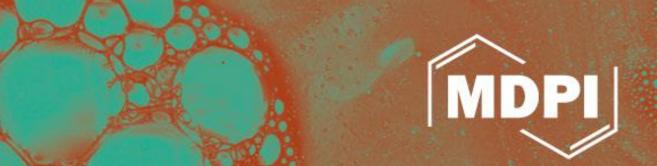
# The 3rd International Online Conference on Toxins



10-12 September 2025 | Online

# Determination of Cannabinoids in Hemp Seeds, Oil and Tea Samples Using LC-MS/MS

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## INTRODUCTION & AIM



Recently, hemp products—seeds, oil, and tea—have been gaining popularity as food due to their high content of essential micro- and macro-nutrients. While hemp products offer significant nutritional benefits, they might pose a risk to consumers due to natural toxins such as cannabinoids. Cannabinoids include both psychoactive substances, such as  $\Delta 9$ -tetrahydrocannabinol ( $\Delta 9$ -THC), and non-psychoactive ones like cannabidiol (CBD). Even though hemp typically contains low levels of  $\Delta 9$ -THC, even trace amounts in food products can be concerning.

Because of this,  $\Delta 9$ -THC content in products for human consumption has been regulated, with the maximum limits introduced under Commission Regulation (EU) 2023/915. The aim of this study was to determine the levels of selected cannabinoids in various hemp products, to verify the profile and content of psychoactive cannabinoids.

### **METHOD**

Nine cannabinoids— $\Delta 9$ -tetrahydrocannabinol,  $\Delta 8$ -tetrahydrocannabinol ( $\Delta 8$ -THC), cannabinol (CBN), cannabidiol,  $\Delta 9$ -tetrahydrocannabivarin (THCV), cannabigerol (CBG),  $\Delta 9$ -tetrahydrocannabinolic acid ( $\Delta 9$ -THCA), cannabidiolic acid (CBDA), and cannabigerolic acid (CBGA)—were determined in 25 samples, including hemp seeds, oil, and teas. Samples were extracted with acetonitrile and diluted before analysis. The compounds were separated on a Kinetex C18 column (1.7  $\mu$ m, 100 × 2.1 mm) using 0.1% aqueous formic acid as mobile phase A and 0.1% formic acid in methanol as mobile phase B. Measurements were conducted using LC-MS/MS, operating in both positive and negative electrospray ionization (ESI+/-).

Table 1. Optimized conditions of the ion source. \*- Quantifier ion.

Compound	Precursor ion [M+H] <sup>+</sup> [m/z]	Fragment ion [m/z]	DP [V]	CE [V]	CXP [V]	EP [V]
Δ9-THC 1		193.1*	61.0	33	10	10
Δ9-THC 2	315.124	123.2	61.0	45	16	10
Δ9-THC 3		135.2	61.0	27	6	10
$\Delta 9$ -THC D <sub>3</sub> (IS)	318.176	123.2	121.0	45	14	10
Δ8-THC 1		193.1*	96.0	33	10	10
Δ8-THC 2	315.112	123.1	96.0	43	14	10
Δ8-THC 3		233.0	96.0	25	12	10
THCV 1		123.0*	109.0	43	15	10
THCV 2	287.20	165.0	109.0	31	12	10
THCV 3		231.0	109.0	28	11	10
CBD 1		193.2*	56.0	31	10	10
CBD 2	315.100	123.1	56.0	41	14	10
CBD 3		135.1	56.0	35	15	10
CBD D <sub>3</sub> (IS)	318.132	196.2	51.0	31	28	10
CBG 1		193.3*	105.0	25	10	10
CBG 2	317.025	123.1	105.0	45	16	10
CBG 3		207.1	105.0	13	16	10
CBN 1		223.1*	101	29	12	10
CBN 2	311.098	195.2	101	37	24	10
CBN 3		208.1	101	42	10	10
Δ9-THCA 1		245.2*	-145	-34	-29	-10
Δ9-THCA 2	357.065	191.2	-145	-44	-21	-10
Δ9-THCA 3	337.003	203.0	-145	-46	-17	-10
Δ9-THCA D <sub>3</sub> (IS)	360.044	316.200	-35	-36	-17	-10
CBDA 1		245.1*	-140	-40	-15	-10
CBDA 2	357.085	107.1	-140	-52	-5	-10
CBDA 3		311.2	-140	-34	-14	-10
CBGA 1		315.3*	126.0	-32	-17	-10
CBGA 2	359.075	136.0	126.0	-44	-15	-10
CBGA 3	333.073	191.1	126.0	-44	-19	-10

#### **RESULTS & DISCUSSION**

The cannabinoid profile varied among the tested materials. Seeds contained lower amounts of most cannabinoids, and only one hemp seed sample exceeded the maximum allowable limit for  $\Delta 9$ -THC equivalents. In oils, cannabinoids were dominated by CBD, with smaller proportions of CBDA. Notably, three out of five oil samples contained  $\Delta 9$ -THC and  $\Delta 9$ -THCA levels significantly above the legal limit, reaching up to 398.3 mg/kg.

Tea (leaves) contained the highest overall levels, particularly of CBD and CBDA, making it the richest source of non-psychoactive cannabinoids. However, it also showed elevated concentrations of  $\Delta 9$ -THC and  $\Delta 9$ -THCA (up to 68.0 mg/kg and 69.7 mg/kg, respectively). Across all samples, CBD and CBDA were the main contributors to the total cannabinoid content

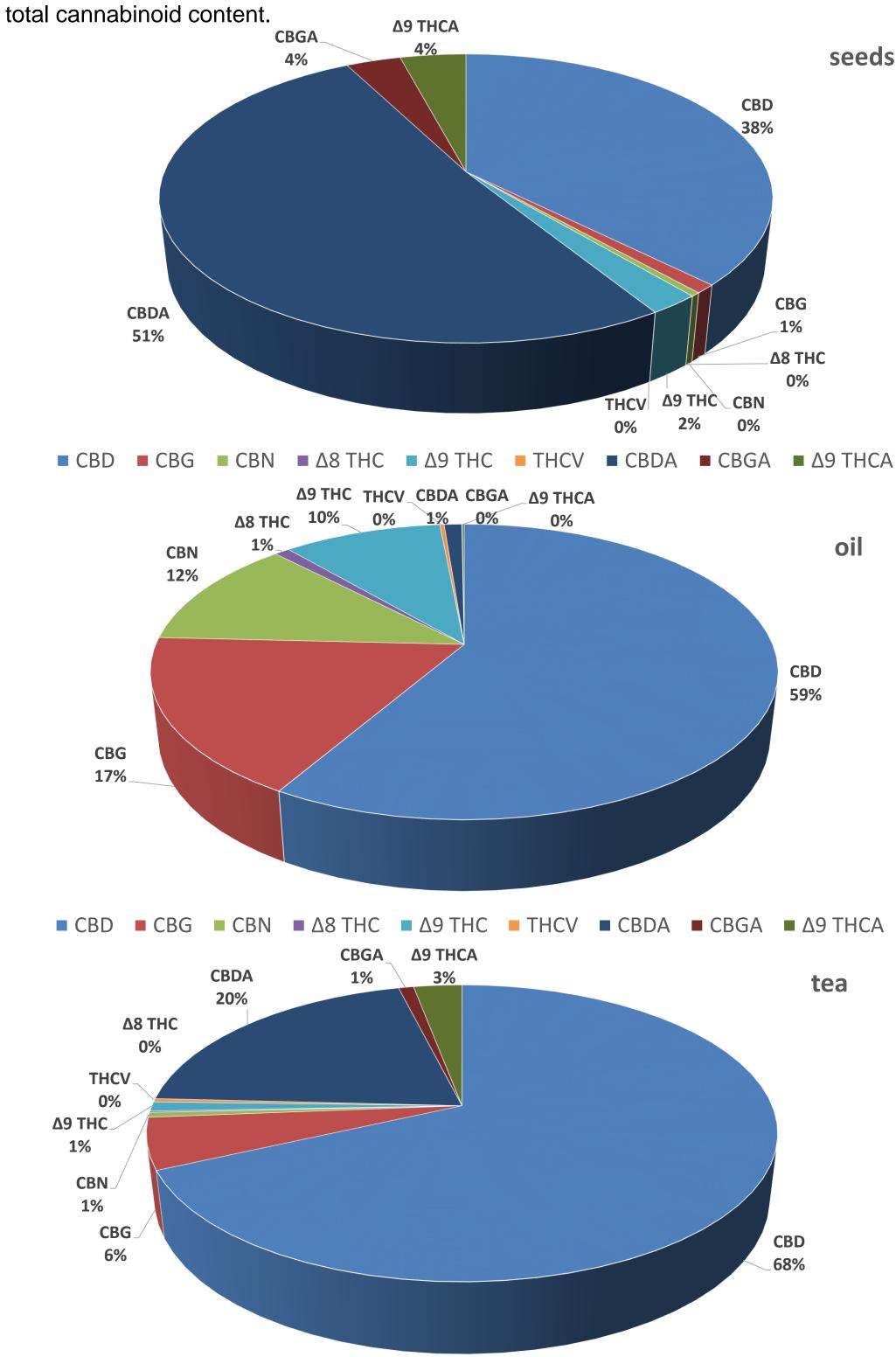


Figure 1. Cannabinoid profiles of seed, oil and tea samples, expressed as the percentage composition of individual compounds.

■ CBD ■ CBG ■ CBN ■ Δ8 THC ■ Δ9 THC ■ THCV ■ CBDA ■ CBGA ■ Δ9 THCA

#### CONCLUSION

While hemp products offer health benefits, the risks from cannabinoids must be managed. Regulatory oversight, standardized testing, and public awareness are crucial for ensuring the safety of hemp-based foods. Although hemp contain low  $\Delta 9$ -THC levels, some tested products exceeded the allowed concentrations, posing a risk to consumers.

### FUTURE WORK / REFERENCES

Di Marco Pisciottano, I., Guadagnuolo, G., Soprano, V., Esposito, M., & Gallo, P. (2021). A survey of Δ9-THC and relevant cannabinoids in products from the Italian market: A study by LC–MS/MS of food, beverages and feed. *Food Chemistry*, *346*, 128898.

McRae, G., & Melanson, J. E. (2020). Quantitative determination and validation of 17 cannabinoids in cannabis and hemp using liquid chromatography-tandem mass spectrometry. *Analytical and Bioanalytical Chemistry*, *412*(27), 7381–7393.