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Original Article

Variability in Color and Phytochemical Properties of Hemp (*Cannabis sativa* L.) upon Drying Techniques; An Opportunity for Industrial Products

Afsaneh Esfandi[1], Ali Mehrafarin[2*], Sepideh Kalateh Jari[1], Hassanali Naghdi Badi[3,4] and Kambiz Larijani[5]

[1]*Department of Horticultural Science and Agronomy, Science and Research Branch, Islamic Azad University, Tehran, Iran*
[2]*Medicinal Plants Research Center, Institute of Medicinal Plants, ACECR, Karaj, Iran*
[3]*Department of Agronomy and Plant Breeding, Faculty of Agriculture, Shahed University, Tehran, Iran*
[4]*Medicinal Plants Research Center, Shahed University, Tehran, Iran*
[5]*Department of Chemistry, Science and Research Branch, Islamic Azad University, Tehran, Iran*

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***Corresponding author**
a.mehrafarin20@gmail.com

ABSTRACT

The drying process can preserve herbal products against pathogens and improve their shelf life and quality; however, drying techniques have different effects on the appearance and quality of final products. Accordingly, the present study assessed various drying techniques viz. sunlight, shade, oven (45, 55, and 65 °C), vacuum (45, 55, and 65 °C), and microwave (20, 400, and 600 W) on color and phytochemicals characteristics of hemp (*Cannabis sativa* L.) plants with respect to total phenolic content (TPC), cannabidiol (CBD), and tetrahydrocannabinol (THC), chlorophyll (Chl) content, and color properties using multivariate analysis. The results revealed that the highest CBD and THC were observed in plants dried in a microwave at 400 and 600 W, respectively. The TPC reached the highest amount in shade drying conditions and was followed by microwave at 400 W, and oven at 45 °C. Although Chl b mainly remained unchanged, Chl a represented the lower amount by increasing the temperature of drying methods, especially over 65 °C. The lightness (L*) and brightness (b*) of fresh leaves were higher than dried samples, while over 65 °C possessed their minimum amount of L*. Agglomerative hierarchical clustering (AHC) showed three different clusters were determined as microwaves at 200, 400, and 600 W were placed in a distinguished cluster. Finally, this experiment suggested shade drying or minimum temperatures of the oven and vacuum techniques to reach constant color and phytochemicals, while microwaves can be recommended for CBD and THC, which can be useful in food and pharmacological industries.

INTRODUCTION

Today, medicinal plants are widely cultivated in different areas of the world to replace synthetic materials due to their primary role in improving the immune system [1]. Pre and post-harvest practices affect the amount and type of active ingredients in medicinal plants [2]. Postharvest techniques like storage conditions, drying methods, packaging, etc. can modify the quality and quantity of final herbal products through changes in main secondary metabolites [3].

Hemp (*Cannabis sativa* L.) is an annual dioecious herbaceous species of Cannabaceae, being cultivated in Iran for hundreds of years [4]. It possesses many medicinal properties for diabetes, pain modulation, obesity, and anorexia. Cannabinol (CBN) is the primary ingredient in hemp that is responsible for its anti-inflammatory, analgesic, and anti-seizure characteristics [5]. Among phytocannabinoids, cannabigerol (CBG), (-)-Δ^9-*trans*-tetrahydrocannabinol (Δ^9-THC), cannabidiol (CBD), and cannabichromene (CBC) are biosynthesized in

Cannabis plants and are considered to be the main or major natural phytocannabinoids [6].

The drying process is a primary agent among postharvest practices, which modifies the appearance and quality of herbal products [7]. The drying technique can inhibit microbial growth and minimize biochemical changes by decreasing the mitigating water content in plant tissues to less than 15%. It can preserve the attributes of aroma and color and also maintain the active ingredients and quality of the final product [8]. Drying is a necessity in food and pharmacological sciences, which (a): mainly preserves the product's quality [9], (b) reduces the volume and weight of the final product to facilitate storage and transportation, and (c) enhances the shelf life of products [10]. Drying techniques have different responses depending on plant species, active ingredient type, and plant tissue [10]. Traditional drying methods like natural drying (drying in the shade and sun) and hot air drying are still widely used for obtaining dried products with the application of minimal equipment and devices [12]. Oven-based drying at different temperatures mostly between 40 and 60 °C has positive effects on plant tissues [12]. Hot air dryers are completely common, but due to the low rate of heat transfer into the food and the long drying time, the energy efficiency is low, which prolongs the humidity drop, and the nutritional value decreases because of the slow drying process [2]. Recently, new drying methods such as microwave drying, freeze drying, infrared drying, and vacuum drying have more attention compared to traditional techniques [2]. The microwave drying technique is currently available in the herb processing industry [13]. It leads to rapid evaporation of water from food with shorter drying time compared to many drying practices, and a reduction of energy consumption in the drying process. Drying factors such as microwave power, drying time, the initial moisture content of the product, and the dielectric properties of the materials can modify the quality of final products [13].

There is a growing interest in the use of different drying techniques to obtain high-quality products of medicinal plants. During the drying, different factors like moisture, time, temperature, and plant species can influence the final product. The changes of main metabolites like chlorophyll (Chl), phenols, flavonoids, and color characteristics have been addressed upon different drying techniques in Thymus daenensis Celak [10], Artemisia judaica L. [14], Hibiscus sabdariffa L. [15], and Kelussia odoratissima [2]. The literature showed different responses of phytochemicals under drying methods, and accordingly, Yilmaz *et al*. [7] represented the decreases in Chl a and increases in TPC and noticeable changes in color properties upon the drying process. Therefore, the present study aimed to evaluate the changes in cannabinoids, phenolic content, photosynthesis content, and color properties under hemp (*C. sativa* L.) plants under drying methods. The findings can be used to obtain the best drying method for a high-quality product, which can be beneficial in food and pharmacological industries.

MATERIALS AND METHODS

Pant preparation and experiential design

Leaves of hemp at the end of the growing stage were collected from Ardabil in the northwest of Iran (32.32° 97′ N, 50.11° 12′ E; 2100 m above sea level). Sample identification was done by expert botanists at Medicinal Plants Research Center, Institute of Medicinal Plants, ACECR, Karaj, Iran). The voucher specimen (No. 207) was deposited at the seed bank of IMP-ACECR. After that, the leaves were transferred to the laboratory under standard conditions for drying, extraction, and measurement of biochemical attributes. To maintain the original fresh quality, the collected above-ground parts were stored in a refrigerator at 4°C until they are used for the drying test. Two sets of 250 g were used for further analysis, where a set of samples was used for fresh tissue analysis and the remaining beaches were applied for drying methods. This work was done according to a completely randomized design (CRD) to study different drying methods on the phytochemicals of hemp leaves.

Drying Methods and Equipment

Eleven drying methods including sunlight, shade, three mechanical oven methods at 45, 55, and 65 °C (capacity 2001; Faraz Electric, Iran), three vacuum methods at 45, 55, and 65 °C, and three microwave methods at 200, 400, and 600 W were used for drying techniques.

Sun and Shade Drying

In the sun-drying process, fresh herbs were placed on well-ventilated drying racks and were exposed directly to the sunlight. Shade drying used solar energy as a heating source. The process was carried

out in almost the same way as sun drying, except that the herbs were placed under the shade in a room with good ventilation, low humidity (25%), and no direct exposure to sunlight [16].

Oven Drying

An oven was used for this method, and it was checked with a desired temperature for achieving a stable status. In this method, the samples were dried at 40 °C, 60 °C, and 80 °C, and their weights were recorded in 20 min intervals of drying process [17].

Vacuum Drying

The vacuum is generated by a vacuum pump (Germany) with a final pressure of 15 bar and a pump speed of 22 L/min. The temperature (45, 55 and 65 °C) was exerted by the IR lamp and adjusted by changing the height of the IR lamp and measured with a thermocouple.

Microwave Drying

The drying experiments were carried out in a microwave-vacuum dryer with a power output of 200, 400, and 600 W and a microwave frequency of 2450 MHz. It consisted of a microwave oven (M 945; Samsung, South Korea). For mass transfer kinetics, the weight changes have been recorded each 5 min for 200 W, 2 min for 400 W, and 0.5 min for 600 W.

Determination of Cannabidiol (CBD) and Tetrahydrocannabinol (THC)

For CBD and THC, 100 mg of ground samples were accurately weighed and extracted with 1 mL ethanol and then centrifuged at 4200 rpm. The ethanol and samples were diluted to 1 mg/mL. After agitating in an orbital shaker for 15 min at room temperature, the samples were centrifuged at 4.200 rpm. They were filtered through a 0.22 µm PTFE syringe filter prior to analysis. The UHPLC/UV (Thermo Scientific, Bremen, Germany) was used to determine Δ9-THC and CBD. Chromatographic separation was obtained using a HALO C18 Fused-Core column (2.7 µm, 150 × 2.1 mm), with a HALO guard column (2.7 µm, 5 x 2.1 mm), and a ternary A/B/C multistep gradient (solvent A: 0.1% acetic acid in water, solvent B: 0.1% acetic acid in acetonitrile, and solvent C: methanol). Flow rate was 0.25 mL/min, column temperature was 30°C, and injection volume was 1 µL. Data acquisition was performed in full UV-Vis scan mode [18].

Determination of total phenolic content (TPC)

The Folin-Ciocalteu reagent was used to determine TPC spectrophotometrically using garlic acid (GA) as a standard [19]. The 0.2 mL Folin-Ciocalteu and 1.8 mL distilled water were added to 0.2 mL of plant extract or standard solution (0-100 mg/mL GA). After 5 min, 3 mL Na_2CO_3 solution was added to the mixture. After 90 min at room temperature, the absorbance of the samples was measured by a Lambda 45-UV / Visible spectrophotometer at 750 nm. TPC was calculated as equivalent to mg of gallic acid (GAE) per gram of dry weight.

Chlorophyll (Chl) assay

The total Chl content was extracted according to Arnon [20]. The 200 mg of fresh samples were homogenized in 8 ml 80% acetone. After that, the mixture was centrifuged at 4 °C for 15 min (3000 rpm). Supernatants were used to analyze total Chl concentration. The 645 and 663 nm were applied at a spectrophotometer.

Color measurement

The color of the hemp leaves was measured by a Minolta Chroma meter CR 400 color meter (Minolta Co., Osaka, Japan) before and after drying. The color meter was calibrated against a standard calibration plate of a white surface and set to CIE Standard Illuminant C. The L^*, a^* and b^* values are the averages of ten readings. The color brightness coordinate L^* measures the whiteness value of a color and ranges from black at 0 to white at 100. The chromaticity coordinates a^* measures red when positive and green when negative, and the chromaticity coordinate b* measures yellow when positive and blue when negative [21].

Statistical Analysis

All data was statistically analyzed SAS software in three replicates. The value of treatments was compared by Duncan's multiple range tests. The data were statistically investigated at 5% probability level. The multivariate analysis was carried out by XLSTAT software.

RESULTS AND DISCUSSION

Cannabidiol (CBD), tetrahydrocannabinol (THC), and total phenolic content (TPC)

The results represented noticeable changes in CBD and THC upon drying techniques ($P \leq 0.05$). The

maximum CBD (431 µg/g mL) was observed in plants dried in microwave 400 W and followed by microwave 600 and 200 W to be 344 and 341 µg/g mL, respectively. However, the minimum CBD was observed in fresh plants and those dried in sunlight and vacuum method at 45 °C (Table 1). THC showed variability under drying techniques with different trends. Fresh plants possessed the lowest THC, while microwaves had the higher amounts. Compared to fresh plants, a ~16-fold increase of THC was obtained in plants experiencing microwave 600 (Table 1). The natural phytocannabinoids in hemp are biosynthesized in plants by specific enzymes [22]. The materials were affected by different factors viz. tissue type, age, growth conditions, and harvest time of plant [22]. In addition, CBD and THC can be modified over postharvest time, as a result of different degradation routes [23]. The major degradation is the heat-induced decarboxylation of Δ9-THCA, into the psychoactive component Δ9-THC. All phytocannabinoid acids are susceptible to degradation when cannabis is smoked, vaporized, or cooked but, importantly, the same processes can occur during storage [6]. Microwave drying was the best technique to reach the maximum CBD and THC, which supports the previous findings [13,24]. This drying method can quickly evaporate the water in the food. Compared with many drying methods, the drying time is relatively short and the energy consumption is low during the microwave-dried process [25]. Compared with hot-air drying, microwave-dried products show less shrinkage, better color, and rehydration capabilities [26]. The quality of microwave-dried products is affected by drying parameters, such as microwave power (W), drying time, initial moisture content of the product, and dielectric properties of the material [24]. We observed that CBD and THC respond differently to microwave power. It is reported that microwave power from 360 to 900 W can shorten the drying time of parsley by 64%, microwave-dried parsley shows good color retention, and the color is only slightly darker than fresh parsley [27]. Similarly, Sarimeseli [28] represented that increasing the microwave power from 180 W to 360 W resulted in an increase in the diffusion coefficient and a decrease in the rehydration capacity of dried coriander leaves.

All dried plants represented higher TPC compared with fresh leaves (Table 1). As a result, the data revealed changes in CBD, THC, and TPC levels based on drying procedures, with higher levels in dried samples compared to fresh plants. Polyphenols are the common natural antioxidant that can be affected by certain thermal treatments during the drying process. Phenols are the main biochemical materials in medicinal plants that have a positive correlation with antioxidant capacity and protect the plant against abnormal conditions [29]. The increased TPC under thermal conditions can be due to the rupture of cell walls as a result of thermo-mechanical effects induced by vapor expansion within hemp leaves resulting in releasing bound phenolic compounds [30]. In line with our results, Yilmaz *et al*. [7] represented the increased TPC by increasing the temperature of the drying process.

Chlorophyll Content

Chl content showed different responses to the drying process (Table 2). Although Chl a possessed no significant differences at a low temperature of oven and vacuum techniques, higher temperatures led to a reduction of Chl a. Chl b represented not noticeable changes among most treatments, being presented in a range of 0.43-0.48 mg/g. Total Chl differed from 1.6 mg/g at oven 65 °C and sunlight to 1.8 mg/g at fresh plants. Similarly, the remarkable reduction in Chl a and Chl b with increasing drying temperatures have been reported on Brassica oleracea L. [31]. Kumar *et al*. [15] showed that thermal dehydration resulted in greater degradation of Chl as compared with non-thermal drying. The green color of plants is due to the presence of Chl in thylakoids memorable of chloroplasts, which is protected by protein through creating Chl-protein complexes. Chl a and Chl b are the main photosynthesis pigments, which are similar in main structures and different in substituent on the C-3 carbon position: Chl a has methyl group, while Chl b has formyl group. The present study showed the higher resistance of Chl b under the drying process relative to Chl a, which was previously mentioned by Oliveira *et al*. [31]. Chl a showed a 13% reduction upon oven 65 °C, while Chl b represented an 8% decrease. The higher reduction of Chl a can be rooted in thermal damage and its conversion into derivate compounds like pheophytin [30]. Accordingly, Mounir *et al*. [30] represented higher degradation of Chl a in Okra pods when dried by instant controlled pressure drop (DIC) compared with shade drying, whereas Chl b remained without significant changes.

Color Characteristics

The color properties showed different amounts when plants were dried upon drying techniques (P≤0.05). The L* value in fresh plants (34.8) was higher than in dried samples, and also over 65 °C (28.2) showed the minimum amount of L* (Fig. 1a). In addition, drying led to reduced b* value, ranging from 18.86 in fresh samples to 16.76 in oven 65 °C (Fig. 1b). For a*, there was found in negative values, varying from -2.46 in 65 °C to -4.83 in fresh samples (Fig. 2a). In addition, a*/b*, an effective parameter in color quality, was obtained in a range of -0.16 to -0.25 (Fig. 2b). Like present study, the changes in color values have been previously reported in various plants. Yilmaz *et al*. [7] represented the decreased L* in thyme plants. In addition, Jing *et al*. [32] noticed that the reduction in L* value can be because the drying process results in the browning response and pigment degradation [33]. The reduction in water content in plants leads to decreased photosynthesis pigments and finally increased a* value, being toward redness. Similarly, increments in a* and a*/b* and decreases in L* and b* under drying techniques have been addressed in Thymys daenensis leaves [34]. In some researches, the drying process could even show a positive value of a*, which represents the higher sensitivity of plants upon the corresponding technique of drying. The number of color properties can be different under drying techniques, temperature, types of species, and plant tissues. Arslan and Ozcan [21] demonstrated that microwaves inhibited color degradation during drying. Temperature and duration of drying are the main parameters affecting color characteristic. Accordingly, in this work, over 65 °C led to noticeable changes in color values, which is in line with the results obtained by Rahimmalek and Goli [34] with a remarkable degradation in the color of Thymys daenensis.

Table 1 Cannabidiol (CBD), tetrahydrocannabinol (THC), total phenolic content (TPC) of hemp plants under drying methods

Drying methods	CBD (µg/g)	THC (µg/g)	TPC (mg GA/g)
Fresh	35.0±1.10 g	25±1.3 j	7.5±0.47 i
Shade	268.3±5.6 c	261.3±2.04 e	34.1±1.18 a
Sunlight	30±2.32 g	231.3±4.77 f	19.4±1.93 h
Oven 45 °C	206.3±5.23 d	337±2.33 c	31.9±1.52 ab
Oven 55 °C	128.3±2.05 e	55.7±12.58 i	26.5±0.9 de
Oven 65 °C	207.7±2.49 d	276.3±3.29 d	23±0.64f
Vacuum 45 °C	54.7±2.04 g	142.3±1.71 g	21.7±1.41 gh
Vacuum 55 °C	86.7±2.06 f	106.7±1.24 h	24.3±0.69 ef
Vacuum 65 °C	234.7±1.7 d	103.3±2.71 h	25.3±1.38 ef
Microwave 200 W	341.0±3.27 b	362.7±13.57 b	28.7±0.87 cd
Microwave 400 W	431.3±1.25 a	350.3±4.01 b	33.4±0.86 a
Microwave 600 W	344.0±2.45 b	398.7±2.49 a	29.7±0.78 bc

Values are means ± standard deviation (±SD) of three replications (n= 3). Different letters show statistically significant differences among treatments at $P \leq 0.05$.

Table 2 Chlorophyll (Chl) content of hemp plants under drying methods

Drying methods	Chl a (mg/g)	Chl b (mg/g)	Total Chl (mg/g)
Fresh	1.17±0.03 a	0.48±0.02 a	1.8±0.02 a
Shade	1.16±0.03 a	0.47±0.02 ab	1.7±0.04 ab
Sunlight	1.11±0.01 bc	0.45±0.02 ab	1.6±0.03 cd
Oven 45 °C	1.15±0.02 ab	0.44±0.01 b	1.7±0.03 bc
Oven 55 °C	1.07±0.02 c	0.43±0.01 b	1.6±0.01d e
Oven 65 °C	1.02±0.01 d	0.44±0.01 b	1.6±0.01 e
Vacuum 45 °C	1.17±0.01 a	0.46±0.01 ab	1.74±0.02 ab
Vacuum 55 °C	1.16±0.01 a	0.45±0.02 ab	1.75±0.02 ab
Vacuum 65 °C	1.16±0.03 a	0.44±0.01 ab	1.74±0.02 ab
Microwave 200 W	1.15±0.02 ab	0.46±0.03 ab	1.74±0.02 ab
Microwave 400 W	1.14±0.01 ab	0.47±0.02 ab	1.74±0.02 ab
Microwave 600 W	1.11±0.01 bc	0.45±0.02 ab	1.7±0.03 bc

Values are means ± standard deviation (±SD) of three replications (n= 3). Different letters show statistically significant differences among treatments at $P \leq 0.05$.

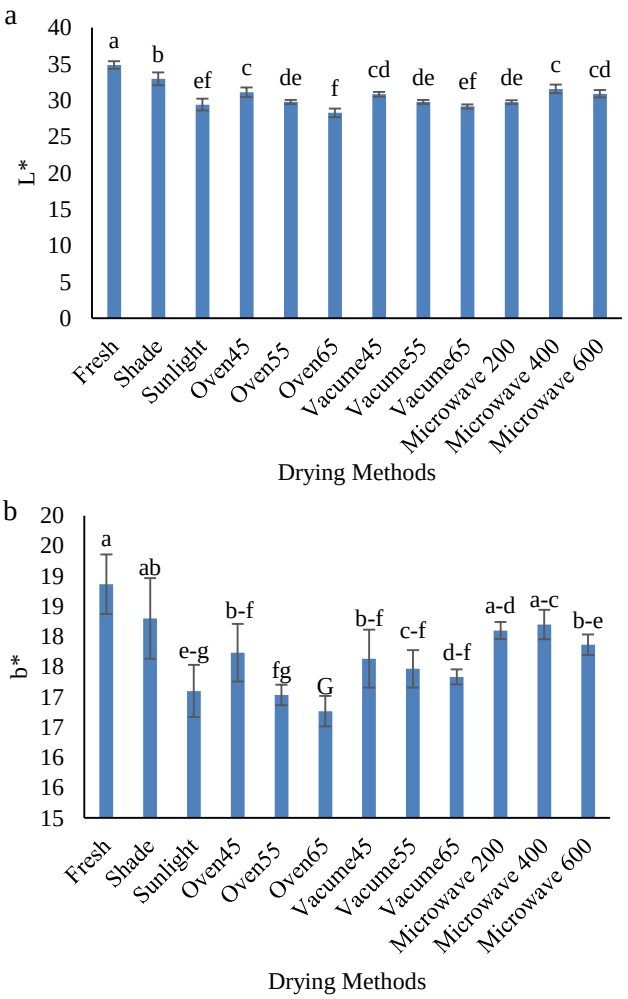

Fig. 1 Lightness (*L**) and blue to yellow (*b**) values under drying methods. Values are means ± standard deviation (SD) of three replications (n= 3). Different letters show statistically significant differences among treatments at $P \leq 0.05$.

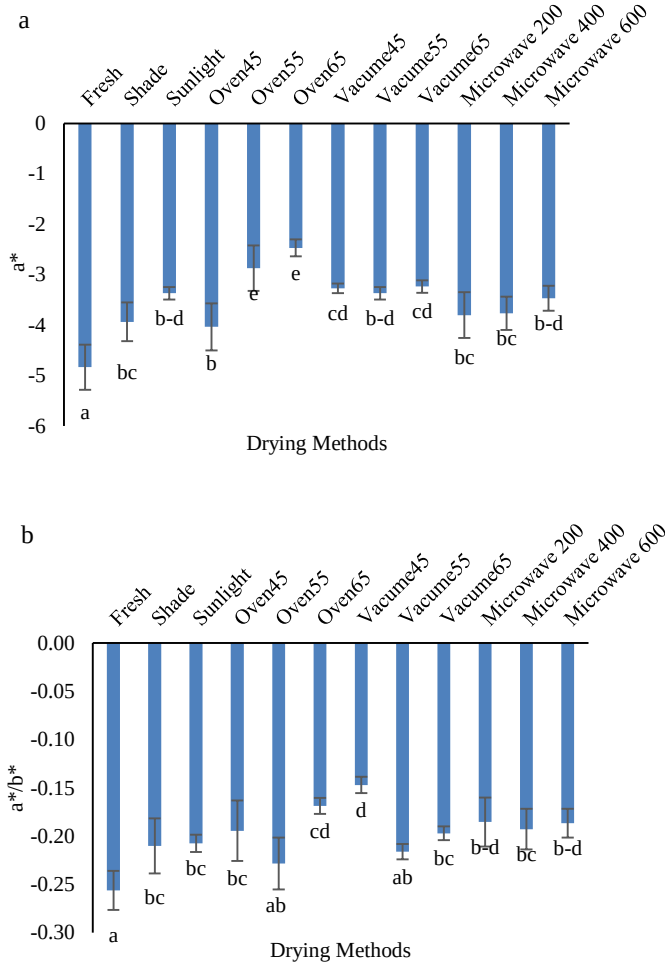

Fig. 2 Green to red (*a**) and *a**/b* values under drying methods. Values are means ± standard deviation (SD) of three replications (n= 3). Different letters show statistically significant differences among treatments at $P \leq 0.05$.

Multivariate Analysis

According to PCA, F1 with 57.22% justified Chl a, Chl b, total Chl, L^*, b^*, a^*, and a^*/b^*, while F2 (24.61%) specified CBD, THC, and TPC. In addition, fresh, shade, oven 55 °C, oven 65 °C, and vacuum 65 °C were justified by F1, and other treatments were determined by F2. CBD, THC, and TPC were identified as primary variables in distinguishing microwave 650 W (Fig. 3). Based on AHC analysis, three different clusters were determined so that cluster 1 included vacuum 65 °C, sunlight, vacuum 45 °C, vacuum 55 °C, fresh and oven 45 °C; cluster 2 consisted of shade, oven 45 °C, and oven 65 °C, and microwave 200, 400, and 600 W were placed in cluster 3 (Fig. 4).

CONCLUSION

This study represented those drying techniques had various effects on the color and biochemical properties of hemp. Generally, shade drying showed higher chlorophyll and phenolic content. In addition, microwaves reached the maximum cannabidiol. In addition, the most closet lightness to fresh plants was observed at shade drying. Therefore, we can select shade drying due to its low investment cost and high-quality dried products in terms of color and phytochemicals. On the other hand, microwaves can be recommended if the purpose is designed on obtaining the maximum cannabidiol and tetrahydrocannabinol.

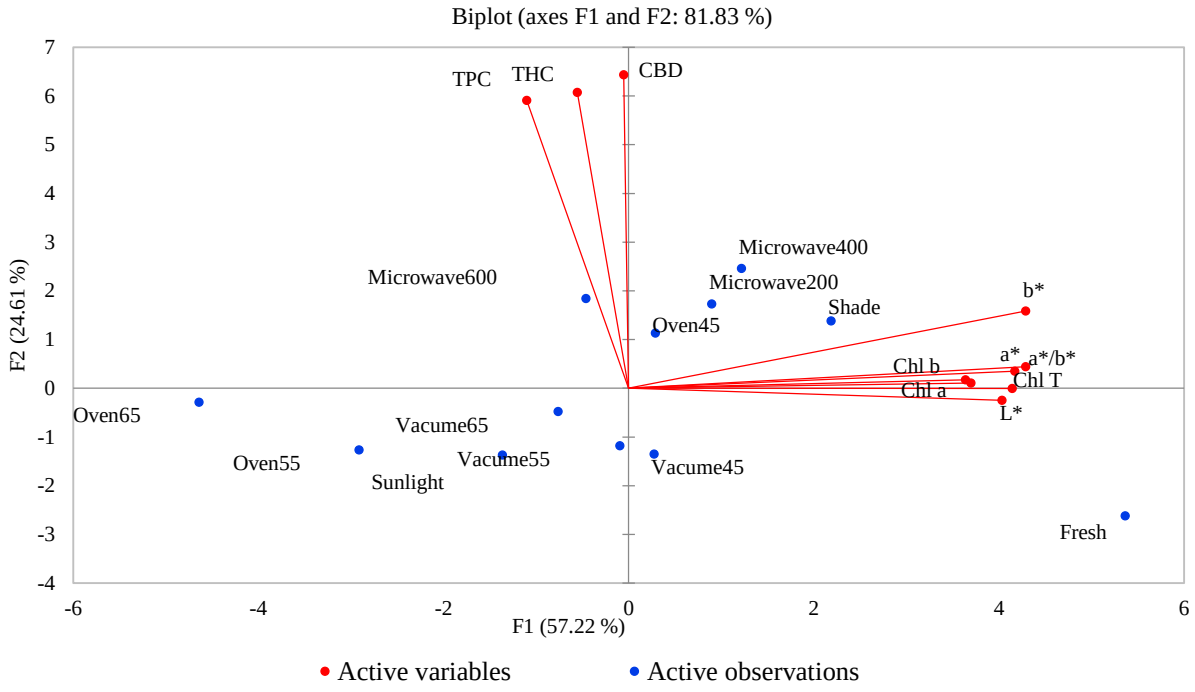

Fig. 3 Principal component analysis (PCA) for biochemical and color properties under different drying methods. TPC: total phenolic content, CBD: cannabidiol, THC: tetrahydrocannabinol (THC), Chl: chlorophyll.

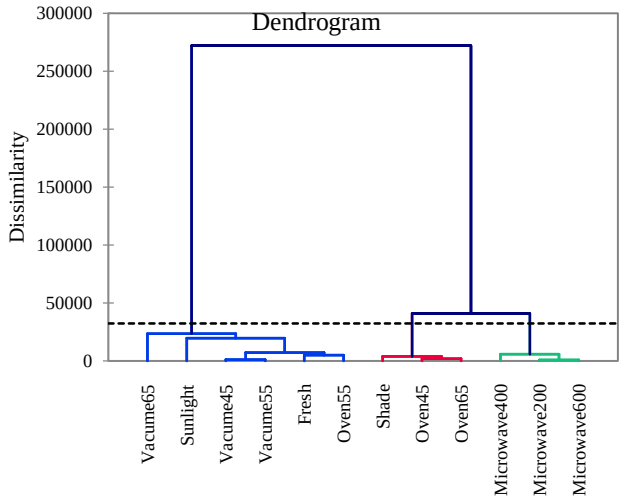

Fig. 4 Agglomerative hierarchical clustering (AHC) for different drying methods

Author Contribution

All authors contributed equally to this article.

Conflict of Interest

The authors declare that they have no conflict of interest.

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