2.2 Carbohydrate metabolic pathways and regulation by key enzymes

Sucrose transported from leaves enters a metabolic network involving sucrose phosphate synthase (SPS), sucrose synthase (SS), and several forms of invertase (cell wall, vacuolar, and neutral), which together determine the balance between sucrose and hexoses in the receptacle (Wang et al., 2025). In Tochiotome fruit, sucrose rises rapidly in late development, while SPS and SS activities are relatively low, suggesting that sucrose accumulation is driven more by limited breakdown than by enhanced synthesis (Khammayom et al., 2022). Acid invertase activity declines early but increases again at ripening, leading to abundant hexoses and explaining why ripe red fruits frequently contain glucose as the major sugar despite strong late sucrose accumulation (Topçu et al., 2022).

Genome-wide analyses reveal a large invertase (INV) gene family in octoploid strawberry, with polyploidy-driven amplification and many members showing fruit-preferential expression. Among them, cell wall invertase FaCWINV1 is markedly upregulated during development, strongly expressed in ripe fruit, and significantly correlated with total sugar content, indicating a central role in sucrose unloading and sink strength establishment (Wang et al., 2025). Transcriptional studies also show that SPS transcripts increase sharply at late stages, while SS and acid invertase transcripts often decrease or fluctuate, supporting a model in which coordinated regulation of these enzymes and their genes channels carbon toward sugar accumulation as fruit ripen (Osatuke and Pritts, 2021).

2.3 The influence of developmental stages and environmental factors on sweetness

Strawberry fruit development from green through white/turning to red stages is accompanied by coordinated changes in sugars, hormones, and other metabolites that shape sweetness. Multi-stage analyses show that sucrose, glucose, and fructose all rise as fruit ripen, but sucrose is most tightly linked to ripening progression and activation of ripening-related genes (Osatuke and Pritts, 2021). In white-fleshed strawberries, total soluble solids, total sugar, and sucrose increase strongly while total acid and anthocyanins decrease, and transcriptomic data highlight enrichment of “starch and sucrose metabolism” and hormone signaling pathways, indicating that sucrose accumulation and plant hormones jointly regulate maturation and flavor development.

Environmental conditions, especially temperature, further modulate sweetness by altering sugar accumulation patterns. In greenhouse-grown strawberries, a larger day-night temperature differential during fruit development is associated with higher soluble sugar content and overall better fruit quality, whereas elevated temperatures late in the season reduce soluble sugars despite similar radiation and humidity, underscoring temperature’s dominant effect on sweetness (del Olmo et al., 2020). Comparative studies across farms likewise find soluble solids and titratable acidity to be positively associated with air temperature differential during ripening, while fertilization, pesticide intensity, and microbial inputs have little consistent influence on sugar levels. These findings emphasize that developmental programming of carbohydrate metabolism interacts strongly with thermal conditions to determine final sweetness.

3 Material Basis and Regulatory Mechanisms of Strawberry Acidity Formation

3.1 Major organic acid species (citric acid, malic acid, etc.)

Strawberry acidity is primarily determined by the composition and concentration of organic acids in the receptacle tissue. In cultivated *Fragaria* × *ananassa*, citric acid is generally recognized as the predominant organic acid, with malic acid as the second major component, and both together account for most of titratable acidity that shapes basic sourness perception. Studies on white-fleshed strawberry further confirm that citric and malic acids are much higher than other detected acids such as 2-oxobutyric, methylmalonic, suberic, and ascorbic acids, reinforcing their central role as the main acidity-forming components in different genetic backgrounds (Wang et al., 2025).

Comparative work in wild species highlights that the organic acid spectrum can diverge markedly from cultivated types. In *Fragaria nilgerrensis*, isocitric, succinic, and methylmalonic acids dominate the profile, while many other organic acids remain relatively stable across development, suggesting that different strawberry species rely on distinct TCA-cycle intermediates to support fruit metabolism and flavor formation (Ikegaya, 2023). Despite this diversity, across fleshy fruits more broadly, citrate and malate emerge as the two organic acids that most