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Research Article

EFFECTS OF BATCH AND CONTINUOUS-FLOW MICROWAVE-ASSISTED PROCESS FOR THE DECARBOXYLATION OF CANNABIDIOLIC ACID IN HEMP EXTRACT

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ABSTRACT. This research aimed to design, optimize, and compare batch and flow-type microwave-assisted processing using temperature, time, and microwave effects to accelerate the decarboxylation of cannabidiolic acid (CBDA) to cannabidiol (CBD) in hemp extract. The conversion yield of decarboxylation was investigated at different heating temperatures and processing times, and the resulting yields were compared with those obtained using two other methods. The optimum conversion yields of the batch and flow-type microwave-assisted processing of decarboxylation were 90.3% (120 °C for 30 min) and 92.8% (90 °C for 30 min) in hemp extract, respectively. As a result, batch and flow-type microwave-assisted processing are effective and viable methods for the decarboxylation of CBDA to CBD at relatively short residence times and high throughput.

Keywords: *Cannabis sativa L., cannabidiol acid, cannabidiol, decarboxylation, microwave*

INTRODUCTION

Cannabis (*Cannabis sativa* L.) has been a valuable resource for over 5000 years and has been cultivated specifically for its industrial uses. According to traditional medical records, cannabis-derived cannabinoids have many biological properties, including anticancer [1], anti-inflammatory [2], anxiolytic [3], antipsychotic [4], antiepileptic [5], neuroprotective [6], vasorelaxant [7], antispasmodic [8], antiproliferative [9], antiemetic [10], antibacterial [11], antidiabetic [12], antipsoriatic [13], analgesic [14], bone-stimulant [15], and antimicrobial properties [16]. Over 140 cannabinoids have been isolated and identified in cannabis thus far. Cannabinoids, especially the nonpsychoactive component cannabidiol (CBD), have been widely studied in the pharmaceutical industry. In addition, cannabis extracts containing cannabinoids, including CBD, were classified as novel foods in the European Union (Regulation 2015/2283 on Novel Food). CBD is obtained via the slow decarboxylation of its acidic form, i.e., cannabidiolic acid (CBDA), which is naturally present in plants (Fig. 1). Therefore, an efficient CBDA decarboxylation process has become crucial in the cannabis industry. This decarboxylation process occurs spontaneously in dry conditions and is accelerated by long-term storage, light exposure, and high temperatures. However, despite the importance of decarboxylation in optimizing the transformation yield, few data are publicly available for reference. The first study, examining the effect of the CBDA decarboxylation parameters (temperature, solvents, sorbent phases, and salts) on CBD yield in an open reactor, was performed by Leisztner et al. [17]. Cannazza et al. studied the decarboxylation of cannabinoid acids, including CBDA, in hemp seed oil in open and closed reactors [18]. Moreno et al. studied the effect of several parameters (temperature, time, presence of oxygen, and amount of plant material) on cannabinoid decarboxylation, including CBDA [19]. Silion and Pinteala et al. recently screened the best decarboxylation conditions to obtain the maximum CBDA decarboxylation yield from hemp oil [20]. Herein, we aimed to investigate the decarboxylation process using the batch and flow microwave irradiation of the cannabis plant extract, mainly CBD.

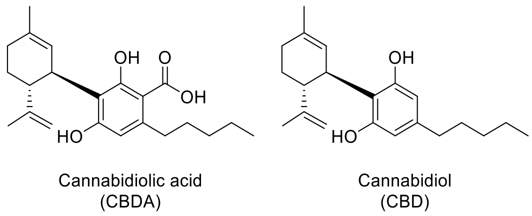

Fig. 1. *Chemical structure of CBDA and CBD*

MATERIALS AND METHODS

Preparation of Korean Hemp Extract

Korean hemp (*Cannabis sativa* L.) was provided by JayHempKorea Ltd. (Andong City, Gyeongsangbuk-do, Republic of Korea) by assignment/transfer approval processes (approval No. 1564) stipulated by the Korean Ministry of Food and Drug Safety and the Seoul Regional Food and Drug Administration. Harvested in October 2018, Korean hemp leaves were finely cut, and 200 g of the leaves were extracted twice with ethanol (2.0 L) using an ultrasonic processor (Sonics, VC505, Sonics & Materials, CT, USA) with 40% power for 1 h. Then, they were extracted at room temperature for 24 hours. Concentrating ethanol under reduced pressure gave the extract (17.6 g), including CBDA and CBD, as dark green syrups.

Isolation, Identification, and Preparation of Calibration Curves of CBDA and CBD

HPLC was performed on an LC-Forte/R (YMC, Kyoto, Japan) equipped with a UV detector (220 nm) using a Phenomenex Luna C18 column (250 mm × 21.2 mm, 10 μm). In contrast, semipreparative LC was conducted using (Gilson, Middleton, WI, USA) a refractive index detector and a Phenomenex Luna C18(2) column (250 mm × 10 mm, 5 μm). The standard CBDA and CBD single ingredients used in all the experiments were directly isolated from the extract of Korean hemp (*Cannabis sativa* L.) raw material with 96.3% and 97.1% purity. The CBDA and CBD spectroscopic data agreed with those reported in the literature [21]. The standard solutions of CBDA and CBD were prepared in water with concentrations of 10, 25, 50, 100, and 250 ppm to construct the calibration curves (Figure 2). An analytical reversed-phase Shimadzu Nexera X2 ultra-performance liquid chromatography (UHPLC) system comprising a solvent degassing unit (DGU–20A), a binary pump (LC–30AD), an autosampler (SIL–30AC), a system controller unit (CBM–20A), photodiode array detector (SPD–M20A), and a column oven unit (CTO–20AC) was used for qualitative and quantitative analyses with a Phenomenex Luna

Omega polar C18 column (150 × 2.1 mm, 1.6 μm). For standard aqueous solutions, elution solvents A and B used in the UPLC system were water and acetonitrile, respectively, and each was pumped using two separate pumps. About 3 μL of the standard aqueous solution was injected into the column using a syringe and an elution solvent mixture of A/B (70:30 *v*/*v*) at a 0.3 mL/min flow rate. After that, the % volume of the elution solvent B was gradually changed to 100% (20 min), 100% (23 min), and 30% (26 min). Each resultant ingredient isolated from the column was analyzed using UV–Visible (UV–Vis) spectroscopy.

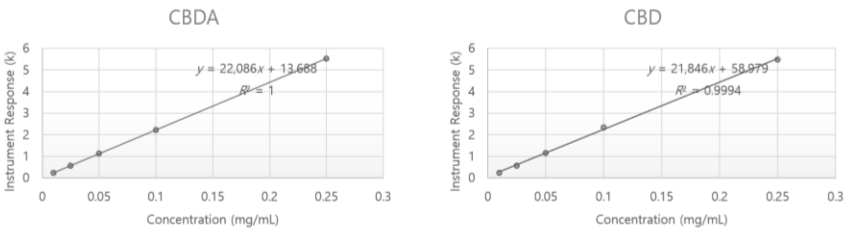

Fig. 2. *The calibration curves of CBDA and CBD*

Analysis of CBDA and CBD

About 3 μL of the resultant product mixture was injected to separate using UHPLC. The mobile phase in UHPLC contained a binary gradient of solvent A (water) and solvent B (MeCN): 70% B, 10.0 min; 85% B, 11.0 min; 95% B, 15.0 min; and 70% B. The flow rate was established at 0.3 mL/min, and a 220 nm detection wavelength was used. The quantitative analysis was performed according to the calibration curves obtained for CBDA and CBD. Electrospray ionization (ESI)–MS (Shimadzu LCMS-2020 system, Kyoto, Japan) was used for qualitative analysis.

Preparation of Processed Korean Hemp Extract using Batch Microwave Irradiation

The Korean hemp extract was dissolved at a concentration of 100,000 ppm in ethanol, and then the reaction temperature of the reactor was set at 80 °C–130 °C. Further, microwave processing was conducted at a maximum microwave power of 200 W and a frequency of 2450 MHz for 5–40 min.

Preparation of Continuous Microwave Irradiation Equipment

A microwave irradiator manufactured by CEM Company (Matthews, NC, USA) was used as continuous microwave processing equipment. A tube comprising PTFE and PFA was inserted into a 10 mL flow cell accessory reaction chamber to manufacture a continuous reactor. The tube had an outer diameter of 1/16 inch, an inner diameter of 1.0 mm, a length of 127.4 cm, and an inner tube volume of 1.0 mL. After that, the chamber of the continuous reactor was filled with water and acted as a temperature controller by transferring the heat of the water-filled chamber to the tube. One end of a liquid-feeding pump (YMC-KP series) was connected to the inserted tube as an inlet through which the reaction mixture was applied, and the other end was connected to a back pressure regulator of 75 psi (UPCHURCH, P-786) as an outlet through which the reaction mixture was discharged.

Preparation of Processed Korean Hemp Extract Using Continuous Microwave Irradiation

The Korean hemp extract was dissolved at a concentration of 200 ppm in ethanol, and then, the reaction temperature of the continuous reactor was set at 70 °C–100 °C. Continuous microwave processing was conducted at a maximum microwave power of 45 W and a frequency of 2450 MHz for 5–60 min. Each reaction time was controlled by controlling the flow rate of the liquid-feeding pump, and the power was 3–45 W during microwave irradiation. The reaction time according to the flow rate is as follows: 5 min at 0.2 mL/min, 10 min at 0.1 mL/min, 20 min at 0.05 mL/min, 30 min at 0.033 mL/min, 40 min at 0.025 mL/min, and 60 min at 0.017 mL/min. The reaction time represents the time the reactants remain in the tube in the continuous reactor.

RESULTS AND DISCUSSION

Batch-Type Microwave-Assisted Processing

Table 1 shows the CBDA and CBD contents expressed in mg/g of the Korean hemp extract after dissolving it in ethanol and irradiating it with batch-type microwave-assisted processing. In the initial Korean hemp extract (Table 1, entry 1), cannabinoids included 68.2 mg of CBDA and 8.9 mg of CBD per 1 g of extract, indicating that the CBD yield and content were 13.0% and 11.5%, respectively. To examine the effect of time and temperature on the CBD yield and content, the experiments were conducted at a microwave processing temperature of 80 °C–120 °C within 30 min. According to microwave irradiation's temperature and treatment time, CBDA in the extract was gradually changed into CBD via a decarboxylation reaction. Consequently, the amounts of CBD in the processed product were increased (Table 1, entries 2–5 and 8). Based on those results, the experiments were conducted at a microwave processing temperature of 120 °C with 10 min intervals (Table 1, entries 6–10). The generated amounts of CBD (62.0 mg per 1 g of extract) reached a maximum after 30 min at 120 °C of microwave irradiation (Table 1, entry 8). Although the % content of CBD was high after 30 min at 120 °C of microwave irradiation, the thermal decomposition of CBDA or CBD occurred after microwave irradiation at 120 °C for 40 and 50 min (Table 1, entries 9 and 10) and high temperatures of over 130 °C (Table 1, entry 11). Therefore, the optimal temperature and treatment time of batch-type microwave irradiation for the decarboxylation of CBDA was 30 min at 120 °C to avoid thermal decomposition.

Table 1. *Effect of temperature and treatment time on CBD-generated amounts after batch-type microwave-assisted processing of Korean hemp extract (mg/g)*

Entries	Temp. (°C) – Time (min)	CBDA (mg), A	CBD (mg), B	CBD Yield (%) [a]	CBD Content% = {B/(A + B)} × 100
1	–	68.2	8.9	13.0	11.5
2	80–30	58.0	16.7	24.3	22.4
3	90–30	49.1	24.5	35.7	33.3
4	100–30	37.5	31.6	46.0	45.7
5	110–30	34.6	34.1	49.6	49.6
6	120–10	15.3	50.4	73.3	76.6
7	120–20	4.5	61.3	89.2	93.2
8	120–30	2.6	62.0	90.3	96.0
9	120–40	0.6	61.4	89.4	99.0
10	120–50	0.5	60.9	88.6	99.3
11	130–5	1.7	56.8	82.7	96.9

[a] CBD yield = B/68.7 mg (theoretical CBD amount at 100% conversion) × 100.

Flow-Type Microwave-Assisted Processing

Subsequently, we examined CBDA decarboxylation in the Korean hemp extract by performing flow-type microwave irradiation. The experiments were conducted at a microwave-assisted processing temperature from 70 °C to 100 °C in 5 °C–10 °C intervals for 5–60 min to examine the effect of treatment time and temperature on the CBD yield and content (Table 2, entries 2–12). The generated amount of CBD reached a maximum after microwave irradiation at 90 °C after 30 min, and CBDA was completely converted to CBD. In addition, the thermal decomposition of cannabinoids occurred after microwave irradiation at a high temperature of 100 °C (Table 2, entries 13–14).

Table 2. *Effect of temperature and treatment time on the generated amounts of CBD after the continuous microwave-assisted processing of Korean hemp extract (mg/g)*

Entries	Temp. (°C) – Time (min)	CBDA (mg), A	CBD (mg), B	CBD Yield (%) [a]	CBD Content% = {B/(A + B)} × 100
1	-	68.2	8.9	13.0	11.5
2	70–10	55.0	17.6	25.6	24.2
3	70–20	46.9	25.1	36.5	34.9
4	80–10	40.6	31.4	45.7	43.6
5	80–20	25.8	41.2	60.0	61.5
6	80–40	10.3	55.3	80.5	84.3
7	80–60	4.3	58.2	84.7	93.1
8	90–10	17.4	50.0	72.8	74.2
9	90–20	6.3	59.3	86.3	90.3
10	90–30	n.d.[b]	63.7	92.8	>99.5
11	90–40	n.d.[b]	62.1	90.4	>99.5
12	90–60	n.d.[b]	58.9	85.7	>99.5
13	100–5	25.4	36.9	53.7	59.2
14	100–10	n.d.[b]	58.7	85.4	>99.5

[a] CBD yield = B/68.7 mg (theoretical CBD amount at 100% conversion) × 100. [b] n.d. = not detected.

CONCLUSION

The decarboxylation of CBDA under continuous-flow microwave-assisted process conditions produced the decarboxylated product CBD in a good yield within a short time compared to batch-type conditions using the Korean hemp extract. However, it is easy to establish a mass production system for industrialization with the batch type, whereas the continuous-flow type requires the development of a system to achieve the same. Nevertheless, the continuous-flow type is economically suitable for industrialization, and it is attractive in continuous manufacturing, which has recently attracted considerable attention.

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REFERENCES

[1] Massi, R., Solinas, M., Cinquina, V., Parolaro, D. (2012): Cannabidiol as potential anticancer drug. The Journal of Clinical Pharmacology 75: 303-312.

[2] Atalay, S., Jarocka-Karpowicz, I., Skrzydlewska, E. (2020): Antioxidative and Anti-Inflammatory Properties of Cannabidiol. Antioxidants 9: 21.

[3] Wright, M., Ciano, P. D., Brands, B. (2020): Use of Cannabidiol for the Treatment of Anxiety: A Short Synthesis of Pre-Clinical and Clinical Evidence. Cannabis and Cannabinoid Research 5: 191-196.

[4] Batalla, A., Janssen, H., Gangadin, S. S., Bossong, M. G. (2019): The Potential of Cannabidiol as a Treatment for Psychosis and Addiction: Who Benefits Most? A Systematic Review. Journal of Clinical Medicine 8: 1058.

[5] Thiele, E., Marsh, E., Mazurkiewicz-Beldzinska, M., Halford, J. J., Gunning, B., Devinsky, O., Checketts, D., Roberts, C. (2019): Cannabidiol in patients with Lennox-Gastaut syndrome: Interim analysis of an open-label extension study. Epilepsia 60: 419-428.

[6] Fernández-Ruiz, J., Sagredo, O., Pazos, M. R., García, C., Pertwee, R., Mechoulam, R., Martínez-Orgado, J. (2012): Cannabidiol for neurodegenerative disorders: Important new clinical applications for this phytocannabinoid? British Journal of Clinical Pharmacology 75: 323-333.

[7] Stanley, C. P., Hind, W. H., Tufarelli, C., O'Sullivan, S. E. (2015): Cannabidiol causes endothelium-dependent vasorelaxation of human mesenteric arteries via CB_1 activation. Cardiovascular Research 107: 568-578.

[8] Capasso, R., Borrelli, F., Aviello, G., Romano, B., Scalisi, C., Capasso, F., Izzo, A. A. (2008). Cannabidiol, extracted from Cannabis sativa, selectively inhibits inflammatory hypermotility in mice. British Journal of Pharmacology 154: 1001-1008.

[9] Lee, H. S., Tamia, G., Song, H. J., Amarakoon, D., Wei, C. I., Lee, S. H. (2022): Cannabidiol exerts anti-proliferative activity via a cannabinoid receptor 2-dependent mechanism in human colorectal cancer cells. International Immunopharmacology 108: 108865.

[10] Rock, E. M., Goodwin, J. M., Limebeer, C. L., Breuer, A., Pertwee, R. G., Mechoulam, R., Parker, L. A. (2011): Interaction between non-psychotropic cannabinoids in marihuana: Effect of cannabigerol (CBG) on the anti-nausea or anti-emetic effects of cannabidiol (CBD) in rats and shrews. Psychopharmacology 215: 505-512.

[11] Appendino, G., Gibbons, S., Giana, A., Pagani, A., Grassi, G., Stavri, M., Smith, E., Rahman, M. M. (2008): Antibacterial Cannabinoids from *Cannabis sativa*: A Structure-Activity Study. Journal of Natural Products 71: 1427-1430.

[12] Weiss, L., Zeira, M., Reich, S., Har-Noy, M., Mechoulam, R., Slavin, S., Gallily, R. (2006): Cannabidiol lowers incidence of diabetes in non-obese diabetic mice. Autoimmunity 39: 143-151.

[13] Muruganantham, N., Basavaraj, K. H., Dhanabal, S. P., Praveen, T. K., Shamasundar, N. M., Rao, K. S. (2011): Screening of *Caesalpinia bonduc* leaves for antipsoriatic activity. Journal of Ethnopharmacology 133: 897-901.

[14] Boyaji, S., Merkow, J., Elman, R. N. M., Kaye, A. D., Yong, R. J., Urman, R. D. (2020): The Role of Cannabidiol (CBD) in Chronic Pain Management: An Assessment of Current Evidence. Current Pain and Headache Reports 24: 4.

[15] Sun, Y. X., Xu, A. H., Yang, Y., Zhang, J. X., Yu, A. W. (2015): Activation of Cannabinoid Receptor 2 Enhances Osteogenic Differentiation of Bone Marrow Derived Mesenchymal Stem Cells. BioMed Research International 2015: 874982.

[16] Blaskovich, M. A. T., Kavanagh, A. M., Elliott, A. G., Zhang, B., Ramu, S., Amado, M., Lowe, G. J., Hinton, A. O., Pham, D. M. T., Zuegg, J., Beare, N., Quach, D., Sharp, M. D., Pogliano, J., Rogers, A. P., Lyras, D., Tan, L., West, N. P., Crawford, D. W., Peterson,

M. L., Callahan, M., Thurn, M. (2021): The antimicrobial potential of cannabidiol. Communications Biology 4: 7.

[17] Veress, T., Szanto, J. I., Leisztner, L. (1990): Determination of cannabinoid acids by high-performance liquid chromatography of their neutral derivatives formed by thermal decarboxylation. Journal of Chromatography 520: 339-347.

[18] Citti, C., Pacchetti, B., Vandelli, M. A., Forni, F., Cannazza, G. (2018): Analysis of cannabinoids in commercial hemp seed oil and decarboxylation kinetics studies of cannabidiolic acid (CBDA). Journal of Pharmaceutical and Biomedical Analysis 149: 532-540.

[19] Moreno, T., Dyer, P., Tallon, S. (2020): Cannabinoid Decarboxylation: A Comparative Kinetic Study. Industrial & Engineering Chemistry Research 59: 20307-20315.

[20] Petrovici, A. R., Simionescu, N., Sandu, A. I., Paraschiv, V., Silion, M., Pinteala, M. (2021): New Insights on Hemp Oil Enriched in Cannabidiol: Decarboxylation, Antioxidant Properties and In Vitro Anticancer Effect. Antioxidants 10: 738.

[21] Choi, Y. H., Hazekamp, A., Peltenburg-Looman, A. M. G., Frédérich, M., Erkelens, C., Lefeber, A. W. M., Verpoorte, R. (2004): NMR Assignments of the Major Cannabinoids and Cannabiflavonoids Isolated from Flowers of *Cannabis sativa*. Phytochemical Analysis 15: 345-354.