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# Quality and bioactive compound accumulation in two holy basil cultivars as affected by microwave-assisted hot air drying at an industrial scale

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Holy basil (Ocimum Tenuiflorum L.) contains several bioactive compounds useful to the pharmaceutical and food industries. Microwave drying (MD) is a powerful technique for rapid drying of food or plant materials while preserving bioactive compounds during the process. However, little is known about the optimal combination of MD power with hot air drying (HAD) that can preserve the quality and yet only consume reasonable energy when drying holy basils. For that purpose, the effects of drying methods using MD combined with HAD at 45°C were examined to prevent losses in quality, antioxidant activities, and volatile flavor compounds in two holy basil cultivars (green and red cultivars). Holy basil leaves were dried at different MD powers of 200, 400, and 600 W combined with HAD and compared with a traditional tray drying (TD) at 45°C. Drying using MD at 600 W with HAD displayed significantly high levels of color retention, chlorophyll, and carotenoid content in both cultivars. The green cultivar showed a greater accumulation of total phenolic compounds (TPC), terpenoids, and DPPH free radical scavenging at 400 W with HAD. However, the red cultivar had the highest TPC, flavonoid, and terpenoid content at 600 W with HAD. The accumulation of major volatile organic compounds (VOCs) was also affected, and treatment at 600 W exhibited the highest methyl eugenol and  $\beta$ -caryophyllene content in both cultivars. The use of the highest power of MD (600 W) with HAD for leaf drying reduced the effective drying time and energy consumption among both cultivars. Taking into consideration the dried quality of antioxidant accumulation and energy consumed for drying, we recommend using MD at 400 or 600 W with HAD for the green cultivar and 600 W for the red.

#### KEYWORDS

*Ocimum Tenuiflorum* L., secondary metabolite, plant factory, bioactive compounds, drying methods, tray drying

# Introduction

The genus Ocimum is a member of the family Lamiaceae, comprising approximately 68 species indigenous to tropical regions of Asia, Africa, and Central and South America (Singh and Chaudhuri, 2018). Holy basil, Ocimum sanctum Linn. synonym Ocimum tenuiflorum L., is the most important species of the genera due to its medicinal, perfumery, religious, ceremonial, cuisine, and essential oil uses (Nandkarni, 1982). This aromatic shrub is commonly identified as two cultivars, namely, the green holy basil with green leaves and red holy basil with purple leaves (Darrah, 1974; Vani et al., 2009). Holy basil is reported to exhibit antidiabetic, wound-healing, antioxidant, radiation protective, immunomodulatory, antifertility, antiinflammatory, antimicrobial, antistress, and anticancer activities (Godhwani et al., 1988; Kelm et al., 2000; Vats et al., 2004; Yanpallewar et al., 2004; Mukherjee et al., 2005; Singh et al., 2005; Gupta et al., 2006; Salles Trevisan et al., 2006). The aroma compounds of holy basil essential oil (methyl eugenol chemotype, 56.18%) have been identified by solid phase microextraction (SPME)/GC-MS/flame ionization detection (FID) and olfactoric evaluations. The spicy-green-notes of essential oil are due to methyl eugenol, β-caryophyllene oxide, and germacrene D, while spicy-peppery-notes correspond to germacrene D (Jirovetz et al., 2003). Moreover, the major pharmacological activities of holy basil essential oil, such as mosquitocidal, antimicrobial, and anthelmintic, were found to be mediated by its marker constituent, eugenol (Kelm and Nair, 1998; Asha et al., 2001; Kumar et al., 2010).

Fresh leaves of plants are highly perishable and tend to lose quality immediately after harvest and thus require preservation against deterioration and spoilage. Water content in fresh Lamiaceae herbs is approximately 75-80% wet basis; however, to preserve its quality, water levels need to be reduced to less than 15% wet basis for preservation (Dixon and Paiva, 1995). Drying is one of the oldest and the most commonly used preservation techniques. Drying the herbs inhibits microbial growth and forestalls certain biochemical changes. Yet, it can cause alterations that affect herb quality, such as changes in structure and alterations in aroma caused by losses in volatiles or the formation of new volatiles as a result of oxidation or esterification reactions (Díaz-Maroto et al., 2002; OrphAnides et al., 2013). Certain compounds have been observed to increase in different herbs after drying-for example, eugenol in bay leaf (Díaz-Maroto et al., 2002; OrphAnides et al., 2013), thymol in thyme (Venskutonis, 1997), and some sesquiterpenes in different herbs (Baritaux et al., 1992; Yousif et al., 1999). Most studies have reported changes in the color and volatile compounds of aromatic herbs after drying (Díaz-Maroto et al., 2002; Di Cesare et al., 2003). The quality of the dried product is mostly influenced by drying conditions as well as the method adopted for drying. Currently, most dehydration techniques that are used to preserve fruits are fluidized bed drying, solar drying, hot air drying, microwave drying, osmotic dehydration, foam mat, spray drying, and freeze-drying (Barrozo et al., 2014).

Tray drying is the conventional method of drying that is widely used on a commercial scale because of its simplicity and low-cost equipment. However, tray drying involves a longer drying time due to the requirements of convective hot air. In addition to longer drying durations, the products are also exposed to undesirable effects such as reductions in bioactive compounds, shrinkage, slow rehydration, and loss of color (Dev et al., 2011; Workneh and Oke, 2012; Horuz and Maskan, 2015; Hihat et al., 2017; Kaveh et al., 2021; Zia and Alibas, 2021). Hence, the use of microwaves has emerged as an interesting alternative technique. In contrast to convective drying, microwaves can generate large amounts of heat inside rather than externally. Heat is generated by water molecules interacting with electromagnetic waves directly, leading to water vapor pressure differences between the interior and surface areas, leading to rapid external migration of moisture.

Microwave-assisted hot air has been widely used for agricultural drying with a positive effect on quality, antioxidant activity, and bioactive compounds (Arslan and Özcan, 2010; Dev et al., 2011; Workneh and Oke, 2012; Chaikham et al., 2013; Ozcan-Sinir et al., 2018; Poogungploy et al., 2018). Sensory evaluation of persimmon chips processed by microwave-assisted hot air microwave and freeze techniques showed higher sensory scores and nutritional values than traditional hot air drying (Jia et al., 2019). This indicated that dried samples using microwave techniques were positively affected by food products. In recent times, the application of microwave hot air rolling drying (MHRD) has developed to incorporate the advantages of microwave drying and hot air drying. The MHRD technique could improve the cooking efficiency of kidney beans, with a reduction of 2.5 times drying time at the same temperature when compared to without microwaves (Li et al., 2022). It has also been applied in ginger drying to determine the appropriate microwave power and drying temperature for the final product (Zeng et al., 2023). Similarly, microwave drying was applied for dewatered sludge, and it was found that the application of microwaves reduced the drying time by at least 37.5% compared to using hot air (Wulyapash et al., 2022). In addition to quality prospectus, concerns that producers are preoccupied with are energy usage that influences drying costs. These concerns may be reduced by microwave-assisted hot air drying (Poogungploy et al., 2018; Kaveh et al., 2021; Yue et al., 2021).

This study, therefore, aimed to evaluate the effect of alternative microwave drying processes under different conditions while protecting and maintaining dried leaf quality and bioactive compound accumulation in the red and green holy basil cultivars. We investigated the energy consumption at an industrial scale for food production and cosmetics industry use.

# Materials and methods

# Plant material

The fresh stems and leaves of two commercial holy basil cultivars (*Ocimum tenuiflorum* L.; green and red holy basil) were collected at the harvesting stage (49 days after transplantation) from a plant factory using artificial light (PFAL) at the National Center for Genetic Engineering and Technology Development Agency (BIOTEC), Thailand Science Park, Klong Lung, Pathum Thani, Thailand. Holy basil plants were grown under controlled environmental conditions with artificial light at 200  $\mu$ molm<sup>-2</sup>s<sup>-1</sup> of light intensity, 25±1°C air temperature, 75±3% relative humidity, and 1,000±150  $\mu$ molmol<sup>-1</sup> CO<sub>2</sub> concentration (Thongtip et al., 2022; Chutimanukul et al., 2022b).

# Drying methods and energy efficiency

The leaves of green and red holy basil were dried under four different conditions, each with four replications. The drying methods comprised commercial tray drying (TD) with hot air at 45°C and

previously unevaluated regimes of microwave drying (MD) assisted hot air drying at 45°C under three different power strengths of 200, 400, and 600 W. The TD with hot air for industrial scale is presented in Figure 1A. We used 12 drying trays with width, length, and height of  $53 \times 72 \times 3$  cm. MD was designed and constructed by staff from the Faculty of Engineering, Mahasarakham University, Thailand. The apparatus for microwave-assisted hot air drying at the pilot scale is shown in Figure 1B. Four magnetrons at 1000W were installed, generating microwaves at a frequency of 2,455 MHz. Under MD, the air was heated by a 9kW electric heater and circulated by a 1.5kW centrifugal fan with an electric motor. The drying chamber was 80 cm each in length, width, and height and featured eight drying trays with a diameter of 60 cm. Plant material samples for each run were 500 g placed on rotated trays, and fresh holy basil leaves were laid out in a single layer for drying with a thickness of less than 2 mm. Samples were dried until water activity (a<sub>w</sub>) was lower than 0.6 (i.e., the recommended safe level for storage). After drying, the weight of each sample was recorded to calculate water evaporation.

# Moisture content and a<sub>w</sub> determination

Fresh leaves were chopped into small pieces before determining moisture content. Approximately 5g samples were placed in an aluminum can and put in the oven at 103°C for 24h. Moisture content was calculated from the water evaporated divided by the fresh weight (AOAC, 1995). The dried samples were measured for  $a_w$  using an AquaLab water activity meter (Aqua-Link 3.0, Pullman, WA). It was calibrated with distilled water to obtain  $a_w$  in the range of  $1.000 \pm 0.003$ . Five dried leaves were used for each measurement.

## Specific energy consumption

SEC is the total energy consumed during drying divided by the mass of water evaporation. Tray drying energy consumption was evaluated from the blower and heater apparatus. The energy consumption of the microwave-assisted hot air drying was determined from four components, namely, microwave, blower, motor, and heater apparatus. A clamp multimeter was used to measure the voltages and electrical currents of all apparatus. The electrical energy consumption of the magnetron and heater was calculated by

*Emicrowave or Eheater* = 
$$(V \times I \times t_1) / 1000$$
 (1)

The electrical energy consumption of the blower and motor was calculated by

Eblower or Emotor = 
$$(V \times I \times t_2)/1000$$
 (2)

where *E* is the energy consumption of each component (kWh), V is voltage (V), I is electrical current (A),  $t_1$  is time (h) when the device is turned on during drying, and  $t_2$  is drying time (h) throughout the experiment.

The SEC of the microwave-assisted hot air dryer was reported in MJ/g of water evaporation using the following expression:

$$SEC = \frac{3.6(E_{microwave} + E_{heater} + E_{blower} + E_{motor})}{m_i - m_f}$$
(3)

The SEC of the tray dryer was reported in MJ/g of water evaporation using the following expression:

$$SEC = \frac{3.6(E_{heater} + E_{blower})}{m_i - m_f}$$
(4)

where 3.6 is the electric energy conversion factor from kilowatt hour to megajoule,  $m_i$  is initial mass and  $m_f$  is the final mass (g).

# Color measurements

Leaf samples of both green and red cultivars were ground to a fine powder before measuring color with a Hunter Lab Colorimeter (type Color Flex, United States). Color coordinate values,  $L^*$ ,  $a^*$ ,  $b^*$ , for each sample were recorded.  $L^*$  represented lightness, varying from 0 (dark) to 100 (light). Positive  $a^*$  and  $b^*$ values indicated redness and yellowness, while negative  $a^*$  and  $b^*$ values indicated greenness and blueness. Chroma (*C*) and hue angle (*h*) were calculated using the following equations (Pathare et al., 2013):



$$C = \sqrt{a^{*2} + b^{*2}}$$
(5)

$$h = \arctan \frac{b^*}{a^*} \tag{6}$$

# **Pigments accumulation**

All pigment contents such as chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids in the holy basil were examined according to Wellburn (1994). The dry samples were ground to a fine powder in a mortar, from which 100 g was extracted with 1 mL of absolute methanol solvent. The mixture was then incubated at 15°C in darkness for 72 h and then centrifuged at 8,000 rpm, 15°C for 5 min. After centrifugation, the supernatant solution was separated completely. The absorbance of the extracted solution was recorded at wavelengths of 470, 646.8, and 663.2 nm using a spectrophotometer (Agilent 8,453 UV–vis Spectroscopy System, Country). The concentration of the pigments was calculated using the following formulae (Wellburn, 1994):

Chlorophyll a (Chl a) = 
$$12.25 \text{ A}663.2 - 2.79 \text{ A}646.8$$
 (7)

Chlorophyll b (Chl b) = 
$$21.5 \, A646.8 - 5.1 \, A663.2$$
 (8)

Carotenoids = (1,000 A470 - 1.82 Chl a - 85.02 Chl b) / 198 (9)

$$Total Chlorophyll (TChl) = Chl a + Chl b$$
(10)

Pigment accumulation was presented as ug per gram dry weight (DW) of the sample.

## Antioxidant activity evaluation

#### Sample extraction

Holy basil samples, after harvesting and drying by different methods, were ground to a fine powder and extracted following a modified method (Chutimanukul et al., 2022a) for analyzing the total content of phenolic compounds (TPC), total flavonoid compounds (TFC), and the scavenging activity of DPPH radicals. Ten microgram of the fine powder sample was thoroughly mixed with 5 mL absolute methanol containing 1% HCL, which was used as a solvent. After incubation at room temperature for 3 h, the mixture solution was centrifuged for 5 min at 12,000 rpm. The supernatant was transferred to another microtube and used for the determination of TPC, TFC, and DPPH radical scavenging activity.

### Total phenolic content

The total phenolic content (TPC) was assayed following modified Folin-Ciocaltea colorimetric methods (Chutimanukul et al., 2022b). Two hundred microliter of extracted solution was mixed with  $200 \,\mu\text{L}$  of 1 N Folin–Ciocalteu reagent and incubated at room temperature for 15 min. Then, the mixture was added to  $600 \,\mu\text{L}$  of 7.5% sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>) and incubated for 1 h before detecting the absorbance at 730 nm by spectrophotometer (MultiskanSky, Thermo Scientific). Gallic acid was used as a standard solution, and the results were presented as milligrams of gallic acid equivalent per gram dry weight of the sample (mg of GAE / g DW).

#### Total flavonoids content

The quantification of total flavonoid content in holy basil was evaluated based on the method of (Chutimanukul et al., 2022b) with minor modification of the colorimeter method. Three hundred and fifty microliter of the extracted solution was added to  $75\,\mu\text{L}$  of 5% sodium nitrite (NaNO<sub>2</sub>) and completely mixed using a vortex. The mixture was incubated for 5min before adding  $75\,\mu\text{L}$  of 10% aluminum chloride (AlCl<sub>3</sub>·6H<sub>2</sub>O) and kept at room temperature for 5min. Lastly, 1 M sodium hydroxide (NaOH) was pipetted to the mixture solution and left to stand for 15min. The absorbance of the homogenate solution was determined at 515 nm by a spectroradiometer. The content of total flavonoids was calculated using rutin as the standard solution and showed as milligrams of rutin equivalent per gram dry weight of holy basil (mg of rutin/g DW).

#### Total terpenoids content

To investigate the content of total terpenoids in the holy basil after drying, 100 mg of fine powder of the dried sample from several drying treatments was extracted by mixing with 1 mL absolute methanol and incubating in the dark for 48 h. After incubation, the mixture was centrifuged at 6,500 rpm for 15 min. The determination of total terpenoids of the extracted solution was performed using the procedures of Ghorai et al. (2012) and Chutimanukul et al. (2022a). Absorbance was measured at a wavelength of 540 nm using a spectrophotometer with 99.9% methanol as a blank control. Linalool was used as the standard solution, and the content of total terpenoids was exhibited as milligrams of linalool per gram dry weight (mg of linalool /g DW).

# DPPH radical scavenging activity

Antioxidant scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals was detected by following the method of Chutimanukul et al. (2022b). Hundred microliter of extracted solution was completely mixed with 900  $\mu$ L of 0.1 mM DPPH and kept in the dark at 25°C for 30 min. The absorbance of the mixture was determined at 515 nm using a spectroradiometer. Trolox was used as a reference antioxidant. The percentage of DPPH scavenging as an antioxidant activity was calculated by the equation (Yen and Duh, 1994): Inhibition% = (A<sub>control</sub> – A<sub>515</sub>)/(A<sub>control</sub>) × 100. Where A<sub>control</sub> and A<sub>515</sub> were the absorbance of 0.1 mM DPPH without sample and absorbance of sample at wavelength 515 nm, respectively.

#### Volatile compound quantification

The analysis of volatile organic compound (VOCs) content was obtained by gas chromatography with mass spectrometry (Agilent; 7890B) combined with a quadruple time-of-flight mass spectrometer (GC/Q-TOF, Agilent, 7250) and PAL autosampler system (CTC Analytics AG, Switzerland). Extraction of the sample and analysis were carried out according to the method described by Chutimanukul et al. (2022a). Hundred milligram of the dried leaf powder was placed in 1 mL of absolute methanol with 10  $\mu$ L of 2000 ppm gammahexalactone, and then uniformly mixed for 1 min. The samples were sonicated by ultrasonic bath for 30 min. To eliminate the insoluble material, the prepared extracts were centrifuged at 10,000 rpm for 5 min. The supernatant was collected and stored in a vial at  $-20^{\circ}$ C for further analysis.

For the analysis of VOCs of dried leaves of holy bail in each MD treatment, 1 µL of the extract was injected into a multimode inlet (MMI). The inlet temperature was controlled at 250°C, and the samples were carried at a constant flow rate of 1.0 mL/min by highpurity helium (>99.999%). Chromatographic separation was conducted with a DB-FFAP column  $(30 \text{ m} \times 0.25 \text{ mm} \times 0.25 \text{ µm})$ (Agilent Technologies, United States). GC operational conditions were as follows: the temperature program was initiated at 60°C for 1 min, increased to 250°C by 10°C/min for 3 min; transfer line, 250°C; ion source (EI) temperature, 240°C; quadruple temperature, 150°C; EI energy 70 eV; and scanning mode from 20 to 350 m/z. Four target compounds, including eugenol, methyl eugenol, β-Caryophyllene, and Linalool, were investigated. The calibration curves were constructed with the relative internal response ratio as a function of the analyte concentration of the mixtures of the four target standard compounds with a range of 0.04-100 ppm.

For data processing, the target compounds, including eugenol, methyl eugenol, and  $\alpha$ -Humulene, were determined by comparing the calibration curves against relative internal response ratios. Concentrations of target analytes were reported as  $\mu$ g per gram of DW.

#### Experimental plan and data analysis

The experiments were designed according to a completely randomized design (CRD) with four replications. Statistical data were analyzed by IBM SPSS (IBM Corporation; Armonk, NY, United States). One-way analysis of variance (ANOVA) was used for sample comparisons in each parameter. Mean separations were analyzed with Duncan's Multiple Range Test (DMRT) at a 95% significance (p < 0.05). The data were presented as means ± S.E (standard error).

# Results

# Energy consumption

To investigate the energy consumption and specific energy consumption of the four different drying treatments on the two cultivars of holy basil, the initial moisture content, final moisture content, and water activity  $(a_w)$  of leaves after drying were calculated. Table 1 indicates the effects of the four drying methods: microwave drying (MD) under three different powers of 200, 400, and 600 W combined with hot air drying (HAD) at 4°C and the tray drying (TD) method using 45°C of hot air affected drying time. The total drying times for TD were substantially longer in comparison to microwave oven drying of both cultivars. Among different powers for MD treatments, the highest MD power caused the lowest average drying time. For a load of 500 g fresh leaves at 200, 400, and 600 W of MD with HAD, green holy basil leaves were dried after 6, 3.5, and 3h, respectively, while the red holy basil leaves took 5, 4, and 2.5h, respectively. Moreover, across drying methods, the drying process

caused reductions in moisture contents of fresh leaves, from between 87.5 and 88.6% at harvest to 8.0-9.9% in 2.5-7h of drying. After drying, the observed  $a_w$  value ranged from 0.30 to 0.45. The appearance of two holy basil cultivar leaves after drying treatments is shown in Figures 2, 3.

Energy efficiency via evaluation of energy consumption and specific energy consumption (SEC) was assessed. Blower ( $E_{blower}$ ), motor ( $E_{motor}$ ), heater ( $E_{heater}$ ), microwave ( $E_{mocrowave}$ ), total energy ( $E_{total}$ ), evaporated water, and SEC were considered. The details are presented in Table 2. After drying two cultivars of holy basil, we found that the MD treatments had the lowest  $E_{blower}$ ,  $E_{heater}$   $E_{total}$ , and SEC as the power of the microwave increased. On the other hand, TD had the highest value. Among different drying treatments, there were no differences in the values of evaporated water. Compared to the values obtained through the TD treatment, the SEC value at 600 W of MD was 3.5 times in green holy basil and 4 times in red holy basil.

# Qualitative properties

The color change of green and red holy basil dried leaf powder is shown in Table 3. Overall, lighter-colored leaf samples were dried with the highest MD power. For dried leaves of both green and red cultivars, the parameters of  $L^*$  (Lightness) value of MD at 600 W were the highest when compared with other methods. When comparing the  $a^*$  values of all dried leaves to those of fresh leaves, the lowest  $a^*$ (redness: green to red) values were observed under MD at 600 W, while TD showed the highest value. Moreover,  $b^*$  (yellowness) of both cultivars showed the lowest value under 600 W. *C* (chroma value: color saturation) and *h* (hue angle: color angle) increased with an increase in the power of MD, with leaf samples becoming clearer after drying. For MD at 200 and 400 W, dried leaf samples of each cultivar appeared lighter and greener than for TD treatment.

# Pigment accumulation

The effect of different drying techniques on pigment content was investigated (see Figure 4 for details). Leaves of green and red holy basils were significantly influenced by drying treatment. A significant accumulation of total chlorophyll (TChl), chlorophyll a (Chl a), and chlorophyll b (Chl b) was identified in both cultivars. It was the highest at 600 W MD (Figures 4A–C). Additionally, MD at 600 W resulted in increased carotenoid content in dried leaves of green holy basil, while carotenoid content was lowest when leaves were dried under TD. However, the red cultivar did not show significant differences across drying treatments (Figure 4D).

# Bioactive compounds and antioxidant capacity

After drying, the content of total phenolic compounds (TPC), total flavonoid content (TFC), and total terpenoids were found to be affected by the different drying treatments (Figure 5). The TPC content of both green and red holy basil leaves showed significant differences. Green holy basil leaves showed the highest TPC levels at 400 W of MD, while red holy basil leaves had the highest TPC content

Holy basil cultivar	Drying treatment	Drying time (h)	Initial moisture content (% w.b.)	Final moisture content (% w.b.)	a <sub>w</sub>
Green	45°C	7	$88.12 \pm 0.45$	$9.66 \pm 0.04$	$0.40\pm0.04$
	$200\mathrm{W} + 45^\circ\mathrm{C}$	6	$88.26 \pm 0.70$	$9.02\pm0.03$	$0.39\pm0.02$
	$400\mathrm{W} + 45^\circ\mathrm{C}$	3.5	87.93±0.06	$9.25 \pm 0.29$	$0.36\pm0.04$
	$600 \mathrm{W} + 45^\circ\mathrm{C}$	3	$87.89 \pm 1.39$	$8.68 \pm 0.97$	$0.33 \pm 0.03$
Red	45°C	6	$88.12\pm0.91$	$9.06 \pm 0.37$	$0.37\pm0.02$
	$200 \mathrm{W} + 45^\circ\mathrm{C}$	5	$88.55 \pm 0.09$	$8.42\pm0.41$	$0.33 \pm 0.01$
	$400\mathrm{W} + 45^\circ\mathrm{C}$	4	$88.25 \pm 0.40$	$8.82 \pm 0.25$	$0.35\pm0.03$
	$600 \mathrm{W} + 45^{\circ}\mathrm{C}$	2.5	88.22±0.37	$9.83 \pm 0.04$	$0.43 \pm 0.02$

TABLE 1 Drying time, initial moisture content, final moisture content, and water activity  $(a_{y})$  of green and red holy basil leaves after drying by tray drying (TD, 45°C) and various microwave powers combined with hot air drying at 45°C (HAD) (200 W + 45°C, 400 W + 45°C and 600 W + 45°C).

Values are represented as mean  $\pm$  SE (n = 4).



at 600 W (Figure 5A). Across different drying treatments, there were no significant differences for TFC in green holy basil. However, different drying treatments affected TFC accumulation in red basil, with 600 W MD showing the highest accumulation (Figure 5B). Different drying treatments significantly influenced the total terpenoid content in both green and red holy basil leaves (Figure 5C). MD at 400 and 600 W revealed the highest values for green holy basil leaves, and the levels were highest at 600 W in red holy basil. The total terpenoid content was lowest in TD treatments for red holy basil. DPPH analysis among drying treatments showed that antioxidant activity, i.e., the scavenging rates of DPPH, in green holy basil leaves had the highest value at MD 200 W. However, it was not significantly different than MD 400 W. Further, MD at 600 W recorded the lowest reading. Drying treatment did not significantly affect the antioxidant capacity among red holy basil leaves (Figure 5D).

# Volatile content

Volatile organic compounds (VOCs) of the two holy basil cultivars were analyzed using gas chromatography coupled with mass spectrometry (GC/Q-TOF). Results were obtained for the following VOCs: eugenol, methyl eugenol, Linalool, and  $\beta$ -caryophyllene (Figure 6). The highest eugenol levels for green holy basil were observed in TD treatments, followed by 200, 400, and 600 W MD, respectively (Figure 6A). However, red holy basil leaves did not show significant differences among drying treatments. In contrast, a significantly greater amount of methyl eugenol was present at 400 and 600 W MD in green holy basil leaves, but it was not significantly affected in red holy basil leaves (Figure 6B). Linalool alone was significantly different in the green holy basil leaves (Figure 6C). Regarding  $\beta$ -caryophyllene, TD and 200 W MD recorded the highest



+ 45°C (**B**), 400 W + 45°C (**C**), 600 W + 45°C (**D**) and tray drying (**E**).

TABLE 2 Energy consumption values of green and red holy basil leaf samples with tray drying (TD, 45°C) and various microwave powers combined with hot air drying at  $45^{\circ}$ C (HAD) (200 W +  $45^{\circ}$ C, 400 W +  $45^{\circ}$ C, and 600 W +  $45^{\circ}$ C).

Holy basil cultivar	Drying treatment	Drying time (h)	E <sub>blower</sub> (kWh)	E <sub>motor</sub> (kWh)	E <sub>heater</sub> (kWh)	E <sub>microwave</sub> (kWh)	E <sub>total</sub> (kWh)	Evaporated water (g)	SEC (MJ/g)
Green	45°C	7	4.95	-	38.78	-	43.73	78.46	2.01
-	$200\mathrm{W} + 45^\circ\mathrm{C}$	6	1.12	0.70	15.94	7.20	24.96	79.24	0.86
	$400W+45^\circ C$	4	0.75	0.47	11.95	6.40	19.57	78.68	0.68
	$600\mathrm{W} + 45^\circ\mathrm{C}$	3.5	0.65	0.41	9.30	7.35	17.71	79.21	0.57
Red	45°C	6	4.24	-	33.24	-	36.48	79.06	1.71
	$200\mathrm{W} + 45^\circ\mathrm{C}$	5	0.93	0.58	13.28	5.00	19.79	80.13	0.71
	$400\mathrm{W} + 45^\circ\mathrm{C}$	4	0.75	0.47	11.95	6.40	19.57	79.43	0.67
	$600\mathrm{W} + 45^\circ\mathrm{C}$	2.5	0.47	0.30	6.64	3.75	11.6	78.39	0.41

values, and MD 400 and 600 W showed the lowest  $\beta$ -caryophyllene values, which were significantly different for both holy basil varieties (Figure 6D). MD 600 W displayed the highest content in green holy basil leaves but was not significantly different from the TD treatment.  $\beta$ -caryophyllene content was highest in MD 400 and 600 W.

# Discussion

Our study indicates that variations in microwave drying treatments have significant impacts on the energy consumption of green and red holy basil leaves at an industrial scale. According to the industrial standards for dried herbal materials, the final moisture content is usually below 12% w.b., depending on the type of plant tissue (Preparations WECoSfP and World Health Organization, 1996). Such moisture content typically enhances shelf life during storage without affecting the quality of the plant products by reducing their susceptibility to post-harvest pathogens (Poós and Varju, 2017). Several studies have shown that a moisture content range of 7–12% is ideal for raw herbal materials in medicine preparations (Tarhan et al., 2011; Jayaraman and Gupta, 2020; Shalaby et al., 2020; Wiset et al., 2021). Consequently, the drying method is an important factor that has a considerable impact on the quality of resulting herbal materials. Our holy basil leaf samples from the two cultivars under plant factory cultivation showed 8.0–9.9% final moisture content after the various drying treatments (Table 1). We dried leaf samples until a<sub>w</sub> was lower than 0.6, which is safe for storage and prevents microbial growth (Rahman and Labuza, 2007).

Overall, the increased power of MD was associated with reduced drying times of the herbal products. Microwaves increase moisture evaporation rates from plant tissues by the rapid activation of energy absorption of water molecules. This leads to the generation of a

Holy basil cultivar	Drying treatment	L*	a*	b*	С	h
Green	45°C	$40.30 \pm 0.24^{a}$	$-4.77 \pm 0.04^{a}$	$20.95\pm0.29^{\circ}$	$21.39\pm0.69^{\circ}$	$101.62 \pm 0.14^{\rm b}$
	$200\mathrm{W} + 45^\circ\mathrm{C}$	$39.79\pm0.62^{\rm b}$	$-5.56 \pm 0.02^{b}$	$22.12\pm0.20^{\rm b}$	$22.76 \pm 0.71^{\rm bc}$	$103.53\pm1.18^{\rm a}$
	400 W + 45°C	$39.30\pm0.32^{\rm b}$	$-5.73 \pm 0.01^{b}$	$23.97 \pm 0.26^{a}$	$24.61\pm0.37^{\rm b}$	$103.52 \pm 0.07^{\rm a}$
	$600 \mathrm{W} + 45^{\circ}\mathrm{C}$	$40.31 \pm 0.16^{a}$	$-6.67 \pm 0.04^{\circ}$	$21.04 \pm 0.12^{\circ}$	$27.11 \pm 0.12^{a}$	$103.85 \pm 0.13^{a}$
F-test	*	*	*	*	*	*
Red	45°C	$40.26 \pm 0.87^{\rm b}$	$-3.61 \pm 0.16^{a}$	$23.98\pm0.06^{\rm b}$	$24.44 \pm 0.07^{\circ}$	$99.17\pm0.87^{\circ}$
	$200 \mathrm{W} + 45^{\circ}\mathrm{C}$	$40.05 \pm 0.81^{\rm b}$	$-4.82 \pm 0.05^{\rm b}$	$23.21\pm0.18^{\circ}$	$23.60\pm0.18^{\rm d}$	$101.83\pm0.06^{\rm b}$
	$400\mathrm{W} + 45^\circ\mathrm{C}$	$40.70\pm0.33^{\mathrm{b}}$	$-5.42 \pm 0.03^{\circ}$	$24.92 \pm 0.21^{a}$	$25.44\pm0.21^{\mathrm{b}}$	$102.35 \pm 0.02^{a}$
	600 W + 45°C	$43.24 \pm 0.14^{a}$	$-5.95 \pm 0.01^{d}$	$21.99\pm0.29^{\rm d}$	$26.53\pm0.29^{\text{a}}$	$102.26 \pm 0.05^{a}$
F-test	*	*	*	*	*	*

TABLE 3 The color parameters ( $L^*$ ,  $a^*$ ,  $b^*$ , C, and h) of green and red holy basil leaves after drying using tray drying (TD, 45°C) and various microwave powers combined with hot air drying at 45°C (HAD) (200 W + 45°C, 400 W + 45°C, and 600 W + 45°C).

Values are represented as mean  $\pm$  SE (n = 4). Different letters indicate significant differences between lines at p < 0.05 according to Duncan's Multiple Range Test (DMRT) at a 95% significant difference. \*Significant difference at p < 0.05.



FIGURE 4

Pigment accumulation, total chlorophyll (A), Chl a (B), Chl b (C), and carotenoid (D) in green and red holy basil leaves after tray drying (TD,  $45^{\circ}$ C) and drying under various microwave power combinations with hot air at  $45^{\circ}$ C (HAD) (200, 400, and 600 W). ANOVA was performed, followed by mean comparisons with DMRT. The different letters above the bars show significant differences between treatments at *p* < 0.05. "ns" indicates no significant difference.

temperature gradient between the sample and the air in the drying chamber that promotes the moisture in the samples to move outwards (Hemis et al., 2011; Doymaz et al., 2015; Hou et al., 2021; Mouhoubi et al., 2022). Previous reports of the Trabzon persimmon fruit (Alibas et al., 2019) and Kaffir lime leaf (Pradechboon et al., 2022) showed that increases in MD power induced a rapid increase in temperature due to the high energy transfer into samples. Our study indicates that MD at 600 W with HAD treatment ensured the shortest drying time for both green and red holy basil leaves of 3 and 2.5 h, respectively. In comparison, holy basil leaves dried by tray drying (TD) showed the longest drying time at 7 and 6 h for green and red holy basil leaves, respectively. This result was consistent with previous reports showing that an increase in MD power causes a dramatic increase in the average drying rates of various plants (Alibas et al., 2019; Liu et al., 2021; Pradechboon et al., 2022).

Energy consumption values among different drying methods of red and green holy basil leaves are shown in Table 2. MD methods had high energy-saving effects when compared with TD, which is widely used in chemical, pharmaceutical, and food industries to preserve herbal products for downstream processing and extended storage (Mujumdar, 2008; Chen and Mujumdar, 2009). Our study shows that all MD treatments have a lower and specific energy consumption (SEC) than TD. Further, microwave drying at 600 W with HAD provided around 2–3-fold energy consumption reduction over TD for



#### FIGURE 5

The content of total phenolic content (TPC) (A), total flavonoid content (TFC) (B), total terpenoids content (C), and DPPH free radical scavenging rate (D) in green and red holy basil leaves after tray drying (TD, 45°C) and various microwave power combinations with hot air at 45°C (HAD) (200, 400, and 600 W). ANOVA was performed, followed by mean comparison with DMRT. The different letters above the bars show significant difference between treatments at p < 0.05. "ns" indicates no significant difference.



The accumulation of eugenol (A), methyl eugenol (B), Linalool (C), and β-caryophyllene (D) in green and red holy basil leaves after tray drying (TD, 45°C) and various microwave power combinations with hot air at 45°C (HAD) (200, 400, and 600 W). ANOVA was performed, followed by mean comparison with DMRT. The different letters above the bars show a significant difference between treatments at p < 0.05. "ns" indicates no significant difference.

green and red holy basil leaves, respectively. The increase in microwave power enhanced the movement of water molecules since microwaves can easily penetrate inside the sample, where the microwave energy is transformed into heat. When the molecules of water are rubbed together, they produce heat, which increases the water temperature. In the case of TD, the heat is transferred from the surface of the sample to inside it by heat conduction, causing a longer drying time (Chen et al., 2019). These results are in accordance with previous findings that energy consumption and SEC decrease as microwave power increases (Varith et al., 2007; Kaveh et al., 2021). This is largely due to the drying time being shorter. Moreover, these results suggest that the highest MD power (600 W with HAD) might increase evaporation rates during the drying process, leading to decreases in energy consumption.

Drying processes can significantly affect the qualitative features of herbal production. Color is a psychological property of food products that directly influences the perception of flavor and quality (García et al., 2009), and the drying process is one of the most important causes of color degradation or dehydration (Lozano and Ibarz, 1997; Demiray et al., 2017). This is likely due to the gradual rising of heat, leading to the decomposition of colored pigments (Orikasa et al., 2014). Studies in various plant leaves and fruits have shown that the temperature during the drying process results in color changes in spearmint leaf (Islam et al., 2019), holy basil leaf (Raksakantong et al., 2011), Kaffir lime leaf (Pradechboon et al., 2022), papaya (Islam et al., 2019), and kiwi (Izli et al., 2017; Taghinezhad et al., 2021).

Likewise, in the current study, we observed that the drying treatment significantly influenced color change in the dried leaf powders of green and red cultivars. Colorimetric parameters, such as  $L^*$ ,  $a^*$ ,  $b^*$ , and C, are widely used to quantify the coloration of food and fruit products (Arias et al., 2000; Pathare et al., 2013).  $L^*$  represents lightness,  $a^*$  represents red (positive value) and green (negative value),  $b^*$  represents yellow (positive value), and blue (negative value), and C represents color saturation. H represents relative amounts of redness and yellowness, with 90° for yellow, 180° for green, and 270° for blue (Francis, 1980; Voss, 1992; Ayala-Silva et al., 2005).

Our results showed that 600 W of MD with HAD caused the highest values of  $L^*$ ,  $a^*$ , C, and h (hue) of green and red holy basils with  $b^*$  at its lowest when compared with other MD treatments and conventional TD (Table 3). This is consistent with previous studies that found MD (increased power) with HAD caused low  $a^*$  values (Yilmaz and Alibas, 2021; Pradechboon et al., 2022). However, there were no significant differences between the L\* and b\* values of green holy basil at 600 W of MD with HAD and under TD conditions. This may be explained by the drying time of TD, which, without a microwave, took longer time to dry than 600 W of MD with HAD. The TD condition had lower heat than microwave power and resulted in brightness and yellow shades that were similar to 600 W of MD with HAD treatment. Similarly, the leaf pigment contents of the dried samples of green and red holy basils showed the highest accumulation of total chlorophyll (TChl), chlorophyll a (Chl a), and chlorophyll b (Chl b) in MD 600W (Figure 4). These findings indicate that MD 600 W with HAD treatment, with its shortest drying duration, is promising in terms of psychologically interpreted properties of herbal products by maintaining greener and lighter colors in dried holy basil leaves of both cultivars.

Secondary metabolites play an important role in plant defense response and are widely used to relieve several ailments in traditional medicine (Afzal et al., 2022; Aziz et al., 2022; Kainat et al., 2022; Khalid et al., 2022). Essential oils, terpenes, phenolics, phenolic acids, and flavonoid compounds are the main constituents of antioxidants in the holy basil plant (Kadian and Parle, 2012; Agarwal et al., 2017; Maqbool et al., 2023; Waliat et al., 2023). The drying process may also induce changes in the secondary metabolites, bioactive compounds, and volatile oils among herbal products. Several studies have demonstrated that high temperature seriously damages secondary plant metabolites such as total phenolic compounds (TPC), total flavonoid compounds (TFC), terpenoids, antioxidant properties, and volatile oils in various plant species (Sang et al., 2014; Hihat et al., 2017; Nguyen and Le, 2018). Phenolic compounds and flavonoids are the most important phytochemical components and have potential against diseases, presumably due to their antioxidant properties (Rice-Evans, 2001). In our study, after drying the green holy basil leaves, levels of TPC, DPPH, and terpenoids were highest at 400 W MD and similar to that of TD treatment. In contrast, red holy basil leaves showed the highest of these secondary metabolites and antioxidants at 600 W of MD with HAD. However, the highest power of MD caused degradation in secondary metabolites and antioxidants in green holy basil leaves, whereas red holy basil leaves were not significantly affected by the highest power of MD. This result is consistent with previous reports that found that phenolics and flavonoids decreased at higher microwave powers in herbal leaves and cereal grains (Xiao et al., 2008; Ragaee et al., 2014; Thiangma et al., 2022; Zia et al., 2022). This may be because high microwave power levels beyond a threshold limit cause oxidation and thermal degradation of aromatic compounds by enzyme inactivation, causing decreases in bioactive compound levels (Nüchter et al., 2004; Thiangma et al., 2022). Optimization of drying and operating conditions mainly depends on the characteristics of plant raw materials as well as processing methods. This necessitates that the drying conditions be chosen based on the type of herbal material. Our findings clearly indicate that dried leaves of green holy basil with the highest TPC, terpenoids, and antioxidant contents were found at 400 W of MD with HAD.

The major components among volatile organic compounds (VOCs) of holy basil are phenylpropanoids and terpenoids (Chutimanukul et al., 2022a,b). Several target compounds have shown beneficial impacts on humans, including antioxidant properties, antibiotic-resistant bacteria, and antifungal and anti-inflammatory activities (Burt, 2004; Pandey et al., 2017). The target compounds required by food, pharmaceutical, and cosmetic industries are methyl eugenol, eugenol, Linalool, and  $\beta$ -caryophyllene content, which are the primary VOC compounds produced in holy basil (Vyas et al., 2014; Chutimanukul et al., 2022b). Eugenol and methyl eugenol are widely used for flavoring foods and have been extensively used for their anti-inflammatory, antioxidant, antibacterial, and analgesic properties (De Vincenzi et al., 2000; Choi et al., 2010; Pramod et al., 2010; Nejad et al., 2017). Linalool is a monoterpene that occurs naturally in aromatic plants and is commonly used as a chemical ingredient for flavor and fragrance in cosmetics (Arctander, 1969; Villa et al., 2007; Kamatou and Viljoen, 2008). β-caryophyllene is the primary sesquiterpene used in medicinal applications due to its anticancer (Fidyt et al., 2016), diabetes (Basha and Sankaranarayanan, 2016), colitis (Dutra et al., 2011), anxiety, and depression properties (Bahi et al., 2014).

Our study showed that VOC content was influenced by drying variables, particularly in green holy basil (Figure 6). For green holy basil leaves, raising the power of MD decreased eugenol and linalool content (Figures 6A,C). However, raising the MD power from 200 to 400 W increased the content of methyl eugenol and  $\beta$ -caryophyllene, especially at the maximum power of MD 600 W (Figures 6B,D). For red holy basil, the drying treatment did not significantly affect the content of eugenol, methyl eugenol, and Linalool. Interestingly, increasing MD power caused higher  $\beta$ -caryophyllene content in red holy basil leaves (Figure 6). This was consistent with previous reports (Harborne et al., 1993) that examined the effect of different drying processes on the yield of volatile products in plants and vegetables. The physicochemical

changes of volatile compounds during the drying process depend on several factors, such as the drying method, post-harvest practice, and the instability of essential properties (Venskutonis, 1997; Thamkaew et al., 2021). One of the most important chemical alterations of VOCs is oxidation and isomerization, which are deterioration processes of secondary products either enzymatically or chemically (Turek and Stintzing, 2013). Moreover, heat promotes the initial formation of free radicals, which catalyzes the autoxidation reaction (Lee et al., 2007). These results suggest the highest power at 600 W of MD with HAD condition could maintain the methyl eugenol and  $\beta$ -caryophyllene content in green holy basil leaves, while 400 and 600 W of MD could induce higher contents of β-caryophyllene in dried leaves of red holy basil. This finding should be of great value in fine-tuning the drying processes of holy basil in terms of food quality preservation and product development. Notably, the application of MD power with the HAD method could be used to produce high-value products while maintaining desired characteristics for the food and cosmetic industries.

# Conclusion

Our study has established that the use of microwave drying (MD) combined with hot air drying at 45°C (HAD) can be successfully used to preserve the quality, bioactive compounds, and volatile organic compounds (VOCs) of the two cultivars of holy basil leaf for food and pharmaceutical industries. Our data clearly indicates that MD power at 600 W with HAD has the highest ability to maintain color change of leaf in both cultivars by decreasing chlorophyll and carotenoid content. While 400 W of MD with HAD can be proposed for stabilization of TPC and DPPH free radical scavenging activity in green holy bail leaves, these bioactive compounds in red cultivars had the highest accumulation at 600 W of MD treatment. Moreover, 600 W of MD with HAD also exhibited the highest VOC content comprising methyl eugenol and  $\beta$ -caryophyllene for both cultivars. Based on the findings of our study, using 600 W of MD proved to be highly efficient in terms of energy savings resulting from significantly shortened drying times. This alternative method of drying shows promise as a viable option for green and red holy basils and could potentially replace conventional drying methods. The adoption of these alternatives could lead to better product qualities and higher bioactive compound retention, as well as lower electrical energy consumption at industrial scales.

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# Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

# Author contributions

PC, TT, and TS: study conception and design. LW, HJ, AT, and PC: data collection. LW, NP-a, KM, and PC: analysis and interpretation of results. LW and PC: writing the original draft. CD: revise the manuscript. PC and CD: writing review and editing. All authors reviewed the results and approved the final version of the manuscript.

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# **Conflict of interest**

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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