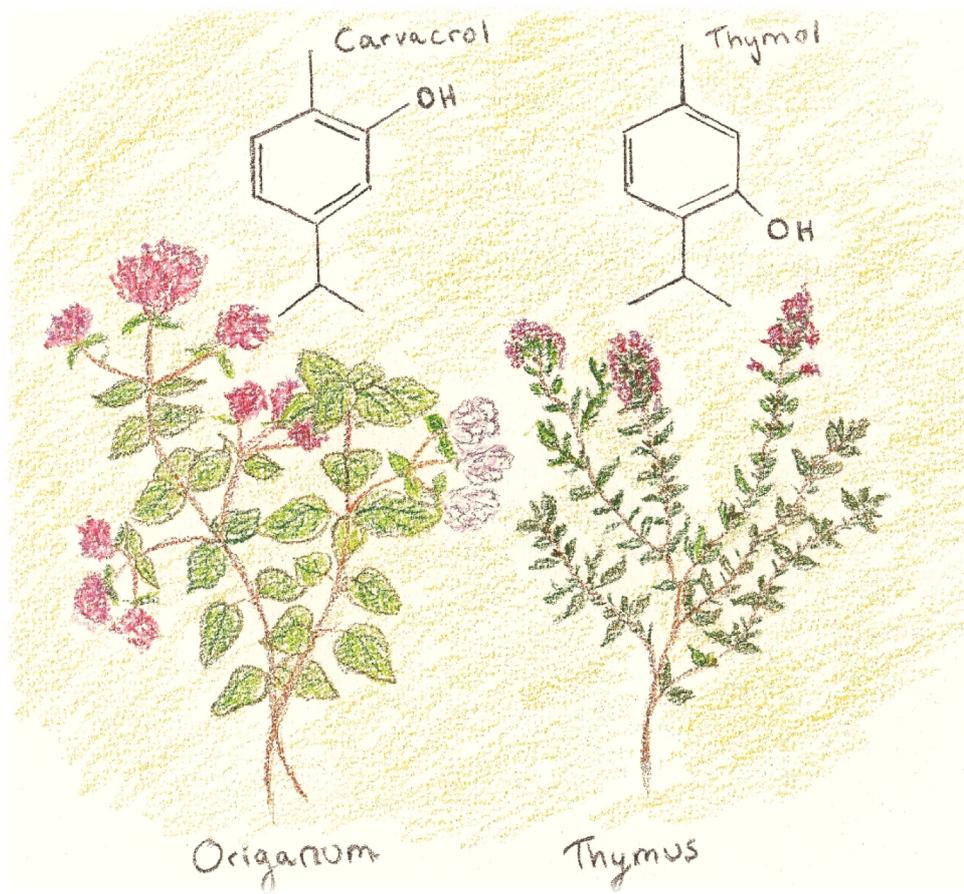


Essential Oils and Phenolic Compounds as Food Preservatives

Eteriska oljor och fenoler i konservering av livsmedel

Erika Häggman



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Abstract

Recent studies have shown that consumers are increasingly choosing food items without synthetic preservatives or additives. In order to find alternatives to address the food industry's need of ensuring food safety, this essay focuses on the possible use of natural preservatives derived from antiseptic plants. The plants in question most often originate from the Mediterranean area and belong to the *Lamiaceae* family. The essential oils from these plants have been examined and found to be useful against foodborne pathogens and bacteria such as *Listeria monocytogenes* and *Escherichia coli*. Particularly the essential oils from *Origanum* and *Thymus* with the antibacterial constituents *carvacrol* and *thymol* have proven to be effective. *Thymol* and *carvacrol* cause destruction of the bacteria's cell membrane. There are, however, several challenges when attempting to use essential oils in food items. For example, plants show large chemical variabilities, which makes it difficult to provide consistent products of high quality. Therefore, cultivation techniques and knowledge are of great importance. Furthermore, efficacy of the essential oil constituents depends on pH and the ingredients of the food. Additionally, the contribution of sensory properties is an issue. Nevertheless, this essay will illustrate that the essential oils from the genus *Origanum* and *Thymus* do show promising results when cultivated in awareness and applied to food items with a low pH and fat content.

Sammanfattning

Ny forskning visar att konsumenter allt mer väljer livsmedel utan syntetiska konserveringsmedel och tillsatser. Livsmedelsindustrin behöver hitta alternativ som kan bibehålla livsmedels säkerheten, därför fokuserar denna uppsats på möjligheten att använda naturliga konserveringsmedel från antiseptiska växter. Växterna som det är frågan om kommer ofta från Medelhavsområdet och tillhör växtfamiljen *Lamiaceae*. De eteriska oljorna från dessa växter har undersökts och visat sig vara effektiva mot matburna patogener och bakterier såsom *Listeria monocytogenes* och *Escherichia coli*. Särskilt effektiva är de eteriska oljorna från släkterna *Origanum* och *Thymus*, som innehåller de antibakteriella ämnena *carvacrol* och *thymol*. *Thymol* och *carvacrol* förstör cellmembranen hos bakterierna. Däremot finns det flera utmaningar när det kommer till att använda eteriska oljor i livsmedel. Till exempel så varierar växternas kemi, vilket gör det svårt att bibehålla en jämn produkt av hög kvalitet. Därför är odlingstekniker och

kunskap viktigt. Utöver det så beror effekten av de eteriska oljorna på pH värdet och ingredienserna i livsmedlet. Bidragandet till smak och lukt är också ett problem. Icke desto mindre visar denna uppsats att de eteriska oljorna från *Origanum* sp. och *Thymus* sp. ger positiva resultat, särskilt efter medveten odling och då de tillsätts i livsmedel med lågt pH och fettinnehåll.

Table of contents

Introduction	6
Background.....	6
Aim	8
Method.....	8
Results	9
Essential Oils	9
Phenolic Compounds	10
Optimising Production and Yield	11
<i>Origanum</i>	11
<i>Thymus</i>	12
Factors Influencing Phenolic Content in Plants	13
<i>Genetics</i>	14
<i>Plant Development Stages</i>	14
<i>Environmental and Meteorological Factors</i>	15
Optimising Cultivation	15
<i>Propagation</i>	16
<i>Temperature</i>	16
<i>Day Length and Light Intensity</i>	17
<i>Irrigation</i>	17
<i>Fertiliser Application</i>	18
<i>Soilless Cultures and Hydroponic Systems</i>	18
<i>Harvest</i>	19
<i>Postharvest</i>	19
Antimicrobial and Antioxidant Activity	20
<i>Antibacterial Activity</i>	20
<i>Antifungal Activity</i>	22
<i>Antioxidant Activity</i>	22
Extraction Methods.....	23
Use as Preservatives.....	24
<i>Gram-Positive and Gram-Negative Bacteria</i>	24

<i>MIC</i>	24
<i>Combining Essential Oils and Major Compounds</i>	25
Challenges	26
Discussion.....	27
Conclusion	30
References	32

Introduction

The last decade has seen a growing demand for nutritious and healthy food (Burt 2004). Consequently, consumers are scanning the store shelves for products without any chemical additives or synthetic preservatives (Davidson 2006). However, these preservatives are highly depended upon when ensuring food safety, minimising loss and additionally prolong the shelf life of food products. Especially since food that is contaminated by foodborne pathogen or has been oxidized may not be safe to consume (Prakash et al. 2012a; Santos-Sánchez et al. 2017). The synthetic chemicals can be found in food items that are ready to be heated in the microwave and other ready-prepared meals (Pesavento et al. 2015), which this essay will focus on. Preservatives can be divided into antioxidants, which prevent oxidation, and antimicrobial preservatives that inhibit pathogen growth (Mani-López, Palou and López-Malo 2016; Lau and Wong 2019). This has sparked an area of research, where the use of antiseptic plants and their essential oils has been in focus (Burt 2004). Antiseptics are defined as substances with antimicrobial and particularly antibacterial activity, which in other words inhibit the growth of microorganisms (Cambridge Dictionary 2019). There are around 3000 known essential oils, and only 10 % are widely used today (Ríos 2016). Ríos (2016) describes that the composition of essential oils may vary depending on the origin of the plant as well as the climate where it is grown. Essential oils are known to have antimicrobial effects, which is defined as inhibiting the growth of bacteria and fungi and are traditionally used in medicine and cosmetics (Burt 2004). Furthermore, essential oils are biodegradable, whereas synthetic antioxidants and preservatives are not (Prakash et al. 2012a). Additionally, the oils are less likely to induce resistance of pathogens, as the antimicrobial activity is a result of several constituents (Prakash et al. 2012a). The extracts are therefore a way to meet the consumer demand at the same time as preserving the food safety of products and ensuring a long shelf life.

Background

The most commonly discussed food-borne pathogens in literature are different bacteria (Burt 2004; Gutierrez, Barry-Ryan and Bourke 2009; Lv et al. 2011). The bacteria usually studied are *Escherichia coli*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella*, *Pseudomonas aeruginosa* and *Bacillus subtilis* (Gutierrez, Barry-Ryan and Bourke 2009). These pathogens can

contaminate and grow in fresh food, processed and ready-to-eat food items, meat and beverages. Some bacteria can thrive in several environments, such as *L. monocytogenes*, whereas some are found particularly in meat or merely on fresh vegetables (Gutierrez, Barry-Ryan and Bourke 2009). If not inhibited, these bacteria can cause outbreaks of illnesses (WHO 2019). For example, *L. monocytogenes* causes listeriosis, which can have serious consequences. *E. coli* and *Salmonella* on the other hand causes diarrhoea and vomiting (WHO 2019).

Today nitrites, nitrates, calcium propionate and sulphites as well as sulphur dioxide are extensively used as synthetic antimicrobial preservatives in food (Abdulmumeen, Risikat and Sururah 2012; Lau and Wong 2019). These have been reported as potentially harmful to consumers (Abdulmumeen, Risikat and Sururah 2012). Especially nitrite, which is most often used as an additive in meat, could be harmful and cause illness and cancer when often consumed (Abdulmumeen, Risikat and Sururah 2012; Fletcher 2014). Furthermore, sulphur dioxide can cause asthma (Abdulmumeen, Risikat and Sururah 2012). In addition, the chemical preservative benzoic acid is commercially used in order to prevent pathogens, however research has shown that the acid can cause physical reactions, such as convulsion (Lau and Wong 2019). Therefore, consumers are requiring other, safer, methods of food preservation.

There is a large number of plant species that produce essential oils (Bhattacharya 2016). About 400 of these species, from around 60 families, are used commercially. A few of these families are of significant importance. These are *Lamiaceae*, *Apiaceae* and *Asteraceae* (Bhattacharya 2016). According to Davidson and Zivanovic (2003) the plants that possess the highest antiseptic properties are some species from the *Lamiaceae* family: *Rosmarinus officinalis*, *Origanum vulgare*, *Thymus vulgaris*, *Ocimum basilicum* and *Salvia officinalis*. Most importantly, *Origanum* and *Thymus* essential oils have been described as the most effective and active antimicrobial against common food bacteria (Lambert et al. 2001; Bagamboula, Uyttendaele and Debevere 2004; Gutierrez, Barry-Ryan and Bourke 2009; Lv et al. 2011).

Essential oils are formed by a vast mix of volatile secondary metabolites (Ríos 2010). The production of plant metabolites is a part of the metabolic pathway and is enzyme regulated and results in primary and secondary metabolites (Dayani and Sabzalian 2016). These serve different

functions in the plants. The primary metabolites are defined as essential for the continued growth and development of the plant. Secondary metabolites do not primarily influence the growth of the plant but are instead important for the defence mechanisms (Devika and Koilpillal 2012). Furthermore, secondary metabolites are produced by plants with the aim of protection against biotic and abiotic stress and to ensure survival (Bassolé and Juliani 2012). Additionally, the secondary metabolites contribute to the characteristics of plants, such as their aroma, physical attributes and taste (Bennett and Wallsgrove 1994). Hence, the essential oils are not primarily essential for the plants, the name instead comes from the aroma and *essence* of the plant (Lubbe and Verpoorte 2011). Secondary metabolites may possess qualities that are antiseptic, antifungal, antioxidant and repellent (Burt 2004). The geographical location and environmental conditions surrounding the plant greatly influence the production and quality of secondary metabolites (Bhatia 2015; Dayani and Sabzalian 2016).

Aim

The aim of this essay is to describe and evaluate the antibacterial secondary metabolites that some plants produce, and subsequently examine if these are applicable in food items. Even though the focus will lie on the antibacterial activity, the antioxidant and antifungal activities will be briefly examined as well. Furthermore, this essay will focus specifically on the use of preservatives in ready-prepared meals. In doing so, two core research questions will be addressed:

1. What functions do essential oils and phenolic compounds possess and how can these be applied in food items?
2. How can the production of essential oils and phenolic compounds in the genus *Origanum* and *Thymus* be maximised?

Method

This study was based on literature, most of which was scientific articles based on research and experiments. The articles were largely obtained from ScienceDirect, ISHS, through library books, Web of Science and the university library website. As this essay contains information

about chemistry, biology and plant physiology as well as agriculture and processing the key words used when searching for information have been vividly different. The initial key words, such as *antiseptic plants*, *secondary metabolites*, *natural preservatives* and *antiseptic essential oils* were later followed by more detailed and specific words. Some examples: *phenolic compounds*, *extraction essential oils*, *thymol mode of action*, *optimising carvacrol*, *essential oil production*, *cultivation of medicinal plants*, *drought stress Origanum*, *thymol chemotypes*, *Gram-negative bacteria*.

Results

Essential Oils

Essential oils are defined as volatile liquids with a low molecular weight, most commonly found in aromatic plants (Hyldgaard, Mygind and Meyer 2012). According to Deans and Ritchie (1987) the essential oils are obtained from different parts of plants, depending on the species and variety and consequently where the oils are produced. All parts of plants can produce oils, the flowers, leaves and seeds to the stem, bark and roots. Where these secondary metabolites are produced depends largely on the plant family (Ríos 2016). In the *Lamiaceae* and *Asteraceae* families the essential oils can be found in glandular hairs, whereas in plants within the *Lauraceae* family the oils are found in cells that are non-differentiated. Essential oils dissolve in fats or lipids; hence they are lipophilic (Prakash et al. 2015; Ríos 2016). Essential oils, and thus the secondary metabolites, have a wide range of functions in the plants (Ríos 2016). Depending on the plant, these substances act as defence mechanisms, as antioxidants and protection against pathogens including fungi and bacteria (Ríos 2016).

Essential oils consist of a mixture of constituents (Burt 2004). These are referred to as major or minor components. The major components are the compounds found in the largest amount and therefore make up most of the oil. The minor components can instead exist merely as traces in the oil. The major chemical components of essential oils mostly consist of terpenoids, usually monoterpenes or sesquiterpenes (Cristani et al. 2007). Terpenes can be divided into hydrocarbons and oxygenated compounds. The oxygenated monoterpenes are assumed to possess the highest antimicrobial activity. Phenols, such as *carvacrol* and *thymol*, belong to the oxygenated monoterpenes (Cristani et al. 2007). In addition, other compounds forming the

essential oils originate from several chemical classes, such as ketones, alcohols and aldehydes (Dhifi et al. 2016).

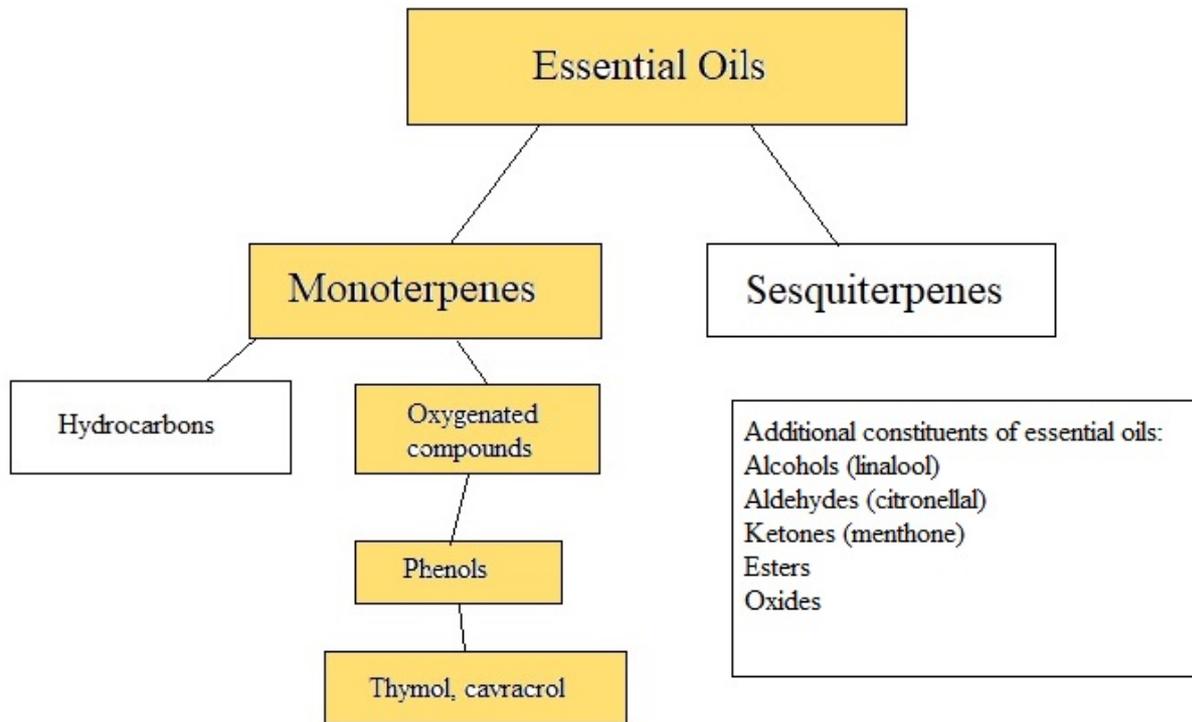


Figure 1. A simple overview of the chemical composition of essential oils. The relevant information for this essay is highlighted. Information is gathered from Cristani et al. (2007) and Dhifi et al. (2016).

Phenolic Compounds

Phenols are one of the most extended groups of secondary metabolites consisting of more than 8000 compounds (Lee Tan and Lim 2015). These compounds consist of a benzene ring attached to at least one hydroxyl group. The complexity of phenols varies from simple structures to larger molecules. Therefore, phenols can be allocated to two main subgroups, simple phenols or polyphenols. The simple phenols have merely one hydroxyl group, whereas polyphenols consist of at least a pair of these groups (Lee Tan and Lim 2015). The biosynthesis of phenols includes two pathways, and these are the acetate pathway and the Shikimate pathway (Dyakov and Dzhavakhiya 2007; Kougan et al. 2013). The Shikimate pathway involves the aromatic amino acids phenylalanine and tyrosine. Eventually, these pathways result in phenolic compounds

(Kougan et al. 2013). According to Kougan et al. (2013) phenolic compounds can be stored in the vacuole of plant cells. In essential oils, phenolic compounds are found in the forms of for example, *thymol*, *carvacrol* and *eugenol* (Cristani et al. 2007; Lv et al. 2011). According to Tognolini et al. (2006) the essential oils from *Thymus* and *Origanum* consist of a large amount of phenols. Furthermore, phenols add properties such as antimicrobial activity to the essential oils (Lambert et al. 2001). Therefore, the two most effective essential oils that possess high antimicrobial activity is said to be from *Origanum* and *Thymus*, because of the high content of *carvacrol* and *thymol* (Gutierrez, Barry-Ryan and Bourke 2009). Moreover, phenols are antioxidants (Lee Tan and Lim 2015) and thus believed to be found in larger amounts when the plant is exposed to stress (Dyakov and Dzhavakhiya 2007).

Optimising Production and Yield

The chemical variability of essential oils is an issue which is difficult to control as it is influenced by genetic factors and life stages as well as the geographical location, environmental and meteorological conditions (Fleuriet and Macheix 2003; Pietta, Gardana and Pietta 2003; Prakash et al. 2015). Thus, the main challenge for the use of natural preservatives appears to be the lack of uniformity of the essential oil. However, the available knowledge and technology of researchers and growers is of great advantage. Hence, the paragraphs to follow will focus on the plants from which these essential oils and compounds are extracted. It will explore what possibilities there are to standardise and optimise the cultivation and production.

Origanum

Origanum is a genus consisting of perennial herbs (Ortega-Ramirez et al. 2016). One of the most common species is *Origanum vulgare*, which grows in the Mediterranean area and on its islands. *O. vulgare* thrives in a dry climate rich in sunshine (Kokkini, Karousou and Vokou 1994). The common height of an *O. vulgare* plant is around 20 centimetres (Kintzios 2004). The plant consists of woody stems and is rather insensitive to periods of drought. The flowering period is usually from June to late summer depending on the climate. In winter, the foliage dies, but fresh growth will emerge from the roots in the next vegetation season. *Origanum* can successfully be propagated as seeds or by cuttings (Kintzios 2004). The optimal period for cuttings is in late spring. Commonly, the *Origanum* plants are replaced every five or perhaps six years. The

preferred growing conditions are in a medium soil in an area with cool temperatures during summer, which usually means in a high elevation. Commercially used *Origanum* is harvested both from the wild and from cultivation sites (Kintzios 2004). The essential oil is stored in glands in the leaves (Kintzios 2004). Apparently, the highest essential oil yield is in a Mediterranean climate during the summer season (Putievsky, Ravid and Dudai 1988; Giannenas et al. 2018). Putievsky, Ravid and Dudai (1988) found that the biosynthesis of the plant is at its optimal level during the summer. According to Kokkini, Karousou and Vokou (1994) *O. vulgare* plants grown in the Mediterranean region possess more oil glands in comparison to plants grown in other areas. The essential oil of *Origanum* mainly consists of *carvacrol* (Kintzios 2004). However, the constituents vary between species, varieties and chemotypes. The most important chemotype, when aiming to use the oil as an antimicrobial agent, is the type containing *carvacrol*. Of further importance is that the production of *carvacrol* appears to increase when the weather conditions are warm and dry (Karamanos and Sotiropoulou 2013). Dudai et al. (1992) found that the highest concentration of *thymol* is in October, whereas *carvacrol* is produced in highest amounts from August until early autumn. Furthermore, experiments have taken place with a focus on increasing the *carvacrol* content in the oil of different *Origanum* species (Van der Mheen 2006). The experiment resulted in plants with a *carvacrol* content well over 60 %. Van der Mheen (2006) suggests the use of white flowering *O. vulgare* plants, specifically *O. vulgare* ssp. *hirtum*. Yet, it is important to mention that Van der Mheen (2006) additionally discovered that there are large differences in oil composition and yield between individual plants.

Thymus

Thymus is a perennial and has its origin around the Mediterranean Sea but grows in large parts of Europe and Asia (Stahl-Biskup and Venskutonis 2004). *Thymus* is cultivated for commercial purposes in many parts of the world. However, the centre of production is in and around the Mediterranean. Both cultivation and foraging from the wild is being practiced. Cultivated *Thymus* has a life span of two to three years (Król and Kieltyka-Dadasiewicz 2015). In colder climates the plants are prone to freeze during winter and therefore planted as annuals. A Mediterranean climate is optimal for *Thymus*. The most commonly cultivated *Thymus*, and the core species when referring to the plant, is *Thymus vulgaris* (Stahl-Biskup and Venskutonis 2004). *T. vulgaris* is a well-known herb, especially for its use in medicine and cuisine. *T.*

vulgaris is an evergreen shrub, with a maximum height of about 30 centimetres and originates from the most southern parts of Europe. *Thymus* thrives in calcareous soils and, thus, lime can be added before sowing (Stahl-Biskup and Venskutonis 2004).

The essential oil of *Thymus* is located in the leaves: more specifically in trichomes (Stahl-Biskup and Venskutonis 2004). Therefore, the leaves are of economic importance (Król and Kieltyka-Dadasiewicz 2015). The oil content of the dried plant is usually about 2,5 % (Shalaby and Razin 1992). Commonly, the main constituent of *Thymus* essential oil is *thymol*, which makes up about 40 % of the oil, closely followed by *carvacrol* (Shalaby and Razin 1992; Stahl-Biskup and Venskutonis 2004). There are, however, several different chemotypes of *Thymus* (*T. vulgaris*). For commercial use, the chemotype with *thymol* as the main constituent is important (Stahl-Biskup and Venskutonis 2004). Król and Kieltyka-Dadasiewicz (2015) found that *thymol* was highest in the end of August, whereas the biomass was higher at the end of September. Jordán et al. (2006) suggested that harvesting should be done between blooming and fruit setting, as the phenolic content is higher at the time. Shalaby and Razin (1992) recommended a moderate to high fertilisation and a shorter spacing (15 centimetres, compared to 30 or 45) between the plants. In this way, the yield of biomass and oil per area can be optimised.

Table 1. The general composition of essential oils from *Origanum* and *Thymus*.

Plant	Main oil constituents ($< \approx 95\%$)	Minor constituents (the most common ones)	Reference
<i>Origanum vulgare</i>	1. <i>Carvacrol</i> 2. <i>Thymol</i>	γ -terpinene, <i>p</i> -cymene, β -caryophyllene, β -myrcene, α -terpinene	Giannenas et al. (2018)
<i>Thymus vulgaris</i>	1. <i>Thymol</i> 2. <i>Carvacrol</i>	<i>p</i> -cymene, γ -terpinene, linalool, myrcene, 1,8 – cineole	Baranauskiene et al. (2003)

Factors Influencing Phenolic Content in Plants

According to Bhatia (2015) the issue with producing and using secondary metabolites is that the concentration of substances varies greatly in response to different factors. This issue has also been described by Prakash et al. (2015) and there have been suggestions on creating standardised

values of the oil content. In order to do so, one needs to understand how the production is influenced.

Fleuriet and Macheix (2003) describe the three main factors determining the biosynthesis of phenolic compounds in plants. Firstly, genetics influence the production of phenols. Secondly, the different plant development stages, the vegetative and reproductive stages, affect the biosynthesis. Thirdly, the surrounding environmental conditions have a great influence on the enzyme activity and biosynthesis of plants. Besides, the total essential oil content also varies according to the three mentioned factors (Yosr et al. 2013; Dhouioui et al. 2016).

Genetics

Studies made on different plants such as *Origanum* (Kintzios 2004), *Thymus* (Bouzidi et al. 2013) and *Rosmarinus* (Zaouali, Bouzaine and Boussaid 2010) have all shown that the phenolic content of the plant varies within the species and between cultivars. Moreover, results have shown that there is a different composition of the essential oils in and between varieties of *Origanum*, which is understood as genetic variability (Dudai 2008). This has led to the creation of chemotypes within species and varieties, both for *Origanum* and *Thymus* (Kintzios 2004; Stahl-Biskup and Venskutonis 2004). The chemotype classification is based on the genetic variability of the essential oil. Hence, the different chemotypes have a different main constituent of the oil. For example, Rota et al. (2008) examined the essential oil composition in three *Thymus* species. Their study revealed that the main constituents varied slightly. All three species tested had *thymol* as the main component and only one oil contained *carvacrol*. Furthermore, this knowledge may enhance the opportunity for breeding varieties with a higher content of *thymol* and *carvacrol* in the essential oils.

Plant Development Stages

Several researchers agree that the essential oil amount increases at the point of flowering (Dudai 2008; Sellami et al. 2009; Yosr et al. 2013). A suggestion provided from Yosr et al. (2013) is that at the flowering stage the apical meristem is transformed into an inflorescencial meristem which seems to be stimulating the production of essential oil. By contrast, the phenolic compounds in *Rosmarinus* essential oil were found in the highest amount in the leaves, though with a significant decrease during flowering. In agreement, Dudai et al. (1992) found that the thymol

concentration in *Origanum syriacum* decreased during the reproductive stage. On the contrary, Dudai (2008) concluded that the essential oil and *thymol* content in *O. vulgare* was maximised during flowering. Furthermore, the composition of the oil has been shown to change between young and older leaves (Dudai 2008). *Origanum* leaves contain more *thymol* when aged. The suggested reason is that the number of glandular hairs increase with age (Dudai 2008).

Environmental and Meteorological Factors

Studies made on different aromatic plants show that the components of essential oils vary depending on cultivation place (Tibaldi, Fontana and Nicola 2011; Hassiotis et al. 2014). The soil composition, the humidity and hours of direct sunlight are all factors that affect the biosynthesis of these phytochemical compounds (Pietta, Gardana and Pietta 2003).

Monoterpenes have been found to increase in summer (Dhouioui et al. 2016) possibly due to that these compounds may protect the plant against high temperatures and long sunny days.

Interestingly, Dudai et al. (1992) suggested that environmental conditions and weather further influence the plant's growth and development. Day length and temperature will in some cases induce flowering, which leads to a change in essential oil production. Therefore, these factors go hand in hand and it is difficult to determine which factor has the highest impact: environmental conditions or the development stage of the plant (Dudai et al. 1992).

Optimising Cultivation

The cultivation of plants makes it possible to enhance and control the essential oil composition and yield, in comparison to collecting plant material from the wild (Tibaldi, Fontana and Nicola 2011). Consequently, cultivation leads to minimising the chemical variability and making standardisation easier to achieve. Another positive factor is that the price and quality will be less fluctuating compared to wild harvesting, however, production will be more expensive (Lubbe and Verpoorte 2011). Cultivation also enhances the traceability factor. Pietta, Gardana and Pietta (2003) suggested that in order to achieve consistency of essential oils and the useful constituents, the initial step is to choose plants carefully. Of interest is the genetics of the species that naturally, hence genetically, possess the highest percentage of the required compounds (Tibaldi, Fontana and Nicola 2011). Canter, Thomas and Ernst (2005) further suggested breeding as a

method of reaching consistency. Using biotechnology methods would solve the issues regarding chemical variability and would increase the possibility of standardisation.

There are several different cultivation systems, ranging from simple ones grown in soil without irrigation to high technological soilless systems. More control over the cultivation system, will subsequently lead to increased impact on the plants' essential oil production (Pietta, Gardana and Pietta 2003). According to Dudai (2008) a plant grown in a favourable environment will produce both biomass and essential oils. On the contrary, some researchers would argue that the essential oil and phenolic compound production is enhanced by stress (Dyakov and Dzhavakhiya 2007; Ríos 2016). The following factors have been found to be of importance to the yield of biomass and oil and can further be controlled when cultivating the plants.

Propagation

Vegetative propagation is an asexual propagation method which can ensure high quality and standardisation of oil (Namdeo 2018). Therefore, propagation by cuttings for both *Origanum* and *Thymus* may be the optimal method. However, the expense of propagation by cuttings is a lot higher compared to propagation by seeds (Van der Mheen 2006).

Temperature

According to Dudai et al. (1992) the climatic factors that mostly influence production of secondary metabolites and essential oils are light and temperature. Dhouioui et al. (2016) suggested that precipitation and the increase or decrease of temperature are the most influential factors for essential oil production. Additionally, high temperatures are assumed to stimulate the production of most essential oils (Bhattacharya 2016). Additionally, the phenolic content has been shown to increase in high temperatures (Kokkini, Karousou and Vokou 1994). As an example, Hassiotis et al. (2014) found that the amount of essential oils in *Lavandula* decreased after precipitation and a subsequent drop in temperature. This strengthens the suggestion mentioned above: that the biosynthesis of different secondary metabolites is influenced by environmental conditions.

Day Length and Light Intensity

It is known that length of daylight influences plants to a great extent (Dudai et al 1992). Dudai et al. (1992) found that *O. syriacum* went into the reproductive stage earlier when grown under long day conditions (8 hours of sunlight and 8 hours of additional light). The plants flowered even earlier when simultaneously grown in warm temperatures. Interestingly, the plants exposed to long day conditions contained higher amounts of phenolic compounds.

Tibaldi, Fontana and Nicola (2011) evaluated different cultivation systems and the effects on *Origanum* (*O. vulgare* ssp. *hirtum*). The results showed that the biomass production was significantly higher when grown in the field while being exposed to full light, in comparison to shade. When grown in a pot the production was lower despite being in full light. However, the interest being essential oils, which were highest when *O. vulgare* was grown in pots in full light. The pots in full light produced more dry matter, which was correlated with a higher production of essential oil. Interestingly, the reason behind this seemed to be the exposure to minor drought stress, which is in agreement with the assumption that the production of some secondary metabolites is triggered by abiotic stress (Dyakov and Dzhavakhiya 2007).

Irrigation

In the case of irrigation, rather unconventional methods may be favourable, such as consciously exposing the plant to drought (Dudai 2008; Tibaldi, Fontana and Nicola 2011). However, Król and Kieltyka-Dadasiewicz (2015) found that species within the genus *Thymus* thrives in weather conditions with regular precipitation. On the contrary, the example in the paragraph above showed that the *O. vulgare* biomass was positively affected by drought stress (Tibaldi, Fontana and Nicola 2011). Moreover, the content of carvacrol in *Origanum* was found to increase as a result of water stress (Dudai 2008). However, the results of another study suggest that the biomass production of *Origanum* is negatively affected by drought (Azizi, Yan and Honermeier 2009). Bernstein, Chaimovitch and Dudai (2009) examined irrigation with treated effluent on *Origanum*. The water in question contained high levels of salts, nutrients and minerals which subsequently gave it a high electrical conductivity in comparison to the commonly used water. Furthermore, using effluent as irrigation is positive in periods of drought, and the authors add that higher levels of salinity may have a positive effect on the production of phenolic compounds, as a reaction to stress (Bernstein, Chaimovitch and Dudai 2009).

Fertiliser Application

Fertilisers are a great advantage in production systems, especially the application of nitrogen (Sotiropoulou and Karamanos 2010). The application amount depends on the cultivation site and the initial fertility and composition of the soil (Baranauskiene et al. 2003). The optimal rate for *Origanum* sp. is reported to be 80 kg nitrogen per hectare (Sotiropoulou and Karamanos 2010) and 45 kg nitrogen per hectare for *Thymus* sp. (Baranauskiene et al. 2003). When examining the effect of nitrogen fertilisers on the essential oil of *Origanum*, it was detected that the fertilisation had a positive impact on the total essential oil yield (Karamanos and Sotiropoulou 2013), merely because the biomass of the plants increased. Hence, fertilisation does not directly affect the composition of essential oil, but the total amount in the plant (Baranauskiene et al. 2003). However, a slight increase in *carvacrol* could be seen in the fertilised plants compared with the control plants (Karamanos and Sotiropoulou 2013).

Soilless Cultures and Hydroponic Systems

In colder climates, as here in the Nordics, hydroponic systems may be favourable. In a hydroponic system the environmental conditions, fertiliser application and irrigation are under precise control (Giurgiu et al. 2017). Therefore, the optimal cultivation conditions can be created and subsequently the production of bioactive compounds can be controlled (Canter, Thomas and Ernst 2005; Giurgiu et al. 2017). Furthermore, hydroponic systems can provide consistency and sustainability beyond the cultivation in fields (Giurgiu et al. 2017).

T. vulgaris have successfully been grown in NFT (Nutrient Flow Technology) hydroponic systems (Udagawa 1995). The optimal electrical conductivity of the nutritional solution proved to be 3,6 mS/cm, which was when the oil and biomass yield was maximised, although *thymol* was found in the highest concentration at 1,2 mS/cm. By contrast, Giurgiu et al. (2017) found that *Thymus* did not thrive in soilless systems, however, mostly due to the present pests. Lubbe and Verpoorte (2011) suggest that a cultivation system with a low resource input is favourable when cultivating plants for essential oils. The reason being that the price of cultivated plants is a lot higher than plants collected from the wild. Therefore, plants cultivated in a highly technological hydroponic system might not be attractive on the market due to the price (Lubbe and Verpoorte 2011).

Harvest

Baranauskiene et al. (2013) reported that the optimal harvesting time for *Origanum*, considering both oil and biomass yield, was at the flowering stage. However, the phenolic compounds were higher in the vegetative stages, compared to during flowering. Therefore, it is clear that depending on the aim of the essential oil, the optimal harvesting time will vary. Putievsky and Ravid (1984) reported that the optimal harvest time of *O. vulgare*, considering both oil and biomass yield, does not really exist. However, they suggested that the harvest time ought to be planned in accordance to the plant variety.

When harvesting *T. vulgaris* it is of importance to take biomass, the percentage of leaves, and oil yield into consideration (Król and Kieltyka-Dadasiewicz 2015). Therefore, Król and Kieltyka-Dadasiewicz (2015) suggest that the optimal harvesting time is somewhere between August and September. More specifically, the optimal harvesting time depends on weather conditions, longitude, altitude and the micro-climate of cultivation place (Dudai 2008; Hassiotis et al. 2014). Thus, careful consideration should be taken to all factors applied at the cultivation place. Harvesting techniques should also consider in which plant parts the oil is stored, for example in young or old leaves (Dudai 2008).

Postharvest

In addition to the cultivation and its effects on the oil yield and composition, it is important to bear in mind that the postharvest activities have further effect on the essential oil (Tibaldi, Fontana and Nicola 2011). For example, postharvest conditions were found to influence the *carvacrol* content. The *carvacrol* content increased while plant material was drying in a hot oven, which according to the authors may mean that the biosynthesis continues after harvest, specifically the oxidation of certain compounds into oxygenated monoterpenes. The compound *carvacrol* was lower in the essential oil obtained from fresh plant material compared to dried material (Tibaldi, Fontana and Nicola 2011).

Antimicrobial and Antioxidant Activity

Antibacterial Activity

It is important to understand the mechanisms of the antimicrobial activity originating from the essential oils and phenolic compounds.

Ultee, Bennik and Moezelaar (2002) were able to determine that it is the attached hydroxyl group of the compounds that have an impact on the antimicrobial property. The compound studied in this research was *carvacrol*, which is found in *Origanum* sp. and *Thymus* sp. In addition to the hydroxyl group, the structure of *carvacrol* includes delocalised electrons, which is believed to influence the antimicrobial activity further. *Carvacrol* is assumed to manifest in the cell membrane of the food pathogen bacteria (in this case *B. cereus*), which displayed an enlargement of the membrane. This enlargement damages the membrane and consequently causes ions to leak, and further, leading to an osmotic imbalance. This, in turn, affects the ion transportation in and out of the cell (Prakash et al. 2015). Research conducted by Prakash et al. (2015) has shown that phenolic compounds influence the ion gradient controlling the potassium and hydrogen ions of the microbe. Consequently, the water balance will be disturbed and the ATP concentration in the cell will drastically decrease. Eventually, the microbe's cells will be destroyed (Prakash et al. 2015).

Thymol and *carvacrol* belong to the simple phenolic compounds (Bouzidi et al. 2013).

Interestingly, these two are understood to possess the most effective and active antimicrobial activity. Their chemical structure is similar, to the extent that *thymol* and *carvacrol* are isomers. Meaning, *thymol* and *carvacrol* have the same molecular formula ($C_{10}H_{14}O$), but a different structure. The hydroxyl group is merely binding to a different atom (Bouzidi et al. 2013). Therefore, it is this structure, the one hydroxyl group and its added polarity, that appears to constitute the effectiveness of the two compounds (Bassolé and Juliani 2012; Bouzidi et al. 2013). Pokorný (2003) agrees with the assumption that the structure of the molecules plays an essential role in the antimicrobial activity.

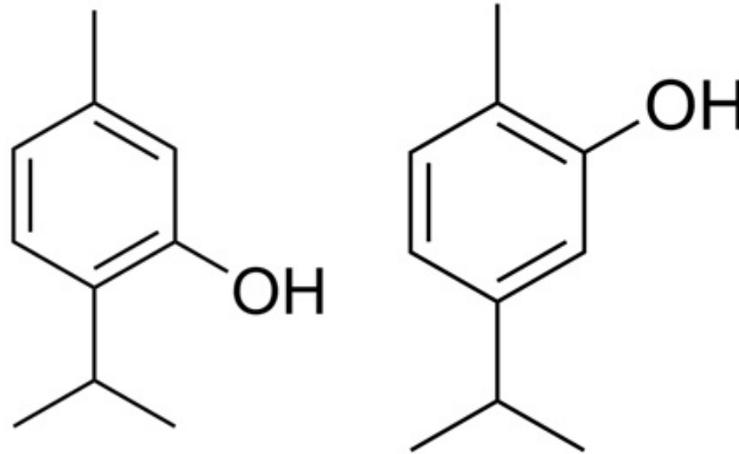


Figure 2 & 3. Chemical structure of *thymol* (left) and *carvacrol* (right). A benzene ring with an attached hydroxyl group. Both structures are made by Edgar181 (2007

https://commons.wikimedia.org/wiki/File:Thymol_structure.png;

https://commons.wikimedia.org/wiki/File:Carvacrol_structure.png).

According to Cristani et al. (2007) the phenolic compounds have a hydrophilic part as well as a hydrophobic part. This enables different actions. The benzene ring is hydrophobic and can, thus, interact with the hydrophobic part of the phospholipids in the membrane, whereas the hydrophilic fragment is attracted to the polarity of the hydrophilic part of the phospholipids. Furthermore, Cristani et al. (2007) see two important reasons behind the phenolic compounds' effectiveness against bacteria. Firstly, the compounds lipophilic character, which enables them to interact with the phospholipids and subsequently damage the membrane. Secondly, a molecule's water solubility (the hydrophilic part) is significant, as it leads to the possibility of the compound to be transported across the cell's water-based mediums (Cristani et al. 2007).

Bouhdid et al. (2009) have investigated the antibacterial effect of the essential oil extracted from *Origanum (O. compactum)*, in which the two main components were *thymol* and *carvacrol*. They found that the oil had a damaging effect on the cell membrane of the bacteria studied, which were *S. aureus* and *P. aeruginosa*. Furthermore, Bouhdid et al. (2009) saw that the cells of the two bacteria cultures were affected in different ways: *P. aeruginosa* reacted more intensely to the essential oil treatment, whereas the cells of *S. aureus* were affected merely in terms of a reduced respiratory activity and loss of cell membrane potential and viability. The cells of *P. aeruginosa*

suffered from vast changes in permeability, respiration and additionally coagulated materials were discovered in the cells (Bouhdid et al. 2009).

Antifungal Activity

Antifungal qualities are also dependent on the structure of the molecule (Kurita et al. 1981).

Kurita et al. (1981) studied the antifungal activity of the most commonly used essential oils. The results showed that phenolic compounds had a remarkably larger antifungal effect compared to alcohols or ketones. It appears that a hydroxyl group adds a lot more to the antifungal activity than what a carbonyl group does. The antifungal activity is similar to the antibacterial one, as it depends on the cell membrane permeability of the compound, which relies on the structure of the molecule and partly by the hydrophobic feature. Moreover, the lipophilic characteristic of essential oils and the major compounds play an important role against fungi as well (Prakash et al. 2012b). As the membrane is destroyed, the intercellular protein is denaturised (Prakash et al. 2012b). Mishra et al. (2013) studied the antifungal activity of different essential oil constituents. The most effective fungicidal was *thymol*, which showed inhibitory effect on *Aspergillus flavus* at a much lower minimum inhibitory concentration as compared to *eugenol* and *menthol*.

According to this study, *carvacrol* showed no antifungal activity (Mishra et al. 2013). This is interesting since the authors assume that it is the free hydroxyl group of thymol that is accountable for the fungicidal effect. Since *thymol* and *carvacrol* are isomers, these compounds share this hydroxyl group (Bouzidi et al. 2013). Nevertheless, the results may have been influenced by factors during the experiment.

Antioxidant Activity

Antioxidants are described as constituents that prevent oxidation and subsequent deterioration of, in this case, food products (Shahidi 2015). Another, more classic, definition of antioxidants is presented by Halliwell (1996) as a substance that slows down or fully averts oxidation of an item. The antioxidant potential of phenolic compounds depends on the hydroxyl group and how many of these the compound possesses (Shahidi 2015). Phenolic compounds can inhibit or prevent the degradation of lipids due to oxidation. Additionally, phenolics may prevent mutations of DNA. In detail, one reason behind the prevention of lipid oxidation lies in that the phenolic compound donates a hydrogen atom. Plants that are mentioned in relation to high

phenolic content are yet again *Rosmarinus*, *Thymus* and *Origanum*. These herbs contain compounds that are effective antioxidants in most kinds of fats. These extracts can be used at up to 1000 mg (powder) per kg food but is effective at around 200 mg as well (Shahidi 2015).

Moreover, Bettaieb et al. (2010) suggest that antioxidants also play an important role in relation to health, as antioxidants can protect the consumer against some diseases.

Extraction Methods

Another significant factor to consider is the mode of extraction of the essential oil and phenolic compounds. According to Ríos (2016) the composition of the essential oil extracted from the same plant will be different depending on the method utilised. This is because some compounds are sensitive to certain factors (Okoh, Sadimenko and Afolayan 2010).

To start with, the extraction method of essential oils depends on the plant characteristics and where the oils are stored (Stratakos and Koidis 2016). Methods that are commonly used in conventional production of essential oils are cold expression, solvent extraction and different distillation techniques. The plant parts can be dried or fresh when being distilled, however, flowers ought to be distilled fresh (Ríos 2016). Among these methods, steam and water distillations are most often used. In these distillation methods, the essential oils are extracted through evaporation and subsequent condensation (Stratakos and Koidis 2016). The plant part is treated with boiling water in the water distillation method and steam during steam distillation. Solvent extraction comprises the use of alcohols, in which the plant material is placed in order to release the essential oil from the plant (Stratakos and Koidis 2016).

In order to be able to choose the most suitable extraction method, it is important to know how the essential oil is affected. For example, the desired monoterpenes (the phenolic compounds *thymol* and *carvacrol*) are negatively affected by solvent extraction and when exposed to steam (Okoh, Sadimenko and Afolayan 2010). Okoh, Sadimenko and Afolayan (2010) compared two different extraction methods of *Rosmarinus* essential oil: solvent free microwave extraction and water distillation. In the solvent free microwave extraction (SFME), the plant part is exposed to heat in addition to pressure. One of the main differences between the methods is the time lapse, which in the SFME is 40 minutes, whereas the extraction lasts for 3 hours in the water distillation. The reported results were that the essential oil obtained through the microwave extraction possessed

higher antibacterial activities. Moreover, it was clear that the essential oil in question contained more oxygenated monoterpenes. In agreement with this investigation, another investigation by Bendahou et al. (2008) noted the fact that the SFME is a useful method, as it offers a higher oil and active compound yield. In their case, the results showed that the extraction from SFME contained higher concentrations of *thymol* as compared to the oil obtained from the water distillation (Bendahou et al. 2008). Depending on the structure and nature of the oil, the water distillation method might cause problems as the exposure to high temperatures may have an impact on the composition of the oil (Stratakos and Koidis 2016).

Use as Preservatives

Gram-Positive and Gram-Negative Bacteria

A brief introduction on bacteria may be beneficial in order to understand the need of preservatives. Bacteria can be either Gram-negative (*E. coli*, *P. aeruginosa*, *Salmonella*) or Gram-positive (*S. aureus*, *B. subtilis*, *L. monocytogenes*) (Cristani et al. 2007; Gutierrez, Barry-Ryan and Bourke 2009). These bacteria possess different qualities and therefore require different assets from the essential oils and phenolic compounds. The Gram-negative bacteria differ from the Gram-positive ones because of an additional outer membrane covering the cell wall (Gutierrez, Barry-Ryan and Bourke 2009). It has been suggested that this membrane restricts the antimicrobial effect of essential oils. However, some essential oils and compounds have shown antimicrobial effects against both Gram-positive and Gram-negative bacteria (Gutierrez, Barry-Ryan and Bourke 2009). For example, essential oils from *Origanum* and *Thymus* have shown antimicrobial effects on Gram-negative bacteria as the main compounds of the oils can be transported across the additional membrane (Gutierrez, Barry-Ryan and Bourke 2009).

MIC

The minimum inhibitory concentration (MIC) defines the lowest effective amount of a compound (Lambert et al. 2001). Lee Tan and Lim (2015) further suggest that MIC is the lowest concentration needed to inhibit further growth of a pathogen. However, it may not be the concentration by which the bacteria decrease or die. Most importantly, the MIC that is needed varies depending on the activity of the compounds and the susceptibility of the microorganism (Lambert et al. 2001; Lv et al. 2011).

Combining Essential Oils and Major Compounds

According to Davidson (2006) one of the issues with regards to essential oils as preservatives is their sensory contribution to the food product. This has to a significant extent been one of the reasons as to why natural preservatives have not been used commercially (Lambert et al. 2001). Therefore, it has been suggested that a combination of compounds and essential oils may be a solution (Lambert et al. 2001; Burt 2004; Gutierrez, Barry-Ryan and Bourke 2009). The MIC can be lowered when combining compounds and therefore the sensory influences will not be a disturbance to the consumer (Nazer et al. 2005; Gutierrez, Barry-Ryan and Bourke 2009; Lv et al. 2011). The sensory issue has been reported by Valero and Giner (2006) when studying *thymol*, *carvacrol*, *cinnamaldehyde* and *eugenol* as well as other phenolic compounds. *Thymol* and *carvacrol* showed significant results as antimicrobial agents when applied to broth. However, the product was rejected by consumers because of the influences on taste and smell (Valero and Giner 2006). Similarly, Bagamboula, Uyttendaele and Debevere (2004) investigated the use of essential oils, and especially the compounds *thymol* and *carvacrol*, as decontamination agents on fresh food items (lettuce), instead of as preservatives in cooked food items. However, the results showed that even though the antimicrobial effect was promising the taste of the lettuce was altered.

Burt (2004) shows interest in the discovery that compounds in the essential oil have synergic effects. This means that the compounds enhance each other's antiseptic effects. The minor components of essential oils contribute in a synergistic way to the antimicrobial activity (Burt 2004). Thus, Rota et al. (2008) suggest that the complete essential oil may possess more antibacterial activity than the main constituents on their own. The reason for this assumption is that there appears to be a synergistic effect between the phenolic compounds (*thymol* and *carvacrol*) and the minor constituents, such as the alcohols *linalool* and *geraniol* and the ketone *camphor*. By contrast, Lambert et al. (2001) suggest that a combination of *thymol* and *carvacrol* can be used instead of *Origanum* essential oil, as the combination is as effective but contributes less to the sensory aspects. Similarly, Bagamboula, Uyttendaele and Debevere (2004) studied the antimicrobial effect of a few essential oils and major compounds. The results showed that *thymol* and *carvacrol* were more efficient as antimicrobial agents than the whole *Thymus* essential oil.

Moreover, Mishra et al. (2013) suggested that the use of pure compounds can be positive, as the compounds are chemically stable and will not change over time. The components of the whole essential oils, however, tend to fluctuate according to different factors. Hence, the antimicrobial activity of the compounds is more reliable.

Another type of combination was tested by Turgis et al. (2012). They investigated if a combination of essential oils and bacteriocin would provide an efficient treatment against food pathogens. The definition of bacteriocins is polypeptides that possess antibacterial characteristics (Liu 2016). Bacteriocins are compounds produced by bacteria. The results showed that especially the combination of *Origanum* essential oil and nisin, as well as *Thymus* essential oil with the same was successful. It appears that the essential oils and nisin had a synergic effect, which enhanced the overall antimicrobial activity. Nisin is an effective antibacterial agent against Gram-positive bacteria and is a bacteriocin (Russell 1998). Consequently, lower concentrations of the essential oils can be used, which solves the issue of sensory contribution. The food pathogens that could be treated with these combinations are the common bacteria *L. monocytogenes*, *E. coli* and *S. typhimurium*. Foodstuff that is usually at risk, and where these combinations could be applied, is fresh food, meat and already prepared meals (Turgis et al. 2012).

Challenges

It is essential to point to the concerns regarding the use of natural preservatives and antioxidants: or more precisely, the way the research is undertaken. Lee Tan and Lim (2015) raise the concern that most of the research is being done *in vitro*, in laboratories. According to them, this may provide unreliable results depending on the methods and material applied. Lee Tan and Lim (2015) provide suggestions, for instance the need for standardisation. Standardisation may be problematic to achieve since there are many factors influencing the phytochemical production in plants. However, Lee Tan and Lim (2015) believe that using the same methods and units might be a start when studying essential oils and their antimicrobial activity.

Another challenge is the pH value of the food. Results gathered by Gutierrez, Barry-Ryan and Bourke (2009) showed that the MIC of the essential oils decreases in a more acidic environment.

Therefore, the effect of the essential oils seems to be higher in food items with lower pH values. When the pH is low the phenol can easily be transported into the membrane of the bacteria cell (Miyague et al. 2015). The phenol then reaches the cytoplasm and contributes to the decrease of the cell's pH value. As a result, the metabolism and the pathways of the cell are disrupted. Moreover, the growth of most food borne pathogens appears to be lower in an acidic environment. The results gained by Gutierrez, Barry-Ryan and Bourke (2009) show that essential oils from *Thymus* and *Origanum* were most efficient in a pH value around 5. In agreement with this, Miyague et al. (2015) found that the lowest MIC of some phenols tested on *L. monocytogenes* was at pH 5. At pH 6 and 7 the concentration had to be increased from 5 mM for pH 5, to 10 mM at pH 6 and the double was needed for pH 7. Therefore, it is likely that there will be sensory consequences on the food items treated with essential oils if pH is higher than 5.

Lastly, the interaction between phenolic compounds and the food elements is problematic. Phenolic compounds are suspected to bind to protein and fat, which restricts its antimicrobial ability (Bagamboula, Uyttendaele and Debevere 2004). Thus, it seems that natural preservatives ought to only be used in food that is low in fat and protein. In contrast, Gutierrez, Barry-Ryan and Bourke (2008) suggest that essential oils can be successfully applied in food items with a high content of protein, as they report that the essential oils from both *Thymus* and *Origanum* showed high antimicrobial activity in food items high in protein. Even though these studies show problematic contrasts, both agree on the fact that the food should be low in fat. Gutierrez, Barry-Ryan and Bourke (2008) add that the food item preferably should contain a low amount of carbohydrates as well.

Clearly, there is a need of further research on the application of essential oils and phenolic compounds in food systems, since results are divided on where and how the use is most effective.

Discussion

The results have shown that antimicrobial plant extracts can be used as preservatives. Nonetheless, there are numerous factors and potential challenges that need to be considered. The use of plant-based preservatives is highly dependent on the standardisation and consistency of

the products. Hence, in order to make it possible to use essential oil and phenolic compounds as commercially used preservatives, the cultivation ought to be optimised and the chemical variability minimised. The first step in achieving this goal is knowledge. Knowledge is needed about the antimicrobial compounds and how these are affected by different growth-related factors. From there on, the cultivation can be adapted with different techniques, such as exposing the plant to stress, controlling nutrition and soil composition, exposure to light and harvesting at the right time. This will enable a standardised high-quality yield of the essential oil and phenolic compound. It is of further importance to choose the right chemotype for the purpose, and perhaps propagate with cuttings from a high-quality mother plant. This will guarantee chemical consistency, which is not possible with propagation from seed. Breeding is a possibility in this area and could be highly beneficial. The goal of the breeding practice ought to be a variety of both *Origanum* and *Thymus* which genetically produce essential oils with high concentrations of *carvacrol* and *thymol*.

Importantly, the core question is how to maximise the yield of phenolic compounds. The biomass, the essential oil and the phenolic compounds all respond slightly differently to abiotic and biotic factors. The key is to find a method where biomass and oil yield is high, and where the oil contains high amounts of phenolic compounds. The glands are situated in the leaves for both *Origanum* and *Thymus* (Kintzios 2004; Stahl-Biskup and Venskutonis 2004), therefore, biomass is important and a large part of the total yield. However, phenols are known to increase when the plant is exposed to stress. The problem is the following: stress is known to decrease the biomass production (Azizi, Yan and Honermeier 2009). Due to this problematic imbalance of biomass and phenolic compound yield, more research as to maximise both biomass and oil yield is deemed necessary.

Furthermore, one aspect to keep in mind is that the essential oils and the compound's antimicrobial effect vary in relation to different bacteria. This means that careful examinations are required in order to find the right inhibitory concentration for the food and specific bacteria in question. More research is, thus, needed on how to inhibit the growth of bacteria in different food systems. For example, it needs to be clear where the different microbes are a risk, such as in certain meat products, ready cooked meals or salads, to name a few, and which MIC is needed

for these specific situations and products. This also means that the MIC must be low enough to be accepted in relation to sensory aspects. Which might be an issue as the MIC of the essential oil is significantly higher when used in food as compared to when investigated *in vitro* (Lee Tan and Lim 2015).

The MIC also depends on the activity of the compounds and whether the essential oil is used wholly, or the main compounds in combinations or alone. More research needs to be conducted on the matter of whether to use the whole essential oil or the major compounds as preservatives, since research have shown conflicting results. The question is if using certain constituents causes a bigger production cost, as less extract will be available with every plant and there are more steps in the process of extracting the pure compound. Therefore, the efficiency of the pure compound in comparison to the whole essential oil will need to be more closely examined.

There are some chemical hurdles as well, such as the pH value dependency and the compounds' interaction with the food ingredients. It needs to be carefully considered in which type of food system these extracts can be added. In food items with a low pH value, the plant-based preservatives would be efficient and most likely fulfil the required qualities of preservatives. Perhaps, this means that essential oils and compounds can be applied only where the efficiency is high enough. However, this might cause problems in the food industry, which may be accustomed to a straightforward way of adding preservatives. Thus, one question to ask is: is it realistic to implement natural preservatives? As it appears, it is possible, yet somewhat restricted in practice.

The use of essential oils and phenolic compounds are sure to be attractive to consumers, but the food industry might not be as convinced. There are more factors to take into account, such as pH, taste and food content. The food safety and shelf life are possibly more important for the food industry, whereas consumers demand healthy and natural products.

Consequently, the whole production chain ought to be communicating, from research to cultivation to consumer. This will enable the creation of a consistent and high-quality preservative, where all needs are understood and met. If this communication is achieved, the cultivation can be optimised, the right postharvest environment and extraction method can be chosen, and the most effective compounds can be applied in suitable food items and, lastly, find

a content consumer. The close communication between the production steps may minimise the cost of production and simultaneously enhance the effectiveness of the used compounds.

It would be interesting to see more research on cultivation of specific compounds. With new technology, the cultivation of medicinal plants and antimicrobial compounds is brought to new levels, where the yield of certain compounds can be optimised. This enables a more cost-effective production and in the long run a more sustainable system. Ongoing research includes nutraceuticals and, thus, more nutritious and healthy food. In the future, more research on the cultivation of plants containing bioactive compounds is sure to be beneficial in many ways, such as health and economy efficiency.

On a final note, one profound question that should not be unasked concerns the bigger picture: should these preservatives added to ready-prepared meals, whether natural or synthetic, in fact be needed? The need has been sparked by a globalisation of food trade and consumerism. There is an established culture of quick food-on-the-go and food stores offering a sheer endless choice of products and ready-prepared meals with a long shelf life. However, if consumer habits changed and more fresh products were consumed and home cooked, we would not be as dependent on preservatives. Instead of adapting the food items to the consumer habits of today, the cultivation systems and crops can be adapted to feed the local area. Consequently, less transportation, less food waste and a fair food trade could be achieved. This implies the work and decisions of national and international politics and individuals' will for vast systematic and personal change.

Conclusion

This essay has reached the conclusion that plant-based preservatives can be an alternative to synthetic ones; specifically, when using the essential oils from *Origanum* and *Thymus*, or the compounds *thymol* and *carvacrol* in combination, in rather acidic ready-prepared food (pH = 5) low in fat and carbohydrates.

An additional conclusion is that antibacterial preservatives used in ready-prepared meals may not be essential. With a change in consumer behaviour and a restriction of accessibility of

foodstuff the food industry will not be as dependent on preservatives. However, the knowledge of how to optimise the phenolic compounds and essential oils in plants is highly valuable. The use of these substances goes far beyond food preservation. The information of how to grow plants in a way which favours the production of phytochemicals can provide more nutritious crops, food and nutraceuticals. Therefore, energy and funding should arguably go into more studies on the biosynthesis of plants and how it is affected by cultivation techniques, in order to maximise yield of desired compounds.

References

Abdumumeen, H.A., Risikat, A.N. and Sururah, A.R. (2012). Food: Its Preservatives, Additives and Applications. *International Journal of Chemical and Biochemical Sciences*, vol. 1, pp. 36-47. Access:

https://www.researchgate.net/profile/Abdumumeen_Hamid/publication/268509774_Food_Its_preservatives_additives_and_applications/links/546dc8a50cf26e95bc3cff6d/Food-Its-preservatives-additives-and-applications.pdf

Azizi, A., Yan, F. and Honermeier, B. (2009). Herbage Yield, Essential Oil Content and Composition of Three Oregano (*Origanum vulgare* L.) Populations as Affected by Soil Moisture Regime and Nitrogen Supply. *Industrial Crops and Products*, vol. 29(2-3), pp. 554-561. DOI: <https://doi.org/10.1016/j.indcrop.2008.11.001>

Bagamboula, C.F., Uyttendaele, M. and Debevere, J. (2004). Inhibitory Effect of Thyme and Basil Essential Oils, Carvacrol, Thymol, Estragol, Linalool and p-cymene Towards *Shigella sonnei* and *S. flexneri*. *Food Microbiology*, vol. 21(1), pp. 33-42. DOI: [https://doi.org/10.1016/S0740-0020\(03\)00046-7](https://doi.org/10.1016/S0740-0020(03)00046-7)

Baranauskiene, R., Venskutonis, P.R., Viškelis, P. and Dambrauskiene, E. (2003). Influence of Nitrogen Fertilizers on the Yield and Composition of Thyme (*Thymus vulgaris*). *Journal of Agricultural and Food Chemistry*, vol. 51(26), pp. 7751-7758. Access: <https://pubs.acs.org/doi/abs/10.1021/jf0303316>

Baranauskiene, R., Venskutonis, P.R., Dambrauskiene, E. and Viškelis, P. (2013). Harvesting Time Influences the Yield and Oil Composition of *Origanum vulgare* L. ssp. *vulgare* and ssp. *hirtum*. *Industrial Crops and Products*, vol. 49, pp. 43-51. DOI: <https://doi.org/10.1016/j.indcrop.2013.04.024>

Bassolé, I.H.N. and Juliani, H.R. (2012). Essential Oils in Combination and Their Antimicrobial Properties. *Molecules*, vol.17(4), pp. 3989-4006. DOI: [10.3390/molecules17043989](https://doi.org/10.3390/molecules17043989)

Bendahou, M., Muselli, A., Grignon-Dubois, M., Benyoucef, M., Desjobert, J.-M., Bernardini, F. and Costa, J. (2008). Antimicrobial Activity and Chemical Composition of *Origanum glandulosum* Desf. Essential Oil and Extract Obtained by Microwave Extraction: Comparison with Hydrodistillation. *Food Chemistry*, vol. 106(1), pp. 132-139. DOI: <https://doi.org/10.1016/j.foodchem.2007.05.050>

Bennett, R.N. and Wallsgrove, R.M. (1994). Secondary Metabolites in Plant Defence Mechanisms. *New Phytol.*, vol. 127, pp. 617-633. Access: <https://nph.onlinelibrary.wiley.com/doi/pdf/10.1111/j.1469-8137.1994.tb02968.x>

Bernstein, N., Chaimovitch, D. and Dudai, N. (2009). Effect of Irrigation with Secondary Treated Effluent on Essential Oil, Antioxidant Activity, and Phenolic Compounds in Oregano and Rosemary. *Agronomy Journal*, vol. 101(1), pp. 1-10.

- Bettaieb, I., Bourgou, S., Wannes, W.A., Hamrouni, I., Limam, F. and Marzouk, B. (2010). Essential oils, Phenolics, and Antioxidant Activities of Different Parts of Cumin (*Cuminum cyminum* L.). *Journal of Agricultural and Food Chemistry*, vol. 58, pp. 10410-10418.
- Bhatia, S. (2015). Chapter 5 – Application of Plant Biotechnology. I: Bhatia, s., Sharma, K., Dahiya, R. and Bera, T. (red.), *Moderna Applications of Plant Biotechnology in Pharmaceutical Science*. Academic Press, Elsevier Inc, pp. 157-207. DOI: <https://doi.org/10.1016/B978-0-12-802221-4.00005-4>
- Bhattacharya, S. (2016). Chapter 3 – Cultivation of Essential Oils. I: Preedy, V.R. (red.), *Essential Oils in Food Preservation, Flavor and Safety*. Academic Press, Elsevier Inc, pp. 19-29. DOI: <https://doi.org/10.1016/B978-0-12-416641-7.00003-1>
- Bouhdid, S., Abrini, J., Zhiri, A., Espuny, M.J. and Manresa, A. (2009). Investigation of Functional and Morphological Changes in *Pseudomonas aeruginosa* and *Staphylococcus aureus* Cells Induced by *Origanum compactum* Essential Oil. *Journal of Applied Microbiology*, vol. 106(5), pp. 1558-1568. DOI: <https://doi.org/10.1111/j.1365-2672.2008.04124.x>
- Bouzidi, L.E., Jamali, C.A., Bekkouche, K., Hassani, L., Wohlmuth, H., Leach, D. and Abbad, A. (2013). Chemical Composition, Antioxidant and Antimicrobial Activities of Essential Oils Obtained from Wild and Cultivated Moroccan *Thymus* species. *Industrial Crops and Products*, vol. 43, pp. 450-456. DOI: <https://doi.org/10.1016/j.indcrop.2012.07.063>
- Burt, S. (2004). Essential oils: their Antibacterial Properties and Potential Applications in Foods – a Review. *International Journal of Food and Microbiology*, vol. 94(3), pp. 223-253. DOI: <https://doi.org/10.1016/j.ijfoodmicro.2004.03.022>
- Cambridge Dictionary (2019). *Antiseptic*. Access: <https://dictionary.cambridge.org/dictionary/english/antiseptic> [2019-05-15]
- Canter, P.H., Thomas, H. and Ernst, E. (2005). Bringing Medicinal Plants into Cultivation: Opportunities and Challenges for Biotechnology. *Trends in Biotechnology*, vol. 23(4), pp. 180-185. DOI: <https://doi.org/10.1016/j.tibtech.2005.02.002>
- Cristani, M., D'Arrigo, M., Mandalari, G., Castelli, F., Sarpietro, M.G., Micieli, D., Venuti, V., Bisignano, G., Saija, A. and Trombetta, D. (2007). Interaction of Four Monoterpenes Contained in Essential Oils with Model Membranes: Implication for Their Antibacterial Activity. *Journal of Agriculture and Food Chemistry*, vol. 55, pp. 6300-6308.
- Davidson, P.M. (2006). Food Antimicrobials: Back to Nature. *Acta Horticulturae*, vol. 709, pp. 29-34. DOI: <https://doi.org/10.17660/ActaHortic.2006.709.3>
- Davidson, P.M. and Zivanovic, S. (2003). Chapter 2 - The Use of Natural Antimicrobials. I: Zeuthen, P. and Bogh-Sorensen, L. (red.), *Food Preservation Techniques*. Woodhead Publishing Limited and CRC Press LLC, pp. 5-30.

- Dayani, S. and Sabzalian, M.R. (2016). Production of Secondary Metabolites in Medicinal Plants through Hydroponic Systems. I: Asaduzzaman, M. (red), *Controlled Environment Agriculture*. Nova Science Publishers, Inc, pp. 33-53. Access: https://www.researchgate.net/publication/322132402_Production_of_Secondary_Metabolites_in_Medicinal_Plants_through_Hydroponic_Systems
- Deans, S.G. and Ritchie, G. (1987). Antibacterial Properties of Plant Essential Oils. *International Journal of Food Microbiology*, vol. 5(2), pp. 165-180. DOI: [https://doi.org/10.1016/0168-1605\(87\)90034-1](https://doi.org/10.1016/0168-1605(87)90034-1)
- Devika, R. and Koilpillai, J. (2012). An Overview on Plant Secondary Metabolites: Its Medicinal Importance. *Journal of Pharmacy Research*, vol. 5(2), pp. 984-986. Access: https://www.researchgate.net/publication/266206147_An_overview_on_plant_secondary_metabolites_Its_medical_importance_RDevika_and_Justin_Koilpillai
- Dhifi, W., Bellili, S., Jazi, S., Bahloul, N. and Mnif, W. (2016). Essential Oils' Chemical Characterization and Investigation of Some Biological Activities: A Critical Review. *Medicines (Basel)*, vol. 3(4). DOI: [10.3390/medicines3040025](https://doi.org/10.3390/medicines3040025)
- Dhouioui, M., Boulila, A., Chaabane, H., Zina, M.S. and Casabianca, H. (2016). Seasonal Changes in Essential Oil Composition of *Aristolochia longa* L. ssp. *paucinervis* Batt. (Aristolochiaceae) Roots and its Antimicrobial Activity. *Industrial Crops and Products*, vol. 83, pp. 301-306. DOI: <https://doi.org/10.1016/j.indcrop.2016.01.025>
- Dudai, N. (2008). Optimization and Improvement of Phenolic Monoterpenes Production in Oregano (*Origanum* spp.). *Acta Horticulturae*, vol. 778, pp. 15-28. DOI: <https://doi.org/10.17660/ActaHortic.2008.778.1>
- Dudai, N., Putievsky, E., Ravid, U., Palevitch, D. and Halevy, A.H. (1992). Monoterpene Content in *Origanum syriacum* as Affected by Environmental Conditions and Flowering. *Physiologica Plantarum*, vol. 84, pp. 453-459.
- Dyakov, Y.T. and Dzhavakhiya, V.G. (2007). Chapter 6 – Horizontal Pathosystem: Resistance factors. I: Dyakov, Y.T., Dzhavakhiya, V.G. and Korpela, T. (red.), *Comprehensive and Molecular Phytopathology*. Elsevier Science, Elsevier Inc, pp. 161-179. DOI: <https://doi.org/10.1016/B978-044452132-3/50010-9>
- Fletcher, N. (2014). Food Additives: Preservatives. *Encyclopaedia of Food Science*, vol. 2, pp. 471-473. DOI: <https://doi.org/10.1016/B978-0-12-378612-8.00226-2>
- Fleuriet, A. and Macheix, J.-J. (2003). Phenolic Acids in Fruits and Vegetables. I: Rice-Evans, C.A. and Packer, L. (red.), *Flavonoids in Health and Disease*, second edition. New York: Marcel Dekker, Inc, pp. 1-41.
- Giannenas, I., Bonos, E., Christaki, E. and Florou-Paneri, P. (2018). Chapter 6 – Oregano: A Feed Additive With Functional Properties. I: Holban, A.M. and Mihai Grumezescu, A. (red.),

Therapeutic Food – Handbook of Food Bioengineering, Academic Press, Elsevier Inc, pp. 179-208. DOI: <https://doi.org/10.1016/B978-0-12-811517-6.00006-4>

Giurgiu, R.M., Morar, G., Dumitraş, A., Vlăsceanu, G., Dune, A. and Schroeder, F.-G. (2017). A Study of the Cultivation of Medicinal Plants in Hydroponic and Aeroponic Technologies in a Protected Environment. *Acta Horticulturae*, vol. 1170, pp. 671-678. DOI: <https://doi.org/10.17660/ActaHortic.2017.1170.84>

Gutierrez, J., Barry-Ryan, C. and Bourke, P. (2008). The Antimicrobial Efficacy of Plant Essential Oil Combinations and Interactions with Food Ingredients. *International Journal of Food Microbiology*, vol. 124(1), pp. 91-97. DOI: <https://doi.org/10.1016/j.ijfoodmicro.2008.02.028>

Gutierrez, J., Barry-Ryan, C. and Bourke, P. (2009). Antimicrobial Activity of Essential Oils Using Food Model Media: Efficacy, Synergistic Potential and Interactions with Food Components. *Food Microbiology*, vol. 26(2), pp. 142-150. DOI: <https://doi.org/10.1016/j.fm.2008.10.008>

Halliwel, B. (1996). Antioxidants: The Basics – What They Are and How to Evaluate Them. *Advances in Pharmacology*, vol. 38, pp. 3-20. DOI: [https://doi.org/10.1016/S1054-3589\(08\)60976-X](https://doi.org/10.1016/S1054-3589(08)60976-X)

Hassiotis, C.N., Ntana, F., Lazari, D.M., Poulis, S. and Vlachonasios, K.E. (2014). Environmental and Developmental Factors Affect Essential Oil Production and Quality of *Lavandula angustifolia* During Flowering Period. *Industrial Crops and products*, vol. 62, pp. 359-366. DOI: <https://doi.org/10.1016/j.indcrop.2014.08.048>

Hyltdgaard, M., Mygind, T. and Meyer, R.L. (2012). Essential Oils in Food Preservation: Mode of Action, Synergies and Interactions with Food Matrix Components. *Frontiers in Microbiology*, vol. 3, pp. 1-24. Access: https://www.researchgate.net/publication/221792983_Essential_Oils_in_Food_Preservation_Mode_of_Action_Synergies_and_Interactions_with_Food_Matrix_Components

Jordán, M.J., Martínez, R.M., Goodner, K.L., Baldwin, E.A. and Sotomayor, J.A. (2006). Seasonal Variation of *Thymus hyemalis* Lange and Spanish *Thymus vulgaris* L. Essential Oils Composition. *Industrial Crops and Products*, vol. 24(3), pp. 253-263. DOI: <https://doi.org/10.1016/j.indcrop.2006.06.011>

Karamanos, A.J. and Sotiropoulou, D.E.K. (2013). Field Studies of Nitrogen Application on Greek Oregano (*Origanum vulgare* L. ssp. *hirtum* (Link) Ietswaart) Essential Oil During Two Cultivation Seasons. *Industrial Crops and Products*, vol. 46, pp. 246-252. DOI: <https://doi.org/10.1016/j.indcrop.2013.01.021>

Kintzios, S.E. (2004). 14 – Oregano. I: Peter, K.V. (red.), *Handbook of Herbs and Spices*. Woodhead Publishing Limited, vol. 2, pp. 215-229. DOI: <https://doi.org/10.1533/9781855738355.2.215>

Kokkini, S., Karousou, R. and Vokou, D. (1994). Pattern of Geographical Variations of *Origanum vulgare* Trichomes and Essential Oil Content in Greece. *Biochemical Systematics and Ecology*, vol. 22(5), pp. 517-528. DOI: [https://doi.org/10.1016/0305-1978\(94\)90046-9](https://doi.org/10.1016/0305-1978(94)90046-9)

Kougan, G.B., Tabopda, T., Kuete, V. and Verpoorte, R. (2013). Chapter 6 – Simple Phenolics, Phenolic Acids, and Related Esters from the Medicinal Plants of Africa. I: Kuete, V. (red.), *Medicinal Plant Research in Africa – Pharmacology and Chemistry*. Elsevier Inc, pp. 225-249. DOI: <https://doi.org/10.1016/B978-0-12-405927-6.00006-0>

Król, B. and Kieltyka-Dadasiewicz, A. (2015). Yield and Herb Quality of thyme (*Thymus vulgaris* L.) Depending on Harvest Time. *Turkish Journal of Field Crops*, vol. 20(1), pp. 78-84. Access: https://www.researchgate.net/publication/275027505_Yield_and_herb_quality_of_thyme_Thymus_vulgaris_L_depending_on_harvest_time

Kurita, N., Miyaji, M., Kurane, R. and Takahara, Y. (1981). Antifungal Activity of Components of Essential Oils. *Agricultural and Biological Chemistry*, vol. 45(4), pp. 945-952. Access: <https://www.tandfonline.com/doi/pdf/10.1080/00021369.1981.10864635?needAccess=true>

Lambert, R.J.W., Skandamis, P.N., Coote, P.J. and Nychas, G.-J.E. (2001). A Study of the Minimum Inhibitory Concentration and Mode of Action of Oregano Essential Oil, Thymol and Carvacrol. *Journal of Applied Microbiology*, vol. 91(3), pp. 453-462. DOI: <https://doi.org/10.1046/j.1365-2672.2001.01428.x>

Lau, J.T.F. and Wong, Y.-C. (2019). Food and Nutritional Analysis | Antioxidants and Preservatives. *Encyclopedia of Analytical Science (Third Edition)*, pp. 374-380. DOI: <https://doi.org/10.1016/B978-0-12-409547-2.14471-3>

Lee Tan, J.B. and Lim, Y.Y. (2015). Critical Analysis of Current Methods for Assessing the *in vitro* Antioxidant and Antibacterial Activity of Plant Extracts. *Food Chemistry*, Vol. 172, pp. 814-822. DOI: <https://doi.org/10.1016/j.foodchem.2014.09.141>

Liu, S.-Q. (2016). Lactic Acid Bacteria: *Leuconostoc* spp. *Reference Module in Food Science*. DOI: <https://doi.org/10.1016/B978-0-08-100596-5.00859-3>

Lubbe, A. and Verpoorte, R. (2011). Cultivation of Medicinal and Aromatic Plants for Speciality Industrial Materials. *Industrial Crops and Products*, vol. 34(1), pp. 785-801. DOI: <https://doi.org/10.1016/j.indcrop.2011.01.019>

Lv, F., Liang, H., Yuan, Q. and Li, C. (2011). In vitro Antimicrobial Effects and Mechanisms of Action of Selected Plant Essential Oil Combinations Against Four Food-Related Microorganisms. *Food Research International*, vol. 44(9), pp. 3057-3064. DOI: <https://doi.org/10.1016/j.foodres.2011.07.030>

Mani-López, E., Palou, E. and López-Malo, A. (2016). Preservatives: Classification and Analysis. I: Caballero, B., Finglas, P.M. and Toldrá, F. (red.), *Encyclopedia of Food and Health*. Academic Press, Elsevier Inc, pp. 497-504. DOI: <https://doi.org/10.1016/B978-0-12-384947-2.00567-5>

Mishra, P.K., Singh, P., Prakash, B., Kedia, A., Dubey, N.K. and Chanotiya, C.S. (2013). Assessing Essential Oil Components as Plant-Based Preservatives Against Fungi that Deteriorate Herbal Raw Materials. *International Biodeterioration & Biodegradation*, vol. 80, pp. 16–21. DOI: <https://doi.org/10.1016/j.ibiod.2012.12.017>

Miyague, L., Macedo, R.E.F., Meca, G., Holley, R.A. and Luciano, F.B. (2015). Combination of Phenolic Acids and Essential Oils Against *Listeria monocytogenes*. *LWT – Food Science and Technology*, vol. 64(1), pp. 333-336. DOI: <https://doi.org/10.1016/j.lwt.2015.05.055>

Namdeo, A.G. (2018). Chapter 20 - Cultivation of Medicinal and Aromatic Plants. I: Mandal, S.C., Mandal, V. and Konishi, T. (red.), *Natural Products and Drug Discovery - An Integrated Approach*. Elsevier Ltd, pp. 523-553. DOI: <https://doi.org/10.1016/B978-0-08-102081-4.00020-4>

Nazer, A.I., Kobilinsky, A., Tholozan, J.-L. and Dubois-Brissonnet, F. (2005). Combinations of Food Antimicrobials at Low Levels to Inhibit the Growth of *Salmonella* sv. *Typhimurium*: A Synergistic Effect? *Food Microbiology*, vol. 22(5), pp. 391-398. DOI: <https://doi.org/10.1016/j.fm.2004.10.003>

Okoh, O.O., Sadimenko, A.P. and Afolayan, A.J. (2010). Comparative Evaluation of the Antibacterial Activities of the Essential Oils of *Rosmarinus officinalis* L. Obtained by Hydrodistillation and Solvent Free Microwave Extraction Methods. *Food Chemistry*, vol. 120(1), pp. 308-312. DOI: <https://doi.org/10.1016/j.foodchem.2009.09.084>

Ortega-Ramirez, L.A., Rodriguez-Garcia, I., Silva-Espinoza, B.A. and Ayala-Zavala, J.F. (2016). Chapter 71- Oregano (*Origanum* spp.) Oils. I: Preedy, V.R. (red.), *Essential Oils in Food Preservation, Flavor and Safety*. Academic Press, Elsevier Inc, pp. 625-631. DOI: <https://doi.org/10.1016/B978-0-12-416641-7.00071-7>

Pesavento, G., Calonico, C., Bilia, A.R., Barnabei, M., Calesini, F., Addona, R., Mencarelli, L., Carmagnini, L., Di Martino, M.C. and Lo Nostro, A. (2015). Antibacterial Activity of Oregano, Rosmarinus and Thymus Essential Oils Against *Staphylococcus aureus* and *Listeria monocytogenes* in Beef Meatballs. *Food Control*, vol. 54, pp. 188-199. DOI: <https://doi.org/10.1016/j.foodcont.2015.01.045>

Pietta, P., Gardana, C. and Pietta, A. (2003). Flavonoids in Herbs. I: Rice-Evans, C.A. and Packer, L. (red.), *Flavonoids in Health and Disease*, second edition. New York: Marcel Dekker, Inc, pp. 43-70.

Pokorný, J. (2003). Chapter 3 – Natural Antioxidants. I: Zeuthen, P. and Bogh-Sorensen, L. (red.), *Food Preservation Techniques*. Woodhead Publishing Limited and CRC Press LLC, pp. 31-48.

Prakash, B., Singh, P., Kedia, A. and Dubey, N.K. (2012a). Assessment of Some Essential Oils as Food Preservatives Based on Antifungal, Antiaflatoxin, Antioxidant Activities and *in vivo* Efficacy in Food System. *Food Research International*, vol. 49(1), pp. 201-208. DOI: <https://doi.org/10.1016/j.foodres.2012.08.020>

Prakash, B., Singh, P., Kedia, A., Singh, A. and Dubey, N.K. (2012b). Efficacy of Essential Oil Combination of *Curcuma longa* L. and *Zingiber officinale* Rosc. As a Postharvest Fungitoxicant, Aflatoxin Inhibitor and Antioxidant Agent. *Journal of Food Safety*, vol. 32, pp. 279-288.

Prakash, B., Kedia, A., Mishra, P.K and Dubey, N.K. (2015). Plant Essential Oils as Food Preservatives to Control Moulds, Mycotoxin Contamination and Oxidative Deuteriation of Agri-Food Commodities – Potentials and Challenges. *Food Control*, vol. 47, pp. 381-391. DOI: <https://doi.org/10.1016/j.foodcont.2014.07.023>

Putievsky, E. and Ravid, U. (1984). Differences in Yield and Essential Oils of Various Types of *Origanum vulgare* L. Grown Under Intensive Cultivation Conditions. *Acta Horticulturae*, vol. 144, pp. 71-76. DOI: [10.17660/ActaHortic.1984.144.9](https://doi.org/10.17660/ActaHortic.1984.144.9)

Putievsky, E., Ravid, U. and Dudai, N. (1988). Phenological and Seasonal Influences on Essential Oil of a Cultivated Clone of *Origanum vulgare* L. *Journal of the Science of Food and Agriculture*, vol. 43(3), pp. 225-228. DOI: <https://doi.org/10.1002/jsfa.2740430304>

Ríos, J.-L. (2016). Chapter 1 – Essential oils: What They Are and How the Terms Are Used and Defined. I: Preedy, V.R. (red.), *Essential Oils in Food Preservation, Flavor and Safety*. Academic Press, Elsevier Inc, pp. 3-10. DOI: <https://doi.org/10.1016/B978-0-12-416641-7.00001-8>

Rota, M.C., Herrera, A., Martínez, R.M., Sotomayor, J.A. and Jordán, M. (2008). Antimicrobial Activity and Chemical Composition of *Thymus vulgaris*, *Thymus zygis* and *Thymus hyemalis* Essential Oils. *Food Control*, vol. 19(7), pp. 681-687. DOI: <https://doi.org/10.1016/j.foodcont.2007.07.007>

Russell, A.D. (1998). 4 – Mechanisms of Bacterial Resistance to Antibiotics and Biocides. *Progress in Medicinal Chemistry*, vol. 35, pp. 133-197. DOI: [https://doi.org/10.1016/S0079-6468\(08\)70036-5](https://doi.org/10.1016/S0079-6468(08)70036-5)

Sánchez-Maldonado, A.F., Schieber, A. and Gänzle, M.G. (2011). Structure-Function Relationships of the Antibacterial Activity of Phenolic Acids and Their Metabolism by Lactic Acid Bacteria. *Journal of Applied Microbiology*, vol. 111(5), pp. 1176-1184. DOI: <https://doi.org/10.1111/j.1365-2672.2011.05141.x>

Santos-Sánchez, N. F., Salas-Coronado, R., Valadez-Blanco, R., Hernández-Carlos, B., Guadarrama-Mendoza, P.C. (2017). Natural Antioxidant Extracts as Food Preservatives. *Acta Sci. Pol. Technol. Aliment.*, vol. 16(4), pp. 361–370. Access: https://www.researchgate.net/publication/321856225_Natural_antioxidant_extract_as_food_preservatives

Sellami, I.H., Maamouri, E., Chahed, T., Wannes, W.A., Kchouk, M.E. and Marzouk, B. (2009). Effect of Growth Stage on the Content and Composition of the Essential Oil and Phenolic Fraction of Sweet Marjoram (*Origanum majorana* L.). *Industrial Crops and Products*, vol. 30(3), pp. 395-402. DOI: <https://doi.org/10.1016/j.indcrop.2009.07.010>

Shahidi, F. (2015). Antioxidants: Principles and Applications. I: Shahidi, F. (red.), *Handbook of Antioxidants for Food Preservation*. Woodhead Publishing, Elsevier Inc, pp. 1-14. DOI: <https://doi.org/10.1016/B978-1-78242-089-7.00001-4>

Shalaby, A.S. and Razin, A.M. (1992). Dense Cultivation and Fertilization for Higher Yield of Thyme (*Thymus vulgaris* L.). *Journal of Agronomy and Crop Science*, vol. 168(4), pp. 243-248. DOI: <https://doi.org/10.1111/j.1439-037X.1992.tb01005.x>

Sotiropoulou, D.E. and Karamanos, A.J. (2010). Field Studies of Nitrogen Application on Growth and Yield of Greek Oregano (*Origanum vulgare* ssp. *hirtum* (Link) Ietswaart). *Industrial Crops and Products*, vol. 32(3), pp. 450-457. DOI: <https://doi.org/10.1016/j.indcrop.2010.06.014>

Stahl-Biskup, E. and Venskutonis, R.P. (2004). 19 – Thymus. I: Peter, K.V. (red.), *Handbook of Herbs and Spices*. Woodhead Publishing Limited, pp. 297-321. DOI: <https://doi.org/10.1533/9781855738355.2.297>

Stratakos, A.C. and Koidis, A. (2016). Chapter 4 – Methods for Extracting Essential Oils. I: Preedy, V.R. (red.), *Essential Oils in Food Preservation, Flavor and Safety*. Academic Press, Elsevier Inc, pp. 31-38. DOI: <https://doi.org/10.1016/B978-0-12-416641-7.00004-3>

Tibaldi, G., Fontana, E. and Nicola, S. (2011). Growing Conditions and Postharvest Management can Affect the Essential Oil of *Origanum vulgare* L. ssp. *hirtum* (Link) Ietswaart. *Industrial Crops and Products*, vol. 34(3), pp. 1516-1522. DOI: <https://doi.org/10.1016/j.indcrop.2011.05.008>

Tognolini, M., Barocelli, E., Ballabeni, V., Bruni, R., Bianchi, A., Chiavarini, M. and Impicciatore, M. (2006). Comparative Screening of Plant Essential Oils: Phenylpropanoid Moiety as Basic Core for Antiplatelet Activity. *Life Science*, vol. 78(13), pp. 1419-1432. DOI: <https://doi.org/10.1016/j.lfs.2005.07.020>

Turgis, M., Dang Vu, K., Dupont, C. and Lacroix, M. (2012). Combined Antimicrobial Effect of Essential Oils and Bacteriocins Against Foodborne Pathogens and Food Spoilage Bacteria. *Food Research International*, vol. 48(2), pp. 696-702. DOI: <https://doi.org/10.1016/j.foodres.2012.06.016>

Udagawa, Y. (1995). Some Responses of Dill (*Anethum graveolens*) and Thyme (*Thymus vulgaris*) Grown in Hydroponic, to the Concentration of Nutrient Solution. *Acta Horticulturae*, vol. 396, pp. 203-210. DOI: <https://doi.org/10.17660/ActaHortic.1995.396.24>

Ultee, A., Bennik, M.H.J. and Moezelaar, R. (2002). The Phenolic Hydroxyl Group of Carvacrol Is Essential for Action against the Food-Borne Pathogen *Bacillus cereus*. *Applied and Environmental Microbiology*, vol. 68(4), pp. 1561-1568. DOI: [10.1128/AEM.68.4.1561-1568.2002](https://doi.org/10.1128/AEM.68.4.1561-1568.2002)

Valero, M. and Giner, M.J. (2006). Effects of Antimicrobial Components of Essential Oils on Growth of *Bacillus cereus* INRA L2104 in and the Sensory Qualities of Carrot Broth. *International Journal of Food Microbiology*, vol. 106(1), pp. 90-94. DOI: <https://doi.org/10.1016/j.ijfoodmicro.2005.06.011>

Van der Mheen, H. (2006). Selection and Production of Oregano Rich in Essential Oil and Carvacrol. *Acta Horticulturae*, vol. 709, pp. 95-200. DOI: [10.17660/ActaHortic.2006.709.10](https://doi.org/10.17660/ActaHortic.2006.709.10)

Walsh, S.E., Maillard, J.-Y., Russell, A.D., Catrenich, C.E., Charbonneau, D.L. and Bartolo, R.G. (2003). Activity and Mechanisms of Action of Selected Biocidal Agents on Gram-Positive and -Negative Bacteria. *Journal of applied Microbiology*, vol. 94(2), pp. 240-247. DOI: <https://doi.org/10.1046/j.1365-2672.2003.01825.x>

WHO (2019). *Foodborne Diseases*. Access: https://www.who.int/foodsafety/areas_work/foodborne-diseases/en/ [2019-05-15]

Yosr, Z., Hnia, C., Rim, T. and Mohamed, B. (2013). Changes in Essential Oil Composition and Phenolic Fraction in *Rosmarinus officinalis* L. var. *typicus* Batt. Organs During Growth and Incidence on the Antioxidant Activity. *Industrial Crops and Products*, vol. 43, pp. 412-419. DOI: <https://doi.org/10.1016/j.indcrop.2012.07.044>

Zaouali, Y., Bouzaine, T. and Boussaid, M. (2010). Essential Oils Composition in Two *Rosmarinus officinalis* L. varieties and Incidence for Antimicrobial and antioxidant Activity. *Food and Chemical Toxicity*, vol. 48(11), pp. 3144-3152. DOI: <https://doi.org/10.1016/j.fct.2010.08.010>