Effects of Drying Temperature and Time on Color, Bioactive Compounds, and Antioxidant Activity in 'Hua Ruea' Chili Fruit (*Capsicum annuum*)

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Abstract

Chilies, fresh or dried, have culinary value and are rich in bioactive compounds with medicinal properties. Drying used as a postharvest preservation strategy influences the amount of bioactive compounds and therefore the effects of drying temperature and time on color, antioxidants and bioactive compounds in chili fruit cv. Hua Ruea were investigated. Fruits were dried at 60 and 90 °C at various drying times of 3, 6, 21 h and at constant weight of at 60 and 90 °C for 23 and 27 h, respectively. Samples were analyzed for color (a*, Hue angle and total color difference), bioactive compounds (capsaicin: CAP; dihydrocapsaicin: DHC; total phenolic contents: TPC, and total flavonoid contents: TFC), and antioxidant activities (ferric ion reducing antioxidant power: FRAP, ABTS and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity). Results indicated that the bioactive compounds and antioxidant activities increased with drying time. These values were increased by approximately 2-fold in FRAP, TPC, TFC, CAP and DHC when compared to final drying time of the fresh fruit. The a* value and Hue angle of chilies dried at 60 °C were higher than those dried at 90 °C indicating that the darkening of chilies was influenced by the drying temperature. Moreover, TPC, TFC, CAP and DHC of chilies dried at 60°C were significantly higher than at 90 °C. These results indicated that drying temperature of 60 °C for 27 h and 90 °C for 23 h is suitable for chilies in order to maintain their physical quality and bioactive compounds and therefore providing a foundation for postharvest processing.

Keywords: Antioxidant, Capsaicin, Total flavonoid, Total phenolics, Pulp color

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Received: 31 July 2018/ Revised: 30 October 2018/ Accepted: 7 March 2019

1. Introduction

Chili (*Capsicum* L.), is one of the most consumed spice in the world whereby it is widely used in food and pharmaceutical industries. Capsicum are known to have vitamin A, B1, B2, Niacin, C, calcium, phosphorous and iron including bioactive compounds with antioxidant potential such as capsaicin, dihydrocapsaicin, nordihydrocapsaicin, homo-capsaicin and homodihydrocapsaicin (Kozukue *et al*., 2005; Maokam *et al*., 2014). These bioactive compounds have been suggested to help in alleviating salivary gland dysfunction and inflammation and to boost metabolic rate (Shin *et al*., 2016), reduce cough symptom (Ternesten-Hasseus *et al*., 2015) and inhibit cholinsterases linked to Alzheimer's disease (Ogunruku *et al*., 2014).

Like other dried food products, chilies are dried to low moisture content prior to postharvest storage. Drying prolongs the shelf-life of products due to low moisture content for microbial activity, and reduces chemical and enzymatic reactions which improve product shelf life. Many drying methods have been reported in chili such as hot air rotary dryer (Mihindukulasuriya and Jayasuriya, 2015), solar drying (Nimrotham *et al*., 2017), freeze dry, and hot air (Toontom *et al*., 2016). Among these even though sun drying is cost effective, hot air oven drying overcomes the disadvantages of sun drying in terms of overall control and uniformity for consistent end product (Tiwari, 2016). However, high drying temperatures may change some physical and chemical characteristics that influence the stability of the bioactive compounds. Several studies investigated the effects of different drying temperature on the bioactive compounds in food. According to Vega-Galvez *et al.* (2009), total phenolic compounds and vitamin C was reduced by almost 4-fold at air-drying temperature of 50, 60, 70, 80 and 90 °C compared to the fresh chilies. Gupta *et al*. (2002) also found that the chili pigment decreases as the drying temperature increased from 55 to 70 °C, but in spite of that the browning pigment increased. The reduction in the bioactive compounds is mainly due to chemical reaction within the chili with other compounds such as binding with proteins and oxidation (enzymatic or non-enzymatic) (Asano *et al*., 1982; Prigent *et al*., 2003). Drying time also influences the amount of compounds present, as reported by Wangcharoen and Morasuk (2009) whereby drying time reduced the total phenolic but increased antioxidant capacity. The same trend can also be seen with increased drying temperature for non-enzymatic browning as reported by Yang *et al*. (2018). In addition, capsaicin degradation was influenced more by the higher drying temperature as compared to drying time (Arifin and Djaeni, 2018). In this study, drying temperature of 60 and 90 °C were chosen based on the results of color from preliminary studies. Previous results showed that at temperatures higher than 90 °C, the chilies were rejected due to the black reddish color upon drying. In contrast, drying at 60 °C showed a bright red color which is acceptable by consumers. Based on the rationale that

postharvest preservation of bioactive compounds requires optimization of drying, the aim of this study was to investigate the effects of temperature and time using oven drying for postharvest preservation of bioactive compounds in 'Hua Ruea' chili fruit (*Capsicum annuum*).

2. Materials and Methods

2.1 Plant materials and sample preparation

The 'Hua Ruea' red chilies used in this experiment were harvested in December 2017 from Sisaket Province, Thailand. The ice-packed chilies were then transported to the King Mongkut's University of Technology Thonburi. Fruits were washed, destalked and air dried to remove excess water. Fresh samples were determined for color, weight, moisture content and frozen with liquid nitrogen kept at -20 °C for further chemical analysis.

2.2 Effect of time and temperature on physico-chemical properties

Chilies were dried in a hot-air oven (Memmert UE200) at 60 or 90 °C for 3, 6, 21 h and at constant weight (23 and 27 h for 60 and 90 °C, respectively), with end moisture content between 13% to 19%. Samples were collected after each drying time.

2.2.1 Moisture content (MC) and color parameters

Ten grams of each sample were dried in an oven at 105 °C for 24 h (AOAC, 2000). Surface color of the samples (a* and hue angle) were recorded as a mean of three determinations at three different locations of the samples using Minolta CR-400 colorimeter. Hue angle and total color differences were calculated using the formula:

$$\text{Hue} = \tan^{-1}\left(\frac{b^*}{a^*}\right)$$

$$\text{Total color differences } (\Delta E) = \sqrt{(L^* - L_o^*)^2 + (a^* - a_o^*)^2 + (b^* - b_o^*)^2}$$

2.2.2 Bioactive compound analysis

Metabolites from the chilies were extracted using the methods of Maokam *et al*. (2014) with some modifications. Sample preparation of 1 mL of extract solution was diluted with 0.8 mL of distilled water and used to inject into the conditioned C18 cartridge for further analysis.

2.2.2.1 Total phenolic content (TPC)

The TPC of extracts were determined by using Folin-Ciocalteau (FC) method of Arnnok *et al*. (2012). Briefly, 0.2 mL of extract was mixed with 2.6 mL distilled water, 2 mL sodium carbonate (7%) and 0.2 mL FC, and incubated for 90 min at room temperature (RT). The absorbance was measured at 745 nm against a prepared blank and results were expressed as dry weight basis (DW) as mg gallic acid equivalents (GAE) per g of sample.

2.2.2.2 Total flavonoid content (TFC)

The TFC of the extracts were determined by using aluminium chloride colorimetric method (Shaimaa *et al*. 2016). Briefly, 500 µL of extract was mixed with 2 mL of distilled water. After 5 min, 150 µL of 10% aluminium chloride was added into the mixture. Next, 2000 µL of sodium hydroxide (1 M) and 1200 µL of distilled water were added after 1 min. The mixture was incubated for 30 min and absorbance was measured at 510 nm against a prepared blank. The results were expressed as DW mg rutin equivalents (RUE) per g of sample.

2.2.2.3 Capsaicin (CAP) and dihydrocapsaicin (DHC) contents

CAP and DHC were analyzed by High Performance Liquid Chromatography (HPLC) using an Agilent 1200 series equipped with a diode array detector. The separation was performed using a RP-18 GP Mightysil column, 250 mm × 4.6 mm × 5 µm. The mobile phase consisted of 60% acetonitrile in distilled water and 0.5% formic acid in distilled water, flow rate was 1 mL/min with an isocratic flow at an ambient temperature for 20 min. The calibration curve was made with the standard marker compounds, CAP and DHC.

2.2.2.4 Capsaicinoid derivatives

The presence of capsaicinoids derivatives were evaluated and confirmed by gas chromatography-mass spectrometry (GC-MS) using a Agilent 7890A gas chromatograph equipped with a flame ionization detector (FID) and silica capillary column HP5-MS (30 m length; 0.25 mm i.d.; 0.25 µm film thickness). A constant flow (2.0 mL/min) of helium was used as carrier gas. The temperature of injector was 250 °C and that of the detector 280 °C. Analyses were performed in a gradient condition whereby initial temperature was 35 °C (constant for 10 min) at a rate of 3 °C/min to 95 °C, next to 270 °C (constant for 10 min) at a rate of 10 °C/min and finally at a rate of 3 °C/min to 300 °C and remained constant for 10 min. The extracts (5.0 µL) were then directly injected. The compounds were compared with Wiley and NIST at 80% quality match.

2.2.3 Antioxidant properties

2.2.3.1 Ferric-reducing antioxidant power (FRAP) assay

The FRAP assay was determined based on the method of Wangcharoen and Morasuk (2009) with some modifications. Briefly, FRAP reagent (950 µL) which consists of 0.02 M $FeCl_3$, 0.01 M 2,4,6-tripyridyl-S-triazine (TPTZ) and 300 M sodium acetate, mixed in the ratio of 10:1:1 were gently mixed in a water bath at 37 °C and extracts (50 µL) were added and incubated for 30 min. The absorbance was read against blank (methanol) at 593 nm and results were expressed as DW as mg Trolox equivalents (TE) per g of sample.

2.2.3.2 ABTS scavenging capacity assay

This assay was performed according to the method described by Sricharoen *et al*. (2015) with some modifications. The radical cation ABTS was produced by reaction of 7.4 mM ABTS and 2.6 mM potassium persulfate in a ratio of 2:1 (v/v). The mixture was then incubated in the dark room at RT for 14 h, then diluted with methanol to obtain an absorbance of 0.7–0.9 at 734 nm. Then, 900 µL of diluted ABTS was added to 100 µL of the extracts, homogenized and incubated for 30 min in the dark at RT. The absorbance was then measured at 734 nm results were expressed as DW mg gallic acid equivalents (GAE), per g of sample.

2.2.3.3 2,2-diphenyl-1-picrylhdrazyl (DPPH) radical scavenging capacity

The DPPH radical scavenging capacity of the extract was determined based on the method described by Arnao *et al*. (1990) and the results were expressed as DW mg gallic acid equivalents (GAE), per g of sample.

2.3 Statistical analysis

The experiment was arranged in randomized complete block design (RCBD) with three replications. All parameters were analyzed in triplicates. The data obtained were subjected to analysis of variance (ANOVA). The means comparisons were separated by using Duncan Multiple Range Test (DMRT) when F-test is significant.

3. Results and Discussion

3.1 Color analysis

The appearance of a produce, especially the color, is vital in influencing the buyer's preferences and decision. Hence, the skin color of chili is an important parameter to be measured. Figure 1(a) showed the a* values of peel color were between 24.53 to 44.09 and 9.23 to 44.09 for 60 °C and 90 °C, respectively. The values showed a declining trend with increasing time for both temperatures. Overall, the a* values of dried chilies at 60 °C were higher than 90 °C by 12%, 30%, 68% and 55% as the drying time increased, indicating that drying at 60 °C retained the red color in chilies better than 90 °C. As for the hue angle, the values were between 32 °C to 38 °C for both 60 °C and 90 °C (Figure 1(b)), which suggested that the color was orange-red (Akoy, 2014). Total color difference (ΔE) increased with drying time and temperature, ranging between 4.4 to 21.6 (60 °C) and 9.0 to 44.0 (90 °C). The drying temperature of 90 °C showed higher ΔE values than that of 60 °C which indicated that the color quality was reduced. This might be due to Maillard reaction, where the reaction between amino acid and sugar was potentially accelerated by high temperatures (Miranda *et al*., 2009), and melanoidins were produced (Wangcharoen and Morasuk, 2009).

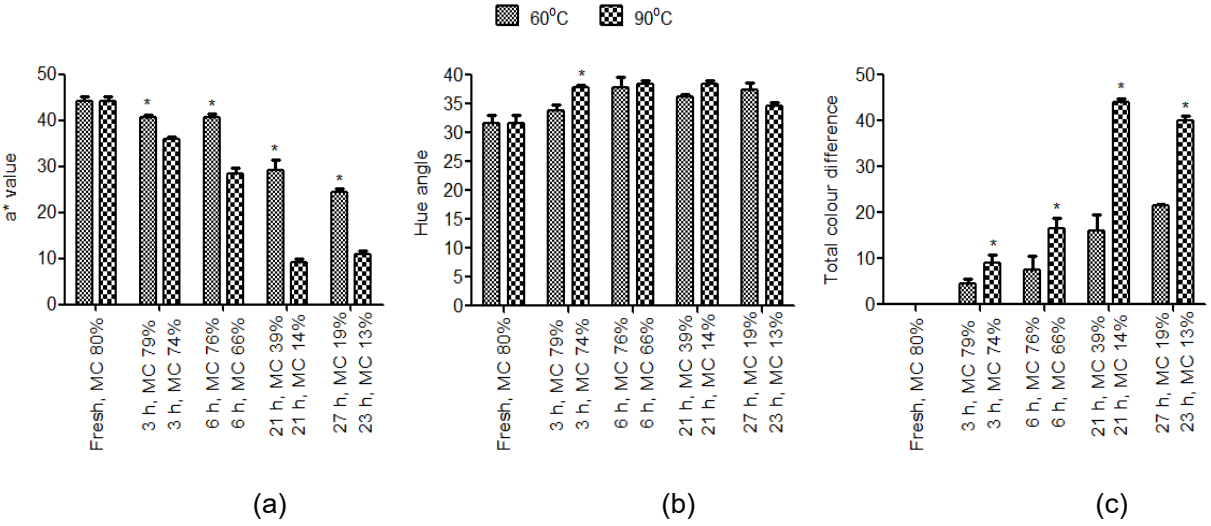

Figure 1 Effects of drying temperature (60°C and 90°C) and time on (a) a* value, (b) hue angle and (c) total color difference. Data are means of three replicates ± SE. *significant at $p < 0.05$ by DMRT.

3.2 Total polyphenol content

Polyphenols are important in our diet as they have antioxidant potential which proofed to be beneficial to our health. In this study, both TPC and TFC increased with drying time and temperature. The TPC of chilies at 6 h and at final drying time (23 and 27 h) was significantly higher at 60 °C than 90 °C by 28% and 22%, respectively (Figure 2(a). As for TFC, both temperatures showed the same trend as TPC but decreased at the final drying time (23 and 27 h) (Figure 2(b). Nonetheless, TFC in chilies dried at 60°C was significantly higher than 90 °C by 23% and 39% at 6 h and 21 h, respectively. Increment of TPC by 2-fold at both drying temperatures were consistent with studies done by Reis *et al*. (2013) and Vega-Gálvez *et al*. (2009). Both studies found that TPC of chilies increased at higher temperatures in contrast to the fresh chilies by 3- and 9-fold. When chilies are subjected to drying at a high temperature, it will undergo several processes such as oxidation (enzymatic or non-enzymatic) and unbinding of polyphenols from the cell wall. During drying at high temperatures, the breakage of ester linkage between phenolic moieties and cell wall causes the increase of free phenolics within the tissue matrix (Liyana-Pathirana and Shahidi, 2006; Yu *et al*., 2001). However, enzymatic oxidation of phenolics occurs when moisture, oxygen and deteriorative enzymes are present, potentially causing TPC reduction in the tissue matrix (Gonçalves *et al*., 2015; Zhou *et al*., 2016). In addition, thermal degradation also produces newly formed products such as protocatechuic acid from rutin and 2,4,6-trihydroxybenzoic acid from quercetin which might have contributed to the increase in TFC in this study (Chaaban *et al*., 2016).

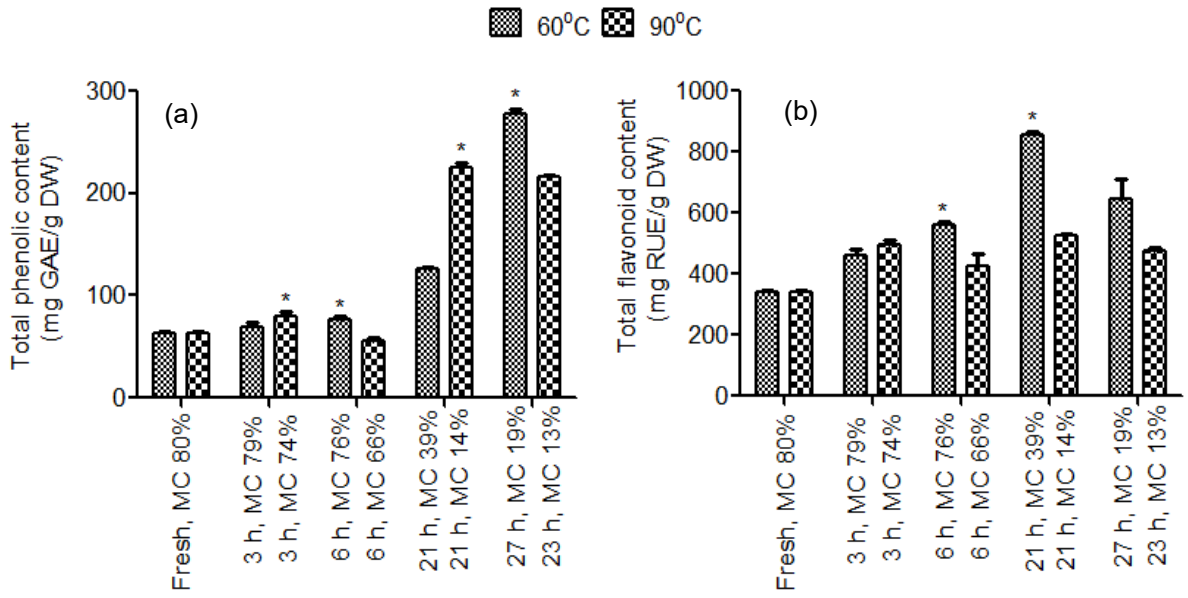

Figure 2 Effects of drying temperature (60 °C and 90 °C) and time on (a) total phenolic content and (b) total flavonoid content. Data are means of three replicates ± SE. *significant at $p < 0.05$ by DMRT.

3.3 Capsaicin (CAP) and dihydrocapsaicin (DHC) contents

Both CAP (Figure 3(a)) and DHC (Figure 3(b)) were at a relatively stable levels for both drying temperatures when dried between 3 and 6 h, but increased by 2-fold at longer drying time (21 h and final (23 and 27 h)). The CAP and DHC contents were significantly higher by 23% and 17%, respectively, at 60 °C as compared to 90°C during final drying time (23 and 27 h). The 2-fold increase in both CAP and DHC at the final drying time were consistent with study reported by Popelka *et al*. (2017) in chilies, whereby the capsaicin content increased by 4 to 10 times in dried chilies versus fresh chilies. The author stated that during thermal process, the matrix was dehydrated which then improved overall content on a dry matter basis and the extractability of the capsaicinoids by cell disruption. Additionally, the increase in capsaicinoid contents is also potentially due to the inactivation of degradative enzymes such as polyphenol oxidase (PPO) when subjected to high temperature (Wang *et al*., 2017). In addition, peroxidase (POD) which is situated in the placental cell layers in chili plays a vital role in the oxidation rate of capsaicinoids. Although peroxidase can be inactivated by means of heating, but reactivation could occur upon removal of the heat source. Nevertheless, Thongsook and Barrett (2005) found that upon heat treatment at 90 °C and incubated at room temperature, broccoli POD had a higher reactivation percentage (50–70%) in comparison with heat treatment at 67.5 °C. Moreover, low heating temperature also prevented the reactivation of broccoli POD due to longer heating time to achieve the desired temperature or moisture content. The reactivation of POD is dependent on the time taken to reach the desired

treatment temperature during heat treatment and also how long the enzymes were exposed to the particular temperature (Adam, 1978; Schwimmer, 1944). These factors justified the increased in both CAP and DHC when subjected to drying at 60 °C in contrast to 90 °C.

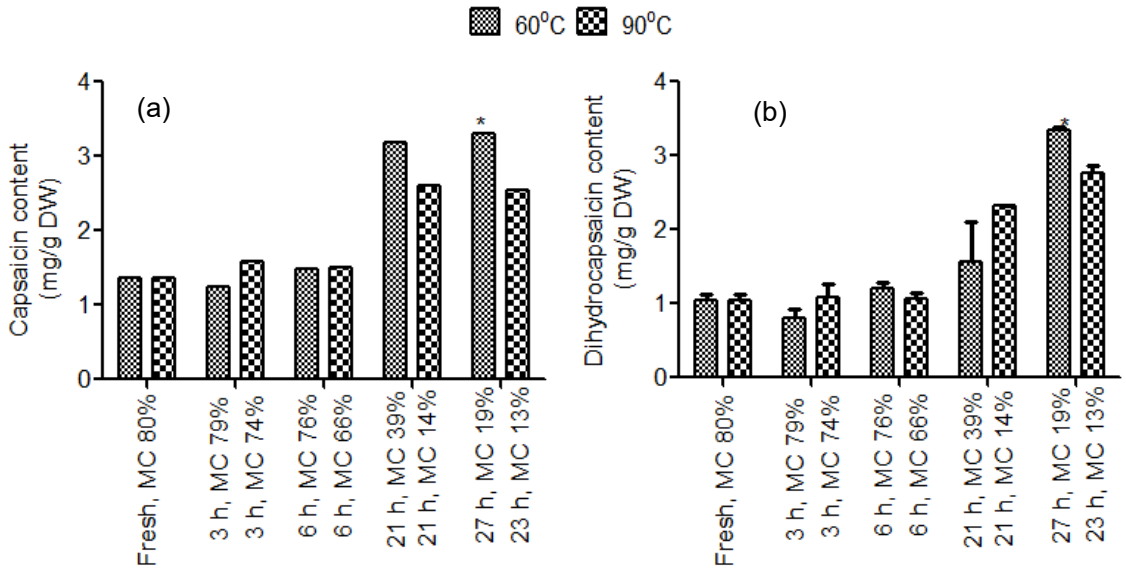

Figure 3 Effects of drying temperature (60 °C and 90 °C) and time on (a) capsaicin and (b) dihydrocapsaicin content. Data are means of three replicates ± SE. *significant at $p < 0.05$ by DMRT.

3.4 Capsaicinoid derivatives

Capsaicinoids derivatives; nonivamide, nordihydrocapsaicin, CAP, DHC and homodihydrocapsaicin for confirmation were identified by GC-MS. The ion chromatograms of CAP standard and chili dried at 60 °C and 90 °C were shown in Figure 4. In total, 28 peaks were found in the extracted samples, however, only five peaks were of interest which are related to capsaicinoids as can be seen in Table 1.

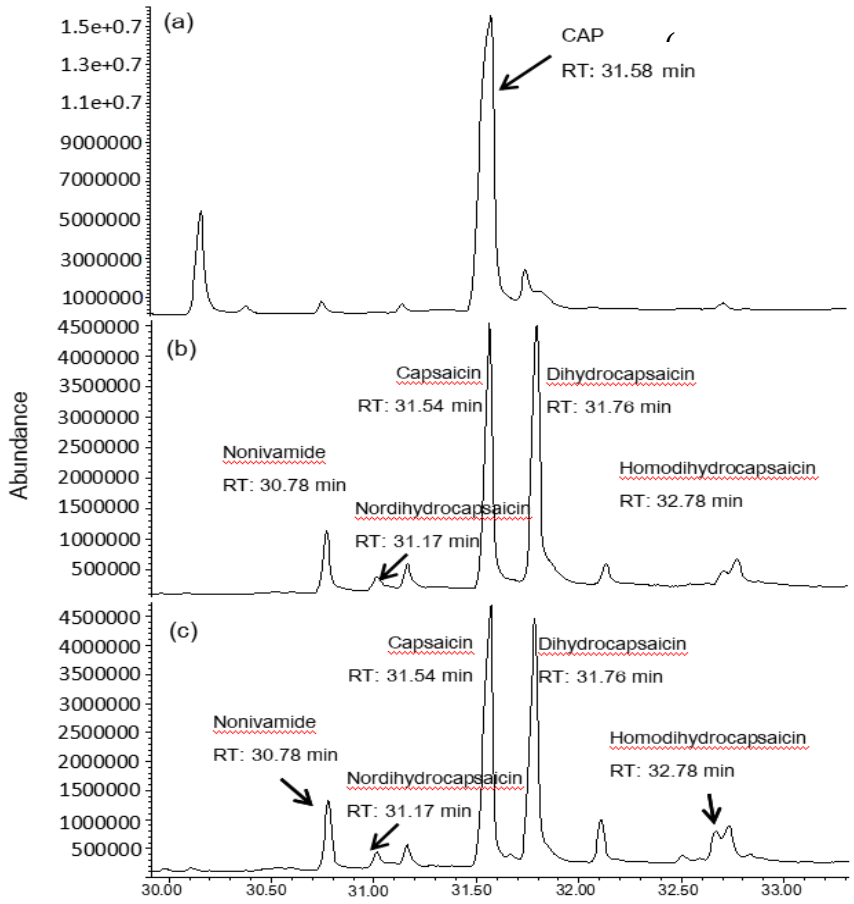

Figure 4 Ion chromatogram of (a) capsaicin standard, (b) extract at 60 °C and (c) extract at 90 °C at 6 h.

The abundance of the five capsaicinoids were summarized in Table 1 and showed an increasing trend with time, with the highest amount observed at 60 °C, which corroborate with studies by Arifin and Djaeni (2018). This is potentially due to the structural changes in CAP to vanillyl nonanoate during the drying process (Katritzky *et al*., 2003). However, homodihydrocapsaicin was not detected at 60 °C in fresh and at drying time of 3 and 6 h, but was detected in 90 °C.

Table 1 The contents of five capsaicinoids found in chili samples with drying temperature of 60°C and 90°C, expressed in peak

Name of compounds	Molecular weight (g/mol)	Retention time (minutes)	Peak area (× 10^8)								
			60°C					90°C			
			Fresh, MC80%	3 h, MC 79%	6 h, MC 76%	21 h, MC 38%	27 h, MC 19%	3 h, MC 74%	6 h, MC 66%	21 h, MC 14%	23 h, MC 13%
Nonivamide	293.40	30.78	13.73	10.74	12.54	13.59	14.57	10.71	2.05	6.34	8.92
Nordihydro capsaicin	293.41	31.17	10.75	13.30	12.39	11.57	15.74	13.33	1.35	13.79	6.67
Capsaicin	305.41	31.54	6.98	13.59	15.80	13.65	24.13	16.23	7.22	20.37	17.61
Dihydro capsaicin	307.43	31.76	9.37	20.61	18.60	20.92	25.03	19.09	5.41	23.31	16.90
Homodihydrocapsaicin	321.46	32.78	N.D.	N.D.	N.D.	3.56	53.04	16.58	6.36	42.53	24.62

Note: N.D. = not detectable

3.5 Antioxidant activity

The FRAP and ABTS activity showed an increase with time for both temperatures (Figure 5(a), (b)). Both FRAP and ABTS at 90 °C showed a significantly higher activity than at 60 °C by 37% and 1.4% at final drying time (23 and 27 h), respectively. The result of ABTS corroborate with studies by Chaaban *et al*. (2016), whereby ABTS increased with temperature and time, either higher or at a constant level with the native flavonoids. In contrast, DPPH radical scavenging activity (Figure 5c) showed an opposite trend. Both temperatures did not exhibit significant differences in all times except at final drying time (23 and 27 h), with 60 °C showing a higher activity by 19%. The mechanism of DPPH is based on Single Electron Transfer (SET), whereas ABTS is Hydrogen Atom Transfer (HAT) (Badarinath *et al*., 2010). Furthermore, DPPH is used to determine antioxidant activity in natural plant extracts while ABTS can determine hydrophilic and lipophilic antioxidants (Shalaby and Shanab, 2013). In this study, TPC, CAP and DHC content increased with drying time which explains the increment in FRAP and ABTS activity. Both CAP and DHC is lipophilic and potentially caused ABTS activity to increase. Antioxidants such as TPC, CAP and DHC are capable of donating a single electron or hydrogen atom for reduction, thus, the increase in FRAP and ABTS activity. As for the reduction in DPPH radical scavenging activity, this potentially reflects the presence of less oxidized phenolic and flavonoid moieties available to scavenge at higher temperature (Réblova, 2012).

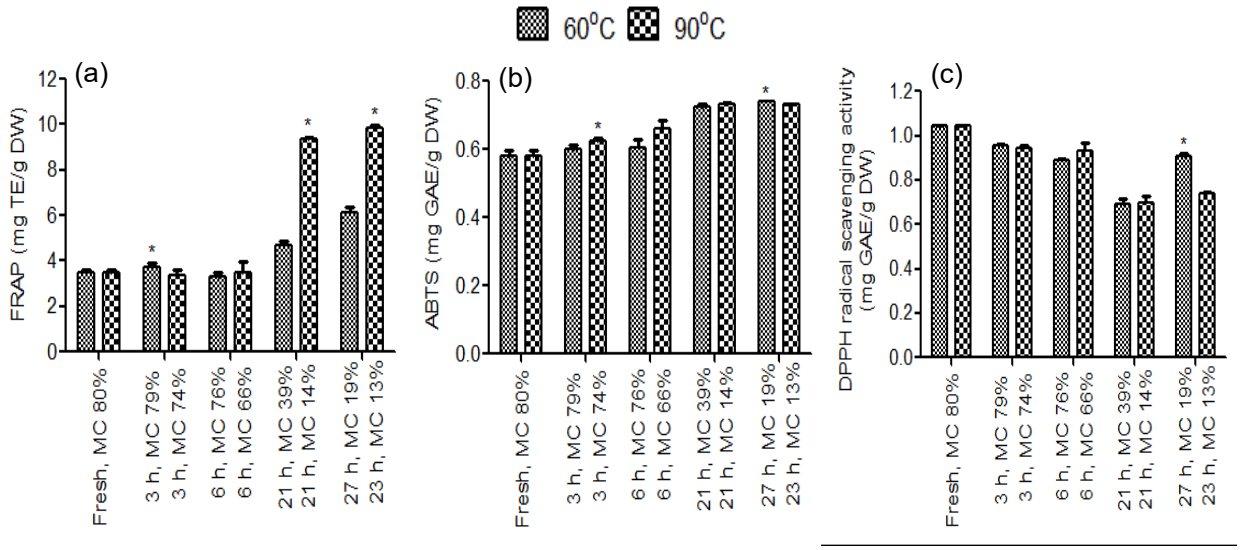

Figure 5 Effects of drying temperature (60 °C and 90 °C) and time on (a) FRAP, (b) ABTS and (c) DPPH radical scavenging activity. Data are means of three replicates ± SE. *significant at $p < 0.05$ by DMRT.

4. Conclusion

In this study, increasing drying temperature from 60 to 90 °C reduced the amount of polyphenols and bioactive compounds in dried 'Hua Ruea' chilies. In addition, drying at 90 °C also reduced the quality of dried chilies in term of color. However, as drying time was prolonged, the amount of polyphenols and bioactive compounds increased. Overall total polyphenols, antioxidant activities and bioactive compounds were all higher in dried samples as compared to the fresh samples. In conclusion, drying temperature of 60 °C for 27 h and 90 °C for 23 h was most suitable for chilies in order to maintain their physical quality and bioactive compounds. Further research is needed to identify bioactive compounds using Liquid chromatography-mass spectrometry (LC-MS).

Acknowledgements

We would like to thank Petch Pra Jom Klao-Ph.D scholarship for financial support and Postharvest Technology Innovation Center, Office of the Higher Education Commission, Thailand for facilities support.

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