

Changes in Phenolic Compounds, Antioxidant and Physical Properties of Mulberry Tea Influenced by Intensity of Far-Infrared Radiation

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Abstract: This study aimed to apply far-infrared radiation and hot-air convection (FIR-HA) drying to improve physical quality and antioxidant properties of mulberry tea. Parameters studied were FIR intensities of 2, 3, 4 and 5 kW/m² with the combination of HA at constant temperature of 40 °C and air velocities of 1.5 m/sec. Drying rate and moisture content reduction were increased with increasing FIR intensities and elapsed times. Changes in the colour values (ΔE) were increased with increasing of FIR intensities (5>4>3>2 kW/m²). The outer surfaces of FIR-HA dried tea were mildly damaged. DPPH radical scavenging activities of mulberry tea were significantly increased with FIR intensities of 4 and 5 kW/m² but were decreased at lower FIR intensities (2-3 kW/m²). Total phenolic and total flavonoid content were increased with increasing FIR intensities. Changes in individual phenolic acid were evaluated. Eleven phenolic compounds were identified, namely *p*-coumaric acid, benzoic acid, (+)-catechin, chlorogenic acid, vanillic acid, syringic acid, sinapic acid, protocatechuic acid, ferulic acid, gallic acid and caffeic acid. The most predominant phenolic was *p*-coumaric acid for all samples of mulberry tea, ranged from 78-200 mg/g in mulberry tea dried under FIR-HA with FIR intensities of 2-5 kW/m² extract. All identified phenolic compounds contents were increased with increasing FIR intensities in all FIR-HA samples compared to fresh leaves except for chlorogenic acid which were found to have greater amount in FIR-HA with FIR intensities of 2 kW/m² samples. This study has demonstrated that intensity of FIR effected not only on total phenolic and flavonoid contents but also on individual phenolic compounds.

Key words: Far-infrared radiation • Intensity • Drying • Antioxidant • *Morus alba* L.

INTRODUCTION

Mulberry (*Morus alba* L.) leaf has been used for various purposes in different cultures throughout the world. Several studies have reported that mulberry leaf has potential antioxidant activity [1-3]. The extract of mulberry leaves scavenged the DPPH radical and inhibited the oxidative modification of rabbit and human low-density lipoprotein (LDL) [4]. Recently in Thailand, mulberry-leaf tea has gained more popularity as a health beverage and is seeking for industrial manufacture.

In the process of tea manufacturing, three main steps are needed namely, blanching, withering and drying. However, the degradation of antioxidant and nutritional quality of mulberry tea occurred during process is a serious problem. Drying processes may affect the quality

and quantity of antioxidant activity in mulberry tea. Traditionally, drying process of tea was done by hot air (HA) however this method results in undesirable quality such as not uniform, dark colour and decreased antioxidant activity, while sun drying has certain limitations such as time consuming and climate dependent. The drying method by using the combination of far-infrared (FIR) radiation and HA is one of the food preservations since the main nutrients of the agricultural products such as proteins, starches and water, have the principal bands of infrared radiation absorption at wavelengths greater than 2.5 μm [5, 6]. The combined infrared radiation and hot air heating (FIR-HA) has been considered to be more efficient over radiation or hot air heating alone as it provides a synergistic effect led to more rapid drying and less energy consumption [7, 8].

FIR creates internal heating with molecular vibration of material, i.e. molecules absorb the radiation of certain wavelengths and energy and cause vibration excitedly. Moreover, the mechanism of far-infrared drying is different from hot air drying [5] and the electromagnetic wave energy is absorbed directly by the dried food with less energy loss. FIR was thought to liberate and activate low molecular weight natural antioxidant compounds, because it heats materials without degrading the constitutive molecules of the surface and contributes to an even transfer of heat to the center of the materials [9]. Since most antioxidative compounds in plant are phenolic compounds which are covalently bound form with insoluble polymers [10, 11] hence, it is necessary to find effective processing methods to liberate the natural antioxidant compounds from plant sources. Far-infrared (FIR) radiation or simple heat treatment could liberate and activate low-molecular-weighted natural antioxidants in plants [12-14]. However there has been no report on effect of FIR-HA on physical and antioxidant properties or specifying amounts of antioxidant in mulberry leaves. Therefore, the main attention of this study was to improve the physical quality and antioxidant properties of mulberry tea by FIR-HA. The effects of FIR intensity in combined FIR-HA were investigated for optimizing the most suitable drying conditions. We hoped to obtain the most appropriate conditions for applying in the mulberry tea drying method.

MATERIALS AND METHODS

Materials: Mulberry (*Morus indica* L.) leaves, cultivar Burirum 60 were harvested from Silk Innovation Center, Mahasarakham University, Maha sarakham Province, Thailand in June 2008. Mulberry leaves were washed, drained and sliced into approximately 0.5 cm x 5 cm. The raw mulberry leaves were washed, blanched at 80°C for 30 seconds and then withering (subjected to mild drying) at 40-45 °C for 30 minutes in a pan. Afterwards, withered leaves were dried under FIR-HA.

FIR-HA dryer: A laboratory scale dryer using combination of far-infrared radiation and hot-air convection used in this study was developed in Research Unit of Drying Technology for Agricultural Product, Faculty of Engineering, Mahasarakham University, Thailand, as shown in Figure 1. The dryer consists of a stainless steel drying chamber with inner dimensions of 30 x 51 x 50 cm. A far-infrared heater was 122x60 mm in area and maximum power of 250 W (Sang Chai Meter Co., Ltd., Bangkok, Thailand). Two sets of three-FIR heaters were installed, one at the top and another one at the bottom of the drying chamber. The FIR intensity was varied by power regulator. The sample tray (25.4 x 37 cm), was placed parallel to the set of FIR heaters at the top and bottom of the drying chamber and the distance between each set of heaters and a tray was fixed at 15 cm.

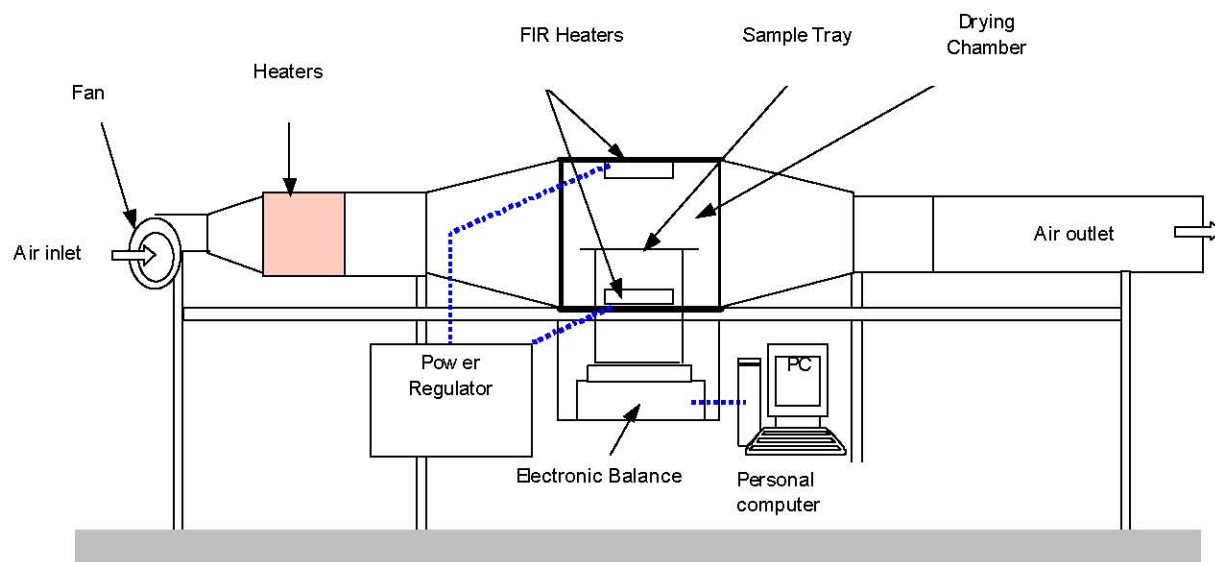


Fig. 1: Combined far-infrared radiation with hot-air convection system

The sample tray was supported on a balance which enabled continuous recording of the mass the product throughout the test. The temperature of inlet air passing through a hot-air heater was controlled by a PID controller (accuracy of $\pm 1^\circ\text{C}$). The air was passed through the drying chamber by a centrifugal fan.

The hot-air temperature was measured by K-type thermocouples connected to a data logger (accuracy of $\pm 1^\circ\text{C}$). Besides, air velocity was measured by hot-wire anemometer (accuracy of ± 0.1 m/s).

Drying Processes:

Laboratory Drying Protocols: Leaves and teas were subject to two different drying methods, i.e., hot air and FIR-HA. For each drying method, 100 g of fresh leaves and tea was used. In FIR-HA drying, the withering mulberry leaves were FIR-HA dried in the combined FIR-HA dryer at FIR intensities of 2-5 kW/m², HA temperature of 40 °C, HA velocities of 1.5 m/s and drying time of 60 minutes. After heating, the sample are called mulberry tea and allowed to cool to ambient temperature before extraction.

Commercial Drying Protocol: Mulberry tea was dried by hot-air drying machine at 60 °C for 6 hr using hot air oven (UFE 600, Memmert, Memmert company, Germany). This process was conducted at Hinherb Silathip Agriculturist Group, Khonkaen, Thailand.

Colour: Colour changes in sample were determined by a Minolta CR-300 Chroma Meter (Minolta, Japan) in *L*, *a*, *b* colour scale. The colour difference ΔE was calculated from the *L*, *a*, *b* parameters, using the Hunter-Scottfield equation:

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}$$

Photographs: The sample was observed in transmitted light with Olympus SZX-12 stereomicroscope at a magnification of 10x with illumination either from a fiber-optic light source. An Olympus SP-12 camera with monitor and controller is attached for digital image capture. Images of the fresh leaves, mulberry tea by FIR-HA dried and hot-air dried samples subjected were captured.

Extraction Procedure of Antioxidant in Mulberry Tea: The extracts prepared from the mulberry tea were made by boiling in distilled water for 5 min. The ratio between

sample and extraction medium was 1:25. The mixtures were filtered through a filter paper (Whatman No. 1) [15] and used for analyzing antioxidant activity *in vitro*. All analyses were performed in three replicates.

DPPH Radical Scavenging Activity: The hydrogen atom or electron-donation ability of the corresponding extracts and some pure compounds was measured from the bleaching of purple coloured methanol solution of DPPH [16]. The antioxidant activity of the extracts, on the basis of the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical, was determined by the method described by Braca *et al.* [17]. Aqueous extract (0.1 ml) was added to 3 ml of a 0.004% MeOH solution of DPPH. Absorbance at 517 nm was determined after 30 min and the percent inhibition activity was calculated as $[(A_0 - A_e)/A_0] \times 100$ (A_0 = Absorbance without extract; A_e = absorbance with extract).

Determination of Total Flavonoid Content: Total flavonoid content was determined using colourimetric method described by Dewanto *et al.* [18] with slight modification. Briefly, 0.5 ml of the extract was mixed with 2.25 ml of distilled water in a test tube followed by addition of 0.15 ml of 5% NaNO₂ solution. After 6 min, 0.3 ml of a 10% AlCl₃•6H₂O solution was added and allowed to stand for another 5 min before 1.0 ml of 1 M NaOH was added. The mixture was mixed well with vortex. The absorbance was measured immediately at 510 nm using spectrophotometer. Results were expressed as mg rutin equivalents in 1 g of dried sample (mg RE/g).

Identification and Quantification of Phenolic Compounds

Phenolics Extraction: The phenolic compounds in test samples were extracted using a modification of the procedure described by Uzelac *et al.* [19]. Each sample (5 g) was mixed with 50 mL methanol/HCl (100:1, v/v) which contained 2% tert-butyl hydroquinone, in an inert atmosphere (N₂) during 12 h at 35 °C in the dark. The extract was then centrifuged at 4000 rpm/min and supernatant was evaporated to dryness under reduced pressure (35-40 °C). The residue was redissolved in 25 mL of water/ethanol (80:20, v/v) and extracted four times with 25 mL of ethyl acetate. The organic fractions were combined, dried for 30-40 min with anhydrous sodium sulfate, filtered through a Whatman-40 filter and evaporated to dryness under vacuum (35-40 °C). The residue was redissolved in 5 mL of methanol/water

(50:50, v/v) and filtered through a 0.45 μm filter before injection (20 μL) into the HPLC aperture. Samples were analyzed in triplicate.

Determination of Total Phenolic Content: The total phenolic contents (TPC) of each extract were determined using the Folin-Ciocalteu reagent [20]. The reaction mixture contained 1 mL of mulberry tea extract, 0.5 mL of the Folin-Ciocalteu reagent, 3 mL of 20 % sodium carbonate and 10 mL of distilled water. After 2 h of reaction at ambient temperature, the absorbance at 765 nm was measured and used to calculate the phenolic contents using gallic acid as a standard. The total phenolic contents were then expressed as gallic acid equivalent (GAE), in mg/g dry sample.

HPLC-DAD System for Analysis of Phenolic Compounds:

HPLC analysis was performed using Shimadzu LC-20AC pumps, SPD-M20A Diode-array detection and chromatographic separation were performed on a LUNA C-18 column (4.6 x 250 mm i.d., 5 μm). The composition of solvents and gradient elution conditions were described previously by Uzelac Uzelac *et al.* [19] with some modifications. The solvent system used was a gradient of mobile phase A containing 3% acetic acid in water; solution B contained a mixture of 3% acetic acid,

25% acetonitrile and 72% water. The following gradient was used: 0-40 min, from 100% A to 30% A-70% B with a flow rate 1 mL/min; 40-45 min, from 30%A-70% B to 20% A-80% B with a flow rate 1 mL/min; 45-55 min, from 20% A-80% B to 15% A-85% B with a flow rate 1.2 ml/min; 55-57 min, from 15% A-85% B to 10% A-90% B with a flow rate 1.2 mL/min; 57-75 min 10% A-90% B with a flow rate 1.2 mL/min. Operating conditions were as follows: column temperature, 40 °C, injection volume, 20 μL , UV-Diode Array detection at 278 nm.

Statistical Analyses: Analysis of variance (ANOVA) in a completely randomized design, Duncan’s Multiple Range Test. All determinations were done at least in triplicates and all were averaged. The confidence limits used in this study were based on 95 % ($p < 0.05$).

RESULTS AND DISCUSSION

Moisture Content Analysis: Drying curves of mulberry tea undergoing FIR-HA drying at various FIR intensity are shown in Fig. 2. As expected, at the same hot-air velocity level the rate of moisture reduction increased with an increase in the FIR intensity because of the increased FIR intensity difference between the drying product and the surrounding as well as to the increased moisture diffusivity.

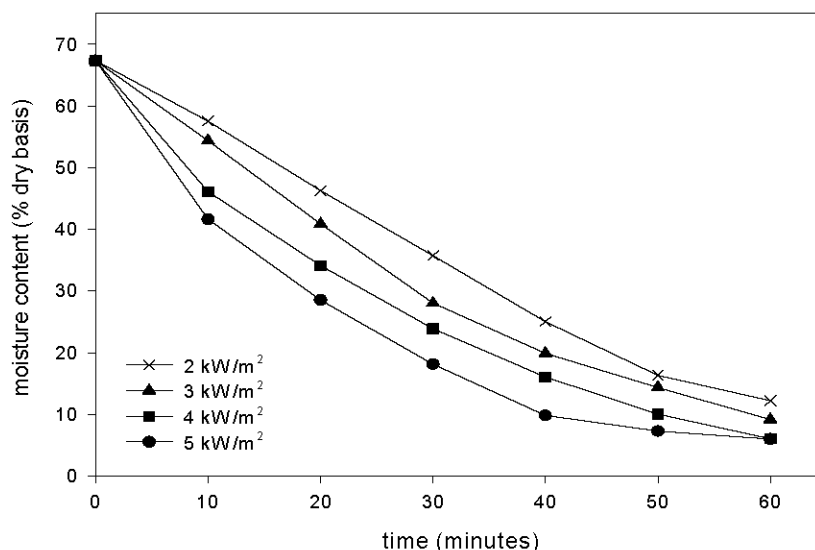


Fig. 2: Effect of FIR intensity on moisture content of mulberry tea.

Values are means (n=3). [Moisture content was fixed to be not access 7% dry basis, in commercial mulberry tea, drying process was conducted by using hot air commercial dryer at 60 °C for 6 hr.]

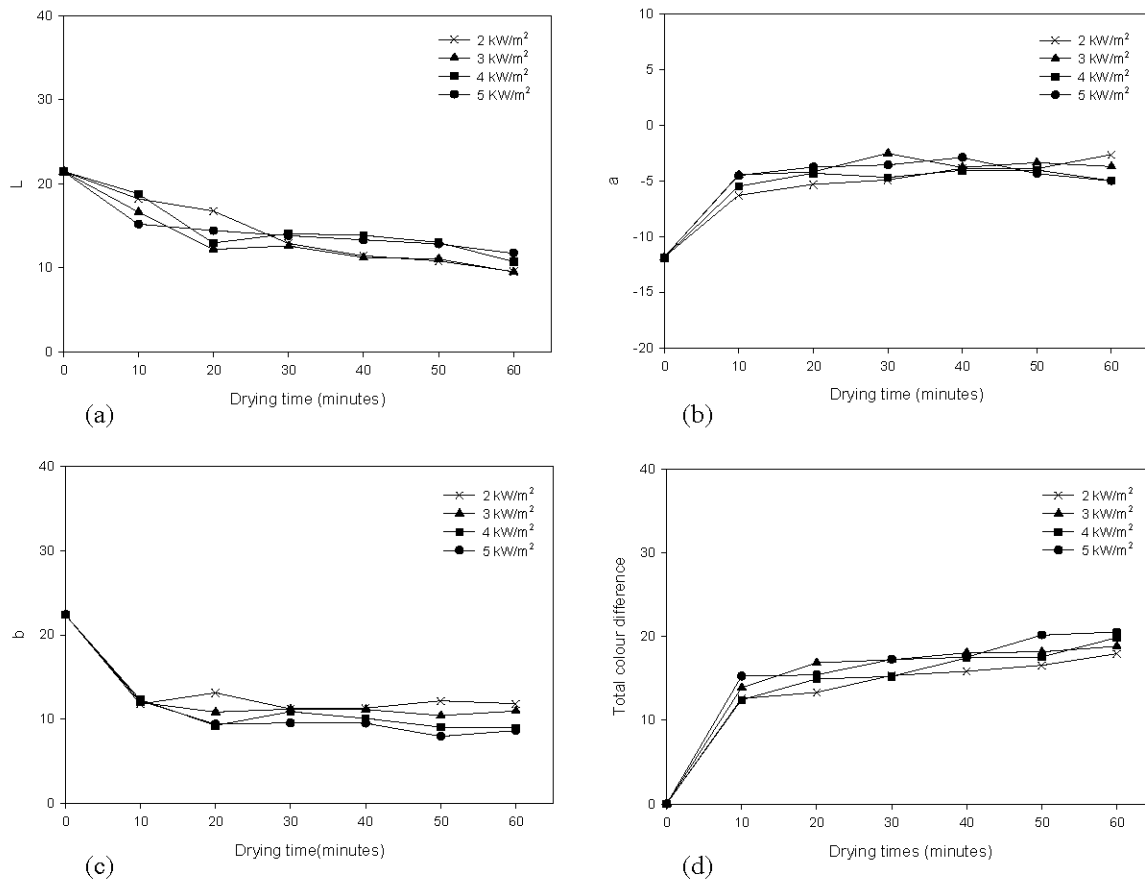


Fig. 3: Effect of FIR intensity on colour of mulberry tea. Values are means (n=10).

Moisture content comparison among the FIR-HA drying samples at various FIR intensities at 2, 3, 4, 5 kW/m² are shown in Fig 2. Moisture contents reduction increased with increasing elapsed times. An increase in drying rate with increasing radiation intensity has been noticed in earlier studies on infra-red (IR) drying [21-27].

In the process of FIR drying, the heat is generated deep inside the kernel and tends to be selectively the highest in the regions having the highest moisture content. Thus the vapour pressure would be largest in these regions and diffusion will be toward the areas of lower vapour pressure such as the kernel surface [8]. Convective drying is a slow process relying on heat conduction from the outer surface towards the interior. It is clear that drying rates are higher and drying time is substantially lower under combined FIR-convection drying than in convection alone. The use of combined FIR-convection provides necessary heat of

vaporization from deep inside the kernel and slightly HA to carry away the evaporated moisture. The use of FIR and air temperature resulted in much faster drying and considerable reduction in energy than at comparable convective temperatures.

Colour and Micrographs: Colour is a psychological property of food products that effects to the enjoyment of eating. Temperature during drying is one of the causes of colour degradation in dehydrated products [28]. Colour parameters of tea dried by different methods compared to fresh leaves are shown Fig. 3. A slight decrease in *L*-value was observed (Fig. 3 (a)). The changes in *L*-value of FIR-HA dried mulberry tea were increased with increasing FIR intensities. In contrast, a slight increase in *a*-value and *b*-value was illustrated in Fig. 3 (b) and (c), respectively. The *a*-value and *b*-value of mulberry tea decreased with FIR intensity increased. The results presented in this work suggest that the change in *L*, *a* and *b*-values of FIR-HA

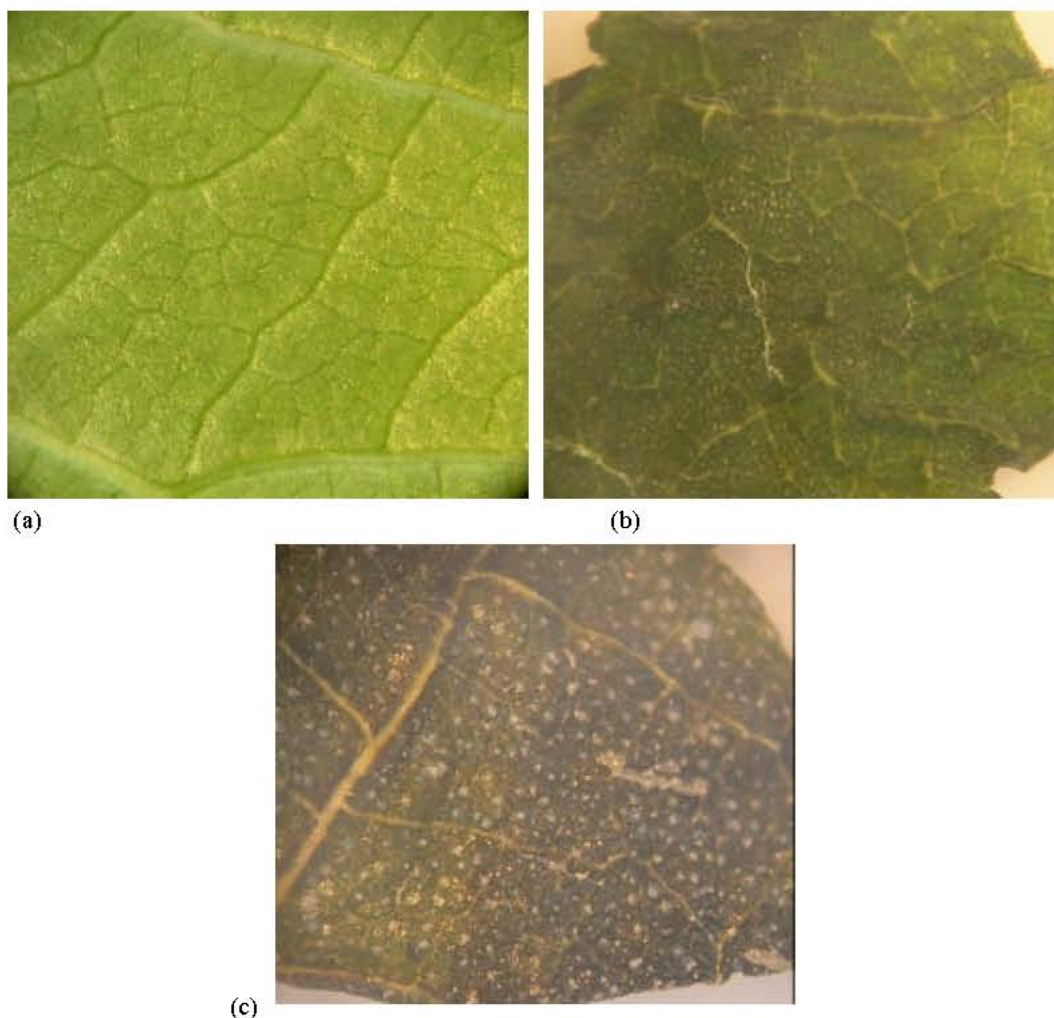


Fig. 4: Appearance of mulberry leaf (a) mulberry tea by FIR dried (b) and hot-air dried (c)

dried tea were small as compared to commercial tea (data not shown). The total color difference ΔE , which is a combination of parameters L , a and b -values, is a colourimetric parameter extensively used to characterize the variation of color in food during processing. A marginal increase in ΔE was observed (Fig. 3 (d)) with drying time. Greater changes in colour (ΔE) were found when higher FIR intensities were applied. A uniform colour of mulberry tea dried by combined FIR-HA drying and commercial tea was observed (data not shown). This could be explained that dried by combined FIR-HA drying induces more uniform colour on the products than commercial tea, HA drying. Physical appearance as demonstrated by the micrograph of the tea from stereomicroscope is shown in Fig. 4. The results indicated that FIR-HA dried samples had uniform and small porous structure similar to fresh mulberry leaf. While the HA

dried commercial tea appeared more damage and disruption of outer surfaces of tea. This could be explained that HA drying harshly removes the water from cellular walls at the heated surface causing large pores, more damage of plant tissues. The changes in pores and outer surfaces of the tea could affect the quality characteristic of the tea.

DPPH Radical Scavenging Activity: DPPH is a free radical compound and has been widely used to test the free radical-scavenging ability of various samples [29-31]. It is a stable free radical with a characteristic absorption at 517 nm, was used to study the radical-scavenging effects of extracts. As antioxidants donate protons to this radical, the absorption decreases. To evaluate the scavenging effects of water extract of mulberry tea, DPPH. inhibition was investigated.

Table 1: Effect of FIR intensity on total phenolic and total flavonoid content of mulberry tea

Sample	TFC (mg RE/g dry sample)	TPC (mg GAE/g dry sample)
FIR-HA, 2 kW/m ²	21.29±0.18 ^d	32.84±0.59 ^d
FIR-HA, 3 kW/m ²	21.61±0.15 ^{cd}	38.23±0.57 ^c
FIR-HA, 4 kW/m ²	22.02±0.18 ^{bc}	44.00±0.40 ^b
FIR-HA, 5 kW/m ²	22.28±0.14 ^a	51.07±0.58 ^a

Effect of combined far-infrared radiation and hot-air convective drying on % DPPH radical scavenging activity in the extract of mulberry tea is shown in Fig. 4. DPPH radical scavenging activities of mulberry tea significantly increased with FIR radiation at FIR intensities of 4 and 5 kW/m² while at lower FIR intensities (2-3 kW/m²) decreased. After FIR radiation for 60 min, the percent of DPPH radical scavenging activity significantly increased to 76 % for FIR intensity of 5 kW/m², HA velocity 1.5 m/sec compared to untreated mulberry leaves (71 %). This indicated that the increase was induced by the FIR ray. This finding supports a previous study that FIR radiation increased the antioxidant activity of rice hull extract [32]. FIR may have capability to cleave covalent bonds and liberate antioxidants such as flavonoids, carotene, tannin, ascorbate, flavoprotein or polyphenols from repeating polymers [9]. According to our data from the present study has demonstrated that the increase of radical scavenging activity was not only dependent upon the exposure time to FIR-radiation but also FIR intensity.

Determination of Total Flavonoid Content: Large amounts of natural phenolic compounds are found in teas, fruits and vegetables [33]. Flavonoids are the most common and widely distributed group of plant phenolic compounds, which are characterized by a benzo- γ -pyrone structure, which is ubiquitous in fruits and vegetables. Total flavonoid can be determined in the sample extracts by reaction with sodium nitrite, followed by the development of coloured flavonoid-aluminum complex formation using aluminum chloride which can be monitored spectrophotometrically at 510 nm. TFC of mulberry tea increased with FIR radiation increased. TFC showed the highest content in mulberry tea with FIR intensities of 5 kW/m² (22.28 mg RE/g dry sample) followed by the FIR intensities of 4 kW/m² (22.02 mg RE/g dry sample), 3 kW/m² (21.61 mg RE/g dry sample) and 2 kW/m² (21.29 mg RE/g dry sample) (Table 1).

Determination of Total Phenolic Content: Phenolic compounds are widely distributed in fruits and vegetables [34], which have received considerable attention, due to their potential antioxidant activities and free-radical

scavenging abilities, which potentially have beneficial implications in human health [34-37]. TPC was determined in comparison with standard gallic acid and the results expressed in terms of mg GAE/g dry sample.

TPC showed the highest content in mulberry tea with FIR intensities of 5 kW/m² (51 mg GAE/g dry sample) followed by the FIR intensities of 4 kW/m² (44 mg GAE/g dry sample), 3 kW/m² (38 mg GAE/g dry sample) and 2 kW/m² (32 mg GAE/g dry sample) (Table 1).

Phenolic Compounds: Phenolic compounds are the most active antioxidant derivatives in plants [38]. They are known to act as antioxidants not only because of their ability to donate hydrogen or electrons but also because they are stable radical intermediates [39, 40]. Generally, the outer layers of plant such as peel, shell and hull contain large amount of polyphenolic compounds to protect inner materials. A number of phenolic acids are linked to various cell wall components such as arabinoxylans and proteins [41, 42]. The HPLC-DAD analysis of mulberry tea aqueous extracts revealed the presence of phenolic compounds. By this means, in the four analyzed samples, it was possible to identify eleven phenolic compounds: *p*-coumaric acid, benzoic acid, chlorogenic acid, vanillic acid, syringic acid, sinapic acid, protocatechuic acid, ferulic acid, gallic acid, caffeic acid, (+)-catechin (Fig. 6). Drying at higher FIR intensity yielded a greater of all phenolic compounds than drying at lower FIR intensity. *p*-Coumaric acid was the most predominant phenolic compounds in all samples of mulberry tea, contributing about 78 mg/g to 200 mg/g in mulberry tea with FIR intensities of 2 to 5 kW/m² extract. Benzoic acid, (+)-catechin, vanillic acid, caffeic acid, ferulic acid and sinapic acid showed the highest content in mulberry tea with FIR intensities of 5 kW/m². Gallic acid and syringic acid was the highest content in sample with FIR intensities of 3 kW/m² (4.4 and 3.9 mg/g respectively). Chlorogenic acid and protocatechuic acid was the highest content in sample with FIR intensities of 2 kW/m² (23.1 and 4.6 mg/g respectively). FIR rays are biologically active and it transfers heat to the center of materials evenly without degrading the constitute molecules of surface [9, 43]. Lee *et al.* [44] reported that stronger antioxidant activity

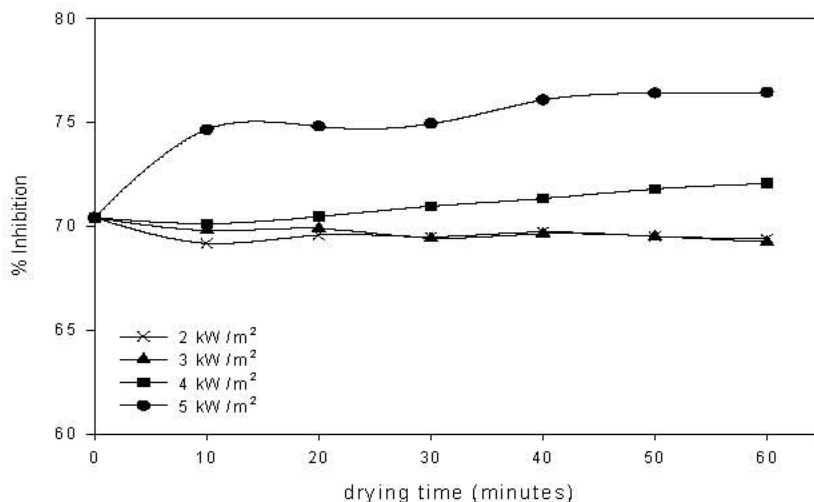


Fig. 5: Effect of FIR intensity on DPPH scavenging activity of mulberry tea. Values are means (n=3)

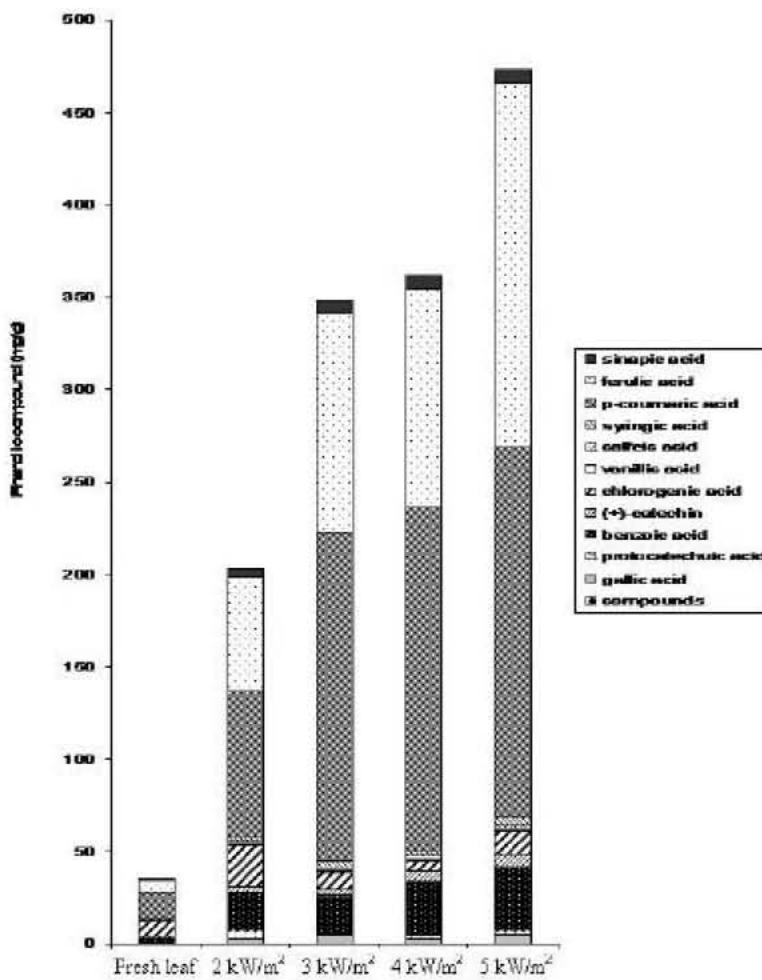


Fig. 6: Phenolic compound (mg/g) in mulberry tea

was observed from methanolic extract of FIR-radiated peanut hull, compared to those of heat treatment (electronic muffle furnace). This has been explained that simple heat treatments could not cleave covalently bound phenolic compounds from mulberry tea but FIR treatments could [44].

In addition, phenolic compounds of plants might be present in different binding status depending on chemical structures. Thus, effective processing steps to liberate phenolic acids from plant tissues may not be the same. Our present study has shown that individual phenolic compounds differently response to different levels of FIR-intensities.

CONCLUSIONS

Our results have demonstrated that FIR-HA drying could improve colour and tissue damage of mulberry tea. An increase of antioxidant activities of mulberry tea was also achieved by FIR-HA drying. This could be explained that FIR liberated phenolic compounds and thus increased the antioxidant activity of mulberry tea extract. The increase of radical scavenging activity depends upon FIR intensity and the exposure time to FIR-radiation. From this feature of combined FIR-HA drying, it is believed that the combined drying method should be appropriate for applying in the mulberry tea drying method. This information is potentially useful if FIR-HA is to be used for mulberry tea drying in industrial scale.

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