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Effect of vegetable oil types on the stability of cannabinoids in cannabis sublingual drops

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Abstract

This work sought to evaluate the stability of cannabis sublingual drops using high-performance liquid chromatography. The cannabis extract was dissolved in different types of vegetable oil–four types of mixed vegetable oils and 11 types of single vegetable oil. They were stored in the dark at 4°C and 30°C for 90 days. The contents of main cannabinoids (cannabidiol, tetrahydrocannabinol, and cannabinol) were analyzed and compared with the contents at the initial time. Results showed that almost all of the vegetable oil types provided the formulation with a shelf-life (remained cannabidiol and tetrahydrocannabinol ≥ 90%) of more than 60 days but less than 90 days at 30°C. Storage of cannabis sublingual drops at 4°C provided more stable formulations than at 30°C. The formulations with the shelf-life of more than 90 days were found when the seven vegetable oils, including mixed oil (no.1), mixed oil (no.3), sesame oil, rice bran oil, olive oil (no.1), coconut oil (no.1), and coconut oil (no.2) were used. In summary, the seven vegetable oil types can be selected as a vehicle to prepare the cannabis sublingual drops due to they could stabilize cannabinoids content.

Keywords**:** *Cannabidiol, cannabinoids, cannabis sublingual drop, stability, tetrahydrocannabinol, vegetable oil.*

1. Introduction

Stability of pharmaceutical and herbal products plays an important role in the pharmaceutical and herbal product development process. Stability data explain several factors affected the shelf-life of the products. The stability evaluation is required for an understanding of the physicochemical properties of drug substances and products (Melveger, & Huynh-Ba, 2009). The degradation of active ingredients affects the quality, efficacy, and safety of pharmaceutical and herbal products (Melo, Homem-de-Mello, Silveira, & Simeoni, 2014). Stability considerations can dictate the suitable condition or environment for drug production, choice of packaging, storage, transportation, and shelf-life of the finished products (Melveger, & Huynh-Ba, 2009).

Cannabis (*Cannabis sativa* L. subsp. *indica*) is a plant in the Cannabaceae family. In Thailand, it is categorized as a narcotic plant. Cannabis contains 750 identified phytochemicals; among these, more than 100 compounds are cannabinoids (Radwan et al., 2015). Recently, more than 150 cannabinoids are identified in the literatures (Hanuš, Meyer, Muñoz, Taglialatela-Scafati, & Appendino, 2016). Furthermore, a

novel phytocannabinoid is still found (Citti et al., 2019). The key cannabinoids are Δ^9-tetrahydrocannabinol (THC), cannabidiol (CBD), and cannabinol (CBN). Nowadays, THC and CBD are two phytocannabinoids mostly used for a medical purpose (Madras, 2015). More than these two are used today for treatment (Bielawiec, Harasim-Symbor, & Chabowski, 2020). The most widely used dosage form of cannabis products in Thailand is a sublingual drop. It has been used for the treatment of many symptoms especially insomnia, epilepsy, and pain. Various vegetable oils are used as a vehicle; however, there are limited number of reports about the stability of cannabinoids in sublingual drop formulations.

2. Objectives

The objective of this work was to evaluate the stability of cannabis sublingual drops—cannabis extract dissolved in vegetable oils—using high-performance liquid chromatography for quantitative analysis of cannabinoids content. The vegetable oils provided the high stability of the cannabis sublingual drops will be suggested to use as a vehicle for the product.

3. Materials and methods

3.1 Materials

Standard CBD, THC, and CBN, the purity was more than 99%, determined by HPLC, were isolated and purified from the seized cannabis bar obtained from the Narcotic Suppression Bureau. It was permitted by the Office of the Narcotics Control Board, Food and Drug Administration, Ministry of Public Health. All oil types (food grades) were coded as shown in Table 1. MIX1, MIX2, and MIX3 were manufactured by Morakot Industries Co., Ltd., Thailand. MIX4 was manufactured by Oh Chin Hing Sesame Oil Factory, Vietnam. SESAME was purchased from Lemon Farm, Thailand. SOY and CANOLA were manufactured by Thai Vegetable Oil PCL, Thailand. PALM was manufactured by Oleen Co., Ltd., Thailand. SUNFLOWER was manufactured by Thanakorn Vegetable Oil Products Co., Ltd., Thailand. RICE BRAN, CORN, and COCONUT2 were manufactured by Lam Soon Co., Ltd., Thailand. OLIVE1 and OLIVE2 were manufactured by Migasa, Spain. COCONUT1 were manufactured by Deena Products Co., Ltd., Thailand. All oil types were purchased from the supermarket in Pathum Thani Province. Olive oil no. 1 was full bodies & mild, while olive oil no. 2 was mild test. Coconut oil no.1 was extra virgin, while coconut no.2 was not extra virgin. Methanol (HPLC grade) was obtained from Duksan Pure Chemicals, Korea. Isopropanol (HPLC grade) was obtained from Fisher Scientific, UK.

3.2 Preparation of cannabis sublingual drops

The crude extract of cannabis was prepared by mixed cannabis (250 g) with 1,500 mL of 95% ethanol in a 5-L beaker (n = 2). The mixture was extracted in water bath at 40°C for 30 min (Monton et al., 2019), and then it was filtered in order to collect the filtrate. They were extracted for three times, then the filtrates were pooled. Ethanol was eliminated by a rotary evaporator (Büchi R-100, Büchi Labortechnik AG, Switzerland). The full spectrum of cannabis extract was obtained. The cannabis sublingual drop containing cannabis extract 3%, by weight, was prepared by mixing 3 g of cannabis extract with 97 g of vegetable oil in a 150-mL beaker under heating at 80°C for 5 min. Then, it was further agitated at 100 rpm for 3 days to ensure that it was mixed homogeneously. The obtain mixture (5 mL) was filled to an amber bottle with dropper. They were kept in dark at 4°C and 30°C for 90 days. They were sampled to determine cannabinoids content every 30 days and compared with the initial data.

3.3 Analysis of cannabinoids content using HPLC

The 150 mg of cannabis sublingual drop was dissolved in isopropanol in a 10-mL volumetric flask and adjusted to the volume. It was filtered and analyzed cannabinoids content by HPLC. The cannabinoids content was calculated according to the calibration curve of each cannabinoid.

The analysis of cannabinoids content was performed by HPLC instrument (Agilent 1260 Infinity, Agilent Technologies, USA). The HPLC method was slightly modified from previous work (Monton et al., 2019). The ACE 3 C18-PFP column (150×3.0 mm, i.d., 3 μm) (Advanced Chromatography Technologies Ltd., UK) was used and controlled at 25ºC. The isocratic elution system of water and methanol (17:83 v/v) in a flow rate of 0.4 mL/min was used. The injection volume was 5 μL. The detection wavelength was 222 nm.

4. Results and discussion

The cannabis sublingual drops were prepared by using different vegetable oils as a vehicle. Then, they were determined the weight per drop, cannabinoids content, and amount of cannabinoids per drop as shown in Table 1. Each drop of the different vegetable oil types was different in its weight. The lowest and the highest weight per drop were found in COCONUT2 (19.23 mg) and OLIVE2 (27.28 mg), respectively. These differences could promote different doses when they were used. The delivery dose per drop was varied from 34 to 50 μg CBD, 101 to 148 μg THC, and 50-71 μg CBN. Moreover, the cannabinoids content in cannabis sublingual drops which contained 3% cannabis extract was varied from 0.16 to 0.19% CBD, 0.52 to 0.55% THC, and 0.25 to 0.26% CBN. However, the limitation of this work was the seized cannabis bar which was stored for several years was used: it could not be specified the exact age, so the high CBN was observed due to THC was oxidized to CBN (Garrett & Tsau, 1974; Harvey, 1990; Repka, ElSohly, Munjal, & Ross, 2006; Zivovinovic, Alder, Allenspach, & Steuer, 2018), and the acid forms of cannabinoids was almost changed to neutral forms.

There are several advantages of sublingual administration, e.g., rapid absorption and fast onset of action, increase bioavailability, easy to use, avoid first-pass metabolism, etc. (Pawar, Ghorpade, & Kokane, 2018). However, the sublingual drops exhibited less accurate dosing style, the suitable administration technique highly affected the dose accuracy. According to the effect of drug vehicle, the different vehicle provided the different viscosity of the formulation, the weight per drops or deliver dosing was varied. So the patient should titrate the dose if the different vehicles of the formulation were used.

Table 1 Weight per drop, cannabinoids content, and amount of cannabinoids per drop of cannabis sublingual drops prepared using various vegetable oil types

Vegetable oils	Coded	Weight per drop (mg)*	Cannabinoids content in sublingual drop (%)**			Amount of cannabinoids per drop (mg)**		
			CBD	THC	CBN	CBD	THC	CBN
Palm oil mixed soybean oil (1:1.8)	MIX1	27.10±1.16	0.181±0.006	0.517±0.019	0.255±0.009	0.049±0.002	0.140±0.006	0.069±0.003
Canola oil mixed sunflower oil (2.3:1)	MIX2	25.67±0.69	0.183±0.015	0.542±0.044	0.252±0.021	0.047±0.001	0.139±0.004	0.065±0.002
Mixed oil (non-identified)	MIX3	21.78±2.47	0.178±0.008	0.526±0.023	0.247±0.011	0.039±0.004	0.115±0.013	0.054±0.006
Sesame oil mixed soybean oil	MIX4	25.19±0.76	0.163±0.002	0.537±0.005	0.253±0.003	0.041±0.001	0.135±0.004	0.064±0.002
Sesame oil	SESAME	19.51±0.83	0.166±0.003	0.542±0.011	0.264±0.005	0.032±0.001	0.106±0.004	0.052±0.002
Soybean oil	SOY	25.86±1.23	0.179±0.001	0.525±0.007	0.251±0.001	0.046±0.002	0.136±0.006	0.065±0.003
Palm oil	PALM	20.39±0.89	0.175±0.002	0.517±0.007	0.246±0.003	0.036±0.002	0.105±0.005	0.050±0.002
Canola oil	CANOLA	25.29±1.49	0.179±0.002	0.525±0.007	0.249±0.001	0.045±0.003	0.133±0.008	0.063±0.004
Sunflower oil	SUNFLOWER	22.88±1.38	0.181±0.004	0.536±0.012	0.255±0.006	0.041±0.003	0.123±0.007	0.058±0.004
Rice bran oil	RICE BRAN	27.03±1.62	0.185±0.001	0.547±0.005	0.263±0.003	0.050±0.003	0.148±0.009	0.071±0.004
Corn oil	CORN	26.36±1.31	0.183±0.005	0.542±0.014	0.261±0.007	0.048±0.002	0.143±0.007	0.069±0.003
Olive oil (no. 1)	OLIVE1	20.30±1.05	0.177±0.002	0.524±0.008	0.252±0.004	0.036±0.002	0.106±0.006	0.051±0.003
Olive oil (no. 2)	OLIVE2	27.28±1.76	0.178±0.006	0.530±0.018	0.254±0.008	0.049±0.003	0.145±0.009	0.069±0.004
Coconut oil (no. 1)	COCONUT1	25.61±1.53	0.180±0.006	0.532±0.016	0.256±0.008	0.046±0.003	0.136±0.008	0.066±0.004
Coconut oil (no. 2)	COCONUT2	19.23±0.97	0.175±0.003	0.524±0.004	0.259±0.002	0.034±0.002	0.101±0.005	0.050±0.003

*Mean±SD (n = 10); **Mean±SD (n = 3)

Figures 1-3 show the stability of CBD, THC, and CBN in various vegetable oils after stored at 4°C and 30°C for 90 days in the dark. The shelf-life of the pharmaceutical products was the time that the active ingredient degraded for 10% or active ingredient remained for 90% of label claim (Capen et al., 2012). Hence, this work selected the CBD and THC as a marker for the determination of stability. Nevertheless, CBN was not considered due to its remaining content might be the effect of its degradation combined with its increment from the degradation of THC in the formulation. Figure 1 and Figure 2 reveal that CBD and THC were stable in all vegetable oils at 30°C in the dark for at least 60 days, while it was lower than 90% at 90 days. Except, OLIVE1, CBD and THC were lower than 90% at 60 days. There were seven vegetable oil types promoted stable of CBD under storage in the dark at 4°C including MIX1, MIX3, SESAME, RICE BRAN, OLIVE1, COCONUT1, and COCONUT2. While there were 11 vegetable oil types promoted stable of THC under storage at 4°C including MIX1, MIX3, SESAME, SOY, PALM, CANOLA, RICE BRAN, CORN, OLIVE1, COCONUT1, and COCONUT2. These data revealed that the storage of the cannabis sublingual drop in the dark at 4°C gave more stable formulation compared with storage at 30°C. However, an increasing CBD and THC in some time points was found, especially at 4°C. Several oils became grease when stored in refrigerator, and non-homogenous formulation could be occurred. So, it could interfere the sample preparation and analysis. There were some reports showed that THC in cannabis oil was degraded in a temperature-dependent manner. THC that contained in cannabis resin and cannabis oil was higher degraded when exposing to light at 22ºC when compared with the data at 4ºC in darkness (Trofin, Dabija, Vaireanu, & Filipescu, 2012a; Trofin, Dabija, Vãireanu, & Filipescu, 2012b). Moreover, THC and CBD were highly degraded by increasing temperature rather than the solvent type used (Citti et al., 2016). According to the above data, it can be concluded that cannabis sublingual drops prepared using seven vegetable oils—MIX1, MIX3, SESAME, RICE BRAN, OLIVE1, COCONUT1, and COCONUT2—and store at 4°C had shelf-life of more than 90 days.

In the case of CBN, it was not selected as a marker because the remained content of CBN could affect by its degradation with the degradation of THC. The CBN content which higher than 90% when store in the dark at 30°C for 90 days, was observed when seven vegetable oils—MIX1, CANOLA, RICE BRAN, CORN, OLIVE2, COCONUT1, and COCONUT2—were used. While, when they were stored in the dark at 4°C, the 11 vegetable oils i.e., MIX1, MIX3, SESAME, SOY, PALM, CANOLA, RICE BRAN, CORN, OLIVE1, COCOCUT1, and COCONUT2, promoted the stable CBN (Figure 3). The authors mentioned that the seize cannabis bar used in this work had the high CBN content that might not be suitable for the treatment. However, it could be used as a theoretical study to determine the stability of cannabis products.

The different vegetable oil types gave different stability of some cannabinoids. It was difficult to describe the mechanistic effect due to both plant extract and vegetable oils composed of several compounds which can be interacted among them. In addition, the natural or synthetic antioxidants in different vegetable oils could prevent the degradation of THC by oxidation reaction. Not only vegetable oils could affect the cannabinoids, but cannabinoids could also alter some fatty acids in the vegetable oil. Our preliminary data showed that the fatty acids profile of the cannabis sublingual drops when stored at 4°C and 30°C was altered when determined by gas chromatography-mass spectrometry technique. However, this topic will be investigated in the future work. This result can be concluded that some interactions between several chemical constituents contained in the cannabis sublingual drop were occurred, so it might affect the shelf-life of the products.

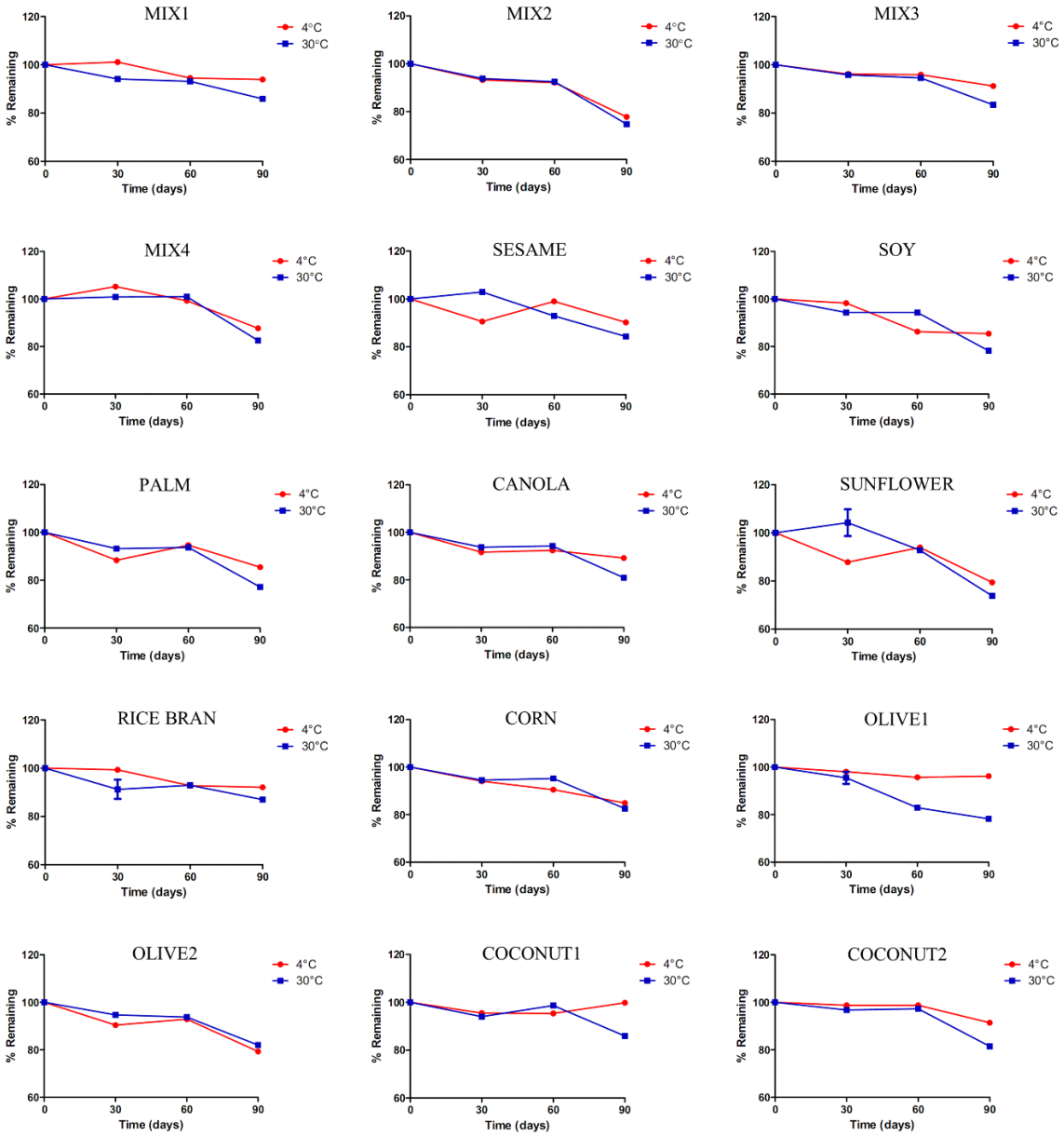

Figure 1 Stability of CBD in various vegetable oils after stored at 4°C and 30°C for 90 days in the dark

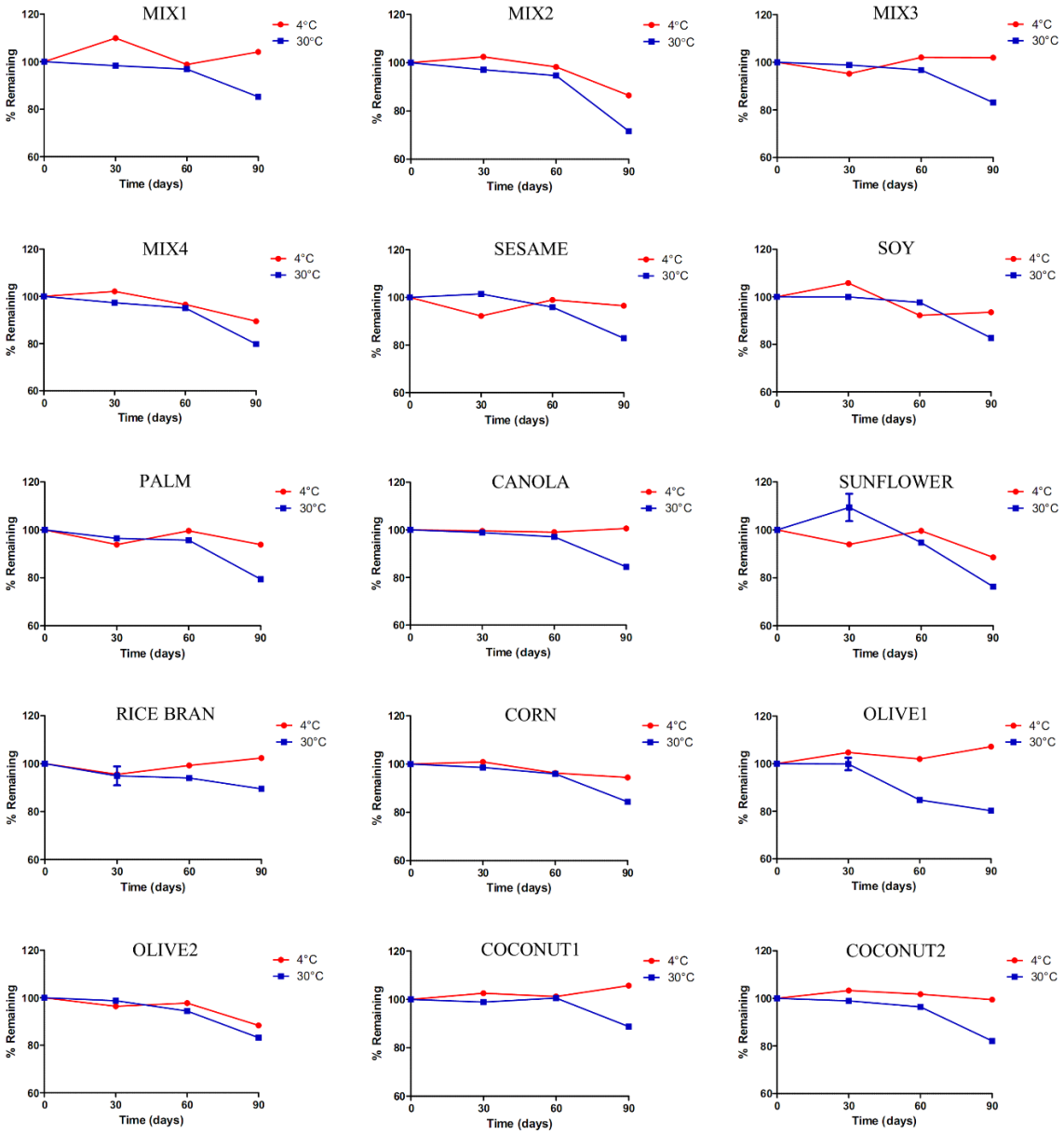

Figure 2 Stability of THC in various vegetable oils after stored at 4°C and 30°C for 90 days in the dark

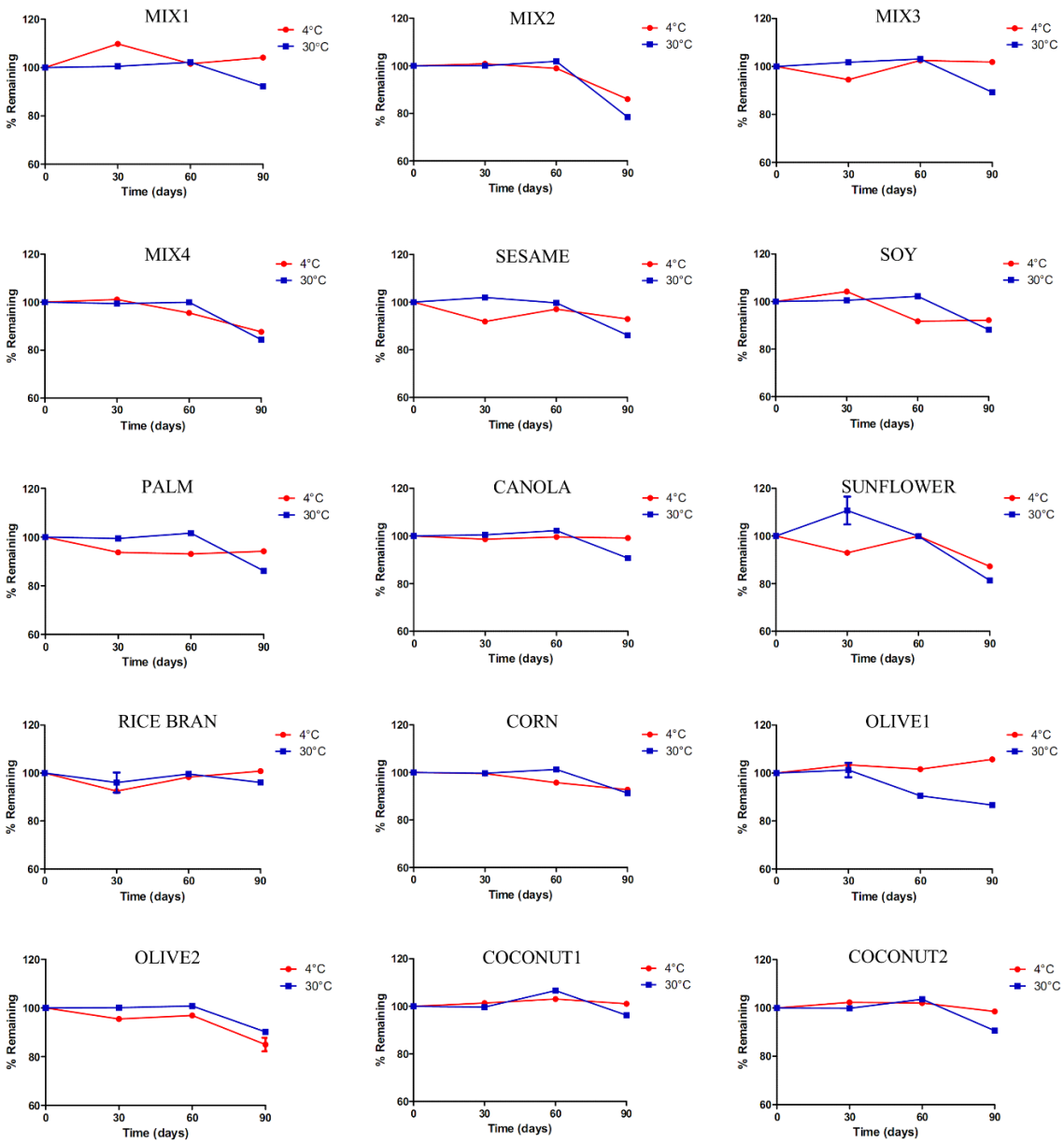

Figure 3 Stability of CBN in various vegetable oils after stored at 4°C and 30°C for 90 days in the dark

5. Conclusions

The stability of cannabis sublingual drops prepared by using different types of vegetable oil as a vehicle was determined. Stoage of cannabis sublingual drop at 4°C gave higher stability than at 30°C. The formulations with the shelf-life of more than 90 days were found when the seven vegetable oil types–MIX1, MIX3, SESAME, RICE BRAN, OLIVE1, COCONUT1, and COCONUT2–were used. In conclusion, the seven vegetable oil types can be selected as a vehicle to prepare the cannabis sublingual drops due to it promoted stable cannabinoid content. However, the sublingual drops could undergo rancidity, which affected the stability of the sublingual formulations. So, it was suggested to evaluate the changing of vegetable oils in the future work.

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7. References

Bielawiec, P., Harasim-Symbor, E., & Chabowski, A. (2020). Phytocannabinoids: Useful drugs for the treatment of obesity? Special focus on cannabidiol. *Frontiers in Endocrinology, 11*, 114. DOI: 10.3389/fendo.2020.00114

Capen, R., Christopher, D., Forenzo, P., Ireland, C., Liu, O., Lyapustina, S., O'Neill, J., Patterson, N., Quinlan, M., Sandell, D., Schwenke, J., Stroup, W., & Tougas, T. (2012). On the shelf life of pharmaceutical products. *AAPS PharmSciTech, 13*(3), 911-918. DOI: 10.1208/s1

Citti, C., Ciccarella, G., Braghiroli, D., Parenti, C., Vandelli, M. A., & Cannazza, G. (2016). Medicinal cannabis: Principal cannabinoids concentration and their stability evaluated by a high performance liquid chromatography coupled to diode array and quadrupole time of flight mass spectrometry method. *Journal of Pharmaceutical and Biomedical Analysis, 128*, 201-209. DOI: 10.1016/j.jpba.2016.05.033

Citti, C., Linciano, P., Russo, F., Luongo, L., Iannotta, M., Maione, S., Laganà, A., Capriotti, A. L., Forni, F., Vandelli, M. A., Gigli, G., &. Cannazza, G. (2019). A novel phytocannabinoid isolated from *Cannabis sativa* L. with an *in vivo* cannabimimetic activity higher than Δ^9-tetrahydrocannabinol: Δ^9-Tetrahydrocannabiphorol. *Scientific Reports, 9*(1), Article number: 20335. DOI: 10.1038/s41598-019-56785-1

Garrett, E. R., & Tsau, J. (1974). Stability of tetrahydrocannabinols I. *Journal of Pharmaceutical Sciences, 63*(10), 1563-1574. DOI: 10.1002/jps.2600631016

Hanuš, L. O., Meyer, S. M., Muñoz, E., Taglialatela-Scafati, O., & Appendino, G. (2016). Phytocannabinoids: a unified critical inventory. *Natural Product Reports, 33*(12), 1357-1392. DOI: 10.1039/c6np00074f

Harvey, D. J. (1990). Stability of cannabinoids in dried samples of cannabis dating from around 1896-1905. *Journal of Ethnopharmacology, 28*(1), 117-128. DOI: 10.1016/0378-8741(90)90068-5

Madras, B. K. (2015). Update of Cannabis and its medical use. Retrieved January 13, 2020, from https://www.who.int/medicines/access/controlled-substances/6_2_cannabis_update.pdf

Melo, S. R., Homem-de-Mello, M., Silveira, D., & Simeoni, L. A. (2014). Advice on degradation products in pharmaceuticals: A toxicological evaluation. *PDA Journal of Pharmaceutical Science and Technology, 68*(3), 221-238. DOI: 10.5731/pdajpst.2014.00974

Melveger, A. J., & Huynh-Ba, K. (2009). Critical regulatory requirements for a stability program. In K. Huynh-Ba (Ed.), *Handbook of stability testing in pharmaceutical development* (pp. 9-19). New York, USA: Springer.

Monton, C., Madaka, F., Settharaksa, S., Wunnakup, T., Suksaree, J., & Songsak, T. (2019). Optimal condition of cannabis maceration to obtain the high cannabidiol and Δ^9-tetrahydrocannabinol content. *Anais da Academia Brasileira de*

Ciências, *91*(3), e20190676. DOI: 10.1590/0001-3765201920190676

Pawar, P. P., Ghorpade, H. S., & Kokane, B. A. (2018). Sublingual route for systemic drug delivery. *Journal of Drug Delivery and Therapeutics*, *8*(6-s), 340-343. DOI: 10.22270/jddt.v8i6-s.2097

Radwan, M. M., ElSohly, M. A., El-Alfy, A. T., Ahmed, S. A., Slade, D., Husni, A. S., Manly, S. P., Wilson, L., Seale, S., Cutler, S. J., & Ross, S. A. (2015). Isolation and pharmacological evaluation of minor cannabinoids from high-potency *Cannabis sativa*. *Journal of Natural Products*, *78*(6), 1271-1276. DOI: 10.1021/acs.jnatprod.5b00065

Repka, M. A., ElSohly, M. A., Munjal, M., & Ross, S. A. (2006). Temperature stability and bioadhesive properties of Δ^9-tetrahydrocannabinol incorporated hydroxypropylcellulose polymer matrix systems. *Drug Development and Industrial Pharmacy*, *32*(1), 21-32. DOI: 10.1080/03639040500387914

Trofin, I. G., Dabija, G., Vaireanu, D. I., & Filipescu, L. (2012a). Long-term storage and cannabis oil stability. *Revista de Chimie*, *63*(3), 293-297.

Trofin, I. G., Dabija, G., Vãireanu, D. I., & Filipescu, L. (2012b). The influence of long-term storage conditions on the stability of cannabinoids derived from cannabis resin. *Revista de Chimie*, *63*(4), 422-427.

Zivovinovic, S., Alder, R., Allenspach, M. D., & Steuer, C. (2018). Determination of cannabinoids in *Cannabis sativa* L. samples for recreational, medical, and forensic purposes by reversed-phase liquid chromatography-ultraviolet detection. *Journal of Analytical Science and Technology*, *9*(1), Article number: 27. DOI: 10.1186/s40543-018-0159-8