven dried at 40°C and sun dried sample groups were also located in close proximity to each other. This indicates that the sample groups that were positioned close to each other had similar taste attributes.

***Origanum majorana* L. 'Egyptian'**

The fresh sample of marjoram 'Egyptian' was remarkably distinct from the other samples treated by the different preservation methods (Figure 47). Thus, it's taste proved to be completely different. The average recognition accuracy and prediction accuracy was 99.00% and 91.98% (Appendix 4/3). All the samples showed 100% classification accuracy after cross-validation, except samples dried at 60°C and microwaved at 700 W. For these treatments, a misclassification was observed, because the oven dried (at 60°C) sample belonged to the microwave dried (at 700 W) one in 19.88% during cross validation. The comparison reveals a certain parallelism between the taste of this two methods.

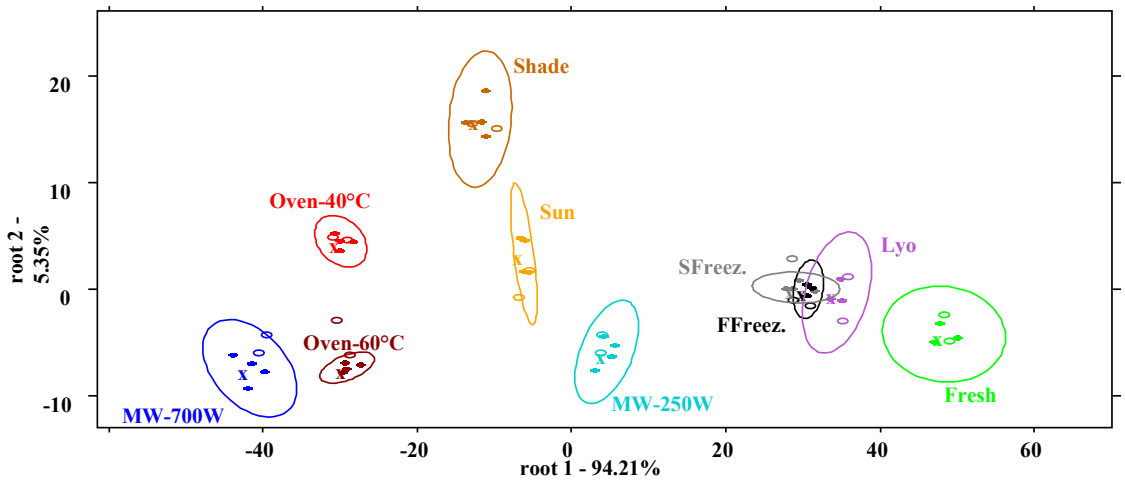

Figure 45. Linear discriminant analysis (LDA) classification model for the grouping of preservation methods based on electronic tongue results applied on *Artemisia dracunculus* L. 'Artemis'

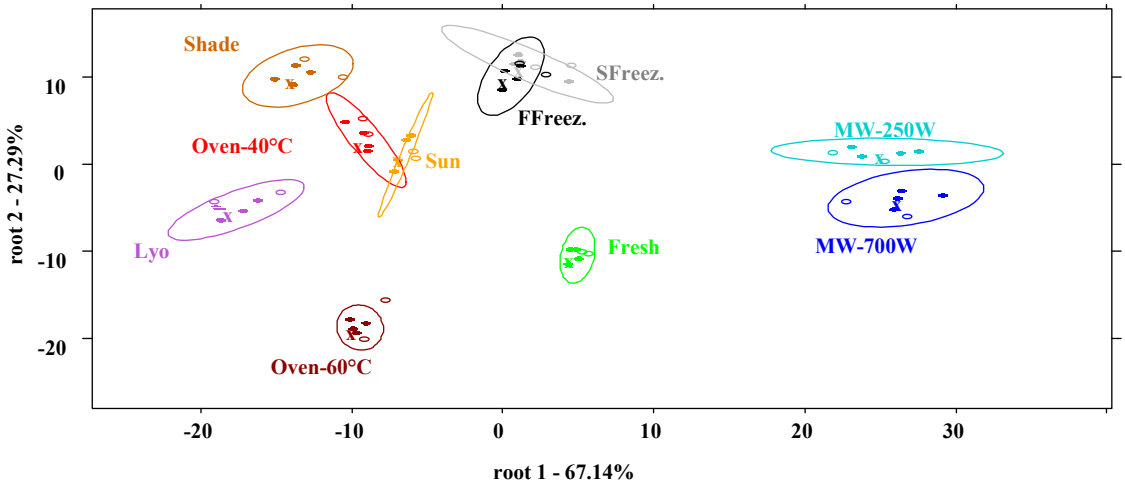

Figure 46. Linear discriminant analysis (LDA) classification model for the grouping of preservation methods based on electronic tongue results applied on *Mentha* x *piperita* L. LAMIMENTA18

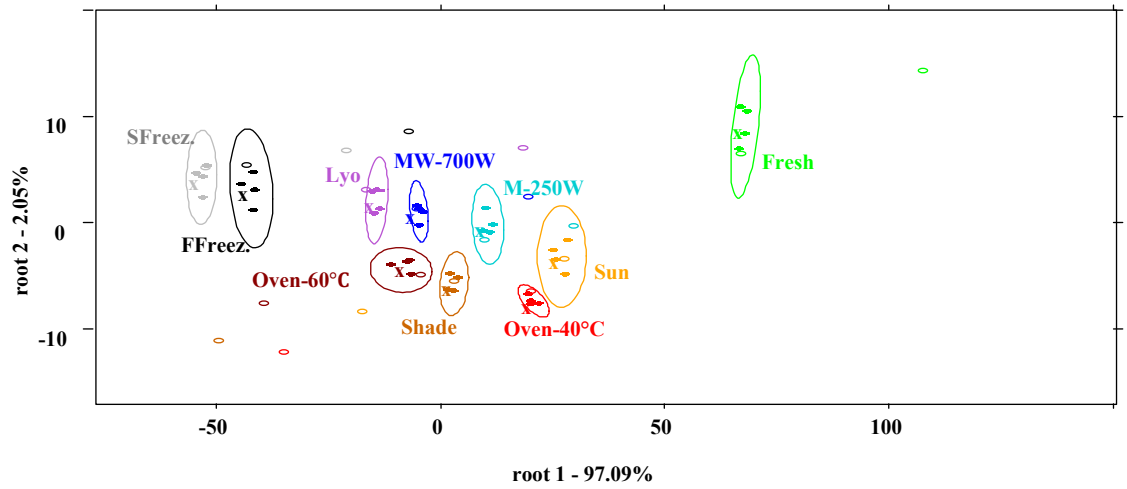

Figure 47. Linear discriminant analysis (LDA) classification model for the grouping of preservation methods based on electronic tongue results applied on *Origanum majorana* L. 'Egyptian'

†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

***Levisticum officinale* Koch. 'Mittelgroblättriger'**

Appendix 4/4 shows the confusion table for the classification of different preservation methods applied in lovage 'Mittelgroblättriger'. The classification accuracy was 100% during the training and cross validation. This means that all the preserved sample groups had different taste characteristics. The fresh sample group was found to be positioned separately from the samples treated by the different preservation methods (Figure 48), but microwave drying at 700 W treatment was located the closest to the fresh sample cluster.

***Satureja hortensis* L. LAMISATU22**

In case of summer savory gene bank accession Nr. LAMISATU22, we observed similar results in the confusion table (Appendix 4/5). All the samples showed 100% classification accuracy during the training and cross-validation. However, the fresh sample group was spectacularly distinct from the clusters of preserved samples (Figure 49).

***Ocimum basilicum* L. 'Genovese'**

The average recognition accuracy and prediction accuracy for sweet basil 'Genovese' was 100% and 95.53%, respectively (Appendix 4/6). The analysis of preservation methods using cross-validation revealed a high degree of accuracy, with 100% classification success in most samples. However, exceptions were noted in case of microwave dried (250 W, 700 W) and naturally dried (sun, shade) samples (Figure 50). Notably, the microwave dried sample at 700 W was misclassified as it was dried at 250 W in 19.88% during cross-validation, suggesting a challenge in distinguishing between the taste of these two preservation methods. Similarly, shade dried sample was incorrectly identified as sun dried in 75.19%, indicating that these two methods were somewhat similar. The fresh and frozen samples were positioned separately and away from the others signifying a difference in taste attributes.

***Origanum vulgare* L. subsp. *vulgare* commercial sample**

The classification accuracy for common oregano was exemplary, achieving a 100% classification accuracy during both training and cross-validation phases, as detailed in Appendix 4/7. The fresh sample group was in a distant position from the other preserved sample groups. However, the analysis revealed a notable proximity between certain sample groups (Figure 51). Specifically, the microwave dried samples were found to be closely aligned with each other, as were the frozen samples with each other. In a similar way, oven dried (at 40°C), sun and shade dried samples were also found to be in close proximity to each other, as illustrated in Figure 53, which indicated their similarity in taste.

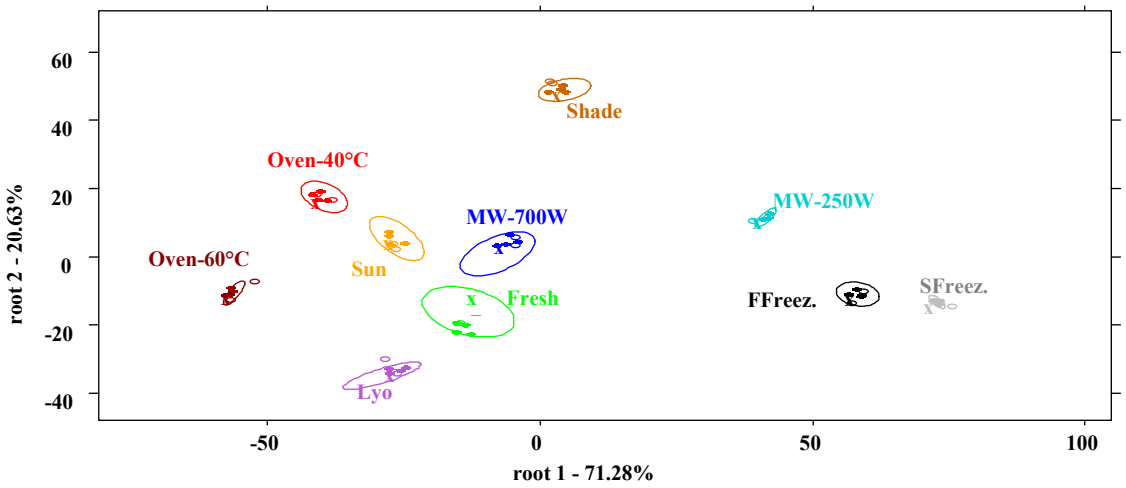

Figure 48. Linear discriminant analysis (LDA) classification model for the grouping of preservation methods based on electronic tongue results applied on *Levisticum officinale* Koch 'Mittelgroblättriger'

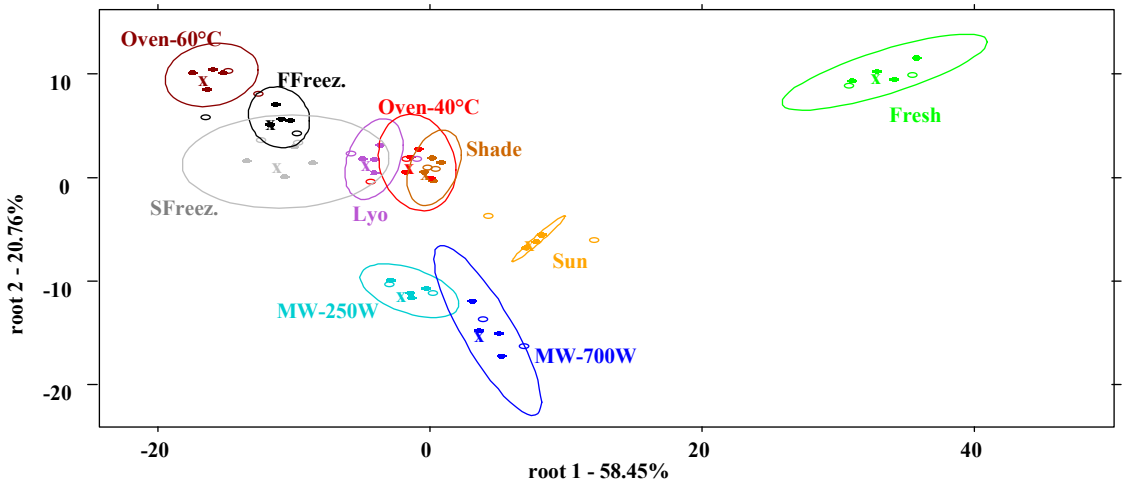

Figure 49. Linear discriminant analysis (LDA) classification model for the grouping of preservation methods based on electronic tongue results applied on *Satureja hortensis* L. LAMISATU22

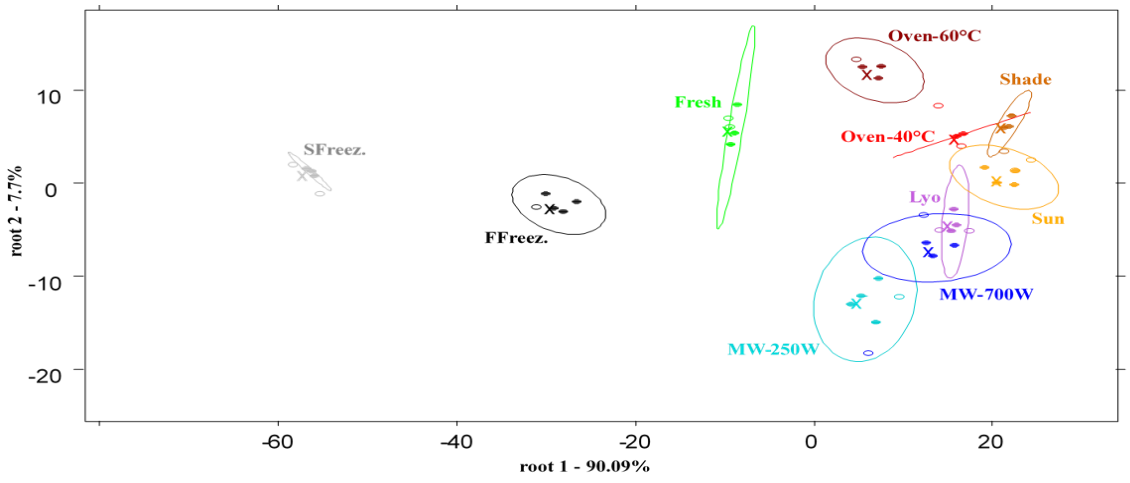

Figure 50. Linear discriminant analysis (LDA) classification model for the grouping of preservation methods based on electronic tongue results applied on *Ocimum basilicum* L. 'Genovese'

†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

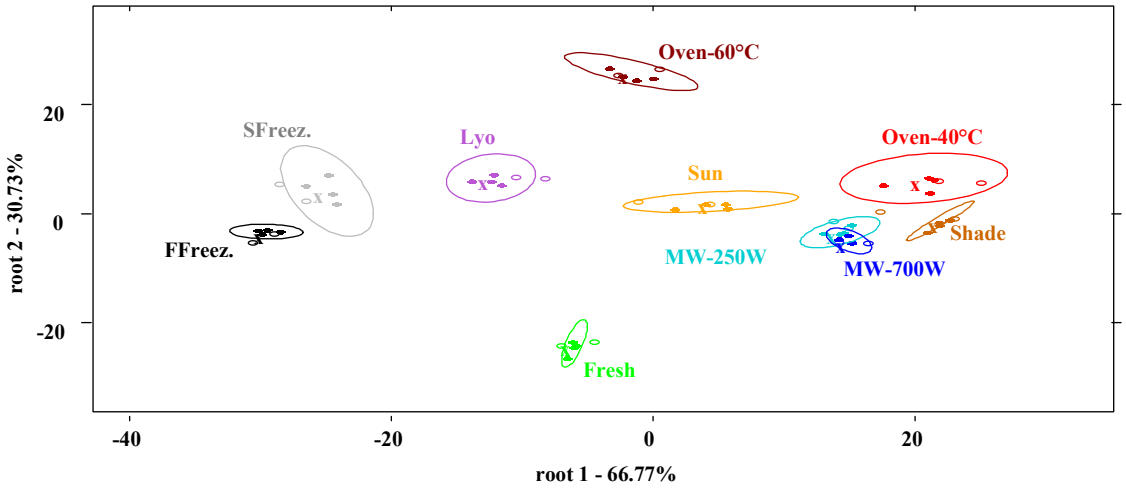

Figure 51. Linear discriminant analysis (LDA) classification model for the grouping of preservation methods based on electronic tongue results applied on *Origanum vulgare* L. subsp. *vulgare*

†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

Conclusions

Electronic tongue (e-tongue) is a sensitive sensor-based instrument used for distinguishing food products and analysing their quality. It is effective in environmental monitoring, pharmaceuticals and food analysis, providing safe, quick and easy-to-use results (Wasilewski et al., 2019; Haddi et al., 2014; Oliveri et al., 2009). Generally, it is used for liquids and has been applied to test the quality of coffee (Arrieta et al., 2020), wine (Haddi et al., 2014; Kovacs et al., 2020; Rudnitskaya, 2018), fruit juices (Haddi et al., 2014), oils (Oliveri et al., 2009; Semenov et al., 2019; Apetrei and Apetrei, 2014), tea (Banerjee et al., 2019) and some semi-solid food like tomato concentrate (Vitalis et al., 2020). However, our findings have clearly demonstrated that the e-tongue method is perfectly suitable for testing the herbal extracts too.

In our experiment, the difference in taste of the alcoholic extracts prepared from samples of herbs and spices was clearly identifiable. The e-tongue results showed that the taste of fresh samples was spectacularly different from that of the treated samples, and each treatment resulted in a distinctly dissimilar flavour character. Fresh samples were the closest in taste to frozen and lyophilised samples in general. We also found that the two types of microwave treatment (250 W and 700 W) had very similar flavoured extracts, and the same was observed for the two types of freezing methods (slow and fast) too.

According to the data, it can be assumed that the flavour of samples is strongly correlated with the EO content and composition of plant materials, but not only these are the influencing factors.

6. 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 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 medicinal and aromatic plants. 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.

7. 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.

8. SUMMARY

The quality of culinary spices, rich in biologically active ingredients (e.g. volatiles and phenoloids), is crucial for their health benefits and organoleptic properties. Every preservation method, both traditional and modern, significantly influences their characteristics. However, research on these methods and their effects on spices has been limited and often contradictory, highlighting the need for further study to optimize preservation techniques for producing products with excellent quality.

The main goal of this research was to describe, how different preservation methods, specifically freezing and drying (using so techniques, which are widespread or perspective in the practice), affect the various properties of spices. In our experiment, nine preservation methods (sun and shade drying, oven drying at 40 and 60ºC, lyophilization, microwave drying at 250 and 700 W, slow and fast freezing) were investigated in comparison to the freshly harvested plant material (control). The properties observed were essential oil (EO) content and composition, total phenolic content (TPC) and total antioxidant capacity (TAC) of aqueous extracts prepared from plants, the rosmarinic acid (RA) and total hydroxycinnamic acid (THA) content, furthermore organoleptic characteristics, such as colour and taste. Eighteen plant species were included in the study.

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. 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 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.

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. Based on SEM photos, 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.

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.

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. 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.

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. 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 spices. 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.

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 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.

For the first time, the *taste* of extracts made from seven 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). 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.

Our results show that each preservation method has its' own advantages and disadvantages. The choice of the appropriate preservation technique should be based on the requirements of final product.

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APPENDICES

Appendix 1

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Publications

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

Appendix 2

Appendix 2/1. The ambient air temperature (°C) during sun drying of the examined plant species for the year 2020

Species	Date	Period	Time	Temperature
Helichrysum italicum* L., *Origanum majorana* L. 'Magyar', *Saturēja hortensis* L. LAMISATU22 and 'Saturn', *Ocimum basilicum* L. 'Ohré', *Origanum vulgare* L. subsp. *vulgare	July 06	Sunny period	11:30	42.2
	July 06		12:00	62.3
	July 06		12:30	45.5
	July 06		13:00	56.1
	July 06		13:30	43.3
	July 06		14:00	40.6
	July 06		14:30	36.7
	July 06-07		15:00-9:30	23.4-26.1
	July 07	Sunny period	10:00	56.8
	July 07		10:30	59.2
	July 07		11:00	60.5
	July 07		11:30	53.4
	July 07		12:00	50.6
	July 07		12:30	38.9
	July 07		13:00	33.4
	July 07		13:30	30.7
	July 07		14:00	27.3
	July 07		14:30	29.8
	July 07-08		15:00-08:00	23-25
	July 08	Sunny period	08:30	30.4
	July 08		09:00	63.2
	July 08		09:30	72.9
	July 08		10:00	52.2
	July 08		10:30	59.4
	July 08		11:00	69.7
	July 08		11:30	63.5
	July 08		12:00	64.6
	July 08		12:30	48.8
	July 08		13:00	49.3
	July 08		13:30	36.8
	July 08		14:00	33.6
	July 08		14:30	30.4
	July 08-09		15:00-11:30	22.5-25.3
	July 09	Sunny period	12:00	47.5
	July 09		12:30	67.7
	July 09		13:00	67.5
	July 09		13:30	59.3
	July 09		14:00	43.8
	July 09		14:30	29.2
	July 09-10		15:00-14:00	22.8-25.7
	July 10	Sunny period	14:30	30.2
	July 10		15:00	31.2
	July 10		15:30	31
	July 10		16:00	29
***Artemisia dracunculus* L. 'Artemis', 'Zöldzamat', *Mentha spicata* L.**	August 01-03		10:00-10:00	22.5-25.3
	August 03	Sunny period	10:30	37.6
	August 03		11:00	52.2
	August 03		11:30	44.4
	August 03		12:00	37.8
	August 03		12:30	32.5
	August 03		13:00	37.1
	August 03		13:30	41.2
	August 03		14:00	36.8
	August 03		14:30	49.8
	August 03		15:00	30.3
	August 03		15:30-23:30	24.5-26.2

Appendix 2/2. The ambient air temperature (°C) during sun drying for the year 2021

Spec	Date	Period	Time	Temperature		Date	Period	Time	Temperature
Levisticum officinale **Koch. 'Mittelgrobblättriger' and ASTLEVI44;** ***Mentha x piperita*** **'Mexián' and LAMIMENTA18**	June 28	Sunny period	11:30	32.5		June 10	Sunny period	13:00	28.3
	June 28		12:00	32.8		June 10		13:30	31.0
	June 28		12:30	32.8		June 10		14:00	28.0
	June 28		13:00	33.1		June 10		14:30	28.0
	June 28		13:30	33.3	***Salvia officinalis*** **L. 'Regula',** ***Melissa officinalis*** **L. 'Lemona',** ***Origanum vulgare subsp. hirtum***	August 03	Sunny period	11:30	25.4
	June 28		14:00	33.0		August 03		12:00	25.3
	June 28		14:30	32.7		August 03		12:30	25.3
	June 28-30		15:00-10:30	22.9-23.1		August 03		13:00	27.0
	June 30	Sunny period	11:00	30.5		August 03		13:30	27.7
	June 30		11:30	31.4		August 03		14:00	26.6
	June 30		12:00	31.9		August 03		14:30	26.3
	June 30		12:30	33.1		August 03-04		15:00-11:00	22.0-25.7
	June 30		13:00	33.4		August 04	Sunny period	11:30	25.7
	June 30		13:30	31.0		August 04		12:00	25.6
	June 30		14:00	34.4		August 04		12:30	25.6
	June 30		14:30	35.8		August 04		13:00	28.0
	June 30		15:00	36.5		August 04		13:30	28.8
	June 30		15:30	36.8		August 04		14:00	30.0
Origanum majorana **L. 'Egyptian',** ***Thymus vulgaris*** **L. 'French Summer', 'Deutscher Winter',** ***Ocimum basilicum*** **L. 'Genovese'**	June 06		11:00-13:00	24.8-27.0		August 04		14:30	37.9
	June 06	Sunny period	13:30	33.0		August 04-05		15:00-11:00	17.0-28.5
	June 06		14:00	32.9		August 05	Sunny period	11:30	19.0
	June 06		14:30	30.8		August 05		12:00	19.0
	June 06		15:00	32.0		August 05		12:30	23.0
	June 06		15:30	31.0		August 05		13:00	23.0
	June 06		16:00	30.6		August 05		13:30	22.0
	June 06		16:30	30.1		August 05		14:00	22.0
	June 06-07		17:00-09:30	22.0-27.0		August 05		14:30	22.0
	June 07	Sunny period	10:00	32.7	***Salvia officinalis*** **L. 'Extrakta',** ***Petroselinum crispum*** **(Mill) (whole and chopped),** ***Anethum graveolens*** **L. (whole and chopped)**	August 06	Sunny period	11:30	23.0
	June 07		10:30	32.4		August 06		12:00	23.0
	June 07		11:00	33.1		August 06		12:30	23.0
	June 07		11:30	32.5		August 06		13:00	23.0
	June 07		12:00	32.8		August 06		13:30	25.0
	June 07		12:30	32.8		August 06		14:00	26.3
	June 07		13:00	33.1		August 06		14:30	34.2
	June 07		13:30	35.0		August 06		15:00	29.0
	June 07		14:00	33.0		August 06-07		15:30-11:00	21.0-29.0
	June 07		14:30	33.0		August 07	Sunny period	11:30	23.6
	June 07-08		15:00-12:00	21.5-27.0		August 07		12:00	23.8
	June 08	Sunny period	12:30	32.0		August 07		12:30	24.0
	June 08		13:00	32.4		August 07		13:00	31.0
	June 08		13:30	36.0		August 07		13:30	28.9
	June 08		14:00	37.5		August 07		14:00	30.1
	June 08		14:30	39.5		August 07		14:30	31.3
	June 08		15:00	30.0		August 07-08		15:00-11:00	22.3-27.6
	June 08-09		15:30-13:00	21.4-26.4		August 08	Study tour	11:30	31.0
	June 09	Sunny period	13:30	31.0		August 08		12:00	28.2
	June 09		14:00	31.0		August 08		12:30	38.0
	June 09		14:30	29.0		August 08		13:00	35.8
	June 09		15:00	49.4		August 08		14:00	37.5
	June 09		15:30	64.8		August 08		14:30	38.7
	June 09		16:00	65.2		August 08-09		15:00-11:00	17.4-26.0
	June 09		16:30	69.8		August 09	Sunny period	11:30	38.0
	June 09		17:00	58.2		August 09		12:00	35.8
	June 09		17:30	59.0		August 09		12:30	37.5
	June 09		18:00	55.2		August 09		13:00	38.7
	June 09		18:30	58.3		August 09		14:00	29.0
	June 09		19:00	39.2		August 09		14:30	28.5
	June 09-10		19:30-11:00	23.0-25.5		August 09-10		15:00-14:30	24.0-26.4
	June 10		11:30	28.0	***Rosm.. officinalis*** **L.**	Sept. 06	sunny per.	10:30-14:30	22.0-24.0
	June 10		12:00	27.4		Sept. 06-07		15:00-10:30	19.0-24.0
	June 10		12:30	27.8		Sept. 07	sunny per.	11:00-15:00	22.9-26.3

Appendix 2/3. The ambient air temperature (°C) during sun drying of the examined plant species for the year 2022

Species	Date	Period	Time	Temperature
***Lavandula angustifolia* 'Budakalászi 80', *Lavandula* x *intermedia* 'Judit'**	June 28	Sunny period	11:30	25.0
	June 28		12:00	40.0
	June 28		12:30	32.0
	June 28		13:00	33.2
	June 28		13:30	32.0
	June 28		14:00	32.0
	June 28		14:30	31.0
	June 28-29		15:00-11:30	19.0-26.0
	June 29	Sunny period	12:00	31.0
	June 29		12:30	31.0
	June 29		13:00	36.4
	June 29		13:30	35.3
	June 29		14:00	33.0
	June 29		14:30	33.4
	June 29		15:00	31.0
	June 29		15:30	33.0
	June 29		16:00	33.0
	June 29-30		16:00-10:30	21.4–27.0
	June 30	Sunny period	11:00	30.0
	June 30		11:30	30.2
	June 30		12:00	34.0
	June 30		12:30	34.1
	June 30		13:00	34.0
	June 30		13:30	37.2
	June 30		14:00	36.0
	June 30		14:30	36.0
	June 30		15:00	35.0
	June 30		15:30	34.0
	June 30		16:00-20:00	27.4–22.4

Appendix 2/4. Dry matter content (%) of the examined plant species

Examined plant species		'Variety' or accession		Dry matter content (%)									
				Fresh	Sun	Shade	Oven-40°C	Oven-60°C	Lyo	MW-250W	MW-700W	SFreez.	FFreez.
Species with glandular hairs or trichomes	Lavender	'Budakalászi 80'		41.74	93.73	93.48	92.88	93.85	92.07	95.03	95.58	41.58	40.37
		'Judit'		36.87	90.25	89.18	89.92	88.95	88.62	96.13	97.27	37.10	37.13
	Lemonbalm	'Lemona'		27.09	94.40	94.15	94.85	95.83	99.53	96.75	97.84	26.61	26.32
	Peppermint	'Mexián'		23.71	93.68	92.97	95.58	95.42	94.08	94.47	95.05	23.51	23.23
	Sweet basil	'Genovese'		13.69	95.95	95.75	96.40	96.00	95.80	96.32	97.05	12.44	14.66
		'Ohré'		16.78	94.30	92.20	95.05	95.37	96.25	96.85	94.77	18.25	15.98
	Marjoram	'Egyptian'		31.90	94.75	94.47	94.48	95.13	98.60	96.05	96.77	32.93	32.80
		'Magyar'		32.21	95.90	93.65	93.78	94.57	96.07	95.85	96.40	32.99	30.55
	Greek oregano	Commercial sample Jellito		24.28	94.63	94.30	93.35	94.90	99.28	93.15	94.15	21.00	21.42
	Rosemary	'Harmat'		38.97	95.08	95.12	95.28	95.88	99.10	96.58	97.08	38.71	38.89
	Garden sage	'Regula'		31.47	94.90	94.88	94.92	95.23	93.23	94.58	94.55	30.69	29.84
	Summer savory	Gene bank accession Nr. LAMISATU22		27.31	94.72	93.80	93.63	94.83	97.23	96.32	96.42	27.59	27.93
		'Saturn'		30.18	94.93	93.40	91.20	92.15	91.33	93.55	95.70	26.86	27.95
	Garden thyme	'French Summer'		35.21	94.30	94.33	94.25	95.98	92.38	96.20	96.45	36.74	36.17
		'Deutscher Winter'		38.25	95.22	94.95	94.87	96.02	91.48	93.83	94.60	37.30	39.76
Species accumulating the EO primarily or exclusively in secretory ducts	French tarragon	'Zöldzamat'		30.49	94.87	93.74	95.42	94.96	96.27	95.20	95.43	29.85	29.64
		'Artemis'		31.03	93.90	93.55	95.68	95.53	93.83	95.87	95.48	30.49	31.33
	Lovage	'Mittelgroblättriger'		19.27	97.52	95.92	96.74	94.89	98.63	95.52	96.25	19.05	18.95
		Gene bank accession Nr. ASTLEVI44		19.41	93.90	93.38	94.25	93.80	95.73	94.90	95.43	16.18	17.41
	Parsley	Commercial sample	Whole	22.75	92.67	92.05	93.12	93.15	98.30	94.35	93.92	21.92	22.38
			Chopped	23.90	95.85	93.15	93.50	93.25	100.45	88.00	92.83	22.60	23.17

***Note*: 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**

Appendix 3

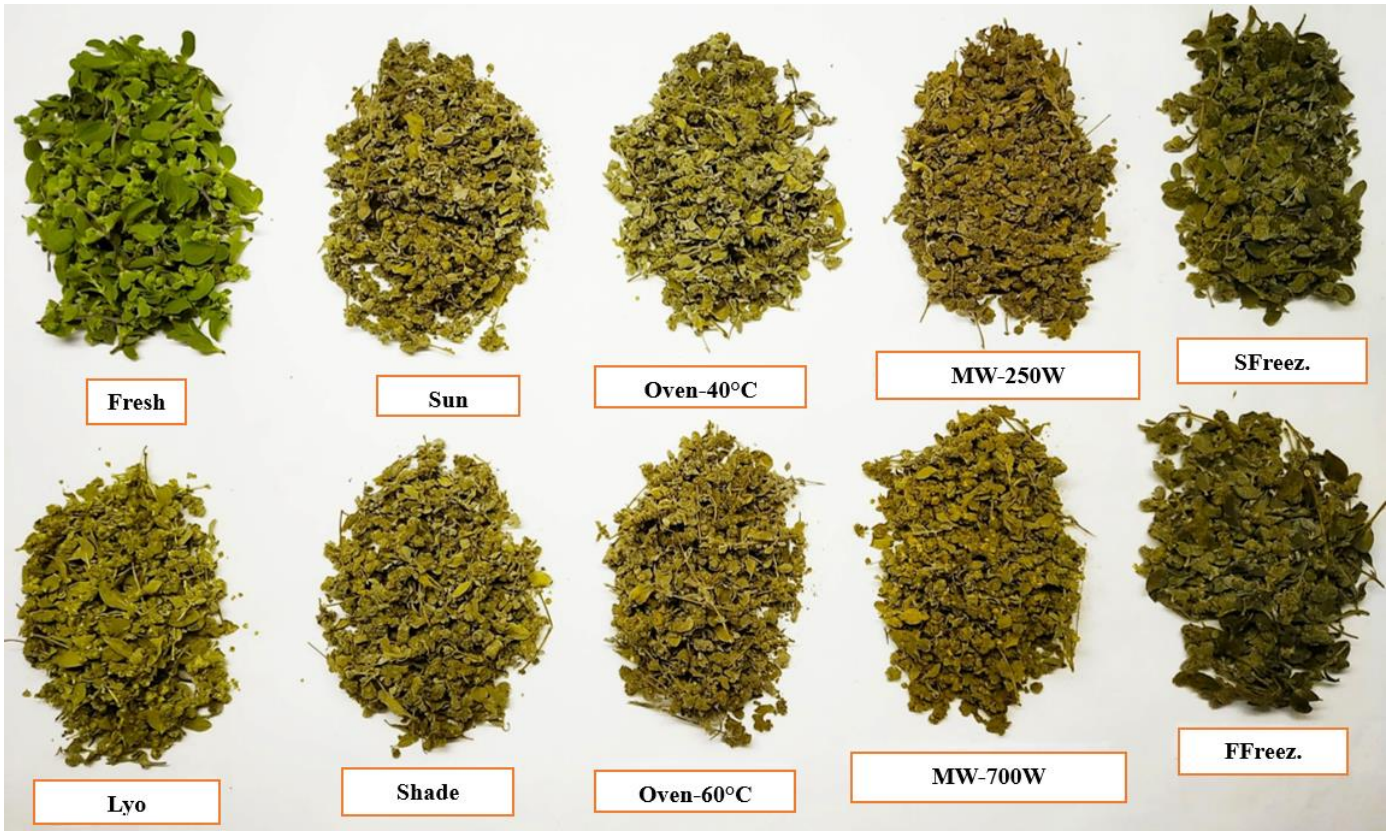
Appendix 3/1. Colour characteristics of fresh and preserved leaves of *Salvia officinalis* L. 'Regula'

Appendix 3/2. Colour characteristics of fresh and preserved leaves of *Ocimum basilicum* L. 'Ohré'

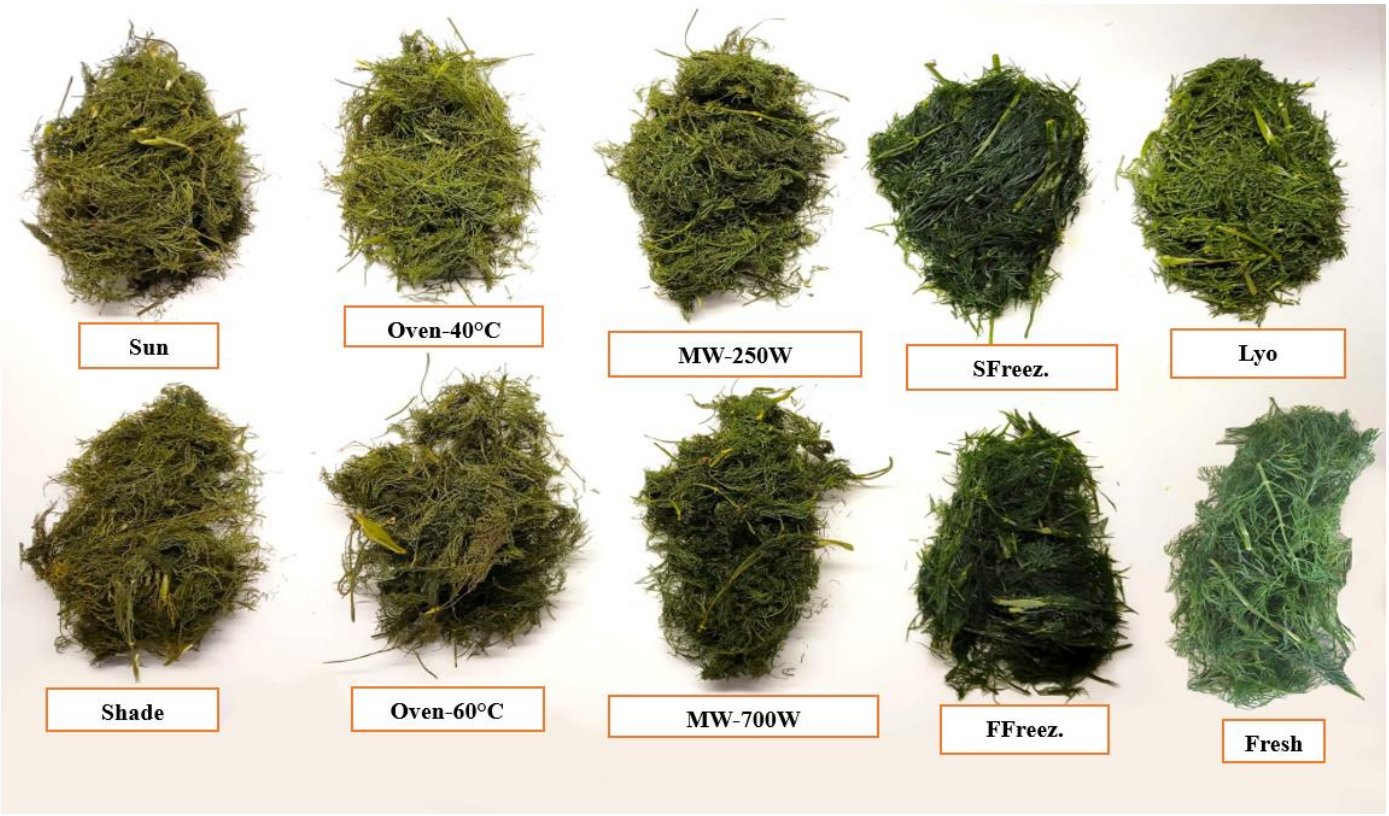
Appendix 3/3. Colour characteristics of fresh and preserved leaves of *Mentha x piperita* 'Mexián'

Appendix 3/4. Colour characteristics of *Levisticum officinale* Koch. 'Mittelgroblättriger'

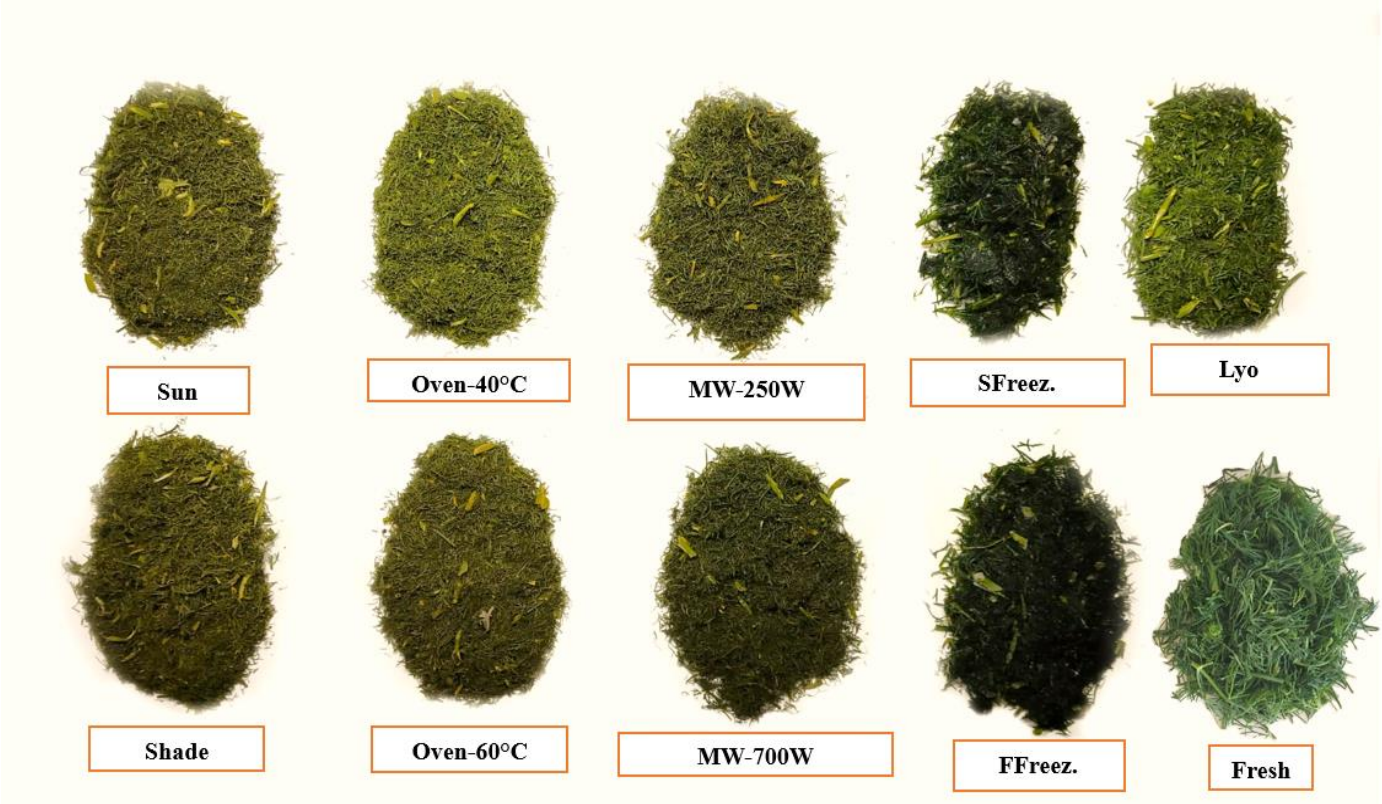
Appendix 3/5. Colour characteristics of fresh and preserved leaves of *Melissa officinalis* L. 'Lemona'



Appendix 3/6. Colour characteristics of fresh and preserved leafy shoots of *Origanum majorana* L. 'Magyar'



Appendix 3/7. Colour characteristics of fresh and preserved, <u>whole</u> leaves of *Anethum graveolens* L.



Appendix 3/8. Colour characteristics of fresh and preserved, <u>chopped</u> leaves of *Anethum graveolens* L.

Appendix 3/9. Colour characteristics of fresh and preserved, <u>whole</u> leaves of *Petroselinum crispum* (Mill) Nym. var. *neapolitanum*

Appendix 3/10. Colour characteristics of fresh and preserved, <u>chopped</u> leaves of *Petroselinum crispum* (Mill) Nym. var. *neapolitanum*

Appendix 4

Appendix 4/1. Confusion table for the classification of different preservation methods applied in *Artemisia dracunculus* L. 'Artemis'. Classifications are expressed as percentages (%)

Average accuracies		FFreez	Fresh	Lyo	MW-250W	MW-700W	Oven-40°C	Oven-60°C	Shade	SFreez	Sun
Recognition 99.00%	FFreez.	90.09	0	0	0	0	0	0	0	0	0
	Fresh	0	100	0	0	0	0	0	0	0	0
	Lyo	0	0	100	0	0	0	0	0	0	0
	MW-250W	0	0	0	100	0	0	0	0	0	0
	MW-700W	0	0	0	0	100	0	0	0	0	0
	Oven-40°C	0	0	0	0	0	100	0	0	0	0
	Oven-60°C	0	0	0	0	0	0	100	0	0	0
	Shade	0	0	0	0	0	0	0	100	0	0
	SFreez.	9.91	0	0	0	0	0	0	0	100	0
	Sun	0	0	0	0	0	0	0	0	0	100
Cross-validation 91.98%	FFreez.	59.88	0	0	0	0	0	0	0	40.12	0
	Fresh	0	100	0	0	0	0	0	0	0	0
	Lyo	0	0	100	0	0	0	0	0	0	0
	MW-250W	0	0	0	100	0	0	0	0	0	0
	MW-700W	0	0	0	0	100	0	0	0	0	0
	Oven-40°C	0	0	0	0	0	100	0	0	0	0
	Oven-60°C	0	0	0	0	0	0	100	0	0	0
	Shade	0	0	0	0	0	0	0	100	0	0
	SFreez.	40.12	0	0	0	0	0	0	0	59.88	0
	Sun	0	0	0	0	0	0	0	0	0	100

Appendix 4/2. Confusion table for the classification of different preservation methods applied in *Mentha x piperita* L. gene bank accession Nr. LAMIMENTA18. Classifications are expressed as percentages (%)

Average accuracies		FFreez	Fresh	Lyo	MW-250W	MW-700W	Oven-40°C	Oven-60°C	Shade	SFreez	Sun
Recognition 99.00%	FFreez.	100	0	0	0	0	0	0	0	0	0
	Fresh	0	100	0	0	0	0	0	0	0	0
	Lyo	0	0	100	0	0	0	0	0	0	0
	MW-250W	0	0	0	100	0	0	0	0	0	0
	MW-700W	0	0	0	0	100	0	0	0	0	0
	Oven-40°C	0	0	0	0	0	100	0	0	0	0
	Oven-60°C	0	0	0	0	0	0	100	0	0	0
	Shade	0	0	0	0	0	0	0	100	0	0
	SFreez.	0	0	0	0	0	0	0	0	100	0
	Sun	0	0	0	0	0	0	0	0	0	100
Cross-validation 91.98%	FFreez.	100	0	0	0	0	0	0	0	0	0
	Fresh	0	100	0	0	0	0	0	0	0	0
	Lyo	0	0	100	0	0	0	0	0	0	0
	MW-250W	0	0	0	100	0	0	0	0	0	0
	MW-700W	0	0	0	0	100	0	0	0	0	0
	Oven-40°C	0	0	0	0	0	100	0	0	0	0
	Oven-60°C	0	0	0	0	0	0	100	0	0	0
	Shade	0	0	0	0	0	0	0	100	0	0
	SFreez.	0	0	0	0	0	0	0	0	100	0
	Sun	0	0	0	0	0	0	0	0	0	100

†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

Appendix 4/3. Confusion table for the classification of different preservation methods applied in *Origanum majorana* L. 'Egyptian'. Classifications are expressed as percentages (%)

Average accuracies		FFreez	Fresh	Lyo	MW-250W	MW-700W	Oven-40°C	Oven-60°C	Shade	SFreez	Sun
Recognition 99.00%	FFreez.	100	0	0	0	0	0	0	0	0	0
	Fresh	0	100	0	0	0	0	0	0	0	0
	Lyo	0	0	100	0	0	0	0	0	0	0
	MW-250W	0	0	0	100	0	0	0	0	0	0
	MW-700W	0	0	0	0	100	0	0	0	0	0
	Oven-40°C	0	0	0	0	0	100	0	0	0	0
	Oven-60°C	0	0	0	0	0	0	100	0	0	0
	Shade	0	0	0	0	0	0	0	100	0	0
	SFreez.	0	0	0	0	0	0	0	0	100	0
	Sun	0	0	0	0	0	0	0	0	0	100
Cross-validation 91.98%	FFreez.	100	0	0	0	0	0	0	0	0	0
	Fresh	0	100	0	0	0	0	0	0	0	0
	Lyo	0	0	100	0	0	0	0	0	0	0
	MW-250W	0	0	0	100	0	0	0	0	0	0
	MW-700W	0	0	0	0	100	0	**19.88**	0	0	0
	Oven-40°C	0	0	0	0	0	100	0	0	0	0
	Oven-60°C	0	0	0	0	0	0	**80.12**	0	0	0
	Shade	0	0	0	0	0	0	0	100	0	0
	SFreez.	0	0	0	0	0	0	0	0	100	0
	Sun	0	0	0	0	0	0	0	0	0	100

Appendix 4/4. Confusion table for the classification of different preservation methods applied in *Levisticum officinale* Koch. 'Mittelgroblättriger'. Classifications are expressed as percentages (%)

Average accuracies		FFreez	Fresh	Lyo	MW-250W	MW-700W	Oven-40°C	Oven-60°C	Shade	SFreez	Sun
Recognition 99.00%	FFreez.	100	0	0	0	0	0	0	0	0	0
	Fresh	0	100	0	0	0	0	0	0	0	0
	Lyo	0	0	100	0	0	0	0	0	0	0
	MW-250W	0	0	0	100	0	0	0	0	0	0
	MW-700W	0	0	0	0	100	0	0	0	0	0
	Oven-40°C	0	0	0	0	0	100	0	0	0	0
	Oven-60°C	0	0	0	0	0	0	100	0	0	0
	Shade	0	0	0	0	0	0	0	100	0	0
	SFreez.	0	0	0	0	0	0	0	0	100	0
	Sun	0	0	0	0	0	0	0	0	0	100
Cross-validation 91.98%	FFreez.	100	0	0	0	0	0	0	0	0	0
	Fresh	0	100	0	0	0	0	0	0	0	0
	Lyo	0	0	100	0	0	0	0	0	0	0
	MW-250W	0	0	0	100	0	0	0	0	0	0
	MW-700W	0	0	0	0	100	0	0	0	0	0
	Oven-40°C	0	0	0	0	0	100	0	0	0	0
	Oven-60°C	0	0	0	0	0	0	100	0	0	0
	Shade	0	0	0	0	0	0	0	100	0	0
	SFreez.	0	0	0	0	0	0	0	0	100	0
	Sun	0	0	0	0	0	0	0	0	0	100

†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

Appendix 4/5. Confusion table for the classification of different preservation methods applied in *Satureja hortensis* L. gene bank accession Nr. LAMISATU22. Classifications are expressed as percentages (%)

Average accuracies		FFreez	Fresh	Lyo	MW-250W	MW-700W	Oven-40°C	Oven-60°C	Shade	SFreez	Sun
Recognition 99.00%	FFreez.	100	0	0	0	0	0	0	0	0	0
	Fresh	0	100	0	0	0	0	0	0	0	0
	Lyo	0	0	100	0	0	0	0	0	0	0
	MW-250W	0	0	0	100	0	0	0	0	0	0
	MW-700W	0	0	0	0	100	0	0	0	0	0
	Oven-40°C	0	0	0	0	0	100	0	0	0	0
	Oven-60°C	0	0	0	0	0	0	100	0	0	0
	Shade	0	0	0	0	0	0	0	100	0	0
	SFreez.	0	0	0	0	0	0	0	0	100	0
	Sun	0	0	0	0	0	0	0	0	0	100
Cross-validation 91.98%	FFreez.	100	0	0	0	0	0	0	0	0	0
	Fresh	0	100	0	0	0	0	0	0	0	0
	Lyo	0	0	100	0	0	0	0	0	0	0
	MW-250W	0	0	0	100	0	0	0	0	0	0
	MW-700W	0	0	0	0	100	0	0	0	0	0
	Oven-40°C	0	0	0	0	0	100	0	0	0	0
	Oven-60°C	0	0	0	0	0	0	100	0	0	0
	Shade	0	0	0	0	0	0	0	100	0	0
	SFreez.	0	0	0	0	0	0	0	0	100	0
	Sun	0	0	0	0	0	0	0	0	0	100

Appendix 4/6. Confusion table for the classification of different preservation methods applied in *Ocimum basilicum* L. 'Genovese'. Classifications are expressed as percentages (%)

Average accuracies		FFreez	Fresh	Lyo	MW-250W	MW-700W	Oven-40°C	Oven-60°C	Shade	SFreez	Sun
ecognition 99.00%	FFreez.	100	0	0	0	0	0	0	0	0	0
	Fresh	0	100	0	0	0	0	0	0	0	0
	Lyo	0	0	100	0	0	0	0	0	0	0
	MW-250W	0	0	0	100	0	0	0	0	0	0
	MW-700W	0	0	0	0	100	0	0	0	0	0
	Oven-40°C	0	0	0	0	0	100	0	0	0	0
	Oven-60°C	0	0	0	0	0	0	100	0	0	0
	Shade	0	0	0	0	0	0	0	100	0	0
	SFreez.	0	0	0	0	0	0	0	0	100	0
	Sun	0	0	0	0	0	0	0	0	0	100
Cross-validation 91.98%	FFreez.	100	0	0	0	0	0	0	0	0	0
	Fresh	0	100	0	0	0	0	0	0	0	0
	Lyo	0	0	100	0	0	0	0	0	0	0
	MW-250W	0	0	0	100	**19.88**	0	0	0	0	0
	MW-700W	0	0	0	0	**80.12**	0	0	0	0	0
	Oven-40°C	0	0	0	0	0	100	0	0	0	0
	Oven-60°C	0	0	0	0	0	0	100	0	0	0
	Shade	0	0	0	0	0	0	0	**75.19**	0	0
	SFreez.	0	0	0	0	0	0	0	0	100	0
	Sun	0	0	0	0	0	0	0	**24.81**	0	100

†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

Appendix 4/7. Confusion table for the classification of different preservation methods applied in *Origanum vulgare* L. subsp. *vulgare* commercial sample. Classifications are expressed as percentages (%)

Average accuracies		FFreez	Fresh	Lyo	MW-250W	MW-700W	Oven-40°C	Oven-60°C	Shade	SFreez	Sun
Recog-nition 99.00%	FFreez.	100	0	0	0	0	0	0	0	0	0
	Fresh	0	100	0	0	0	0	0	0	0	0
	Lyo	0	0	100	0	0	0	0	0	0	0
	MW-250W	0	0	0	100	0	0	0	0	0	0
	MW-700W	0	0	0	0	100	0	0	0	0	0
	Oven-40°C	0	0	0	0	0	100	0	0	0	0
	Oven-60°C	0	0	0	0	0	0	100	0	0	0
	Shade	0	0	0	0	0	0	0	100	0	0
	SFreez.	0	0	0	0	0	0	0	0	100	0
	Sun	0	0	0	0	0	0	0	0	0	100
Cross-validation 91.98%	FFreez.	100	0	0	0	0	0	0	0	0	0
	Fresh	0	100	0	0	0	0	0	0	0	0
	Lyo	0	0	100	0	0	0	0	0	0	0
	MW-250W	0	0	0	100	0	0	0	0	0	0
	MW-700W	0	0	0	0	100	0	0	0	0	0
	Oven-40°C	0	0	0	0	0	100	0	0	0	0
	Oven-60°C	0	0	0	0	0	0	100	0	0	0
	Shade	0	0	0	0	0	0	0	100	0	0
	SFreez.	0	0	0	0	0	0	0	0	100	0
	Sun	0	0	0	0	0	0	0	0	0	100

†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