

Modified Atmospheric Packaging and Its Effect on Postharvest Cannabis Quality

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How to cite this paper: MacLaughlin, L.L. and MacDonald, M.T. (2024) Modified Atmospheric Packaging and Its Effect on Postharvest Cannabis Quality. *American Journal of Plant Sciences*, 15, 222-234.
<https://doi.org/10.4236/ajps.2024.153016>

Received: January 18, 2024

Accepted: March 26, 2024

Published: March 29, 2024

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Abstract

Cannabis sativa L. is used as fiber, food, and medicine in several countries. Though it is illegal for recreational use in most of the world, there are some countries that have legalized production and sale. There is a lot of research on production of cannabis, but less so on storage technologies. Cannabis contains several high value compounds, such as cannabinoids and terpenoids, that are susceptible to degradation via light, temperature, and oxygen. Several studies have explored temperature and light, and industry has adjusted accordingly. However, less is known about oxygen-induced degradation. Biochemical studies have demonstrated oxidative degradation of high value compounds, and many producers use some form of modified atmospheric packaging (MAP) for storage. However, the efficacy of MAP is unclear. The objective of this paper is to review our current understanding of MAP in postharvest cannabis storage and identify avenues where additional research is needed.

Keywords

Cannabinoids, *Cannabis sativa*, Marijuana, Nitrogen Packaging, Oxidation, Postharvest, Terpenoids, THC

1. Introduction

Cannabis sativa L. is an herbaceous annual plant that has been used as a source of fibers, food, and medicine in various countries for centuries [1]. Paleobotanical evidence suggests that *C. sativa* originated in central Asia over 10,000 years ago and spread to Europe and then the remainder of the world largely due to human cultivation [2]. *C. sativa* is a complex plant with over 400 biochemical entities, including cannabinoids, terpenoids, flavonoids, and alkaloids [3]. Cannabinoids, a class of terpene phenolic compounds, are the most active biochem-

ical compounds [4]. Delta-9-tetrahydrocannabinol (THC) is likely the most studied cannabinoid since it is the main compound associated with the psychoactive effects of *C. sativa* [5]. It is this psychoactive effect that had *C. sativa* as the most widely used illicit drug in the world [6], though use of *C. sativa* is now legal in several countries, Uruguay was the first country to regulate cannabis at the national level in 2013 [7]. Since regulation by Uruguay, several other countries have legalized cannabis for medicinal or recreational use. As of early 2023, Canada and 21 states in the United States of America have legalized cannabis for recreational use [8].

Cannabinoids and terpenoids are both high value compounds occurring in cannabis. Volatile organic compounds are an important component of cannabis' organoleptic properties [9] [10], while cannabinoids contribute to cannabis' psychoactive properties [11] [12]. Yet volatile terpenes decrease by 10 to 50% within 1 month of postharvest storage [13]. Cannabinoids are also susceptible to degradation during storage, generally through decarboxylation [14] [15] [16].

Retention of these high value compounds of dried cannabis inflorescence during storage has seen industry adoption of N₂ modified atmosphere packaging (MAP) technologies. Literature demonstrating the efficacy of MAP for the preservation of quality, aroma, and flavour, spans numerous agricultural products such as coffee (*Coffea arabica*), where aroma is important for customer satisfaction [17]. High N₂ conditions improve preservation of several volatile aromatic compounds over storage with atmospheric air [18]. Similar research has also demonstrated the efficacy of MAP for retention of aromatics, finding high N₂ storage of the aromatic plant lemon verbena (*Aloysia citrodora*) results in increased extractable essential oils content compared to atmospheric conditions [19]. Potato chip seasoning stored under high N₂ MAP has even been found to retain higher levels of volatile disulphides and terpenes compared to atmospheric control [20]. Use of MAP technologies for the storage of milk powder, a confectionary ingredient prone to oxidation and development of off-flavors, sees increased customer acceptance of downstream chocolate products [21].

Industry interest in the retention of these high-value compounds under storage has seen the adoption of MAP technologies within the Canadian market space. However, MAP infrastructure represents a significant cost to producers and limited research on its efficacy currently exists. This review discusses current research of cannabis stored under MAP, addressing limitations of previous work, and identifying gaps in our knowledge.

2. Modified Atmospheric Packaging (MAP)

2.1. History and Application

MAP alters the gaseous composition of atmospheric air surrounding perishable items to extend shelf life and preserve product quality [22]. Early research investigating modifying gaseous composition of apple storage environments for extended shelf life during the 1920s later saw the technology utilized for the trans-

port of hanging beef in the 1930s [23]. Industrialization and commercialization of MAP infrastructure eventually led to its adoption in the packaging of retail meats to prevent the development of aerobic bacteria and improve color retention in red meat via decreased oxidation [24]. Implementation by the seafood industry for inhibition of spoilage reportedly doubled or tripled shelf life in some circumstances [25]. However, widespread employment of MAP has been somewhat limited due to concerns around the potential development of anaerobic microorganisms, such as *Listeria monocytogenes* in red meats and *Clostridium botulinum* in seafood products [24] [25]. The microbial pros and cons of using MAP for plant products are presented in other reviews [26].

The historical applications of MAP for products like baked goods, chips, coffee, and tea, offers examples seemingly aimed more towards the preservation of product quality and flavour, rather than prevention of microbial spoilage [23]. Improved organoleptic qualities like aroma and flavour under MAP in various food products has been reported in consumer panels [27] and studies exploring postharvest changes in the physical and chemical properties of plant products under MAP continue to emerge. The link between oxidation, degradation of lipids, and reduction in flavour is well documented, and presented in several reviews [28]. MAP technologies have demonstrable efficacy in delaying oxidation of lipids to improve the stability and shelf life of various food products including potato chips, red meats, and fish [24] [29] [30]. Molecules of oxygen present in atmospheric air react with unsaturated fatty acids, producing free radicals in the form of unstable hydroperoxides which undergo a cascade of further reactions [31].

2.2. Challenges

MAP technologies are immensely popular and have a global value of more than \$6 billion USD [32]. MAP slows deteriorative processes and limits microbial growth and therefore greatly extends product shelf life [32]. However, consumer preferences have shifted in recent years in favor of product quality as opposed to freshness [33]. The effect of MAP on many aspects of cannabis quality is not yet known.

The primary objective of MAP is to decrease the concentration of O₂ in storage packaging [32]. This can be done through a variety of methods, but one popular method is to add an inert gas, such as N₂, to the storage atmosphere to displace O₂ [32]. But the exact amount of O₂ displacement is critical and can pose a challenge. If O₂ is too low, anaerobic respiration can occur and postharvest quality rapidly decreases [32]. Taking MAP beyond tolerable limits has altered postharvest texture and aroma in other products, to the extent that consumers start to question freshness as well [34] [35].

MAP represents an additional cost for industry. Gases may be added directly from compressed gas cylinders or gas emitters may be added [36]. In the case of N₂ MAP, there is also the possibility of using a nitrogen generator or displace-

ment with liquid nitrogen [36]. Each application method has a fixed infrastructure cost that can range from tens to hundreds of thousands of dollars. There is also a residual cost, that would vary regionally with compressed gas or liquid N prices. As an example, in Nova Scotia it can cost up to \$0.90 CAN per can to modifier the storage atmosphere using liquid N [37]. The challenge of the MAP additional cost is magnified considering there is a lack of knowledge in effectiveness of MAP in preserving postharvest quality of cannabis, including high value compound.

3. High Value Compounds and Preservation Targets

3.1. Identification

Cannabinoids and terpenoids account for most of cannabis' therapeutic effects, either individually and synergistically [38] [39] [40]. Cannabinoids and terpenoids are secreted from specialized disc cells and are then subsequently stored within trichomes on the surface of female inflorescence [41] [42] [43]. Many of these biosynthesized compounds, like cannabinoids and terpenoids, are lipidic in their nature and remain stored as oil-in-water emulsions contained within hydrophilic apoplast [44]. Cannabinoids are typically associated with cannabis, but are also found in some liverworts, rhododendron, and fungi [45]. Terpenoids are widespread throughout the plant kingdom [14].

3.2. Cannabinoids

The term cannabinoid refers to meroterpenoids, which comprise of a resorcinylic center with attached isoprenyl, alkyl, or aralkyl side chains [46]. Cannabis typically produces alkyl type cannabinoids with a 10-carbon monoterpene isoprenyl moiety and pentyl side chain [46]. The most abundant cannabinoids in *C. sativa* are THC, cannabidiols (CBDs), Cannabichromenes (CBCs), and cannabigerols (CBGs) and their respective acidic forms [47]. Natural biosynthesis usually forms acidic cannabinoids that contain a carboxyl group (COOH), but chemical processes such as oxidation, decarboxylation, or cyclization result in non-acidic forms [48] [49]. Such chemical processes are caused or accelerated by exposure to light, oxygen, or heat [14] [50].

Degradation of cannabinoids can result in loss of bioavailability, such as THC to cannabinol (CBN) [51]. However, cannabinoid oxidation to lesser understood compounds with unknown psychotropic and biological activity, or cannabinoids for which analytical testing does not exist presents some experimental design challenge. Oxidation to underappreciated and understudied cannabinoids that may even be beneficial under prolonged storage is also possible. Efforts to identify psychoactive forms of cannabinoids are still under way with recent research even seeing the application of machine learning [52]. "It is crucial to understand how cannabinoids are related with each other when studying cannabis, considering that degradation (including decarboxylation, isomerization, irradiation, and oxidation) can affect the chemical components through improper opera-

tions or during long-term storage with unsuitable conditions” [53]. Yet as reviewed by Rupasinghe *et al.* [54] the complex pharmacology and interactions of even the most well studied cannabinoids THC, and CBD, are still being unraveled.

3.3. Terpenoids

Terpenes and terpenoids are characterized by their strong aroma. These molecules are volatile hydrocarbons [14]. Terpenes are classified by the number of 5-carbon building blocks they contain. For example, sesqui-terpenes contain 15 carbons [55]. Terpenoids are modified terpenes that have incorporated various oxygen arrangements. Typically, terpenoids is an umbrella term including both terpenes and terpenoids [40].

Biosynthesis of terpenoids within the cannabis plant starts with isoprene diphosphate precursors that feed into the plastidial methylerythritol or cytosolic mevalonate pathway [9]. Cannabis essential oils are composed almost entirely of mono- and sesquiterpenes, accounting for 98% of constituents [56]. Characterization of both mono- and sesqui-terpenes classes across three different chemovars has identified a nearly even mixture of 46.5% monoterpenes and 53.5% sesquiterpenes [57]. The eventual fate of terpenes is dynamic, potentially ending up as cannabinoids via the addition of a phenol group or remaining stored as one of the many known endogenous terpenes present in the plant [58]. Natural degradation can also occur through chemical processes like isomerization, oxidation, dehydrogenation, polymerization, and thermal rearrangement [59]. The ultimate fate of any particular terpenes can be difficult to determine due to the number of products and functional groups that are formed. In cannabis alone, terpenes can be oxidized into alcohols, ketones, and aldehydes [60].

The roles and functions of plant terpenes are extremely diverse. As reviewed by Pichersky and Raguso [61], they act as antimicrobial, antifungal, signaling molecules, and contribute to interactions with their other organisms, such as pollinator attraction and deterring herbivory. Terpenes typically account for 3% - 5% of dried cannabis inflorescence biomass [62]. Potential medical benefits of terpenes, particularly those in cannabis, represent an area of great research interest. Consumption of plants rich in terpenes has a rich human history, and in-depth medical benefits of plant terpenes have been presented in other reviews [62] [63].

4. Location of High Value Compounds in Cannabis

4.1 Trichomes Structural and Functional Chemistry

Classified as multi-cellular appendages, the glandular trichomes of cannabis secrete and accumulate many economically important compounds, including cannabinoids, monoterpenes, and sesquiterpenes [41] [42] [43]. The metabolite storage cavities of *C. sativa* trichomes are subcuticular in nature and form via delamination of the primary wall [64] [65]. An anatomical arrangement that sees

two biological layers theoretically inhibit oxidation, and aid retention of high-value compounds under prolonged storage. The cell wall itself is rich in weakly bonded polysaccharides, and its composition has only recently been deduced via glycomic profiling and monosaccharide analysis by researchers investigating cell wall remodeling for prevention of metabolite leakage during trichome maturation [44]. However, the cuticle still represents the outermost layer of the modified epidermal cell, acting as a protective layer against water loss and oxidation, while serving as the interface for potential interactions with gaseous atmospheric environment.

From a structural perspective the glandular trichomes of cannabis are encapsulated by the cuticle, which is composed of varying lipidic layers [42] [66]. Structure of the cuticle varies amongst land plants, and working models of architecture vary, but the recent review from [67] defines the cuticle as three distinct layers: the cuticular layer, cuticle proper, and epicuticular waxes. With a composition of cellulose, polysaccharides, cutin, and waxes, the cuticular layer is constructed first and its assembly is adherent directly to the primary wall, or outermost layer of the polysaccharide rich epidermal cell wall [67]. The cuticle proper follows and is constituted mainly of cutin, intracuticular waxes, and, is typically considered void of polysaccharides, while a layer of epicuticular wax serves as the interface for potential interactions between the organ and the atmospheric environment [67]. The cuticle also contains additional embedded non-lipid polysaccharides and polyester linked phenolic compounds with their architectural and structural functionality under review [68].

4.2. Cuticle Degradation: A Potential Mechanism for Accelerated Degradation

The major macromolecular component of the cuticle is cutin, a polyester of covalently bound C16 and C18 hydroxylated fatty acids [69]. Reactions of the cuticle with oxygen species present in atmospheric air and generation of additional reactive oxygen species (ROS), offers a potential mechanism for the oxidation of the cuticular layer and subsequent oxidation of the primary cell wall (Figure 1). Accelerating degradation of the trichomes high value contents, however, mapping all potential interactions presents a challenge as the molecular assembly and mechanisms of linkage within the cuticle's constituents are complex [70] [71].

5. Cannabis Literature Conundrum

Most literature regarding postharvest degradation of high value compounds in cannabis have focused on other storage conditions, such as temperature and light. As one example, THC decreased by 63% when stored in light at 20°C for 98 weeks [72]. THC decreased by only 25% when stored in darkness instead, which was further reduced to 10% if storage temperature decreased to 5°C [72]. More recent studies have reported similar results [73] [74]. Most recently, it was shown that freezing samples in darkness could completely alleviate degradation of cannabinoids during storage even over several years [16].

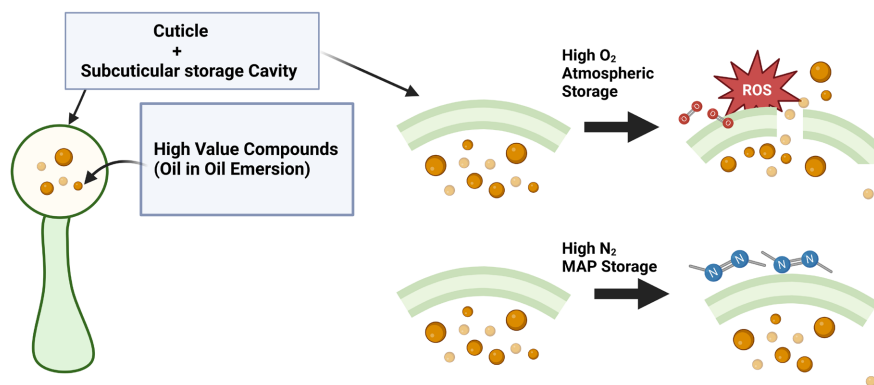


Figure 1. Graphical illustration for potential mechanism accelerated loss of high value compounds via oxidation of the cuticle and primary wall, resulting in escape of metabolites from the apoplastic storage cavity and their subsequent oxidation. Figure created with BioRender.com.

The presence of oxygen during storage is another factor that contributes to postharvest degradation of high value compounds. The addition of hydrogen peroxide, a strong oxidizing agent, greatly increased postharvest degradation of THC [75]. Conversely, storage with terpenes with established antioxidant properties effectively decreased degradation of THC [76]. Though terpene MAP was effective, it is more common for industry to use alternative MAP, such as N₂. The strategy with N₂ MAP is to greatly decrease the partial pressure of O₂ during storage, which should decrease the opportunity for oxidation. Industry currently does not typically include antioxidants with storage.

Despite commercial use, little is known on the effectiveness of N₂ MAP on preservation of high value targets in cannabis. One study investigated post-harvest changes in dried cannabis inflorescence terpene content under MAP using comparably unreactive argon but found no improvement versus storage in atmospheric conditions [13]. Storage at 2 weeks, and 4 weeks, saw terpene losses of 39.2% and 50.2% respectively under argon MAP, while losses of 40.5% and 51.6% were observed at the same time points for the control [13]. However, the flower sample used in this study had a very low terpene content of 0.170% w/w, due to its age at the time of sampling. With such a significant loss of volatile compounds having already occurred the potential for further loss would likely have been greatly reduced. A second study offered little experimental detail but reported storage in the absence of light to be more important for cannabinoid stability over storage with N₂, with major cannabinoids THC, CBD, and CBN measured [77].

N₂ MAP infrastructure represents a significant cost for industry. Further, liquid or gaseous N₂ dosage represents another incurred operational cost, yet little evidence for its efficacy over atmospheric storage exists. Other better-known factors can be addressed through cooling and storage in opaque containers. However, MAP requires more work to determine whether N₂ is effective. Even if N₂ MAP is effective, the exact effectiveness needs to be quantified and other potential strategies explored.

6. Conclusion

Though MAP has been used for almost 100 years commercially, it has only been used within the last few decades for legalized commercial production of cannabis. There is sufficient evidence that N₂ MAP or similar technologies are effective as increasing the shelf life of postharvest cannabis. However, there is less information available on effectiveness of N₂ MAP on the preservation of high value compounds, such as cannabinoids and terpenoids, in cannabis. The little information that's available academically supports the idea that N₂ MAP does not preserve those high value compounds. The lack of information available underscores a major gap in our knowledge that would be of value to the cannabis industry.

Acknowledgements

We thank Dr. Chijioke Emenike for reviewing an early draft of this manuscript.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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