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Biological pathways and enzymatic mechanisms in phytochemical biosynthesis: A review

Olusola Ifedolapo WATTI *

Department of Chemistry, University of Ilorin, P.M.B. 1515, Ilorin, Kwara State, Nigeria.

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Abstract

Essential oils are complex mixtures of volatile secondary metabolites derived from plants, with significant ecological and therapeutic roles. Their chemical diversity arises from intricate biosynthetic pathways involving monoterpenoids, sesquiterpenoids, and phenylpropanoids. This review explores the biosynthetic mechanisms underlying the formation of these compounds in *Citrus sinensis*leaves, focusing on the mevalonate and non-mevalonate pathways. Additionally, how enzymatic activity and post-harvest metabolic processes influence the qualitative and quantitative variations in essential oil constituents was discussed. Understanding these pathways provides insights into optimizing plant-based products for pharmaceutical, cosmetic, and agricultural applications.

Keywords: Essential oils; Biosynthesis; Citrus sinensis; Monoterpenoids; Enzymatic activity; Plant-based products

1. Introduction

Essential oils are odorous, hydrophobic, and highly volatile secondary metabolites found in approximately 10% of the Plant Kingdom (Bauer *et al*., 2001). These oils are complex mixtures of volatile organic compounds that play crucial roles in plant defense, allelopathy, pollinator attraction, and protection against pathogens (Shawe, 1996; Lawrence, 2000). Various plant families, including Rutaceae, Ericaceae, Betulaceae, Valerianaceae, and Myrtaceae, contain species that produce essential oils (Sukhdev *et al*., 2008; Liolios *et al*., 2010). These oils are typically stored in specialized secretory structures such as glandular trichomes, oil cells, or resin ducts, depending on the plant family (Buhner, 2000).

Essential oils are typically obtained from different parts of plants, including flowers (e.g., rose and jasmine), leaves (e.g., *Ocimum* spp. and lemongrass), stems and bark (e.g., cinnamon), wood (e.g., cedar and pine), roots (e.g., vetiver and valerian), seeds (e.g., fennel and nutmeg), and fruits (e.g., bergamot and lemon) (Usman *et al*., 2010; Campelo *et al*., 2011). These oils are extracted using various techniques such as steam distillation, hydrodistillation, solvent extraction, and cold pressing, each of which influences the yield and composition of the extracted oil (Baker *et al*., 2000; Kondo *et al*., 2002).

Among the diverse plant families bearing essential oils, Rutaceae stands out due to its economic importance, particularly through species like *Citrus sinensis* (sweet orange). Essential oils from *Citrus sinensis* are widely used in food, cosmetics, perfumery, and pharmaceutical industries. The chemical composition of these oils varies significantly due to factors such as seasonal variations, developmental stages, post-harvest drying methods, and enzymatic activity within the plant (Kuster *et al*., 2003; McGimpsey *et al*., 2006). Studies have shown that essential oil yield and composition in plants like *Thymus vulgaris*, *Origanum vulgare*, and *Tagetes minuta* are significantly influenced by environmental factors such as temperature, soil conditions, and light exposure (McGimpsey *et al*., 2006; Skoula and Harborne, 2002; Abdelrazzaq *et al*., 2013).

*Corresponding author: WATTI OLUSOLA IFEDOLAPO

The biosynthesis of essential oils primarily involves two major pathways: the mevalonate (MVA) pathway and the non-mevalonate (MEP/DOXP) pathway. These pathways lead to the production of key terpenoid precursors such as isopentenyl pyrophosphate (IPP) (**1**) and dimethylallyl pyrophosphate (DMAPP) (**2**) (Berg *et al*., 2007; Swanson and Hohl, 2006). These precursors undergo further enzymatic modifications to form monoterpenoids, sesquiterpenoids, and other volatile constituents. The chemical diversity of essential oils is largely determined by the activity of terpene synthases, which catalyze a range of cyclization, oxidation, and rearrangement reactions (Croteau, 1987; Jorg *et al*., 2009).

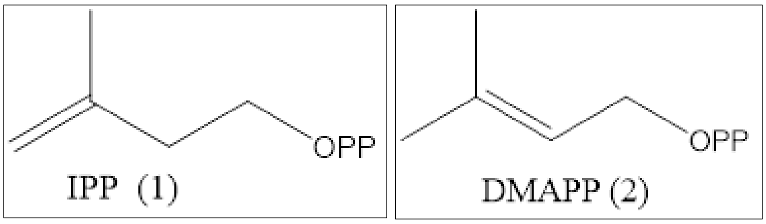

Figure 1 IPP and DMAPP

Post-harvest handling and processing conditions also influence the composition of essential oils. Drying methods such as sun-drying, shade drying, and oven drying can alter the volatile profiles of essential oils due to enzymatic and oxidative changes. For instance, the oil composition of *Salvia officinalis* varied significantly when cultivated under different soil conditions, leading to distinct chemotypes dominated by either camphor or 1,8-cineole (Abdelrazzaq *et al*., 2013). Similarly, the activity of key biosynthetic enzymes such as thymol synthase in *Thymus vulgaris* fluctuates with seasonal variations, affecting the final chemical profile of the oil (Atti-Santos *et al*., 2004).

This review aims to elucidate the phytochemical pathways responsible for the biosynthesis of monoterpenoids and sesquiterpenoids in *Citrus sinensis* leaves. By analyzing the enzymatic mechanisms involved, this work highlights the dynamic interplay between biosynthetic precursors, intermediates, and final products. Furthermore, we examine how environmental and post-harvest conditions alter these pathways, leading to changes in essential oil profiles. The findings of this review will contribute to a deeper understanding of the biochemical and ecological significance of essential oils, as well as their industrial applications.

2. Phytochemical Pathways in Essential Oil Biosynthesis

2.1. Mevalonate Pathway

The mevalonate pathway(Reaction Scheme 1), also known as the classical isoprenoid pathway, plays a crucial role in the biosynthesis of terpenoids in many plants. This pathway begins with the condensation of two acetyl-CoA (3) molecules to form acetoacetyl-CoA (4), catalyzed by acetyl-CoA transferase. Another condensation step involving acetyl-CoA and acetoacetyl-CoA leads to the formation of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) (5), catalyzed by HMG-CoA synthase.HMG-CoA (5) is then reduced to mevalonate (6) using NADPH, forming mevalonic acid (MVA) (5) in the cytosol. This reduction is followed by sequential phosphorylation catalyzed by mevalonate kinase, resulting in the formation of 5-phosphomevalonate (8). This compound is further phosphorylated to yield mevalonate-5-diphosphate (9), which undergoes decarboxylation by mevalonate-5-diphosphate decarboxylase to produce isopentenyl pyrophosphate (IPP) (1). IPP (1) is then isomerized by IPP isomerase into dimethylallyl pyrophosphate (DMAPP)(2).

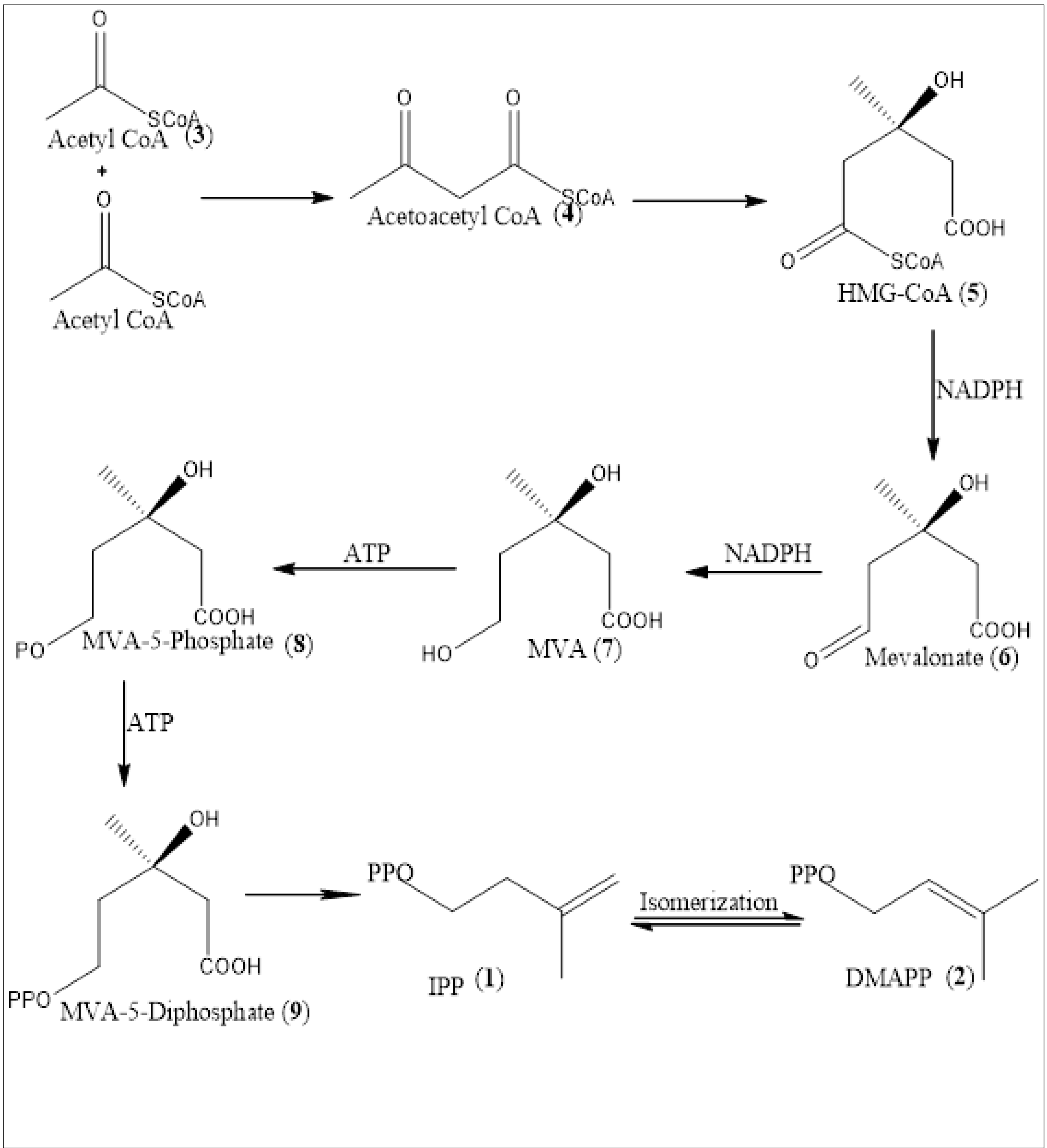

Figure 2 Reaction Scheme 1 Mevalonate pathway

2.2. Non-Mevalonate Pathway

The non-mevalonate pathway (Reaction Scheme 2), also referred to as the methylerythritol phosphate (MEP) or deoxyxylulose phosphate (DOXP) pathway, is an alternative route for isoprenoid biosynthesis. This pathway occurs primarily in plastids and is widespread in bacteria, algae, and higher plants.The pathway is initiated by the condensation of pyruvate (10) and glyceraldehyde-3-phosphate (11), catalyzed by DOXP synthase (DXPS), forming 1-deoxy-D-xylulose-5-phosphate (DOXP) (12). DOXP is then reduced in the presence of DOXP reductase to produce 2-C-methylerythritol-4-phosphate (MEP) (13). This intermediate undergoes additional enzymatic transformations, including phosphorylation and cyclization reactions (14-16), leading to the formation of IPP (1) and DMAPP (2)(Rohmer and Rohmer, 1999; Zingle *et al.*, 2010; William, 2007).

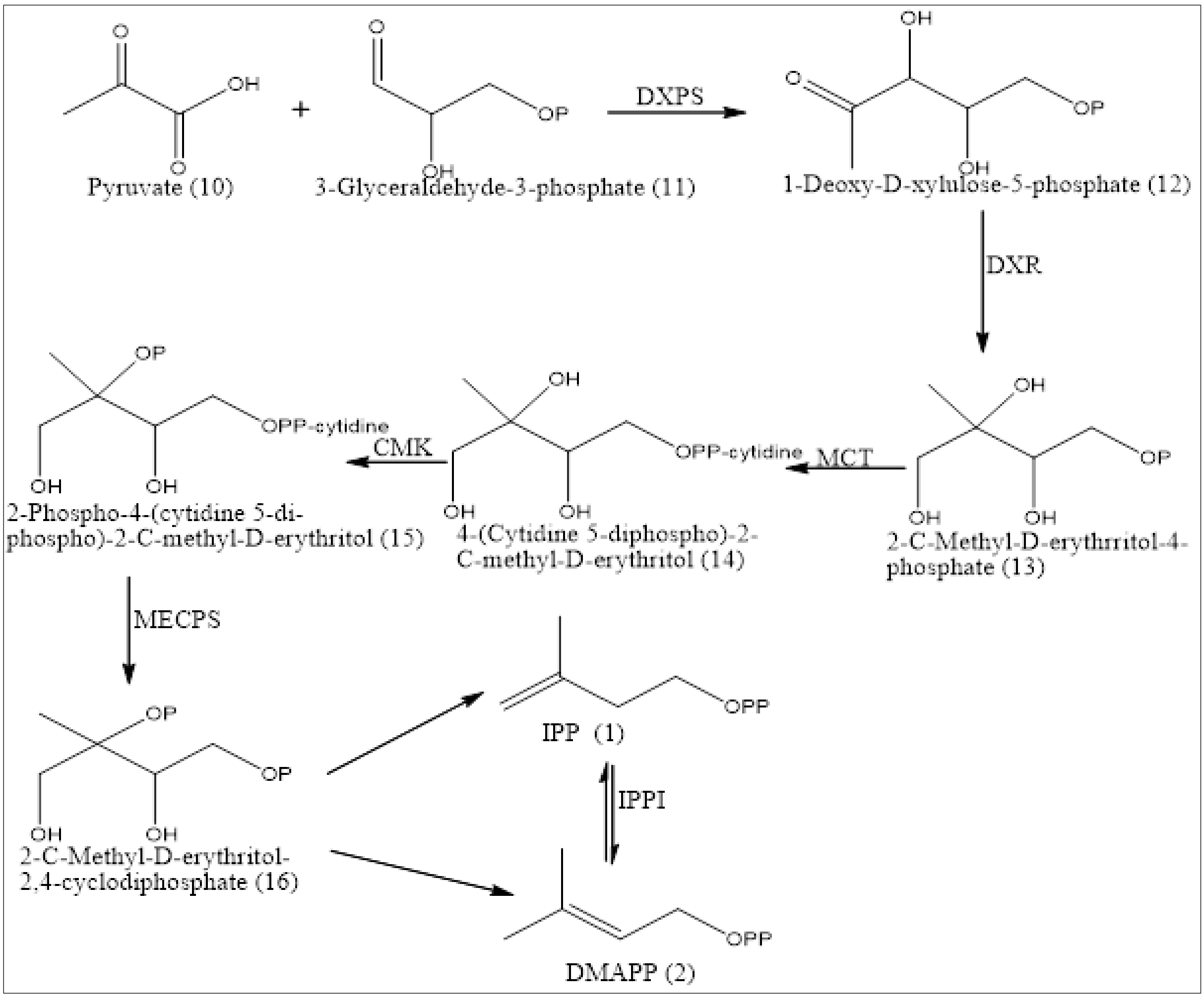

Figure 3 Reaction Scheme 2: Non-mevalonate pathway

These two key intermediates, IPP (**1**) and DMAPP (**2**), serve as the fundamental building blocks for all terpenoids, including monoterpenoids and sesquiterpenoids (Fig. 3). In *Citrus sinensis*, the mevalonate pathway significantly contributes to the production of geranyl pyrophosphate (GPP) (**17**) and farnesyl pyrophosphate (FPP) (**18**), which are precursors for monoterpenes and sesquiterpenes, respectively. GPP (**17**) undergoes ionization to form the geranyl cation, which then participates in cyclization and rearrangement reactions catalyzed by terpene synthases to generate diverse monoterpenoids. Similarly, FPP serves as a precursor for sesquiterpenoid biosynthesis through a series of enzymatic reactions.

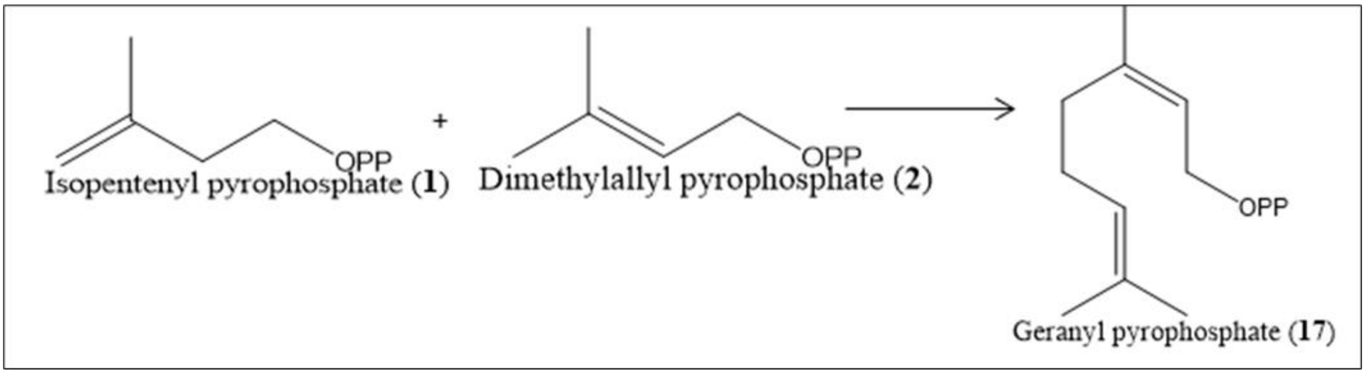

Figure 4 Formation of GPP

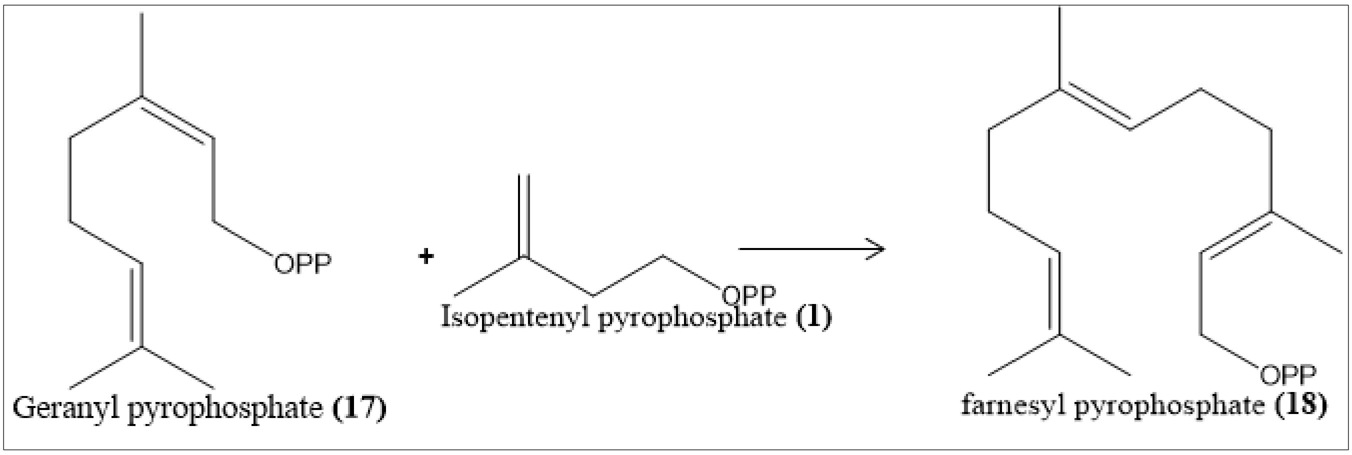

Figure 5 Formation of FPP

Both the mevalonate and non-mevalonate pathways play vital roles in essential oil biosynthesis, either operating independently or synergistically. Their regulation ensures the dynamic production of terpenoids, which contribute to the characteristic aroma, flavor, and potential pharmacological properties of plant-derived essential oils.

3. Biosynthetic Mechanisms of Monoterpenoids and Sesquiterpenoids

3.1. Monoterpenoid Biosynthesis

Monoterpenoids constitute a major class of bioactive compounds in essential oils, contributing significantly to their aromatic and therapeutic properties. In *Citrus sinensis*, the biosynthesis of monoterpenoids is initiated by the conversion of isopentenyl pyrophosphate (IPP) (1) and dimethylallyl pyrophosphate (DMAPP) (**2**) to geranyl pyrophosphate (GPP) (**17**), which serves as the universal precursor for monoterpene synthases.

3.1.1. Mechanism of Monoterpenoid Biosynthesis

The conversion of GPP (**17**) to monoterpenoids is catalyzed by a diverse group of monoterpene synthases, which facilitate a sequence of reactions, including:

- Ionization and Cyclization: GPP (**17**) undergoes ionization to form the geranyl cation, which can rearrange to form the linalyl cation, a key branching point in monoterpenoid biosynthesis.
- Hydride Shifts and Ring Closure: The linalyl cation can further undergo 6,1-ring closure to produce the α-terpinyl cation, a crucial intermediate leading to cyclic monoterpenes like limonene and 3-carene.
- Structural Modifications: These include oxidation, reduction, and hydrolysis, yielding compounds such as geraniol, citronellol, and neral.

3.1.2. Key Monoterpene Synthases in Citrus sinensis

Several monoterpene synthases have been identified, each playing a role in determining the chemical composition of the essential oils:

- α-Fenchene Synthase: Converts geranyl cation into α-fenchene via the intermediate fenchyl cation, influencing the characteristic aroma of fresh leaves. This enzyme is particularly active in morning-harvested leaves.
- 3-Carene Synthase: Facilitates the biosynthesis of 3-carene, a dominant monoterpenoid in leaves harvested in the afternoon, especially during the rainy season.
- Limonene Synthase: Converts the α-terpinyl cation into limonene, a key component influencing the oil's flavor and insecticidal properties. It also serves as a precursor for other oxygenated monoterpenes such as carvone and menthol.

3.1.3. Temporal and Seasonal Variations

The activity of monoterpene synthases varies with environmental factors such as time of harvest and seasonal changes. For instance, α-fenchene is most abundant in morning-harvested leaves, whereas 3-carene predominates in afternoon-harvested samples. Such variations are attributed to differences in enzyme expression and precursor availability.

3.2. Sesquiterpenoid Biosynthesis

Sesquiterpenoids, with their larger molecular structures and greater structural diversity, are derived from farnesyl pyrophosphate (FPP) via the action of sesquiterpene synthases. These enzymes catalyze complex rearrangements, leading to the formation of diverse sesquiterpenoid skeletons.

3.2.1. Mechanism of Sesquiterpenoid Biosynthesis

The formation of sesquiterpenoids follows a pathway analogous to monoterpenoid biosynthesis but involves FPP as the primary substrate. The process includes:

- Ionization and Cyclization: FPP is ionized to form the farnesyl cation, which undergoes multiple cyclizations to generate various carbocation intermediates such as germacradienyl and humulyl cations.
- Rearrangements and Hydride Shifts: These intermediates undergo rearrangements, including 1,2- and 1,4-hydride shifts, Wagner–Meerwein rearrangements, and ring contractions, yielding structurally diverse sesquiterpenes.
- Termination Reactions: The reaction is completed by proton loss or nucleophilic attack, leading to the formation of final sesquiterpenoid products.

3.2.2. Key Sesquiterpene Synthases in Citrus sinensis

Several sesquiterpene synthases play critical roles in defining the sesquiterpenoid profile of essential oils:

- β-Elemene Synthase: Catalyzes the conversion of germacradienyl cation to β-elemene, which is particularly prevalent in dry-season essential oils.
- Caryophyllene Synthase: Converts humulyl cation to β-caryophyllene, a sesquiterpene with well-documented antimicrobial and anti-inflammatory properties.
- α-Sinensal Synthase: Facilitates the transformation of farnesyl cation into α-sinensal, an oxygenated sesquiterpenoid known for its antioxidant activity.

3.2.3. Structural and Environmental Influences on Sesquiterpenoid Biosynthesis

- The structural diversity of sesquiterpenoids arises from variations in cyclization pathways and subsequent rearrangements.
- The biosynthetic output of sesquiterpene synthases is influenced by plant age, geographical location, and seasonal variations. For example, β-caryophyllene dominates in afternoon-harvested oils, while α-sinensal is more prominent in morning-harvested samples

4. Influence of Enzymatic Activity on Essential Oil Composition

Enzymatic activity plays a crucial role in defining the composition of essential oils. Terpene synthases catalyze the conversion of acyclic prenyl diphosphates into various cyclic and acyclic forms, leading to the diverse chemical profiles observed in essential oils (Jörg *et al*., 2009). Several factors, including seasonal and diurnal variations, significantly impact enzyme expression, thereby altering oil composition.

4.1. Effect of Seasonal Variation on Enzymatic Activity

Studies have shown that the enzymatic activity of specific terpene synthases varies with seasonal changes. For example, in *Thymus vulgaris*, thymol and carvacrol contents were lower during autumn but increased in other seasons due to the activity fluctuations of thymol synthase (McGimpsey *et al*., 2006). Similarly, *Origanum vulgare* exhibited a richer profile of oxygenated compounds in the spring, followed by summer, autumn, and winter (Atti-Santos *et al*., 2004).

In Salvia officinalis, soil conditions influenced the enzymatic pathways leading to different chemotypes. Plants cultivated under protected soilless conditions primarily produced camphor due to camphor synthase activity, whereas those grown in soil-based conditions had a higher concentration of 1,8-cineole, attributed to enhanced 1,8-cineole synthase activity (Abdelrazzaq *et al*., 2013).

4.1.1. Case Studies in Citrus sinensis

The influence of enzymatic activity on essential oil composition is evident in *Citrus sinensis*, where variations are observed based on season, time of harvest, and drying duration.

- **Rainy Season**
  - Morning Harvest: *α-fenchene synthase* exhibited peak activity, leading to a significant production of α-fenchene (93%) and related derivatives (Table 1).

Table 1 Enzymatic Activity in *Citrus sinensis* During Rainy Season

S/N	Compound	KI	% Composition (RMF)	% Composition (RAF)	Mass Spectral Data
1	α-thujene	931	0.4	1.2	136, 121, 105, 93, 77
2	α-pinene	939	1.5	1.8	136, 121, 93, 91, 77
3	α-fenchene	951	12.7	-	136, 121, 93, 79, 41
4	β-thujene	971	0.6	0.3	136, 121, 105, 93, 77
5	β-pinene	980	-	3.0	136, 121, 93, 69, 41

- (Key: RMF - Fresh leaves harvested in the morning; RAF - Fresh leaves harvested in the afternoon)
- Afternoon Harvest: 3-carene synthase became dominant, resulting in an increased concentration of 3-carene (18.0%).
- **Dry Season**
  - *β-pinene synthase* showed increased +ctivity, particularly in leaves dried for five days. This suggests that under arid conditions, enzymatic priorities shift to produce β-pinene and related terpenoids (Table 2).

Table 2 Enzymatic Shifts in *Citrus sinensis* During the Dry Season

Compound	DSMF	DSM1	DSM2	DSM3	DSM4	DSAF	DSA1	DSA2	DSA3	DSA4	DSA5
β-thujene	0.3	0.5	0.2	-	0.8	0.3	-	0.6	-	-	1.2
α-pinene	0.9	1.1	0.5	0.2	1.4	0.9	1.6	1.4	1.0	0.9	1.2
α-fenchene	-	13.7	12.6	-	-	-	-	-	-	-	-

(Key: DSMF - Fresh leaves harvested in the morning during the dry season; DSAF - Fresh leaves harvested in the afternoon during the dry season)

4.1.2. Post-Harvest Metabolic Activities and Oil Composition

Post-harvest metabolic processes further modify essential oil profiles. Some compounds initially present in glycosidic forms undergo hydrolysis upon drying, releasing their active terpenoid components and enhancing oil potency (Whish & Williams, 1996).

- *cis-β-Ocimene* and *citronellal* were absent in fresh leaves but emerged after two days of drying, likely due to enzymatic hydrolysis of their precursors (Table 3).

Table 3 Post-Harvest Emergence of Terpenoid Components

S/N	Compound	DD1	DD2	DD3	DD4
1	α-thujene	0.3	0.3	-	-
2	α-pinene	0.9	1.1	0.5	0.2
3	β-pinene	2.9	-	1.8	2.6
4	3-carene	9.1	6.3	7.0	2.6

(Key: DD1 - Leaves dried for one day; DD2 - Leaves dried for two days; DD3 - Leaves dried for three days; DD4 - Leaves dried for four days)

- Leaves dried for five days contained 39 identified compounds, compared to 36 in fresh leaves, indicating that enzymatic transformations and volatilization significantly influence the final chemical profile.
- Certain compounds, such as decanal, β-pinene, and neryl acetate, were found in fresh leaves but absent in dried samples, suggesting volatilization effects, whereas 2-carene, α-fenchene, and linalool appeared only after drying, supporting the role of post-harvest metabolic activities (Table 4).

Table 4 Percentage Composition of Monoterpenoids in Fresh and Dried *Citrus sinensis* Leaves

Compound	RMF	RM1	RM2	RM3	RM4	RM5	RAF	RA1	RA2	RA3	RA4	RA5
β-thujene	0.6	0.5	0.7	-	0.5	0.6	0.3	-	0.4	-	0.8	0.4
α-pinene	1.5	6.4	3.1	3.4	2.4	1.7	1.8	1.6	3.6	1.5	1.8	4.4
α-fenchene	12.7	8.3	-	17.7	-	-	-	12.7	-	8.8	-	1.2

(Key: RMF - Fresh leaves harvested in the morning during the rainy season; RAF - Fresh leaves harvested in the afternoon during the rainy season)

5. Effects of Post-Harvest Conditions on Biosynthetic Pathways

Post-harvest processing significantly influences the composition and quality of essential oils by modulating the enzymatic activity of terpene synthases. Variations in drying methods, seasonal conditions, and storage duration alter the biosynthetic pathways, affecting both yield and chemical profile of the essential oils.

5.1. Influence of Drying Methods on Terpene Synthase Activity

Drying enhances oil concentration by promoting moisture loss, but excessive drying leads to volatilization and degradation of sensitive compounds. This has been observed in various plants, including *Citrus sinensis*, *Eucalyptus*, and *Melaleuca alternifolia*, where controlled post-harvest drying can even increase oil yield by mobilizing stored metabolites

5.2. Drying Duration and Compound Formation

5.2.1. Different drying durations lead to specific variations in the chemical composition of essential oils:

- One-Day Drying: Enhances the formation of β-pinene and sulcatone, likely due to early dehydration-stimulated enzyme activity (Table 3.8).
- Two to Four Days: Increases oxygenated monoterpenoid content, such as citronellol and citronellal, suggesting active oxidation and hydration reactions (Table 3.5).
- Five-Day Drying: Reduces the presence of certain volatiles like sulcatone and phytol while promoting the formation of stable compounds like linalool and nerolidol (Table 5).

Table 5 Effect of Drying on Major Essential Oil Constituents

Drying Duration	Major Monoterpenoids (%)	Major Sesquiterpenoids (%)
1 Day	β-Pinene (4.2), Limonene (10.6)	β-Caryophyllene (2.8), α-Sinensal (1.1)
2 Days	Citronellal (5.3), Linalool (4.8)	β-Elemene (3.1), β-Guaiene (1.5)
3 Days	Citronellol (7.8), Nerol (4.4)	β-Elemene (4.2), Cis-β-Farnesene (2.2)

4 Days	Terpinen-4-ol (7.3), Citral (5.7)	β-Caryophyllene (3.8), α-Sinensal (1.9)
5 Days	Linalool (6.0), Nerolidol (5.2)	β-Caryophyllene (2.5), α-Sinensal (1.3)

5.3. Seasonal Variations in Essential Oil Composition

The biosynthetic pathways of essential oil constituents are highly responsive to seasonal changes. For instance, the limonene content of *Citrus sinensis* peaks during the rainy season (8.2–11.0%) but is nearly absent in dry-season afternoon-harvested leaves. In contrast, β-elemene and α-sinensal become dominant in dry-season oils, reflecting a shift in enzymatic activity influenced by environmental conditions.

Table 6 Seasonal Variation in Monoterpene and Sesquiterpene Content

Season	Limonene (%)	Citral (%)	β-Elemene (%)	α-Sinensal (%)
Rainy	8.2 - 11.0	3.7 - 5.3	1.4 - 3.6	0.9 - 1.4
Dry (Morning)	3.2 - 5.4	2.1 - 3.8	3.8 - 4.6	1.5 - 2.2
Dry (Afternoon)	0 - 2.1	1.4 - 3.0	4.2 - 5.1	2.0 - 2.8

6. Role of Phytochemicals in Biological Activities

The bioactivity of *Citrus sinensis* essential oils is closely linked to their chemical composition, with major compounds such as limonene, β-caryophyllene, and α-sinensal exhibiting antimicrobial, antioxidant, and insecticidal properties.

6.1. Antioxidant Properties

Oxygenated monoterpenoids such as linalool and citronellol play a crucial role in free radical scavenging, protecting cells from oxidative stress. Additionally, α-sinensal contributes significantly to the antioxidant potential of the oil.

Table 7 Antioxidant Activity of Key Essential Oil Compounds

Compound	Antioxidant Activity (%)	Reference
Linalool	78.5	Venkutonis *et al*., 2005
Citronellol	72.3	Campelo *et al*., 2011
α-Sinensal	81.7	Majnooni *et al*., 2012

6.2. Insecticidal Activity

Essential oils from *Citrus sinensis* have been shown to be effective against storage pests such as *Callosobruchus maculatus*. The insecticidal properties depend on the balance between hydrocarbon and oxygenated terpenoids, which in turn is influenced by harvest timing and drying conditions.

Table 8 Insecticidal Activity of *Citrus sinensis* Essential Oils

Compound	Target Pest	Mortality Rate (%)
β-Elemene	*Callosobruchus maculatus*	85.3
β-Caryophyllene	*Sitophilus oryzae*	78.9
Citronellal	*Tribolium castaneum*	82.5

7. Conclusion

The biosynthesis of essential oil constituents in *Citrus sinensis* involves intricate enzymatic pathways operating via the mevalonate and non-mevalonate routes. These pathways lead to the production of monoterpenes and sesquiterpenes, whose profiles are influenced by various biotic and abiotic factors. Seasonal, diurnal, and post-harvest conditions significantly modulate the activity of terpene synthases, resulting in fluctuations in essential oil yield and chemical composition. Studies have demonstrated that drying techniques, exposure to sunlight, and soil conditions impact oil production by altering enzyme activity and volatile compound retention.

Post-harvest metabolic activities have also been observed to modify the chemical constituents of essential oils, with certain compounds forming due to enzymatic conversion during drying. This highlights the importance of optimizing extraction protocols to preserve key bioactive constituents, such as β-elemene and α-sinensal, which have demonstrated insecticidal, antioxidant, and antimicrobial properties. A better understanding of the interplay between environmental factors and enzymatic activity will enhance the efficiency of essential oil production and support its applications in medicine, agriculture, and industry.

Recommendations

To maximize the utility and commercial potential of *Citrus sinensis* essential oils, the following recommendations are proposed:

- Optimization of Harvest Timing: Conduct in-depth studies on the diurnal and seasonal variations in enzymatic activity to determine the optimal harvest periods that maximize oil yield and bioactive compound retention.
- Standardization of Drying Protocols: Develop and validate drying methods that minimize volatilization losses while promoting beneficial transformations of key constituents. Studies have shown that post-harvest drying under controlled conditions can improve essential oil yield while minimizing microbial growth and biochemical degradation.
- Characterization of Bioactive Compounds: Isolate and evaluate the biological properties (insecticidal, antioxidant, and antimicrobial) of major essential oil components such as β-elemene and α-sinensal for targeted industrial and pharmaceutical applications.
- Genetic and Biotechnological Enhancements: Explore the genetic regulation of terpene synthases and investigate potential biotechnological interventions to enhance the biosynthesis of desirable essential oil compounds.
- Soil and Environmental Management: Conduct field trials to assess the impact of soil type, nutrient availability, and environmental stressors on essential oil composition. Studies indicate that soil conditions significantly influence the production of certain chemotypes due to the differential activity of specific synthases.
- Sustainable Extraction Techniques: Promote the adoption of green extraction methods, such as supercritical CO_2 extraction, to improve oil purity and yield while reducing environmental impact.
- Future research should focus on isolating and characterizing novel synthases responsible for the formation of high-value bioactive compounds. Additionally, advances in molecular biology and genetic engineering could pave the way for the development of improved *Citrus sinensis* cultivars with enhanced essential oil profiles.

By implementing these strategies, researchers and industry stakeholders can optimize *Citrus sinensis* essential oil production for enhanced economic and therapeutic value.

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