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Research Article

Antioxidant capacity changes in Chili Spur Pepper (*Capsicum annuum* Linn. var. acuminatum Fingerh.) during drying process

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Abstract: The antioxidant capacity of chili spur pepper (*Capsicum annuum* Linn. var. acuminatum Fingerh.) during the drying process at 70, 100, and 121°C, was analyzed by 3 different methods, including Ferric reducing antioxidant power (FRAP) assay, Improved ABTS radical cation decolourization assay and DPPH free radical scavenging activity, together with the analysis of total phenolic content and browning pigment formation. It was found that total phenolic content might decrease at the beginning of the drying process, but would be increased when browning pigments were developed. The decrease of total phenolic content affected the reducing power (FRAP), however, there was no effect on free radical scavenging activities (ABTS and DPPH). This meant the reducing power of chili spur pepper was directly influenced by phenolic compounds both in chili spur pepper and those developed from the browning reaction, whilst its free radical scavenging activities were not only affected by phenolic compounds but also influenced by other compounds developed during the drying process. In addition, it was found that the antioxidant capacity of dried chili spur pepper was dependent on the degree of browning and the drying temperature. The reducing power of dried products might be lower than fresh ones if they were dried at 70°C, but their free radical scavenging activities would be higher than the fresh product.

Keywords: food, FRAP, ABTS, DPPH, antioxidant capacity, dried chili, phenolic content, browning reaction products

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Introduction

Chili spur pepper (*Capsicum annuum* Linn. var. acuminatum Fingerh.) is one of two chili types widely used in Thailand. It has a finger shape of 4 to 6" in length, with a heat of 30,000 - 60,000 Scoville units and is known in Thai as "*Prik Chee Fah*", from the upright growth habit of the fruit. Chilies are high in vitamins A and C, but low in calories and sodium and contain amounts of potassium, magnesium and folic acid. It is the capsaicinoids, (vanillylamides of monocarboxyl acids) which are responsible for the pungency or bite that are considered as active compounds in chilies. Capsaicin accounts for about 50 to 70% of total capsaicinoids. It provides the bite but has no odour. Other components contributing to the pungency are 20 to 25% dihydrocapsaicin which, together with capsaicin, provides fiery notes from mid-palate to throat, 7% nordihydrocapsaicin which is fruity and sweet and has the least burning sensation and 1% homocapsaicin and 1% homodihydrocapsaicin which give a numbing and prolonged burn (Uhl, 2000).

Chilies have been recognized by many cultures around the world for their medicinal qualities. When chilies are eaten, capsaisin stimulates the release of endorphins, which give a pleasurable feeling. Moreover, chilies are believed to increase circulation, relieve rheumatic pain, treat mouth sores and infected wounds, reduce blood clots and aid digestion by stimulating saliva and gastric juice flow (Uhl, 2000). Capsaicin has been tested by many investigators for its effects on experimental carcinogenesis and mutagenesis. There is no solid evidence showing that chili and capsaicin are carcinogenic in humans. In contract, many studies reveal substantial antioxidant, antigenotoxic and anticarcinogenic effects of chili extracts and capsaicin (Surh *et al*., 1998; Prasad *et al*., 2004). As a result, capsaicin is suggested to be an important dietary phytochemical with antioxidant and chemopreventive activities.

Antioxidant values and total phenolic content of chili were reported in some previous papers. Pellegini *et al*. (2003) showed that FRAP and ABTS values were 23.54 mmol Fe^{2+} equivalent per kg of fresh weight and 7.62 mmol Trolox equivalent per kg of fresh weight, respectively. Wangcharoen and Morasuk (2007) showed that FRAP, ABTS and DPPH values of chili spur pepper were 0.62 – 1.71, 1.32 – 3.82 and 0.36 – 1.23 mg Vitamin C equivalent per gram of fresh weight, respectively and total phenolic content was 0.85 – 1.81 mg Gallic acid equivalent per gram of fresh weight .

Both fresh and dried chilies are used as food ingredients or seasoning. They provide not only heat but also flavour, colour and visual appeal to food. During the heating process, non-enzymatic browning reaction, including Maillard reaction, caramelisation and chemical oxidation of phenols occur. The antioxidant activity of Maillard reaction (Yanagimoto *et al*., 2002; Yilmaz and Toledo, 2005; Osada and Shibamoto, 2006) and caramelisation products (Benjakul *et al.*, 2005) have been reported. In this paper we report on the antioxidant capacity of chili spur pepper during drying at 70, 100, and 121°C by three different methods, including Ferric reducing antioxidant power (FRAP) assay, Improved ABTS radical cation decolorization assay and DPPH free radical scavenging activity, together with total phenolic content and browning pigment

formation, absorbance at 420 nm. The correlations of % antioxidant capacity, total phenolic content and absorbance at 420 nm were analyzed.

Materials and Methods

Chemicals and instruments

TPTZ (2,4,6-tripyridyl-s-triazine), DPPH (2,2-diphenyl-1-picrylhydrazyl) [Sigma], ABTS (2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)), Folin-Ciocalteu phenol reagent, ferric chloride, gallic acid, glacial acetic acid, hydrochloric acid, sodium acetate, potassium persulphate, sodium carbonate, vitamin C [Fluka] were of analytical grade.

Sample extraction

Fresh green and red chili spur peppers (*Capsicum annuum* Linn. var. acuminatum Fingerh.), were purchased from fresh food markets. The edible portions of the fresh samples were homogenized using a blender. Two grams of blended fresh sample were transferred into a 25 cm x 150 cm tube and were then dried at 70, 100 and 121°C. Sample tubes were collected 10 times during the drying period. Sample extraction method of Leong and Shui (2002) was modified. Ten ml of a solvent (60% (v/v) of 95% ethanol) was added to the collected sample tube. The extraction was done by using a vortex mixer for 1 min. The mixture was filtered through a Whatman filter paper No 1. The filtrate was adjusted to 10 ml by deionized water and then was used for all assays including FRAP, ABTS, DPPH, total phenolic content and formation of browning pigments (absorbance at 420 nm). Extracts of blended fresh green and red chili spur pepper were prepared for comparison and the weight change during the drying process was also recorded.

Ferric reducing antioxidant power (FRAP) assay

FRAP, a method for measuring total reducing power of electron donating substances, was assessed according to Benzie and Strain (1999). Briefly, 6 ml of working FRAP reagent (0.1 M acetate buffer: 0.02 M $FeCl_3$: 0.01 M TPTZ = 10 : 1 : 1) prepared daily was mixed with 20 µl of extract sample. The absorbance at 593 nm was recorded after a 30-min incubation at 37°C. Absorbance increases were calculated as FRAP values for comparison with the fresh sample. Vitamin C (0 - 15 µg) was used as a standard.

ABTS radical cation decolorization (ABTS) assay

The method, based on the ability of antioxidant molecules to quench the long-lived ABTS radical cation ($ABTS^{\bullet+}$), of Re *et al*. (1999) was modified. The $ABTS^{\bullet+}$ was produced by reacting 7 mM ABTS stock solution with 2.45 mM postassium persulphate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12 - 16 hours before use. The $ABTS^{\bullet+}$ solution was diluted with deionized water and

95% ethanol (1 : 1) to an absorbance of 0.70 (± 0.02) at 734 nm. Twenty to one hundred µl of the extract was mixed with 6 ml of diluted $ABTS^{\bullet+}$ solution. The decrease of absorbance was recorded at 1 min after mixing. Absorbance decreases were calculated as ABTS values for comparison with the fresh sample. Vitamin C (0 - 20 µg) was used as the standard.

DPPH free radical scavenging activity (DPPH)

The method of Brand-Williams *et al.* (1995), based on the reduction of DPPH radical solution in the presence of hydrogen donating antioxidants, was used with some modifications. 0.8 mM DPPH radical solution in 95% ethanol was prepared. One hundred to four hundred µl of the extract was diluted to 5.4 ml using deionized water and 95% ethanol (1 : 1) before 0.6 ml DPPH radical solution was added and shaken vigorously. The decrease of absorbance was recorded at 1 min after mixing. Absorbance decreases were calculated as DPPH values for comparison with the fresh samples. Vitamin C (0 - 40 µg) was used as the standard.

Total phenolic content (TPC)

The Folin-ciocalteau micro method of Waterhouse (n.d.) was used. Sixty µl of the extract was diluted with deionized water to 4.8 ml and 300 µl Folin-ciocalteau reagent was added and shaken. After 8 min, 900 µl of a solution of 20% sodium carbonate was added and mixed. The solution was left at 40°C for 30 min before reading the absorbance at 765 nm. Absorbance increases were calculated as total phenolic content for comparison with the fresh sample. Gallic acid (0 - 50 µg) was used as the standard.

- **Values of FRAP, ABTS, DPPH, and TPC** (mg standard equivalent per gram of dried weight)

$$Value.of.FRAP, ABTS, DPPH, and.TPC = \frac{\frac{[SA - BA]}{Slope} \times [10/U]}{[2][1 - MC][1{,}000]}$$

When: SA = Sample absorbance for FRAP value and TPC or
absorbance decrease of sample for ABTS and DPPH values
BA = Blank (no extract) absorbance for FRAP value and TPC or
absorbance decrease of blank for ABTS and DPPH values
(extract was substituted by deionized water for blank)
Slope = Slope of standard curve
[10/U] = Total volume of extract (10 ml) / Used volume of extract (ml)
[2] = Weight of blended sample (g)
MC = % moisture content of sample / 100
[1,000] = Factor for changing µg to mg.

- **Change of antioxidant capacity, TPC, and weight (%)**

$$Change.of.antioxidant.capacity, TPC, and.weight = \frac{Value.of.collected.sample}{Value.of.blended.fresh.sample} \times 100$$

Formation of browning pigment

The formation of browning pigments for all extracts were determined by the official method (ADOGA, 1976), the measurement of absorbance at 420 nm for each diluted extract.

Statistical analysis

The experiment was repeated three times and was conducted on separate marketing purchases (triple measurements for each marketing purchase). The bivariate correlations between percentage of antioxidant capacity, total phenolic content and absorbance at 420 nm were analyzed.

Results and Discussion

Green chili spur pepper

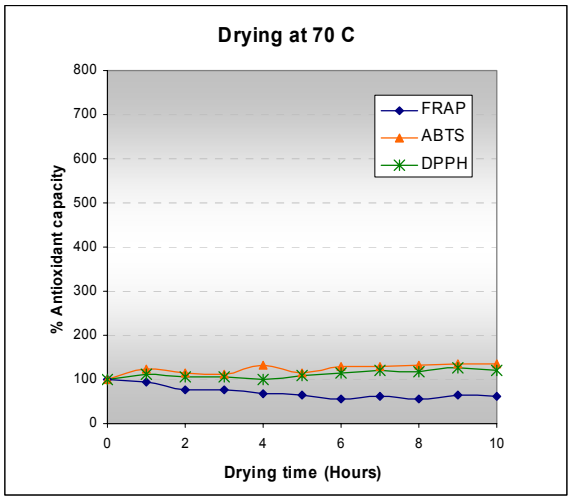
FRAP, ABTS, and DPPH values, and total phenolic content of fresh green chili spur pepper were 6.71 $\pm$ 1.02, 14.51 $\pm$ 0.53, and 3.91 $\pm$ 0.43 mg Vitamin C equivalent per gram of dried weight and 9.33 $\pm$ 1.58 mg Gallic acid equivalent per gram of dried weight, respectively. The different value of each antioxidant assay from the same sample was also found in previous papers (Wang *et al*., 1998; Pellegrini *et al*., 2003; Yang *et al*., 2006), and it could be caused by the unique mechanism of each assay and variations in antioxidant capacity and mechanisms of compounds in natural samples.

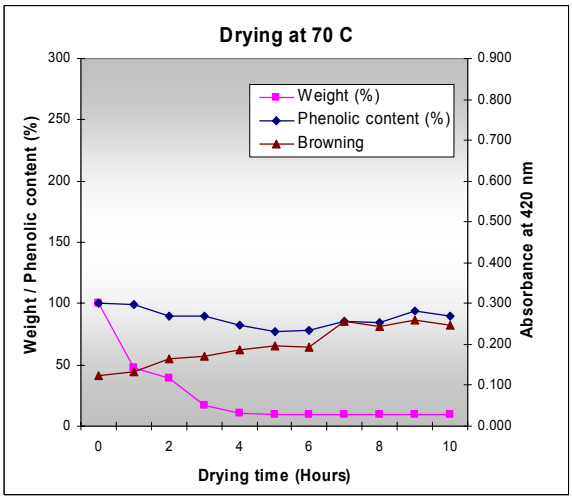
During the drying process at 70°C, the FRAP value decreased when drying time was increased, however, this seemed to increase after 6 hours. This trend was similar to the change of total phenolic content ($r = 0.786$). The change within the first 6 hours could be explained by the chemical oxidation of phenolic compounds during heat treatment (Manzocco *et al*., 2001), and after that some phenolic compounds would be developed from non-enzymatic browning reaction. This could be confirmed by the good correlation between total phenolic content and absorbance at 420 nm after 6 hours ($r = 0.835$). ABTS and DPPH values increased during drying and were similar to the change of absorbance at 420 nm and the formation of browning pigments ($r = 0.787$ and 0.804, respectively). It is possible that there were some compounds, both phenolic compounds and others with free radical scavenging activities, developed from non-enzymatic browning reaction (Yanagimoto *et al*., 2002; Yilmaz and Toledo, 2005; Osada and Shibamoto, 2006), as well as other reactions during drying.

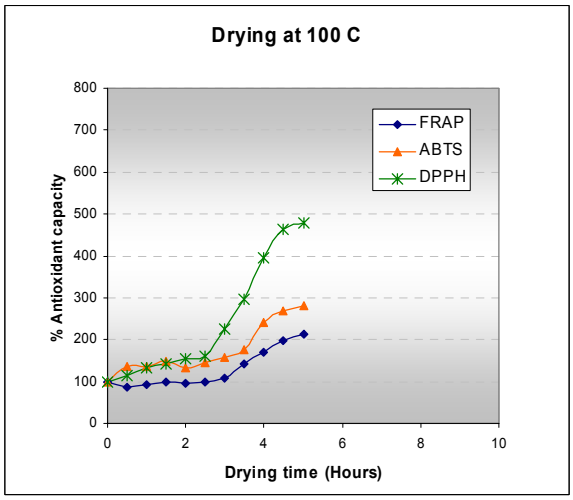
Results of drying at 100 and 121°C were similar to the those at 70°C, but the decreasing period of FRAP value and total phenolic content was much shorter, and the

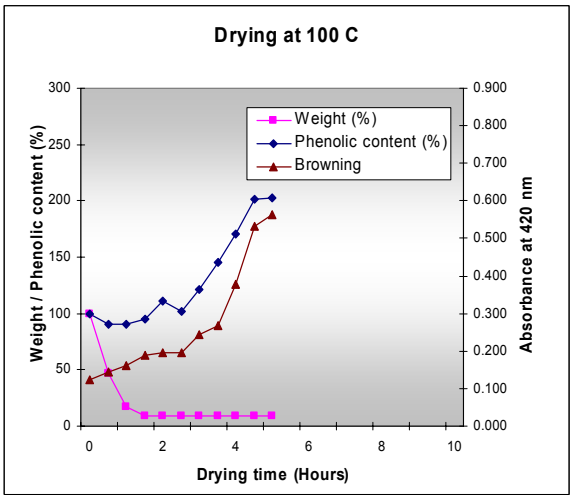
increase of ABTS and DPPH values, and absorbance at 420 nm, was faster and higher. All correlations between % antioxidant capacity, total phenolic content, and absorbance at 420 nm were highly significant ($r \geq 0.957$) and it was found that the antioxidant capacity

of dried brown chili spur pepper was higher than that of fresh green samples, depending on the degree of browning (Figure 1).


Drying at 70 C
% Antioxidant capacity
Drying time (Hours)
FRAP
ABTS
DPPH


Drying at 70 C
Weight / Phenolic content (%)
Absorbance at 420 nm
Drying time (Hours)
Weight (%)
Phenolic content (%)
Browning


Drying at 100 C
% Antioxidant capacity
Drying time (Hours)
FRAP
ABTS
DPPH


Drying at 100 C
Weight / Phenolic content (%)
Absorbance at 420 nm
Drying time (Hours)
Weight (%)
Phenolic content (%)
Browning

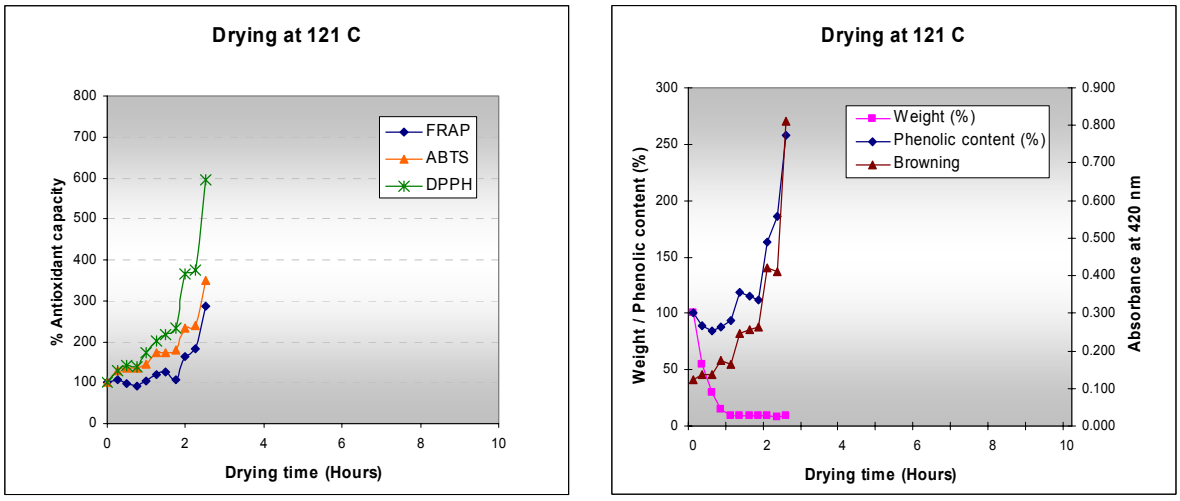


Figure 1. Antioxidant capacity, phenolic content, absorbance at 420 nm and weight of green chili spur pepper during drying at 70, 100 and 121°C.

Red chili spur pepper

FRAP, ABTS, DPPH values, and total phenolic content of fresh red chili spur pepper were 10.19 $\pm$ 1.61, 24.31 $\pm$ 2.34, and 7.85 $\pm$ 0.99 mg Vitamin C equivalent per gram of dried weight, and 11.57 $\pm$ 1.17 mg Gallic acid equivalent per gram of dried weight, respectively. These values were significantly higher than those found with the green peppers ($p \leq 0.05$).

Otherwise, almost all of the red chili spur pepper results did not differ greatly from the green ones, except that there was a decrease of absorbance at 420 nm during the beginning of the drying process (Figure 2). The reason for this could be that some pigments which absorbed 420 nm light in red chili spur pepper could be decomposed by heating and some decomposed pigments might be phenolic compounds because the correlation between total phenolic content and absorbance at 420 nm at the beginning of drying at 70°C was found ($r = 0.781$). This decomposition affected the FRAP value, considered by the correlation between FRAP value and total phenolic content at the beginning of drying at 70°C ($r = 0.838$), but it did not influence the ABTS and DPPH values because both values increased once the drying process commenced. These results showed that the FRAP value, or reducing power, was affected by phenolic compounds, but ABTS and DPPH values, free radical scavenging compounds, were affected by both phenolic compounds and others developed during drying.

For drying at 100 and 121°C, all correlations between % antioxidant capacity, total phenolic content and absorbance at 420 nm were also highly significant ($r \geq 0.958$). The antioxidant capacity of dried brown chili spur pepper was also higher than the fresh red one, depending on the degree of browning (Figure 2) and it was higher than the results obtained with the green chili spur pepper (Figures 1 and 2).

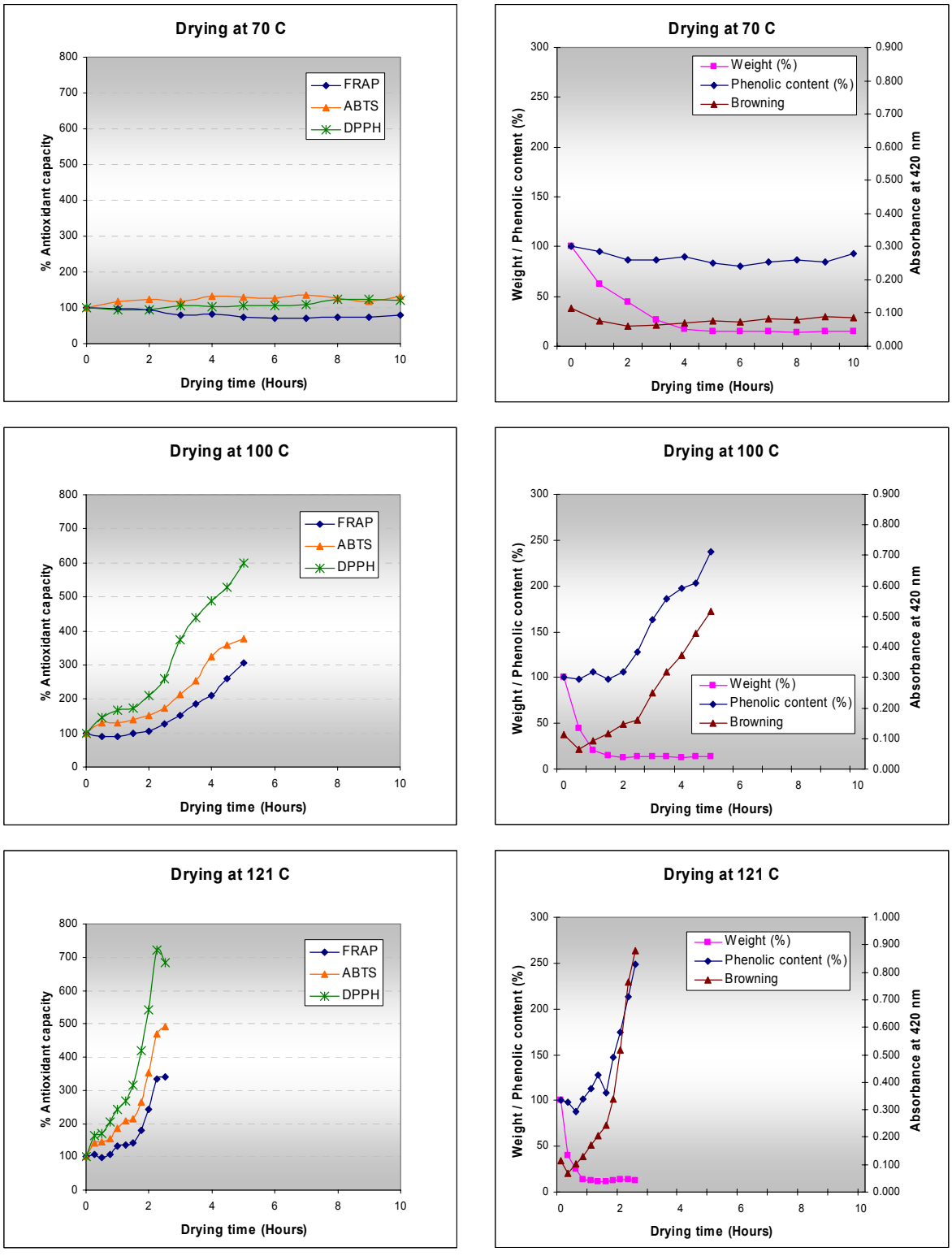


Figure 2. Antioxidant capacity, phenolic content, absorbance at 420 nm and weight of red chili spur pepper during drying at 70, 100 and 121°C.

Conclusion

Fresh and dried chili spur peppers are normally used as ingredients or seasonings in many Thai recipes. Phenolic compounds in fresh chili spur pepper, as well as some browning reaction products developed during drying, could be a good source of antioxidant compounds. Drying at 70°C might decrease the reducing power (FRAP value) of chili spur pepper, while drying at 100 and 121°C did not. Drying at all 3 temperatures in this study (70, 100, and 121°C) could increase free radical scavenging activities (ABTS and DPPH values) of chili spur pepper. The antioxidant capacity of dried chili spur pepper was higher than that found for fresh samples, depending on the degree of browning and the drying temperature.

Acknowledgment

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