Fast-field-cycling NMR Relaxometry and Biometric Analyses for the Evaluation of Hemp Inflorescences Traceability

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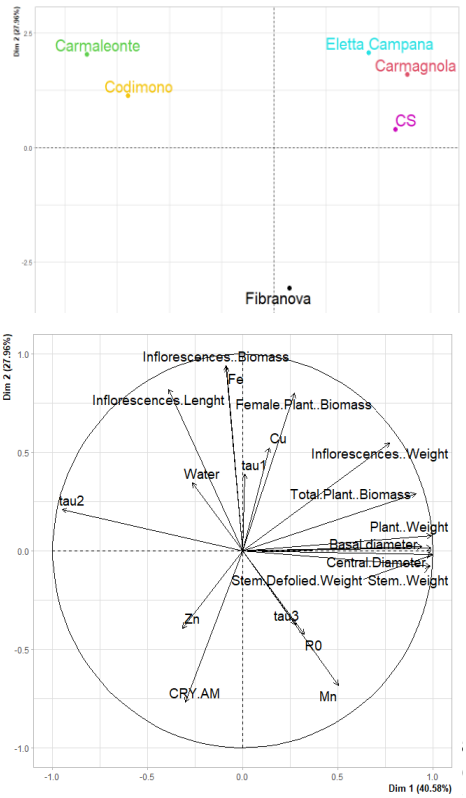
Hemp (*Cannabis sativa L.*), a versatile crop, offers numerous environmental advantages, including carbon sequestration, disease cycle disruption, soil erosion control, biodiversity support, reduced pesticide reliance, and alignment with the European Green Deal goals [1]. Industrial hemp varieties, adhering to the EU-mandated 0.2% Δ9-tetrahydrocannabinol (THC) limit, are freely cultivable. Beyond its conventional use as a source of stem-derived bast fibers, hemp finds applications in non-textile sectors. Notably, its inflorescences yield valuable essential oils (EO). However, EO composition and aroma profiles vary significantly across cultivars [2], necessitating raw material authentication. To address this, we employed various NMR techniques and biometric data to distinguish among seven hemp cultivars. High-resolution NMR spectroscopy and fast field cycling NMR relaxometry have proven effective in differentiating plant tissues based on origin [3]. NMR techniques offer advantages over other analytical methods due to their speed and non-destructive nature [4]. Furthermore, combining NMR data with biometric analyses through statistical methods enhances their potency [5].

During 2021, two monoecious (*Codimono* and *Carmaleonte*) and five dioecious hemp cultivars (*Carmagnola*, *Carmagnola Selected* (CS), *Eletta Campana*, *Fibrante and Fibranova*) were cultivated in an open field setting in Battipaglia, Italy. Biometric measurements and inflorescence sampling were conducted at the end of the crop cycle. Following a 65°C heat treatment, inflorescences underwent grinding in preparation for analysis. FFC NMR experiments were carried out at a constant 25°C using a Stelar Spinmaster FFC 2000 relaxometer, using a previously described experimental setup by Conte et al. [6]. Experimental data processing employed the ModelFreeFFC software developed at the University of Bologna (Bologna, Italy) to derive correlation time (τ_C) distributions. CPMAS ^{13}C NMR experiments were conducted using a Bruker Avance 400 spectrometer operating at 100 MHz on the ^{13}C nucleus with a rotor spin rate of 13 kHz. Cellulose crystallinity was determined by deconvolution of signals within the 95-80 ppm range, following the method described by Park et al. [7]. Total recoverable levels of Cu, Fe, Mn, and Zn in inflorescences were quantified using a Perkin Elmer AAnalyst 400 with flame atomization (Waltham, MA – USA) after nitric-perchloric acid digestion. Principal Component Analysis (PCA) was performed using the R software (v4.1.2) and the FactoMineR package.

The τ_C distributions derived from the FFC NMR study (not shown) revealed the presence of two or three bands across three distinct relaxation domains for all samples. Recognizing the complex molecular makeup of inflorescences, higher τ_C values can be attributed to components with low molecular mobility, such as polysaccharides, proteins, fibers, and adsorbed water. Conversely, intermediate and lower τC values likely represent more mobile components, including polyphenols, cannabinoids, terpenes, and others. Notably, the peak τ_C values

within each domain (τ_{C1}< τ_{C2} <τ_{C3}) varied among cultivars, suggesting differences in inflorescence molecular composition.



principal components: tau1 τ_{C1}, tau2 τ_{C2}, tau3=τ_{C3}; CRY.AM=crystalline-to-amorphous ratio

In contrast, the CPMAS ^{13}C NMR spectra did not reveal inter-cultivar differences except within the 95-80 ppm interval. Variations in this region were attributed to differences in the amorphous and crystalline forms of cellulose. Significant differences among cultivars were also observed in biometric measurements and metal concentrations (data not shown).

To further investigate these distinctions, principal component analysis was performed using parameters derived from the NMR techniques, metal levels, and biometric measurements. Three distinct cultivar groups emerged from the PCA analysis. Along PC1, which accounted for 40.6% of the variability, Codimono and Carmaleonte were clearly distinguished from Eletta Campana, Carmagnola, and CS. This separation was driven by higher τ_{C2} values in Codimono and Carmaleonte, indicating a different composition of intermediate molecules, as well as by smaller stem dimensions.

Along PC2, explaining 24.8% of the variability, Fibranova and Fibrante were set apart by their higher crystalline-to-amorphous ratios and, to some extent, higher τ_{C3} values. Reduced inflorescence and female plant growth also contributed to the differentiation of these cultivars.

Conclusions

This study highlights the potential of combining FFC NMR relaxometry, CPMAS ^{13}C NMR, and biometric analyses as a comprehensive approach for differentiating among hemp cultivars. The distinct clustering of cultivars based on these parameters underscores the sensitivity of these techniques to variations in molecular composition, cellulose structure, and growth patterns. Future research expanding upon these findings by correlating relaxometric characteristics with the biochemical composition of hemp inflorescences will further strengthen the value of this approach for cultivar identification and quality control in hemp production.

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