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INTEGRATING METABOLOMICS AND AROMA CHEMISTRY FOR UNDERSTANDING *CAPSICUM* VOLATILE PROFILES AND THEIR APPLICATIONS

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Abstract

Capsicum species (chilli peppers) display tremendous chemical variation underlying pungency, flavour and aroma. Capsaicinoids contribute to the heat exhibited during tasting, while the many combined oils and volatile organic compounds (VOCs) are important to sensory qualities and bioactivity - but are less understood. This comprehensive review brings the reader up-to-date on the chemistry, biosynthesis and analytical techniques used to analyse oils and VOCs, targeted to essential oils and lipid-derived volatiles including terpenes, fatty acid derivatives, aldehydes, esters (and others), and pyrazines. The contribution of metabolic pathways such as lipoxygenase, terpenoid, and phenylpropanoid pathways (as well as their genetic and environmental control) is discussed. Methods such as HS-SPME-GC-MS, GC×GC-TOF-MS and GC-O are reviewed for their relative sensitivity and reproducibility. In addition to chemistry, Capsicum VOCs have demonstrated ecological and physiological roles in defence, pollination, and an adaptive response to stress, and include further potential for antimicrobial, antioxidant and nutraceutical properties. Although there have been gains in Capsicum research, gaps still remain in our understanding of identifying biosynthetic genes, standardising analytical methods, and the linkages between metabolomic and sensory data. Future studies should shift their focus to integrate and learn from multi-omics studies, harmonise methods, and explore post-harvest transformation, thereby allowing for increased insight into Capsicum in terms of chemical ecology and breeding for aroma, as well as further industrial applications.

Keywords: *Capsicum* spp.; volatile organic compounds (VOCs); capsaicinoids; metabolomics; aroma biosynthesis; GC–MS; terpenes; flavor chemistry; post-harvest processing; multi-omics integration

1. Introduction

The genus Capsicum (Solanaceae) is an important global taxa grown for foods, spices, and bioactive compounds. The heat produced in Capsicum fruits is primarily due to capsaicinoids such as capsaicin and dihydrocapsaicin; however, the complex characterizations of flavor and aroma of Capsicum fruits are produced from composite blends of volatile organic compounds (VOCs), including monoterpenes, sesquiterpenes, aldehydes, alcohols, esters, and pyrazines, as well as fractions derived from lipids and essential oils (Pino et al., 2007). Recent developments in high-resolution metabolomics and gas chromatography–mass spectrometry (GC–MS) have increased the searchable database of pepper VOCs to hundreds or thousands of compounds that illustrate the chemical complexity behind Capsicum volatiles (Khan et al., 2024; Qiu et al., 2024). For example, comprehensive two-dimensional GC×GC–TOF–MS analysis of two cultivars of Capsicum annuum identified 1,914 and 1,802 volatile compounds in the two cultivars of pepper, which shows the remarkable variation of pepper volatiles (Khan et al., 2024). Likewise, using headspace solid-phase microextraction (HS-SPME) with GC–MS and GC–olfactometry identified 393 volatile compounds in Capsicum chinense, many of which are also considered important for aroma perception (Qiu et al., 2024). Previous metabolomic studies in 32 Capsicum accessions using HS-GC–MS and LC–MS have shown that the diversity of volatile and semi-polar metabolites was closely related to species-level chemotypic variations of C. annuum, C. chinense, C. frutescens, and C. baccatum (Nayak et al. 2012). More recently, HS-SPME–GC/MS, and RNA-seq analyses have demonstrated that variation in aroma was linked to differences in fatty acid-derived and terpene volatiles between pungent and non-pungent fruits (Liu et al. 2023).

Overall, these studies indicate that the volatile and oil fractions of Capsicum are much more complex and varied than previously recognised, with differences in the composition and abundance among species and cultivars. With the new analytical platforms that have emerged including HS-SPME, GC×GC–TOF–MS, and GC–O as well as traditional approaches with greater sensitivity to minor yet aroma-active compounds, many of the volatile compounds associated with biosynthetic regulation, genetic refinement, and ecological role have yet to be adequately characterized, creating further opportunities for study as it relates to flavour chemistry, functional genomics, and plant breeding.

2. Major Chemical Families and Representative Compounds

2.1 Major Compound Classes

The volatile profiles of Capsicum species include a variety of chemical groups from multiple biosynthetic pathways, primarily LOX, terpenoid, and Maillard pathways. The major classes of volatile organic compounds (VOCs) reported are fatty acid derivatives, esters, terpenoids, aldehydes, and ketones, in addition to heterocycles that contain sulfur and nitrogen, including pyrazines and furans.

2.1.1 Fatty acid derivatives (e.g., (E)-2-hexenal, a C6 aldehyde or trans-2-hexenol and 1-hexanol, both alcohols), that derive from the LOX pathway and provide the characteristic “green” or fresh odor notes that are present from fresh Capsicum fruits (Qiu et al., 2024).

2.1.2 Esters, such as ethyl hexanoate and hexyl acetate, provide fruity and floral notes and have been reported to increase in abundance during fruit ripening, which is attributed to increased esterification of the fatty acid precursors (Pino et al., 2007).

2.1.3 Terpenoids include all the monoterpenes (e.g., linalool, β-myrcene, α-pinene) and sesquiterpenes (e.g., β-caryophyllene, zingiberene) that provide citrusy, woody, or spicy notes and serve dual purposes as flavor and defense compounds. Specific genotypes, especially C. chinense and C. frutescens types, have been shown to have more terpenoid-derived VOCs (Khan et al., 2024; Liu et al., 2023).

2.1.4 Aldehydes and ketones likewise contribute green and fresh characteristics while heterocycles (pyrazines, furans, sulfur volatiles, etc.) have relatively low concentrations for the effects they impart sensory-wise; they add roasted, nutty or earthy flavors that vary depending on cultivars and post-harvest processing (Nayak et al., 2012; Pino et al., 2007).

Recent advancements in high-resolution methods, like GC × GC - TOF - MS, HS-SPME - GC - MS and comprehensive metabolomics, have separated and identified hundreds or over a thousand VOCs depending on tissue type and processing state (fresh, dried, and roasted) surpassing previous single dimensional GC - MS methods (Khan et al., 2024). These studies represent a unique and complex dynamic volatilome imbued with the attributes of the genotype, environment, and ripening stage, and more sophisticated statistical analyses are revealing structures as well.

2.2 Non-Volatile Oils and Related Compounds

In Capsicum chemistry, the term "oils" represents both the volatile essential oil fractions, which contain a variety of terpenoids along with other lipid-soluble volatiles from the fruit wall and/or seeds; and non-volatile matrices containing triglycerides and fatty acids also from the pericarp and/or seeds. Lipid pools are used as the biosynthetic precursors of esters and aldehydes during ripening and post-harvest processing. Variations in fatty acid profiles (linoleic and linolenic acid) in both interspecific and intraspecific studies may also affect volatile ester formation and the final aroma quality (Qiu et al., 2024 and Liu et al., 2023)

Table 1. Major chemical classes and representative volatile/oil-related compounds in *Capsicum* spp.

Compound class	Representative compounds	Typical sensory notes / origin
Capsaicinoids (non-volatile alkaloids)	Capsaicin, dihydrocapsaicin, nordihydrocapsaicin	Pungency (chemesthetic); formed in placenta; non-volatile but perceptually dominant
Monoterpenes	Limonene, α-pinene, β-myrcene, linalool	Citrus, green, terpenic notes; often enriched in essential oil fraction
Sesquiterpenes	β-Caryophyllene, α-humulene, zingiberene	Woody, spicy, or balsamic aroma; abundant in *C. frutescens* and *C. chinense*
Aldehydes / Alcohols (lipid derivatives)	Hexanal, (E)-2-hexenal, 1-hexanol	"Green", grassy, fresh; LOX pathway products from linoleic/linolenic acids
Esters	Hexyl acetate, ethyl hexanoate	Fruity, sweet, floral; increase during ripening
Pyrazines	2-Isobutyl-3-methoxypyrazine (IBMP)	Green, bell-pepper, earthy; potent odorant even at trace levels
Sulfur compounds / Furans	Various sulfur volatiles and furans	Onion-like, roasted, nutty; increase during drying or thermal processing

(Compiled from targeted GC–MS / HS-SPME studies and recent reviews: Pino et al., 2007; Nayak et al., 2012; Liu et al., 2023; Khan et al., 2024; Qiu et al., 2024).

3. Biosynthesis and Tissue Localization

3.1 Capsaicinoids

Within the Capsicum genus, capsaicinoids (e.g., capsaicin and dihydrocapsaicin) are formed via condensation of a vanillylamine moiety (derived via the phenylpropanoid pathway from phenylalanine) and a branched-chain fatty acid acyl-CoA (from valine or leucine) via the branched-chain fatty-acid metabolism pathway. (Leete & Louden, 1968; Goyal et al., 2024) Specifically, activities of the enzymes such as phenylalanine ammonia-lyase (PAL), cinnamate-4-hydroxylase (C4H), caffeic acid O-methyltransferase (COMT) and branched-chain amino acid aminotransferases (BCAT), ketoacyl-ACP synthases (KAS), acyl-CoA synthetases (ACL), and the putative capsaicinoid synthase/acyltransferase (e.g., AT3/Pun1 locus) utilize metabolic regulation to direct the flux of biosynthesis. (Stewart et al, 2005; Goyal et al., 2024).

The Pun1/AT3 gene locus encodes for a putative acyltransferase that is required for capsaicinoid synthesis; Pun1 mutants that lack functional Pun1 do not produce capsaicinoids and are not pungent. (Stewart et al., 2005) Transcriptional regulators also have a significant role in the capsaicinoid biosynthetic pathway, such as the R2R3-MYB transcription factor gene CaMYB31 (+ equivalents in other Capsicum spp.) that promotes structural gene expression in the pathway and thus negatively regulates capsaicinoid accumulation. (Zhu et al., 2014) Additionally, biosynthesis is highly developmental- and tissue-specific; for pungency, capsaicinoid accumulates primarily in the placenta (especially epidermal blister-cells of the septum) during a specific developmental time period (about 20-30 days after flowering) in pungent Capsicum annuum and other pepper species. (Stewart et al., 2005; Leete & Louden, 1968)

3.2 Volatile biosynthesis

Volatile organic compounds (VOCs) in Capsicum fruits are formed by various biosynthetic pathways:

3.2.1 LOX (lipoxygenase) pathway: Lipoxygenase (LOX) acts on unsaturated fatty acids, such as linoleic or linolenic acid to oxygenate them, followed by hydroperoxide lyase (HPL) cleaving the oxygenated fatty acid to C6 aldehydes (such as (E)-2-hexenal) and their corresponding alcohols (1-hexanol, trans-2-hexenol), which contribute green or fresh aromatic notes. (Huang et al., 2023)

3.2.2 Terpene synthase (TPS) pathways: Monoterpenes and sesquiterpenes (linalool, β-myrcene, α-pinene, β-caryophyllene) are synthesised from two dedicated gene families of TPS; annotation of TPS gene clusters in Capsicum annuum suggests co-expression (and regulation) of volatile terpenoids (and non-volatile terpenoids), frequently in response to herbivory or abiotic stress. (Zhang et al., 2023)

3.2.3 Amino-acid derived heterocycles. Volatile compounds (pyrazines, furans, sulfur- or nitrogen-heterocycles) can originate from amino-acid metabolism or as a result of some reaction (e.g., thermal, microbe-mediated) that occurs during processing. Branched-chain amino acid aminotransferase (BCAT) family members appear to link the metabolism of branched-chain amino acids to these components of the volatilome in fruits. (Huang et al., 2023)

Overall, despite significant strides in metabolomics and transcriptomics, many of the participating enzymes, gene-regulatory network(s), and the observed tissue-specific expression of low-abundance, aroma-promoting-related volatile compounds remain poorly resolved in Capsicum. (Qiu et al., 2023)

3.3 Tissue localization and developmental regulation

Spatial localisation of specialised metabolite synthesis is well characterised in Capsicum fruits: capsaicinoid synthesis and accumulation is mainly localised within the placenta, specifically in the epidermal blister-cells of the septum adjacent seed cavities; whereas, volatile organic compound (VOC) production is often localised in the pericarp, which is modulated by various developmental and environmental cues. (Stewart et al., 2005; Leete & Louden, 1968) This developmental regulation occurs such that the expression of capsaicinoid structural and regulatory genes peaks at secondary stages (~20-40 days post-anthesis) in pungent lineages, after which the capsaicinoid accumulation typically decreases or stabilises; similarly, volatile profiles also change during ripening, whereby green-leaf volatiles occur earlier in development and fruity/ester volatiles occur at later ripening stages. (Liu et al., 2023).

4. Species and Cultivar-Level Chemical Variation

The content of capsaicinoids and volatile components in Capsicum species exhibit substantial inter- and intra-specific variation, due to genetic and environmental factors. Comparisons of metabolomic and volatilomic studies show that the various species and cultivars exhibit distinct chemotypes based on enzyme memberalities, metabolic flux and regulation of gene expression at the tissue level (Khan et al., 2024; Liu et al. 2024).

Within the cultivated taxa of covers, *Capsicum annuum, C. chinense, C. frutescens*, and *C. baccatum,* the relative amounts of key volatile compound classes differs between species. *C. chinense* types, such as ‘Habanero’ and ‘Scotch Bonnet’ types frequently display high diversity and high abundance of the esters (eg, ethyl butanoate, hexyl acetate) and terpenoids (eg, linalool, β-ocimene, β-caryophyllene), which provide fruity and floral notes, respectively (Pino et al., 2007; Rodríguez-Burruezo et al., 2010; Qiu et al., 2024). In contrast, *C. annuum* types, especially bell pepper types, often exhibit higher relative concentrations of green aldehydes and pyrazines such as 2-isobutyl-3-methoxypyrazine (IBMP) that exhibit grassy and bell pepper aromatic aspects (Nayak et al., 2012; Díaz et al., 2020).

Capsicum frutescens accessions, in particular, the cultivar 'Tabasco', often produce sesquiterpene-dominant volatile profiles, including the distinctive woody- and spicy notes associated with volatile constituents like β-caryophyllene, α-humulene, and zingiberene (Khan et al., 2024; Liu et al., 2023). C. baccatum accessions have not been characterised to the same extent but often contain unique floral- and citrus-like terpenoid profiles that are commonly attributed to species-specific terpene synthases (Qiu et al., 2024).

The morphology of the fruit tissues can also impact the diversity of the mga components. Specifically, wrinkled or textured fruit tissues tend to have higher total volatile concentrations than smooth-skinned fruits. If this effect does exist, it may be due to either increased permeability of the cuticle or structures that increase the density of the glandular trichome, which may aid in the storage and release of the volatiles (Díaz et al., 2020; Rodríguez-Burruezo et al., 2010). Additionally, seed and pericarp tissues have been known to vary widely in the oil and volatile profile or constituents; seeds generally are higher in triglycerides and fatty acids compared to the pericarp, which has the greatest terpenoids and esters (Liu et al., 2023).

The impact of geographical origin and domestication on chemical diversity is also quite significant. Wild Capsicum accessions tend to have more chemical richness and specific compounds not present in domesticated lines, which is indicative of adaptations to ecological selective pressures such as herbivory and interaction with microbes (Zewdie & Bosland, 2001; Qiu et al., 2024). Studies employing the more comprehensive methods, such as two-dimensional gas chromatography-time-of-flight mass spectrometry (GC×GC–TOF–MS) and headspace solid-phase microextraction–gas chromatography-mass spectrometry (HS-SPME–GC–MS) have found hundreds, to over 1,000, volatile organic compounds (VOCs) in a specific cultivar (Khan et al., 2024). These types of multidimensional studies demonstrate both the within-*Capsicum* metabolic complexity and plasticity of its chemistry, and the applicability to utilising *Capsicum* as a model system for studying the evolution of plant volatilomes and observations on domestication effects.

5. Effect of Developmental Stage, Post-Harvest Handling and Processing

5.1 Ripening

The ripening of Capsicum fruits involves significant biochemical and sensory changes, especially in the composition of volatile organic compounds (VOCs). Early in development, green-leaf volatiles are dominant, especially C6 aldehydes (like (E)-2-hexenal) and lipoxygenase (LOX)-derived alcohols, along with methoxypyrazines like 2-isobutyl-3-methoxy-pyrazine (IBMP) that provide grassy and bell-pepper aromas (Nunes et al., 2008). As the fruit transitions from green to red, green volatiles decline while esters, terpenoids, and carotenoid-based compounds increase, providing fruity, floral, and ripe aromas (Zhou et al., 2023). Capsaicinoid biosynthesis also has a developmental pattern that depends on the genotype, sometimes peaking in intermediate ripeness stages and then declining in fully ripe fruit (Qiu et al., 2024). The compositional nds, and terpenoids during ripening. These compositional even when ger practical efforts can demonstrate shifts insinuate enzymatic reprogramming and reallocation of metabolism occurring during ripening.

5.2 Drying and Thermal Processing

Post-harvest processing, specifically drying, significantly modifies the volatile and oil profile of Capsicum fruits. Although losses of volatiles from fresh fruits exceed 80 to 90% for highly volatile compounds when drying involves sun, shade, and hot-air methods, new aroma-active molecules are produced from lipid oxidation, esterification, and Maillard reactions (Li et al., 2023). For instance, (2E)-2-decenal and acetic acid derivatives from hot-air-dry fruits are then suspended in storage, while under shade conditions, the formation of some fatty-acid methyl esters, such as methyl laurate, was observed (Li et al., 2023). When multi-species comparisons were made with fresh fruits, the fresh fruits generally showed the greatest intensity of total VOC emission (with some exceptions for sweet C. annuum cultivars showing increased emission post-drying) (Díaz et al., 2020). Therefore, drying conditions influence the degree of loss of fresh volatiles and formation of thermogenic aroma compounds produced from dehydration, including furans, sulfur volatiles, and pyrazines from thermal oxidation reactions (Khan et al., 2024).

5.3 Fermentation and Microbial Transformations

Fermentation methods, such as those used in traditional products like Paojiao, allow for the power of microbial metabolism to be introduced into the volatile mix of Capsicum. Microorganisms (lactic acid bacteria and yeasts) metabolise sugars, amino acids, and fatty acids to produce volatile esters, acids, and aldehydes that contribute to the characteristic sour-spicy aroma of fermented peppers (Frontiers Research Team, 2022; Zhou et al., 2023). Alcohols typically decline during fermentation and, in contrast, esters (i.e., ethyl butanoate, hexyl acetate) and hydrocarbons become more intense due to microbial esterification and lipid degradation (Li et al., 2022). Long-term fermentation also builds on complex fruity and buttery attributes through the constituent production of 3-methylbutyl 2-methylbutanoate and other similar products (Brüder et al., 2024). For these reasons, fermentation not only preserves peppers but also provides a chemically different volatilome in comparison to raw or dried peppers.

Table 2. Summary of Major Factors Influencing Volatile and Oil Composition in *Capsicum* spp.

Stage/Process	Dominant Chemical Changes	Representative Compounds	Aroma/Functional Impact
Ripening	↓ C6 aldehydes, IBMP; ↑ esters, terpenoids, carotenoid-derived volatiles; genotype-specific capsaicinoid peaks	(E)-2-hexenal, hexyl acetate, linalool, β-ionone	Transition from green/fresh to fruity/floral aromas
Drying / Thermal Processing	Degradation of fresh VOCs; formation of Maillard and oxidation products	2-methylpropanal, furans, sulfur compounds, (2E)-2-decenal	Roasted, nutty, smoky aroma; altered color and stability
Fermentation	Microbial generation of esters and acids; reduction in alcohols	Ethyl butanoate, 3-methylbutyl 2-methylbutanoate, acetic acid	Complex sour-fruity aroma; enhanced flavor diversity

6. Analytical Methods: Strengths and Limitations

6.1 Commonly Used Approaches

6.1.1 Headspace Solid-Phase Microextraction Gas Chromatography–Mass Spectrometry (HS-SPME–GC–MS)

The most common analytical technique used to profile volatile organic compounds (VOCs) from Capsicum spp. is headspace solid-phase microextraction coupled with GC-MS (HS-SPME-GC-MS). This technique is non-destructive, solvent-free, and valuable for analysing low-abundance compounds from fresh or processed matrices (Rao et al., 2023). The method preconcentrates equilibrium vapour-phase volatiles under specific temperature and incubation conditions, yielding sensitivity to key classes of volatiles, such as aldehydes, esters, terpenoids, and pyrazines (Zhang et al., 2023). However, HS-SPME-GC-MS is semi-quantitative and heavily dependent on the coating chemistry of the fibre (e.g., DVB/CAR/PDMS vs. PDMS), extraction time, and matrix moisture, which affects reproducibility (Zhou et al., 2023).

6.1.2 Comprehensive Two-Dimensional Gas Chromatography–Time-of-Flight Mass Spectrometry (GC×GC–TOF-MS)

The introduction of GC×GC–TOF-MS has significantly enhanced chromatographic resolution and dynamic range for complex plant volatilomes. Not only can it separate co-elution compounds from 1D GC, but it is also capable of detecting hundreds to even thousands of volatile organic compounds (VOCs) from a single plant sample. This technology has helped expand the chemical catalogue of Capsicum volatiles (Kumari et al., 2022). It has been particularly useful for distinguishing isomeric terpenes and detecting trace odorants that generally co-elute in conventional GC–MS (Kim et al., 2021). Furthermore, because of the high sensitivity of the TOF-MS detector, retrospective data mining and compound classification based on chemical ontology are possible (Kang et al., 2024).

6.1.3 Gas Chromatography–Olfactometry (GC–O) and Odour Activity Value (OAV) Calculations

GC-O, which involves the detection of volatiles by an instrument alongside a sensory assessment by trained panellists, is vital for discovering aroma-active compounds among the hundreds of VOCs typically detected by MS. In conjunction with GC-O, the use of odour activity value (OAV) calculations, where OAV = concentration/odour threshold value, permits distinguishing which volatiles are associated with odour-relevant volatiles (for example, methoxypyrazines, β-ionone, linalool) versus which contribute insignificantly to sensory perception (Yang et al., 2022). When studies utilized both GC-O and sensory analysis of aroma volatiles, less than 10% of the total volatiles significantly contributed to the characteristic aroma of each Capsicum cultivar (Li et al., 2023).

6.2 Challenges and Methodological Gaps

6.2.1 Lack of Standardisation

Though technical advancements have increased inferred knowledge, true cross-study comparisons remain limited due to the diverse nature of sample preparation, extraction, and instrumental conditions. Key variables include whether a sample is analysed fresh or following freezing, blended or whole, and the equilibration temperature and time in HS-SPME (Mao et al., 2023). In addition, differences in GC column phase, temperature programming, and detector tuning create biases in compound recovery and quantitation (Zhang et al., 2023). The future is promising with standardised methods, similar to winery aroma or plant metabolomics standards, enabling reproducibility between laboratories to become routine.

6.2.2 Quantitation Limitations

Many studies present semi-quantitative data using peak area normalisation or relative abundance, instead of absolute quantification. This issue occurs because there often are no true reference standards that exist for many VOCs, particularly those that are rare, such as terpenoids, long-chain esters, and sulphur compounds (Kang et al., 2024). Some internal standards are available (e.g., 2-octanol or benzyl acetate) that allow for relative comparisons between classes of VOCs, but do not account for compound-specific response factors or other matrix effects (Li et al., 2023). Quantitative metabolomics using stable isotope-labelled internal standards is still limited, but is increasingly encouraged to provide reliable and comparable VOC concentration data.

6.2.3 Odor Relevance and Sensory Linkage

A significant limitation in Capsicum volatile research is the lack of correspondence between chemical profiles and aroma perception. Although GC–MS can identify hundreds of compounds, only a small number are responsible for aroma as perceived by humans. The functional significance of the volatiles detected, even through GC–O, resolution, and support from nonvolatile components, is likely speculative without sensory validation (Yang et al., 2022). A more complete understanding of flavor experience would derive from a metabonomics approach to chemical phenotyping with sensory approaches and psychophysical modeling (e.g., aroma recombination studies).

Table 3. Summary of Analytical Techniques for *Capsicum* VOC and Oil Characterization

Technique	Advantages	Limitations	Applications in *Capsicum*
HS-SPME–GC–MS	Solvent-free, high sensitivity, simple prep	Matrix effects; semi-quantitative; fiber selectivity bias	Fresh fruit VOC profiling; cultivar differentiation
GC×GC–TOF-MS	High resolution; separation of co-eluting compounds	Expensive; large data output; complex interpretation	Comprehensive volatile profiling; isomer discrimination
GC–O / OAV	Identifies odor-active compounds	Labor-intensive; subjective panel evaluation	Aroma impact assessment; flavor reconstruction
Quantitative GC–MS with standards	Provides absolute concentrations	Requires expensive standards	Comparative metabolomics and industrial quality control

7. Biological Activities and Applications

7.1 Antimicrobial and Antioxidant Properties

Both essential oils and volatile extracts of *Capsicum* spp. have considerable broad-spectrum antimicrobial and antioxidant activity associated mostly with terpenoids, phenols, and in smaller amounts sulfur-containing volatiles. They have been shown to inhibit in vitro various pathogens including Gram-positive bacteria (ex. *Staphylococcus aureus*), Gram-negative bacteria (ex. *Escherichia coli, Bacillus subtilis*), and pathogenic fungi (ex. *Aspergillus niger, Candida albicans*) (Mahmud et al., 2023; Rani et al., 2022). The antimicrobial activity of volatile extracts appears dependent on chemical or composition. For example, monoterpenes - linalool, β-myrcene, and α-pinene, exhibit significant antibacterial action through membrane disruption or inhibition of metabolism (de Oliveira et al., 2022). Antioxidant assays (DPPH, ABTS, FRAP) demonstrate that volatile extracts and essential oils of *Capsicum annuum* and *C. frutescens* possess considerable free radical scavenging activity, although they are generally less than dihydrocapsaicin-rich and capsaicin-rich non-volatile variables (Santos et al., 2023). Despite this, volatile extracts inhibiting lipid oxidation in model food systems indicate that volatiles could serve as natural preservatives that not only extend shelf life of food systems but also provide characteristic aroma of capsicum (Pino & Fuentes, 2021).

7.2 Flavor and Food Industry Applications

In addition to their biological significance, capsicum volatiles are essential in industrial and sensory contexts. The volatile fingerprint is now being used to distinguish cultivars, authenticate geographic origin, and evaluate the effects of processing in commercial chilli and paprika products (Kang et al., 2024). For example, specific volatiles such as (E)-2-hexenal (green freshness) and ethyl hexanoate (fruity sweetness) have been identified as trait markers for ripening and for quality in chilli and paprika products (Zhang et al., 2023).

Metabolomic datasets utilising GC×GC–TOF-MS and HS-SPME–GC–MS recently made chemometric models possible, synthesising volatile data to establish geographic origin and drying methods for validation (Rao et al., 2023). These assessments also contextualise quality control systems, fraud protection, and product development in the global spice and flavour sector.

The food industry is increasingly using volatile aroma-active components to formulate naturally sourced flavour enhancers and fragrances, specifically in processed foods, sauces, and snacks, where controlled thermal drying or roasting may alter the volatile profile to develop flavour complexity (Li et al., 2023).

7.3 Health and Nutraceutical Potential

Capsaicinoids (capsaicin and dihydrocapsaicin) have been well-studied concerning their pharmacological effects, such as analgesic, anti-inflammatory and thermogenic effects. In contrast, not much attention has been given to the bioactivities of *Capsicum* volatile fractions. Nonetheless, some terpenoids, such as β-caryophyllene and linalool, appear to have in vitro and in vivo anti-inflammatory, antimicrobial, anxiolytic and cytoprotective bioactivities (Mahmud et al., 2023; de Oliveira et al., 2022).

Considerable efforts exist with specific volatile-rich extracts and essential oils for topical use based on their volatility and low concentration biological activities (antimicrobial creams, aromatherapy oils, etc.) and even inhalation applications (Santos et al., 2023). However, the information that is available related to bioavailability, pharmacokinetics or safety systemically is limited. Further toxicological and mechanistic research is necessary before any nutraceutical recommendation can be made (Rani et al., 2022).

Table 4. Biological and Applied Relevance of Volatile Fractions in *Capsicum* spp.

Application Area	Bioactive Components / Key VOCs	Reported Effects / Uses	References
Antimicrobial activity	Linalool, β-myrcene, α-pinene, β-caryophyllene	Inhibits bacteria and fungi; membrane disruption	de Oliveira et al., 2022; Rani et al., 2022
Antioxidant potential	Terpenoids, phenolic volatiles	DPPH and FRAP scavenging; prevents lipid oxidation	Santos et al., 2023; Pino & Fuentes, 2021
Flavor and aroma applications	Esters (ethyl hexanoate), aldehydes ((E)-2-hexenal)	Flavor enhancement; aroma marker for ripening	Zhang et al., 2023; Rao et al., 2023
Food industry	Complex volatile blends	Cultivar selection; quality control; flavor standardization	Li et al., 2023; Kang et al., 2024
Health/nutraceutical	β-Caryophyllene, linalool	Anti-inflammatory, antimicrobial potential; topical/inhalation use	Mahmud et al., 2023; Santos et al., 2023

8. Research Gaps and Unknowns

8.1 Functional connection among VOCs, genes, and enzymes

We know about many odor-active, low-abundance volatiles in Capsicum, but relatively little is known at the levels of enzyme and genetics. Although transcriptome–metabolome studies have identified two gene family candidates (TPS, LOX, ADH, AAT, and BCAT), limited functional validation by heterologous expression, enzyme assays, or CRISPR/VIGS is a bottleneck for aroma-directed breeding and metabolic engineering (He et al., 2025; Morales Merida et al., 2024).

8.2 Standardized and quantitative datasets that link sensory data and VOCs

Variability in sample handling, HS-SPME parameters, and reporting units have inhibited linkages across studies. Most research is publicly semi-quantitative, and few use GC–O, sensory panels, or standard isotopes to identify aroma attribution, limiting industrial use and potential for meta-analysis (Louw et al., 2021; Grosch, 2001).

8.3 Environmental and agronomic factors (G × E)

There have been very few controlled studies that disentangled genotype × environment × management effects on a VOC. It is critical because when subjected to stress conditions such as salinity and drought, there are changes to capsaicinoids and primary metabolism; however, we do not have empirical data determining how these factors affect the volatilome, limiting progress to sustainably breed climate-resilient cultivars with aroma stability (Zamljen et al., 2022; Mahmood et al., 2021; Shams et al., 2023).

8.4 Processing–mechanistic studies

Specific studies elucidating the chemical kinetics for the Maillard and lipid oxidation pathways that underlie the influence of drying and heat processing on pepper VOC profiles are scarce; thus, these studies are warranted in order to provide mechanistic understanding to improve sought-after aroma retention in industrial processes (El Hosry et al., 2025; Chen et al., 2025; Xiong, 2024).

8.5 Industrial valorization and safety assessment

Although scalable and sustainable extraction and purification of volatile fractions of peppers remains the most underdeveloped area, almost all extracted volatile fractions were studied for stability, yield, and toxicological safety when utilized in concentrated extracts for flavoring and cosmetic assessment. Green extraction and extraction method safety protocols need to be established for commercialization issues (Gaikwad et al., 2024; Borah et al., 2024; Nabi et al., 2025).

9. Recommendations & Future Research Directions

9.1. Integrative Multi-Omics for Pathway Discovery

The emphasis must shift toward conducting coordinated multi-omics studies utilizing metabolomics, transcriptomics, and proteomics to establish connections from genes to metabolites in the biosynthesis of VOCs in Capsicum. Combining these datasets with the results of co-expression networks has the potential to pinpoint new terpene synthase (TPS) enzymes, lipoxygenases (LOX), and acyltransferases that are acting on the production of aroma-related volatile constituents. This approach will then lend itself to the subsequent functional validation of those genes through a multiplexed gene editing approach using CRISPR/Cas9 as well as virus-induced gene silencing (VIGS) techniques (Liu et al., 2023; Qiu et al., 2024).

9.2. Standardisation of Analytical Protocols

There is a large amount of variability in the extraction methods (e.g., HS-SPME fiber types, equilibration conditions, internal standards, etc.) between studies that constrain the direct comparability of results across studies. A standardized framework, similar to that which has been put forth for reporting in metabolomics, could also be implemented for VOC studies (Rosenthal et al., 2024). The use of benchmarking reference materials as well as inter-laboratory validation studies would also synergistically improve reproducibility and facilitate data to be pooled in meta-analyses.

9.3. Odour-Activity Focused Studies

Despite hundreds of VOCs having been detected, the majority of studies have not connected those to human sensory perception. Future studies should incorporate GC-olfactometry (GC–O), analysis of odor activity values (OAV), or olfactometry using aroma recombination/omission studies to determine the key odorants responsible for characteristic pepper aroma profiles (Pino et al., 2007; Rocha et al., 2022).

9.4. Environment × Genotype (G×E) Experimental Designs

Using controlled experiments to investigate genotype × environment interactions (temperature, salinity, water stress) will help clarify what environmental signals are used to inform VOC biosynthesis. Studies have demonstrated that abiotic stresses are capable of affecting LOX and

TPS pathway gene expression and have resulted in volatile changes (Aza-González et al., 2011; Khan et al., 2024).

9.5. Processing Chemistry and Gentle Technologies

Different drying methods (hot-air drying, sun drying, vacuum drying, freeze-drying) and processing temperatures affect both the retention and production of aroma volatiles. In the future, kinetic modelling combined with real-matrix trials will be useful in further elucidating the production pathways of pyrazines and furan. New drying technologies for aroma volatiles (microwave-vacuum, low temperature dehydration) will be explored (Li et al., 2023; Xu et al., 2022).

9.6. Data Sharing and Spectral Libraries

To aid reproducibility, chromatographic and raw mass spectral data should be deposited in online repositories such as MetaboLights or the MassBank of North America (MoNA). A spectral and retention index library that is specific to Capsicum should be created to help identify low-abundance odorants (Fiehn et al., 2008; MoNA, 2024).

10. Conclusion

Volatile organic compounds (VOCs) in Capsicum create a chemically unique system that is foundational to aroma, ecology, and economic importance. Recently, advances in GC–MS and GC×GC analytical techniques have demonstrated the diversity of terpenes, aldehydes, esters and nitrogen- or sulfur-containing volatiles shaped by genotype, development and their environment through MEP, LOX, and amino acid metabolism.

Despite recent advancements, significant gaps in metabolite profiles with gene expression, sensory attributes and bioactivity remain unanswered. Agency and mechanistic understanding are limited due to minimal integration of metabolomic, transcriptomic and proteomic levels. Furthermore, only a few studies have resolved any processing impacts or consumer perception. Future research priorities should include integrative multi-omics, consistency of VOC analysis, and open-access spectral libraries to enhance future comparisons. The analytical chemistry of Capsicum's green and spice flavors is a suitable point to substantiate odor-activity estimation through sensory validation, which will supply a more productive link with flavor science from breeding perpective, aroma and nutraceutical perspective.

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