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Potential of plant-derived compounds to enhance the efficiency of conventional preservatives

Bachelor thesis

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Výživa a potraviny

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Declaration

I hereby declare that I have authored this bachelor thesis carrying the name „Potential of plant-derived compounds to enhance the efficiency of conventional preservatives“ independently under the guidance of my supervisor. Furthermore, I confirm that I have used only professional literature and other information sources that have been indicated in the thesis and listed in the bibliography at the end of the thesis. As the author of the bachelor thesis, I futher state that I have not infringed the copyrights of third parties in connection with its creation.

In Prague on 21. 4. 2023 ____________________________

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Potenciál využití rostlinných látek ke zvyšování účinnosti konvenčních potravinářských konzervantů

Shrnutí:

Cílem bakalářské práce bylo zhodnocení dostupných dat o synergickém účinku mezi konvenčními konzervanty a rostlinnými látkami. Literární rešerše dále pojednává o konzervaci potravin, konvenčních konzervačních látkách a jejich vedlejších účincích, rostlinných silicí, jejich chemickém složení, metodách extrakce, o použití silic jako potravinářských aditiv a jejich vlivu na senzorické vlastnosti, dále je uveden stručný popis rostlinných extraktů.

Nejpoužívanějším konvenčním konzervantem byl nisin, který vykazoval synergický účinek se všemi použitými sloučeninami rostlinného původu. Kombinace nisinu a sloučenin rostlinného původu, konkrétně rozmarýnového extraktu, je zároveň alternativou, která umožňuje snížit potřebnou dávku samotného použitého konzervačního prostředku a zároveň se jeví jako potenciální řešení problému bakteriální rezistence. Práce rovněž uvádí možnou alternativu konzervace mléka pomocí nisinu a jeho kombinace s cinnamaldehydem, kyselinou citronovou a silicí *Ziziphora clinopodioides*. Dále byl dvojnásobně potrvzen synergický efekt lysozymu při konzervaci ryb, kdy kombinace lysozymu a extraktu z kůry granátového jablka vykazovala i senzorickou přijatelnost a oproti neošetřeným vzorkům prodloužila přijatelnost vzorků makrely o dalších 6 dnů. Na základě zhodnocení dostupných dat lze potvrdit, že sloučeniny rostlinného původu skutečně zvyšují účinnost konvenčních konzervačních látek vzhledem k tomu, že všechny kombinace konzervačních látek a sloučenin rostlinného původu vykazovaly synergický účinek. Účinnost byla prokázána v různých potravinových modelech, od masa a masných výrobků, mléka, omáček a polévky, mrkvové šťávy a šťávy z cukrové třtiny, po ryby až po čerstvé jahody a brokolici. Je tedy vidět velká rozmanitost potenciálního využití synergie ke konzervaci potravin.

Klíčová slova: konvenční konzervační látky, rostlinné látky, silice, synergie, zvýšení účinnosti, trvanlivost

Potential of plant-derived compounds to enhance the efficiency of conventional preservatives

Summary:

The aim of the bachelor thesis was to evaluate the available data on the synergistic effect between conventional preservatives and plant-derived compounds. The literature search further discusses food preservation, conventional preservatives and their side effects, plant essential oils, their chemical composition, extraction methods, the use of essential oils as food additives and their effect on sensory properties, and a brief description of plant extracts.

The most frequently used conventional preservative was nisin, which showed a synergistic effect with all the plant-derived compounds reported. The combination of nisin and plant-derived compounds, specifically rosemary extract, is both an alternative that allows the required dose of the preservative used alone to be reduced and also appears to be a potential solution to the problem of bacterial resistance. The thesis also attributes a possible alternative to milk preservation using nisin and its combination with cinnamaldehyde, citric acid and *Ziziphora clinopodioides* essential oil. Furthermore, the synergistic effect of lysozyme in fish preservation was confirmed, where the combination of lysozyme and pomegranate peel extract also displayed sensory acceptability and prolonged the acceptability of mackerel samples by an additional 6 days compared to untreated ones. Based on the evaluation of the available data, it can be confirmed that plant-derived compounds indeed enhance the efficiency of conventional preservatives, given that all combinations of preservatives and plant-derived compounds demonstrated a synergistic effect. Efficiency has been shown in different food models, varying from meat and meat products, milk, sauces and soup, carrot and sugar cane juice and fish to fresh strawberries or broccoli. Therefore, a great variety of potential uses of synergy for food preservation can be seen.

Keywords: conventional food preservatives, plant compounds, essential oils, synergy, enhancement, shelf life

Content

1 Introduction

The means to prolong the shelf life of food were fundamental to the functioning of civilization back in the old days. Conventional preservation techniques are used to maintain food and extend its shelf life. The use of food additives to prolong the shelf life is also one of the means of preservation (Amit et al. 2017).

Nowadays, minimally processed foods without the use of synthetic preservatives are frequently preferred by consumers. Additives currently used for food preservation are predominantly synthetic in nature. The safety and possible negative effects of synthetic preservatives are under debate due to their potential side effects on human health (Risikat et al. 2012; Anand & Sati 2013; Dwivedi Sangeeta et al. 2017).

The use of natural substances for food preservation is a possible alternative instead of the application of the synthetic ones. Alternative substances suitable for food preservation include, among other things, essential oils. As well as perservation of food, the use of essential oils dates to ancient times. Essential oils are aromatic substances of plant origin that possess antibacterial, antifungal and antiviral properties that are important for their use in the food industry and are also known for their medicinal properties. However, their use is broader and not limited to these areas (Bakkali et al. 2008; Dima & Dima 2015; Nazzaro et al. 2017).

Essential oils are mixtures of volatile organic compounds, which are among other things, the products of the distillation process of a chemical precursor in plants. The main organic compounds found in essential oils would include for example terpenes, which can modify the flavour and aroma of food and beverages. At the same time, the origin and method of extraction of essential oils must also be taken into account, with consumers again preferring the traditional method of extraction and synthetic essential oils being less favoured (Bakkali et al. 2008; Ben Salha et al. 2021; Sadgrove et al. 2022).

Other plant-derived substances suitable as alternatives to synthetic preservatives include plant extracts. Plant extracts contain biomolecules that possess mechanisms effective against resistant pathogens (Malik & Mandal 2022; Abdullahi et al. 2022).

However, both essential oils and plant extracts can have an impact on the sensory attributes of foods, especially the flavour (Yamazaki et al. 2004; Akhtar et al. 2015).

The use of plant-derived compounds as food additives along with synthetic substances is an alternative option to potentially increase sensory acceptability due to the use of lower concentrations of the plant-derived substances. And at the same time there is the potential effect of increasing the efficiency of conventional preservatives (Thomas & Isak 2006; Solomakos et al. 2008).

2 Scientific hypothesis and aims of the thesis

The aim of the bachelor thesis was to evaluate available data on synergistic effect of conventional preservatives with plant-derived compounds in food models with special emphasis on the shelf life and sensory properties of the foods.

3 Literature research

3.1 Preservation

Food preservation and its history goes back to ancient civilization when knowledge of food preservation was one of the first most important steps in the foundation of civilization. The aim of food preservation is to prolong the shelf life of food and preserve its original properties such as nutritional value, colour, taste, and texture. Food processing for preservation involves also the stages related to growing, harvesting and distribution. Food preservation therefore involves the processing of food to maintain its quality and prevent chemical and microbial degradation, using techniques such as dehydration, refrigeration, freezing or heating. In addition to the abovementioned traditional methods, new technologies such as hurdle technology, irradiation and high-pressure technology are currently being used (Amit et al. 2017).

As such, foods subject to preservation can be classified according to several factors, such as their nutritional value, their function, and their shelf-life. For food, the process of spoilage is natural, resulting in changes in the food's colour, texture, taste, nutritional value and, of course, loss of edibility, which can lead to various diseases or even death for the consumer. The division of foods according to their shelf life is into perishable (their shelf life ranges from a few days to 3 weeks, this category would include food such as meat, milk and its products, and seafood), semi-perishable (their shelf life can be up to 6 months, if proper storage conditions are maintained, this would include vegetables, fruit or cheese, for example) and non-perishable (these are natural and processed foods that can be stored for several years and would include nuts, flour, sugar, beans or canned fruit, for example).

The main factors of food spoilage mentioned above include air, nutrients, temperature, pH, and the presence of various chemicals. These factors can be divided into several processes - chemical, physical and microbial. These processes can occur simultaneously and are not mutually exclusive (Steele 2004; Doyle 2009; Amit et al. 2017).

Chemical spoilage

Chemical and biochemical processes occur naturally in food. Fresh food may undergo quality changes such as pH changes due to the growth of microorganisms and their metabolism or changes due to toxic substances or lipid oxidation. Oxidative changes also depend on temperature changes. In addition to oxidation, chemical processes would include, for example, proteolysis, Maillard reaction, hydrolytic rancidity, or hydrolysis of pectin (Steele 2004; Amit et al. 2017).

Physical spoilage

This spoilage process causes physical changes or instability, such as loss, increase or migration of moisture between different ingredients, or physical separation of ingredients. The main factors affecting this kind of spoilage include temperature, moisture content, crystallization, and crystal growth (Steele 2004; Amit et al. 2017).

Microbial spoilage

This form of spoilage occurs under the action of microorganisms when it is one of the most common causes of consumer diseases. This type of deterioration can be prevented or delayed by preservation - by adjusting the storage temperature, reducing water activity and pH, or, for example, by using preservatives (Tianli et al. 2014; Amit et al. 2017)

Methods of food preservation and processing

Dehydratation

This is one of the oldest and least expensive preservation methods, the principle of which is to evaporate water from solid or liquid foods, the principle being to obtain a solid product with a low water content to the extend that the activity of microorganisms is inhibited (which for most microorganisms would be below the water activity value of 0.88). Advantages of this process include reduction in weight and volume of food products, ease of packaging, storage, and transport. Disadvantages include loss of flavour and aroma, loss of some nutritional components of the food such as loss of vitamin C, B_1, proteins and/or lipids (Rayaguru & Routray 2010; Agrahar-Murugkar & Jha 2010; Berk 2013; Syamaladevi et al. 2016; Amit et al. 2017).

Drying methods would include pasteurisation. Pasteurisation destroys spoilage microorganisms and enzymes. Pasteurisation is a process in which food is subjected to a mild heat treatment with temperatures averaging between 65 and 75 °C, the exception being the UHT technique. The ultra high temperature (UHT) method is based on continuous exposure to temperatures between 135 and 150 °C for several seconds and is typically used for milk preservation. Other well-known heat preservation methods include sterilisation. It is a severe heat treatment process at temperatures from 135-140 °C up to 150 °C for foods with a pH greater than 4.6 (Shenga et al. 2010; Amit et al. 2017).

Freezing

The principle of the freezing technique as food preservation is based on the reduction of water activity and its content in food. Freezing stops the growth of pathogenic microorganisms. During freezing, ice formation occurs, the process involved is nucleation. Nucleation refers to the formation of an ice crystal which then grows. The time required to lower the initial temperature of the food is called the freezing time. Freezing can be slow and fast, with slow freezing generally resulting in the formation of larger crystals in the extracellular tissue and fast freezing resulting in the formation of smaller crystals throughout the tissue (George 2008; Amit et al. 2017).

Chilling

The temperature of food in the preservation method of chilling varies from -1 to 8 °C, the principle being that this method lowers the initial temperature of the food and then maintains it. In addition to slowing down microbial and biochemical changes, this method also increases the shelf life of the food. This method of preservation is relatively expensive as it requires specialised equipment such as a plate heat exchanger, a vacuum attribution system or an air cooler, for example (Saravacos & Kostaropoulous 2002; Amit et al. 2017).

Chemical processes

Preserving food using chemical agents is one of the traditional methods of preservation. However, the chemicals used for preservation have raised concerns among consumers because of the potential impact on their health. These are substances that are capable of preserving the appearance, texture and taste of the food, thereby preserving the shelf life of the food along with the ability to inhibit microorganisms. Preservatives can be divided into the above mentioned chemical and natural preservatives (Adams & Moses 2008; Amit et al. 2017).

Figure 1 is showing a simple graph with methods and classification of food preservation.

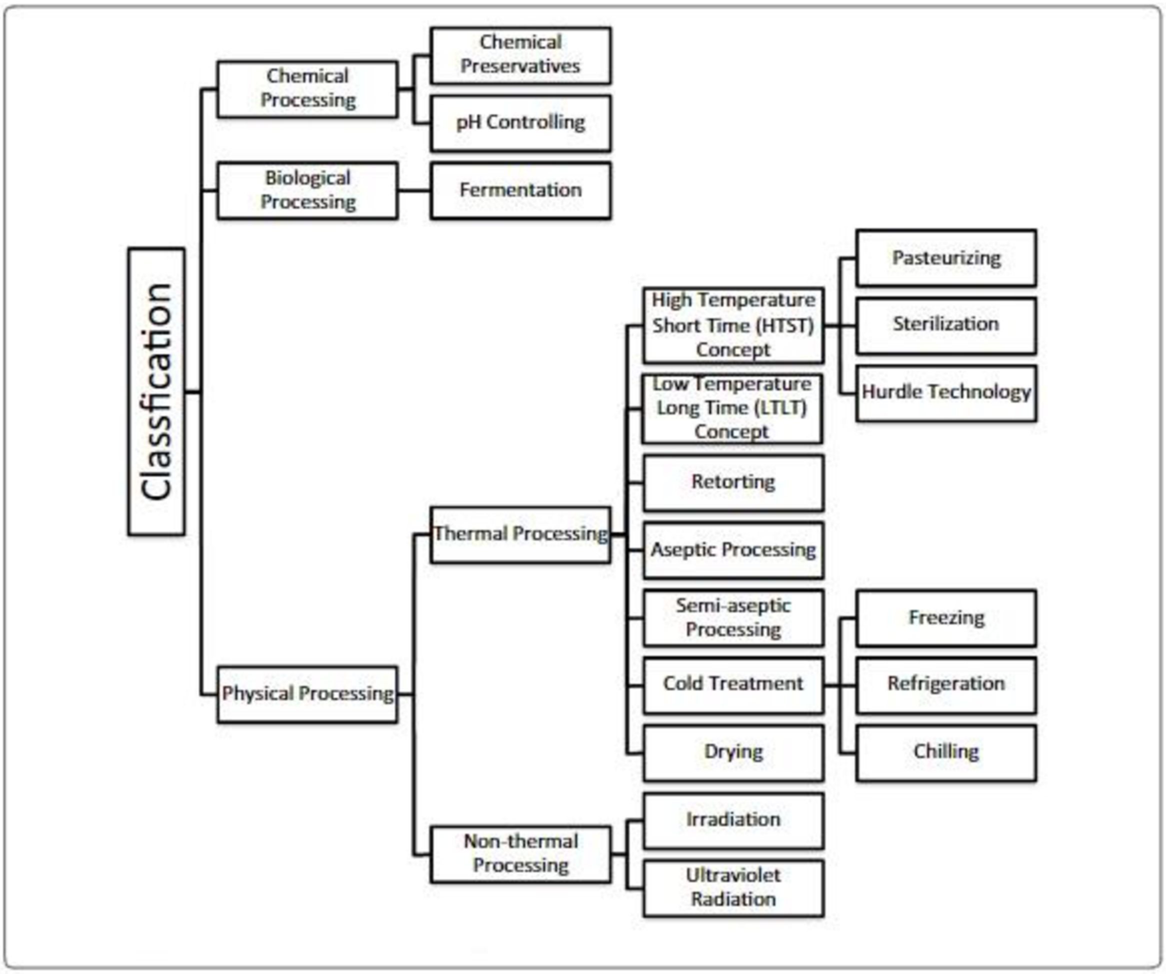

Figure 1: Methods of food preservation (Amit et al. 2017).

3.1.1 Conventional preservatives

Food additives are used to extend the shelf life of food and to help improve the colour, taste and nutritional value of food. Depending on their composition, additives can be divided into two groups, namely natural preservatives and synthetic preservatives. Chemically synthesised additives are based on chemical raw materials. Preservatives could be further divided according to their function into, for example, preservatives, antioxidants, sweeteners, colouring agents, thickeners, or bleaching agents. Some substances may overlap in their function and so, for example, the European Union has a classification list for them, the so-called E number, where each substance is assigned a number unique to it. This subchapter deals with E-numbered preservatives (Wu et al. 2022).

Natural preservatives would include, for example, nisin (E234), which is abundant in the Table 1 of my thesis. Nisin is a substance produced by the bacterium *Lactococcus lactis*. It has broad bactericidal effects against a large number of gram-positive bacteria. Nisin inhibits the growth of germinating spores, so bacteria against which it is effective include (among others, lactic acid bacteria, *Lysteria monocytogenes*) *Clostridium* and *Bacillus*. Nisin is generally regarded as safe, being approved in over 50 countries as a preservative. Nisin is being added to meat, dairy products, tea, soy products, coffee beverages, canned foods, as well as in processed cheeses, for example. The maximum amount of nisin added is 0.15 - 0.5 g/kg of food. Artificial preservatives often used include sorbic acid (E200). Sorbic acid is also used for the preservation of dairy products and, for example, processed vegetables or soya products. Its maximum permitted level is 0.075 – 2.0 g/kg (Esteban & Palop 2011; Wu et al. 2022).

The maximum dosage of benzoic acid (E210) and its salts is 0.2 - 2.0 g/kg. Benzoic acid is being used as a preservative and as well as antioxidant. Benzoic acid occurs in nature as a glucoside, which is found in some fruits such as blueberries or plums, as well as in cinnamon or cloves, but is usually synthesized artificially. Its antimicrobial properties affect mainly yeast, fungi and bacteria to a lesser extent. It is often used in combination with other preservatives, especially in acidic foods, as it is pH dependent. Benzoic acid and its alkaline salts - sodium, potassium, calcium benzoate (E211; E212; E213) are used as preservatives in fruit juices and soft drinks, in marmalades, jams, products based on fish and eggs, in sauces or, for example, in spices and so on (Silva & Lidon 2016; Wu et al. 2022).

Sulfur dioxide (E220) is a non-flammable, colorless preservative with a suffocating odor. Among its drawbacks is the potential destruction of vitamin B_1 (thiamine). Sulfur dioxide and its salts - sodium, potassium, and calcium sulfites (E221 - E226; E226 - E228), are used for their inhibition of yeast, bacteria and fungi. They are also used as antioxidants. As far as specific foods are concerned, sulfur dioxide and its salts are used in the production of wine, to prevent the growth of harmful microorganisms, as well as in meat products, in fruits and dehydrated vegetables, in sweets, jams, jellies, in fillings for pies, or, for example, in beverages, both alcoholic and non-alcoholic, in mustard and in many other cases (Silva & Lidon 2016).

Nitrites and nitrates include potassium nitrite (E249), sodium nitrite (E250) are being used as preservatives and as color fixates mainly in meat products. These preservatives are synthesized artificially, but they also occur to a lesser extent in nature. They are a potent inhibitor of *Clostridium botulinum*.

Acetic acid (E260) is used as an acidity regulator and also as a preservative, acetic acid is either a solid crystalline substance or a colorless liquid. Acetic acid is able to inhibit yeast, bacteria and fungi. Acetic acid and its salts are used as additives in mustard, fruit and vegetable, canned fish, cheese, bread, instant puddings and, for example, in baby food. Acetic acid and its acetates have no side effects (Silva & Lidon 2016).

Another antibiotic that is used to preserve food is pimaricin or natamycin (E235). Natamycin is a natural preservative produced by the mycorrhizal organism *Streptomyces natalensis*, natamycin is colourless and odourless. Natamycin is effective against all moulds and yeasts but is ineffective against bacteria and viruses. The use of natamycin in food is recognized as safe, at the same time natamycin is used to treat fungal infections in humans. Natamycin is used to preserve dairy products such as cheese and yoghurt, and to preserve juices and wines (Dalhoff & Levy 2015; Silva & Lidon 2016; Meena et al. 2021).

Figure 2 shows a list of selected conventional preservatives along with their E-numbers.

E-Number	Name of Preservative
E 200	Sorbic acid
E 202	Potassium sorbate
E 203	Calcium sorbate
E 210	Benzoic acid
E 211	Sodium benzoate
E 212	Potassium benzoate
E 213	Calcium benzoate
E 214	Ethyl p-hydroxybenzoate
E 215	Sodium ethyl p-hydroxybenzoate
E 216	Propyl p-hydroxybenzoate
E 217	Sodium propyl p-hydroxybenzoate
E 218	Methyl p-hydroxybenzoate
E 219	Sodium methyl p-hydroxybenzoate
E 220	Sulphur dioxide
E 221	Sodium sulphite
E 222	Sodium hydrogen sulphite
E 223	Sodium metabisulphite
E 224	Potassium metabisulphite
E 226	Calcium sulphite
E 227	Calcium hydrogen sulphite
E 228	Potassium hydrogen sulphite
E 230	Biphenyl, diphenyl
E 231	Orthophehyl phenol
E 232	Sodium orthophenyl phenol
E 233	Thiabendazole
E 234	Nisin
E 235	Natamycin
E 239	Hexamethylene tetramine
E 242	Dimethyl dicarbonate
E 249	Potassium nitrite
E 250	Sodium nitrite
E 251	Sodium nitrate
E 252	Potassium nitrate
E 281	Sodium propionate
E 282	Calcium propionate
E 283	Potassium propionate
E 284	Boric acid
E 285	Sodium tetraborate (borax)
E 1105	Lysozyme

Figure 2: The list of selected preservatives with their E-numbers (Anand & Sati 2013).

3.1.2 Side and adverse effects of conventional preservatives

A daily dose of sodium benzoate of up to 0.5 g/day is harmless to humans, but at higher concentrations it can accumulate. The preservatives benzoic acid (E210) and calcium benzoate (E213) have been shown to cause allergic reactions, probably due to the synthesis of certain compounds with other substances in the food, particularly in persons suffering from asthma or urticaria, in persons using anti-inflammatory drugs or in persons intolerant to acetylsalicylic acid. In addition to the previously mentioned allergic reactions, neurotoxic waste substances and gastric irritation are also present.

E215 and E219 have also shown allergic effects (mainly at the oral level) and have been shown in animal experiments to have numbing effects, to induce convulsions and even teratogenicity (which has therefore not been definitively confirmed).

In addition, sodium benzoate (E211) appears to exacerbate the symptoms and course of asthma and appears to have neurotoxic, carcinogenic effects and effects on the fetus, where it may cause fetal abnormalities or hyperactivity. For example, in Australia, additives E214; E215; E217 and E219 are banned (Risikat et al. 2012; Silva & Lidon 2016).

Sulphur dioxide (E220) can cause allergic reactions such as asthma, headaches, nausea, diarrhoea, eczema, skin and stomach irritation in sensitive individuals. Sulphites have also been linked to the possible development of cancer and the mutagenicity they show towards certain bacteria. However, their mutagenicity and carcinogenicity have not yet been confirmed (European Commision 1999; Silva & Lidon 2016).

Nitrites are able to form nitrosamines by reacting, for example, with hydrochloric acid in the stomach, leading to the formation of nitrous acid, which can then react with amines to form nitrosamines, which are genotoxic carcinogens, where any amount can cause cancer cells and tumors. They can also have toxic effects, particularly in infants, and have side antihypertensive effects (European Commision 1999; Silva & Lidon 2016).

Nitrite reacting with hemoglobin can form methemoglobin, which can cause unconsciousness and death, especially in infants. There is reportedly some association with increased nitrate in food with increased deaths from Alzheimer's and Parkinson's disease or type 2 diabetes mellitus, for example (Anand & Sati 2013).

3.2 Essential oils

Essential oils are highly concentrated aromatic oils that are extracted from plants using steam distillation, hydrodiffusion, or pressure. The undiluted form of essential oils is referred to as “neat“, but this is rarely used. Carrier oils such as sunflower, jojoba, canola, olive, and sweet almond oils are used to dilute essential oils. In aromatherapy literature, essential oils are also referred to as plant “essences“. The method of extraction is crucial to the classification of essential oils (Manion & Widder 2017).

Essential oils are hydrophobic secondary metabolites in plants, which are showing antimicrobial qualities. Essential oils are volatile aromatic substances, hydrophobic oil liquids that contain mono and sesquiterpenes as main components. Mono and sesquiterpenes are phenolic substances that are the main antibacterial components in essential oils (Natu & Tatke 2019).

Essential oils are characterized by a strong odour. Essential oils are known for their bactericidal, virucidal, fungicidal and medicinal properties and also for their fragrance. They are also used for food preservation and they also have analgesic, sedative and anti-inflamatory remedies. Essential oils can be used in many ways, however in nature, the role of essential oils is mainly to protect the plants againts pests due to their properties and can also reduce the herbivore’s appetite for such plants or it can as well attracts insects to help the spreading of pollens and seeds or repel the inconvenient ones (Bakkali et al. 2008).

It is not true that aromatic plants contain essential oils, as essential oils are mixtures of volatile organic compounds that have either been formed as a result of a distillation process from chemical precursors in the plant material or have been biosynthesized in specialized plant cells. Aromatic plants therefore contain precursors and components that are needed for the production of essential oils, called essential oil components. These extracts are referred to as “concretes (hexane extracts), fat extracts, absolutes (ethanol extract of concretes), andenfleurage perfumes“.

However, there are exceptions. For example, oils produced by mechanical pressing of citrus peel (such as bergamot) can be referred to as essential oils. Nevertheless, with the exception of citrus fruits, pressed oils are referred to as 'fixed' or 'expressed' oils (Sadgrove et al. 2022).

The chemical composition of essential oils varies greatly and depends on several factors, including the source of the raw material, climate conditions of plantations, and the methods used to obtain the oils. These variations in composition can affect the antibacterial activity of essential oils (Białoń et al. 2017).

3.2.1 Chemical composition of essential oils

The components that form essential oils are found in high concentrations of around 20-70%. These components could be divided into two main groups, which differ in their biosynthesis, however, essential oils are composed of up to a maximum of 60 components overall (Bakkali et al. 2008).

1. **Terpenes**

The distillation of essential oils provides terpenes. We may also encounter the term terpenoid, which is the form in which terpenes occur in nature. It refers to an oxygen-containing terpene. These are mostly compounds formed by alcohols, ketones and aldehydes. Terpenes and terpenoids are strongly aromatic substances that are used in the food, pharmaceutical and cosmetic industries, for example. Terpenes are one of the largest groups containing more than 55.000 different structures. Isoprene, a five-carbon unit, forms terpenes by its combinations. The terpenes found in essential oils could be divided into two groups, namely mono (C10) and sextupenes (C15) (Bakkali et al. 2008; Ben Salha et al. 2021).

Monoterpenes

The simplest group, making up up to 90% of essential oils, are monoterpenes, which occur in a variety of structures. Monoterpenes can be divided into three groups according to their structure: acyclic (linear), monocyclic (with one ring), bicyclic (Bakkali et al. 2008; Ben Salha et al. 2021).

Acyclic monoterpenes: Among the most widespread acyclic monoterpenes is geraniol, whose precursor is geranyl pyrophosphate (GPP). (Ben Salha et al. 2021)

Geraniol has a characteristic odour and flavour. Geraniol is the main product and component of the essential oils of *Cymbopogon martinii* (66.2-76.9%), *Pelargonium graveolens* (21.08%), *Rosa Damascena* (18.7-21.2%), *Rosa centifolia* (7.4-11.3%), and *Cymbopogon nardus* (22.77%). Geraniol has several properties, in particular antifungal and antibacterial effects, biological and pharmacological properties such as antidiabetic, cardioprotective, antidepressant action, anti-inflammatory, antioxidant, insecticidal and/or repellent activity and in vivo and in vitro antitumor activity are also reported (Lira et al. 2020).

Monocyclic monoterpenes: The precursor is also the GPP or neryl pyrophosphate. Monocyclic monoterpenes are divided into two groups: $C_{10}H_{16}$ (or $C_{10}H_{18}$) hydrocarbons – the best known representatives of this groups are D-limonene, the phe-landers or the terpinol derivates.

And $C_{10}H_{20}$ hydrocarbons – with the best known derivates of menthane, which are menthol and menthone and the derivates containing oxide such as eucalyptol or cineole (Ben Salha et al. 2021).

Menthol and menthone are main components in peppermint oil (from *Mentha piperita*) (menthol 30.0-55.0%, menthone 14.0-32.0%, limonene 1.0-5.0%, cineole 3.5-4.0%), and also in cornmint oil (from *Mentha arvensis*) (menthol 30.0-50.0%, menthone 17.0-35.0%, limonene 1.5-7.0%, cineole max. 1.5%). *Mentha piperita* essential oil is the most crucial of the mint oils due to its antibacterial properties. It's being used in therapy, for treatment of chronic or acute gastritis and entritis, for respiratory and oral disorders and inflamatory. *Mentha piperita* essential oil has minty flavour and cooling effect and is also widely used in chewing gums, toothpastes, perfumes, soaps and so on. It can be replaced with *Mentha arvensis* essential oil due to its cheaper prize (Kalemba & Synowiec 2020).

Bicyclic monoterpenes: These monoterpenes are bicyclic cyclopropanes, carane, thujane, bicyclic, cyclobutane pinane, camphane, fenchane, α-pinene, and β-pinene mostly found in essential oils from conifers (Ben Salha et al. 2021).

Some monoterpenes show activity on the central nervous system. These monoterpenes include the aforementioned β-pinene. The leaves of *Litsea glaucescens* are used in traditional Mexican medicine for the treatment of central nervous system related diseases such as epilepsy, fright and sadness. The antidepressant activity is assigned to main components of the essential oil of *Litsea glaucescens*, β-pinene and linalool. Antidepressant activity of the essential oil was demonstrated at doses of 100 and 300 mg/kg (Guzmán-Gutiérrez et al. 2012).

Sesquiterpenes

Sesquiterpenes are most commonly found in plants such as celery, lavender, lemon, lemongrass, peppermint, pine, rosemary, sage, thyme, eucalyptus, coriander, or orange. Sesquiterpenes are made up of three isoprene units. Sesquiterpenes are divided into acyclic, monocyclic, bicyclic, tricyclic and polycyclic groups. The precursor of acyclic sesquiterpenes is trans-farnesyl pyrophosphate. Monocyclic sesquiterpenes are subdivided into four other groups: bisabolan, germacran, eleman, humulan. The most important of the polycyclic sesquiterpenes is caryophyllene, which is most abundant in pepper and other spices (Bakkali et al. 2008; Ben Salha et al. 2021).

Caryophyllene has strong antioxidant activity, which may be related to its antibacterial action against gram-positive bacteria. β-caryophyllene also exhibits selective cytotoxic qualities against human colorectal cancer cells (Dahham et al. 2015).

2. Second group composed of aromatic and aliphatic constituents

This is the less dominant group of substances contained in essential oils. These compounds are found abundantly in botanical families such as *Lamiaceae*, *Myrtaceae*, *Rutaceae*, and specific botanicals would include, for example, cloves, cinnamon, anise and so on. The biosynthetic pathways of these compounds and terpenes are usually separate in plants, for example, in cinnamon essential oil, the major component is cinnamaldehyde and the minor component is eugenol (Bakkali et al. 2008).

Cinnamaldehyde is the main compound of cinnamon essential oil and possesses antibacterial activity (Lu et al. 2011).

Figure 3 presents chemical structures of some selected compounds described above.

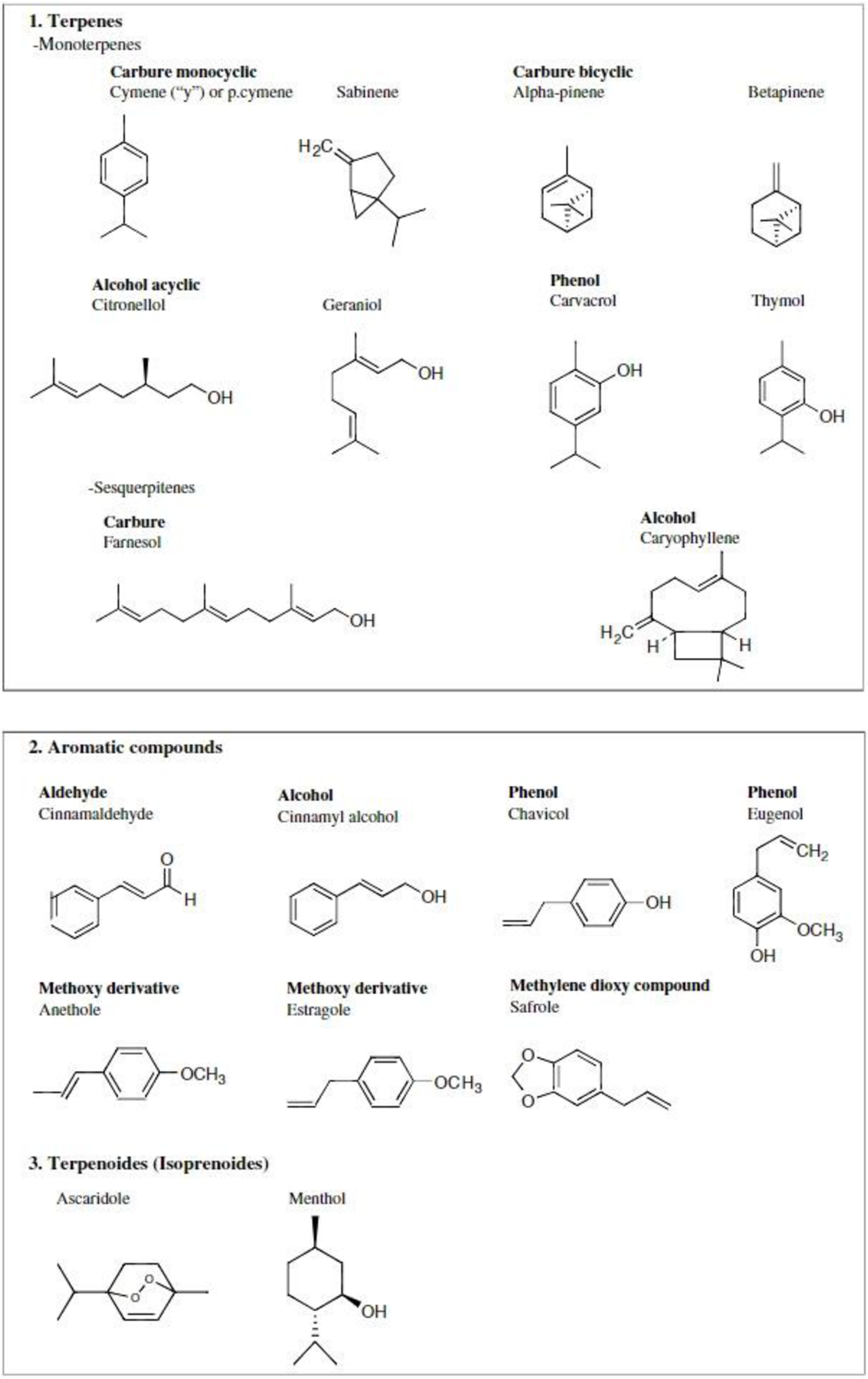

Figure 3: Selected components of essential oils (Bakkali et al. 2008).

3.2.2 Methods of obtaining essential oils

It appears that the method of extraction and origin of essential oils is important to consumers, consumers prefer essential oils to be extracted from plants using the traditional methods, these essential oils are the most preferred. However, synthetic essential oils are also tolerated among consumers, but only if they have been produced in a bioreactor using genetically modified yeast or using plant cell structures. The method of extracting essential oils using chemicals that can be potentially dangerous is not popular among consumers, and at the same time consumers have concerns about the authenticity of the essential oil, which probably stems from the fact that it is quite common to add a few drops of fragrance to fixed oils, which would include, for example, sunflower oil, and then sell this oil as a 'pure essential oil' (Sadgrove et al. 2022).

Traditional methods of extracting essential oils include water distillation, steam distillation, cold pressing, or solvent extraction, for example. Nevertheless, these methods have disadvantages such as hydrolysis, heat-induced degradation, low extraction efficiency, thermal or hydrolytic effects causing decomposition of ester compounds, and more specifically, the solvent method may evaporate the essential oil from the solvent or may leave some of the solvent in the essential oil. Because of these disadvantages, other methods are being used and tested that could contribute to environmental protection by reducing the production of hazardous substances and reducing the use of solvent sand fossil energy. These methods include extractions by using ultrasound, pressurized liquid, pressurized hot water, microwave assistation, application of solvent supported by membrane and for example, microexcraction in solid phase (Reyes-Jurado et al. 2015; Akdağ & Öztürk 2019).

Steam distillation

The most used method of extracting essential oils is steam distillation, which produces up to 93% of essential oils. In this process, it is crucial that only water vapour passes through the plant material. The water steam in question softens the cells of the plant material and helps the essential oil to evaporate to form a vaporised form. The steam generator is located outside the distillation unit, and it is important to keep its temperature below 100 °C. The essential oil in vaporized form is then cooled in a condenser, and after it cools, two layers are formed, with the oil, which has a lower density than water, being the top layer (Akdağ & Öztürk 2019; Shankar et al. 2021).

Water distillation

This method is typical for extracting essential oils specifically from plant material, such as flowers and wood, which will not become damaged by boiling. In this method, water has the function of a barrier, which prevents overheating that can cause damage to the material. The essential oil is in the vapour form, and it goes the same way as in the distillation by steam (Shankar et al. 2021).

Maceration

Maceration is a method of extracting essential oils from, for example, strawberries, brown mustard, chokeberry, garlic, bitter almonds or, for example, boxwood leaves, which contain thermolabile compounds that are extracted by maceration in warm water to release their essential oils. This method is based on the principle of placing the roughly ground powdered material and a suitable solvent, such as water, alcohol, or oil, in a closed container where it is kept at a room temperature for three days, with frequent stirring, while the substances dissolve. One of the main disadvantages of extracting essential oils by maceration is apparent from the preceding text and that is the relatively long time required to extract the essential oil, while other disadvantages would include low extraction efficiency (Basavegowda & Baek 2021).

Enfleurage

The problem with some flower essential oils is that they can be damaged due to hydrolysis and polymerization, which reduces the quality and yield of the oil product. Enfleurage is one method of extracting flower essential oils. Simply put, this method is based on the use of cold fat, where the fragrance of the flowers is being imbibed using this fat. This method is also used because the essential oil content of fresh petals is so low that other methods of extraction are commercially unviable. The oil or fat in this method should be stable, odourless, tasteless, and applied in a thin layer, forming a fat corps, to the glass plates. The petals are then placed on this thin layer of fat and replaced after a several hours (up to 24 hours specially for jasmine). After absorption of the fragrance by the fat, the essential oil is extracted with alcohol (Soe et al. 2016).

Cold-pressing extraction

The cold pressing method is a typical method for extracting essential oils from the peel of citrus fruits. During extraction, volatile oils are released, which are found in oil sacs, which are then obtained using this method, followed by centrifugation (Asbahani et al. 2015).

Solvent extraction

The solvent extraction method of essential oil extraction is mainly used for frail and dainty floral materials that are unable to withstand steam distillation extraction.

The solvents used include, but are not limited to, hexane, petroleum ether or, for example, methanol. The principle of this method is based on mixing the solvent with the plant material and then heating the mixture to extract the essential oil. Afterwards, filtration is carried out, where the resulting filtrate is further concentrated by evaporation of the solvent. The resulting concentrate is called either 'resin' or 'concrete', which is the name given to the mixture made up of a combination of extracted essential oil, wax, and fragrance (Tongnuanchan & Benjakul 2014).

3.2.3 Essential oils as food additives

Essential oils of plants can be used in many sectors, since their discovery, essential oils have been used in various rituals, or as aphrodisiacs, medicines, as part of cosmetics, in aromatherapy and agriculture, as food additives and flavorings, since essential oils are exceptional, among other things, for their antimicrobial and antioxidant effects. The activity of these essential oils depends on the type of plant, the chemical composition of the plant, the methods of extraction of the essential oil, which also determines the price of these products.

When using essential oils as additives in food, it is necessary to consider their specific aroma, which just accompanies its volatile components, whereby this smell affects the resulting smell of the food and its taste, respectively, by preserving with essential oils we can change the typical smell and taste of the food (Tongnuanchan & Benjakul 2014; Dima & Dima 2015).

Consumer demand for more natural foods is increasing, leading to a rise in the production of foods with natural antimicrobials, which could include the aforementioned essential oils as potential direct antimicrobials that can also improve the quality of food products. Nevertheless, the use of essential oils alone shows that, for example, in the case of *Escherichia coli* avoidance, higher concentrations must be added to the food to avoid its occurrence, but this leads to a change in the sensory properties of the food due to the typical properties of essential oils mentioned above, such as strong aroma and taste. The strength of the antibacterial effects of essential oils has been established by dissolving the essential oil in agar or broth, however, it has been found that *in vivo* tests and tests in real food just show a difference in concentration, with several times higher concentrations having to be added to real food than in *in vivo* tests. The only exception was the occurrence of *Aeromonas hydrophila*, where higher concentrations of essential oils did not need to be added when treating cooked pork, lettuce as well as in the case of *in vivo* tests. Some preservatives containing essential oils are commercially available, such as 'DMC Base Natural', which is composed of 50% glycerol and the remaining 50% just essential oils from rosemary, sage and citrus (Burt 2004; Kusalaruk & Nakano 2021).

Thyme and oregano essential oil

The main monoterpenoid compounds found in the essential oils of thyme and oregano, which are herbs of the *Lamiaceae* family, are thymol and carvacrol. They exhibit antibacterial effects on many strains of bacteria, including *Escherichia coli*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Salmonella enterica*. Due to their antibacterial properties, they are used in the food industry for food preservation and as flavourings. Carvacrol is used for the preservation and treatment of a variety of fresh fruits and vegetables or, for example, to preserve milk or rice. It is also used, for example, in alcoholic beverages. Thymol is mainly used as an additive of mouthwashes (Kachur & Suntres 2020).

Rosemary essential oil

Rosemary essential oil is extracted from the *Rosmarinus officinalis* plant, whose glands containing essential oils are found in the leaves and flowers of the plant, with the best quality essential oil being obtained mainly from the leaves. The main components found in rosemary essential oil include 1,8-cineole, myrcene, α and β-pinene, camphene, camphor, and so on. Rosemary essential oil is characterised by its aroma, which is why it is used, for example, as a component of perfumes or disinfectants, and it also has balsamic properties. It is also used as a food flavouring (Presti et al. 2005).

Rosemary essential oil exhibits antioxidant activity and are suitable for food preservation where they are better perceived by consumers due to their natural origin. The ability to prolong the shelf life of cream cheese is attributed to the fact that essential oils prevent lipid oxidation and thus the development of a rancid taste (Olmedo et al. 2013).

Alvarez et al. (2019) found that rosemary essential oil showed antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella typhimurium*, which was attributed to components of the essential oil that would include camphor, 1,8-cineole, and α-pinene, with α-pinene showing antibacterial activity against both gram-negative and gram-positive bacteria.

Cinammon essential oil

Cinnamon essential oil obtained from the *Cinnamomum zeylanicum* plant of the *Lauraceae* family, containing cinnamaldehyde as one of its main components, demonstrating antibacterial activity effective against both gram-positive and gram-negative bacteria. *Cinnamomum zeylanicum* is a medicinal herb, a tropical evergreen tree native to Sri Lanka. The essential oil of this plant is mainly used as a food seasoning, in cosmetics, in medicine, as it has antibacterial properties in addition to antioxidant, aromatic and anti-carcinogenic activity (Pathirana et al. 2019).

The essential oil is extracted mainly from the bark and leaves of the tree. The essential oil obtained from the bark of *Cinnamomum zeylanicum*, in addition to the aforementioned cinnamaldehyde, contains eugenol and linalool, which together with cinnamaldehyde account for 82.5% of the total essential oil. In addition to the abovementioned properties of the essential oil, it also exhibits antifungal activity, specifically against *Candida* spp. Thus, it is indicated that this essential oil is suitable for the potential treatment of oral candidiasis. Additionally, in traditional Ayurvedic medicine, it is used as an alternative remedy for respiratory, gynecological, and digestive disorders (Ferreira et al. 2021).

Lemongrass essential oil

Lemon grass (*Cymbopogon* spp.) from the *Poaceae* family is a plant rich in vitamins, proteins and antioxidants. One of the key indicators of the plant's medicinal potential is its citral terpene content. The essential oil possesses antioxidant, antimicrobial, antihypertensive properties, and has shown inhibition of various cancer cells. Lemongrass extract has shown antibacterial activity against both gram-positive and gram-negative bacteria and fungi. Assuming that a variety with a higher citral content is improved, potential uses for this variety could be in food preservation and safety, in surgery or for possible alternative cancer treatments (Mukarram et al. 2022).

Cymbopogon citratus, also known as lemongrass, has been traditionally used in folk medicine to treat various conditions such as nervous and gastrointestinal disturbances, as well as being an antispasmodic, analgesic, anti-inflammatory, antipyretic, diuretic, and sedative. Studies have shown that extracts from the leaves of *Cymbopogon citratus* possess antioxidant, antimicrobial, and antifungal properties. The drying method of lemongrass leaves significantly affects the essential oil content, with oven-dried leaves having the highest oil content. The major components of the essential oils are geranial, neral, and myrcene, which determine the quality of lemongrass. *Cymbopogon* is an important genus with around 120 species grown in tropical and subtropical regions worldwide and is widely cultivated for various industries such as pharmaceuticals, cosmetics, food, and agriculture (Mohamed Hanaa et al. 2012).

3.2.4 Effect of essential oils on sensory attributes

The use of essential oils in food preservation is limited by their volatility, their interactions with the treated food and, most importantly, their odour or taste (Alvarez et al. 2019).

Essential oils have major limitations as food preservatives due to their poor water solubility as well as abovementioned strong flavour (Yazgan 2020).

Massoud & Sharifan (2020) found that the addition of *Rosmarinus officinalis* essential oil to probiotic yogurt decreased the rate of bacterial growth over 21 days and improved the sensory attributes and overall acceptability. *Rosmarinus officinalis* essential oil also exhibited antimicrobial properties that could enhance the shelf life of foods and inhibit the growth of pathogens and spoilage microorganisms. The high total phenolic content of *Rosmarinus officinalis* essential oil positively affected the sensory perception. The addition of plant extracts to yogurt may also improve organoleptic attributes and yogurt texture, which plays a crucial role in product development, quality control, and consumer satisfaction. It can be concluded that *Rosmarinus officinalis* essential oil has the potential to deliver *Bifidobacterium bifidum* in sufficient population in yogurt, as well as starter cultures.

Tornambé et al. (2008) aimed to investigate how adding essential oils obtained from plants collected from a mountain pasture affected the sensory properties of milk and cheese. The essential oil was primarily made up of terpenoid compounds and was obtained from 46 plant species, with *Festuca rubra*, *Agrostis capillaris*, and *Carex caryophyllea* making the largest contributions. Low concentrations of essential oils were not easily detected by the assessors, and the concentration threshold for perception was found to be between 0.1 - 1.0 µL/L. Samples containing 1.0 µL/L of essential oil were rated as having a "thyme" and "mint" aroma, a stronger taste, and a sweeter flavor.

It was found out by Asensio et al. (2012) that adding oregano essential oil to olive oil in darkness improved its positive taste and aroma, while reducing the negative attribute of rancid flavour. The color of olive oil is also an important sensory property, which is largely determined by the presence of chlorophylls and carotenoids in olive oil. The addition of oregano essential oil not only protects against lipid oxidation, but also preserves the taste, aroma, and health benefits of the oil. Samples exposed to light showed a higher decrease in quality compared to those kept in the dark. It was also found that the positive attributes of bitterness and pungency in olive oil are related to its phenolic compounds. Oregano essential oil can prolong the shelf life of extra virgin olive oil by preserving its sensory quality and reducing lipid oxidation due to its antioxidant activity.

3.2.5 Plant extracts

In addition to essential oils, plant extracts are also used in the food industry. For instance, some of their most important components would be polyphenols and carotenoids, which have several effects, including an antimicrobial effect that is important for the food industry. They also prolong shelf life and, among other things, help to maintain the colour stability of foods (Proestos 2020). Biomolecules contained in plant extracts have antibacterial properties in addition to antifungal, anti-inflammatory, anti-cancer and antioxidant effects, among others. These biomolecules have mechanisms that are effective against resistant pathogens, which helps food safety. The way that are plant extracts obtained is important. Traditional methods of obtaining plant extracts would include mainly methods such as percolation, maceration or, for example, Soxhlet extraction (Malik & Mandal 2022; Abdullahi et al. 2022).

However, solvent extraction is most commonly used, with the choice of solvent being crucial. The solvents are selected based on their polarity, which should ideally be similar to that of the solute. The most widely used universal solvents include, for example, ethanol or methanol (Zhang et al. 2018).

Chapter 5 in Table 1 presents examples of food preservation using essential oils, but also the plant extracts just mentioned. The plant extracts used in Table 1 include, for example, pomegranate peel extract, rosemary extract and grape seed extract.

Pomegranate peel extract

Pomegranate, *Punica granatum* L., and its extracts possess several positive effects, ranging from inhibitory and preventive effects against diseases such as cancer or diabetes mellitus type 2, whereby they possess free radical inhibiting properties, while also being antiteratogenic. The benefit of the pomegranate could be attributed to the fact that the aforementioned properties do not only apply to the edible part of the fruit, but also to the inedible part, such as its flowers, peel, leaves, etc. Besides their health-promoting properties, the pomegranate, its peel and its extract, is also a suitable preservative that improves the quality of food. However, its downside is its strong negative effect on sensory attributes. It is also used in the food industry as a food colourant or flavour enhancer. Ethanol or water is most used for extraction of pomegranate extract. The extract has been found to have a strong antibacterial effect against gram-positive bacteria in meat products and can be used to preserve vegetable oils in order to stabilise them. It has also proven its effectiveness in preserving dairy products without adversely affecting the beneficial probiotic *Lactobacillus casei* Shirota (Akhtar et al. 2015).

Grape seed extract

Grape seeds from *Vitis vinifera* plant are waste product for instance in wine production. However, grape seeds possess several beneficial properties, which would include, for example, antioxidant, anti-inflammatory, anti-allergic, antimicrobial anti-cancer properties and many others. Red wine grape seeds are most often used to obtain the extract by maceration in ethanol. The antioxidant activity of grape seeds is notable for being higher than, for example, vitamin C, E and provitamin A. Other beneficial properties of grape seed would include its positive anti-ageing effect on the skin and also its ability to heal wounds as its enzymes catalyse histamine in allergic and inflammatory reactions. It possesses antibacterial properties mainly against gram-negative bacteria, however, it has also shown efficacy against gram-positive bacteria such as staphylococcus aureus or bacillus subtilis. In addition, grape seeds are also used as a dietary supplement or, for example, in the cosmetic industry (Gupta et al. 2020).

3.3 Synergy between essential oils and conventional preservatives

In simplified terms, synergy can be explained as a reflection of an increase in effect relative to the agents themselves, or also as a cooperative, non-antagonistic action. Synergy, or supraadditive synergy, is commonly described as "1+1>2". Using the specific example of drugs, synergy is explained as the possibility of combining several types of drugs to increase the effectiveness of the original component (Geary 2013).

Another example for me could be the synergy of plants. In plants, synergy can involve, for example, protecting active substances from enzymatic degradation or for facilitating transport across barriers (Gilbert & Ferreira Alves 2003).

The use of essential oils as food preservatives is not advisable because they do not show sufficient antimicrobial activity and at higher concentrations, they affect the organoleptic properties of food. The combination of essential oils and conventional preservatives, with a synergistic effect between them, has been suggested as one solution. The combination and interaction between antibacterial agents can have 3 different consequences, synergistic, additive or antagonistic. We speak of synergy when the antibacterial activity of the combination of substances is higher than the individual substances used alone. To determine the synergistic effect, the use of the fractional inhibitory concentration index calculation, which is based on the measurement of the minimum inhibitory concentration (MIC), is used to analyse the combined effect of a mixture. The calculation is performed using the following formula. A synergistic effect is considered when the FIC values are < 0,5.

$$\mathrm{FIC}_{Index} = \mathrm{FIC_A} + \mathrm{FIC_B},\ \textit{when: } \mathrm{FIC_A} = \frac{\mathrm{MIC_{A+B}}}{\mathrm{MIC_A}};\ \mathrm{FIC_B} = \frac{\mathrm{MIC_{A+B}}}{\mathrm{MIC_B}}$$

It is not well understood what exactly drives the synergy between the components of essential oils. Nevertheless, there are three theoretical mechanisms producing synergy: (i) the site and mode of action of each ingredient, (ii) the mechanisms leading to synergy and antagonism between compounds, and (iii) the way each ingredient interacts with the food that affects antibacterial properties. Encapsulation and controlled release of potent synergistic combinations could be used to reduce the influence of essential oils on organoleptic properties while enhancing antibacterial potential (Burt 2004; Rattanachaikunsopon & Phumkhachorn 2010; Nguefack et al. 2012; Hyldgaard et al. 2012).

4 Methodology

The data was collected through literature search in Web of Science database using appropriate key words whereas the use of a conventional preservative in combination with a plant-derived compound was the main inclusion criterion.

5 Effect of plant-derived compounds to enhance the efficiency of preservatives

The following chapter contains data obtained by searching the Web of Science database, which are entered and arranged in Table 1. The search focused on several key points of information, including a basic indication of the preservative used, the compound of plant origin and the food model on which the study was conducted. The search then focused on obtaining the most effective active concentration of substances that the preservative and the plant-derived compound exhibited in combination. The effect of the combination on a specific microorganism was also noted. It is mainly aimed at indicating the decrease in the number of microorganisms during storage. Another point that was investigated involves performing a sensory analysis of the resulting application of the combination of compounds in the food model. The method of application procedure of the compounds was also recorded.

Table 1. Combinatory effect of conventional food preservatives with plant-derived compounds in food models

Combination preservative + plant compound *(References)*	**Food**	**Microorganisms**	**Method**	**Active concentration**	**Effect**
nisin + thymol (Punyauppa-Path et al. 2015)	sugarcane juice	*Shigella spp.*	inoculation with 10^5 CFU/mL, addition of agents into inoculated juice, storage for 4 h at 4 °C	0.5µM + 0.75 mM	no detectable viable cell count of all *Shigella* species Sensory analysis All attributes rated between "like moderately" and "like very much" on the hedonic scale → the addition of thymol and nisin to sugarcane juice would be acceptable to consumers.
nisin + rosemary extract *(Rosmarinus officinalis L.)* (Thomas & Isak 2006)	chicken soup	*Listeria monocytogenes* *Bacillus cereus*	pasteurization at 80 °C for 2 min, cooling followed by inoculation (10^3 CFU/mL), storage at 8 °C	100 IU/mL + 0.00084 % phenolic diterpenes	250 IU/mL + 0.35% phenolic diterpenes → < 1.0 ×10^2 CFU/mL 150 IU/mL + 0,00168% p. d., pH 4.6 → no detection 150 IU/mL + 0,00168% p. d., pH 6.7 → 6 × 10^3 CFU/mL *(L. monocytogenes)*
	carbonara souce				100 IU/mL + 0.00084 % p. d. → 2 log CFU/mL (*B. cereus,* 15 °C)
	Bolognese sauce				100 IU/mL + 0.00084 % p. d. → 2 log CFU/mL (*L. monocytogenes,* 8 °C) Sensory analysis was not performed.

Combination preservative + plant coumpound *(Referrences)*	**Food**	**Microorganisms**	**Method**	**Active concentration**	**Effect**
nisin + cinnamaldehyde (Shi et al. 2017)	pasteurised milk tryptic soy broth	*Staphyloccocus aureus*	cultivation at 37 °C in tryptic soy broth, direct inoculation of 10^6 CFU/mL	8 µg/mL + 0.25 mg/mL	inoculum 10^6 CFU/mL → drop of 7.2 log CFU/mL inoculum 10^6 CFU/mL → drop of 5.62 log CFU/mL Sensory analysis was not performed.
nisin + citric acid (Zhao et al. 2017)	pasteurized milk	*Staphylococcus aureus* *Listeria monocytogenes*	inoculation of 10^6 CFU/mL, direct application of agents, incubation at 37 °C without shaking	16 µg/mL + 0.25 mg/mL	≥ 2 log CFU/mL after 24 h reduction of 4.7 log CFU/mL after 24 h *(S. aureus)* Sensory analysis was not performed.

Combination preservative + plant coumpound *(References)*	**Food**	**Microorganisms**	**Method**	**Active concentration**	**Effect**
nisin + carvacrol (Churklam et al. 2020)	sliced Bologna sausage	*Listeria monocytogenes*	inoculation of 2×10^3 CFU/mL togethet with agents, storage at 4 °C for 7 days in clean plastic box in refrigerator	25 µg/ml + 62.5 µg/ml	day 0 – 3 showed 0.1×10^2 CFU/g day 7 showed 1.5×10^2 CFU/g Sensory analysis was not performed.
nisin + carvacrol (Esteban & Palop 2011)	carrot juice	*Listeria monocytogenes*	addition of agents into growth medium, followed by inoculation of 10^3 CFU/mL, application of agents into 4.5 mL of carrot juice with pH = 6.14	0.13 µM + 0.67 mM	heat treated samples (15 min, 55 °C) – 0.5 log CFU/mL non-treated samples – 3.2 log CFU/mL Sensory analysis was not performed.

Combination preservative + plant coumpound *(References)*	**Food**	**Microorganisms**	**Method**	**Active concentration**	**Effect**
nisin + thyme essential oil (Solomakos et al. 2008)	minced beef meat	*Listeria monocytogenes*	inoculation in stomacher bags with subsequent homogenization, followed by direct application	1000 IU/mL + 0.6%	*Listeria monocytogenes* strain was kept below 1 log CFU/g at 4 °C or 10 °C throughout storage. Scott A and cocktail strains were kept below 1 log CFU/g at 4 °C; at 10 °C the value below 1 log CFU/g lasted up to day 8. Increase up to 1.7 log CFU/g by the end of storage. Sensory analysis The sensory attributes (color, odor, overall acceptability) of the samples were evaluated by a trained panel, and those treated with 0.6% thyme EO received scores above the point of rejection.
nisin + rosemary extract (Akhter et al. 2021)	mutton meat emulsion	total plate count *(not specified)*	mincing of mutton, followed by addition of agents, packaged in low density polyethylene pouches, followed by irradiation (at 1 kGy)	5 g + 0.4 g *(/ 1000 g of meat emulsion)*	non-irradiated – 5.02 log CFU/g irradiated – 1.29 log CFU/g Sensory analysis was not performed.

Combination preservative + plant coumpound *(References)*	**Food**	**Microorganisms**	**Method**	**Active concentration**	**Effect**
nisin + *Ziziphora clinopodioides* essential oil (Shahbazi 2016)	milk	*Salmonella typhimurium* *Staphylococcus aureus*	prior inoculation of 10^5 CFU/mL samples were boiled in warm bath, followed by addition of agents, followed by homogenization, storage at 4 °C for 9 days	500 IU/mL + 0.2 %	5 log CFU/mL → complete inhibition on day 5 *(S. typhimurium)* 5 log CFU/mL → complete inhibition on day 5 *(S. aureus)* Sensory analysis was not performed.
nisin + grape seed extract, green tea extract (Theivendran 2006)	turkey frankfurters	*Listeria monocytogenes*	sterilization by linear electron akcelerátor (30 kGy), followed by inoculation of 10^6 CFU/mL, followed by addition of agents, storage in sterile Whirl Pak bags for 28 days at 4 °C and 10 °C	10000 IU/mL + 0.1 %, 0.1%	the growth was suppresed by 2.8 and 2.3 log CFU/mL by the end of storage (day 28) Sensory analysis was not performed.

Combination preservative + plant coumpound *(References)*	**Food**	**Microorganisms**	**Method**	**Active concentration**	**Effect**
benzoic acid + *Rosmarinus officinalis* essential oil (REO) (Hadian et al. 2017)	beef cutlet	*Salmonella typhimurium*	UV light treatment (20 min) before inoculation of 10^9 CFU/g, encapsulation of REO in chitosan-benzoic acid nanogel, addition of nano-encapsulated REO, storage in sterile bags at 4 °C for 12 days	2000 µg nano-encapsulated REO	2000 µg nano encapsulated REO: day 1 – reduction of 3.3 CFU/g day 12 – reduction of 2.5 CFU/g Sensory analysis was not performed.
acetic acid, citric acid (Schirmer et al. 2009)	fresh salmon	*Enterobacteriaceae* *Lactic acid bacteria*	inoculation followed by application of brine solution with agents, followed by CO_2 packaging, storage at 4 °C for 14 days	1% + 3% + 100 % CO_2 packaging	no detectable bacterial growth Sensory analysis Samples showed significant acetous odor and flavor. Samples marinated with citric acid + acetic acid remained fresher than control samples after nine days of storage.

Combination preservative + plant coumpound *(References)*	**Food**	**Microorganisms**	**Method**	**Active concentration**	**Effect**
potassium sorbate + carvacrol (Pereira Batista et al. 2019)	tomato paste	*Salmonella typhimurium*	pasteurization, followed by addition of agents, ionculation of 10^6 CFU/mL, storage at room temperature for 10 days	39 µg/mL + 78 µg/mL	time-kill assay: no detectable viable cell counts after 48 h experimentally inoculated: day 1 – reduction of 1.5 log CFU/g day 2 – reduction of 3 log CFU/g day 3 – complete inhibition Sensory analysis Attributes of taste and overall acceptability showed differences. Treated samples were evaluated at 65 – 63 % of overall acceptability, compared to untreated group (80 %).
sodium acetate + *Zataria multiflora* essential oil (Ehsani et al. 2014)	vacuum-packaged trout burgers	viable mesophilic bacteria	addition of agents into minced meat, followed by pressing the mixture with manual press machine, vacuum packaged, freezed for 2 h at – 80 °C, followed by storage at 4 °C for 21 days	2 % + 0.1 %	Control group: day 0 - 3.82 log CFU/g; day 15 - 6.82 CFU/g Treated group: day 0 - 3.67 log CFU/g; day 15 - 4.21 CFU/g Sensory analysis Results showed that treated samples had high overall acceptaility with values of 8.1; 8.125; 8.35 and 8.425 with the 9-point scale.

Combination preservative + plant coumpound *(References)*	**Food**	**Microorganisms**	**Method**	**Active concentration**	**Effect**
lactic acid + *Origanum vulgare* essential oil (De Barros et al. 2012)	bovine meat model	*Staphylococcus aureus*	sterilization (121 °C; 15 min; 1.21 atm), followed by inoculation of 10^7 CFU/mL, air dried for 30 min, then dipped in agents, storage in refrigerator at 7 ± 1 °C in polypropylene cups	0.62 µL/mL + 0.15 µL/mL	day 1 – 5.29 log CFU/mL day 2 – 4.5 log CFU/mL Sensory analysis was not performed.
natamycin, sodium diacetate + lemon grass essential oil (Ben-Fadhel et al. 2017)	broccoli florets	*Escherichia coli* O157:H7 *Listeria monocytogenes* *Salmonella typhimurium* *Aspergillus niger*	irradiation of samples with 10.4 kGy, application of alginate antibacterial film (30 s), followed by drying (30 min), application of CaCl2 (15 min), followed by inoculation	0.008% + 0.5% + 0.125%	application of 0.4 kGy → reduction to 3.4 log CFU/g during 4 days, undetectable level from 7th day of storage irradiation of 0.4 kGy → 3.4; 3.3; 2.4 g CFU/g (0, 7th, 10th day) irradiation of 0.4 kGy → complete reduction during storage irradiation of 0.8 kGy → 2.3 – 2.0 log CFU/g (day 1), after that undetectable level Sensory analysis was not performed.

Combination preservative + plant coumpound *(References)*	**Food**	**Microorganisms**	**Method**	**Active concentration**	**Effect**
acetic acid + thymol + carvacrol (Zhou et al. 2007)	Mueller-Hinton broth	*Salmonella typhimurium*	inoculation of 5 × 10^5 CFU/mL, incubation for 24 h at 37 °C, addition of agents	0.10%, 150 µg/mL + 200 µg/mL, 0.1 µl/mL; 0.2 µl/mL	logarithmic reduction of microbial count: - 3.83 (carvacrol (0.2 µl/mL + acetic acid) - 3.67 (thymol + acetic acid) - 3.27 (carvacrol 0.1 µl/mL + acetic acid) Sensory analysis was not performed.
lysozyme + cinnamon essential oil (Feng et al. 2017)	strawberries	*Listeria monocytogenes* *Salmonella enteritidis* *Aspergillus niger* *Penicillium*	9 groups of strawberries; unpacked, packed in fresh-keeping film, packed in PVA/β-CD/CEO/LYS nanofilm, stored at 20 °C	0.25% + 2%	packed with fresh-keeping film decayed on day 4 unpacked rotten on day 6 packed with PVA/β-CD/CEO/LYS nanofilm showed no sign of decay on day 6 Sensory analysis was not performed.

Combination preservative + plant coumpound *(References)*	**Food**	**Microorganisms**	**Method**	**Active concentration**	**Effect**
lysozyme + pomegranate peel extract (Khodanazary 2019)	mackerel fillets (*Scomberomorus commerson)*	total mesophilic and psychotropic bacteria counts	heat treatment of the solution (40 °C, 24 hours), followed by direct application, storage in polyethylene bags (12 days at 4 ± 1 °C)	0.1 g w/v + 1.5% w/v	day 0 day 6 TMC: control – 2.10 log CFU/g; 7.08 log CFU/g combination – 2.08 log CFU/g; 4.81 log CFU/g PTC: 1.17 log CFU/g; 4.31 log CFU/g 1.16 log CFU/g; 3.17 log CFU/g Sensory analysis All samples were evaluated with the highest score at the begining of storage. After 6 days, untreated samples were evaluated as unacceptable due to odour, treated samples were unacceptable after 12 days.
lysozyme + cinnamaldehyde (Xu et al. 2020)	olive flounder fillets *(Paralichthys olivaceus)*	*Shewanella putrefaciens* *Pseudomonas fluorescens*	solutions of lysozyme and β-Cyclodextrin were made, addition of cinnamaldehyde into β-CD solution, dipped into solutions for 30 min at 4 °C, packaging with air-proof polyethylene bags, storation at 4 ± 1 °C	0.1% w/v + 0.1% w/v + β-CD 0.86% w/v	6.24 log CFU/g by the end of storage. Control samples were up to7.89 log CFU/g. Sensory analysis was not performed.

5.1 Evaluation of the effect of plant-derived compounds to enhance the efficiency of preservatives

The most frequent preservative used in Table 1 was nisin, which was repeatedly found in combination with rosemary extract, for the preservation of foods containing meat, namely mutton meat emulsion, chicken soup, carbanora sauce and bologna sauce. Nisin was further tested twice in combination with carvacrol, namely for the preservation of sliced Bologna sausages and carrot juice. The application of nisin for the preservation of pasteurized milk has been used in combination with cinnamaldehyde and citric acid. For the preservation of unpasteurized milk, nisin was combined with essential oil from *Zataria clinopodiodes*. The combination of nisin and thymol was used for the preservation of sugarcane juice and the combination of nisin with thyme essential oil was used for the preservation of minced beef. Finally, nisin was used to preserve turkey frankfurters in combination with grape seed extract and green tea extract.

The synergistic antibacterial effect of nisin and thymol against *Shigella* species in sugarcane juice at 4 °C was investigated by Punyauppa-Path et al. (2015). Although nisin alone was ineffective, the application of nisin along with thymol enhanced the inhibitory effect of thymol against all tested *Shigella* species. The antimicrobial activity of thymol and nisin increased with increasing nisin concentration.

The synergy of the combination of nisin and rosemary extracts has been shown to enhance the activity of nisin against *Listeria monocytogenes* and *B. cereus* in vitro as well as in real food models. Low amounts of rosemary extract increased the bacteriostatic and sporostatic activity of nisin against cocktails of *L. monocytogenes* and *B. cereus* in chicken soup, carbonara sauce and bolognese sauce. This synergism is mainly attributed to the presence of the antioxidant phenolic diterpenes, carnosol and carnosic acid in rosemary extracts (Thomas & Isak 2006).

Shi et al. (2017) explored the antibacterial effects of a combination of nisin and cinnamaldehyde against *Staphylococcus aureus* in pasteurized milk and tryptic soy broth. The study found that the combination of nisin and cinnamaldehyde had a stronger bactericidal effect than either compound separately, with a significant decrease in bacterial counts. The combination showed synergistic antibacterial activity and might potentially help minimize the problem of bacterial resistance. The combination of nisin and cinnamaldehyde in pasteurized milk had a better bactericidal effect than in tryptic soy broth. This combination led to a significant decrease in bacterial counts of 7.2 log CFU/mL in 24 hours compared to nisin used alone.

The synergistic effect of nisin and citric acid in pasteurized milk against *Listeria monocytogenes* and *Staphylococcus aureus* was investigated by Zhao et al. (2017). The combination of nisin and citric acid showed a decrease of $\geq$ 2 log CFU/mL and no further appearance of viable cells was observed after 24 hours. The combination has a stronger synergistic bactericidal effect in pasteurized milk against *L. monocytogenes* than against *S. aureus*. The combination was found to be more effective than the use of these agents individually.

Churklam et al. (2020) found that nisin and carvacrol used in ready-to-eat slices of sausages stored at 4 °C for 7 days, significantly reduced the growth rate of *Listeria monocytogenes* compared to control samples and samples treated with carvacrol or nisin alone. The combination significantly decreased the growth of bacterial cells after day 4.

Effect of different concentrations of nisin and carvacrol, with or without heat pre-treatment, on the growth of *Listeria monocytogenes* in carrot juice was tested during the storage at 37 °C. The combination of 0.66 mM carvacrol and 0.13 μM nisin resulted in a bacteriostatic effect. The heat pre-treatment significantly decreased the viable counts of *Listeria monocytogenes* compared to non-treated cells incubated with the same amounts of antimicrobial agents (Esteban & Palop 2011).

The inhibitory effects of nisin and thyme essential oil on *Listeria monocytogenes* strain in minced beef meat was researched at 4 °C as well as at 10 °C. Sensory analysis showed that the samples treated with 0.6% thyme essential oil received scores above the point of rejection, therefore, these samples might be acceptable by consumers. The results show that the combination is an effective way to inhibit *Listeria monocytogenes* in minced meat, as it keeps its population below 2 log CFU/g during storage at 4 °C, which was the official limit set by the European Union (Solomakos et al. 2008).

The combination of nisin and rosemary extract with or without radiation treatment in mutton emulsion was evaluated for its preservative effect by (Akhter et al. 2021). The samples treated with both the combination and γ-irradiation reduce bacterial growth better than non-irradiated samples. The treatment did not reduce the physicochemical quality of the bovine meat emulsions. The study indicates that the use of combination together with γ-irradiation can be used to maintain the quality of the meat emulsions.

Shahbazi (2016) investigated the use of the combination of nisin and *Ziziphora clinopodioides* essential oil to treat milk against *Salmonella typhimurium* and *Staphylococcus aureus* during storage for 9 days at 4 °C. The combination showed significant antimicrobial effects with longer storage period. There is a synergistic effect between nisin and ZEO, probably because the essential oil enhances the effect of nisin by increasing the pore size in the cytoplasmic membrane. Sensory analysis has not been carried out, however, based on these findings the combination is suitable as a preservative in milk and dairy products.

The use of the combination of nisin with grape seed extract and green tea extract against *Listeria monocytogenes* in turkey frankfurters was investigated by Theivendran (2006) for 28 days at 4 °C and 10 °C. There was a reduction in the growth of *Listeria monocytogenes* by 2.8 and 2.3 log CFU/mL at both temperatures on the last day of storage. This combination demonstrates safety and quality improvement of ready-to-eat meat products; however, sensory analysis of frankfurters was not performed, thus not confirming consumer acceptability.

Hadian et al. (2017) investigated the synergistic effect between the application of chitosan-benzoic acid nanogel and *Rosmarinus officinalis* essential oil in the preservation of beef cutlets against *Salmonella typhimurium* inoculation at 4 °C for 12 days. The combination showed higher efficiency in reducing microorganisms than using rosemary essential oil alone for treatment, furthermore, the combination showed promise in terms of maintaining the pH level of the samples.

Antibacterial effect between each acetic acid, citric acid and CO_2 treatment was investigated on uncooked salmon fillets by Schirmer et al. (2009). The combination of acetic acid, citric acid treated with CO_2 reduced bacterial activity during storage at detection level of 10^3 CFU/ml for 14 days at 4 °C. A parallel sample was taken after 21 days of storage and no growth occurred even then. It is also important to include the sensory analysis in evaluation, which showed that the appearance of the salmon was not altered by the treatment, however, the treatment did affect the flavour and odour, whereby they were rated as acetous.

Pereira Batista et al. (2019) evaluated the use of potassium sorbate and carvacrol against *Salmonella typhimurium* in tomato paste. Application of the combination at concentrations of 39 μg/ml (potassium sorbate) + 78 μg/ml (carvacrol), the growth of the bacteria was completely inhibited on the third day of storage, while the physico-chemical properties were not altered. The sensory properties were considered acceptable.

The effect of a combination of sodium acetate and *Zataria multiflora* essential oil on vacuum-packed trout burgers during 21 days of storage at 4 °C was investigated by Ehsani et al. (2014). The combination demonstrated overall acceptability up to 21 days, whereby the usual shelf life was approximately 13 days. The extended shelf life of the trout burgers due to the application of the combination is related to the synergistic effect demonstrated by the compounds. Sensory analysis rated the treated samples as highly acceptable with values of 8.1; 8.125; 8.35 and 8.425 on a nine-point scale.

De Barros et al. (2012) showed inhibitory effect of lactic acid and *Origanum vulgare* essential on the growth of *Staphylococcus aureus* in bovine meat model and meat broth at 7 °C.

Synergistic and additive effect between lemongrass essential oil and sodium diacetate treated with γ-irradiation was investigated in broccoli florets by (Ben-Fadhel et al. 2017). The combination alone, without the use of γ-irradiation of 0.4 kGy showed a decrease in the number of *Salmonella typhimurium*, however, with the use of radiation treatment, there was a complete reduction in the incidence of colonies in broccoli florets stored at 4 °C. Thus, there was a manifestation of a synergistic effect, not only in combination between antimicrobial agents, but also between agents and γ-irradiation. In the case of *Listeria monocytogenes*, there was also a reduction, and after the application of γ-irradiation, no colonies were detected during storage. The most resistant was *Aspergillus niger*, the dose of γ-irradiation had to be increased to 0.8 kGy, then no detection of *Aspergillus niger* was found from the first day to the rest of the storage.

Zhou et al. (2007) used several combinations of different agents, especially acetic acid, were used in the study to inhibit *Salmonella typhimurium* in Mueller-Hinton broth for 24 h at 37 °C. Of the 22 combinations, 9 showed a synergistic effect between the plant-derived substance and the conventional preservative, the most significant of which would be the combination of carvacrol and acetic acid, followed by the combination of thymol with acetic acid. The combination resulted in a 3.19 - 3.83 log decrease of *S. typhimurium*. The study indicates that the combination with thymol and carvacrol provides adequate antimicrobial effect and reduces the required concentration.

The treatment of strawberries with lysozyme and cinnamon essential oil as active compounds was investigated by Feng et al. (2017). A nanogel was prepared from the given compounds, consisting of β-Cyclodextrin and polyvinyl alcohol in addition to the above-mentioned compounds. The nanogel formed was applied to the surface of strawberries at a storage temperature of 20 °C. Strawberries treated with PVA/β-CD/CEO/LYS nanogel showed no signs of decay at day 6, whereas untreated strawberries were rotten at day 6. Nanogel showed strong antibacterial activity, making it a suitable candidate for active food packaging. O'Callaghan & Kerry (2015) also suggest that active packaging prolongs and maintain the shelf life of food, as well as it can improve the sensory properties.

Combination of lysozyme and pomegranate peel extract demonstrated synergistic antibacterial effect in mackerel fillets against mesophilic and psychotropic bacteria as well as the lowest sample weight loss compared to using the subsatnces individually. The combination was poured onto the surface and then heat treated in air forced oven at 40 °C for 24 hours, after the treatment glycerol was added. Then the mackerel was stored at 4 ± 1 °C for 12 days. In combination, the synergistic antibacterial effect of lysozyme and the inhibitory effect of pomegranate peel extract were demonstrated. The results prove that the combination can extend the shelf life up to 9 days and preserve the quality of mackerel, as well as its sensorical acceptability (Khodanazary 2019).

Xu et al. (2020) investigated incidence of *Shewanella putrefaciens* and *Pseudomonas fluorescens* in oliver flounder fillets at 4 ± 1 °C using the combination of lysozyme and cinnamaldehyde for 20 days of storage. A higher antibacterial effect was demonstrated between lysozyme and cinnamadehyde, as well as the effect of slowing down lipid oxidation.

6 Conclusion

Based on the studies available on Web of Science database, data on the synergistic effect between plant-derived compounds and conventional preservatives tested in food models were evaluated. However, only a minority of the testing was also subjected to sensory analysis, which has a major impact on consumer acceptability.

Nisin proved to be the most effective as well as the most tested conventional preservative demonstrating synergy with plant-derived compounds. It demonstrated synergy in all cases tested and specifically in combination with rosemary extract is proving to be a possible natural food preservation solution that has the potential to reduce the required dose of a single preservative. It should also be mentioned that this combination has even greater potential with subsequent low γ-irradiation, nevertheless, we can conclude that this form of preservation would not be well accepted by consumers. The success of nisin and its combinations with various plant-derived compounds has been demonstrated multiple times in the same food models, specifically in milk, where due to its effectiveness, we can evaluate nisin and its combinations specifically with cinnamaldehyde, citric acid and *Ziziphora clinopodioides* essential oil as a suitable potential preservative for milk and dairy products, while demonstrating a potential reduction in the problem of bacterial resistance.

The results prove the existence of synergy in all the combinations studied, thus we can conclude that selected plant-derived compounds do indeed enhance the efficiency of conventional preservatives. However, it is essential to perform a series of further studies dealing not only with the synergy, but also with the impact on the sensory properties of foods and therefore their acceptability by consumers. One of the solutions to the adverse effects on the aroma and flavour of food that plant-derived compounds exhibit is the use of nanofilm or microencapsulation. The use of nanofilm was demonstrated in the results and indicated both a synergistic effect between the combination of lysozyme and cinnamon essential oil, but also suppressed the typical strong aroma of the essential oil.

Other possibilities to eliminate the strong aromatic properties of potentially used plant-derived compounds would include the use of lower concentrations, but this would reduce their preservative effect. Deodorising agents could be added to foods, or alternatively, flavour enhancing agents such as umami flavour enhancers or flavour masking agents. Still, the use of microencapsulation seems to be the most suitable potential solution to this issue.

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