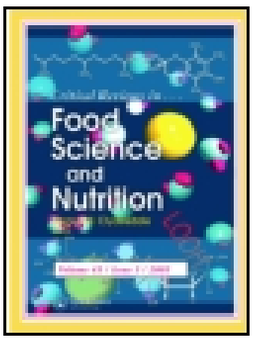

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Citrus essential oils: Extraction, authentication and application in food preservation

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ABSTRACT

Citrus EOs is an economic, eco-friendly and natural alternatives to chemical preservatives and other synthetic antioxidants, such as sodium nitrites, nitrates or benzoates, commonly utilized in food preservation. Citrus based EOs is obtained mainly from the peels of citrus fruits which are largely discarded as wastes and cause environmental problems. The extraction of citrus oils from the waste peels not only saves environment but can be used in various applications including food preservation. The present article presents elaborated viewpoints on the nature and chemical composition of different EOs present in main citrus varieties widely grown across the globe; extraction, characterization and authentication techniques/methods of the citrus EOs; and reviews the recent advances in the application of citrus EOs for the preservation of fruits, vegetables, meat, fish and processed food stuffs. The probable reaction mechanism of the EOs based thin films formation with biodegradable polymers is presented. Other formulation, *viz.*, EOs microencapsulation incorporating biodegradable polymers, nanoemulsion coatings, spray applications and antibacterial action mechanism of the active compounds present in the EOs have been elaborated. Extensive research is required on overcoming the challenges regarding allergies and obtaining safer dosage limits. Shift towards greener technologies indicate optimistic future towards safer utilization of citrus based EOs in food preservation.

KEYWORDS

Citrus essential oils; limonene; nanoemulsion; extraction and authentication of essential oils; antimicrobial action

1. Introduction

Essential oils (EOs) are aromatic compounds found in great quantities in oil sacs or oil glands present at different depths in the fruit peel, mainly flavedo part and cuticles. These are soluble in alcohol, ether, and natural oils, but insoluble in water. Citrus EO is a complex mixture of approximately 400 compounds and their content as well as composition depends on (a) species, variety and cultivar, (b) cultivation, (c) extraction and (d) separation methods (Nannapaneni, *et al.*, 2009). Citrus EOs contains 85 to 99% volatile and 1 to 15% non-volatile components (K Fisher and Phillips, 2008). The active compounds in EOs arehighly volatile and labile to oxygen, heat, or light (Muriel-Galet, *et al.*, 2015).The volatile constituents are a mixture of monoterpene (such as limonene) and sesquiterpene hydrocarbons and their oxygenated derivatives, including aldehydes, ketones, acids, alcohols and esters (Flamini, Tebano, and Cioni, 2007). The volatile compounds are further categorized as alcohols, ethers, aldehydes, ketones, esters, amines, amides, phenols, heterocycles, and mainly the terpenes. The non-volatile fraction includes long chain hydrocarbons, fatty acids, sterols, carotenoids, and oxygenated heterocyclic compounds. EOs formation occurs by mainly two distinct biosynthetic pathways, viz., (a) Phenylpropanoids and (b) Mevalonate pathway. Biogenetically, formation of terpenoids and phenylpropanoids take place via different primary metabolic precursors. One of the biosynthesis routes for terpenoid formation is shown in figure 1. The pathways involved in terpenoidsare (a) mevalonate and (b) mevalonate-independent pathway. On the other hand, phenylpropanoids originate from shikimate pathway (Dewick, 2002). Terpenoid derivatives are created from intermediate acetates, viz., malonic acid and aromatic compounds derived from shikimic acid and phenylpropanoid pathways. Terpenes and their oxygenated derivatives (Terpenoids) are the most important categories of EOs. Depending on the number of isoprene units in the structure, terpenes are classified into hemiterepens (C5), menoterpenes (C10), sesquiterpenes (C15), diterpenes (C20), sesterterpenes and, (C25) triterpenes (C30), tetra-terpenes (C40) and poly-terpenes $(C5)_n$(where n is greater than 8).

Essential oils are widely usedin cosmetics, perfumery, toiletries and other personal hygiene products. It is used in aromatherapy including massage, inhalations, and baths since ancient times. It acts as a masking agent towards unpleasant odour in textile, plastics and paint industries. It is also very important in gradient in pharmaceutical formulations (Lawless, 2002). Recently, EOs as natural antimicrobial agents have received great attention in food and packaging industries due to increasing consumers' concerns and demand for safety of food stuffs (Calo, Rivera, Crandall, O'Bryan, and Ricke, 2015).

2. Main components of citrus essential oils

The compounds present in citrus oil are grouped into five major classes, viz., hydrocarbon monoterpenes, oxygenated monoterpenes, hydrocarbon sesquiterpenes, oxygenated sesquiterpenes,

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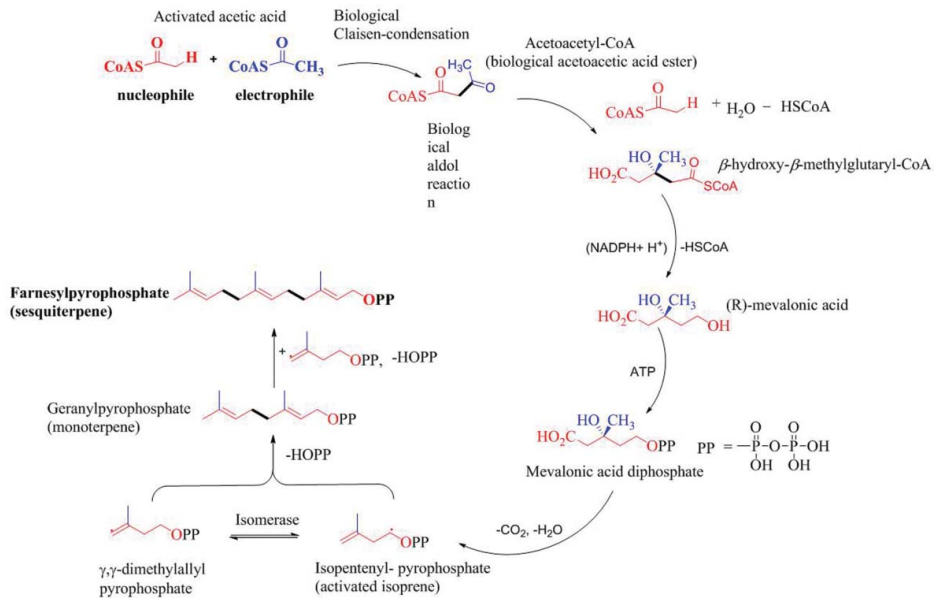

Figure 1. Biosynthesis pathways of monoterpenes and sesquiterpenes through mevalonate pathway.

etc. The approximate chemical compositions of these five major classes are shown in figure 1a (Supplementary). Limonene is the major chemical component of citrus Eos; amount ranging from 32 to 98% (Svoboda and Greenaway, 2003). It is a single loop monoterpene (Briitmaier, 2006). The distribution of limonene in various citrus varieties is shown in figure 1b (Supplementary). Apart from limonene, the distribution of other chemical constituents including the minor components in four major citrus varieties, viz., grapefruit, mandarin, lemon and sweet orange are shown in figure 1c-f (Supplementary).

The most important groups of compounds present in citrus essential oils are aliphatic aldehydes and oxygen-containing mono- and sesquiterpenes. So far, thousands of important compounds belonging to the terpenes family have been identified as functionalized derivatives of alcohols, (geraniol, α-bisabolol); ketones (menthone, *p*-vetivone); aldehydes (citronellal, sinensal); esters (γ-tepinyl acetate, cedryl acetate); and phenols (thymol) (Modzelewska 2005). The specific aroma of these compounds is characterized especially by the length of unsaturated straight chain aldehydes C_8 –C_{14}, acetate, nootkatone and α-selinenone. Song *et al.* 2000 showed that the main odor-active components of peel oil of *Citrus aurantium* L. *cyathifera* Y. Tanaka are geraniol, octanal, linalyl acetate and (*E*)-limonene oxide. Tamura *et al.* 1993 characterized the aroma quality of the kabusu (*Citrus aurantium* f. kabusu) using GC and reported a list of general aroma producing compounds, namely octanal, nonanal, decanal, undecanal, dodecanal, linalool and perillaldehyde. Furthermore, bitter orange contains geraniol, linalyl acetate, geranyl acetate and linalool as main aroma producing compounds.

Distillation is the most widely used methods for the extraction of aroma producing compounds. Other methods used for the extractions are fractionated distillation, steam distillation at atmospheric pressure or vacuum distillation. Low temperature distillation or a petroleum ether extraction can remove all the odour and flavour producing compounds from the citrus juice, i.e., unsaturated hydrocarbons and alcohols. Different extraction methods, as well as analysis result in different percentage composition of aroma producing compounds in the essential oils. Figure 2 represents some of the major water soluble and water insoluble aroma producing compounds present in citrus EOs.

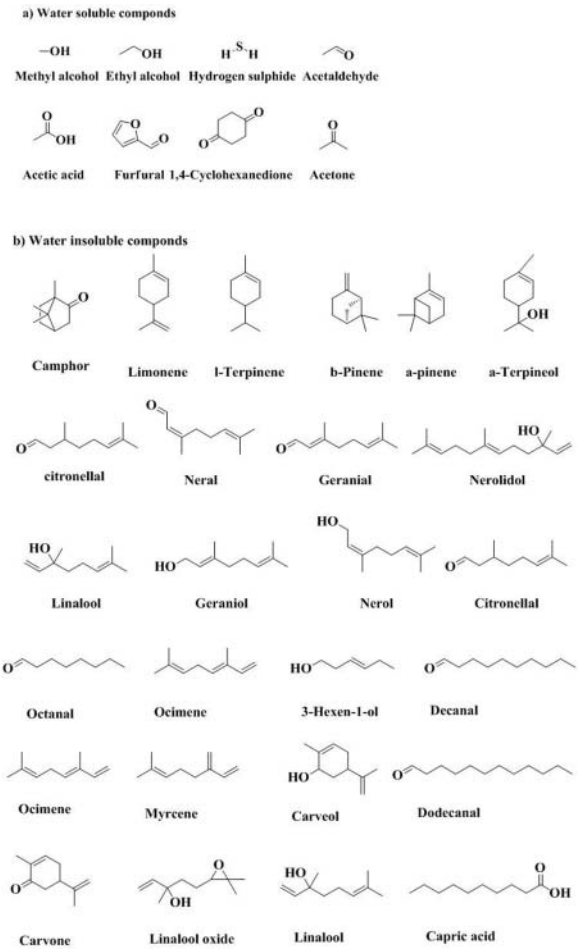

Figure 2. Major water soluble and water insoluble aroma producing compounds present in citrus EOs.

Fig. 1(a). Approximate composition of different chemical constituents in five major classes of citrus EOs (Bausbia, Vian, Ferhat, Meklati, and Chemat, 2009); (b) Distribution of limonene in different citrus varieties (Dugo and Mondello, 2010); (c-f) Distribution of different constituents other than limonene and minor components (Arce and Soto, 2008)(Supplementary).

3. Extraction, characterization and authentication of citrus EOs

EOsis mainly present at different depths in the peel and cuticles of the fruit. The amount of major classes of EOs varies in fresh and dried citrus peels as shown in figure 3a. EOs is released when oil sacs are crushed during juice extraction. The major component of the EOsis *d*-limonene, which is used as a green

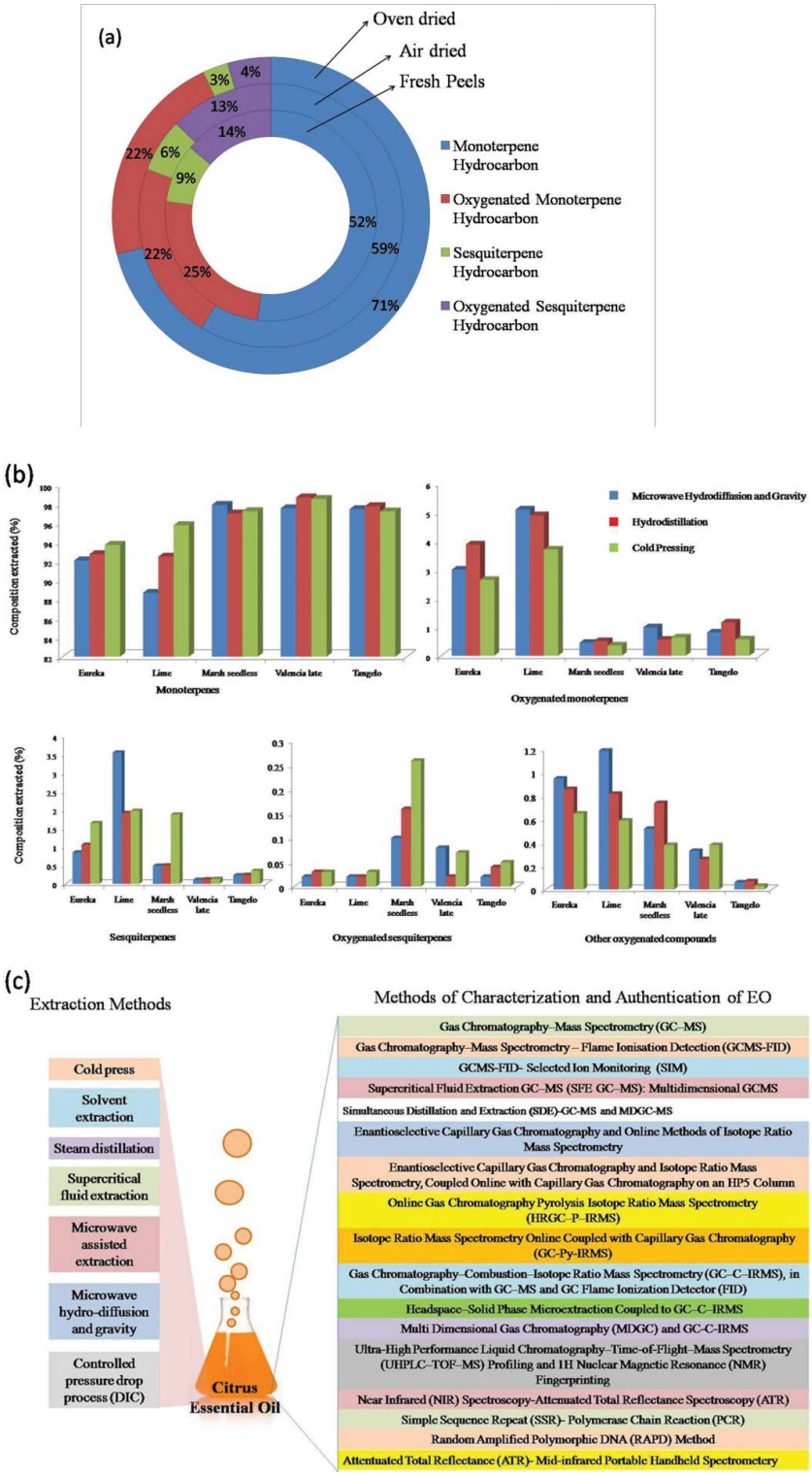

Figure 3. (a) The amount of major classes of EOs varies in fresh and dried citrus peels(G.M. Kamal, F. Anwar, A.I. Hussain, N. Sarri, and M.Y. Ashraf, 2011; Yang and Kang, 2013) (b).Effect of different extraction techniques on the yields and the composition of the major classes of citrus EOs in different citrus varieties (Bausbia, *et al.*, 2009); (c) Summary of extraction, characterization and authentication techniques for citrus EOs.

solvent for the determination of fats and oils and considered safer than petroleum solvents (Ueno, Tanaka, Hosino, Sasaki, and Goto, 2008). EOsis commonly extracted by cold pressing. Traditional and semi-industrial processes include cold pressing and distillation. In cold pressing, the peel and cuticle oils are removed mechanically. The yield is a watery emulsion, which is then centrifuged to recover the EOs (Ferhat, Meklati, and Chemat, 2007). The EOs are best removed immediately after the generation of wastes as bacteria present in the waste bring in compositional changes, i.e., conversion of *d*-limonene into α-terpineol (Murdock and Allen, 1960). A relatively simpler extraction method is stream stripping and distillation method. This is an effective method for removing oil components from oil-milled sludge. Distillation is sometimes considered as an economical way to recover the oils (with better yield of 0.21%) compared to cold pressing (yield-0.05%) (Ferhat, *et al.*, 2007). During distillation, the citrus peels are exposed to boiling water or steam. The oils are released into water and then collected through distillation. The steam and EO vapours are condensed and collected in specialized vessel called "Florentine flask" (Guenther, 1974). Compared to conventional steam distillation (SD), the modern distillation technique is considered effective as it saves time and energy. However, the EOs obtained by distillation deteriorate easily and develop off-flavours because of the instability of the terpene hydrocarbons, particularly *d*-limonene (Yamauchi and Sato, 1990).Microwave Steam Distillation (MSD) is a highly efficient method which not only accelerates the extraction process many folds, but also enables recovery of Eos without causing any changes in the oil composition. The effectiveness of MSD over SD is attributed to the more rapid rupture of the cell wall under strong microwaves and release of the cell cytoplasm. Effect of different extraction techniques on the yields and the composition of the major classes of citrus Eos in different citrus varieties are shown in figure3b (Bausbia, *et al.*, 2009). The main extraction, characterization and authentication methods employed for citrus EOs have been listed in figure 3c and elaborated in table 1.

4. Applications of EOs

EOs has been extensively used in many applications since long time. In recent years, it has been explored for many diversified applications in insect/pest control for stored food products, cereals and grains; processed food preservation; antimicrobial packaging for food products; edible thin films and antimicrobial packaging films; nanoemulsions for preservation of vegetables and fruits; ingredients in soda/citrus concentrates; flavouring agent in carbonated colas, soft drinks; meat, fish and seafood preservation. The recent progress in this direction has been elaborated in the following sections.

4.1. Role as preservatives: Thin films and packaging, edible films

Nowadays, eco-friendly materials from natural polymers are gaining attention for the development of biodegradable packaging materials to reduce environmental pollution (Petersen, *et al.*, 1999). Packaging is widely used for the protection of food quality, thereby ensuring hygiene and extended shelf-life (Coma, 2008). Development of biodegradable packaging materials is an effective alternative to synthetic packaging material obtained from petrochemical products. The latter are non-biodegradable and impart negative impact on the environment. Moreover, biodegradable materials are eco-friendly, nontoxic and shown to have many desirable physico-chemical characteristics over synthetic counterparts. Proteins, lipids and polysaccharides are generally employed for the production of bio-based packaging materials for food industry (Bao, Xu, and Wang, 2009).Among these biopolymers, proteins have been extensively used due to their abundance and good film-formability. Proteins are heteropolymers containing a variety of amino acids, which can undergo a wide range of interactions and chemical reactions (Stevens, 1999). The protein-based films have excellent barrier properties for gases like oxygen and carbon dioxide, and volatile compounds (Limpan, Prodpran, Benjakul, and Prasarpran, 2010). EOs are categorised as GRAS (generally recognised as safe) by U.S. Food and Drug Administration (Persico, *et al.*, 2009). Citrus Eos appear as interesting natural compounds with great potential use in foodstuffs preservation. The incorporation of EOs into gelatin films offers antimicrobial activity as well as improves the physicochemical properties.figure 4 (i) represent schematic diagrams for the formation of chitosan/gelatin based polymeric materials incorporating citrus EOs.

Chitosan-EOs composite edible coatings have demonstrated to be effectiveat extending the shelf-life of some fruits and vegetables, such as sweet pepper (Xing, *et al.*, 2011) and table grapes (Sánchez-González, *et al.*, 2011b). The antifungal effect of bioactive coatings prepared with modified chitosan and limonene (and/or EO) on the fungal decay of cold-stored strawberries has been evaluated by (Vu, Hollingsworth, Leroux, Salmieri, and Lacroix, 2011). Antimicrobial activity of chitosan–lemon EOs composite coatings on the postharvest quality of cold-stored strawberry has been reported (A. L. Perdones, A. Sánchez-González, A. Chiralt, and M. Vargas, 2012).

4.2. Description of chitosan/gelatin-EO polymer blends: Reaction mechanism

Chitosan is a biodegradable, biocompatible, muco-adhesive and nontoxic natural polymer obtained by alkaline deacetylation of chitin. The latter is a component of the protective cuticles of crabs, shrimps and other crustaceans and fungal mycelia (Sinha, *et al.*, 2004). It is soluble in acid conditions, but it has poor solubility above pH 6.5. It is a linear polysaccharide molecule composed of repeating units of β-(1–4)-2-amino-2-deoxy-D-glucopyranose (*D*-glucosamine), and has free amino groups on its polymeric chains. The amino groups protonate to give chitosan its cationic character which determines its main properties, e.g., controlled drug release for anionic substances, muco-adhesive properties, in-situ gelling properties, etc. (Kumbar, Kulkarni, and Aminabhavi, 2002). It has great potential for pharmaceutical application as drug and therapeutic enzyme carriers (Smelcerović, Knežević-Jugović, and Petronijević, 2008; Žuža, Obradović, and Knežević-Jugović, 2011). The physical and chemical properties of chitosan make it an appropriate material for the encapsulation of EOs. The latter adds antimicrobial properties to the resultant material. Chitosan

Table 1. Techniques of extraction, characterization and authentication of citrus EOs.

Methods /Techniques	Reports	Remarks	References
		Extraction	
(i) Hydrodistillation (HD)	HD- 500 g peel in 3L deionised water for 3 h; 3 kWhCP: 1 kg, Mechanical pressMHG: 500 g; 500 W; 15 min; 0.2 kWh	Eureka lemon (*Citrus limon* L.), Villa Franca (*Citrus limon* L.), Lime(*Citrus aurentifolia* Chrism. Swing), Marsh Seedless (*Citrus paradisi* L.), Tarocco(*Citrus sinensis* L.), Valencia late (*Citrus sinensis* L.) Washington Navel (*Citrus sinensis* L.), Tangelo seminole (*Citrus paradise* Macf.)	Bausbia, *et al.*, 2009
(ii) Cold Pressing (CP) (iii) Microwave Hydrodiffusion and Gravity (MHG)		MHG is simplified working mechanism, faster technique and requires lesser energy; Yields high purity final products; Post treatment of the waste water.	
(i) Hydrodistillation (HD)	Sweet orange peels(*Citrus sinensis*)	(i) HD: 200 g dried peel in 2 litres dist. H_2O; 4 h; Yield = 1.97 mg/g dry matter	(Allaf, Tamao, Ruiz, and Chemat, 2013)
(ii) Instant Controlled Pressure Drop Technique (DIC)		(ii) DIC: Saturated steam;5 kPa to 1 MPa; 2 min; Yield = 16.57 mg/g dry matter	
(i) Cold pressing (CP)	*Citrus limon* L.	(i) CP: 1 kg of whole lemon utilized for cold pressing for 1 h followed by centrifugation, dried over anhydrous sodium sulphate: Electricity consumed- 1kWh; Yield: 0.05%; CO_2rejected: 800 g	(Ferhat, *et al.*, 2007)
(ii) Hydrodistillation (HD)		(ii) HD: 200 g peel in 2 litres H_2O distilled for 3 h; Electricity consumed: 4.33kWh; Yield: 0.21%; CO_2rejected: 3464 g	
(iii) Microwave dry distillation (MD)		(iii) MD: 200 g peel, microwave irradiation power 200 W for 30 min; Electricity consumed- 0.25 kWh; Yield: 0.24%; CO_2rejected: 200 g Microwave dry distillation is energy and time saving, requires no solvent and the extracted EOs has higher amounts of oxygenated compounds	
(i) Microwave Steam Distillation (MSD)	Orange (*Citrus sinensis*),	EOs; limonene	(Shakir and Salih, 2015)
(ii) Steam Distillation (SD)	Lemon (*Citrus limon*), Mandarin (*Citrus reticulata*)	DI Water (i) Microwave power 135 W, 35 min (ii) Distillation for 45 min Yield = SD/ MSD Orange: 83.22%/ 80.97%; Mandarin: 83.03%/ 84.39%: Lemon: 65.29%/ 59.16%	
	Orange peel (*Citrus aurentium* L.)	DI water (i) Microwave oven working at 800 W, 2.45 GHz, 140 min (ii) SD: Steam Distillation for 7 h (i) MSD: Extracted essential oil contain 18 detectable components (ii) SD: Extracted essential oil contain 7 detectable components	(Kusuma, Putra, and Mahfud, 2016)
		Characterization and authentication	
Gas Chromatography–Mass Spectrometry (GC–MS)	Chemical composition and principal constituents of the citrus essential oil	Identification of compounds, *viz*., oxygenated sesquiterpenes, octane, monoterpenes, and oxygenated terpenes: Volatile Flavour Compounds in Lemon Peel Extract: α-thujene, α-pinene, camphene, sabinene, (β-pinene, myrcene, *p*-cymene, limonene, γ-terpinene, linalool, nonanal, *trans*-1 imonene-1,2-epoxide, *cis*-l imonene-1,2-epoxide, citronellal, terpinen-4-ol, α-terpineol, decanal, neral, geranial, neryl acetate, geranyl acetate, caryophyllene, *trans*-α-bergamotene, β-bisabolen	(Zhou, Hai-Yan, Tun-Hai, and Tian, 2010)
Multidimensional Gas Chromatography – Flame Ionization Detection (MDGC-GC-FID)	Determination of Enantiomeric composition in Lemon peel	MDGC-GC-FID:	(Blanch and Graeme, 1998)
Multidimensional Gas Chromatography – Flame Ionization Detection – Selected Ion Monitoring (MDGC-GC-MS-SIM)	Determination of enantiomeric excess of *R*-(+)-limonene in Lemon peel oil (97.1 and 97.4%)	MDGC-GC-MS-SIM:Quantitative determination of enantiomeric excess of *cis*-(+)- and *trans*-(+)-limonene-1,2-epoxide (88.0 and 91.9%)	
Enantioselective Gas*Chromatography (esGC) and* Multidimensional Gas Chromatography (MDGC)	Enantiomeric composition and distribution in different citrus EOs	Citrus varieties: *C. deliciosa* Ten., *C. limon* (L.) Burm.,*C. bergamia, C. aurantifolia* (Christm.) Swing.,*C. latifolia* Tan., *C. sinensis* (L.) Osbeck, and *C. aurantium* L. esGC: α-thujene, α-pinene, camphene, β-pinene, sabinene, α-phellandrene, β-phellandrene, limonene, linalool, camphor, citronellal, linalyl acetate, terpinen-4-ol, α-terpineol	(Bonaccorsil, *et al.*, 2011)

(*Continued on next page*)

Table 1. (*Continued*)

Methods /Techniques	Reports	Remarks	References
		MDGC: separation of the enantiomers of camphor and citronellal, determine the enantiomeric distribution of camphene, α- and β-phellandrene:	
Simultaneous Distillation and Extraction (SDE)-GC-MS and MDGC-MS	Analysis of volatile flavour compounds of yuzu, lemon and lime; Enantiomeric ratios of chiral and authentication of quality	*Citrus junos* Sieb. ex Tanaka (yuzu), *Citrus limon* BURM. f. (lemon) and *Citrus aurantifolia* Christm. Swingle (lime)	(Hong, *et al.*, 2017)
		volatile compounds were identified with limonene, γ-terpinene and linaloolStereochemical analysis (Yuju: Lemon: Lime) 1*S*,4*R*-(-) camphene (94.74, 98.67, 98.82), *R*-(+)-limonene (90.53, 92.97, 99.85) and *S*-(+)-β-phellandrene (98.69, 97.15, 92.13) in oil samples *R*-(+)-sabinene (88.08) in C. junos; and *S*-(-)-sabinene (81.99, 79.74) in *C. limon* and *C. aurantifolia*, respectively	
Supercritical Fluid Extraction GC–MS (SFE GC–MS) Involving Use of Multidimensional GC	Extraction and determination of composition; separation of enantiomers	qualitative and quantitative determination of the compositions of thepredominant volatile aroma constituents in EOs and separation of the target enantiomeric compounds	(Flores, Blanch, Castillo, and Herraiz, 2005)
Enantioselective capillary gas chromatography (enantio-cGC) and comparative isotope ratio mass spectrometry (IRMS), coupled on-line with conventional GC	Authenticity control of flavours and EOs	Enantiomeric Ratio (*S, R*) of α-Pinene, β- Pinene and Limonene in citrus EOs	(Mosandl, 2004)
Enantioselective Capillary Gas Chromatography and Isotope Ratio Mass Spectrometry, Coupled Online with Capillary Gas Chromatography on an HP5 Column	Analysisand authenticity investigations of lemon (*Citrus limon*) EOs	Isotope data (δ^{13}C PDB) and δ2H (V-SMOW) for citral (neral + geranial) and citronellal from isotope ratio mass spectrometry online coupled with capillary gas chromatography (GC-Py-IRMS) and chiral data for citronellal in the EOs; yield information on the origin of EOs and disclose adulterants possibly with EOs from other sources	(Nhu-Trang, Casabianca, and Grenier-Loustalot, 2006a)
Online Gas Chromatography Pyrolysis Isotope Ratio Mass Spectrometry (HRGC–P–IRMS)	Authentication of the Flavor Compounds	Decanal, Linalool, Linalyl Acetate, *E*-2-Hexenal, and *E*-2-Hexenol in EOs	(Hör, Ruff, Weckerle, König, and Schreier, 2001)
Isotope Ratio Mass Spectrometry Online Coupled with capillary Gas Chromatography (GC-Py-IRMS)	Authentication and genuineness of mandarin *Citrus reticulata* Blanco EOs	$\delta^{13}C_{PDE}$ values of characteristic flavour components of mandarin EOs: α-sinensal, limonene, γ-terpinene, α-thujene, β-pinene/ sabinene myrcene, terpinolene, methyl N-methylanthranilate, linalool and octanal in authentic and commercial mandarin oils	(Faulhaber, Hener, and Mosandl, 1997)
Enantioselective Gas Chromatography in Flavor and Fragrance Analysis	Authentication of fragrance compounds	Enantiomer separation of linalool and linalyl acetate in natural bergamot and adulterated bergamot oil	(König and Hochmuth, 2004)
Headspace–Solid Phase Microextraction Coupled to GC–C–IRMS	Authentication of some citrus EOs	authenticity and enantiomeric distribution of selected chiral volatiles of Italian liqueurs, bergamot, lemon, and mandarin EOs	(Schipilliti, Bonaccorsi, Cotrone, Dugo, and Mondello, 2013)
Multi Dimensional Gas Chromatography (MDGC) and GC-C-IRMS	Authentication of Neroli) and Lime Oils and determine the level of adulteration	Egyptian Bitter Orange Flower Oil (or Neroli): GC–FID and GC–MS–linear retention index were employed to investigate the enantiomeric distribution of 12 volatile compounds; GC-C-IRMS was employed to ascertain the δ^{13}C(VPDB) values of some alcohols, esters, and monoterpene and sesquiterpene hydrocarbons	(Bonaccorsi, *et al.*, 2012)
		Lime oils (*Citrus aurantifolia* Swingle and *Citrus latifolia* Tanaka): MDGC to study the enantiomeric distribution of camphene, limonene, linalool, α-phellandrene, β-phellandrene, β-pinene, terpinen-4-ol, α-terpineol, sabinene, and α-thujene; GC–C–IRMS was used to ascertain the isotopic ratios of β-caryophyllene, geranial, germacrene B, limonene, neral, α-pinene, β-pinene, α-terpineol, and trans-α-bergamotene.	
Ultra-High Performance Liquid Chromatography–Time-of-Flight–Mass Spectrometry (UHPLC–TOF–MS) Profiling and 1H Nuclear Magnetic Resonance (NMR)	Fingerprinting for Lemon Oil	to reveal variances in metabolites in lemon oil samples that find application in the flavour and fragrance industry, viz., Flavonoids, furocoumarins, fatty acids, terpenoids, citropten and bergamottin	(Marti, *et al.*, 2014)
Near-Infrared Spectroscopy	Quantification of citral in lemongrass and lemon oils	rapid, simple, non-destructive and accurate determination of the citral (Citral (3,7-dimethyl-2,6-octadienal)) content of lemon oils	(Wilson, Ivanova, Watt, and Moffat, 2002)

(*Continued on next page*)

Table 1. (*Continued*)

Methods /Techniques	Reports	Remarks	References
ATR (attenuated total reflectance)/FT-IR as well as NIR-FT Raman spectroscopy	Determination of composition of citrus oils	Quantitative distinction between different oil types on the basis of characteristic compounds like limonene, myrcene, α-pinene, β-pinene, sabinene, γ-terpinene, aldehydes and carotenoids in grapefruit, sweet orange, mandarin, lemon, bitter orange, lime (distilled and pressed)	(Schulz, Schrader, Quilitzsch, and Steuer, 2002)
Simple Sequence Repeats (SSR) and single nucleotide polymorphisms	Genetic analysisof of 47 citrons (*Citrus medica*L.)	An array of simple sequence repeat markers is used to accurately attribute a citrus essential oil to a certain citrus cultivar. This is important in assigning certification labels for protected designation of origin (PDO) and protected geographical indication (PGI) for assuring the authenticity of food products. Furthermore it furnishes valuable information to understand the genetic diversity and relationships between different citrus varieties within the species	(Ramadugu, *et al.*, 2015)
SSR; Inter-Simple Sequence Repeat (ISSR) markers	Identification of closely related citrus cultivars	Different cultivars or citrus species and hybrids have characteristically different fingerprint patterns or genetic profiles originating from selection of mutants. ISSR-markers generated by 22 primers were tested for distinguishing among samples from 94 trees of 68 citrus cultivars; Determination of parentage of citrus hybrids	(Shimizu, *et al.*, 2016)
Inter-Simple Sequence Repeat Polymerase Chain Reaction (ISSR-PCR)	Identification characterization and screening technique for*Citrus*somatic hybrids	Rapid method of characterizing allotetraploid somatic hybrids of mandarins with high reproducibility, technical simplicity and the requirement for low quantities of DNA, viz., between Redblush and Duncan grapefruit (*Citrusparadisi*Macfadyen) with Avana, Tardivo di Ciaculli mandarin (*C. deliciosa*Tenore) and Fortune mandarin (*C. reticulata*Blanco)	(Scarano, Abbate, Ferrante, Lucretti, and Tusa, 2002)
Isotope data (δ13C(Pee Dee Belemnite (PDB)) and δ2H(Vienna standard mean ocean water (V-SMOW))			

biopolymer based microparticles can be prepared by different techniques: (a) cross-linking with different cross-linking agents (glutaraldehyde, formaldehyde or genipin), (b) spray drying, (c) ionotropic gelation, (d) simple and complex coacervation (Al-Helw, Al-Angary, Mahrous, and Al-Dardari, 1998). Among all these techniques, the emulsion cross-linking method is a method of choice. Other methods may involve elevated temperature, high pressure, etc. The cross-linking method is based on the reaction between the amino group of chitosan and the aldehyde group of the cross-linking agent (Meng, Sturgis, and Youan, 2011).Chitosan films possess relatively high permeability for water vapours(Vargas, Albors, Chiralt, and González-Martínez, 2009),which is a major drawback since adequate barrier properties are desirable to avoid dehydration of fruits during storage. The addition of lipid materials or EOs to chitosan based films can improve their moisture barrier properties.

4.2.1. Chitosan film formation

Preparation of chitosan microparticles emulsion cross-linking method. Preparation of chitosan microparticles involving emulsion cross-linking has been carried out by (A. L. Perdones, *et al.*, 2012) via two different routes, viz., (a) varying the EO concentration, and (b) varying the concentration of cross-linking agent (gluteraldehyde). In the first route, the chitosan, along with lactic acid, and EO is first homogenised in liquid paraffin and then added a calculated amount of gluteraldehyde. Soon after the addition of gluteraldehyde, the microparticles are formed and collected post centrifugation as pellets. In the second route, chitosan is dispersed in glacial acetic along with EO and subjected to homogenisation followed by microfluidization. The reaction results into chitosan-EO film dispersed in the solvent media. The film is later separated and washed to obtain the desired material.

Multiple mechanisms may be involved in the formation of chitosan based thin films containing EOs. A few hypothetical mechanisms are shown in figure 4 (ii) a-c. In the first mechanism, the free amino group present on chitosan attacks on the aldehyde group of geranial (active compound present in citrus EO) and sits as a flanked side chain on the polymer. Glutaraldehyde molecules may undergo self-polymerization and the resultant polymer participates in the cross-linking between chitosan polymeric chains as shown in figure 4 (ii) a. In the second hypothesis, the free glutaraldehyde molecule reacts with free -NH_2 group of the chitosan molecule thereby joining the polymeric chitosan chains by cross-linking. The geranial molecules are supposed to block the free –NH_2 groups present on the chitosan molecule as shown in figure 4(ii) b. In third hypothesis, all the –NH_2 groups might be attacked by the glutaraldehyde molecules giving rise to both, cross-linking between chitosan polymeric chains as well as flanked side chains (figure 4(ii)c).

Gelatin is another versatile biomaterial obtained by the controlled hydrolysis of the fibrous insoluble collagen present in the bones and skin generated as waste during animal slaughtering and fish processing (Ahmad, Mehraj, Benjakul, Ovissipour, and Prodpran, 2011). It possesses excellent film formability (Hoque, Sazedul, Benjakul, and Prodpran, 2010). Gelatin-based films for coating or packaging can secure the quality of foods during storage, due to its barrier properties against gases, like

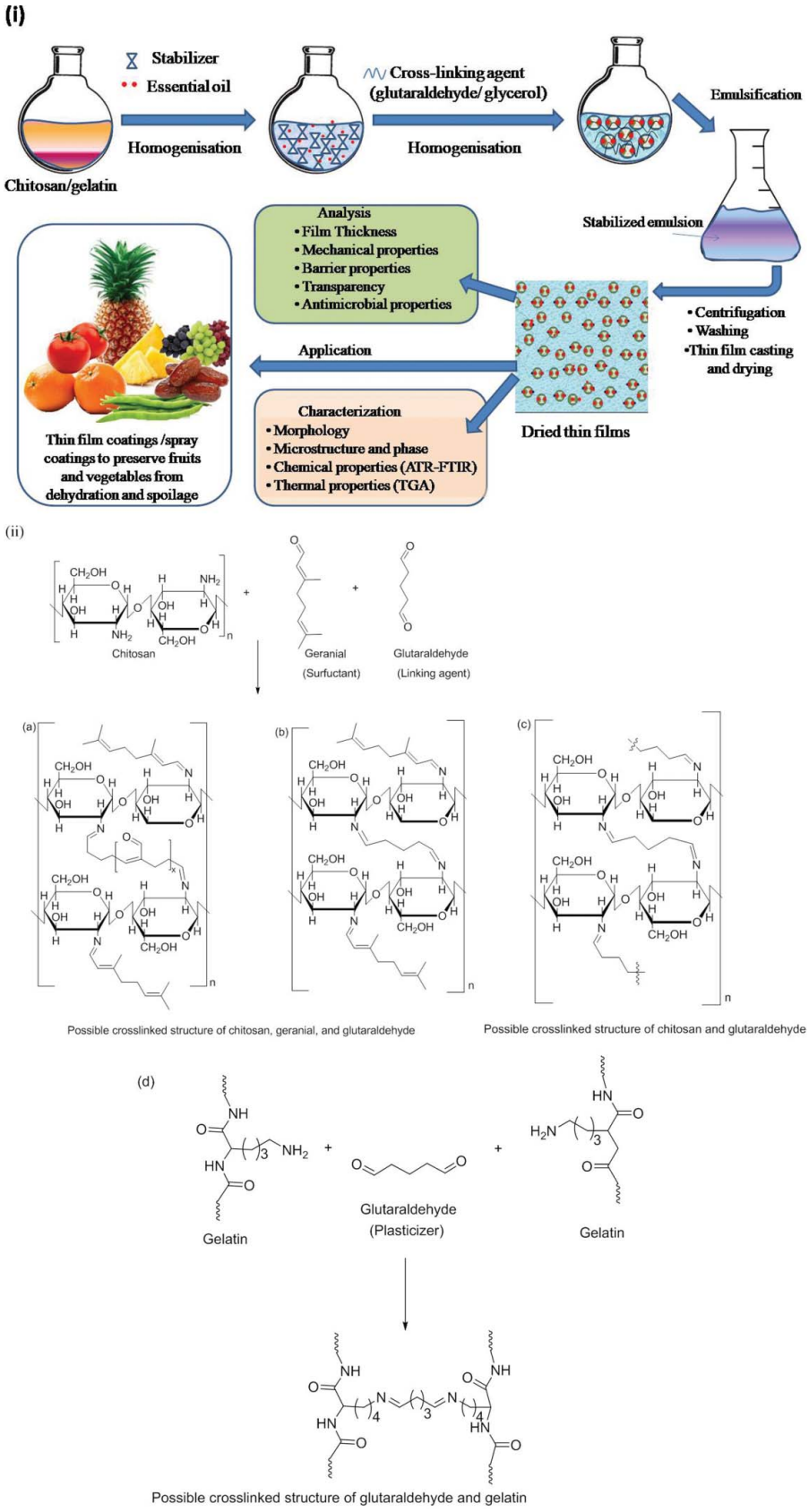

Figure 4. (i) Schematic diagrams illustrating the formation of chitosan/gelatin based polymeric materials incorporating citrus EOs. (ii) Possible cross-linked structures of (a-b) chitosan, geranial and gluteraldehyde, (c) chitosan and gluteraldehyde and (d) gluteraldehyde and gelatin, link through imine formation.

oxygen, moisture, and protection against light and lipid oxidation (Jongjareonrak, Benjakul, Visessanguan, and Tanaka, 2011). Moreover, gelatin films exhibit good mechanical properties, antimicrobial activity as well as antioxidant properties which are further influenced by the addition of active substances (Pires, *et al.*, 2009). Addition of citrus EOs to the film as antimicrobial agent has been reported by (Pereda, Ponce, Marcovich, Ruseckaite, and Martucci, 2011).

4.2.2. Gelatin film formation

To a gelatin solution in distilled water (3% concentration) is added EO and glycerol (or gluteraldehyde) and homogenised to obtain a stabilised emulsion. Glycerol acts as a plasticizer. The ratio of plasticizer and EO (antimicrobial agent) can be varied to obtain the required properties in the final product. To the stabilised emulsion with 25% of protein concentration, tween-20 is added as an emulsifier and the resultant mixture is homogenised to obtain the desired consistency for film casting followed by drying. The dried film samples are analysed for film thickness, mechanical properties, water vapour permeability, film solubility, colour, light transmission and transparency, electrophoretic analysis and antimicrobial properties. These are further characterized by Attenuated Total Reflectance-Fourier Transforms Infrared-spectroscopy (ATR-FTIR), Thermo-gravimetric analysis (TGA), Microstructure, etc.Hypothetical mechanism of gelatin thin film formation is shown in figure 4(ii) d. In this mechanism, the gelatin polymeric molecules are cross-linked by glutaraldehyde through imine formation by a reaction between free amino part of gelatin and aldehyde group of glutaraldehyde.

5. Citrus EO based microencapsulation and nanoemulsion

EOs in edible food packaging materials is an interesting and innovative measures in food industries. These are generally made of proteins, carbohydrates or polysaccharides and lipids. The main challenge is their hydrophilic nature which can be improved by the addition of sodium caesinate. The latter is obtained from acid precipitation of cow milk protein 'caesin'. Addition of EOs is supposed to enhance the flavour and aroma to these edible films.

The phenolic compounds present in EOs not only protect the meat, fish and processed products from microbial spoilage, but also protect from lipid oxidative degradation. Furthermore, citrus EOs adds gustatory and aroma value to the food stuffs. The mechanism of anti-bacterial action of citrus EOs is explained in figure 5a. The active components (lypolytic moieties) present in citrus EOs disrupt the cell membrane structure by inhibiting H^+ ion-dependent movement of solutes across the cell membrane, consequently disturbing the pH environment inside the cell and leading to cell lysis and death. Meat, flesh, fish and their processed food stuffs perish because of lipid peroxidation. The conventional methods of preservation of these food stuffs include salting, refrigeration, radiation, addition of chemicals preservatives, etc. Irradiation treatments have disadvantages in terms of bringing change to the colour of the food and breakdown of the proteins and this require addition of artificial chemical preservatives, such as BHA (Butylhydroxyanisole), BHT (Butylated hydroxytoluene), sodium nitrites and nitrates. These may add undesirable components to the food, thereby risking its safety and raising health concerns. Factors affecting the freshness and shelf-life of fresh meat, fish and processed products are shown in figure 4b. The physic-chemical targets of food preservation, conventional methods of preservation and advantages of citrus EOs in preservation have also been summarized in figure 5b. The lipid peroxidation results in off-flavours to the meat which can be reduced by EOs.

5.1. Microencapsulation

Microencapsulation is packaging of active components of EOs in the form of tiny droplets of solids or liquids as a core. The packaging shell may be a continuous film or porous or perforated preferable made of biodegradable materials, such as chitosan, gelatine, alginate or poly(L-lactide). The droplet size ranges between 3–800 μm in diameter containing 10–90% of its core material supposed to remain within the capsule for a specific period of time and controlled release. Microencapsulation is achieved by two methods, viz., coacervation in aqueous phase, i.e., encapsulation of water –insoluble or hydrophobic core materials, and coacervation in organic phase, i.e., encapsulation of water soluble compounds. Encapsulation of EOs ensures the stability of EOs by prohibiting direct reaction between the active compounds and food proteins.

5.2. Nanoemulsification

EO based nanoemulsions are droplets of size ranging between 20–200 nm. These are basically created by three methods, namely low energy emulsification, high energy emulsification and combined emulsification. A typical emulsification has two different immiscible liquids dispersed in a continuous phase. The low energy methods include (a) Phase Inversion Temperature (PIT); (b) Emulsion Inversion Point (EIP) and (c) Spontaneous Emulsification method(Preedy, 2015).

5.2.1. Low energy nanoemulsification

The low energy methods are non-destructive, require high concentration of surfactants, and rely on the spontaneous formation of emulsion droplets by either changing its composition or the environment. PIT method is based on the changes in the solubility of the surfactants in the continuous phase with temperature. Surfactant's affinity towards water and oil changes with rise in temperature thereby change in the solubility. The surfactant is hydrophilic in nature at low temperatures which alters to hydrophobic upon increase in temperature. This alteration in the nature of surfactants is due to the dehydration of the polyoxyethylene chains present in non-ionic surfactants. The nanoemulsion formed by PIT method with a rapid cooling and heating the emulsion at an optimized hydrophilic-hydrophobic balance temperature are kinetically stable. In PIC method surfactant concentration is altered which ultimately alters the composition of the emulsion. Salt may be added to the emulsion which is stabilised by the anionic charges on the surfactant and the combination inverts an oil-water emulsion system to a water-oil emulsion. The latter can again invert into the oil-water emulsion upon dilution with water which reduces the ionic strength of the surfactant. In the EIP method, one

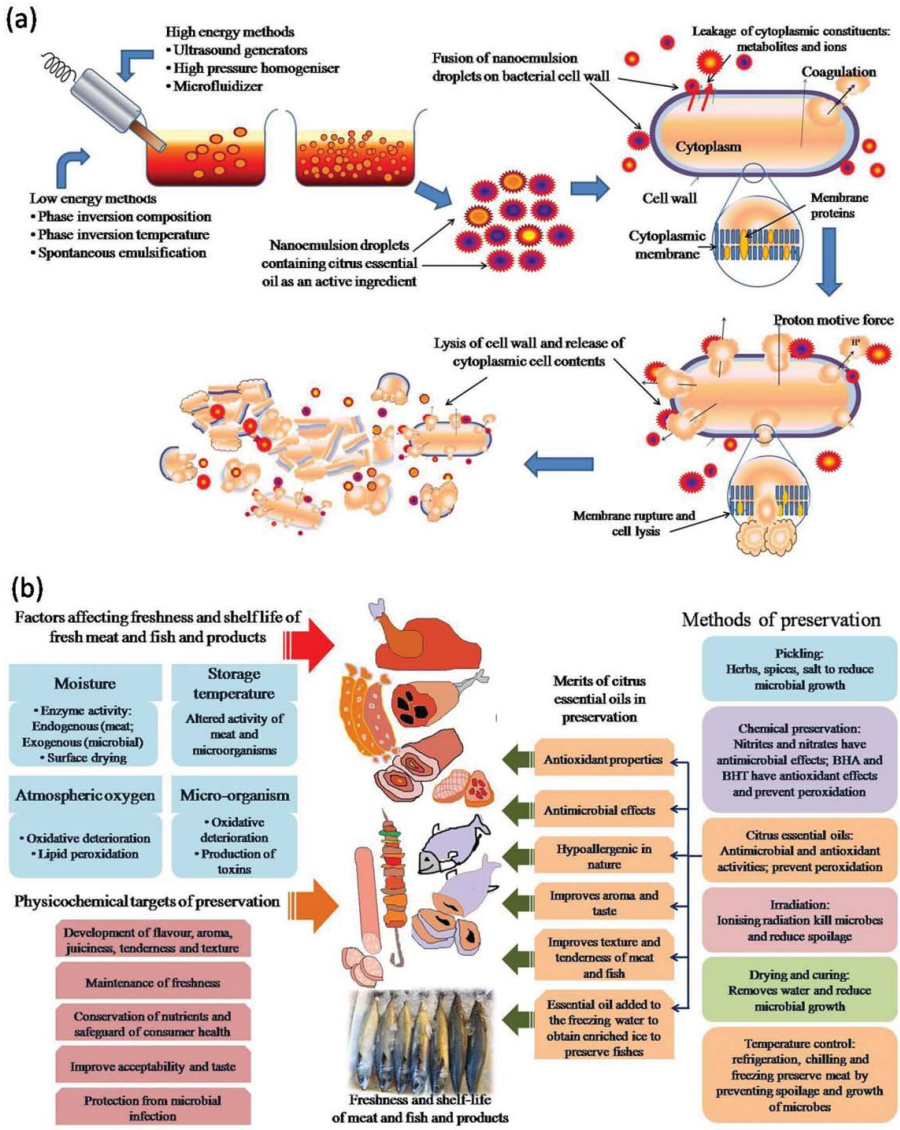

Figure 5. (a) Method of preparation and mechanism of antimicrobial action of citrus EOs based nanoemulsions, (b) Utilization of citrus EOs in preservation of meat, fish and processed food products.

type of emulsion system is inverted into another through a catastrophic pulverization of the bulk emulsion. The latter is brought upon by increasing the volume of the dispersed phase possessing high affinity towards the surfactant. Addition of another surfactant to the emulsion formation has been observed to provide a more stable nanoemulsion (Preedy, 2015).

5.2.2. High energy nanoemulsification

These methods utilize intense disruptive mechanical forces, e.g., microfluidization, high pressure homogenization, ultrasonication to pulverize large emulsion drops into tiny droplets. High pressure homogenization is a widely utilized technique popular in food industries. In this method, a coarse emulsion of appropriate composition is subjected to a very high pressure and propelled through a very restrictive valve orifice resulting into very fine submicron emulsion. Microfluidized technique achieves emulsification of desired EO-matrix composition via collision of flow streams of immiscible liquids moving out of the microchannels through narrow orifice. This method creates emulsion droplets of highly reduced size and very appropriate for the production of food grade emulsion. Ultrasonication technique utilizes high intensity sonic waves. The tip of the sonicator probe made of titanium alloy is placed into the homogenised mixture of EO and matrix/solvent and intense mechanical vibrations are generated. The latter creates cavitation, turbulence and interfacial waves. Nanoemulsion droplets possess larger surface area compared to the bulk or micron emulsion and have shown effective antimicrobial actions. To obtain kinetically stable nanoemulsions with desired consistency and properties, both, composition of the feed material as well as the synthesis methodology has to be optimised (Preedy, 2015). Citrus based EOs is a cost effective, environmentally-friendly and relatively a non-toxic approach, requiring less amount of surfactant with potentially beneficial advantages for food and beverage industries. The recent reports on the applications of citrus EOs, EO based microemulsion and thin film composites with chitosan or gelatin are listed and summarized in Table 2.

Table 2. Application of citrus EOs for food safety, flavouring and preservation.

Citrus essential oil/ Formulation	Application	References
Bergamot EOs	EOs added to chitosan and HPMC edible filmsshow antibacterial activity against *E. coli, Listeria monocytogenes* and *S. aureus*	(Sánchez-González, Cháfer, Hernández, Chiralt, and González-Martínez, 2011a)
EOs added to gelatin edible films	Antibacterial activity against *S. aureus* and *Listeria monocytogenes*	(M. Ahmad, Benjakul, Prodpran, and Agustini, 2012)
EOs added to chitosan and locust bean gum edible films	Antimicrobial activity against *Aspergillus flavus*	(Aloui, *et al.*, 2014)
Citrus EOs	Antimicrobial activity against 8 different strains of *Fusarium* spp., *Aspergillus flavus* and *Aspergillus niger*	(Stević, *et al.*, 2014)
EOs added to chitosan nanoemulsions	Antimicrobial activity against *E. coli* O157:H7 and *Samonella* Typhimurium	(Severino, *et al.*, 2015)
EOs in vapour phase	In food matrix Cabbage leaf and chicken skin *to inhibit the growth of Listeria monocytogenes,L. monocytogenes, S. aureus, B. cereus, E. coli* O157:H7, *Campylobacter jejuni*, and three *Arcobacter butzleri* strains.	(K. Fisher, Rowe, and Phillips, 2007)
EOs in Chitosan and HPMC coatings (hydroxypropylmethyl cellulose)	In grapes to inhibit the growth of Molds, yeasts, and mesophiles	(Sánchez-González, *et al.*, 2011b)
EOs in Chitosan coatings	In oranges to protect from *Penicillium italicum*	(Cháfer, Sánchez-González, González-Martínez, and Chiralt, 2012)
EOs in chitosan and locust bean gum coatings	To protect the Dates from *Aspergillus flavus*	(Aloui, et al., 2014)
EOs in modified chitosan coatingnanoemulsion	To protect the Green beans from *E. coli* O157:H7 and *Samonella* Typhimurium	(Severino, *et al.*, 2015)
Bitter Orange (*Citrus aurantium* L.) EOs	Bactericidal activities against / quantitative bactericidal activity (BA_{50}) value: In apple juice with ascorbic acid: *Escherichia coli* O157:H7 (0.11%), *Salmonella enterica* (0.19%) In apple juice without ascorbic acid:*Escherichia coli* O157:H7 (0.037%), *Salmonella enterica* (0.019%)	(Friedman, Henika, Levin, and R.E., 2004)
Lime juice (*Citrus aurantifolia*)/ EOs	Common ingredient of alcoholic and non-alcoholic cocktails and typical dishes of Mexican, Indian, Vietnamese, and Thai cuisine	(Ubando-Rivera, Navarro-Ocaña, and Valdivia-López, 2005)
Citral and limonene	As flavouring agent in perfumes, creams, soaps, household cleaning products, and in some food products such as fruit beverages and ice creams	(Espina, Gelaw, de Lamo-Castellví, Pagán, and García-Gonzalo, 2013)
200 μL of lime EOs per 100 mL of apple juice	Inhibition of *Listeria monocytogenes* growth in apple juice (food matrix) at 5°C and at 37°C. Extension in the lag time at least 292.7% compared to juice without lime EOs	(Carrizo, Audicio, Sanz, and Ponzi, 2014)
Limonene at 100 ppm	Reduction in inactivation time of *Escherichia coli* in natural orange Juice (food matrix)	(Espina, Condón, Pagán, and García-Gonzalo, 2014)
Gellan-based edible coating incorporated with limonene at 0.3%	Reduction in total plate count in fresh-cut pineapple (food matrix) after 16 days of cold storage	(Azarakhsh, Osman., Ghazali, Tan, and Adzahan, 2012)
Chitosan-based edible coating incorporated with lime EOs at 0.1%	Inhibition of *Rhizopus stolonifera* growth in fresh tomato (food matrix)	(Ramos-García, *et al.*, 2012)
Mosquite gum-based edible coating incorporated with lime EOs at 0.05%	Reduction in disease incidence caused by *Colletotrichum gloeosporioides* and *Rhizopus stolonifer in* fresh papaya (food matrix) by 100% and 60%, respectively.	(Bautista Baños, 2013)
Lime EOs (terpenoid content: γ-terpinene and terpinolene)	Antiradical and antioxidant activities (multiple antioxidant mechanisms): Radical scavenger activity of 158.5 mg TE/mL against the free synthetic radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). Films from fish skin gelatin containing lime EOs possess DPPH and 2,2′-azino-di-[3-ethylbenzthiazoline sulfonate] diammonium salt radical scavenging activity and ferric reducing antioxidant power of 0.09, 0.46, and 4.24 μmol TE/g dried film, respectively.Inhibitory effects against linoleic acid peroxidation	(Tongnuanchan, Benjakul, and Prodpran, 2012)
Sweet Orange (*Citrus sinensis*) EOs (terpenes and limonene)	Used in foods and beverages, as well as cosmetics and drugs, due to their wide spectrum of biological activities such as being effective antimicrobial and antifungal agents	(Velázquez-Núñez, Avila-Sosa, Palou, and López-Malo, 2013)
C. sinensis (L.) Osbeck EOs	Antibacterial effects on against food-borne pathogenic bacteria (*Staphylococcus aureus, Listeria monocytogenes*, and *Salmonella enterica*. Sweet orange EOs can replace commercial detergents on surface of kitchen utensils has been reported, thus being a green sanitizer.	(Settani, *et al.*, 2012)
Emulsions *of C. sinensis* (L.) Osbeck var. Liucheng EOs	Inactivate Gram-negative bacteria, such as *Salmonella* Typhimurium, *Escherichia coli*, and *Vibrio parahahemolyticus* inoculated in stainless steel and plastic surfaces. these bacteria usually survive on the surfaces after washing with detergents	(Lin, Sheu, Hsu, and Tsai, 2010)
Citral, monoterpenes in EOs	Inhibitory to the growth of molds commonly associated with food spoilage, such as *Aspergillus niger, Aspergillus flavus, Penicillium chrysogenum*, and *Penicillium verrucosum*;Antifungal activity	(Espina, *et al.*, 2011)
Sweet orange EOs natural terpenes	Inactivate organisms related with deterioration of fruit at harvest as well as insects related to dissemination of pathogens in fresh fruits and vegetables; Insecticidal activity against a variety of insects via topical application; Insecticidal effects against adult housefly (*Musca domestica* L.) with a LC_{50} value of 3.9 mg/dm^3 after 30 min of exposure	(Palacios, Bertoni, Rossi, Santander, and Urzúa, 2009)

(*Continued on next page*)

Table 2. (*Continued*)

Citrus essential oil/ Formulation	Application	References
Citrus reticulata EOs (pinene, limonene, and nerol) concentrations of 1–2 μL	100% inhibition of the growth of fungal species of the genus *Aspergillus* responsible for spoilage and poisoning of food *A. ochraceus, A. niger*, and *A. flavus*; inhibit ochratoxigenic and aflatoxigenic molds	(Mihai and Popa, 2014)
Citrus reticulata EOs at a concentration of 0.2 mL/100 mL/ 0.15 mL/100 mL	Complete inhibition of fungal growth of *A. alternata, R. solani*, and *C. lunata:* complete inhibition of spore formation and production of *A. alternata, R. solani*, and *C. lunata;*Inhibitory to foodborne and phytopathogenic fungi—*Alternaria alternata, Rhizoctonia solani, Curvularia lunata, Fusarium oxysporum*, and *Helminthosporium oryzae*	(Chutia, Bhuyan, Pathak, Sarma, and Boruah, 2009)
EOs from (Lemon, lime, neroli, and orange oils are collectively called citrus oils); neroli—*Citrus aurantium* (Rutaceae); lemon—*Citrus limon* (Rutaceae); lime—*Citrus limetta* (Rutaceae); orange—*Citrus sinensis* (Rutaceae)	As flavors in carbonated cola and citrus soft drinks, fizzy drinks and mineral drinks: Lemon-lime sodas contain lemon and lime EOs as the main flavorings constituent, with or without orange oil as a minor component. Orange sodas contain orange oil as the main flavoring constituent, with or without lemon and lime oils as minor components; Main constituent in citrus soda concentrates and citrus concentrates, e.g., lemon lime concentrates, orange concentrates	(Ameh and Obodozie-Ofoegbu, 2016)
Citrus EOs incorporated into a low-density polyethylene films	As flavoring films for packaging biscuit due to its ability to prevent changes in water vapor permeability and mechanical properties through time	(Dias, *et al.*, 2013)
Lime EOs	Natural antimicrobial preservative in cream-filled cakes and pastries to lower the risk of food poisoning	(Jafari, *et al.*, 2011)
Bergamot EOs	Direct incorporation of bergamot oil amounts 0.5, 1, 2, and 3 (w/w) by dissolution method on chitosan packaging material for Fruits	(Sánchez-González, Cháfer, Chiralt, and González-Martínez, 2010)
Bergamot EOs	Direct incorporation of 5, 10, 15, 20, and 25% (w/w) to bergamot EOs in the gelatin film by dissolution method to package unicorn leather jacket	(M. Ahmad, *et al.*, 2012)
Lemon peel oil	Lemon peel extract incorporated into PCL, PLA, LDPE in amounts 7, 10, and 15% (w/w) for food packaging	(Del Nobile, *et al.*, 2009)
Limonene	Limonene incorporated on to modified chitosan in amount 0.2% (w/w) for edible coating of strawberries	(A. Perdones, L. Sánchez-González, A. Chiralt, and M. Vargas, 2012)
Neroli—*Citrus aurantium*; lemon—*Citrus limon* lime—*Citrus limetta*; orange—*Citrus sinensis*	Important constituent in soda, "fizzy drink" and "mineral drink", Soda concentrates, citrus concentrates; Neroli oil is obtained from flowers of bitter orange as well as other citrus flowers	(Palazzolo, Laudicina, and Germanà, 2013)
Bergamot and lemongrass EOs	Bergamot and lemongrass EOs incorporated into gelatin edible films to inhibit the growth of microorganisms, viz., *Staphylococcus aureus, Escherichia coli, Listeria monocytogenes*, and *Salmonella thyphimurium*	(M. Ahmad, *et al.*, 2012)
Bergamot, lemon, and tea tree EOs	Bergamot, lemon, and tea tree EOs incorporated into chitosan and hydroxypropylmethylcellulosebased edible films to inhibit the growth of *Staphylococcus aureus, Escherichia coli*, and *Listeria monocytogenes;* as coating oftable grapes	(Sánchez-González, *et al.*, 2011b)

6. Challenges and future perspectives

Although, EOs is gaining much attention in food research due to its natural origin, it is difficult to standardise the amounts of application for optimal in-vitro effects in meat or fish preservation. One of the biggest practical challenges in this direction is that the active volatile compounds found in EOs may interact with the proteins present in the meat, resulting in the destabilization of EOs and formation of new and probably undesirable compounds, thereby reducing its active antimicrobial effects. This requires a greater amount of EOs to ensure preservation of meat. But application of a greater amount of EOs into meat has been observed to alter the taste, aroma and quality of the meat and processed food stuffs and consequently less acceptable for consumption. However, strong aroma and flavour of citrus EOs enable good effects at low concentration simultaneously limiting negative organoleptic changes. Citrus EOs are considered as safe for consumption due to their non-toxic nature and hypoallergenicity, but, in some cases, it has been found to cause skin irritation and allergy. One of the probable solutions to this problem is encapsulation of EOs in a suitable biodegradable shell capable of a controlled release of the active compounds. This not only reduces EOs' instability, i.e., reactivity towards substrate proteins, but also ensures the antimicrobial properties through controlled release. This aspect is required to be focused in food research in order to ensure safe dosage limits, and composition for food preservation. Another challenge is incorporation of EOs in meat may eliminate target microbial population, but in turn, may produce favourable conditions to promote growth and virulence of other undesirable microbes leading to spoilage. Therefore, extensive study is required in order to achieve a critical understanding on (a) safe dosage limit, (b) physico-chemical stability and bioactivity of EO compounds in isolation as well as in combination, (c) Interaction of bioactive compounds present in EOs with surface proteins on food stuffs, (d) Allergic reactions, (e) optimal dosage limit to prevent the food stuff from spoilage and deterioration of quality, taste and aroma of the same because of excess amount, (f) Improved methods of encapsulation of EOs and controlled release to ensure enhanced shelf-life of the food stuffs.

7. Summary and conclusions

Citrus EOs is an economic, eco-friendly and natural alternatives to BHA, BHT and other synthetic antioxidants, sodium nitrites, nitrates or benzoates, commonly utilized in food preservation. Citrus based EOs is obtained mainly from the peels of citrus fruits. With increasing production of citrus crops and wide spread consumption of the processed citrus based products, the quantity of discarded waste is mounting every year and causing environmental problems. The extraction of citrus oils from the waste peels not only saves environment but can be used in various applications including food preservation.

The present article reviews the recent advances in the application of citrus EOs for the preservation of fruits, vegetables, meat, fish and processed food stuffs. EOs based thin films, microencapsulation incorporating biodegradable polymers, nanoemulsion coatings, spray applications and antibacterial action mechanism of the active compounds present in the EOs have been elaborated. Extensive research is required on overcoming the challenges regarding allergies and obtaining safer dosage limits. Shift towards greener technologies indicate optimistic future towards safer utilization of citrus based EOs in food preservation.

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