COMPARATIVE STABILITY OF DRIED CANNABIS SATIVA L. FLOWER IN N_2-FLUSHED ALUMINUM LAMINATE BAGS VERSUS HDPE JARS UNDER ICH LONG-TERM STORAGE CONDITIONS

Katerina Muvcheska Paneva
Faculty of Medical Sciences, Goce Delcev University, Stip, North Macedonia
katerince_f.oh@hotmail.com, katerina.436s@student.ugd.edu.mk
Bistra Angelovska
Faculty of Medical Sciences, Goce Delcev University, Stip, North Macedonia
bistra.angelovska@ugd.edu.mk
Ivana Cocovska
Nedcann North Macedonia LLC, Ohrid, Republic of North Macedonia ivana_cocovska@yahoo.com
Jasmina Ristov
Nedcann North Macedonia LLC, Ohrid, Republic of North Macedonia jasminaristov@gmail.com
Katerina Ristanchevska
Nedcann North Macedonia LLC, Ohrid, Republic of North Macedonia farmacijanova@gmail.com

Abstract: This study evaluates the impact of packaging configuration and oxygen exposure on the chemical and microbiological stability of dried *Cannabis sativa* L. flower under controlled long-term storage conditions. Two commercially relevant packaging systems were compared: multilayer aluminum laminate bags packaged under nitrogen-modified atmosphere and high-density polyethylene jars sealed without inert gas. Four batches from two commercial strains with comparable cannabinoid potency were stored at 25 ± 2 °C and 60 ± 5 % relative humidity and analyzed at predefined time points up to nine months. Stability was assessed using validated pharmacopoeial and accredited analytical methods for active compound content, degradation indicators, moisture-related parameters, and microbiological quality. Descriptive trend analysis was applied due to single-sample testing per time point. The results demonstrate improved retention of Δ^9-tetrahydrocannabinol in nitrogen-packaged laminate bags compared with ambient packaging, with all nitrogen-packaged samples remaining within specification throughout the study, whereas an out-of-specification result was observed in one ambient-packaged batch at the ninth month. Microbiological parameters remained within regulatory limits for both packaging systems, indicating maintained microbiological safety under controlled storage. The observed stability differences are attributed to the combined effect of reduced headspace oxygen and the superior barrier properties of aluminum laminate materials, which limit oxygen and water vapor ingress and reduce oxidative potential during storage. These findings support the use of nitrogen-modified atmosphere packaging as an effective strategy for improving the chemical stability of dried cannabis flower without compromising microbiological compliance. From a practical perspective, the results provide a performance-based framework for packaging selection in good manufacturing practice environments and support informed decision-making related to shelf-life definition and quality risk management. Future studies including identical packaging formats with and without nitrogen are recommended to further isolate the independent contribution of atmosphere modification.
Keywords: Cannabis sativa L., stability, modified atmosphere packaging, nitrogen, Δ^9-tetrahydrocannabinol, packaging performance

1. INTRODUCTION

Cannabis sativa L., belonging to the family Cannabaceae, despite its widespread cultivation, trade, and use, is still considered one of the most controversial plants in the world (Stefkov et al., 2022). The phytochemical profile of cannabis is exceptionally complex, with more than 750 compounds identified, over 100 of which are classified as cannabinoids. In addition to cannabinoids, the plant contains terpenes, flavonoids, phenolic compounds, alkaloids, fatty acids, and other secondary metabolites, which collectively contribute to the pharmacological and organoleptic properties of the plant material (Lowe et al., 2021).

Control of drying and storage conditions is essential for preserving the chemical and pharmacological integrity of products based on *Cannabis sativa* L. According to Spadafora et al. (2024), optimal drying conditions include a temperature of 20–25°C and a relative humidity of approximately 50%, with indirect lighting and adequate ventilation, whereby a stable moisture content of 10–12% is achieved after six days, minimizing microbial activity and chemical instability (Grafström et al., 2019).

After drying, the stability of cannabinoids and terpenes largely depends on storage conditions. Critical factors include temperature (above 25°C accelerates THC oxidation), relative humidity (below 40% leads to excessive drying, while above 60% increases microbial risk), light exposure (initiates photo-oxidation), and packaging material, whose permeability to O_2 and H_2O directly affects product stability.

Modified atmosphere packaging represents a process of controlling and replacing the gaseous composition inside the package. The principle is based on removing air and introducing N_2 to create an inert environment that inhibits oxidative degradation of active substances. Oxidative degradation represents a major pathway of cannabinoid potency loss in stored cannabis inflorescence, particularly affecting Δ^9-THC levels during prolonged storage (Bueno et al., 2023). Packaging properties such as permeability, hermeticity, and resistance to pressure further influence the effectiveness of the N_2 atmosphere. HDPE packaging, although lightweight and economical, exhibits higher oxygen permeability compared with glass. Although HDPE provides good mechanical stability and moisture barrier properties, its limited resistance to oxygen and light makes it less suitable for photosensitive and oxidatively sensitive products such as cannabinoids and terpenes. According to MacLaughlin and MacDonald (2024), this factor may lead to a gradual increase in O_2 inside the package after six months of storage.

2. MATERIALS AND METHODS

This study was designed as a comparative study to evaluate the effect of nitrogen-modified atmosphere packaging (N_2-MAP) on the stability of dried *Cannabis sativa* L. flower during controlled storage.

Four batches of dried *Cannabis sativa* L. flower from two commercial strains (King's Kush and Original Blitz) with comparable cannabinoid potency were selected to minimize batch-related variability. Samples were packed into two packaging configurations: 10 g multilayer aluminum laminate bags packaged under nitrogen modified atmosphere, and 10 g high-density polyethylene jars sealed without inert gas.

Nitrogen packaging was performed using a controlled vacuum–gas flush system. For nitrogen modified atmosphere packaging (N_2-MAP), multilayer laminate bags with the following structure were used: an inner layer of 75 µm LDPE, a composite barrier layer of 12 µm aluminum, and an outer layer of 12 µm PET. The bag dimensions were 125 × 125 × 205 mm (internal) and 140 × 230 mm (external). The efficiency and reproducibility of atmosphere modification were verified by measuring residual headspace oxygen during process qualification. Typical residual oxygen levels ranged between approximately 5.0 and 6.6 %, confirming stable establishment of a reduced-oxygen environment prior to stability storage.

All samples were stored under controlled environmental conditions (25 ± 2 °C, 60 ± 5 % RH) in accordance with ICH Q1A(R2). Stability monitoring was conducted at predefined intervals, and critical chemical and microbiological quality attributes were evaluated using validated pharmacopoeial and accredited analytical methods (Δ^9-THC, CBN, loss on drying, TAMC, TYMC, specified microorganisms, heavy metals, mycotoxins; European Pharmacopoeia; ISO/IEC 17025).

3. RESULTS

This paper compares two commercially relevant packaging systems for dried medical cannabis flower: an aluminum laminate bag with nitrogen-modified atmosphere and a plastic child-resistant container without inert gas. The objective is to evaluate the influence of packaging material and the presence or absence of oxygen on the chemical and microbiological stability of the product.

Due to the single-sample design (n = 1 per time point), the comparison between packaging configurations was performed using descriptive trend evaluation.In King's Kush, nitrogen-modified atmosphere packaging showed improved Δ^9-THC retention over 9 months compared to ambient packaging, with THC retention of 90.3% for N_2-MAP laminate bags versus 83.9% for HDPE jars. Furthermore, only the ambient HDPE jar configuration resulted in an out-of-specification (OOS) Δ^9-THC result at T_9, while N_2-MAP samples remained within specification at all time points. Since only one sample per stability time point was analyzed, the results should be interpreted as indicative trends rather than statistically confirmed differences; nevertheless, the consistent direction of change and the occurrence of OOS results in ambient packaging support the practical superiority of the N_2-MAP laminate system for maintaining Δ^9-THC within specification during storage.

Table 1. Summary of Δ⁹-THC retention and degradation trends by packaging system and strain

Strain	Packaging	THC T_0 (%)	THC T_9 (%)	Retention T_9 (%)	Δ% (T_9 vs T_0)	Rate (ΔTHC / 9 months)	Linear slope (per month)	OOS at T_9
King's Kush	N_2-MAP laminate bag	21.54	19.46	90.3	−9.7	−0.231	−0.246	No
King's Kush	HDPE jar (ambient)	21.34	17.91	83.9	−16.1	−0.381	−0.323	Yes
Original Blitz	N_2-MAP laminate bag	27.49	26.09	94.9	−5.1	−0.156	−0.223	No
Original Blitz	HDPE jar (ambient)	26.86	22.81	84.9	−15.1	−0.450	−0.425	No

Source: Authors research

The microbiological stability of dried *Cannabis sativa* L. flower was evaluated by monitoring the total aerobic microbial count (TAMC) and total yeast and mold count (TYMC at time points T_3, T_6, and T_9 for both packaging systems (N_2-MAP laminate bags and HDPE jars without nitrogen).

The results indicate that, for all analyzed samples, TAMC and TYMC remained within the defined microbiological limits for dried cannabis flower (TAMC ≤ 1×10^5 CFU/g; TYMC ≤ 1×10^4 CFU/g) throughout the entire storage period.

For the King's Kush variety, TAMC values were low in both packaging systems, with the highest measured value being 10 CFU/g in the non-nitrogen packaging at T_3. With respect to TYMC, the highest value for King's Kush was 2,800 CFU/g (HDPE jar without nitrogen, T_6), which, although relatively higher compared with other time points, remained within the TYMC acceptance limit.

For the Original Blitz variety, TAMC and TYMC values were higher compared with King's Kush, which may be attributed to the natural variability of the plant matrix and the initial microbial load. The highest TAMC and TYMC values were 3,000 CFU/g (N_2-MAP bags, T_3), while for the non-nitrogen packaging the highest TYMC value was 1,200 CFU/g (HDPE jar, T_3). All results remained within specification, and no trend of progressive microbial increase over time was observed.

Table 2. Microbiological compliance summary (TAMC and TYMC) by packaging system and strain

Strain	Packaging	Max TAMC (CFU/g)	Max TYMC (CFU/g)	$\log_{10}$(Max TAMC)	$\log_{10}$(Max TYMC)	Margin to TAMC limit (log)	Margin to TYMC limit (log)	Status
King's Kush	10 g bags (N_2-MAP)	5	200	0.699	2.301	4.301	1.699	Pass
King's Kush	10 g jars (ambient)	10	2,800	1.000	3.447	4.000	0.553	Pass
Original Blitz	10 g bags (N_2-MAP)	3,000	3,000	3.477	3.477	1.523	0.523	Pass
Original Blitz	10 g jars (ambient)	400	1,200	2.602	3.079	2.398	0.921	Pass

Source: Authors research

Overall, the microbiological results confirm that both packaging systems provide a stable microbiological profile under controlled long-term storage conditions (25 ± 2 °C / 60 ± 5 % RH), and that the application of N_2-MAP does not exert a negative effect on microbiological safety. These findings support the interpretation that the primary difference between the two packaging systems is manifested mainly in the domain of chemical stability (Δ⁹-THC), while microbiological parameters remain compliant with specifications.

The obtained microbiological results (TAMC and TYMC) indicate that both packaging systems—N_2-MAP laminate bags and HDPE jars without the use of inert gas—ensure satisfactory microbiological stability of dried *Cannabis sativa* L. flower during storage under controlled long-term conditions (25 ± 2 °C / 60 ± 5 % RH). At none of the analyzed time points were the specification limits for TAMC (≤ 1×10^5 CFU/g) and TYMC (≤ 1×10^4 CFU/g) exceeded, confirming that the packaging, regardless of nitrogen application, does not lead to microbiological instability or progressive increase in microbial load over time.

From a scientific perspective, it is important to emphasize that TAMC and TYMC parameters in dried flower primarily reflect the initial microbial contamination and the drying and handling conditions, whereas after drying and packaging, microbial growth is limited provided that water activity and moisture remain low. The controlled stability conditions (60 ± 5 % RH) and the use of barrier packaging materials likely contribute to maintaining a relatively stable internal microenvironment and preventing conditions favorable for microbial proliferation.

Although, theoretically, packaging systems with different water vapor transmission rates may influence moisture dynamics within the product, no trend of gradual microbiological increase was observed in the present study, indicating that moisture remained controlled in both systems. Furthermore, the application of N_2-MAP is expected to exert a dominant effect on chemical stability (through reduction of oxygen and oxidative potential), while microbiological stability under low-moisture conditions is more strongly influenced by process-related factors (drying, hygiene, initial contamination) than by the presence of an inert gas.

In summary, the microbiological data confirm the safety of both packaging systems with respect to TAMC and TYMC under the defined conditions and provide additional assurance that the observed differences between the packaging systems are manifested primarily through chemical stability parameters (Δ^9-THC), rather than through microbiological non-compliance. This is particularly important from a good manufacturing practice perspective, as it demonstrates that optimization of packaging using N_2-MAP can deliver benefits in potency and compliance without compromising the microbiological conformity of the final product.

4. DISCUSSIONS

The results of the comparative analysis of the stability study of dried *Cannabis sativa* L. flower packaged in 10 g nitrogen-filled bags and 10 g jars without nitrogen indicate potential differences in the retention of Δ^9-THC during storage. In packaging under a modified atmosphere (N_2), THC content exhibits a slower and more controlled decreasing trend, remaining within the defined specifications in most cases up to time point T_9.

In contrast, jars packaged without the application of inert gas show a more pronounced decline in THC, with the occurrence of out-of-specification (OOS) results in some batches at T_9. These differences can be explained by the different microenvironments formed inside the packages. It is well established that Δ^9-THC is sensitive to oxidation, and that the presence of oxygen, light, and elevated temperature accelerates its gradual degradation into cannabinol (CBN) and other degradation products. With respect to microbiological quality, the results demonstrate that both nitrogen-packaged products and jars without nitrogen remain within pharmacopoeial limits for TAMC, TYMC, and the presence of specified pathogenic microorganisms up to T_9.

Additionally, the observed differences in stability are logically associated with the barrier properties of the packaging materials, since multilayer aluminum laminate structures are generally recognized as high-barrier packaging systems with extremely low oxygen transmission rate (OTR) and water vapor transmission rate (WVTR) compared with polymer containers such as HDPE (Robertson, 2016). This contributes to minimizing oxygen ingress from the external environment and reducing oxidative potential during storage. Such barrier performance, in combination with reduced headspace oxygen, most likely creates a synergistic protective effect reflected in improved retention of Δ^9-THC.

The packaging system used for N_2-MAP bags combines two protective mechanisms: (i) reduction of headspace oxygen through nitrogen flushing, and (ii) high-barrier packaging based on aluminum foil. The presence of a 12 μm aluminum layer in the PET/Al/LDPE structure significantly limits the diffusion of oxygen and water vapor from the external environment during storage, thereby creating a more stable environment with reduced oxidative potential. In comparison, HDPE packaging, despite providing good mechanical protection and the use of a lift-and-peel liner, remains a polymer-based system with higher oxygen permeability. Moreover, the headspace at T_0 is ambient (~21% O_2), and oxidative risk may persist over time. This provides a rational technical and scientific basis for the observed differences in Δ^9-THC stability, particularly in the case of King's Kush, where an out-of-specification result was observed at T_9 in HDPE jars, whereas Δ^9-THC remained within specification in N_2-MAP laminate bags.

5. CONCLUSIONS

It is important to emphasize that the observed differences in stability between the two packaging systems cannot be attributed exclusively to the presence of nitrogen as a single factor. Rather, they most likely result from a combined and synergistic effect of: (1) reduced oxygen concentration in the headspace of the N_2-MAP system, and (2) differences in the barrier properties of the packaging materials. Multilayer aluminum laminate materials are known to provide significantly superior barriers to oxygen and water vapor compared with polymer containers such as HDPE, thereby reducing oxygen ingress from the external environment and minimizing oxidative potential during storage. Consequently, the differences observed in Δ^9-THC stability most likely reflect the integrated protective performance of the packaging system as a whole, rather than the isolated effect of the modified atmosphere alone.

Nevertheless, this system-based approach represents a significant strength of the study, as it enhances the practical applicability of the results in the context of real-world good manufacturing practice decisions related to packaging selection. The packaging systems were evaluated from a performance-based perspective, focusing on: (i) retention of potency (Δ⁹-THC), (ii) risk of degradation-related changes, and (iii) microbiological stability during storage. Furthermore, this approach provides relevant input for quality and risk management in decision-making processes related to packaging selection, shelf-life definition, and the prevention of potential out-of-specification stability outcomes.

In future studies, to more precisely isolate the effect of nitrogen as an independent variable, it is recommended to include an additional comparative group using identical laminate bags packaged without nitrogen (ambient headspace). This would enable a statistically and experimentally robust differentiation between the effect of atmosphere modification and the barrier properties of the packaging materials. Nevertheless, the results of the present study provide a strong basis for practical conclusions and scientific support for the superiority of N_2-MAP laminate packaging with respect to Δ^9-THC retention and overall product stability.

ACKNOWLEDGEMENTS

The author would like to express sincere gratitude to Nedcann for providing the organizational and technical support necessary for the realization of this study. Special thanks are extended to the Quality Control (QC), Production and Quality Assurance (QA) teams for their contribution to sample handling, stability monitoring, and implementation of the associated processes. The author also acknowledges all colleagues who participated in the operational activities and documentation-related tasks supporting this work.

The author is especially grateful to the mentor for professional guidance, constructive feedback, and continuous support throughout the development of the study. Finally, heartfelt thanks are extended to the family for their encouragement, patience, and understanding

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