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REVIEW PAPER

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LYOPHILIZATION AND ITS EFFECTS ON THE ESSENTIAL OIL CONTENT AND COMPOSITION OF HERBS AND SPICES – A REVIEW

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ABSTRACT

Herbs and spices have a prolonged history of use in culinary purposes, as preservatives and also as pharmaceuticals. Their characteristic flavour and aroma is mainly due to the volatiles accumulated within them. These compounds are very sensitive to different factors (e.g. high temperature, moisture or oxygen), therefore the methods used for preserving herbs and spices can influence not only their healing characteristics but also organoleptic properties and enjoyment value. During the drying process, the temperature of plant parts is increased, which results in the loss of essential oils. Among the artificial drying methods, lyophilization or freeze-drying helps to obtain dried products with good organoleptic properties, as this method uses a low temperature and a shorter processing time. In recent times, lyophilization has become an accepted method for preserving different herbs and spices as it has many advantages compared with other drying and preservation techniques. However, only relatively little and contradictory information is available in relation to the formation of essential oils during freeze-drying. This review aims to summarize the effects of lyophilization on the essential oil content and composition of herbs and spices.

Keywords: herbs, spices, essential oil, composition, drying, lyophilization

INTRODUCTION

There is a basic presumption that lyophilization properly preserves the quality and quantity of active substances present in herbs and spices; therefore it is superior to other preservation techniques. A few systematic studies have been conducted to verify these assumptions, and according to these results, lyophilization has significant effects on the volatile profiles of herbs and spices (Abascal et al., 2005). Due to its low operating temperature, lyophilization has been recommended by several research findings as an appropriate drying method for preserving the fresh aroma of spices (Antal, 2010). However, in some other studies the negative, modifying effects of freeze-drying were also described.

AROMATIC HERBS AND SPICES

According to the European Spice Association, culinary herbs and spices are the consumable parts of plants that are traditionally added to foodstuffs for either their natural flavoring, aromatic and/or visual properties (ESA, 2018). These special plants have been known and exploited since ancient times for culinary purposes, as preservatives and also as pharmaceuticals. They are the sources of different phytochemicals, many of which contain essential antioxidants and possess remarkable pharmacological and medicinal properties apart from making food taste good (Kähkönen et al., 1999; Newman and Cragg, 2012; Velioglu et al., 1998). In general,

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herbs and spices contain volatile oils, which determine their organoleptic properties and value to consumers.

Volatile compounds are mainly composed of terpenes biogenerated by the mevalonate pathway, which is also known as the isoprenoid pathway. Besides containing hydrocarbon and oxygenated monoterpenes and sesquiterpenes, they also contain phenolic compounds, which are derived via the shikimate pathway (Dhifi et al., 2016). Volatile oils can be synthesized by different plant organs, both internally and externally. Internally, these oils are present in secretory cells (e.g. bay laurel) or in intercellular secretory ducts (e.g. lovage, dill, etc.), and externally in glandular hairs (e.g. sweet basil, marjoram, tarragon, etc.). Essential oils are widely used in aromatherapy, cosmetics and perfumery industries. They also possess antioxidant, antifungal, antibacterial and chemopreventive properties (Bassolé and Juliani, 2012; Iqbal et al., 2013; Victoria et al., 2012). Essential oils are sensitive to sunlight, high temperature, moisture and oxygen. Hence, processing and preserving plant parts containing volatiles requires a great deal of expertise.

DRYING – AN IMPORTANT POST-HARVEST OPERATION

The physical and chemical properties of herbs and spices after harvest are determined by the amount of moisture present in them. Protecting plant materials against deterioration and spoilage is usually carried out by reducing their water content to the appropriate minimum level. Drying is one of the most important post-harvest operations, which lowers the plant moisture content and 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). The process also results in a reduction in the weight and volume of plant materials, with a positive outcome for transport and storage (Calixto, 2000).

The main aims of drying herbs and spices are as follows:

- To obtain a final product without any change or with the desired change in the amount and composition of active substances present or in a favourable standard quality that can be accepted by the consumers.
- To ensure no negative changes in 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.

Drying can be carried out in a natural way (using the energy of sun or in the shade) and in an artificial way, with the help of different drying instruments.

LYOPHILIZATION

Among artificial drying methods, lyophilization, also known as freeze-drying is a rather new, non-convective drying technique which uses a low temperature and a shorter processing time (Gaidhani et al., 2015). It seems to be very effective and promising, but is rather cost-intensive and hence prevents the extensive use of this method (Díaz-Maroto et al., 2002; Özcan et al., 2005).

Lyophilization is a process of drying in which water in the sample is first frozen and subsequently removed by sublimation under a vacuum. Sublimation is the main principle involved in this drying process, where water passes directly from a solid state (ice) to a vapour state without passing through the liquid state. The lyophilization process consists of three major stages: freezing – to transform most of the water into ice; primary drying – to sublime the ice; and secondary drying – to separate unfrozen water by desorption. Annealing is an optional step in lyophilization, which is occasionally used to crystallize the formulation component (Lavakumar et al., 2013).

The entire process of lyophilization is conducted at a low temperature and pressure, hence it is suitable for drying compounds which are thermolabile or otherwise unstable in aqueous solutions for prolonged storage periods, but that are stable in their dry state. The aspect of the lyophilization process which makes it different from other dehydration techniques is that most of the deterioration and microbiological reactions are stopped due to the absence of liquid water and the required low temperatures, which lead to a final product of excellent quality (Ratti, 2001).

The introduction of lyophilization as a practical commercial process dates back to World War II and its first application was found by Greaves in 1944 for the preservation of blood plasma (Greaves, 1954; Kumar et al., 2011). There are numerous applications of lyophilization, most commonly in the food and pharmaceutical industries. Despite many advantages, lyophilization has always been identified as a cost-intensive process. However, its cost varies depending on the type of raw material used, the packaging, the capacity of the plant, duration of cycle, etc.

EFFECT OF LYOPHILIZATION ON THE ESSENTIAL OIL CONTENT OF HERBS AND SPICES

In recent times, lyophilization has become an accepted method for preserving different herbs and spices, as it has many advantages. According to Thamkaew et al. (2020), lyophilization helps in better preservation of the essential oil content in many types of herbs and spices compared with other drying and preservation techniques. However, the effect of freeze-drying on the active compounds of plants is not entirely unanimous, and the data in the literature are often contradictory.

In the case of basil (*Ocimum basilicum* L.), the lyophilized samples recorded a higher essential oil yield in the 'Green' and 'Purple' landraces examined when 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., 2013). On the other hand, another study concluded that the total essential oil content of basil and bay 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 basil had lower retention of volatile compounds than microwave dried samples (Di Cesare et al., 2004).

In relation to coriander (*Coriandrum sativum* L.), the highest essential oil yield was also obtained from lyophilized aerial parts (0.18 ml/100 g dry matter) and no significant differences were observed among oil yields distilled from fresh plant materials and those dried by lyophilization (Pirbalouti et al., 2017). Likewise, in case of aerial parts of Bakhtiari savory (*Satureja bachtiarica* Bunge), freeze-drying recorded a higher percentage of essential oil yield (2.1%) than oven drying (65ºC), sun-drying or shade-drying (Pirbalouti et al., 2013). In the case of purple perilla (*Perilla frutescens*) leaves, the lowest essential oil yield (0.32%) was observed in case of lyophilization compared to oven-drying, shade-drying, sun-drying or mid-infrared-drying. The expanded porous structure of lyophilized leaves could cause the diffusion of essential oil to the air (Xing et al., 2017).

The amount of isolated essential oil is greatly influenced by the chamber pressure and the freezing temperature. Freeze-dried lemon balm leaves at high pressure (250–300 Pa for 14 hours) exhibited a higher content of essential oil (0.252 v/w%) compared with those freeze-dried at low (50–80 Pa for 12 hours) pressure (0.191 v/w%). This is due to the fact that volatiles of lemon balm are found in peltate glandular trichomes on the surface of leaves, and low pressure in the freeze dryer chamber strongly affects the glandular hairs, which results in significant loss of essential oils (Antal et al., 2014). According to another study, the extremely low temperature used during freeze-drying could be responsible for the high losses of essential oils of *M. spicata* leaves (Díaz-Maroto et al., 2003).

EFFECT OF LYOPHILIZATION ON THE ESSENTIAL OIL COMPOSITION OF HERBS AND SPICES

According to many studies, in comparison with other drying methods, lyophilization gives better retention of the major volatile compounds. In sage (*Salvia officinalis* L.) and thyme (*Thymus vulgaris* L.), lyophilization preserved the total volatile compounds relatively well. The character of the changes in thyme constituents isolated by simultaneous distillation-extraction (SDE) is very similar to that of sage, although some twists were observed. For instance, the amount of β-caryophyllene in the freeze-dried herb increased by 37% (Venskutonis, 1997). Similar results were obtained in the case of freeze-dried thyme, where the major volatile compounds β-caryophyllene and thymol were found to be increased (282 and 6907 mg/kg respectively) when compared to the fresh plant materials (β-caryophyllene – 213 mg/kg and thymol – 6465 mg/kg) (Venskutonis et al., 1996). In the case of Mexican oregano (*Lippia berlandieri* Schauer), there was a noticeable and significant increase in the concentration of a major aroma volatile β-myrcene upon lyophilization. Greater hydrolysis of the non-volatile conjugates of the lyophilized samples during the extraction process in the purge and trap apparatus may be responsible for this increase in concentration. Statistical analysis also showed a significant reduction in thymol concentration in air-dried samples, while its concentration remained unchanged in lyophilized samples (Yousif et al., 2000). The study confirmed that lyophilized fennel (*Foeniculum vulgare* Mill.) maintained its volatile composition much better than conventionally dried samples. The GC-MS analysis confirmed that *trans*-anethole, a phenyl propanoid derivative and the major volatile compound found in fennel plants, was better preserved in freeze-dried samples for 24 hrs. The amount of isoanethole, another important aroma compound of fennel was found to be higher for the freeze-dried sample than in the fresh one (Gardeli et al., 2010). Similarly, the retention of the major compounds of the fresh dill herb (*Anethum graveolens* L.), α-phellandrene and 3,6-dimethyl-2,3,3a,4,5,7a-hexahydrobenzofuran in the freeze-dried samples was better compared to the hot air-dried samples. However, in the process of freeze--drying, high amounts of phytadienes (neophytadiene) were also produced and the formation of these secondary aroma compounds during processing may alter the flavour of the final products (Houpalathi et al., 1985). The ratio of thymol, which is present in the leaves of Mexican oregano (*Lippia berlandieri* Schauer) and thyme (*Thymus vulgaris* L.), was relatively higher in 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 leaves (Sárosi et al., 2013). In comparison to the fresh, sundried and oven-dried samples of Bakhtiari savory (*Satureja bachtiarica* Bunge), the carvacrol content was the highest (41.5%) in freeze-dried samples than other major compounds like γ-terpinene (18.3%), thymol (13.0%) and *p*-cymene (12.7%) (Pirbalouti et al., 2013). Lyophilization preserved a larger amount of citral (64.7%) than oven-drying at 40ºC (55.4%) in the case of lemon verbena (*Lippia citriodora* Kunth) (Ebadi et al., 2015).

In relation to the basil (*Ocimum basilicum* L.) landraces 'Green' and 'Purple', the major compound methyl chavicol was found to be highest in freeze-dried samples (44.5% and 60.7%, respectively) (Pirbalouti et al., 2013). In another study, the ratio of eugenol (the main compound) was also found to be the highest in the freeze-dried samples of cultivar *Ocimum basilicum* 'Mesten' (7.0%) and *Ocimum sanctum* 'Local' (5.3%) (Bowes and Zheljazkov, 2004). In bay leaf, freeze-drying led to an increase in the concentration level of eugenol (445.5 μg/g dry weight) compared to air-drying and oven-drying at 45ºC (Díaz-Maroto et al., 2002).

Lyophilization can also cause a significant changing in the aroma compounds of dried herbs and spices (Cálin-Sánchez et al., 2013). Freeze-drying resulted in a significant change in the volatile constituents of *Anthriscus sylvestris* (L.) Hoffm. (Bos et al., 2002) and *Petroselinum crispum* L. (Díaz-Maroto et al., 2003) compared to fresh plant materials. In a study by Díaz-Maroto et al. (2002), a significant change was observed in the essential oil composition of lyophilized parsley leaves (*Petroselinum crispum* L.) compared with the air-dried sample: the ratio of *p*--mentha-1,3,8-triene and apiole, which are responsible for the characteristic odour in parsley, decreased, while myristicin, another active substance found abundantly in parsley, showed no significant differences (Díaz-Maroto et al., 2002).

Drying time and essential oil composition are also strongly influenced by the chamber pressure. A decrease in drying chamber pressure reduces the freeze-drying time but increases the release of volatile compounds. In lemonbalm (*Melissa officinalis* L.) the retained volatile compounds such as citral, citronellal, geraniol and limonene were significantly higher at a high pressure (250–300 Pa) than at a low pressure (50–80 Pa) (Antal et al., 2014). In another study, the higher chamber pressure tended to lengthen the drying time but preserved the major volatile compounds (carvone and citronellol) of spearmint (*Mentha spicata* L.) better in the final product. 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 (Antal et al., 2011).

Although many studies have been conducted on the compositional changes in terms of the bioactive compounds of aromatic herbs and spices caused by lyophilization, there are still many plant species (for instance, *Borago officinalis*) where no such studies have been conducted and it could be interesting to determine if their bioactivity is retained with this preservation technique (Fernandes et al., 2018).

CONCLUSIONS

The rising demand for herbs and spices has encouraged researchers to develop new drying techniques which can preserve the essential oils and other biologically active compounds in high amounts, which do not damage the appearance, flavour, colour, and texture of the dried plant material. According to the literature data, it seems that lyophilization can be an advantageous technique for preserving the volatile profile of plants, but its effects depend on the plant species (the place of the volatiles' accumulation, the type of volatile components, etc.). In addition, the technical parameters of lyophilization also have a significant influence on the quality of the final product. Information on the negative effects of lyophilization on volatiles from herbs and spices is still scarce and insufficient to explain the contradictory effects of lyophilization on the essential oils of herbs and spices. Therefore it is difficult to draw firm conclusions about its effects.

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