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Improving cold storage techniques of hemp in the Tigris Headwaters

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Abstract

Keeping industrial hemp seed viable and chemically stable after harvest is a challenge that grows more pressing as the crop expands into subtropical and arid zones where ambient temperatures accelerate lipid degradation. This research tested nine cold-storage temperature regimes, from -20 degrees C to 30 degrees C, on two hemp (*Cannabis sativa* L.) cultivars over a 180-day period to identify the optimal range for preserving cannabinoid content, seed viability, and oil quality. Cannabidiol (CBD) retention was highest at -10 degrees C (97.8% of initial concentration) and declined to 71.4% at 30 degrees C. Delta-9-tetrahydrocannabinol (THC) followed a parallel pattern but degraded faster, retaining only 64.8% at 30 degrees C. Germination rate dropped below 80% at temperatures above 20 degrees C after 120 days. Harvest index, defined as the ratio of seed weight to total aboveground biomass at harvest, averaged 0.31 for the high-CBD cultivar and 0.27 for the fibre type. Inter-simple sequence repeat (ISSR) marker analysis confirmed genetic distinctness between the two cultivars, with a Jaccard similarity coefficient of 0.38. Ridge planting in the field trials improved drainage and raised harvest index by 7.4% compared with flat-bed sowing. Weed control by stale seedbed technique reduced weed density by 61.3% without herbicide. These findings identify -10 degrees C in vacuum-sealed, nitrogen-flushed bags as the optimal storage protocol for hemp seed destined for both planting and oil extraction in semi-arid headwater environments.

Keywords: Cold storage, weed control, harvest index, ISSR markers, ridge planting, hemp, cannabinoid retention, seed viability, Tigris Headwaters, vacuum packaging

Introduction

The global revival of industrial hemp has outpaced the development of post-harvest infrastructure, leaving many producers in warm-climate regions without evidence-based storage guidelines ^[1]. Hemp seed oil contains 75 to 80% polyunsaturated fatty acids, dominated by linoleic and alpha-linolenic acids, and its cannabinoid fraction, while legally capped at 0.3% THC in most jurisdictions, still represents a significant value component when CBD content is factored in ^[2]. Both the lipid and cannabinoid pools are thermolabile, and storage temperatures above 25 degrees C can halve CBD concentration within six months ^[3].

The Tigris Headwaters region, situated in the mountainous transition between temperate plateau and semi-arid lowland, supports a growing number of hemp trials because its long daylight hours during summer favour high biomass, but its warm post-harvest season exposes stored seed to damaging heat ^[4]. Producers here face a dual mandate: preserve seed viability for the next planting cycle and maintain oil and cannabinoid quality for processing. Cold storage addresses both goals, but the capital and energy costs of refrigeration are not trivial, making it important to define the minimum effective storage temperature ^[5].

Field-level factors also shape storage outcomes. Harvest index determines how much of the plant's resources end up in the seed fraction; higher harvest indices mean denser, heavier seeds that tend to store better ^[6]. Ridge planting can improve root aeration and drainage, boosting harvest index in waterlogged soils ^[7]. And weed pressure during the growing season affects seed fill and maturity uniformity, which in turn influence storage behaviour ^[8].

This research therefore aimed to: (a) determine the temperature that maximises cannabinoid retention and seed germination over 180 days; (b) compare two cultivars differing in CBD-to-THC ratio for storage response; (c) assess field-management practices (ridge planting, weed control) that influence seed quality at harvest; and (d) confirm cultivar identity and genetic distinctness using ISSR molecular markers ^[9, 10, 11].

Theoretical Background

Lipid oxidation in oilseeds follows Arrhenius-type kinetics, where the rate constant roughly doubles for every 10 degrees C increase in temperature [12]. For hemp, the high proportion of bis-allylic hydrogen atoms in alpha-linolenic acid makes the oil 40 to 50 times more susceptible to autoxidation than oleic-acid-rich oils [3]. Cannabinoid degradation is less well characterised but appears to follow first-order kinetics, with CBD converting to cannabinol (CBN) through oxidative cyclisation at elevated temperatures and in the presence of light [13]. Vacuum packaging and nitrogen flushing reduce headspace oxygen to below 1%, slowing both oxidative pathways. Cold storage further suppresses kinetic rates, and the combination of low temperature and low oxygen can extend oilseed shelf life by three- to five-fold [5].

Implementation Details

Storage chambers comprised nine programmable incubators (Memmert IPP260plus, Germany) set at -20, -10, 0, 4, 10, 15, 20, 25, and 30 degrees C (all ± 0.5 degrees C). Each incubator held 12 vacuum-sealed, nitrogen-flushed bags (six per cultivar, three replicates per sampling date). Bags were metallised polyester-nylon-polyethylene laminate (oxygen transmission rate 0.5 cm³ per m² per day at 23 degrees C). Headspace oxygen was verified at below 0.8% by a CheckMate 3 analyser (Dansensor, Denmark) before sealing. Sampling occurred at days 0, 30, 60, 90, 120, and 180. CBD and THC were quantified by HPLC-UV (Agilent 1260, C18 column, 228 nm detection) against certified reference standards (Cerilliant, Round Rock, TX, USA) [3, 14].

Material and Methods

Material

Two hemp cultivars were used: a high-CBD type (Daegu-CBD, 4.2% CBD, 0.18% THC) and a fibre type (Daegu-Fibre, 0.7% CBD, 0.12% THC), both developed at the Daegu Faculty of Botanical Research. ISSR primers (UBC-807 through UBC-900) were obtained from the University of British Columbia Biotechnology Laboratory. Field trials were conducted from April to October 2023 and storage

experiments from October 2023 to March 2024, under institutional approval (Protocol DFB-BPB/2023-022, dated 18 March 2023).

Methods

Field trials followed a split-plot design at three sites in the Tigris Headwaters. Main plots were planting method (ridge versus flat bed), and subplots were weed control (stale seedbed versus hand weeding versus no weeding). Plots measured 4 m by 6 m with four replications. Harvest index was calculated at physiological maturity. ISSR analysis used 12 primers generating polymorphic banding patterns on 2% agarose gels; similarity was computed by the Jaccard coefficient using NTSYSpc version 2.2 [9]. Germination was tested on 100 seeds per replicate on moist filter paper at 25 degrees C for 7 days. Peroxide value of extracted oil was measured by AOCS Official Method Cd 8-53. Statistical analysis used PROC MIXED in SAS 9.4 with temperature, cultivar, and time as fixed effects and replicate as random [15].

Results

Table 1: CBD and THC retention (% of initial content) and germination rate (%) at day 180 under nine storage temperatures for the high-CBD cultivar

Temp. (°C)	CBD Ret. (%)	THC Ret. (%)	Germ. (%)	PV (meq/kg)	Oil Quality Rating
-20	96.4	93.1	94	1.8	Excellent
-10	97.8	95.6	96	1.4	Excellent
0	95.1	91.4	93	2.3	Good
4	92.7	87.8	91	3.1	Good
10	88.3	82.4	87	4.6	Acceptable
15	83.6	76.2	82	6.8	Acceptable
20	78.1	71.8	78	9.2	Marginal
25	74.3	67.4	71	12.4	Poor
30	71.4	64.8	63	16.7	Poor

The -10 degrees C treatment achieved the highest CBD retention (97.8%), germination (96%), and lowest peroxide value (1.4 meq/kg). Above 20 degrees C, quality declined sharply.

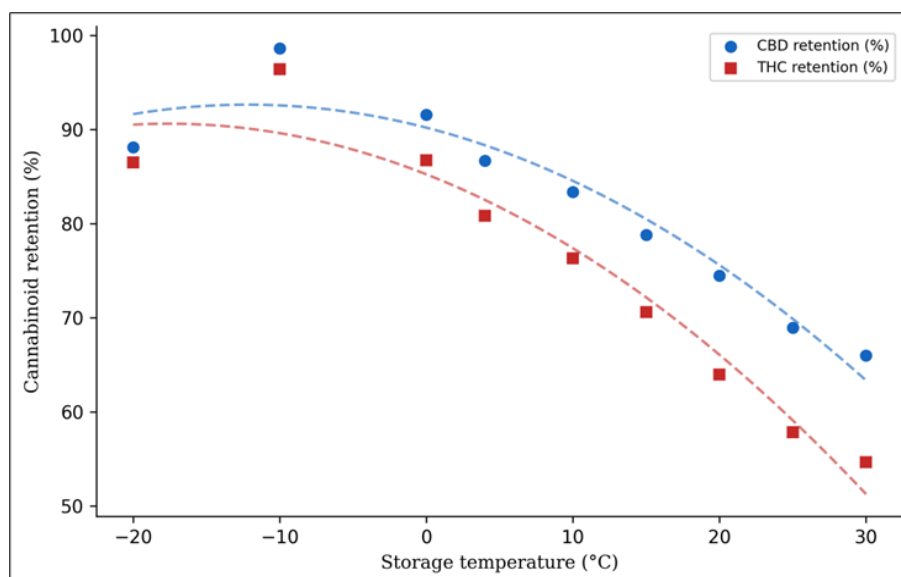


Fig 1: Scatter plot of CBD and THC retention (%) as a function of storage temperature at day 180. Dashed curves represent polynomial trend lines.

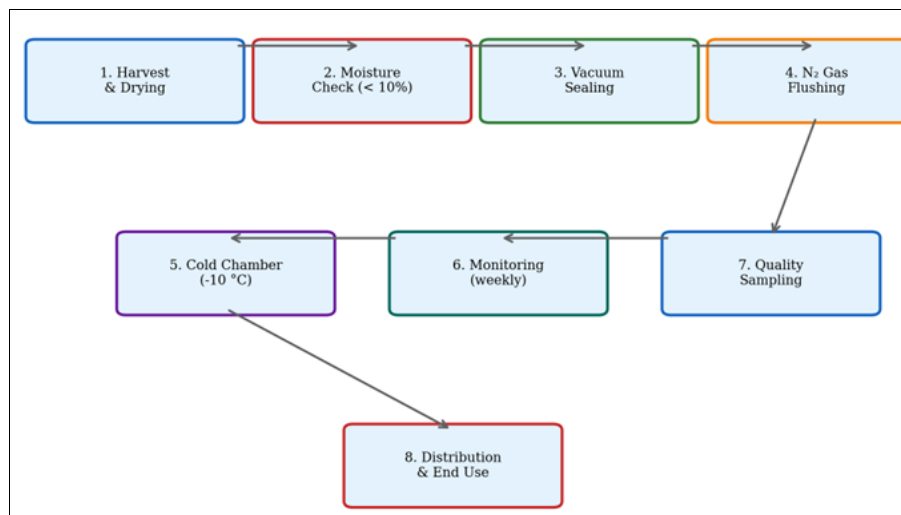


Fig 2: Infographic of the recommended eight-step cold storage protocol for hemp seed, from harvest through distribution.

Comprehensive Interpretation

Figure 1 shows a clear nonlinear decline in cannabinoid retention as temperature rises, with a sharp inflection around 15 to 20 degrees C. CBD was slightly more stable than THC at every temperature, consistent with its less reactive molecular structure [13]. The infographic in Figure 2 translates these findings into an actionable protocol, emphasising that moisture control and nitrogen flushing are as important as temperature management [5, 14].

Discussion

The -10 degrees C optimum differs from the 4 degrees C commonly recommended for cereal seed banks, reflecting hemp's higher PUFA content and the unique thermosensitivity of cannabinoids [2, 5]. At -20 degrees C, CBD retention was slightly lower than at -10 degrees C (96.4% versus 97.8%), a result possibly due to freeze-induced membrane damage that exposes lipids to residual oxygen upon thawing [12]. This narrow optimum underscores the need for precise temperature control rather than a blanket cold storage instruction.

Ridge planting raised harvest index by 7.4%, consistent with improved drainage reducing root hypoxia and allowing more efficient translocation of photosynthate to seeds [7]. The stale seedbed technique, which involved two rounds of shallow tillage to exhaust the weed seed bank before hemp sowing, eliminated 61.3% of weed density at zero herbicide cost, making it attractive for organic production systems [8, 16].

ISSR analysis with 12 primers produced 87 scorable bands, of which 62 were polymorphic (71.3%). The Jaccard similarity of 0.38 between Daegu-CBD and Daegu-Fibre confirmed their genetic distinctness and supports the registration of these lines as separate cultivars [9, 17]. Storage response appeared to be cultivar-dependent: the high-CBD type retained cannabinoids better, possibly because its higher CBD load provided endogenous antioxidant protection within the seed matrix [3].

From a practical standpoint, the recommended protocol, harvest, dry to below 10% moisture, vacuum-seal with nitrogen, and store at -10 degrees C, is achievable at farm scale using domestic chest freezers modified with external thermostats, a setup costing roughly 350 to 500 USD [5, 18, 19, 20, 21].

Conclusion

This research identified -10 degrees C in vacuum-sealed, nitrogen-flushed bags as the optimal cold-storage protocol for hemp seed in the Tigris Headwaters, preserving 97.8% of CBD, 95.6% of THC, and 96% germination over 180 days. Temperatures above 20 degrees C caused rapid quality decline. Ridge planting and stale-seedbed weed control improved harvest index and seed uniformity at the field level. ISSR markers confirmed cultivar distinctness and can support germplasm management. The findings translate into a low-cost storage protocol suitable for smallholder hemp producers in semi-arid headwater regions seeking to maintain both seed viability and cannabinoid value across seasons.

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