Terpenes: A Building Block of Cannabis

Many chemical compounds can be responsible for scent and flavor in botanicals. These chemicals are transformed into neural impulses and travel along the various facial and major nerves to centers in the brain which then interpret the impulses and create taste perception. These perceptions of taste, along with texture, smell and the sensation associated with temperature, pain and pressure (chemesthesis) combine to create the impressions of flavor.

The most common functional group in flavors is carbonyls such as esters, aldehydes, ketones, etc. Other groups which produce flavors are carbohydrates, acids, salts, proteins, and terpenes. Terpenes are the common term for a large group of compounds that contribute to the flavor and smell of botanical products. Isoprene or 2-methyl-1,3-butadiene (Figure 1) and its polymers is the main base of natural rubber and the structural base for terpenes and terpenoids, even though isoprene is not part of the reactions which produce terpenes.

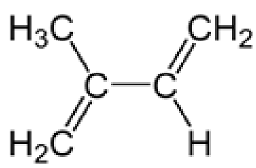

Figure 1. Isoprene Unit

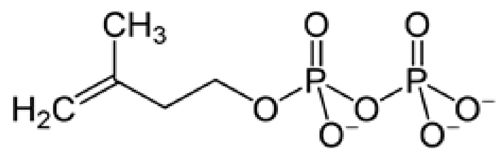

Figure 2. Isopentenyl pyrophosphate

The actual mechanisms for the synthesis of terpenes are derived from units of isopentenyl pyrophosphate (Figure 2). The two metabolic pathways to synthesize terpenes are the mevalonic acid pathway (MVA) or the MEP/DOXP pathway. The pathways are usually exclusive to the type or organism with green algae producing terpenes via the MEP pathway; humans & fungi via the MVA pathway and plants producing terpenes from both pathways[1].

In biological processes, there are essential and nonessential terpenes. Essential terpenes are usually terpenes C15 and higher which are required by the plant, insect or algae to support life and growth. The lower weight terpenes (some C15 and below) are nonessential terpenes which assist in other biological processes or contribute to the defense and functioning of the organism but are not critical to survival. Removal of an essential terpene will damage and ultimately kill the organism, whereas the removal of a nonessential terpene will not.

Terpenes are classified based on the number of isoprene units they contain. Starting with hemiterpenes that have five carbons; monoterpenes have ten carbons, sesquiterpenes have 15 carbons, etc. (Table 1). The classification is based on the C5 rule which is the isoprene synthesis route that organisms employ for the production of terpenes.

Table 1. Terpene Groups & Examples

Terpene Group	# Isoprene Units	# Carbons	Terpene Example	Terpenoid Example	Notes
Hemiterpene	1	5	Isoprene	Isovaleric acid	Isoprene is the only hermiterpene
Monoterpene	2	10	Limonene	Terpineol	Large group of volatile and semivolatile compounds
Sesquiterpene	3	15	Humulene	Farnesol	Large group of volatile and semivolatile compounds
Diterpenes	4	20	Taxadiene	Cafestol	Precursor compounds for production of retinol and retinal
Sesterterpenes	5	25	Ophiobolin A	Geranylnerolidol	Rare group of terpenes mostly from marine sources
Triterpenes	6	30	Squalene	Sterols	Squalene is shark liver oil and the precursor to some steroids
Sesquarterpenes	7	35	Tetraprenylcurcumene	Ferrugicadiol	Mostly produced by microbes
Tetraterpenes	8	40	Lycopene	Cartenoids	Family also includes carotenes

Monoterpenes are lower molecular weight terpenes and are responsible for lighter floral fragrances. These lighter weight terpenes can volatilize quickly during processing involving heat and decarboxylation. Sesquiterpenes are larger molecular weight terpenes and have a heavier fragrance, such as sandalwood or musk, and volatilize at higher temperatures and remain after many processing steps.

The diversity of terpenes and terpenoids is recognized by the range of scents and flavors they produce from the pine scent of Pinene (the most widely encountered terpene in nature) to the lavender and mint notes associated with Linalool. The flavor and aroma of hops are critical to beer, especially Myrcene, beta-Pinene, beta-Caryophyllene, and alpha-Humulene. The terpene abundances can differ greatly between the strains of cannabis, from major terpenes sch as myrcene often found in the percent level, to the minor terpenes such as geraniol which is mostly found in cannabis strains in very small amounts in ppm levels or smaller.

Table 2. Terpene & Terpenoid Groups Found in Cannabis

Terpene Group	Subgroup	MW	BP	Formula	Abundance in Cannabis	Aroma	Cannabis Variant Example
Myrcene	Monoterpene	136.2	168 °C	$C_{10}H_{16}$	High ppm - %	Clove-like, musky, earthy	Levorin 110, Skunk XL
Terpinolene	Monoterpene	136.2	187 °C	$C_{10}H_{16}$	High ppm - %	Pine with herbal and floral notes	Pineapple Jack, Durban Poison
Pinenes (alpha and beta)	Monoterpene	136.2	156 °C	$C_{10}H_{16}$	High ppm - %	Pine	Bubba Hash, Strawberry Cough
beta-Caryophyllene	Sesquiterpene	204.4	264 °C	$C_{15}H_{24}$	High ppm - %	Citrus	Gorilla Glue, Skywalker
Limonene	Mononterpene	136.2	176 °C	$C_{10}H_{16}$	ppm	Lemon, citrus	Lemon Haze, OG Kush
Ocimene	Mononterpene	136.2	100 °C	$C_{10}H_{16}$	ppm	Herbal	White Fire OG, Purple Haze
Humulene	Sesquiterpene	204.4	107 °C	$C_{15}H_{24}$	ppm	Hoppy	Sour Diesel, Pink Kush
Phellandrenes	Mononterpene	136.2	172 °C	$C_{10}H_{16}$	ppm	Citrus and mint	Super Lemon Haze, Super Silver Haze
Linalool	Mononterpene Alcohol	154.2	199 °C	$C_{10}H_{18}O$	ppm	Lavender and floral	Sour OG
Camphene	Mononterpene Alcohol	136.2	159 °C	$C_{10}H_{16}$	ppm – ppb	Damp mint, pine notes	Indica Species
Terpineol	Mononterpene Alcohol	154.3	271 °C	$C_{10}H_{18}O$	ppm – ppb	Floral	Girl Scout Cookies, OG Crush
Carenes	Mononterpene	136.2	172 °C	$C_{10}H_{16}$	ppm – ppb	Pungent, earthy sweet	Super Silver Haze, Skunk #1

The most common terpenes found in cannabis variants are myrcene, caryophyllene and many others (Table 2). Current research is investigating the possible synergistic effects of cannabinoids and terpenes. Research has suggested the presence of terpenes in cannabis products can alter the pharmacokinetics of cannabinoids [(2,3)]. There are still questions being researched as to which cannabis product could contain these healthful or synergistic effects. The growing popularity of cannabis-derived products, especially the increasing popular vapes, are now bringing different groups and sources of terpenes into the cannabis world.

In the world of cannabis products, terpenes within the product can be either cannabis-derived, other botanically-derived, or artificial (synthetic). Products where flavor and fragrance are heavily dependent, such as in vapes, concentrates and oils, some manufacturers will supplement or add other sources of terpenes to create a characteristic smell or taste especially when the extraction processes for some of these products can strip away many naturally cannabis-derived compounds. This process becomes more common for cannabis products, the questions arise if the added compounds are safe. In a 2015 study of e-cigarette flavor and fragrance additives by Allen et al. it was found that some of the added compounds were changing during heating and vaporization into other harmful compounds.

Sample Processing & Analysis for Terpenes

Cannabis compounds can be degraded by high temperatures and oxidation. In ambient temperature grinding processes, heat and energy are generated which can raise the temperature of materials to almost 100 °C and cause up to a 60% loss of critical aromatic components [(4,5)]. Reduction of temperature during processing can prohibit the breakdown of volatile compounds. In one study it was found that cryogenic conditions showed better retention of monoterpenes (myrcene, limonene and pinene) than grinding at ambient temperature [(6,7)].

The extraction and analysis of terpenes in the analytical laboratory from the various cannabis product matrices can be challenging, especially in regard to sample preparation, clean-up and matrix effects. In many cases, related terpenes have the same or similar masses (Table 2) making them difficult to identify in complex mixtures where many isomers or similar compounds are present. The most common method of analysis for terpenes is gas chromatography (GC) with either a flame ionization detector (FID) or mass spectrometer (MS).

The principle of gas chromatography is that samples are vaporized in an inlet at high temperatures of usually over 250 °C and transported via a carrier gas to a chemically infused column. The column material is composed of various chemical binding groups which interact with the vaporized analytes forcing the analytes out of the column phase over time and increasing temperature until the analytes are released into the carrier gas then detected. The result is a chromatogram which displays graphical responses over time when each analyte is detected. Many GCMS column chemistries are based on boiling point or molecular size, meaning that molecules of similar weight or similar boiling points could co-elute or appear overlapped in the chromatogram. Is it this fact which can make the analysis of terpenes challenging since many terpenes have the same molecular weight and formula and relatively low boiling points, which mean they appear very quickly in the chromatogram and often co-elute (Table 2). Instrument and column manufacturers over the decades have become the experts on the separation of compounds and have given analytical labs many methods and specialized columns to aid in analysis.

Table 3. Example of SPEX CertiPrep CAN-TERP-MIX GC/MS Instrument Conditions

Instrument Conditions	
Column	DB-624 UI Column
Size	30 m x 0.25 mm diameter
Program	50 C x 3 minute hold; 15 C/min ramp to 240; hold 20 minutes
Injection Volume	1 µL

Terpene testing and profiling can be difficult since the range of abundance across the profile can vary dramatically. One approach is to segregate sample extractions for different dilutions and therefore separate testing at the percent, ppm and ppb levels. The analyst then creates standards to match each dilution level. Another approach is to reduce the number of analyses by combining standards to match the native levels of terpenes in the cannabis samples.

Terpene analysis has always been an important component of many research areas including atmospheric chemistry, agricultural science, biochemistry, and environmental science to name a few. In industries dependent upon flavor and fragrance products, terpene profiles are part of their routine analytical testing procedures. The cannabis industry is just the newest industry to investigate the role of terpenes for flavor, fragrance and health benefits. As the industry continues to engineer cannabis strains to enhance specific chemical profiles, the importance of terpenes will increase.

For an in-depth look at terpenes, please read “Beyond Potency: The Importance of Terpenes” [8] in the June, 2019 issue of Cannabis Science and Technology Magazine.

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