

# Identification and Determination of Cannabinoids in both Commercially Available and Cannabis Oils Stored Long Term

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Among cannabinoids (CNs) in two commercially available and long-term stored cannabis oils obtained from the seeds of *Cannabis sativa L.* in Japan, tetrahydrocannabinol (THC), cannabidiol (CBD), cannabinol (CBN), and cannabichromene were identified and determined using high-performance thin-layer chromatography and capillary gas chromatography-mass spectrometry after partitioning extraction with *n*-hexane/acetone. CNs were determined in two commercially available cannabis oils, designated “Hemp oil” (THC, 16.3  $\mu\text{g/g}$  and CBN, 5.9  $\mu\text{g/g}$ ) and “Taima-yu” (CBD, 26.1  $\mu\text{g/g}$ ). The concentration of CBD in a cannabis oil that had been stored for 20 years was 107  $\pm$  4  $\mu\text{g/g}$ . However, no other CNs including THC were detected in the oil. The CBD content in the original oil was estimated to be about 130  $\mu\text{g/g}$  from the determination values of the two periods. These results suggest that cannabis oils generally contain CNs to some extent.

**Key words** — commercially available cannabis oil, long-term stored cannabis oil, cannabidiol, tetrahydrocannabinol, cannabinol, cannabichromene

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## INTRODUCTION

Cannabis seeds have been excluded from the legal regulations under the Cannabis Control Law in Japan. However, tetrahydrocannabinol (THC) and cannabinol (CBN), an oxidative product of THC, were found in cannabis seeds sold on the market as feed for birds.<sup>1)</sup>

Cannabis oil was used as boiled oil, for lacquer tree paint and for lighting in the past, but it has not been used recently. In Tochigi prefecture, it was noted that cannabis oil is rich in unsaturated fatty acids and it was attempted to use it as food.<sup>2)</sup>

We previously reported that 121  $\pm$  17  $\mu\text{g/g}$  of cannabidiol (CBD) was detected in cannabis oil made from a cannabidiolic acid (CBDA)-strain cannabis after 8 years of storage after pressing.<sup>2)</sup> Cannabichromene (CBC) is also present in the CBDA-strain cannabis leaf along with CBD. Therefore it is possible that cannabinoids (CNs) are contained in oils made from cannabis seeds in Japan. Cannabis oil is also excluded from the Japanese Cannabis Control Law.

In the present study, we identified and determined CNs in two commercially available cannabis oils sold as feed and cosmetics and in cannabis oil stored for 20 years at room temperature using high-performance thin-layer chromatography (HPTLC) and capillary gas chromatography-mass spectrometry (GC/MS). In addition, we estimated the CNs content in the original cannabis oil since there was no previous report on it.

## MATERIALS AND METHODS

**Chemicals and Reagents** — The authentic CNs were isolated and purified from leaves of *Cannabis sativa L.*<sup>3)</sup> The purities of THC, CBD, CBN, and CBC, were found to be 99.1, 96.8, 99.7, and 71.5%, respectively, using GC.<sup>4)</sup> 5 $\alpha$ -Cholestane as an internal standard was purchased from ICN Co. Ltd. The other chemicals used were of reagent grade.

**Commercially Available Cannabis Oils** — Commercially available cannabis oils were used for this study. One designated “Hemp oil” (30 ml volume) and the other designated “Taima-yu” (500 ml volume) were purchased from a T-shop in Tokyo and an Internet site in Osaka, respectively.

**Long-Term Stored Cannabis Oil**<sup>5)</sup> — Eighteen hundred milliliters of a cannabis oil supplied by Dr. A. Kenmoku, Department of Education, Utsunomiya

University, Tochigi, Japan, which was made from CBDA-strain cannabis seeds was used for this study. This oil was prepared by the following process. In 1983, cannabis seeds (21 kg) produced in the previous year were parched in an iron pot for 5 min and pressed with the expeller. To remove the cake, the crude oil was filtered through six folded sheets of filter cloth with heating at 80°C, and the purified oil (4.5 kg) was obtained. This cannabis oil was stored at room temperature in a brown bottle to avoid direct sunlight.

**Preparation of Samples from Cannabis Oils** — Cannabis oil (1.0 g) was transferred into a 100-ml separating funnel with 15 ml of *n*-hexane and partitioned by vigorously shaking with 30 ml of *n*-hexane-saturated acetonitrile for 10 min. The *n*-hexane layer was extracted twice again in the same manner. The combined acetonitrile layer was washed with 600 ml of water containing 2% sodium chloride and 100 ml of *n*-hexane. After evaporation of acetonitrile, the residues were dried under a stream of nitrogen. The residues were dissolved in 1 ml of the internal standard solution (5 $\alpha$ -cholestane, 0.2 mg/ml) to prepare sample solutions. CNs in the extracts were identified and determined using HPTLC and GC/MS.

**HPTLC Analysis** — The sample solutions (1  $\mu$ l) were placed on HPTLC plates (RP-18, Merck 15037) using 100% acetonitrile as a developing solvent. After development, the plates were sprayed with Echtblausalz B dissolved in NaOH 0.1 M as a coloring reagent.<sup>3)</sup> The detection limit for THC, CBD, CBN, and CBC was 50  $\mu$ g/g in the HPTLC method.

**GC/MS Analysis** — GC was performed using an AutoSystem XL (Perkin Elmer, CT, U.S.A.) instrument equipped with a fused silica gel column (0.25 mm i.d., 30 m length, 0.25- $\mu$ m film thickness; MDN-5S, Supelco, PA, U.S.A.). The column temperature was maintained at 50°C for 2 min, then increased by 10°C/min to 300°C and maintained at that temperature for 3 min. The injection port temperature was set at 250°C. The flow rate of He gas was 1.0 ml/min. The injection type and volume were splitless and 1  $\mu$ l, respectively. MS was performed using a TurboMass (Perkin Elmer) instrument. The temperature of interface and ion source were 280°C. The ionization voltage was 70 eV.

The quantitative analysis was carried out in a selective ion monitoring (SIM) mode of GC/MS. The ions monitored were:  $m/z$  231 for THC, CBD, and CBC;  $m/z$  295 for CBN; and  $m/z$  217 for 5 $\alpha$ -cholestane as an internal standard. A GC/MS mea-

surement in a scan mode ( $m/z$ : 40–400) was also carried out.

This GC-MS method determined CBD and CBC simultaneously owing to good separation of these peaks.<sup>6)</sup> In the present study, the determination limit for THC, CBD, and CBC was 5  $\mu$ g/g and that for CBN was 4  $\mu$ g/g.

**Calibration Curves for THC, CBD, CBN, and CBC** — The calibration curves were prepared in a concentration range from 0.0046 to 0.29 mg/ml for THC, from 0.0047 to 0.75 mg/ml for CBD, from 0.0039 to 0.031 mg/ml for CBN, and from 0.0051 to 0.33 mg/ml for CBC. An equivalent amount of 5 $\alpha$ -cholestane solution as an internal standard was added to each standard solution. Then, 1  $\mu$ l was applied to GC/MS measurement.

The contents of CNs were calculated as followed:

$$\text{Contents of CNs } (\mu\text{g/g}) = \frac{0.2 \times R \times V \times 1000}{W}$$

where  $R$  is the weight ratio of CNs to 5 $\alpha$ -cholestane estimated from the calculation curves,  $V$  the volume of 5 $\alpha$ -cholestane solution (ml), and  $W$  the weight of samples (g).

## RESULTS AND DISCUSSION

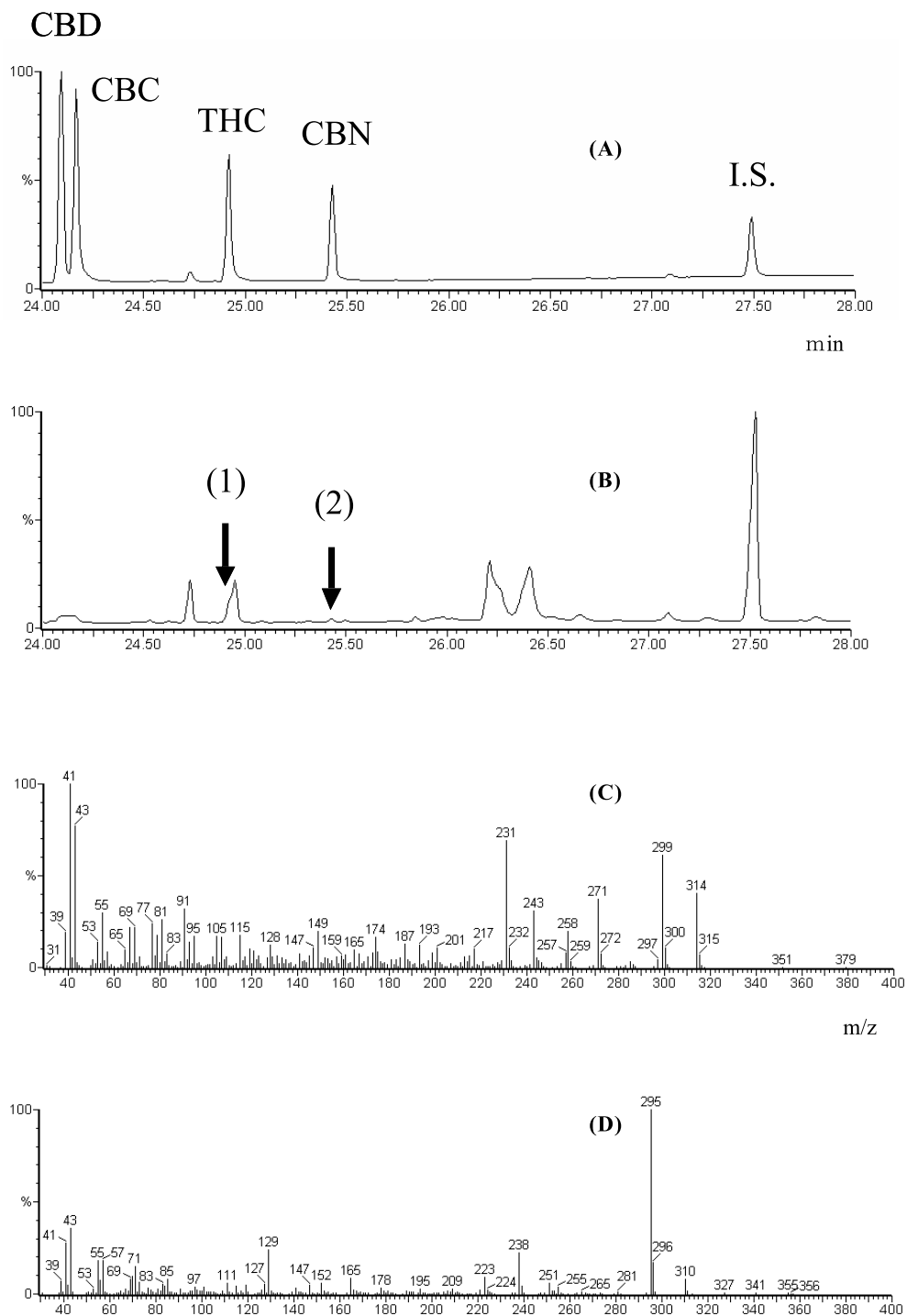
### HPTLC Analysis of CNs in Commercially Available Cannabis Oils and Stored Cannabis Oil

No spot of CNs was detected in the extracts from the commercially available cannabis oils sold as “Hemp oil” (30 ml volume) and “Taima-yu” (500 ml volume) due to the low detection sensitivity of the HPTLC analysis (50  $\mu$ g/g).

An orange spot was recognized at  $R_f$  value 0.65 in the extract from the stored cannabis oil. This spot was considered to be either CBD or CBDA based on a comparison with authentic samples which gave similar  $R_f$  values. However, CBDA in the cannabis oils could be easily converted to CBD by decarboxylation in the heating process and during storage.<sup>7)</sup> Therefore the orange spot appeared to be CBD. No other CNs (THC, CBN, or CBC) were detected in the oil.

### Determination of CNs by GC/MS in Commercially Available Cannabis Oils

Total ion chromatograms (TICs) of authentic CNs and the internal standard (I.S.) are shown in Fig. 1(A). Each CN and internal standard were sepa-



**Fig. 1.** Total Ion Chromatogram (TIC) of CBD, CBC, THC, CBN, and the Internal Standard (I.S.) (A), TIC of Commercially Available "Hemp Oil" (B) and Mass Spectra of THC (C) Indicated by an Arrow (1) and CBN (D) Indicated by an Arrow (2) in "Hemp Oil"

rated sufficiently. The TIC and mass spectra of "Hemp oil" in the commercially available cannabis oil are shown in Fig. 1(B–D), respectively. Mass spectra indicated by the arrows (1) and (2) in Fig. 1(B) corresponded to those of THC and CBN, respectively. Therefore THC and CBN were identi-

fied in the "Hemp oil." THC (16.3  $\mu\text{g/g}$ ) and CBN (5.9  $\mu\text{g/g}$ ) were determined in the "Hemp oil" along with a small amount of CBD and CBC (< 5  $\mu\text{g/g}$  for each compound). CBD (26.1  $\mu\text{g/g}$ ) was detected in "Taima-yu" along with a small amount of THC, CBC (< 5  $\mu\text{g/g}$  for each compound), and CBN (< 4  $\mu\text{g/g}$ )

(Table 1). The production of CBN may be more dependent on the content of THC than on the content of CBD.

The latter appeared to have components similar to those of the stored cannabis oil. These results indicate that the seeds utilized for the production of the two types of cannabis oils were from different strains. "Hemp oil" and "Taima-yu" appeared to be prepared from the seeds of THCA-strain and CBDA-strain cannabis, respectively.

### Determination of CNs by GC/MS in Stored Cannabis Oil

TICs of the stored cannabis oil and mass spectrum of CBD are shown in Fig. 2. The mass spec-

trum indicated by an arrow in Fig. 2(A) corresponded to that of CBD. Therefore CN in the stored cannabis oil was identified and determined to be CBD. The mean concentration of CBD was  $107 \pm 4 \mu\text{g/g}$  ( $n = 3$ ). In addition, a small amount of CBC, THC ( $< 5 \mu\text{g/g}$  for each compound), and CBN ( $< 4 \mu\text{g/g}$ ) was also detected in GC/MS of the oil. The peaks of retention time (25.5–26.5 min) of Fig. 2(A) were unknown compounds.

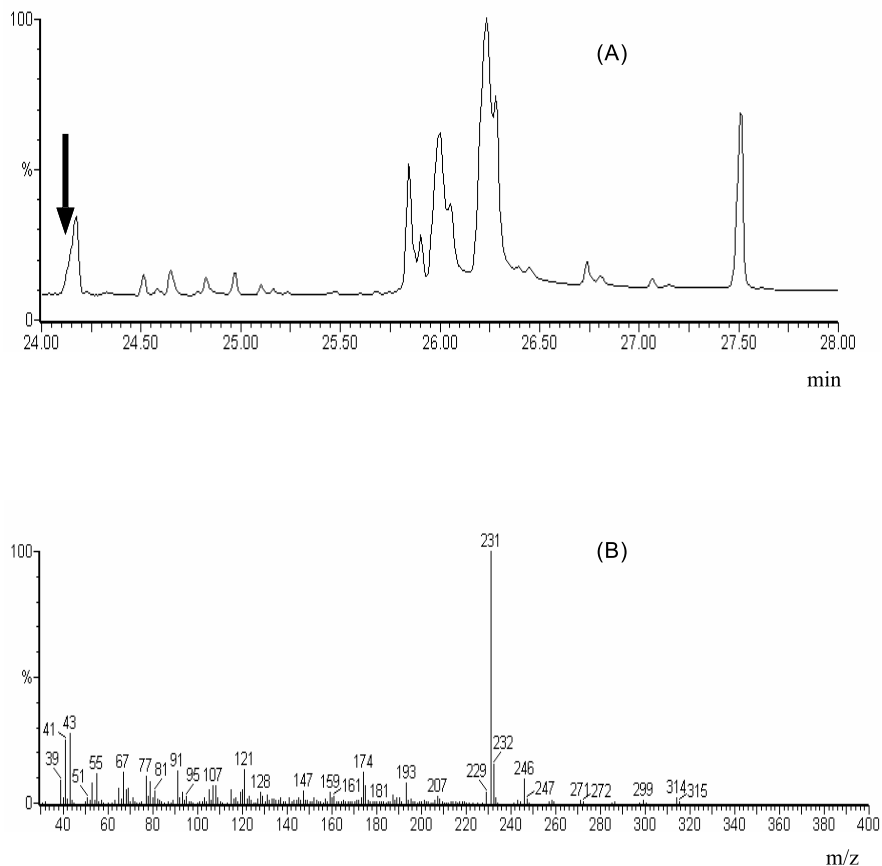
### Estimation of CBD Content

The change in CBD content during storage was estimated based on the present results and the previous study.<sup>2)</sup> The CBD concentration was found to decrease with time. The CBD content at the time of oil expression was estimated to be  $130 \mu\text{g/g}$  from the regression line in Fig. 3. As stated in MATERIALS AND METHODS, because 1 g of cannabis oil corresponded to 4.7 g of seeds, the estimated CBD concentration in the seeds was approximately  $28 \mu\text{g/g}$ . Matsunaga *et al.*<sup>1)</sup> reported that THC and CBN in birdseed were at the level of  $18 \pm 4 \mu\text{g/g}$  and  $14 \pm 3 \mu\text{g/g}$ , respectively. CN contents in fresh seeds are

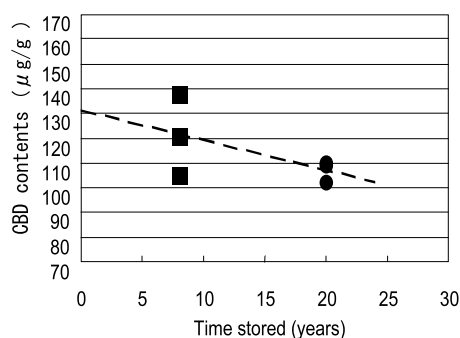
**Table 1.** Cannabinoid Contents in Commercially Available Cannabis Oil ( $\mu\text{g/g}$ )

Sample	THC	CBD	CBN	CBC
Hemp oil (30 ml)	16.3	ND <sup>a)</sup>	5.9	ND <sup>a)</sup>
Taima-yu (500 ml)	ND <sup>a)</sup>	26.1	ND <sup>b)</sup>	ND <sup>a)</sup>

a) ND  $< 5 \mu\text{g/g}$ , b) ND  $< 4 \mu\text{g/g}$ .



**Fig. 2.** TIC of Stored Cannabis Oil (A) and Mass Spectrum of CBD Indicated by an Arrow in Stored Cannabis Oil (B)



**Fig. 3.** Estimation of CBD Contents in Cannabis Oil Stored for 20 years  
Stored for 8 years (■) and 20 years (●).

estimated to be about 30  $\mu\text{g/g}$ . The estimated CBD content in cannabis oils is nearly equal to that of CNs in seeds.

Although CBN in the cannabis seeds appeared to be formed from THC and CBD by oxidative conversion during storage,<sup>8)</sup> CBN production did not appear to progress owing to the low level of CBN in the oil. It is believed that the environment does not allow CBD to undergo an oxidative reaction due to the conditions under which the oil was bottled and shielded from sunlight. ElSohly *et al.*<sup>9)</sup> reported that  $\Delta^9$ -THC contents in confiscated marijuana from 1980–1997 showed potency trends, while hashish oil showed no specific trends and other major CNs (CBD, CBN, and CBC) showed no significant change in concentration over the years. Therefore CNs in cannabis oil are considered to be stable in the oil.

In conclusion, the two commercially available cannabis oils “Hemp oil” and “Taima-yu” contained 16.3  $\mu\text{g/g}$  of THC, 5.9  $\mu\text{g/g}$  of CBN, and 26.1  $\mu\text{g/g}$  of CBD. The concentration of CBD in cannabis oil stored long term was  $107 \pm 4$   $\mu\text{g/g}$  20 years after oil expression. It was assumed that the CBD content in the original cannabis oil was 130  $\mu\text{g/g}$  from the determination values of the two periods. Thus cannabis oil generally contains CNs to some extent. These results suggest that the concentration of CNs in cannabis oils and their psychopharmacologic effects must be examined before marketing.

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