Technological Approach for the Development and Optimization of Decarboxylation Processes in Dried Flower and Ethanolic Extracts of Cannabis for Medical Purposes

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INTRODUCTION

Cannabis is an annual, dioecious, self-pollinating plant, from the genus of flowering plants belonging to the Cannabaceae family. Three main species of cannabis, are commonly recognized: *Cannabis sativa* spp. sativa L., *Cannabis sativa* spp. indica L. and *Cannabis sativa* spp. ruderalis L. Preparations obtained from cannabis are used for various medical purposes, including the treatment of stress, nervousness, anxiety, depression, epilepsy, inflammatory processes, pain and others. Cannabis has also been shown to support maintain intestinal flora, alleviate nausea and vomiting—particularly in cancer patients during chemotherapy or radiation therapy and relieve pain of various origins. Cannabis-based preparations can also be used for muscle spasms and for the prevention of cardiovascular diseases (Hourfane et al., 2023). Phytocannabinoids the bioactive compounds naturally synthesized in the cannabis plant, are the key components responsible for many of these therapeutic effects. The most important of these phytocannabinoids include: Δ^9-tetrahydrocannabinol (Δ^9-THC) and Δ^8-tetrahydrocannabinol (Δ^8-THC) – a psychoactive cannabinoids, cannabidiol (CBD) – a non-psychoactive cannabinoid, cannabigerol (CBG), Δ^9-tetrahydrocannabivarin (Δ^9-THCV), cannabivarin (CBV), cannabidivarin (CBDV), cannabinol (CBN), cannabichromene (CBC), cannabinodiol (CBND), cannabielsoin (CBE), cannabicyclol (CBL), cannabitriol (CBT), tetrahydrocannabinolic acid (THCA) and cannabidiolic acid (CBDA), which are synthesized from a common precursor – cannabigerolic acid (CBGA) (Singh, Yadav et al., 2023).

What differentiates THC from THCA, and CBD from CBDA?

The distinction is related with a process known as "decarboxylation", where raw cannabis material is heated, causing the chemical structure of the acidic cannabinoids to changes into their neutral (non-acidic, active) form. THCA (tetrahydrocannabinolic acid) decarboxylates to form Δ^9-THC (tetrahydrocannabinol), and CBDA (cannabidiolic acid) decarboxylates to form CBD (cannabidiol). Exposure to oxygen and light during the decarboxylation process, can cause Δ^9-THC to easily oxidize to cannabinol (CBN), a compound with different properties.

Important parameters and factors that are crucial for achieving quality decarboxylation including temperature, time, fineness of the plant material and moisture content of the plant material (particularly when decarboxylating of dried cannabis flower). It is advisable to keep decarboxylation temperatures lowin order to preserve the terpenes (Aleksandrov and Janevik-Ivanovska, 2022).

In the Republic of North Macedonia, the production, use and sale of cannabis are regulated by the amendments to the Law on the Use of Narcotic Drugs and Psychotropic Substances (Official Gazette of the Republic of North Macedonia No. 37 of 26.02.2016). This law allows the cultivation of hemp intended for the production of narcotic drugs for medical or scientific purposes, only allowed for legal entities that hold a Cannabis Cultivation Permit issued by the Ministry of Health, and require prior consent from the Government of the Republic of North Macedonia (Aleksandrov et al., 2023).

The aim of this research is to develop the decarboxylation processes for dried (ground) cannabis flower and cannabis crude oil/ethanolic extract, using modern technology and validated methods.

MATERIALS AND METHODS

The following materials and equipment were used in this research: dried cannabis flower (CBD Mazar strain, THC<0.1%; Bioherbalist, Czech Republic); Ethanol 96% *v/v* (Alkaloid AD, North Macedonia); crude oil obtained by ethanol extraction of the CBD Mazar strain; ethanol extraction equipment (ACE Alliance, USA); decarboxylation oven (Binder, Germany); double-layer glass reactor for decarboxylation (ACE Alliance, USA); mill with different sizes of grinding sieves (Sensi Shredder, USA); stainless steel pans/tins for decarboxylation and stainless steel vessels with hermetic closure (Ting Inox, North Macedonia); precision scales (Kern DE, Germany); hygrometer (Ohaus MB 90, USA); binocular (OPTIKA® Microscopes, Italy) and digital microscope (BW Optics, China), as well as protective equipment for work safety. Using these materials and equipment, the following methods were developed: grinding/crushing of plant material before decarboxylation; decarboxylation of dried ground plant

material; ethanol extraction (cold maceration) to obtain crude oil; decarboxylation of crude oil/ethanol extract; as well as microscopic analysis of plant material.

High-performance liquid chromatography (HPLC-MS) was used to determine the content of the main cannabinoids throughout the processes. For the decarboxylation processes of dried (ground) cannabis flower and crude oil (ethanol extract), various optimization modules were used. These modules included different programs for temperature settings, time intervals, temperature gradient, size of plant material grinding, amount of plant material/ethanol extract for decarboxylation and sampling time for analysis during the processes. These parameters were critical points for ensuring the quality and efficiency of the decarboxylation processes. Evaluation was based on data collected on moisture content, cannabinoid content and color of trichomes, both before and after the completion of the decarboxylation process.

RESULTS AND DISCUSSION

During the decarboxylation of dried (ground) cannabis flower, microscopic examination of the plant material is essential, as there is a significant change in the color of the capitular (glandular) trichomes. This change indicates the activation of neutral forms of cannabinoids from their acidic forms. Namely, the color of the trichomes changes from white or light yellow to golden brown or dark brown. The fastest conversion of CBD-A to CBD occurs at a temperature of 120°C to 135°C for 55 minutes. Meanwhile, complete conversion of CBD-A to CBD is achieved at a temperature of 135°C to 145°C for 45-50 minutes. In this case, the total concentration of CBD remains unchanged from the beginning to the end of the process. The fastest conversion of THC-A to Δ^9-THC occurs at a temperature of 110°C to 125°C for 55 minutes. While, the complete conversion of THC-A to Δ^9-THC is achieved at a temperature of 125°C to 135°C for 45-50 minutes. In this case, the total concentration of Δ^9-THC remains unchanged from the beginning to the end of the process. The fastest conversion of CBG-A to CBG occurs at a temperature of 120°C to 130°C for 60 minutes. While, the complete conversion of CBG-A to CBG is achieved at a temperature of 130°C to 140°C for 50 minutes. Some of the results obtained during the research are presented in Figure 1 and Chart 1 below.

Figure 1. Color differences in glandular trichomes of plant material (CBD Mazar dried flower) before (left) and after (right)decarboxylation (145°C for 30 Minutes), evaluated by digital microscope.

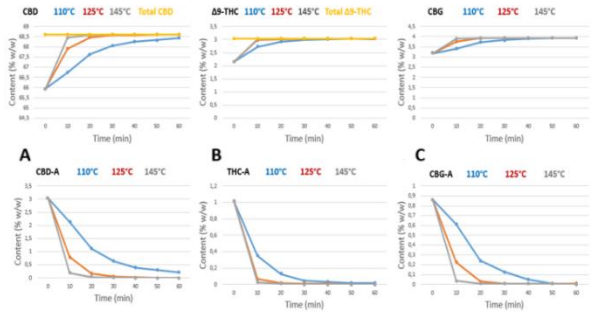

Chart 1. Decarboxylation of cannabis crude oil / ethanolic extract (CBD Mazar crude oil); Program#1: 110°C±5°C / 60 minutes; Program#2: 125°C±5°C / 60 minutes and Program#3: 145°C±5°C / 50 minutes – correlation between the contents of: (A) CBD / CBD-A; (B) Δ^9-THC / THC-A and (C) CBG / CBG-A.

CONCLUSION

In general, after performing the decarboxylation processes according to the different modules and process programs, no significant increase in the CBN concentrations was observed in the products after their decarboxylation (0.293% *w*/*w* before decarboxylation and 0.309% *w*/*w* after decarboxylation). This suggests that the set temperature and time intervals are appropriate for maintaining the stability of CBN levels, as well as the sensitivity of THC to its degradation. Based on these findings, it is recommended that the decarboxylation process not exceed a of 145°C, and should not last longer than 60 minutes.

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