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Full Paper

Antioxidant activity changes during hot-air drying of *Moringa oleifera* leaves

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Abstract: Dried *Moringa oleifera* leaf powder in capsule is now a popular food supplement in Thailand. To investigate its health benefits, antioxidant activities of *M. oleifera* leaves (3 varieties: Num Phrae, Ang Thong and PKM1) during drying in hot-air oven at 50°C and 100°C were studied by 3 different methods, viz. ferric reducing antioxidant power (FRAP), DPPH free radical scavenging activity and ABTS radical cation decolourisation, together with the determination of total phenolic content and browning pigment formation. It was found that the antioxidant activities and total phenolic content tend to decrease in the early stage of drying and then increase in the later stage, and that the dried leaves still have at least 60% of antioxidant activities compared to fresh leaves.

Key words: *Moringa oleifera*, antioxidant activity, total phenolic content, browning pigment formation, hot-air drying

INTRODUCTION

Moringa oleifera, Lam. Syn. *Moringa pterygosperma* Gaertn. (family Moringaceae) is commonly known as drumstick tree or horseradish tree and widely cultivated in the tropics and subtopics of Asia and Africa [1]. Almost all the parts of this plant are used for various ailments in the indigenous medicine of South Asia [2]. Its young leaves and green pods are common vegetables in some countries such as India [3] and Thailand, and the dried leaf powder has now become popular among many Thais.

M. oleifera leaves are highly nutritious. In 100 g dry matter, they contain 29±6 g of protein, 28±6 mg of iron, 1,924±288 mg of calcium, 15,620±6,475 IU of vitamin A and 773±91 mg of vitamin C. This is at least twice the protein in milk and half the protein in egg, and has more iron than in beef, more calcium than in milk, equal vitamin A to carrot and more vitamin C than in orange

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[5]. In addition, the leaves of this plant are reported to have various biological activities such as diuretic [6], immune boosting and hypotensive [7], antiinflammatory [8-10], antiulcer [11], antihepatotoxic [10], antitumour [12], thyroid hormone status regulating [13], hypocholesterolaemic [14-15], radioprotective [16], hypolipidaemic [17], antiatherosclerotic [17], antidiabetic [18] and antioxidant [4, 17, 19-23].

The free radical accumulation or oxidative stress in the human body plays multiple important roles in tissue damage and loss of function in a number of tissues and organs [24], which consequently may adversely affect the immune functions and contribute to pathological conditions and chronic human diseases such as aging, carcinogenesis, diabetes, gastric ulcer and rheumatic joint inflammation [25-26]. Phenolic compounds in plants have been shown to be effective antioxidant constituents and many polyphenolics have a more powerful antioxidant effect than that of vitamin E [27].

M. oleifera leaves with a high content of phenolics and flavonoids [4, 21, 28] show greater antioxidant activity, anti-radical power, reducing power, inhibition of lipid peroxidation, protein oxidation and OH·-induced deoxyribose degradation, and scavenging power of superoxide anions and nitric oxide radicals than do its fruits and seeds [4, 21]. The antioxidant activity of *M. oleifera* leaf extract was found to be higher than that of standard vitamin E [20, 21] and remain unaffected at pH 4 and pH 9 in the dark at 5°C and 25°C respectively for 15 days, although the activity significantly decreases when heated at 100°C for 15 min [20]. In addition, the activity can vary with such factors as variety [4], season and production location [19] and stage of maturity [22].

Dried *M. oleifera* leaf powder in capsules has become a popular food supplement for many people in Thailand [4]. However, heat treatment may affect the stability of *M. oleifera* extracts [20]. This study aims to follow changes in the antioxidant activity of *M. oleifera* leaves during hot-air drying, a conventional process for their drying. Three varieties of *M. oleifera* (Num Phrae, Ang Thong and PKM1) were studied, and the total phenolic content (TPC) and browning pigment formation, which may affect the antioxidant activities, were also determined.

MATERIALS AND METHODS

Chemicals and Apparatus

2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tripyridyl-s-triazine (TPTZ), 2,2'-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), ferric chloride, Folin-Ciocalteu phenol reagent, gallic acid, glacial acetic acid, hydrochloric acid, potassium persulphate, sodium acetate, sodium carbonate and vitamin C were obtained from Sigma-Aldrich and all of them were of analytical grade.

A spectronic 20D+ spectrophotometer (Milton Roy) was used for all assays.

Preparation of Leaf Extract

Fresh leaves of *M. oleifera* (variety: Num Phrae, Ang Thong and PKM1) grown in Chiang Mai province were harvested in April–June 2010. Num Phrae and Ang Thong are local varieties from Amphoe Mueang, Phrae province and Amphoe Pamok, Ang Thong province respectively, while PKM1 is an Indian variety available from Chaipattana Foundation in Bangkok.

Fresh leaves (71-78% moisture content) were macerated using a blender. Two grams of the blended leaves were transferred into each 25x150 cm sample tube and dried in a hot-air oven at 50°C for 12.5 hr or at 100°C for 2.5 hr. Sampling of the leaves was done before drying and then every 2.5 hr for leaves heated at 50°C and every 0.5 hr for leaves heated at 100°C. To prepare a crude extract,

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10 mL of deionised water was added to the collected sample tube and the mixture was shaken by a vortex mixer for 60 seconds [29] and filtered. The filtrate was adjusted to 10 mL and the resulting extract solution was used for the determination of antioxidant activities, total phenolic content and browning pigment formation.

Ferric Reducing Antioxidant Power (FRAP) Assay

The total reducing power of electron donating substances was determined according to Benzie and Strain [30]. Briefly, 6 mL of FRAP reagent (0.1M acetate buffer : 0.02M $FeCl_3$: 0.01M TPTZ = 10:1:1) prepared daily were mixed with 20 µL of the extract solution. The absorbance at 593 nm was recorded after a 30-min. incubation at 37°C. FRAP values were obtained by comparison with a standard curve created using vitamin C (0-15 µg) and reported as mg vitamin C equivalent per gram (dried leaves).

DPPH Free Radical Scavenging Activity

The method of Brand-Williams et al. [31] based on the reduction of DPPH radical solution in the presence of hydrogen donating antioxidants was used with some modifications. Twenty µL of the extract solution were diluted with deionised water : 95% ethanol (1:1) to 5.4 mL and then 0.6 mL of DPPH radical solution (0.8 mM in 95% ethanol) was added and the mixture was shaken vigorously. The decrease of absorbance at 517 nm was recorded at 1 min. after mixing. Vitamin C (0-40 µg) was used as standard and the results were reported as mg vitamin C equivalent per gram (dried leaves).

ABTS Radical Cation Decolourisation Assay

The method of Re et al. [32] based on the ability of antioxidant molecules to quench the long-lived ABTS radical cation ($ABTS^{\bullet+}$) was modified. $ABTS^{\bullet+}$ 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 hr before use. The $ABTS^{\bullet+}$ solution was diluted with deionised water : 95% ethanol (1:1) to an absorbance of 0.70 ($\pm$0.02) at 734 nm. Twenty µL of the extract solution were mixed with 6 mL of the diluted $ABTS^{\bullet+}$ solution. The decrease of absorbance was recorded at 1 min. after mixing. Vitamin C (0-20 µg) was used as standard and the results were reported as mg vitamin C equivalent per gram (dried leaves).

Total Phenolic Content (TPC)

The Folin-Ciocalteau micro method of Waterhouse [33] was used. Twenty µL of the extract solution were diluted with deionised water to 4.8 mL and 300 µL Folin-Ciocalteau reagent were added and shaken. After 8 min., 900 µL of 20% sodium carbonate were added with mixing. The mixture was allowed to stand at 40°C for 30 min. before the absorbance at 765 nm was read. Gallic acid (0-50 µg) was used as standard and the results for TPC were reported as mg gallic acid equivalent per gram (dried leaves).

Formation of Browning Pigments

Browning pigment formation was determined by the official method of the Association for Dehydrated Onion and Garlic Products (ADOGA) [34]. Three mL of the extract solution were mixed with 3 mL of deionised water and its absorbance was measured at 420 nm.

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Experimental Values for Antioxidant Assays and TPC

The experimental values for TPC and antioxidant assays by FRAP, ABTS and DPPH methods (mg standard equivalent per gram of dried leaves) were calculated as:

$$= \frac{\frac{[SA - BA]}{Slope} \times [10/U]}{\left[2 * \left[1 - \frac{\%mc}{100}\right]\right][1{,}000]}$$

where: SA = Sample absorbance for FRAP method or TPC, or absorbance decrease of sample for ABTS or DPPH method
BA = Blank absorbance for FRAP method or TPC, or absorbance decrease of blank for ABTS or DPPH method
Slope = Slope of standard curve
10/U = Total volume of extract solution (10 mL) / Used volume of extract (mL)
2 = Weight of blended fresh leaves (g)
% mc = % moisture content
1,000 = Factor for converting µg to mg

Statistical Analysis

The relation between the three antioxidant activities and total phenolic content or browning pigment formation was analysed by Bivariate correlation. All statistical analysis was done by SPSS 16.0 Family.

RESULTS AND DISCUSSION

Changes at 50°C

As shown in Figure 1, the values from the results of FRAP assay and TPC gradually declined while some values slightly increased in the later stage of the drying process. On the other hand, the values from the results of DPPH and ABTS assays rapidly decreased in the first 2.5 hours and then were quite constant, although some values slightly increased at the end of the drying process.

The drop in TPC could be explained by thermal oxidation and decomposition of phenolic compounds [35], which might be the main reason for the antioxidant activity decrease of *M. oleifera* leaves, as shown by a significant correlation between TPC and all antioxidant activities in Table 1. The FRAP, rather than the DPPH and ABTS assay values, was highly dependent on the TPC. The decrease in the capacity to allay DPPH and ABTS radicals at the first 2.5 hours was faster than the decrease in TPC, possibly due to thermal oxidation and decomposition of non-phenolic antioxidant substances in *M. oleifera* leaves, especially vitamin C [5].

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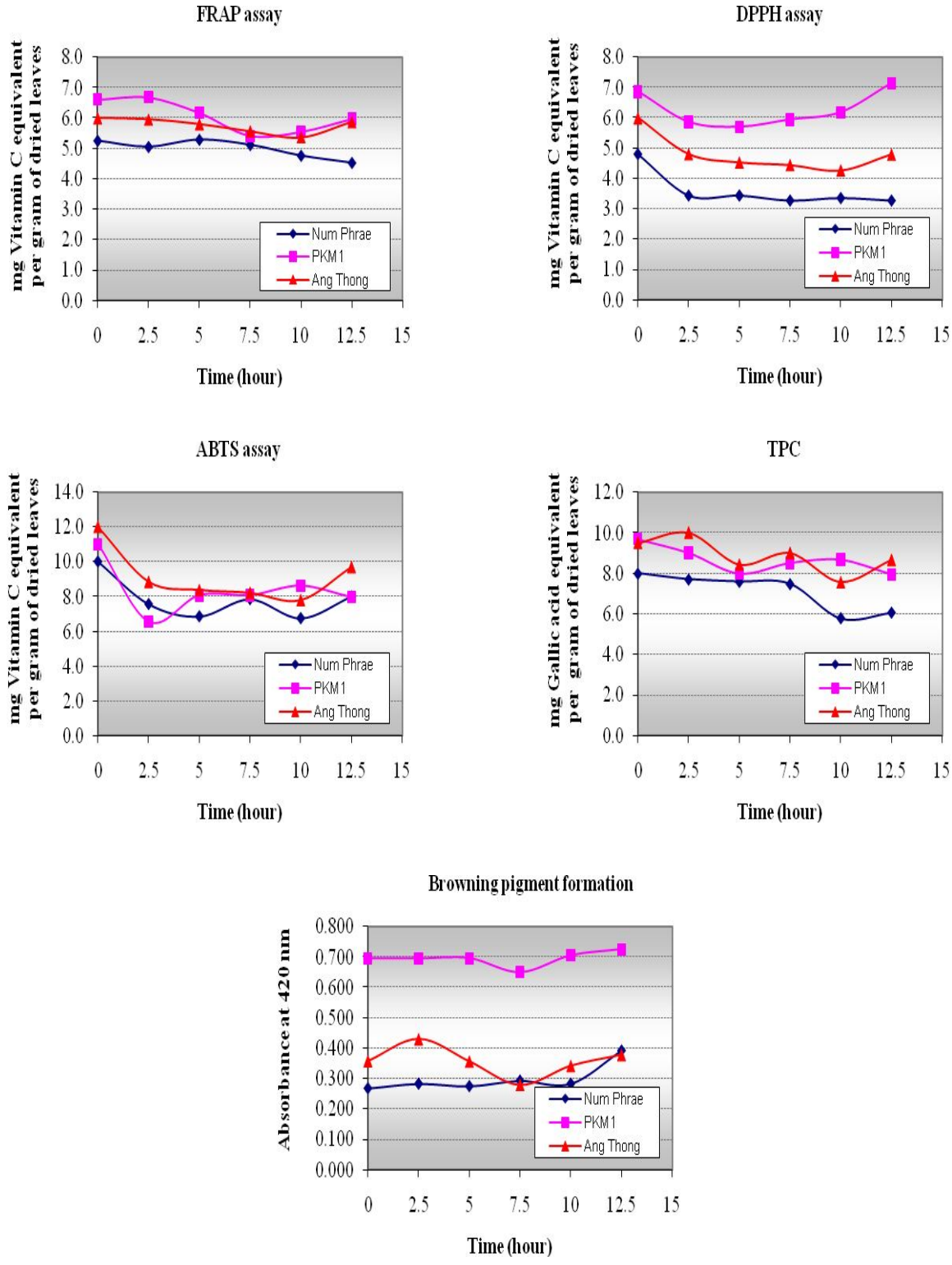

Figure 1. Antioxidant activities (FRAP, DPPH and ABTS assays), TPC and browning pigment formation of *M. oleifera* leaves during drying in hot-air oven at 50°C (Coefficient of variation = 4.76 -13.82%)

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Table 1. Correlation coefficients between TPC/browning pigment formation and antioxidant activities of *M. oleifera* leaves during drying in hot-air oven at 50°C and 100°C

	Correlation coefficient	
	TPC	Browning pigment formation (absorbance at 420 nm)
At 50°C		
FRAP	0.790**	0.627**
DPPH	0.626**	0.834**
ABTS	0.561*	0.035
At 100°C		
FRAP	0.366	0.055
DPPH	0.586*	-0.005
ABTS	0.587*	-0.395
Pooled data		
FRAP	0.621**	0.354*
DPPH	0.587**	0.563**
ABTS	0.558**	-0.037

* Correlation is significant ($p<0.05$). ** Correlation is highly significant ($p<0.01$).

At the later stage of the drying process, some observed increase in antioxidant activity could be attributable to the occurance of antioxidant substances or phenolic compounds by thermal reactions such as non-enzymatic browning reactions [35-39]. This is supported by a significant correlation between the browning pigment formation and results of DPPH or FRAP assay in Table 1. The capacity to trap DPPH radicals was more dependent on the browning pigment formation than the electron donating power (FRAP) results. This high correlation between browning pigment formation and results of DPPH or FRAP assay was also found in our previous work [40]. However, the increase in browning pigment formation (0.020-0.124) during drying at 50°C was not much. Thus, the antioxidant activities in this case could be considered to stem more from natural heat-stable antioxidant substances in *M. oleifera* leaves than from non-enzymatic browning products. As for the capacity to trap ABTS radicals, it was not related to the browning pigment formation but correlated with the TPC only. The increase in the ABTS values could therefore be the results of phenolic compounds from other thermal reactions such as the thermal degradation of insoluble and bound phenolic compounds [41].

From Figure 1, it is evident that the antioxidant activities and TPC of *M. oleifera* leaves significantly decreased during drying at 50°C. Although some of the values were regained in the later stage of the drying process, almost all of them were significantly lower than those for the fresh leaves. However, the antioxidant activity of all dried leaves was at least 60% of that of the fresh leaves. Moreover, the values for antioxidant activities and phenolic content of the three leaf varieties during drying at 50°C were similarly changed: compared to those from fresh leaves (treated as 100%) the values varied between 68-101% (for Num Phrae), 60-104% (for PKM1) and 65-105% (for Ang Thong).

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Changes at 100°C

As shown in Figure 2, the electron donating power (FRAP) value appeared to decrease except that for Num Phrae variety, whose FRAP showed little change. The values for DPPH and

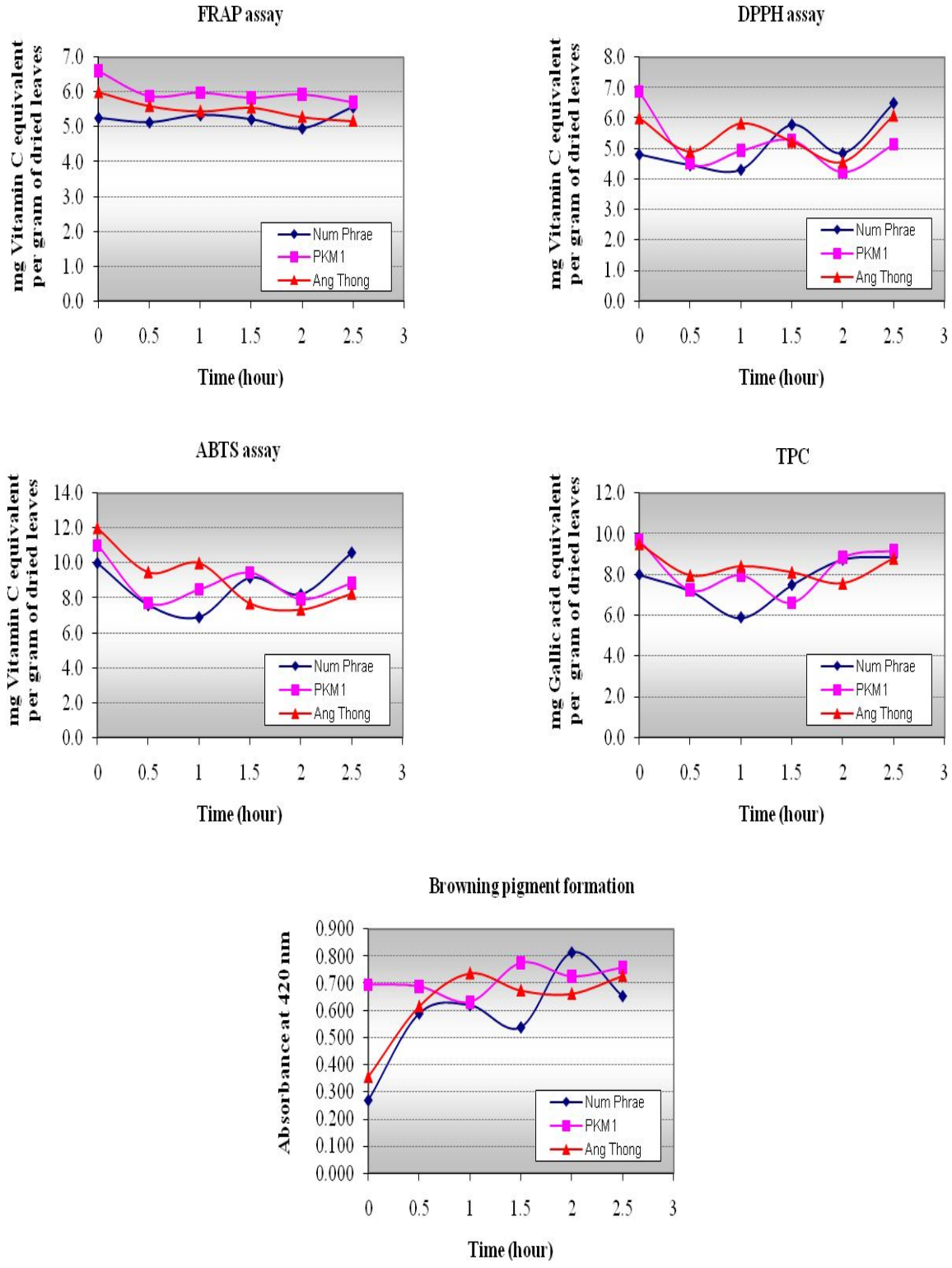

Figure 2. Antioxidant activities by FRAP, DPPH and ABTS assays, TPC and browning pigment formation of *M. oleifera* leaves during drying in hot-air oven at 100°C (Coefficient of variation = 4.44-13.94%)

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ABTS assays as well as TPC appeared to decrease in the early stage of drying and then increase in the later stage. A result similar to the early stage of heating, where there is a significant decrease of antioxidant activity of *M. oleifera* leaves extract heated at 100°C for 15 min., was also reported [20].

As with the changes at 50°C, all decreases in antioxidant activities in Figure 2 could be explained by thermal oxidation and decomposition of materials including phenolic compounds [35] and other non-phenolic antioxidants such as vitamin C [5] in *M. oleifera* leaves, while all increased values could be explained by the formation of antioxidant products of thermal reactions such as non-enzymatic browning reactions [35-39], and also by the thermal degradation of insoluble and bound phenolic compounds [41]. Examples of the latter are the breaking of linkages between *p*-coumaric acid and lignin and between ferulic acid and arabinoxylans [42]. It appears that, compared with values for fresh leaves, the variation in antioxidant activities and phenolic compounds during drying at 100°C for Num Phrae variety (69-135%) were more than those for Ang Thong (61-101%) and PKM1 (61-94%) varieties.

At 100°C drying, there was a lower correlation than in the case of drying at 50°C and only between TPC and results of DPPH or ABTS assays; there was no correlation between browning pigment formation and any antioxidant activities (Table 1). This may be explained by the fact that the bivariate correlation is used to measure the direction and strength of a linear relationship between two variables [43]. However, when the two changes, i.e. thermal oxidation/ decomposition/ degradation and non-enzymatic browning reactions, occurred faster at 100°C, it might be more difficult to correlate them by a linear relationship compared to the situation at 50°C, in which the reaction rates were slower.

Compared to drying at 50°C, TPC of Num Phrae variety's leaves dried at 100 °C was higher than that in its fresh leaves (Figure 2). These phenolic compounds occurring during the later stage of drying by such thermal reactions as the degradation of insoluble and bound phenolic compounds at high temperature could also imply that the leaves of Num Phrae variety probably contain more insoluble and bound phenolic compounds than do Ang Thong and PKM1 varieties.

CONCLUSIONS

Antioxidant activities of *Moringa oleifera* leaves dried by hot-air oven at both 50°C and 100°C were found to be at least 60% that of fresh leaves. Some of the variation in antioxidant activity values were found to be significantly related to the total phenolic content and the formation of browning pigments. Similar changes in antioxidant activities were observed in the three varieties of *M. oleiferea*.

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