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Plant volatiles: Using Scented molecules as food additives

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ABSTRACT

Background: Secondary metabolism of plants produces molecules that are widely used in the food industry for many years. The demand for these molecules has been increasing, and special focus has been given to volatile molecules.

Scope: The study of secondary metabolites with special focus on the volatile molecules is overviewed, explaining their bioactivities and examples of application in the food industry.

Approach: Systematic analysis of volatiles and their applications. Specific analysis of volatile components of plants and their use as active packaging and airborne food preservatives.

Key findings: Some work has been done regarding volatiles in terms of food preservation but is focused mostly on postharvest protection. Considering the number of studies regarding the use of volatiles, the industry should start implementing these molecules for more sustainable approaches to preservation.

Conclusions: The food industry has researched plant volatiles for some years, but the focus has been almost exclusively on postharvest protection, and more research can be directed to other food categories. Technology to overcome limitations of plant volatiles is expected to improve, placing pressure on regulatory bodies to allow these molecules in foods, on par with consumer and industry preference for natural-based molecules used in foods.

1. Airborne metabolites from plants

Plants can synthesize tens to hundreds of thousands of metabolites with different functions and biological properties. Plant volatile organic compounds produced in both the primary and second metabolic pathways constitute about 1% of all plant secondary metabolites. There are about 1700 volatile compounds with great diversity of chemical structures that have been identified in over 90 gymnosperm and angiosperm plant families, and this number is expected to grow as more plants are discovered and analyzed with innovative detection methods. Plant volatiles comprise a lipophilic liquid with low molecular weight and high vapor pressures that can traverse membranes and be distributed into the atmosphere or soil in the absence of a diffusion barrier. They are generated in plant tissues at developmental stages (flowering, ripening, or maturation) and in different plant organs, namely roots, stems, leaves, fruits, and seeds, being flowers the part that contains the highest quantity and largest diversity of volatiles (Muhlemann et al., 2014). Most volatile compounds are synthesized from four metabolic pathways: the lipoxygenase (LOX) pathway, for fatty acid derivatives; the mevalonic acid and methylerythritol phosphate pathways for isoprenoids (terpenoids); the shikimic acid pathway for benzenoids and phenylpropanoids; and finally, the amino acid derivatives pathway. For instance, volatile compounds namely aliphatic aldehydes, alcohols, ketones, and esters of alcohols are synthesized mostly by the LOX (Baldwin, 2010; Kritioti et al., 2020, p. 109543; Pichersky et al., 2006). Plant volatiles are represented by three major groups: terpenoids, phenylpropanoids/benzenoids, and fatty acid derivatives (Table 3). Terpenoids are the main class of volatile compounds which are synthesized from the mevalonic acid and the methylerythritol-phosphate pathways, and are structured on five-carbon building blocks (isoprene), which include many subclasses: hemiterpenes (5C), monoterpenes (10C – more than 25.000 compounds are known), sesquiterpenes (15C), diterpenes (20C), sesterterpenes (25C), triterpenes (30C – steroids), tetraterpenes (40C – limonoids and quassinoids, glycosides, saponins, carotenoids, resins) and polyterpenes (n C), that are contained from a larger number of isoprene units >45 (Carocho & CFR Ferreira, 2013; Muhlemann et al.,

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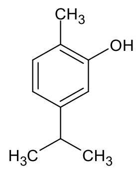
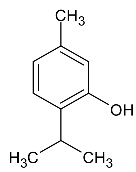
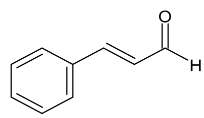
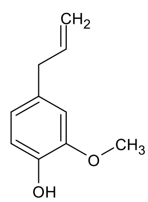
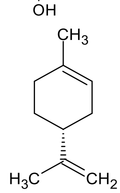
Table 3
Example molecules of the three major groups of volatile compounds (Ramos et al., 2012).

Class	Examples	Chemical structure
Terpenoids	Carvacrol Thymol	 
Phenylpropanoids/Benzenoids	Cinnamaldehyde Eugenol	 
Fatty acid derivatives	Limonene Menthone	

2014; Petrović et al., 2019).

The second largest classes of volatiles, phenylpropanoids and benzenoids, with origin in the phenylalanine amino acid, and are synthesized through the shikimic acid amino acid derivatives pathways. This group is distributed into three subclasses according to the structure of their carbon skeleton: benzenoids (C6–C1), phenylpropanoids (with a C6–C3 backbone), and phenylpropanoid-related compounds (C6–C2) (Yoo et al., 2013). The biosynthesis of benzenoids (C6–C1) and phenylpropanoids is catalysed by commonly distributed enzymes, L-phenylalanine ammonia-lyase, that transform phenylalanine to *trans*-cinnamic acid, and compete with phenylacetaldehyde synthase for phenylalanine use, to then be converted to numerous C6–C1 benzenoid compounds (Orlova et al., 2006). The *trans*-cinnamic acid is converted to the precursors of coumaryl- and coniferyl alcohol and softwood lignin before being reduced, methylated, and acetylated to produce C6–C3 volatile compounds by the monolignol biosynthesis. The production of phenylpropanoids and benzenoids in plants are widely related to progressive activities as formation of secretory structures in young leaves or flowering (Dudareva et al., 2013). The variety of phenylpropanoid/benzenoid compounds in flowers is further enlarged by modifications such as hydroxylation, methylation, and acetylation of direct aromatic precursors that improve the volatility or olfactory properties of volatile compounds. Carboxyl methyltransferases or *O*-methyltransferases responsible of methylation reactions, lead to a varied range of benzenoids/phenylpropanoids, namely veratrole in the *Silene* genus, 3,5-dimethoxytoluene and 1,3,5-trimethoxybenzene in roses, isomethyleugenol and methyleugenol in the *Clarkia* genus (Gupta et al., 2012; Schiestl & Ayasse, 2002). Volatile esters like methylsalicylate in *Clarkia* and *Petunia* genus and methylbenzoate in snapdragon and petunia flowers are synthesized by reactions catalysed by carboxyl methyltransferases (Negre et al., 2003). Acetylated compounds such as benzoylbenzoate, benzylacetate and phenylethylbenzoate are generated by reactions catalysed by acyltransferases (Boatright et al., 2004; Orlova et al., 2006).

The third class of volatiles are the fatty acid derivatives that include the green leaf volatiles (GLV) as well as methyl jasmonate, both obtained from the LOX pathway. There, linolenic, linoleic, and unsaturated C18 fatty acids by means of the lipoxygenase enzyme, lead to the generation of 9- and 13-hydroperoxy intermediates. These intermediates, after joining the two distinct branches of the LOX pathway, are responsible of the generation of all volatile compounds. These intermediates undergo the division form C6 and C9 unsaturated volatile aldehydes that can be additionally reduced to alcohols and then acetylated for producing GLV (Matsui et al., 2006). On the other hand, by sequential β-oxidation in the peroxisome, the 13-hydroperoxide intermediate is transformed to methyl jasmonate. All volatile fatty acid derivatives are synthesized as an answer form attack by necrotrophic pathogens or herbivores (Ameye et al., 2018; Dudareva et al., 2013; Scala et al., 2013).

In nature, all organisms are under pressure to increase their reproductive success. For this, several flowering species use volatiles to attract pollinators and seed disseminators to help them towards reproductive success. Although flowers may be similar in their color and shape, volatile profiles may not be identical, as a result of the huge diversity of volatiles. Floral scent bouquets could contain from 1 to 100 volatiles, but most flowers release between 20 and 60 compounds (Knudsen & Gershenzon, 2006). Some compounds like (*E*,*E*)-α-farnesene and (*E*)-β-farnesene (sesquiterpenes), linalool and (*E*)-β-ocimene (monoterpenes), nonatriene and tridecatetraene (homoterpenes), and indole and (*Z*)-3-hexenyl acetate, have a low emission at night, and high emission during periods of maximal photosynthesis. The flower scents represent a signal that pollinators can use to distinguish a specific flower among different species. Some volatiles take hours after the beginning of the damage to be emitted by the plant, namely the terpenes, methyl salicylate, and indole. Terpenoid volatiles as (*S*)-linalool, myrcene, ocimene, (*E*)-β-farnesene, (*E*)-nerolidol (intermediate to dimethyl-nonatriene) and GLV such as 13-hydroperoxy-linolenic acid (intermediate to GLV), 1-hexenal, (3*Z*)-hexen-1-al, 9-oxononanoate, (3*Z*)-nonen-1-a, and also other volatiles such as indole are volatiles induced after herbivore damage and undergo a selective biosynthetic step as a result of natural selection, being named herbivore-induced plant volatiles (HIPV) (Pichersky & Gershenzon, 2002), which can generate behavioral changes in parasitic plants, neighboring plants, parasitic nematodes, insectivorous birds, and carnivorous arthropods (Dicke & Baldwin, 2010). The influence of neighboring plants can be explained by the role of HIPV's mediating in plant-plant interactions, which stimulate the expression of defense genes and the production of carnivore attracting volatiles in unburdened leaves of neighboring plants (Arimura et al., 2004; Ruther & Kleier, 2005). Furthermore, beyond protecting plants against herbivores, volatiles also defend plant from bacterial infection, phytopathogenic fungi, and viruses (Hammerbacher et al., 2019). A proof of defense by terpenes against microbes is found in garlic infection by *Sclerotium cepivorum*, where a high concentration of fungistatic terpenes are emitted once the infection is detected by the plant (Pontin et al., 2015). Methyl jasmonate and methyl salicylate as volatile plant defense hormones play an important role in offering means to stimulate defense responses in parts of plants that are far from the initial site of infection (Song & Ryu, 2018), for instance, Tanaka et al. (2014) showed that the role of jasmonic acid (JA) in stimulating the accumulation of volatiles, and the role of monoterpene linalool in JA-induced resistance to rice bacterial blight, triggered by *Xanthomonas oryzae* pv. *oryzae*. In addition to emissions by leaves, flowers, and fruits, the roots also release diverse chemicals, inclusing volatiles, responsible for the underground defense system against microbes and herbivores, while also attracting predators of root herbivores. Moreover, volatiles offer plant protection from abiotic stresses, in which volatile isoprenoids can keep plants safe from heat, helping maintain photosynthetic capabilities, thus improving plant thermotolerance at higher temperatures (Peñuelas et al., 2005), while isoprenoids also act as antioxidants, protecting against a variety of oxidative damage.

1.1. Volatiles in the food industry

To solve the problems of changing food attributes, contamination by toxic compounds and oxidation, plant volatiles represent alternatives to synthetic molecules (Hubert et al., 2008). In the food industry, there are many volatile compounds used as flavoring and aromatizing agents, registered as safe food flavorings by the European Food Safety Authority (EFSA), namely citral, *p*-cymene, limonene, carvone, cinnamaldehyde, menthol and thymol (Pellegrini et al., 2018). Carvacrol is extensively used in drinks and sweets as a preserving and flavoring agent (Bhavaniramya et al., 2019), while monoterpenes like 1,8-cineole, limonene, and linalool are used as flavor and fragrance agents for the aroma of lime beverages (Hausch et al., 2015). Despite many essential oils being approved by the Food and Drug Administration of the United States (FDA) as generally regarded as safe (GRAS), Europe and EFSA have tighter regulations in terms of their use in foods. Still, during 2019 and 2020, EFSA has ruled many of the most known essential oils as safe for use in all types of feed of animal husbandry, which might constitute a step towards their wider use in the food industry, especially their use to preserve food, which seems to be the most significant bioactivity that volatiles can present.

1.1.1. Plant volatiles as food preservatives

As natural antimicrobials, volatiles act using different mechanisms against microorganisms and oxidation. *Trans*-cinnamaldehyde, for instance, affects bacteria by hydrolyzing the cellular adenosine triphosphate pool. Carvone, carvacrol, eugenol, and thymol act by disrupting the cell wall structures which permeabilize membranes and leak ions and other materials, that result in a high intercellular and metabolism damage (alteration of electron transport system, coagulation of cytoplasm). Thymol and carvacrol are important monoterpenes reported to inhibit the growth of bacteria, namely *Bacillus cereus*, *Salmonella* spp., *Shigella sonnei* and phylamentous fungi (Bhavaniramya et al., 2019; Pellegrini et al., 2018). Just 5–20 μL/g of coriander, clove, oregano or thyme essential oil (EO) were enough to inhibit *Aeromonas hydrophila*, *Listeria monocytogenes* and autochthonous spoilage flora in certain meat foods. Cinnamaldehyde and thymol preserved alfalfa seeds from *Salmonella* spp. contamination. 1500 ppm of ferulic acid was used in ready-to-eat foods like cheese and smoked salmon for the inhibition of pathogenic bacteria like *L. monocytogenes* (Takahashi et al., 2013). Protection of food is important against rancidity during storage, and thus, many molecules have been used to halt oxidation and extend shelf-life, namely hydroxycinnamic acids which have been exploited as inhibiting lipid oxidation in frozen minced fish (Medina et al., 2009). Mixtures of volatile compounds, mainly terpenes (monoterpenes (C10) and sesquiterpenes (C15)) could lead to synergic or antagonistic effects, but nevertheless constitute endless possibilities in preserving food. Cinnamon EO's, that contains polyphenols, terpenoids, lectins and polyacetylenes, eugenol and cinnamaldehyde have been used as antimicrobial additives (MIC values ranged from 2.9 to 4.8 mg/mL), while oregano EO's (carvacrol and thymol) have been used in the treatment of tomato, apple, and mango juices to inhibit *Escherichia coli* growth (MIC values ranged between 80 and 0.003 μL/mL) (Nabavi et al., 2015; Rodriguez-Garcia et al., 2016). Citrus EO contains around 400 compounds, 85–99% of them are volatiles, including monoterpenes (such as limonene) and sesquiterpene hydrocarbons and their oxygenated derivatives, namely ketones, aldehydes, acids, esters, and alcohols, making this EO a very interesting potential preservative for the food and packaging industries (Calo et al., 2015; Flamini et al., 2007). Applications of this EO include its addition to antimicrobial packaging films, as an ingredient in soda/citrus concentrates, and direct addition to fish, meat, and seafood conservation (Mahato et al., 2019).

Bacteria such as *Salmonella* spp., *Escherichia coli*, *Campylobacter jejuni, Clostridium* spp, *L. monocytogenes*, *Pseudomonas* spp., *Lactobacillus* spp., as well as yeasts and molds are related to the spoilage of meat and meat products, and depend on the storage conditions such as oxygen availability, temperature, moisture, and other parameters, which can accelerate intrinsic factors of meat, leading to its degradation, posing a threat to consumers (Jayasena & Jo, 2013; Ji et al., 2021). Regarding meat products, EO's of rosemary, oregano, clove, thyme, ginger, basilica, balm, coriander, marjoram, and basil have showed a high antimicrobial activity *in vitro*, extending their shelf-life (Jayasena & Jo, 2013). Individual EO's such as thyme (*Thymus vulgaris*), oregano (*Origanum vulgare*), orange (*Citrus sinensis* var. Valencia) have been tested as antimicrobials, for instance 2000 mg/L of oregano EO reduced 1.97 log CFU/g of *Salmonella* spp. populations in 144 h of sausage storage. Furthermore, several studies clearly provided that the addition of oregano EO at 0.6% or 0.9%, showed antibacterial activity against *Salmonella enteritidis* during storage at 4 °C and 10 °C, with a MIC against *S. enteritidis* at 0.25% (v/v) (Jayasena & Jo, 2013). Also, Pateiro et al (2018), showed that the essential oil of basil (*Ocimum basilicum*) could be used as a natural food preservative in beef burgers, decreasing lipid oxidation during twelve days of storage at 4 °C with different concentrations (0.062, 0.125, and 0.25%). Besides, concentrations of 0.1% and 0.5% of black pepper EOs (*Piper nigrum*) have been showed to inhibit *Piper* spp. and other Enterobacteriaceae on fresh pork (Ji et al., 2021). Furthermore, thyme oil encapsulated in liposomes might improve the antibacterial effect on *S. enteritidis* in chicken and keep food safety for a longer time (Pateiro et al, 2018; Smaoui et al, 2021). Five to twenty μL/g of coriander, clove, thyme, and oregano EOs were enough to reduce *L. monocytogenes*, *A. hydrophila* and autochthonous spoilage flora in various meat products. Moreover, in comparison with controls, during 96 h of storage, the addition of *Satureja horvatii* EO (major compounds were *p*-cymene and thymol) to pork meat considerably limited *L. monocytogenes* growth (Falleh et al, 2020). On the other hand, the synergistic effects of EO's are greater than the sum of the individual effects, in which thyme and oregano EOs showed significant synergistic effects against many pathogenic microorganisms like *Penicillium chrysogenum*, *Salmonella* spp., *Staphylococcus aureus, E. coli, B. cereus, Aspergillus flavus,* and *Aspergillus parasiticus* (Ji et al., 2021). Additionally, Jayasena and Jo (2013) showed that the natural microflora counts present in chicken breast meat could clearly be reduced by thyme and lemon balm EO's, and the shelf-life of the fresh product stored at 4 °C was extended. Besides, 0.5 μL/mL and 2 μL/mL were found to be the MIC values for marjoram and thyme EO's for minced pork against *E. coli*, as well as at levels lower than the MIC (2.3 mg/g) of marjoram EO which showed a bacteriostatic effect against *E. coli* in fresh sausages.

One of the main advantages of volatiles when compared to other antioxidant or antimicrobial preservatives is the fact that the former can excerpt their effect in the headspace of packages, reducing processing of foods, and thus reducing costs. On the other hand, most of the effect of volatiles is restricted to the surface of foods, making them less effective in thick foods. Another drawback of using some types of EO as preservatives in *stricto sensu*, is the aroma and taste that can be imparted to the food. This drawback conflicts with the definition of food additives, which should not alter any other aspect of the food beyond the one it is added for. This drawback is one of the reasons these EO are being used as volatile solutions rather than traditional preservatives (Bhavaniramya et al., 2019; Falleh et al., 2020; Myszka et al., 2019; Pellegrini et al., 2018). Active packaging is a concept that delegates functions beyond its protective role, to a food package. Thus, active packaging is now being used in combination with molecules or extracts to have specific effects on food. In this manner, volatiles have been used in combination with these packages to act as preserving agents (Kapetanakou & Skandamis, 2016; Taghavi et al., 2018; Chen et al., 2020; Sharma et al., 2021). For these reasons, volatiles and EO's are becoming a "hot topic" in food science, being used as natural preservatives, but their instability could pose problems for a practical approach, furthermore the aroma they exhibit affects the food's organoleptic characteristics. One solution to overcome the high odor of volatiles is to add mixtures of different extracts that potentiate the preserving activity, thus greatly reducing the needed quantity needed, which would lead to no odor at all within the packaging. Another solution is to impregnate the actual package and let the preserving effect act by contact rather than relying on the headspace of packaging. Finally, encapsulation through spray-drying and other techniques may have a positive effect in controlling the release of the volatiles, further reducing the odor concentration within the package (Falleh et al., 2020; Tiwari et al., 2020). Table 4 shows specific plant volatiles as well as essential oil mixtures from different plants and their

Table 4
Examples of essential oils and volatiles used as active packaging and/or volatile food preservatives.

Source Plant or Volatile Molecule	Food Matrix	Target Effect/Microorganisms	MIC values	Reference
Litsea cubeba (Lour.) Pers. extract and citral	Strawberries	*S. aureus*, *E. coli* - bactericidal effect *Aspergillus niger*, *Saccharomyces cerevisiae* - antimicrobial effect	80 μg/mL and 250 μg/mL, 450 μg/mL	Thielmann et al. (2021)
Green tea oil and carvacrol	Strawberries and pumpkin	*E. coli*, *L. monocytogenes*, *Alternaria alternate*, *Penicillium commune*, *Pseudomonas putida* - antimicrobial effect	-	Rozenblit et al. (2018)
Eucalyptus globulus Labill. and *Cinnamomum zeylancium J.Presl*	Strawberry and tomato	*Colletotrichum coccodes* - Firmness, total soluble solids, weight loss, organic acid content, sweetness and total phenolics	-	Tzortzakis (2007a)
O. vulgare L.	Tomato	*C. coccodes* - Fruit lesions, fungal spore development, fruit cracking,	-	Tzortzakis (2010)
Methyl jasmonate	Tomato	*C. coccodes* – spore germination, fruit lesion,	-	Tzortzakis (2007b)
C. zeylancium	Pepper fruit and tomato	*Botrytis cinerea*, *Cladosporium herbarum*, *Rhizopus stolonifer*, *A. niger* – Sporulation	25 ppm	Tzortzakis (2009)
Cymbopogon citratus L.	–	*C. coccodes*, *B. cinerea*, *C. herbarum*, R. *stolonifer*, *A. niger* – Colony size *in vitro*, sporulation	500–1000 ppm	Tzortzakis & Economakis, 2007c
Methyl jasmonate, *Salvia officinalis* L.	Pepper fruit	*B. cinerea* – Lesion development, spore production, spore germination, colony growth	-	Tzortzakis., 2016
C. zeylanicum, *O. vulgare*, *Syzygium aromaticum* (L.) Merr. & L.M.Perry	–	*E. coli, Yersinia enterocolitica, Pseudomonas aeruginosa, Salmonella enterica* serotype *choleraesuis, L. monocytogenes, S. aureus, B. cereus, E. faecalis, Penicillium islandicum, Penicillium roqueforti, Penicillium nalgiovense, Elymus repens, A. flavus, Candida albicans, Debaryomyces hansenii, and Zygosaccharomyces rouxii* – Gowth inhibition	–	López et al. (2007)
O. vulgare and *T. vulgaris*	Lettuce and carrot	Enterobacteria, lactic acid bacteria and *Pseudomonas* spp. – viability,	20 μg/g	Gutierrez et al. (2009)
T. vulgaris, *C. zeylanicum* and *S. aromaticum*	Strawberry	*Colletotrichum acutatum* – mycelial growth, conidial germination, appressoria formation	15.3 and 76.5 μL/L	Duduk et al. (2014)
Eugenol and thymol	Grapes	Sensory, nutritional, and functional properties	75 and 150 μL	Valero et al. (2006)
Ocimum sanctum Linn., *Prunus persica (L.) Batsch, Zingiber cassumunar* Roxb. *and Zingiber officinale* Roscoe, 1807	Grapes	*B. cinerea*	200 and 100 ppm	Tripathi et al. (2007)
Thymol, menthol, and eugenol	Strawberry	Sugars, organic acids, phenolics anthocyanins, flavonoids, antioxidant activity	-	Wang et al. (2007)
Carvacrol, anethole, cinnamic acid, perillaldehyde, cinnamaldehyde, and linalool	Raspberries	Antioxidant capacity, individual flavonoids	-	Jin et al. (2012)
Trans-2-hexenal, carvacrol, citral, *trans*-cinnamaldehyde, hexanal, (−)-carvone, eugenol, 2-nonanone, and p-anisaldehyde	Stone fruit	*Monilinia laxa* - Conidial germination, mycelial growth	12.3 μL/L 6.1 μL/L	Neri et al. (2006)
Vitis labrusca L.	Kiwi fruit	*B. cinerea* – disease development	-	Kulakiotu et al. (2007)
Trans-2-hexenal, carvacrol, *trans*-cinnamaldehyde, citral, hexanal, (−)-carvone, *p*-anisaldehyde, eugenol and 2-nonanone	Pears	*Penicillium expansum* – mycelial growth and spore germination	12.5 μL/L 200 μL/L	Neri et al. (2005)
Hexanal and *trans*-2-hexenal	Sliced apples	*Pichia subpelliculosa* enzymatic browning, gowth of naturally occurring microflora	-	Corbo et al. (2000)
Cinnamaldehyde	Bread and cheese spread foods	*P. expansum, A. niger*	-	Balaguer et al. (2013)
C. zeylanicum, *O. vulgare*, S. *aromaticum* and *C. citratus*	Apples	*Dendryphion penicillatum, Helminthosporium solani, A. alternata, A. niger, Cladosporium cucumerinum, Claviceps purpurea, Monilinia fructigena, Penicillium digitatum, P. expansum*	128 μL/L, 32 μL/L, 64 μL/L, 16 μL/L	Frankova et al. (2016)
Oregano	Sausages	*Salmonella* spp.	2000 mg/L	Jayasena and Jo (2013)
Marjoram and thyme	Pork meat	*E. coli*	0.5 μL/mL and 2 μL/mL	Jayasena and Jo (2013)
Marjoram	Fresh sausages	*E. coli*	2.3 mg/g	Jayasena and Jo (2013)
S. horvatii	Pork meat	*L. monocytogenes*	–	Falleh et al (2020)
Coriander, clove, thyme and oregano	Meat products	*L. monocytogenes*, *A. hydrophila*	5–20 μL/g	Falleh et al (2020)
Thyme	Chicken meat	S. *enteritidis*	–	Pateiro et al (2018)
P. nigrum	Pork meat	*Pseudomonas* spp. and Enterobacteriaceae	–	Ji et al. (2021)

applicability as active packaging agents and food preservatives. Most of the references in Table 4 relate to diffusion of volatiles, although some have forced air from the essential oil container to the container with the food, and thus, the efficacy is quite different.

Impregnation of volatiles in coatings and films rather than diffusion in the headspace has been tested on grapes, using films made from of chitosan, and bergamot EO and hydroxypropylmethylcellulose to fight *R. stolonifer* and *A. niger* (Santos et al., 2012), in cold stored grapes (Sánchez-González et al., 2011). Other essential oils have been impregnated in chitosan films to preserve blueberries and other fruits (Sun et al., 2014).

2. Extraction methodologies

Volatile extraction and analysis have varied fundamentally over the past 15–20 years. The combination of conventional techniques with new methodologies has increased yields, purity, and quality of volatiles. Several distillation techniques have been improved, namely dry-, vacuum-, steam- or hydro-distillation, offline solvent extraction, and simultaneous distillation–extraction are examples. In addition, efficient extraction techniques like ultrasound or microwave-assisted extraction–hydrodistillation, supercritical fluid extraction or selective accelerated solvent extraction have been combined to improve EO yields (Pavlić et al, 2021; Rubiolo et al, 2010). The main advancements in the sampling of volatile fractions have been accomplished in the 'vapor' phase, identified as headspace sampling (dynamic or static mode). There are many other lesser-known methodologies, namely headspace–solid phase microextraction, in-tube sorptive extraction, headspace sorptive extraction, static and trapped headspace, solid-phase aroma concentrate extraction, headspace liquid-phase microextraction, and large surface area high concentration capacity headspace sampling. The analysis of the volatile fraction has other sequential steps, namely separation, identification, and quantification. Due to the complexity of volatile fraction components, characterized by low and medium polarity compounds, gas chromatography has been the method of reference for their identification and quantification. The most widespread polar stationary phases used in routine analysis are those with polyethylene glycol, while the most common used apolar phases are those based on methyl-phenyl-polysiloxanes and methyl polysiloxanes. Usually, identification and confirmation rely on mass spectrometry coupled to the gas chromatographer (GC-MS), and combination of chromatographic tools (linear retention indices, relative retention time) that result in a specific and reliable identification. The main new achievements for this analysis are fast-GC combined with flame ionization detectors and MS, enantioselective-GC combined with flame ionization detectors (FID) and MS, multidimensional GC, and comprehensive two-dimensional gas chromatography (Rubiolo et al, 2010). The FID is most common GC detector, and can provide information by peak area proportion, while the combined system of GC-FTIR-MS (Fourier transformed infra-red - FTIR) is useful for flavor analysis due to higher complexity of the sample (Kawakami, 1997).

3. Trends and conclusions

It is clear that plant volatiles are going to be a trend in the coming years, be it may as preserving molecules in active packaging, injected into the headspace of food packaging, or mounted on food coatings and films. Furthermore, essential oils and other extracts with volatile molecules are expected to be further used directly in foods. Thus, for this to come to fruition, regulatory issues must be overcome, especially in Europe, where restrictions are tighter for essential oils to be used as preservatives. The pressure can be increased by academia and industry by further using volatiles as matrices for solving food related issues. Examples of volatiles used in the industry as preserving agents are somewhat common, but mostly restricted to the use in fruits and vegetables and targeting microbes. Other types of foods can be studied, namely dairy, meat, fish, pastry, bakery, processed goods, and many others, which constitute endless possibilities to apply volatile metabolites for their preservation, both in terms of microbial decay and oxidation. To solve the problems of high volatility and the impact of volatile aroma and odor, many strategies can be used, namely encapsulation into micro- or nano emulsions as an option for the stabilization, reduction of the organoleptic effects, protection from interacting with the food components, but also combination with other odorless molecules, potentiating their effect and further reducing their relative quantities. Further research is also needed in terms of toxicity; Firstly, toxicity towards the food itself, if migration of volatiles into food is detected, and better understand the metabolization of these molecules in the human digestive tract. Many tools are available to improve this research in terms of reducing costs and time consumption, namely transcriptomic and proteomic techniques, X-ray crystallography, spectroscopy, and computer modeling. Computer modelling is a tool that can be crucial in widespread testing of volatile compounds against targets. Molecular docking has played an important role as preliminary research to understand hypothetical bioactivities *in silico* and can also be of great value for the volatile area. Overharvesting of plants with high valued essential oils and volatiles is a risk that must not be overlooked. To maintain the trend of sustainability in the food industry, cell culture of plants or plant organs should also be investigated to prevent overexploitation and excessive use of land and water, but also the use of plant by-products or residues.

Volatiles as plant secondary metabolites have multiple important roles in plants, from defense of plant as their ancestral function, to the attraction of pollinators and in plant-plant interactions. Out of plant-life, they represent valuable compounds for scientistsm and have different applications mainly in pharmaceutical, cosmetic, and food industry. Many volatile compounds are used as flavoring and aromatizing agents, registered as safe food flavorings by EFSA (citral, *p*-cymene, limonene, carvone, cinnamaldehyde, menthol and thymol). Furthermore, for their strong antimicrobial and antioxidant activity they can be used as preservatives that extent the food shelf life, maintain food stability and nutritional value, and protecting it from microbial spoilage. Now, more than ever, awareness about climate change, pollution, sustainability, and human impact on the environment are, finally and rightfully, in the spotlight. Taking advantage of this global awareness is the key point, in which oil-based plastics are being reduced, recycled, and substituted for plant-based and degradable alternatives, when single use plastics are demonized and their use condemned to reduction, the industry tries to find ways of reducing its impact. This green revolution can be helped by plant volatiles and their low impact on food processing.

CRediT authorship contribution statement

Nabila Ben Derbassi: Writing – original draft. **Mariana C. Pedrosa:** Writing – original draft. **Sandrina Heleno:** Writing – review & editing, Conceptualization. **Marcio Carocho:** Writing – review & editing, Conceptualization, Funding acquisition. **Lillian Barros:** Writing – review & editing, Funding acquisition.

Declaration of competing interest

The authors state no conflict of interest.

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