Applications of Essential Oils as Antibacterial Agents

Subjects: Food Science & Technology

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Microbial foodborne diseases are a major health concern. In this regard, one of the major risk factors is related to consumer preferences for "ready-to-eat" or minimally processed (MP) fruits and vegetables. Essential oil (EO) is a viable alternative used to reduce pathogenic bacteria and increase the shelf-life of MP foods, due to the health risks associated with food chlorine. Indeed, there has been increased interest in using EO in fresh produce. However, more information about EO applications in MP foods is necessary. For instance, although in vitro tests have defined EO as a valuable antimicrobial agent, its practical use in MP foods can be hampered by unrealistic concentrations, as most studies focus on growth reductions instead of bactericidal activity, which, in the case of MP foods, is of utmost importance.

essential oils vegetables fruits

1. Introduction

1.1. Minimally Processed Fruits and Vegetables

The fast pace of modern life has led to a shortage of time, particularly regarding meal preparations; there is an increase in consumer preferences for food that is healthy, fast, and easy to prepare [1][2][3][4]. The food industry—in effort to meet consumer demands—is continuously developing a wide range of ready-to-eat, fresh-cut, refrigerated foods with prolonged shelf-lives [1]. Preservation techniques, such as refrigeration, moderate heating, specific packaging, and antimicrobial disinfectants are usually applied to maintain a product's freshness. Ready-to-eat fresh foods, with minimal alterations and without strong preservatives are referred to as minimally processed (MP) foods [1][2][4]. These new MP foods are marketed and packaged in a ready-to-eat state for ease and convenience, and they comprise a wide range of products, such as fresh cut vegetables, meat, and fish [4][5]. MP foods have emerged in response to a new market tendency, i.e., a concomitant increasing demand for efficient preservation techniques that lack the need for chemical preservatives [3]. MP vegetables/fruits are a particular branch in the MP food industry; this branch has gained much interest from consumers since MP vegetables/fruits are considered healthier than processed food products. Minimally processed fruits and vegetables (MPFVs) include any fresh vegetable or fruit that has been minimally altered (usually cut, peeled, shredded, and washed) and packaged, in a ready-to-use state, whilst remaining fresh [4][6][7][8] (**Figure 1**).

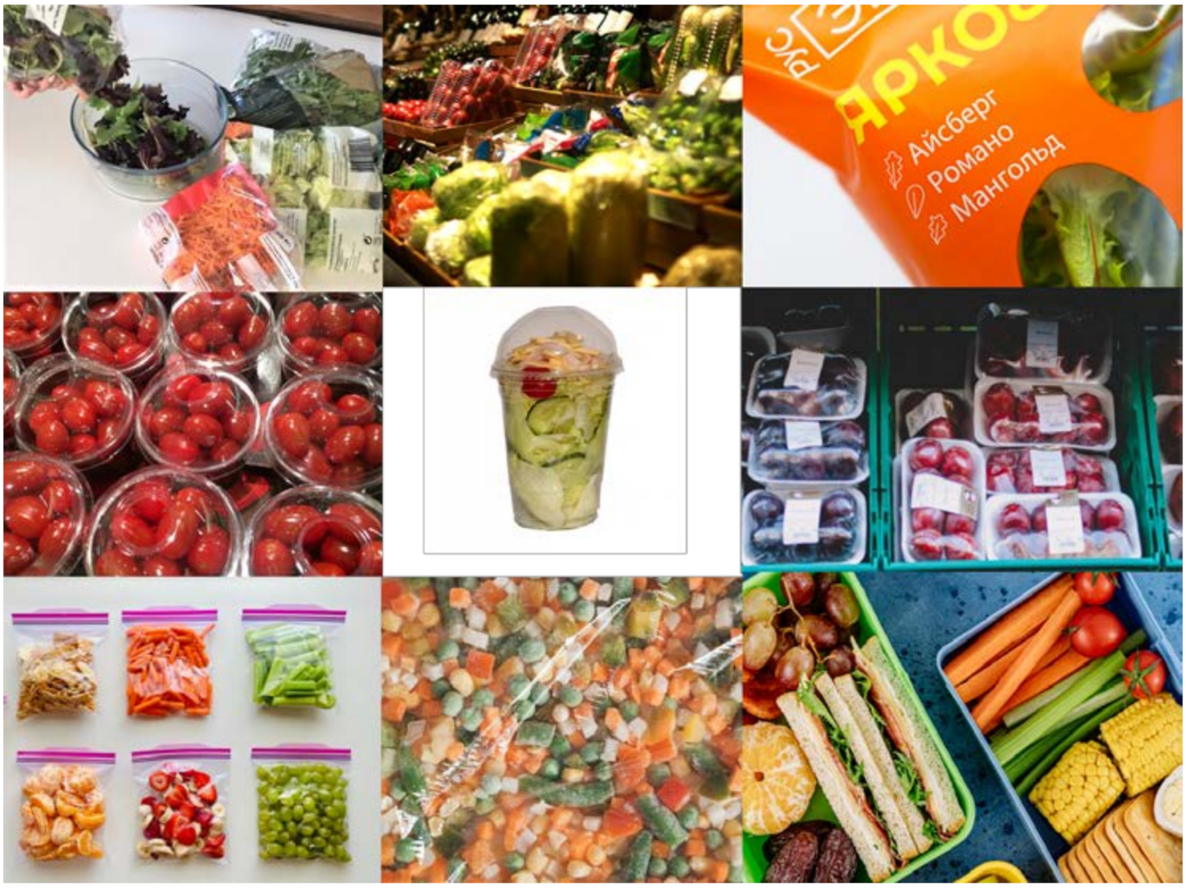

Figure 1. Examples of minimally processed (ready-to-eat) fruits and vegetables.

These types of products simplify everyday life, allowing for the preparation of healthy, enjoyable, and diversified meals, in a time-saving fashion, with reduced food waste. In the United States (US), MPFV sales grow by approximately USD 15 billion per year and represent 15% of sales in all plant products [9][10]. The best-selling product is a ready-to-eat salad, in which sales increased from USD 2.7 to 3.2 billion between 2001 and 2003. In Europe, consumption varies widely among countries, with the United Kingdom (UK) being the largest consumer, having exceeded 120,000 tons of sales in 2004 [9][10].

Nonetheless, MP foods are not sterile. As vegetables are raw and of agricultural origin, MPFVs contain microorganisms (often pathogenic) [11][12][13][14][15]. It is therefore not surprising that some of the most nutritionally recommended foods are also those with the greatest food preservation and safety challenges. Indeed, fruits and vegetables are often incriminated in foodborne diseases worldwide. In recent decades, foodborne outbreaks associated with raw fruit and vegetable consumption have increased. This has led to researchers and health authorities (in food safety areas) analyzing the microbial contamination of fresh produce [16][17][18][19][20]. There is growing concern about the potential risks of microbiological proliferation, owing to the high-levels of manipulation that these types of products are subject to and the increase in MPFV consumption worldwide. Vegetables may become contaminated in the pre-harvest stage (e.g., as a plant in the field or during harvesting) and in the post-harvest phase (e.g., during transportation, processing, and packaging) [14][21][22][23]. Thus, the microbial quality and safety of MPFVs is a serious concern.

Over the years, extensive studies have been carried out on the antimicrobial activities of essential oil (EO) and its application in food systems. The use of EOs—specifically in MPFVs—has been garnering more attention of late, but there are a lack of consolidated appraisals on this issue. Most studies focus on in vitro testing; few show applications in realistic scenarios. Therefore, the present research focuses on the effect of EO in MP food pathogens, focusing on their more realistic applications, particularly on promising innovative solutions for their safe usage. Overall, due to this type of information, EO could become an "added value" to the food industry.

1.2. Major Pathogens Related to Foodborne Diseases in MP Foods

In the last three decades, the epidemiology of foodborne infectious diseases has undergone a radical change; vegetal products have "arisen" as new vehicles of microorganisms [12]. There have been numerous outbreaks, as shown in the scientific literature, describing situations that have resulted in the death of hundreds of people [20][21][22][23][24]. *Salmonella* spp., *Escherichia coli* O157:H7, and *Listeria monocytogenes* are the pathogenic microorganisms that cause the most concerns in outbreaks of this nature [14][18][25]. Several of these outbreaks have led to widespread public health concerns. For example, between May and July 2011, a major outbreak occurred as result of the high number of cases and the difficulties in detecting the source of the infection. The outbreak occurred in Germany; out of a total of 3816 cases, 845 patients developed hemolytic uremic syndrome (HUS) and 54 died. Most of the patients (88%) who developed HUS were adults, contrary to what usually occurs in VTEC strain infections. Likewise, the female gender (aged between 30 and 34 years) was the most affected (68% of cases with HUS and 58% of gastroenteritis). The epidemic strain of this outbreak was an *E. coli* O104:H4 enteroaggregative that acquired the Shiga toxin 2 (Stx2a) converting bacteriophage. This outbreak disseminated worldwide, with reports in 15 European countries and in the USA. In France, eight cases occurred in people who had been present at a community event, and the isolated strain had a genetic profile compatible with the epidemic strain from Germany. Given that it was a common event, it was possible to identify the suspected food as fenugreek sprouts imported from Egypt in 2009 [26][27][28][29]. According to data from the USA, fruits and vegetables account for an estimated 46% of foodborne illnesses, most of which are caused by norovirus, *Salmonella* spp., and *E. coli* O157:H7, with leafy vegetables being the most frequent vehicle. Vegetables are responsible for 2.2 million foodborne illness cases per year (22%), corresponding to the food product responsible for the largest number of patients. It is estimated that 24,000 people (41%) are hospitalized annually due to the consumption of products of plant origin, of which, 38% are attributed to fruits and vegetables and 16% to leafy vegetables, just behind dairy products, which occupy first place (in terms of hospitalizations). Regarding the number of deaths—fruit and vegetable consumption is related to 333 foodborne illnesses per year (23%), far below the 43% from animal product consumption (terrestrial). In summary, leafy vegetables account for the largest number of patients with foodborne diseases (22%), being the second cause of hospitalization (14%) and the fifth most frequent cause of death (6%) [19].

2. Essential Oils as Alternative Food Disinfectants

Since ancient times, the antimicrobial properties of plants and spices have been exploited as food preservatives [30][31][32][33][34]; scientific interest in this area has recently re-emerged [35]. In recent decades, essential oils (EOs) from aromatic and medicinal plants have been used as novel alternatives to common food antibacterial agents, as they are natural products, inherently well tolerated, and present fewer side effects when compared to other food preservatives or disinfectants.

EOs the result of plant secondary metabolites; they are known to present intense odors, being extremely volatile and hydrophobic [36]. They are produced by specialized excretory structures and can be found in several parts of these plants, namely leaves, fruits, flowers, buds, seeds, branches, and roots, and their compositions may vary according to the location [37].

In nature, these metabolites have two distinct functions: (1) they protect plants against pests or infections through their insecticidal, antibacterial, and antifungal actions; (2) they attract certain insects, so that they remove pollen from the plant, facilitating pollination [38]. The amount and composition may vary, both genetically and physiologically, as well as due to external factors, such as growing conditions, harvesting, post-harvest conditions, and environmental factors, among others [36][39].

2.1. Composition of Essential Oils

EOs are volatile, natural, complex compounds formed by aromatic plants as secondary metabolites; they are characterized as having strong odors [38]. In nature, EOs play an important role in the protection of plants through their antibacterial, antiviral, antifungal, and insecticides actions, as well as against herbivores by reducing their appetite for such plants. EOs may attract some insects, to favor the dispersion of pollen and seeds, or repel others that are undesirable [38]. EO chemical compositions can widely differ, according to several factors, such as the soil composition, the organ of the plant from which it is extracted, the time of the year it is harvested, the plant and organ age [38][40], and the extraction method used [30]. The different EO compositions result in different responses in their antimicrobial activities, even when they are tested under the same conditions. Thus, obtaining/extracting in a standardized manner is important in order to obtain a constant composition of EO [30][38].

EOs are complex natural mixtures that could contain approximately 20–60 components at quite different concentrations. They are characterized by two or three major components at fairly high concentrations (20–70%) in combination with other components that are only present in trace amounts [30][38][41]. Generally, it is the component in the greatest concentration (major constituent) that confers the biological activity to the EO; however, this activity is often the result from the synergy between several components [30][41][42]. In a study carried out in the control of *Botrytis cinerea* using several EOs, the authors verified that, in most cases, those with the highest concentrations of the major constituents had higher fungicidal activities [43].

Table 1 shows the major components of some of the most known EOs used in foods. These active compounds have different chemical groups, composed of alcohols, esters, aldehydes, ketones, phenols, and phenolic ethers, with terpene compounds being the most abundant [44]. The components include two groups of distinct biosynthetic origins [30][38]. The main group is composed of terpenes and terpenoids and the other of aromatic and aliphatic constituents, all characterized by low molecular weight [38]. Terpenes form structurally and functionally different classes. They are made from combinations of several 5-carbon-base (C5) units called isoprene [41] and they have been extensively reviewed [41]. The main terpenes in EO are monoterpenes (C10) and sesquiterpenes (C15), but monoterpenes are the most representative molecules, constituting 90% of essential oils and allowing for a large variety of structures [38], although they usually do not represent a group of constituents with high inherent antimicrobial activity [41]. Hemiterpenes (C5), diterpenes (C20), triterpenes (C30), and tetraterpenes (C40) also exist [41]. Examples of plants containing these compounds are angelica, bergamot, caraway, celery, citronella, coriander, eucalyptus, geranium, juniper, lavender, lemon, lemongrass, mandarin, mint, orange, peppermint, petitgrain, pine, rosemary, sage, and thyme [38].

Terpenoids are terpenes that undergo biochemical modifications via enzymes that add oxygen molecules and move (or remove) methyl groups [38][41][44]. Terpenoids can be subdivided into alcohols, esters, aldehydes, ketones, ethers, phenols, and epoxides. Examples of terpenoids in EOs with food applications are: thymol, carvacrol, linalool, citronellal, piperitone, menthol, and eugenol (**Table 1**). The antimicrobial activities of most terpenoids are linked to their functional groups; the hydroxyl group of phenolic terpenoids is recognized as the most important for antimicrobial activity [41].

Besides terpenes and terpenoids, aromatic compounds occur less frequently, but are also noteworthy. They are derived from phenylpropane and include cinnamaldehyde, chavicol, eugenol, myristicin, and safrole, among others [41][44]. The main plant families for these compounds are *Apiaceae*, *Lamiaceae*, *Myrtaceae*, and *Rutaceae*, which include plant species, such as anise, cinnamon, clove, fennel, nutmeg, parsley, sassafras, star anise, and tarragon, among others [41]. Sulfur-based components from plants, such as garlic and mustard oils (e.g., glucosinolates or isothiocyanate derivatives) are also secondary metabolites often found in diverse source plants for EO [41].

Secondary Effects Induced by EO Components

Because of the great number of constituents, essential oils can induce secondary effects to consumers, depending on their concentrations. The use of EO in foods—besides odor and taste—can induce some secondary effects in consumers, although there are restrictions on the doses used for food applications and, most of all, for food safety issues (please see Section 3). The biological effects of EOs have been extensively reviewed elsewhere [38], mostly focusing on cytotoxicity, nuclear mutagenicity, and carcinogenicity. Cytotoxicity occurs mostly due to membrane damage [45][46][47][48][49][50][51], cytoplasm coagulation [52], and overall damage to lipids and proteins [52][53][54][55][56]. Essential oil cytotoxicity in mammalian cells is caused by the induction of apoptosis and necrosis [38]. For example, eugenol, isoeugenol, methyl eugenol, and safrole induce cytotoxicity and genotoxicity in rat and mouse hepatocytes [57], and estragole also induces cytotoxicity in hamster fibroblastic V79 cells [58]. Many studies using EO or their main components have also shown that, grosso modo, most of them do not induce nuclear mutations [38]; however, there are a few exceptions, particularly in the case of some EO constituents that can act as secondary carcinogens after metabolic activation [59]. Specific EO constituents that have been shown to induce carcinogenic metabolites in rodents include safrole (from *Sassafras albidum* EO) [57][60][61], methyl eugenol (from *Laurus nobilis* and *Melaleuca Leucadendron* EO) [57], d-Limonene (from *Citrus* EO), and estragole (from *Ocimum basilicum* and *Artemisia dracunculus* EO) [60][62]. Moreover, the EO from *Salvia* *sclarea* and *Melaleuca quinquenervia* can induce estrogen secretion, which in turn can trigger estrogen-dependent cancers. Moreover, the EO components containing photosensitizing molecules can also cause skin erythema or cancer [63][64].

Table 1. Major components of some essential oils with food application.

Common Name	Scientific Name	Major Constituent	2nd Constituent	3rd Constituent	4th Constituent	5th Constituent	References
Amaryllidaceae							
Garlic	*Allium sativum*	Diallyl disulfide	Allyl methyl trisulfide	Diallyl trisulfide	Diallyl sulfide	Allyl methyl disulfide	[65]
Onion	*Allium cepa*	Dipropyl disulfide	Dipropyl trisulfide	Propenyl propyl disulfide	Methyl propyl trisulfide	Allyl propyl trisulfide	[66]
Asteraceae							
Chamomile	*Matricaria chamomilla*	Bisabolol oxide	Camphene	Sabinene	Limonene	Cineole	[67]
Cupressaceae							
Juniper	*Juniperus communis*	Pinene	Myrcene	Sabinene	Limonene	Caryophyllene	[68]
Lauraceae							
Cinnamon	*Cinnamomum zeilanicum*	Eugenol	α-Himachalene	Bicyclogermacrene	Linalool	Nerolidol	[43]
Lamiaceae							
Basil	*Ocimum basilicum*	Linalool	Geraniol	Eugenol	Eucalyptol	Humulene	[69]
English Lavender	*Lavandula angustifolia*	Linalool	Linalyl acetate	Geraniol	Caryophyllene	Lavandulyl acetate	
Lavender	*Lavandula hybrida*	Octyl Acetate	Linalool	Isobornyl acetate	Camphor	α-Himachalene	[43]
Lemon Balm	*Melissa officinalis*	Neral	Nerol	Geranial	Geraniol	Caryophyllene	[70]
Marjoram	*Origanum majorana*	Terpineol	Sabinene	Cymene	Terpinene	Limonene	[71]
Oregano	*Origanum vulgare*	Thymol	Terpinene	Cymene	Carvacrol	Myrcene	[71]
Peppermint	*Mentha piperita*	Menthol	Menthone	Menthyl acetate	α-Himachalene	Eucalyptol	[43]
Rosemary	*Rosmarinus officinalis*	Eucalyptol	Camphor	Pinene	Camphene	α-Terpineol	[43]

Common Name	Scientific Name	Major Constituent	2nd Constituent	3rd Constituent	4th Constituent	5th Constituent	References
Sage	*Salvia officinalis*	Camphor	Thujone	Cineole	Camphene	Borneol	[72]
Thyme	*Thymus vulgaris*	α-Terpinene	Cymene	Thymol	Linalool	Carvacrol	[73]
Myrtaceae							
Eucalyptus	*Corymbia citriodora*	Citronelal	7-Octen-1-ol	Isopulegol	Fenchyl acetate	Eucalyptol	[43]
Tea Tree	*Melaleuca alternifolia*	Terpinenol	γ-Terpinene	Eucalyptol	α-Terpinene	Cymene	[43]
Clove Tree	*Syzygium aromaticum*	Eugenol	α-Humulene	δ-Cadinene	Caryophyllene oxide	Eugenyl acetate	[43]
Piperaceae							
Black Pepper	*Piper nigrum*	α-Pinene	β-Phellandrene	Terpinene	Cubebene	Farnesene	[43]
Poaceae							
Lemon grass	*Cymbopogon citratus*	Geranial	Neral	Myrcene	Geraniol	Verbenol	[43]
Citronella	*Cymbopogon nardus*	Citronelal	Geraniol	Octenol	Elemol	Citronellyl isobutyrate	[43]
Palmarosa	*Cymbopogon martini*	Geraniol	Geranyl Acetate	Linalool	β-Ocimene	α-Himachalene	[43]
Rutaceae							
Bergamot	*Citrus bergamia*	Linalool	Limonene	Linalyl acetate	Terpinene	Pinene	[74]
Citron	*Citrus medica* var. *sarcodactylis*	Limonene	γ-Terpinene	Terpineol	Bisabolene	Cymene	[75]
Grapefruit	*Citrus paradisi*	Limonene	Myrcene [41]	Pinene	Sabinene	Carvone	[76]
Lemon	*Citrus lemon*	Limonene	Pinene	Linalool [30]	[30][32][41][81][82][83][84][85] Terpineol	Linalyl acetate [41]	[77]
Orange	*Citrus sinensis* var. *dulcis*	Limonene	Myrcene	Pinene	Caproaldehyde	Sabinene	[43]
Tangerine	*Citrus nobilis* var. *tangerine*	Limonene	Linalool	Pinene	Myrcene	Terpineol	[78]
Zingiberaceae							

…nderlying …acteria is …e to their …bacterial …anism to …c nature, …ations in permeability and eventually disruption, culminating in the release of ions and intracellular components [86], resulting in cellular death. The overall antibacterial mechanisms encompassed by EO have been extensively reviewed elsewhere [87]. Overall, the main EO constituents (**Figure 2**, **Table 1**) are those playing the key roles in antibacterial activities, namely terpenes and other compounds, including ketones (e.g., β-myrcene, α-thujone, or geranyl acetate) and phenols (e.g., cinnamaldehyde, carvacrol, eugenol, or thymol) [88]. Carvacrol, eugenol, and thymol have been recognized as some of the major antibacterial compounds in EO [88], although many others are being reported on.

Common Name	Scientific Name	Major Constituent	2nd Constituent	3rd Constituent	4th Constituent	5th Constituent	References
Cardamom	*Elettaria cardamomum*	Terpinyl acetate	Cineole	Sabinene	Terpineol	Limonene	[79]
Ginger	*Zingiber officinale*	Zingiberene	Citronellyl	Phellandrene	Camphene	A-Pinene	[80]

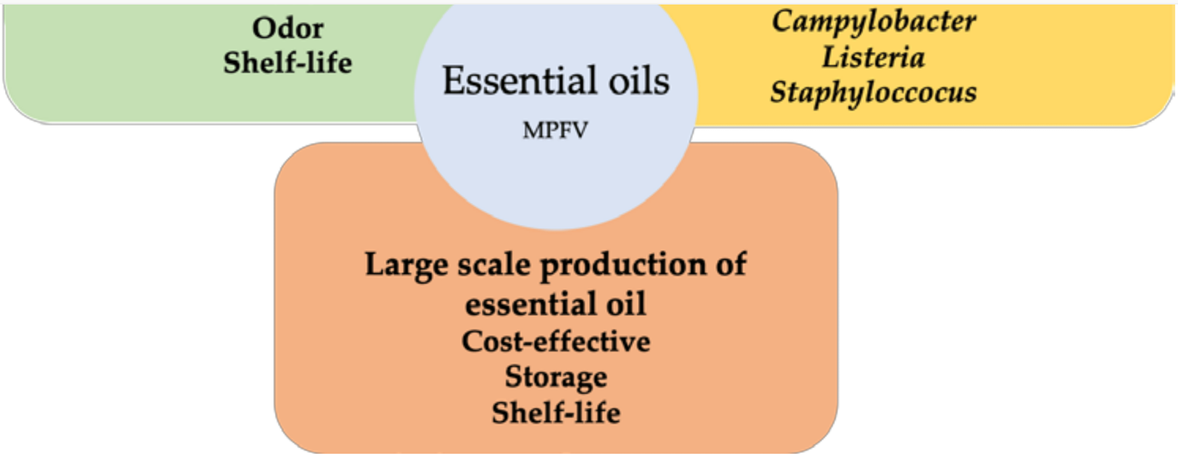

Figure 2. Factors affecting the practicality of essential oil antibacterial activity of minimally processed foods in the food industry.

The detection of antibacterial activity in EO is of extreme importance in the food industry, to tackle the growing concerns about pathogenic and/or resistant bacteria dissemination worldwide, including via food chain transfers. Concerning the concentration range—there are several terms used in the literature to define the antimicrobial activities of EO, which are summarized in **Table 2**. The different definitions differ among the studies, often making it difficult to compare the results reported in various works. In the context of food safety, however, it is important to evaluate the minimum bactericidal concentration (MBC) as well as the (usually much lower) minimum inhibitory concentration (MIC) values, since the elimination of the inoculum is desirable and not only a reduction of its growth.

Table 2. Terms used to define the antimicrobial activities of essential oils.

Terms	Definitions	References
Minimal inhibitory concentration	Lowest concentration resulting in maintenance or reduction of inoculum viability of the tested organism.	[89]
	Lowest concentration inducing a significant decrease in inoculum viability (>90%).	[90]
	Lowest concentration inducing a complete inhibition of the tested organism, up to 48 h of incubation.	[91]
	Lowest concentration inducing visible growth reduction of the tested organism.	[44]
	Lowest concentration reducing visible growth of the tested organism	[92]

Terms	Definitions	References
	Lowest concentration inhibiting visible growth of the tested organism over 18 to 24 h.	[93]
Minimal bactericidal concentration	Lowest concentration at which no growth is observed after subculture.	[94]
	Concentration inducing death of 99.9% or more of the initial inoculum.	[90]
	Lowest concentration that results in the death of 99.9% of the tested organism.	[92]
	Minimum concentration that induces a bactericidal effect, determined by re-culturing broth dilutions that inhibit bacterial growth (i.e., those at or above the MIC).	[93]
Bacteriostatic concentration	Lowest concentration stopping bacterial growth in broth, but cultured when broth is plated onto agar.	[95]
Bactericidal concentration	Lowest concentration stopping bacterial growth in broth; not cultured when broth is plated onto agar.	[95]

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minimally processed vegetables and packaged fruits. Food Qual. Prefer. 2004, 15, 259–270.

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