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Review

The Volatile Composition, Biosynthesis Pathways, Breeding Strategies, and Regulation Measures of Apple Aroma: A Review

Yuying Tang [1,†], Yijun Yao [2,†], Yangyun Wu [1] and Shunbo Yang [1,*]

[1] School of Ecological Technology and Engineering, Shanghai Institute of Technology, Shanghai 201418, China; tayying08@163.com (Y.T.); 13816349570@163.com (Y.W.)
[2] Shanghai Wells Seed Co., Ltd., Shanghai 201899, China; yao_yj@wellsseed.com
* Correspondence: yangsb@sit.edu.cn
† These authors contributed equally to this work.

Abstract: Aroma is an important characteristic of apples, contributing significantly to fruit flavor and consumer acceptance. The aroma profile in apple fruits results from the interaction of multiple volatiles, including esters, alcohols, aldehydes, terpenoids, and others, which are mainly derived from the fatty acid, amino acid, terpenoid, and phenylpropanoid metabolic pathways. With progress in omics technologies, it is of practical significance to uncover the biosynthetic pathway and regulatory mechanism underlying the formation of volatiles, not only for elucidating the apple molecular mechanisms underlying key genetic pathways and in advancing the development of novel apple varieties with optimized fragrance profiles through precision breeding techniques. In this review, the aroma composition in apple fruits and the biosynthesis pathways for volatile formation are summarized. Furthermore, the breeding strategies with molecular techniques and the regulation measures about application engineering on apple aroma are also discussed. This review provides valuable insights for the improvement of apple aroma quality in the future.

Keywords: apple; aroma; biosynthesis pathway; breeding strategy; regulation measure

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1. Introduction

Apple (*Malus* × *domestica*), a globally distributed fruit crop, possesses a unique flavor, superior appearance, and nutritional value, which contribute significantly to its overall quality and consumer appeal. There are approximately 7500 to 8000 documented apple cultivars in the world, though only a fraction (around 100–200) are commercially significant. This count includes heirloom, regional, and wild varieties, with many preserved in gene banks or research orchards. In China, the cultivation of apples is of vital importance to the fruit industry, with an annual output of about 49 million tons, accounting for more than half of the world production. Aroma is a key quality attribute of fruits, and the significance of aroma in apple cannot be understated, as it is a crucial aspect that influences flavor characteristics and sensory quality [1,2]. Apple aroma is a complex trait, comprising a diverse array of volatile compounds that contribute to its distinctive and appealing scent [3,4]. These compounds, often referred to as aroma volatiles, are responsible for the unique sensory attributes that define the olfactory experience of an apple [5]. A diverse array of over 350 volatile compounds are produced by apple fruits, spanning esters, alcohols, aldehydes, carboxylic acids, ketones, and terpenoid derivatives [6]. However, it is only a subset of the 15–20 compounds (e.g., hexyl acetate, 2-methylbutyl acetate, hexyl hexanoate, butyl acetate, 1-hexanol, and (*E*)-2-hexenal) that make a significant contribution to the typical apple aroma [7,8]. The aroma compounds in apple fruits can be influenced by a multitude of

factors, ranging from genetic makeup to environmental conditions (e.g., temperature, light, soil) and harvest handling practices. The sensory expression of aroma strength hinges on both the structural complexity and quantitative ratios of signature compounds in volatile organic mixtures and differs among apple varieties [9,10]. The 'Golden Delicious', 'Fuji', 'Gala' and 'Granny Smith' apple cultivars are commonly used as controls or references due to their well-characterized aroma profiles. Nowadays, headspace solid-phase microextraction coupled with gas chromatography–mass spectrometry (HS-SPME-GC-MS) is a widely adopted method for apple aroma analysis due to its simplicity, speed, and sensitivity in extracting and identifying apple volatile compounds. Moreover, multiple analytical methods such as dynamic headspace sampling (DHS), solvent-assisted flavor evaporation (SAFE), proton transfer reaction mass spectrometry (PTR-MS), electronic nose (E-Nose) and GC-olfactometry (GC-O) can be employed to capture and characterize apple aroma [4,8]. In general, the current studies on apple aroma are marked by a multidisciplinary approach, such as plant biochemistry, molecular genetics, and sensory science. This integrated effort has led to significant advancements in identifying the key volatile compounds that contribute to apple aroma.

Volatile compounds are organic molecules characterized by their low boiling points and high vapor pressures at ambient temperatures, which allow them to evaporate readily and disperse into the air [11,12]. These compounds play a crucial role in the sensory attributes of various fruits, including apples [13]. In apple, the volatile compounds are responsible for fresh, fruity, and sometimes floral notes that enhance the overall eating experience [10,14]. These compounds are synthesized through a series of biochemical routes involving enzymes and precursors, and their production involves several metabolic pathways [15]. For example, aldehydes are primarily produced through the oxidative degradation of unsaturated fatty acids [16]. The production of alcohols often occurs via the oxidation of fatty acids or the decarboxylation of amino acids [17]. Alcohol acyltransferases (AATs) catalyze the conversion of various alcohols synthesized by these pathways into esters [18]. Terpenes are synthesized through the mevalonate (MVA) and methylerythritol phosphate (MEP) pathways, which generate isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP), the important building blocks for terpenoid biosynthesis [15,19]. Therefore, understanding these biosynthesis pathways is essential for developing strategies to improve apple aroma through molecular breeding and genetic engineering.

The genetic basis of apple aroma has been a focal point of numerous studies, revealing intricate relationships between specific genes and the production of volatile compounds that contribute to the characteristic scent of apples [20,21]. Transcriptomic analyses, such as RNA sequencing (RNA-seq), can identify changes in gene expression patterns in response to genetic modifications, while metabolomic profiling can reveal alterations in the levels of volatile compounds [22,23]. Genomics has provided valuable insights into the regulatory networks governing volatile compound production in apples [24,25]. Obtaining nuclear genomes of diverse apple varieties enables the precise identification of structural genes and regulatory elements governing volatile biosynthesis. Comparative genomics reveals single nucleotide polymorphisms (SNPs), copy number variations (CNVs), and transposons linked to aroma divergence, while genome-guided metabolic networks map enzyme–substrate relationships. By correlating these data, researchers can gain insights into the regulatory networks governing volatile compound biosynthesis in apple. Furthermore, the regulation of volatile compound production in apples involves an intricate interplay between genetic and environmental factors. In addition, optimizing growing conditions, such as adjusting temperature, light, water, and agricultural practice or postharvest management, can further enhance volatile compound production. Integrating these approaches with traditional breeding methods holds promise for developing new apple varieties with

superior aroma characteristics. This review summarized recent progress in the volatile composition, biosynthesis pathways, breeding strategies and regulation measures in apple, with the purpose of providing a robust foundation for advancing the understanding of the complex interplay between genetics, metabolism, and environment in shaping the aroma of apples. These findings have significant implications for both fundamental research and applied breeding programs, ultimately contributing to the improvement of apple flavor quality and consumer satisfaction.

2. Volatile Composition in Apple Fruit

Apple aroma, a complex and captivating sensory attribute, plays a pivotal role in determining the flavor quality and consumer acceptance. The aroma of apples is a mixture composition of various volatile compounds, which produced within the fruit during ripening and maturation, including esters, alcohols, and aldehydes, among others. Aldehydes, such as hexanal and 2-hexenal, are produced at relatively high amounts in immature apple fruits [26]. During apple maturation, the volatile organic compound (VOC) profile undergoes a compositional shift from aldehyde-dominant to alcohol-enriched profiles, with 2-methyl-1-butanol emerging as the predominant species alongside elevated 1-butanol, 1-hexanol and 1-propanol concentrations [5]. The characteristic ripe aroma arises primarily from even-carbon-numbered esters derived from biosynthetic coupling between short-chain carboxylic acids (acetic/butanoic/hexanoic acids) and alcohol moieties (ethyl/butyl/hexyl groups), forming the core aroma-active metabolites in mature fruits [18]. These compounds, when combined, create the distinctive and recognizable scent that is synonymous with fresh apples. Each apple variety possesses a unique aroma profile, contributing to its specific flavor and appeal. In the 'Fuji' apple, the compounds contributing mostly to the characteristic aroma were ethyl 2-methylbutanoate (67.1 μg/kg), 2-methylbutyl acetate (200.4 μg/kg), and hexyl acetate (9.9 μg/kg) [27]. Meanwhile, hexyl acetate (549.31 μg/kg), hexyl 2-methylbutanoate (382.45 μg/kg), hexyl hexanoate (187.67 μg/kg), hexyl butanoate (142.74 μg/kg), 2-methylbutyl acetate (255.15 μg/kg), and butyl acetate (196.75 μg/kg) were the prominent volatiles produced by the 'Pink Lady' fruit [28]. During the ripening period, 1-butanol-2-methyl-acetate, 2-hexenal, and 1-hexanol were the most abundant aroma components in the 'Starkrimson' apple [29]. Moreover, hexyl 2-methylbutyrate, hexanol, 2-methyl-butanol, hexanal, (*E*)-2-hexenal, and α-farnesene were detected the most potent odor compounds in the 'Honeycrisp' apple [5]. By principal component analysis (PCA), the research conducted by Zhu et al. [4] showed that hexyl butanoate, (*E*)-2-hexenal and α-farnesene were the vital volatile compounds in 'Ralls', 'Jonagold', 'Orin', 'Indo', and 'Hanfu' apples. Yang et al. [8] evaluated the aroma profiles in 85 apple cultivars and found that the compounds of hexyl acetate, butyl acetate, 2-methylbutyl acetate, 1-hexanol, and (*E*)-2-hexenal were the most abundant volatiles. With Shapley's additive explanatory value analysis, Shimizu et al. identified ethyl 2-methyl butyrate, 2-methylbutyl acetate, α-farnesene, and (*Z*)-3-hexenol as the key aroma compounds among 174 apple genotypes [30]. These efforts focused on identifying the chemical compounds responsible for the distinct scents emitted by different apple varieties. Over time, it became clear that apple aroma was not the result of several compounds but rather a complex mixture of volatiles that interact to create a unique olfactory profile.

3. Biosynthesis Pathways of Apple Volatiles

Volatile compounds of apples play a crucial role in determining their aroma and flavor, which are key attributes influencing consumer preference and market value. These compounds mainly synthesized from fatty acid, amino acid, and carbohydrate metabolism by the specific pathways (Figure 1). The biosynthesis of straight-chain aldehydes, alcohols,

and esters originates from lipid precursors (predominantly linolenic/linoleic acids) via β-oxidative cleavage coupled with lipoxygenase (LOX) mediated catalysis [16]. Branched-chain variants specifically emerge through the isoleucine catabolic cascade, forming characteristic secondary metabolites [31]. Terpenoid production operates through dual enzymatic systems involving both mevalonate and methylerythritol phosphate metabolic frameworks, while phenylpropanoid compounds are generated via the shikimic acid-derived phenylpropanoid network [17,32].

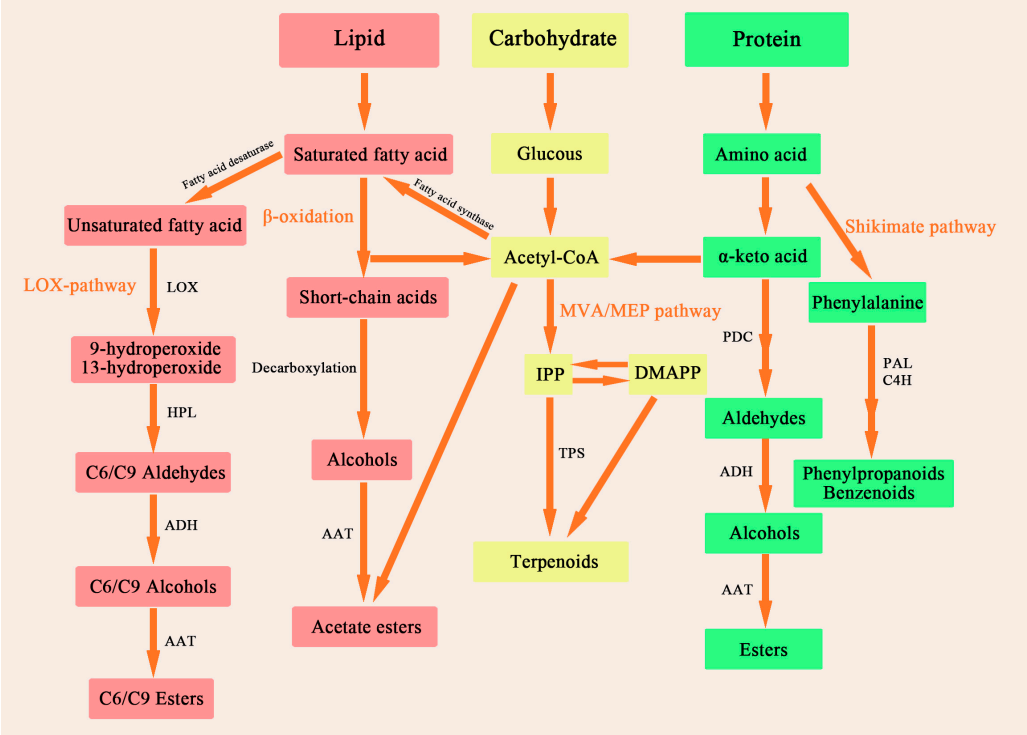


Figure 1. Main formation pathways of volatile compounds in apple fruit. Abbreviations: PDC, pyruvate decarboxylase; ADH, alcohol dehydrogenase; AAT, alcohol acyltransferase; TPS, terpene synthases; LOX, lipoxygenase; HPL, hydroperoxide lyase; DMAPP, dimethylallyl pyrophosphate; IPP, isopentenyl pyrophosphate; PAL, phenylalanine ammonia lyase; and C4H, cinnamate 4-hydroxylase.

3.1. Fatty Acid-Derived Compounds

Fatty acids are the major precursors of aroma volatiles in apple fruit. β-Oxidation and the lipoxygenase (LOX) pathway are both the main enzymatic systems in the catabolism of fatty acids for the formation of straight-chain aldehydes, alcohols, ketones, acids, and esters ranging from C1 to C20, which are apple important character-impact aroma compounds [17]. Aroma volatile compounds in intact apple fruit are formed via the β-oxidation biosynthetic pathway, whereas, when fruit tissue is disrupted, volatiles are produced though the LOX pathway [33]. Nevertheless, at the stage of apple ripening, the increasing availability of fatty acid, along with higher membrane permeability and the breakdown of chloroplasts might allow the LOX pathway to make an alternative to β-oxidation of the whole fruit [34].

β-Oxidation is a major process for the degradation of fatty acids to the formation of flavor molecules, and,, in plants it is mainly performed in peroxisomes [35]. During β-oxidation, fatty acids are activated to their corresponding acyl coenzyme A (CoA) by acyl-CoA synthase. Fatty acid acyl-CoA derivatives can be converted to shorter chain acyl CoAs by losing two carbons in each round of the β-oxidation cycle, requiring flavinadenine dinucleotide (FAD), nicotinamide adenine dinucleotide (NAD), and free CoA. Acyl CoAs are reduced by acyl CoA reductase to aldehyde and then reduced to alcohol by alcohol

dehydrogenase (ADH) for alcohol acyltransferase (AAT) to produce esters [36]. Specifically, the ester production relies upon the supply of acyl CoAs formed during β-oxidation and alcohols. AATs are capable of combining various alcohols and acyl CoAs, resulting in the synthesis of a wide range of esters, thus accounting for the diversity of esters [17].

The LOX pathway is also important for the production of aroma compounds in apple fruit (Figure 2). Linoleic (C18:2) and linolenic (C18:3) acids are the main substrates of LOX in plant tissues [37]. Depending on the carbon targeted for deoxygenation in the polyunsaturated fatty acid, LOX enzymes are classified into two major subfamilies, 9- and 13-LOXs, corresponding to the production of unsaturated 9- or 13-hydroperoxides [15]. Thereafter, 9-hydroperoxide lyase (HPL) and 13-hydroperoxide lyase convert 9- and 13-hydroperoxides to C9 and C6 aldehydes, respectively, which are commonly referred to as green leaf volatiles [15,37]. Moreover, Jasmonic acid, acting as phytohormones, can be generated from 13-hydroperoxy through a separated branch via the production of an unstable epoxide by allene oxide synthase, followed by a series of cyclization reduction reactions [38,39]. Finally, these C9 and C6 aldehydes are metabolized by ADH to form the corresponding C9 and C6 alcohols, followed by further conversion to their esters via the action of AAT [40,41]. Subsequently, the divergence of the esters is dependent on substrate availability, enzyme specificity, and variation in the AAT gene [33].

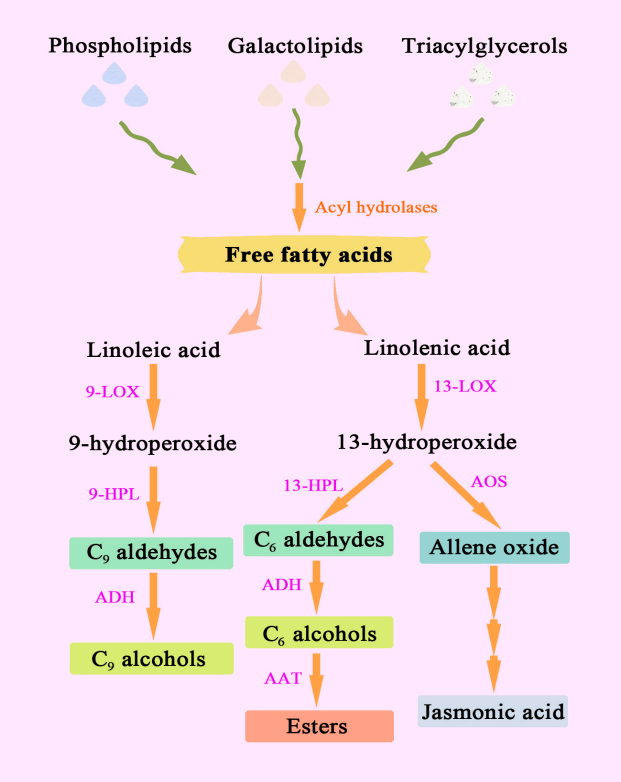


Figure 2. The biosynthetic pathway of fatty acid derived compounds. Abbreviations: AOS, allene oxide synthase; 9-LOX, 9-lipoxygenase; 13-LOX, 13-lipoxygenase; 9-HPL, 9-hydroperoxide lyase; and 13-HPL, 13-hydroperoxide lyase.

3.2. Amino Acid-Derived Compounds

There are a lot branched-chain volatiles in apple fruit that are derived from amino acids such as serine, valine, leucine, alanine, isoleucine, and methionine, or intermediates in their biosynthesis [15]. The biosynthesis of amino acid-derived compounds in plants is thought to be similar to that in bacteria or yeast, where these pathways have been studied more extensively [42,43]. Amino acids undergo an initial deamination or transamination

catalyzed by aminotransferases, which are the key intermediates to convert amino acids into volatiles, leading to the formation of the corresponding α-keto acids [44]. These α-keto acids can be further decarboxylated, followed by reduction or oxidation to form aldehydes, acids, or acyl-CoA in the substrate of α-keto acid decarboxylase or α-keto dehydrogenase (Figure 3). Subsequently, aldehyde and acyl-CoA are used in alcohol esterification reactions by AAT and finally converted to ester compounds [45].

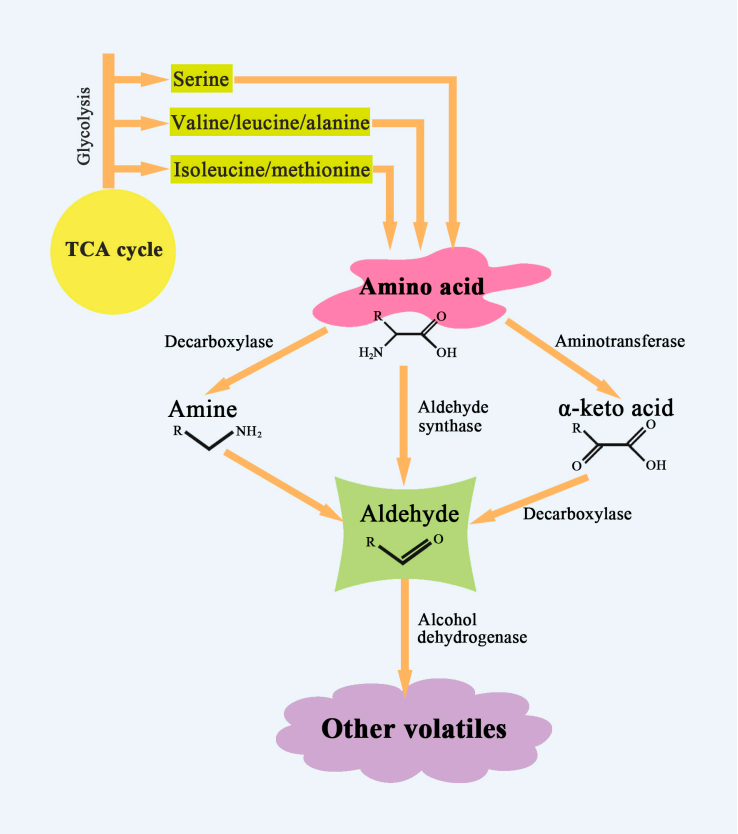


Figure 3. Synthesis of volatile compounds derived from amino acids.

3.3. Terpenoid Pathway

Terpenoids are the most abundant secondary metabolites in plants with many volatile constituents, and enzymatically synthesized from acetyl CoA and pyruvate provided by the carbohydrates [17]. The production of terpenoids is mediated by two parallel pathways, the mevalonate (MVA) pathway operating in the cytosol and the methylerythritol phosphate (MEP) pathway active in the plastids (Figure 4). The MVA pathway gives rise to volatile triterpenes and sesquiterpenes, while the MEP pathway provides precursors to volatile hemiterpenes, monoterpenes, diterpenes, and tetraterpenes [19]. Both MVA and MEP routes result in the formation of IPP and its isomer DMAPP. Initial research indicated that the cytosolic IPP serves as a precursor of farnesyl diphosphate (FPP) for sesquiterpenes and triterpenes, whereas the plastidial IPP provides the precursors for geranyl diphosphate (GPP) and geranylgeranyl diphosphate (GGPP) for mono-, di-, and tetra-terpenes [46]. However, cross-talk between these two IPP biosynthetic pathways is prevalent, particularly in the direction from plastids to cytosol [47]. In ripe apple fruit, the acyclic branched sesquiterpene α-farnesene is the most prominent terpenoid [48–50]. Other terpenes are found at low levels in fruit and vegetative tissues of apples.

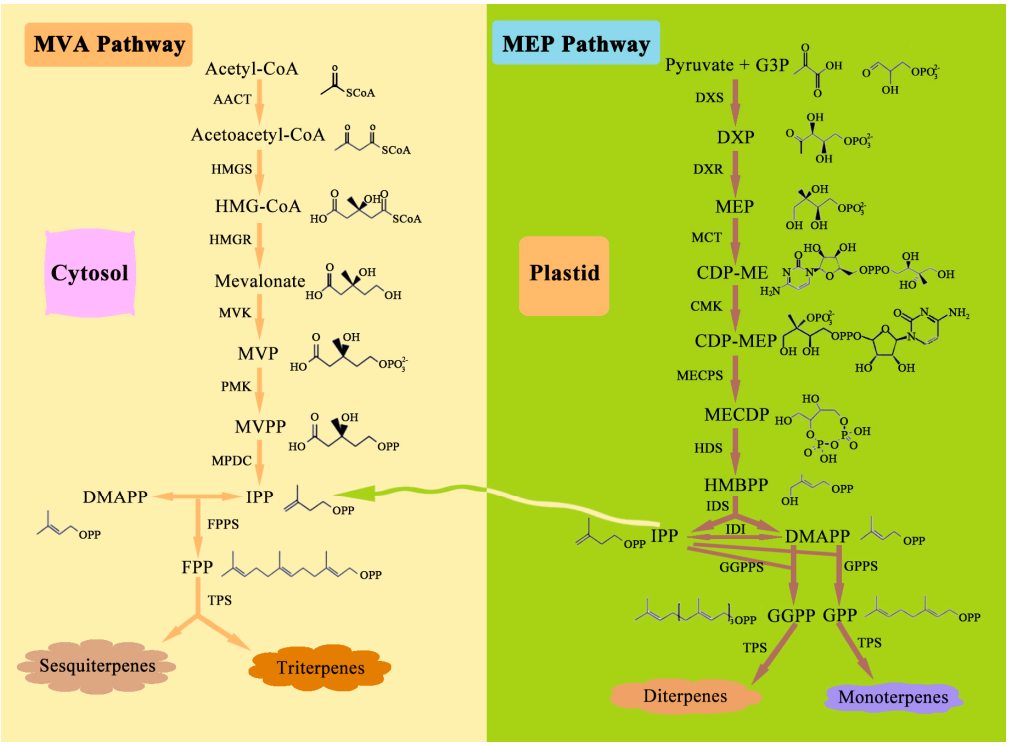


Figure 4. Synthesis of terpenoid volatile compounds. Abbreviations: AACT, acetyl-CoA acetyltransferase; HMGS, HMG-CoA synthase; HMG-CoA, hydroxymethylglutaryl CoA; HMGR, HMG-CoA reductase; MVK, mevalonate kinase; MVP, mevalonate 5-phosphate; PMK, phosphomevalonate kinase; MVPP, mevalonate 5-pyrophosphate; MPDC, mevalonate diphosphate decarboxylase; FPP, farnesyl pyrophosphate; FPPS, FPP synthase; G3P, glyceraldehyde 3-phosphate; DXP, 1-deoxy-D-xylulose 5-phosphate; DXS, DXP synthase; DXR, 1-deoxy-D-xylulose 5-phosphate reductoisomerase; CDP-ME, 4-diphosphocytidyl-2-C-methyl-D-erythritol; CDP-MEP, CDP-ME 2-phosphate; MECDP, 2-C-methyl-D-erythritol 2,4-cyclodiphosphate; HMBPP, (*E*)-4-hydroxy-3-methylbut-2-en-1-yl diphosphate; HDS, 4-hydroxy-3-methylbut -2-en-1-yl diphosphate synthase; IDS, isopentenyl diphosphate synthase; IDI, isopentenyl pyrophosphate isomerase; GPPS, GPP synthase; GGPPS, GGPP synthase; GGPP, geranylgeranyl pyrophosphate; GPP, geranyl pyrophosphate; and TPS, terpene synthase.

3.4. Phenylpropanoid Pathway

Phenylpropanoids that contain a reduced carboxyl group at C9 or an alkyl addition to hydroxyl groups of the benzyl ring or to the carboxyl group (esters and ethers) are considered volatile compounds [47]. The phenylpropanoid pathway, primarily derived from phenylalanine (Phe), is an anabolic pathway that requires several enzymatic reactions including phenylalanine ammonia-lyase (PAL), cinnamate 4-hydroxylase (C4H) 4-coumaroyl-CoA ligase (4CL), and o-methyltransferase (OMT) [51,52]. In apple, the compounds of phenylpropenes, such as eugenol, estragole and isoestragole, also contribute to apple flavor and aroma, which are derived from the shikimate pathway [53]. Previous studies have reported that estragole imparts a spicy and aniseed flavor to apple varieties like 'Royal Gala', 'Ellison's Orange', 'Ralls', and 'Orin' [4,53,54].

4. Breeding Strategies on Apple Aroma

Molecular techniques have revolutionized the approach to improving apple cultivars, particularly in enhancing traits such as aroma. These techniques leverage advanced genetic tools and methodologies to identify and manipulate specific genes responsible for desirable characteristics, thereby accelerating the breeding process and ensuring more precise outcomes. One of the fundamental principles of molecular breeding is the use of molecular markers, which are DNA sequences associated with specific traits. By identifying these

markers, breeders can screen large populations of plants for the presence of desired genes without waiting for the phenotypic expression of those traits.

In apple breeding, molecular markers have been instrumental in identifying genes related to aroma. SSR (Simple Sequence Repeat) and SNP markers are both pivotal tools for assessing genetic divergence among apple cultivars. While SSR markers, often located in non-coding regions, reflect genetic diversity and kinship through linkage disequilibrium (e.g., associations with ester-synthesis genes like *AAT*), SNP markers directly target coding or regulatory regions, enabling the precise identification of functional variants (e.g., LOX gene SNPs linked to hexanal production in 'Granny Smith'). Moreover, the use of SSR/SNP markers to screen the parents carrying the target aroma genes can accelerate the cultivation of new varieties with outstanding aroma. In addition, studies have identified quantitative trait loci (QTLs) associated with volatile compound biosynthesis, which are crucial for the development of aromatic apples. These QTLs are regions of the genome that contain genes influencing the production of volatile compounds, which contribute significantly to the aroma profile of apples. By mapping these QTLs, researchers can select parent plants with high levels of volatile compound-producing genes, thereby increasing the likelihood of producing offspring with enhanced aroma. Several studies have identified molecular markers associated with volatile compound biosynthesis in apples. Based on the saturated genetic linkage map, Zini et al. reported preliminary results of volatile QTL detection in the 'Fiesta' × 'Discovery' apple progeny [55]. Using headspace solid-phase microextraction (HS-SPME) coupled with gas chromatography–mass spectrometry (GC-MS), Dunemann et al. investigated QTL mapping related to aroma compounds in the apple progeny 'Discovery' × 'Prima' and found that the QTLs were mainly clustered on linkage groups (LG) 2, 3, and 9 [56]. Through further research, it was found that the candidate gene encoding an AAT enzyme located on LG 2 was responsible for the impact of four ester compounds (pentyl acetate, butyl acetate, hexyl acetate, and 2-methyl-butyl acetate) in apple fruit. Moreover, Costa et al. validated a set of QTLs associated with apple volatiles in three different environments of Switzerland and confirmed that QTLs relevant to esters and hormone ethylene were located on LG 2 and 15, respectively [57]. Souleyre et al. scanned 46 QTLs for ester and alcohol production in the 'Royal Gala' × 'Granny Smith' population and found the major QTL for ester volatiles was located on LG 2, co-located with *AAT1*, which catalyzes the biosynthesis of ester compounds, contributing to the 'ripe apple' flavor [58]. With the release of the 'Golden Delicious' apple new reference genome [59], aroma profiles in ripe apple fruit of 'Fuji' × 'Cripps Pink' were evaluated, and 87 QTLs were detected for 15 volatile compounds on 14 linkage groups [60]. Despite the significant advancements in marker-assisted selection (MAS) for apple aroma, there are still challenges to overcome. One of the primary challenges is the identification of markers that are consistently associated with aroma traits across different genetic backgrounds and environments. Environmental factors, such as temperature and soil conditions, can influence the expression of volatile compounds, making it essential to validate markers under diverse conditions. Additionally, the complexity of the genetic architecture underlying aroma traits necessitates the identification of multiple markers and the development of multi-locus models to capture the full range of genetic variation.

Moreover, the integration of genomic and transcriptomic data has further refined molecular breeding approaches. High-throughput sequencing technologies, such as RNA-seq, have enabled the identification of differentially expressed genes (DEGs) involved in volatile compound biosynthesis. By analyzing the expression patterns of these genes across different developmental stages and environmental conditions, researchers can gain insights into the regulatory mechanisms controlling aroma production. This information can be used to develop more sophisticated breeding strategies, such as the selection of parents with

complementary gene expression profiles to maximize the inheritance of favorable aroma traits. LOX is an important contributor to the formation of aroma-active C6 aldehydes in apple fruit upon tissue disruption. Schiller et al. explored the expression of 22 putative LOX genes in apple fruit and found that the *MdLOX1* showed a high expression level throughout ripening and probably regulated the precursors for ester production [61]. Vogt et al. reported that the *MdLOX2b* and *MdLOX5e* genes were up-regulated during fruit ripening in the 'Golden Delicious' apple, while *MdLOX1a* was detected only at the last ripening stage of the 'McIntosh' apple [62]. In the research conducted by Yang et al., twelve *MdLOX* genes showed significantly higher expression levels in the 'Jonagold' than that in the 'Granny Smith' apple, and, combined with the measurement results of volatile profiles, the up-regulated expression pattern of *MdLOX* genes might promote the volatile accumulation [23]. AAT, as the vital enzyme involved in the last step of ester synthesis, catalyzes the transfer of an acyl group from the CoA donor to an alcohol acceptor. Zhu et al. characterized and identified a few *AAT* genes for their putative expression patterns and biochemical functions, and they reported that the gene expression levels of *MdAAT1* and *MdAAT2* increased with apple fruit ripening, consistent with the total ester content accumulation pattern [20]. In a previous study conducted by Souleyre et al. [63], the *MdAAT1* gene was shown to be responsible for the synthesis of the esters (hexyl acetate, butyl acetate, and 2-methyl-butyl acetate) in the 'Royal Gala' apple fruit. Li et al. isolated a highly homologous gene *MdAAT2* from the 'Golden Delicious' apple, which was also found to be positively correlated with AAT enzyme activity and the ester formation [64]. Dunemann et al. reported four SNPs of *MdAAT1* linked to variation in ester production, and the 468 bp region was used to screen a set of apple cultivars for association with ester content [18]. Furthermore, the regulatory role of transcription factors (TFs) in aroma compound synthesis has been established in apple. Li et al. investigated the volatile profiles and transcriptomes of the 'Qinguan' apple fruit during development and verified that the MdMYB94 transactivated the *MdAAT2* promoter and participated in the regulation of ester biosynthesis [65]. With transcriptional and epigenetic analysis, the apple TF MdNAC5 was proved to activate *MdAAT1* transcription via binding to its promoter, regulating the biosynthesis of flavor-related esters [66]. The investigation performed by Li et al. revealed that MdMYC2 and MdMYB85 can directly interact with the promoter region of *MdAAT1*, thereby enhancing its transcriptional activity to promote ester synthesis in apples [67]. Additionally, epigenetic modifications, such as DNA methylation and histone acetylation, have also been implicated in the regulation of gene expression related to volatile compound biosynthesis.

Overall, molecular techniques have significantly advanced the field of apple improvement, particularly in the area of aroma enhancement. By combining the precision of molecular markers, the power of genetic engineering, and the depth of genomic and transcriptomic analyses, breeders can develop apple varieties with superior aroma in the future.

5. Regulation Measures for Apple Aroma

The regulation of apple aroma is a crucial aspect in ensuring the optimal quality and consumer appeal of this popular fruit. Various measures including environmental condition, agricultural practice, and postharvest management have been explored to manipulate apple aroma, aiming to enhance or maintain its desirable scent profile.

5.1. Environmental Condition

Environmental conditions play a crucial role in regulating apple aroma, influencing both the production and quality of the fruit scent. Temperature, in particular, is a significant

determinant in the biosynthesis of aroma compounds. Apple aroma, primarily shaped by esters, aldehydes, and alcohols, is temperature-sensitive across growth, storage, and processing stages. Optimal growth temperatures (20–25 °C) enhance the apple aroma volatile precursor accumulation, while excessive heat reduces aldehyde synthesis (e.g., hexanal) and accelerates ripening, potentially diminishing freshness. Postharvest, low temperatures (0–2 °C) inhibit ester production (e.g., ethyl butanoate) but preserve aldehydes, whereas ambient storage (20 °C) promotes esterification and aroma intensity. In addition, temperature can regulate the activity of apple aroma components through enzyme activity (e.g., LOX and ADH). A previous study has shown that optimal temperature ranges during apple maturation can enhance the production of certain esters like 2-methyl butyl acetate, butyl 2-methyl butanoate, and hexyl acetate, which contribute to the fruity aroma of apples [68]. Conversely, extreme temperatures can inhibit the formation of these compounds, negatively impacting the aroma profile. Light exposure is another key environmental factor. Adequate sunlight during the growing season promotes the synthesis of photosynthetic pigments and other compounds that indirectly affect aroma. For instance, increased light intensity has been linked to higher levels of ethylene, resulting in a more intense aroma [69]. Moreover, soil quality and moisture content also contribute to apple aroma. Soil rich in organic matter and essential nutrients supports healthy plant growth, which in turn affects the quality and quantity of apple fruits [70]. Similarly, maintaining an optimal level of soil moisture is vital for ensuring proper apple fruit development and aroma formation [71].

5.2. Agricultural Practice

Agricultural practices also play a significant role in regulating apple aroma. For instance, controlled irrigation, fertilization, and bagging can influence the concentration and composition of aroma compounds in apples. By adjusting these agricultural inputs, farmers can potentially optimize the aroma profile of apples. Deficit irrigation could enhance the apple aroma volatiles quantitatively in terms of concentrations and qualitatively in terms of odor units [72]. Given that nitrogen and calcium did not result in significantly higher levels of N and Ca contents, the content of hexanal was found increased in the treated apple fruits. In addition, the cultivation technique of apple bagging can protect the fruit from pests, diseases, and birds and reduce the amount of insecticides or fungicides on the fruit surface. It will also affect the intrinsic qualities of apple fruits, such as sugar, acid, and aroma. The research conducted by Wang et al. showed that the shading effect after bagging inhibited the photosynthesis on the apple fruit, leading to a reduction in the content of sugars, acids, phenolics, and aroma compounds in the apple fruit [73]. Understanding and managing these agricultural practices is essential to optimize the production of apples with desirable aromas.

5.3. Postharvest Management

Postharvest management also plays a crucial role in influencing apple aroma, as they can significantly affect the quality and shelf life of the fruit. One such practice is storage conditions, which have a direct impact on the preservation and development of apple aroma. Optimal storage temperatures, typically between 0 °C and 4 °C, are essential to maintain the freshness and aroma of apples [74]. Another important postharvest management practice is the use of controlled atmosphere storage (CAS). This technology involves modifying the composition of the atmosphere within the storage facility to reduce oxygen levels and increase carbon dioxide concentrations. By slowing down the respiratory rate of the apples, CAS can extend their shelf life and preserve their aroma for longer periods. Notably ultra-low oxygen ($p(O_2) < 1$ kPa) conditions could lead to reducing of the synthesis about the linear chain volatile compounds due to the reduction in the concentrations of alcohols

and esters in apples [75]. Moreover, 1-methylcyclopropene (1-MCP) treatment was widely used in the storage of apple fruits. As an inhibitor of ethylene perception, 1-MCP prevents ethylene-induced ripening by binding to ethylene receptors, resulting in the reduction in apple aroma production [14,76]. Postharvest treatments such as irradiation can also affect apple aroma. Ultraviolet-C (UV-C) irradiation, although effective in extending shelf life and reducing microbial growth, can sometimes have undesirable effects on apple aroma [77]. Therefore, it is important to carefully assess the benefits and drawbacks of using irradiation as a postharvest treatment. Overall, postharvest management that focuses on maintaining optimal storage conditions, utilizing controlled atmosphere storage, and some postharvest treatments can significantly influence apple aroma. By implementing these practices, it is possible to preserve or enhance the aroma of apples, ensuring the full flavor experience of apple fruits.

6. Issues and Prospects

The studies in the field of volatile compound biosynthesis, aroma molecular breeding, and the genetic basis of apple aroma hold significant promise for advancing both the scientific understanding and practical applications in agriculture and food industries. One critical area that warrants further exploration is the identification and characterization of novel enzymes or regulatory genes related to the biosynthesis of volatiles. While considerable progress has been made in elucidating key pathways, many gaps remain in our knowledge of the specific enzymes and transcription factors that control these processes. High-throughput sequencing technologies and functional genomic approaches can be leveraged to identify new candidate genes and validate their roles in volatile compound production. By combining data from these different levels, researchers can gain insights into the regulatory networks and metabolic pathways that govern aroma production.

Another important direction is the development of more precise and efficient molecular breeding techniques. Current MAS methods have shown promise in improving apple aroma, but there is room for enhancement. Next-generation sequencing technologies can facilitate the discovery of additional molecular markers linked to aroma traits, enabling the more accurate and rapid selection of desirable genotypes. Additionally, genome editing tools such as CRISPR-Cas9 offer the potential to precisely modify genes involved in aroma biosynthesis, allowing for the creation of apple varieties with enhanced aromatic profiles. Advanced artificial intelligence frameworks integrated with machine learning techniques enabled us to analyze large datasets and predict the effects of genetic modifications on volatile compound profiles, thereby optimizing breeding strategies.

Environmental factors also play a crucial role in the production of volatile compounds in apples. Future studies should focus on elucidating the interactions between genetic and environmental factors, particularly in the context of climate change. Understanding how temperature, light, water availability, and soil conditions influence volatile compound biosynthesis can inform the development of cultivation practices that maximize aroma quality. Field trials and controlled environment experiments can be used to assess the impact of various environmental conditions on volatile compound production and to identify genotypes that are resilient to changing climates.

Moreover, consumer preferences and market demands are evolving, and there is a growing interest in apples with unique and diverse aroma profiles. Future research should explore the genetic diversity within wild apple species and traditional cultivars to identify novel sources of aroma traits. This can be achieved through germplasm collection and evaluation programs, which can help in the discovery of new alleles and haplotypes associated with desirable aroma characteristics. Collaborative efforts between breeders, geneticists, and sensory scientists can ensure that the developed apple varieties not only

possess enhanced aroma but also meet consumer expectations in terms of taste, texture, and appearance.

Finally, the commercialization of new apple varieties with improved aroma requires careful consideration of intellectual property rights, regulatory compliance, and market acceptance. Future research should address these issues by developing strategies for protecting new cultivars, ensuring that they meet safety and quality standards, and promoting their adoption by growers and consumers.

7. Conclusions

Apple aroma is a complex yet crucial element in the sensory experience of consuming apples. This aromatic quality not only influences consumer preferences but also plays a pivotal role in the overall marketability and economic value of apple varieties. As the demand for high-quality fruits continues to grow, understanding the intricate mechanisms behind apple aroma has become increasingly important. This review outlines the current knowledge on the formation mechanisms of apple aroma, summarizes the biosynthesis pathway of apple volatiles, analyzes the molecular strategies on improving flavor quality, and discusses the potential regulatory measures to enhance or preserve desirable aromas, aiming to provide the valuable perspectives for future improving aroma quality of apples.

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References

1. Harker, F.R.; Kupferman, E.M.; Marin, A.B.; Gunson, F.A.; Triggs, C.M. Eating quality standards for apples based on consumer preferences. *Postharvest Biol. Technol.* **2008**, *50*, 70–78. [CrossRef]
2. Klee, H.J. Improving the flavor of fresh fruits: Genomics, biochemistry, and biotechnology. *New Phytol.* **2010**, *187*, 44–56. [CrossRef] [PubMed]
3. Abbott, J.A.; Saftner, R.A.; Gross, K.C.; Vinyard, B.T.; Janick, J. Consumer evaluation and quality measurement of fresh-cut slices of 'Fuji', 'Golden Delicious', 'GoldRush', and 'Granny Smith' apples. *Postharvest Biol. Technol.* **2004**, *33*, 127–140. [CrossRef]
4. Zhu, D.; Ren, X.; Wei, L.; Cao, X.; Ge, Y.; Liu, H.; Li, J. Collaborative analysis on difference of apple fruits flavour using electronic nose and electronic tongue. *Sci. Hortic.* **2020**, *260*, 108879. [CrossRef]
5. Yan, D.; Shi, J.; Ren, X.; Tao, Y.; Ma, F.; Li, R.; Liu, X.; Liu, C. Insights into the aroma profiles and characteristic aroma of 'Honeycrisp' apple (*Malus* × *domestica*). *Food Chem.* **2020**, *327*, 127074. [CrossRef]
6. Song, J.; Forney, C.F. Flavour volatile production and regulation in fruit. *Can. J. Plant Sci.* **2008**, *88*, 537–550. [CrossRef]
7. Dixon, J.; Hewett, E.W. Factors affecting apple aroma/flavour volatile concentration: A review. *N. Z. J. Crop Hortic. Sci.* **2000**, *28*, 155–173. [CrossRef]
8. Yang, S.; Meng, Z.; Fan, J.; Yan, L.; Yang, Y.; Zhao, Z. Evaluation of the volatile profiles in pulp of 85 apple cultivars (*Malus domestica*) by HS-SPME combined with GC-MS. *J. Food Meas. Charact.* **2021**, *15*, 4215–4225. [CrossRef]
9. Young, J.C.; Chu, C.; Lu, X.; Zhu, H. Ester variability in apple varieties as determined by solid-phase microextraction and gas chromatography-mass spectrometry. *J. Agric. Food Chem.* **2004**, *52*, 8086–8093. [CrossRef]
10. Aprea, E.; Corollaro, M.L.; Betta, E.; Endrizzi, I.; Dematte, M.L.; Biasioli, F.; Gasperi, F. Sensory and instrumental profiling of 18 apple cultivars to investigate the relation between perceived quality and odour and flavour. *Food Res. Int.* **2012**, *49*, 677–686. [CrossRef]

11. Liu, Z.; Wang, M.; Wu, M.; Li, X.; Liu, H.; Niu, N.; Li, S.; Chen, L. Volatile organic compounds (VOCs) from plants: From release to detection. *TrAC-Trends Anal. Chem.* **2023**, *158*, 116872. [CrossRef]
12. Haick, H. Advances in volatile organic compounds detection: From fundamental research to real-world applications. *Appl. Phys. Rev.* **2024**, *11*, 040401. [CrossRef]
13. Bizzio, N.; Tieman, M.; Munoz, P. Branched-chain volatiles in fruit: A molecular perspective. *Front. Plant Sci.* **2022**, *12*, 814138. [CrossRef] [PubMed]
14. Shoffe, A.; Rudell, D.; Park, D.; Algul, E.; Qin, M.; Shi, M.; Dando, R.; Watkins, B. Aroma volatiles and sensory quality of organically-grown apple cultivars after dynamic controlled atmosphere (DCA) storage and comparison with CA-stored fruit with and without 1-methylcyclopropene (1-MCP). *Postharvest Biol. Technol.* **2024**, *218*, 113162. [CrossRef]
15. Dudareva, N.; Klempien, A.; Muhlemann, J.K.; Kaplan, I. Biosynthesis, function and metabolic engineering of plant volatile organic compounds. *New Phytol.* **2013**, *198*, 16–32. [CrossRef] [PubMed]
16. Rowan, D.D.; Allen, J.M.; Fielder, S.; Hunt, M.B. Biosynthesis of straight-chain ester volatiles in Red Delicious and Granny Smith apples using deuterium-labeled precursors. *J. Agric. Food Chem.* **1999**, *47*, 2553–2562. [CrossRef]
17. Schwab, W.; Davidovich-Rikanati, R.; Lewinsohn, E. Biosynthesis of plant-derived flavor compounds. *Plant J.* **2008**, *54*, 712–732. [CrossRef]
18. Dunemann, F.; Ulrich, D.; Malysheva-Otto, L.; Weber, W.; Longhi, S.; Velasco, R.; Costa, F. Functional allelic diversity of the apple alcohol acyl-transferase gene *MdAAT1* associated with fruit ester volatile contents in apple cultivars. *Mol. Breed.* **2012**, *29*, 609–625. [CrossRef]
19. McGarvey, D.J.; Croteau, R. Terpenoid metabolism. *Plant Cell* **1995**, *7*, 1015–1026.
20. Zhu, Y.; Rudell, D.R.; Mattheis, J.P. Characterization of cultivar differences in alcohol acyltransferase and 1-aminocyclopropane-1-carboxylate synthase gene expression and volatile ester emission during apple fruit maturation and ripening. *Postharvest Biol. Technol.* **2008**, *49*, 330–339. [CrossRef]
21. Liu, X.; Hao, N.; Feng, R.; Meng, Z.; Li, Y.; Zhao, Z. Transcriptome and metabolite profiling analyses provide insight into volatile compounds of the apple cultivar 'Ruixue' and its parents during fruit development. *BMC Plant Biol.* **2021**, *21*, 231. [CrossRef] [PubMed]
22. Zhang, B.; Shen, J.; Wei, W.; Xi, W.; Xu, C.; Ferguson, I.; Chen, K. Expression of genes associated with aroma formation derived from the fatty acid pathway during peach fruit ripening. *J. Agric. Food Chem.* **2010**, *58*, 6157–6165. [CrossRef] [PubMed]
23. Yang, S.; Li, D.; Li, S.; Yang, H.; Zhao, Z. GC-MS metabolite and transcriptome analyses reveal the differences of volatile synthesis and gene expression profiling between two apple varieties. *Int. J. Mol. Sci.* **2022**, *23*, 2939. [CrossRef]
24. Klee, H.J.; Tieman, D.M. The genetics of fruit flavour preferences. *Nat. Rev. Genet.* **2018**, *19*, 347–356. [CrossRef]
25. Schaffer, R.J.; Friel, E.N.; Souleyre, E.J.; Bolitho, K.; Thodey, K.; Ledger, S.; Bowen, J.H.; Ma, J.; Nain, B.; Cohen, D.; et al. A genomics approach reveals that aroma production in apple is controlled by ethylene predominantly at the final step in each biosynthetic pathway. *Plant Physiol.* **2007**, *144*, 1899–1912. [CrossRef]
26. Mattheis, J.P.; Fan, X.; Argenta, L.C. Interactive responses of gala apple fruit volatile production to controlled atmosphere storage and chemical inhibition of ethylene action. *J. Agric. Food Chem.* **2005**, *53*, 4510–4516. [CrossRef]
27. Echeverria, G.; Fuentes, T.; Graell, J.; Lara, I.; Lopez, M. Aroma volatile compounds of 'Fuji' apples in relation to harvest date and cold storage technology: A comparison of two seasons. *Postharvest Biol. Technol.* **2004**, *32*, 29–44. [CrossRef]
28. Villatoro, C.; Altisent, R.; Echeverria, G.; Graell, J.; Lopez, M.; Lara, I. Changes in biosynthesis of aroma volatile compounds during on-tree maturation of 'Pink Lady' apples. *Postharvest Biol. Technol.* **2008**, *47*, 286–295. [CrossRef]
29. Liu, Y.; Chen, N.; Ma, Z.; Che, F.; Mao, J.; Chen, B. The changes in color, soluble sugars, organic acids, anthocyanins and aroma components in 'starkrimson' during the ripening period in China. *Molecules* **2016**, *21*, 812–825. [CrossRef]
30. Shimizu, T.; Hattori, Y.; Ogami, S.; Imayoshi, Y.; Moriya, S.; Okada, K.; Sawamura, Y.; Abe, K. Identification of aroma compounds responsible for apple flavor via palatability score prediction in tree-based regression models. *LWT* **2024**, *192*, 115737. [CrossRef]
31. Matich, A.J.; Rowan, D.D. Pathway analysis of branched-chain ester biosynthesis in apple using deuterium labeling and enantioselective gas chromatography-mass spectrometry. *J. Agric. Food Chem.* **2007**, *55*, 2727–2735. [CrossRef] [PubMed]
32. Vogt, T. Phenylpropanoid biosynthesis. *Mol. Plant* **2010**, *3*, 2–20. [CrossRef]
33. Espino-Diaz, M.; Sepulveda, D.; Gonzalez-Aguilar, G.; Olivas, G. Biochemistry of apple aroma: A review. *Food Technol. Biotechnol.* **2016**, *54*, 375–394. [CrossRef]
34. Park, S.; Sugimoto, N.; Larson, M.D.; Beaudry, R.; van Nocker, S. Identification of genes with potential roles in apple fruit development and biochemistry through large-scale statistical analysis of expressed sequence tags. *Plant Physiol.* **2006**, *141*, 811–824. [CrossRef]
35. Goepfert, S.; Poirier, Y. β-Oxidation in fatty acid degradation and beyond. *Curr. Opin. Plant Biol.* **2007**, *10*, 245–251. [CrossRef]
36. Baker, A.; Graham, I.A.; Holdsworth, M.; Smith, S.M.; Theodoulou, F.L. Chewing the fat: β-oxidation in signalling and development. *Trends Plant Sci.* **2006**, *11*, 124–132. [CrossRef]

37. Mostafa, S.; Wang, Y.; Zeng, W.; Jin, B. Floral scents and fruit aromas: Functions, compositions, biosynthesis, and regulation. *Front. Plant Sci.* **2022**, *13*, 860157. [CrossRef]
38. Muhlemann, J.K.; Klempien, A.; Dudareva, N. Floral volatiles: From biosynthesis to function. *Plant Cell Environ.* **2014**, *37*, 1936–1949. [CrossRef]
39. Ramya, M.; An, H.R.; Baek, Y.S.; Reddy, K.E.; Park, P.H. Orchid floral volatiles: Biosynthesis genes and transcriptional regulations. *Sci. Hortic.* **2018**, *235*, 62–69. [CrossRef]
40. Bartley, I.M.; Stoker, P.G.; Martin, A.D.E.; Hatfield, S.G.S.; Knee, M. Synthesis of aroma compounds by apples supplied with alcohols and methyl esters of fatty acids. *J. Sci. Food Agric.* **1985**, *36*, 567–574. [CrossRef]
41. Jerkovic, I.; Kus, P.M.; Carbonell-Barrachina, A. Volatile organic compounds as artefacts derived from natural phytochemicals sourced form plants and honey. *Phytochem. Rev.* **2019**, *18*, 871–891. [CrossRef]
42. Dickinson, J.R.; Harrison, S.J.; Dickinson, J.A.; Hewlins, M.J.E. An investigation of the metabolism of isoleucine to active amyl alcohol in *Saccharomyces cerevisiae*. *J. Biol. Chem.* **2000**, *275*, 10937–10942. [CrossRef]
43. Tavaria, F.K.; Dahl, S.; Carballo, F.J.; Malcata, F.X. Amino acid catabolism and generation of volatiles by lactic acid bacteria. *J. Dairy Sci.* **2002**, *85*, 2462–2470. [CrossRef]
44. Gonda, I.; Bar, E.; Portnoy, V.; Lev, S.; Burger, J.; Schaffer, A.A.; Tadmor, Y.; Gepstein, S.; Giovannoni, J.J.; Katzir, N.; et al. Branched-chain and aromatic amino acid catabolism into aroma volatiles in *Cucumis melo* L. fruit. *J. Exp. Bot.* **2010**, *61*, 1111–1123. [CrossRef]
45. Beekwilder, J.; Alvarez-Huerta, M.; Neef, E.; Verstappen, F.W.A.; Bouwmeester, H.J.; Aharoni, A. Substrate usage by recombinant alcohol acyltransferases from various fruit species. *Plant Physiol.* **2004**, *135*, 1865–1878. [CrossRef]
46. Wang, Q.; Quan, S.; Xiao, H. Towards efficient terpenoid biosynthesis: Manipulating IPP and DMAPP supply. *Bioresour. Bioprocess.* **2019**, *6*, 6. [CrossRef]
47. Dudareva, N.; Pichersky, E. Biochemical and molecular genetic aspects of floral scents. *Plant Physiol.* **2000**, *122*, 627–633. [CrossRef]
48. Yang, S.; Hao, N.; Meng, Z.; Li, Y.; Zhao, Z. Identification, comparison and classification of volatile compounds in peels of 40 apple cultivars by HS-SPME with GC-MS. *Foods* **2021**, *10*, 1051. [CrossRef]
49. Zhao, P.; Yang, Y.; Wang, X.; Guo, Y. Evolution of typical aromas and phenolic compounds of a red-fleshed apple throughout different fruit developmental periods in Xinjiang, China. *Food Res. Int.* **2021**, *148*, 110635. [CrossRef]
50. Ma, N.; Zhu, J.; Wang, H.; Qian, M.; Xiao, Z. Comparative investigation of aroma-active volatiles in ("Ruixue", "Liangzhi", "Crystal Fuji", and "Guifei") apples by application of gas chromatography−mass spectrometry−olfactometry (GC−MS−O) and two-dimensional gas chromatography-quadrupole mass spectrometry (GC × GC-qMS) coupled with sensory molecular science. *J. Agric. Food Chem.* **2024**, *72*, 25229–25250.
51. Ferrer, J.; Austin, C.S.; Noel, J.P. Structure and function of enzymes involved in the biosynthesis of phenylpropanoids. *Plant Physiol. Biochem.* **2008**, *46*, 356–370. [CrossRef]
52. Neelam; Khatkar, A.; Sharma, K.K. Phenylpropanoids and its derivatives: Biological activities and its role in food, pharmaceutical and cosmetic industries. *Crit. Rev. Food Sci.* **2020**, *60*, 2655–2675. [CrossRef]
53. Yauk, Y.K.; Chagne, D.; Tomes, S.; Matich, A.J.; Wang, M.Y.; Chen, X.Y.; Maddumage, R.; Hunt, M.B.; Rowan, D.D.; Atkinson, R.G. The *O*-methyltransferase gene *MdoOMT1* is required for biosynthesis of methylated phenylpropenes in ripe apple fruit. *Plant J.* **2015**, *82*, 937–950. [CrossRef]
54. Yang, S.; Meng, Z.; Li, Y.; Chen, R.; Yang, Y.; Zhao, Z. Evaluation of physiological characteristics, soluble sugars, organic acids and volatile compounds in 'Orin' apples (*Malus domestica*) at different ripening stages. *Molecules* **2021**, *26*, 807. [CrossRef]
55. Zini, E.; Biasioli, F.; Gasperi, F.; Mott, D.; Mark, T.D.; Patocchi, A.; Gessler, C.; Komjanc, M. QTL mapping of volatile compounds in ripe apples detected by proton transfer reaction-mass spectrometry. *Euphytica* **2005**, *145*, 269–279. [CrossRef]
56. Dunemann, F.; Boudichevskaia, A.; Grafe, C.; Weber, W.; Ulrich, D. QTL and candidate gene mapping for aroma compounds in the apple progeny discovery × prima. *Acta Hortic.* **2009**, *839*, 433–440. [CrossRef]
57. Costa, F.; Cappellin, L.; Zini, E.; Patocchi, A.; Kellerhals, M.; Komjanc, M.; Gessler, C.; Biasioli, F. QTL validation and stability for volatile organic compounds (VOCs) in apple. *Plant Sci.* **2013**, *211*, 1–7. [CrossRef]
58. Souleyre, E.; Chagne, D.; Chen, X.; Tomes, S.; Turner, R.; Wang, M.; Maddumage, R.; Hunt, M.; Winz, R.; Wiedow, C.; et al. The *AAT1* locus is critical for the biosynthesis of esters contributing to 'ripe apple' flavour in 'Royal gala' and 'Granny smith' apples. *Plant J.* **2014**, *78*, 903–915. [CrossRef]
59. Daccord, N.; Celton, J.; Linsmith, G.; Becker, C.; Choisne, N.; Schijlen, E.; Geest, H.; Bianco, L.; Micheletti, D.; Velasco, R.; et al. High-quality De Novo assembly of the apple genome and methylome dynamics of early fruit development. *Nat. Genet.* **2017**, *49*, 1099–1106. [CrossRef]
60. Yang, S.; Yu, J.; Yang, H.; Zhao, Z. Genetic analysis and QTL mapping of aroma volatile compounds in the apple progeny 'Fuji' × 'Cripps Pink'. *Front. Plant Sci.* **2023**, *14*, 1048846. [CrossRef]

61. Schiller, D.; Contreras, C.; Vogt, J.; Dunemann, F.; Defilippi, B.G.; Beaudry, R.; Schwab, W. A dual positional specific lipoxygenase functions in the generation of flavor compounds during climacteric ripening of apple. *Hortic. Res.* **2015**, *2*, 15003. [CrossRef] [PubMed]
62. Vogt, J.; Schiller, D.; Ulrich, D.; Schwab, W.; Dunemann, F. Identification of lipoxygenase (LOX) genes putatively involved in fruit flavour formation in apple (*Malus* × *domestica*). *Tree Genet. Genomes.* **2013**, *9*, 1493–1511. [CrossRef]
63. Souleyre, E.; Greenwood, D.; Friel, E.; Karunairetnam, S.; Newcomb, R. An alcohol acyl transferase from apple (cv. Royal Gala), *MpAAT1*, produces esters involved in apple fruit flavor. *FEBS J.* **2005**, *272*, 3132–3144. [CrossRef] [PubMed]
64. Li, D.; Xu, Y.; Xu, G.; Gu, L.; Li, D.; Shu, H. Molecular cloning and expression of a gene encoding alcohol acyltransferase (*MdAAT2*) from apple (cv. golden delicious). *Phytochemistry* **2006**, *67*, 658–667. [CrossRef]
65. Li, R.; Yan, D.; Tan, C.; Li, C.; Song, M.; Zhao, Q.; Yang, Y.; Yin, W.; Liu, Z.; Ren, X.; et al. Transcriptome and metabolomics integrated analysis reveals MdMYB94 associated with esters biosynthesis in apple (*Malus* × *domestica*). *J. Agric. Food Chem.* **2023**, *71*, 7904–7920. [CrossRef]
66. Cao, X.; Wei, C.; Duan, W.; Gao, Y.; Kuang, J.; Liu, M.; Chen, K.; Klee, H.; Zhang, B. Transcriptional and epigenetic analysis reveals that NAC transcription factors regulate fruit flavor ester biosynthesis. *Plant J.* **2021**, *106*, 785–800. [CrossRef]
67. Li, L.X.; Fang, Y.; Li, D.; Zhu, Z.H.; Zhang, Y.; Tang, Z.Y.; Li, T.; Chen, X.S.; Feng, S.Q. Transcription factors MdMYC2 and MdMYB85 interact with ester aroma synthesis gene *MdAAT1* in apple. *Plant Physiol.* **2023**, *193*, 2442–2458. [CrossRef]
68. Qin, L.; Wei, Q.; Kang, W.; Zhang, Q.; Sun, J.; Liu, S. Comparison of volatile compounds in 'Fuji' apples in the different regions in China. *Food Sci. Technol. Res.* **2017**, *23*, 79–89. [CrossRef]
69. Wang, J.; Sun, Q.; Ma, C.; Meng, W.; Wang, C.; Zhao, Y.; Wang, W.; Hu, D. MdWRKY31-MdNAC7 regulatory network: Orchestrating fruit softening by modulating cell wall-modifying enzyme MdXTH2 in response to ethylene signalling. *Plant Biotechnol. J.* **2024**, *22*, 3244–3261. [CrossRef]
70. Zhang, B.; Yan, S.; Wu, S.; Feng, H.; Siddique, K. Organic and inorganic fertilizers combined with a water-saving technique increased soil fertilities and apple production in a rainfed hilly orchard. *J. Clean. Prod.* **2023**, *414*, 137647. [CrossRef]
71. Wang, Y.; Liu, L.; Wang, Y.; Tao, H.; Fan, J.; Zhao, Z.; Guo, Y. Effects of soil water stress on fruit yield, quality and their relationship with sugar metabolism in 'Gala' apple. *Sci. Hortic.* **2019**, *258*, 108753. [CrossRef]
72. Mpelasoka, S.; Behboudian, H. Production of aroma volatiles in response to deficit irrigation and to crop load in relation to fruit maturity for 'Braeburn' apple. *Postharvest Biol. Technol.* **2002**, *24*, 1–11. [CrossRef]
73. Wang, Z.; Feng, Y.; Wang, H.; Liu, X.; Zhao, Z. Effects of different pre-harvest bagging times on fruit quality of apple. *Foods* **2024**, *13*, 1243. [CrossRef] [PubMed]
74. Watkins, B. Storage technology for apples and pears. *Postharvest Biol. Technol.* **2002**, *24*, 193–194. [CrossRef]
75. Argenta, L.C.; Mattheis, J.P.; Fan, X.; Finger, F.L. Production of volatile comp ounds by Fuji apples following exposure to high CO_2 or low O_2. *J. Agric. Food Chem.* **2004**, *52*, 5957–5963. [CrossRef]
76. Dias, C.; Ribeiro, T.; Rodrigues, A.C.; Ferrante, A.; Vasconcelos, M.W.; Pintado, M. Improving the ripening process after 1-MCP application: Implications and strategies. *Trends Food Sci. Tech.* **2021**, *113*, 382–396. [CrossRef]
77. Yan, R.; Yun, J.; Gurtler, B.; Fan, X. Radiochromic film dosimetry for UV-C treatments of apple fruit. *Postharvest Biol. Technol.* **2017**, *127*, 14–20. [CrossRef]

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