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PERSPECTIVE

The Molecular Basis for Wine Grape Quality—A Volatile Subject

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Volatile organic compounds are important flavor components of finished wines. In addition to winemaking practices, which shape wine quality, cultivation of the grape berries in the vineyard each season affects the production of volatile organic compounds as well as other chemical components that ultimately contribute to our perception of flavor in finished wines. By studying how berry flavor components are determined by the interplay of vine genotypes, the environment, and cultivation practices at the molecular level, scientists will develop advanced tools and knowledge that will aid viticulturalists in consistently producing balanced, flavorful berries for wine production.

Many of us like to relax with a nice glass of wine, but have you ever considered the complex chemistry at play on your nose and palette when you first raise the glass? Whether you find the bouquet and entry from a newly opened bottle to be a pleasant experience or the basis for a scowl and a wrinkle of the nose partly depends on the relative assortment of volatile compounds in the wine. The many volatile organic compounds (VOCs) and other chemical compounds contributing to flavor (taste and aroma) in wines are determined in part in the vineyard through a complex and poorly understood interplay between the natural environment, vineyard management practices, and vine genotypes, including the rootstocks (*1*, *2*). Thus, the consistent production of high-quality grapes for winemaking has traditionally been more art than science. This art is increasingly guided by science for many wine producers, and this trend will continue with a growing contribution from molecular-based technologies and knowledge. Here, we focus on viticulture and grape berry biochemistry, but we acknowledge that enology (the science of winemaking) and human olfactory reception also play critical roles in determining flavor and individual human perception of quality in wines.

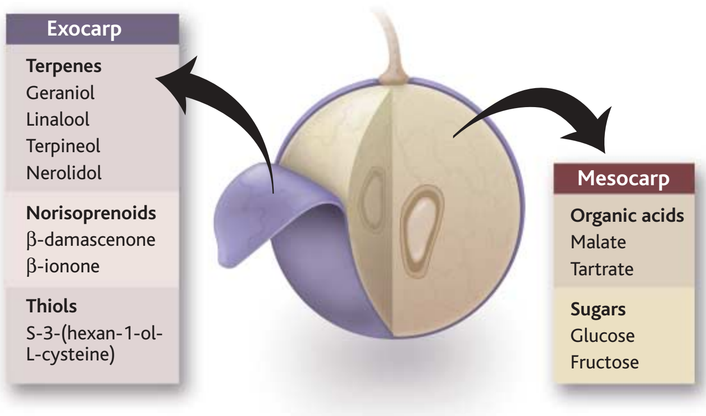

Fig. 1. Major chemical determinants of flavor and wine quality in grape berries are predominantly localized to mesocarp (flesh) or exocarp (skin) tissues. Only a small number of the dozens of known grape compounds important for flavor are represented here. Potentially volatile compounds such as terpenes, norisoprenoids, and thiol precursors are stored as sugar or amino acid conjugates in vacuoles of exocarp cells. The compounds are volatilized through physical crushing and subsequent cleavage by grape, yeast, and/or industrial enzymes (glycosidases and peptidases) during the winemaking process.

Metabolic changes throughout the biphasic growth of grape berries lay the groundwork for flavor. After flowering and fruit set in the first phase, there is an initial burst of growth in the pericarp (flesh plus skin) and seed. The accumulation and storage of organic acids, chiefly malate and tartrate, in mesocarp (flesh) cell vacuoles occurs during this time. The tartness imparted by these acids in the pericarp likely evolved as a safeguard against mammalian and avian foraging while the seeds developed. The first phase is followed by a lag period in which expansion slows while seed maturation is completed. Finally, the second phase of berry ripening is initiated and maturation of the pericarp begins—a process termed "veraison" by the French. Maturation is marked in red cultivars by accumulation of anthocyanins (red pigments) in the exocarp (skin), down-regulation of glycolysis coupled with glucose and fructose accumulation, metabolism of malate as the major carbon source for respiration, and biosynthesis of VOCs and other metabolites important for flavor (*3*). Thus, after seed maturation, the berry becomes more visually attractive and flavorful, promoting geographical seed distribution by foraging animals. For winemaking, harvest dates are chosen to optimize the balance between sweetness, acidity, flavorfulness, and phenolic ripeness. Harvest for winemaking usually occurs 12 to 14 weeks after fruit set.

The most important grapevine compounds contributing to flavor are organic acids, proanthocyanidins (tannins), terpenoids (monoterpenoids, sesquiterpenoids, and C13-norisoprenoids), and various precursors of aromatic aldehydes, esters, and thiols detectable in finished wines. Glucose, fructose, malic acid, and tartaric acid are stored primarily in the vacuoles of mesocarp cells, although some glucose and fructose can be detected in the exocarp. Proanthocyanidins and other polyphenolic compounds, terpenoids, esters, and other less abundant sensory compounds are primarily stored in exocarp cells (Fig. 1). Plant-derived volatile terpenoid compounds occurring in wines are mainly stored as nonvolatile, water-soluble glycoside derivatives (sugar conjugates) in exocarp cell vacuoles, although some terpenoids may also be present as free volatiles. Unlike other aromatic plants that sequester large amounts of lipophilic terpenoid VOCs in specialized anatomical structures (such as glandular trichomes on the surfaces of peppermint leaves, glandular structures in the peels of citrus fruits, or resin

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ducts in pine bark), grape berries seem to lack such anatomical structures for physical sequestration of lipophilic VOCs. Instead, conjugation as water-soluble glycosides provides an alternate means of biochemical trapping and storage of the VOCs. Other important volatiles for wine flavor may be stored as amino acid conjugates in grape cells, such as cysteinylated precursors of aromatic thiols (*4*). Volatilization of compounds stored as conjugates in grape cells is essential for our perception of these as flavor. Glycosidases and peptidases, enzymes that cleave the sugar and amino acid conjugates, thus play a vital role in the timing and production of natural fruit flavors. For winemaking, physical crushing and processing through fermentation can introduce grape and yeast enzymes, respectively, to conjugated substrates. Winemakers may also add exogenous enzyme mixtures to the fermentation to further stimulate volatilization of compounds to improve sensory characteristics of VOCs in the finished wines (*5*, *6*).

The relative assortment of compounds in the berries of each grape variety define what is known as "varietal character" to wine enthusiasts. An excellent example is the vegetative character conferred to Sauvignon Blanc grapes and wines by methoxypyrazine compounds, chiefly 2-methoxy-3-isobutylpyrazine (*7*). We are fine-tuned to perceive methoxypyrazines and can detect them at parts per trillion (femptomolar) levels, possibly as a deterrent to feeding on unripe, acidic fruits. Unlike most compounds important for flavor, methoxypyrazines accumulate during green stages of berry development and are gradually metabolized during maturation, the extent to which is dependent upon sun exposure and other microclimate factors. Subtle herbaceousness is generally held as a desirable character in Sauvignon Blanc wines, whereas it can be considered a defect in most red varietals such as Merlot. In contrast, dozens of different terpenoid compounds contribute nuances of floral or fruity characters to wines, depending on the varietal. Muscat varieties such as Gewürtztraminer, for example, are rich in monoterpene compounds, chiefly geraniol and citronellol, which contribute a distinctive floral character to the wines (*8*). Linalool is another monoterpene compound that not only imparts a floral character to berries but has also been implicated in flower aroma as well as a signaling response to insect feeding, suggesting multiple biological roles for this VOC in plants. One of the most important norisoprenoid compounds for red wine quality is β-damascenone, which imparts a honeylike, fruity character at femptomolar levels (*9*).

There may be dozens to hundreds of chemical compounds in grape berries that, similar to the methoxypyrazines and norisoprenoids, exist in exceedingly small quantities but have yet to be discovered and characterized. Advances in extraction protocols and analytical techniques with the use of increasingly sensitive detection equipment technologies such as Fourier transform ion cyclotron resonance (FT-ICR), mass spectrometry, and nuclear magnetic resonance are pushing the envelope of plant metabolomics and will undoubtedly aid in the discovery of new compounds in grape berries. Relatively few of the dozens of *Vitis* (grapevine) species have been domesticated by humans for wine production. Metabolomics research in grapes should not be limited to commercially important wine grape cultivars but could be extended to further characterize the rich diversity inherent amongst *Vitis* species (*10*). In considering such analyses in grapes, however, human sensory analyses should continue to be coupled with the discovery of new compounds and analogs of known ones in order to characterize if and how they impact berry flavor (*11*). The same considerations should be made for metabolomic analyses of yeast compounds or grape compounds modified by yeasts and detected in finished wines.

An important current research challenge is determining how environmental cues affect the regulation of the genes and enzymes of various metabolic pathways leading to the diverse bouquet of VOCs and other important compounds for wine flavor. Previous experiments have shown how varied environmental conditions affect berry ripening and quality at harvest. The limitation is that such research has been focused on cause and effect—i.e., the "what"—but the mechanisms underlying these processes—i.e., the "how"—remain largely unknown and unexplored. A better understanding of how temperature, light, and water and nutrient availability to the berry qualitatively and quantitatively affect allozyme (i.e., enzyme variant) production and activity will help to develop molecular diagnostic tools that will assist viticulturalists in fine-tuning pruning, cluster thinning, irrigation, and fertilization practices from season to season in each vineyard. To achieve this, complex networks of signaling and metabolic pathways must be characterized at the gene, protein, and metabolite levels in varied, controlled environments in order to begin to clarify how accumulation of sensory compounds is regulated at the molecular level in the grapevine. As a first step, gene cloning and functional characterization of enzymes important for the formation of VOCs in grapes have recently been reported (*12*–*14*). As with other systems of plant VOC formation, the numerous terpenoid synthase (TPS) (*12*, *13*) and carotenoid cleavage dioxygenase (CCD) (*14*) enzymes likely orchestrate much of the complex metabolic profiles of terpenoid or isoprenoid VOCs in *Vitis* species. Curiously, TPSs contribute not only to berry VOC formation but also to the diurnal emissions of VOCs from grapevine flowers. A functional, biochemical genomics approach of TPS and CCD discovery should support metabolite profiling and molecular mapping of related grape quality traits.

Recent and ongoing molecular investigations hold promise in revealing the biological secrets underlying berry ripening and flavor that are currently inaccessible to viticulturalists and winemakers. We expect that increased mechanistic knowledge and the development of new molecular tools (e.g., DNA and protein chip–based technologies) will help guide viticulturalists in clonal selection (matching genotypes to specific mesoclimates), as well as vineyard management to more consistently produce high-quality grapes for wine production from season to season.

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