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Short communication

Accelerated stability of *Cannabis sativa* L. inflorescence: microbiological quality and LOD profile under two packaging configurations

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Introduction

Moisture is a quality-critical parameter in dried Cannabis sativa L. flowers: residual moisture governs susceptibility to microbial proliferation – particularly aerobic mesophiles and moulds (Gwinn et al., 2023) – and influences cannabinoid and terpene stability. Accelerated-stability data linking starting moisture and headspace atmosphere to microbiological and moisture-loss outcomes remain scarce (EMA/HMPC/95714/2013; Ph. Eur. 3028). Conducted alongside a commercial stability programme, this study assessed the microbiological quality and loss on drying (LOD) in the bulk-transport packaging system (LDPE inner / PET-ALU-LLDPE outer) at four starting moisture levels and two packaging configurations. Strain M. Runtz was selected as worst case for its dense inflorescence morphology, associated with reduced internal airflow and elevated post-harvest microbial risk (Mahmoud et al., 2023).

Materials and methods

Plant material and post-harvest processing

Cannabis sativa L. (strain M. Runtz) was propagated from vegetative cuttings and grown under controlled temperature, relative humidity (RH), photoperiod and CO_2 with stage-appropriate fertigation. Harvested inflorescences were dried and cured in dedicated rooms (15–20 °C) with trays rotated twice daily; final curing used two RH set-points (52% and 65%) to generate two distinct starting moisture levels (European Commission, 2008).

Stability programme and sampling

Samples were collected at four decreasing moisture stages: pre-conditioning (groups 1, 2; >14% moisture content (MC)), mid-drying (groups 3, 4; 12–14% MC), cured at 52% RH (groups 5, 6; <10% MC) and cured at 65% RH (groups 7, 8; <12% MC). Each stage was packed in duplicate 25 g units: one under ambient atmosphere (even groups) and one under inert-gas overlay (odd groups). Units were held in a climate chamber (HPP260L Memmert, Germany) at 40 °C ± 2 °C / 75% RH ± 5% RH per ICH Q1A(R2) and analysed at 0, 1, 2 and 3 months.

Loss on drying

LOD was determined gravimetrically per Ph. Eur. 2.2.32 in a vacuum oven (VO29 Memmert, Germany) over 4 Å (type 4A) molecular sieves –325 mesh (Sigma-Aldrich, USA).

Microbiological analysis

Microbial enumeration was performed per Ph. Eur. 2.6.12 under the Ph. Eur. 5.1.4 / 5.1.8 Category C acceptance criteria (TAMC ≤ 10^5 CFU/g; TYMC ≤

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10^4 CFU/g; bile-tolerant gram-negative bacteria ≤ 10^4 CFU/g; absence of Escherichia coli per 1 g; absence of Salmonella per 10 g); Escherichia coli, Salmonella spp. and aflatoxins were determined at release only (T = 0). Testing used a Class II Type A2 biosafety cabinet (BIO II Advance Plus 4, Telstar, Spain) in an ISO Class 7 background.

Results and discussion

Microbial purity

All TAMC and TYMC determinations across the eight groups and four time points were below the analytical LOQ (< 1 000 CFU/g), two to three log units below the Ph. Eur. 5.1.8 Category C limits. One determination (sample 7, month 2) returned TYMC = 1 000 CFU/g, within specification but flagged. Even the worst-case pre-conditioning group (1, 2) showed no detectable growth at any time point, supporting the bulk-transport system for short-term in-process holding.

Loss on drying

Pre-conditioning samples (1, 2; MC >14%) had the highest LOD (~20–23% at T=0), still ~18–21% at month 3. No significant decline was observed ($p \geq 0.79$; $r^2 \leq 0.05$), consistent with high moisture causing atmospheric saturation.

Samples with starting MC 12–14% (3, 4) showed variable LOD: ~9.8% at T=0, rising to 12.29–14.94% at months 1–2, then falling to ~7.4% by month 3, likely reflecting matrix heterogeneity, incomplete homogenisation and moisture redistribution.

Cured samples (5–8) were most stable. RH 52% samples (5, 6) declined from ~8–9% to 5.05–5.68% and RH 65% samples (7, 8) from ~9–10% to ~7.7% by month 3, all within the Ph. Eur. 3028 limit (LOD ≤12.0%).

No regression slopes were statistically significant ($p \geq 0.07$). Variability was higher in in-process samples (26–30% RSD) than cured samples (11–22%), reflecting matrix heterogeneity rather than true product drift. Paired comparison of LOD slopes within each moisture group showed no significant effect of inert-gas overlay (slope CIs overlap); its protective rationale is retained on theoretical grounds pending long-term data. Both groups satisfied Ph. Eur. 3028 at every pull; selection between curing conditions should follow terpene/cannabinoid retention.

Conclusion

All cured Cannabis sativa L. flower material was microbiologically and moisture-stable for three months under ICH Q1A(R2) accelerated conditions, satisfying Ph. Eur. 3028 (LOD ≤ 12.0%) and Ph. Eur. 5.1.4 / 5.1.8 Category C criteria. Even in-process material at LOD up to 22.7% maintained full microbiological compliance. No significant differential effect of inert-gas overlay was demonstrated. These results justify a formal ICH Q1A(R2) primary stability programme on three production-scale cured batches in the commercial container at long-term (25 °C/60% RH), intermediate (30 °C/65% RH) and accelerated (40 °C/75% RH) conditions.

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