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Full Paper

Effect of heat treatment on the antioxidant capacity of garlic

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Abstract: The determination of the antioxidant capacity of dry and wet-heated garlic, at 70, 100, and 121 °C by 3 different methods, namely ferric reducing antioxidant power (FRAP) assay, improved ABTS radical cation decolourization assay, and DPPH free radical scavenging activity, together with the determination of total phenolic content and formation of browning pigments of the same materials was carried out. The result showed that the antioxidant capacity of heated garlic was decreased by the decomposition of some phenolic and sulfur-containing compounds. However, when browning pigments developed, the antioxidant capacity of the heated brown garlic increased with the degree of browning, provided that it was not too dark. In addition, this study showed that ABTS and FRAP assay were better methods for expressing the antioxidant capacity of garlic due to its total phenolic content, although FRAP and DPPH assay were better if the antioxidant capacity of garlic was mainly caused by browning pigments.

Key words: garlic, antioxidant capacity, heat treatment

Introduction

Garlic (*Allium sativum* Linn.) is widely used as a food ingredient and a medicinal plant in many countries, especially in Asia. It is also a recommended Thai medicinal plant for the primary health care system [1]. Epidemiologic studies show an inverse correlation between garlic consumption and progression of cardiovascular disease. Garlic has been shown to inhibit enzymes involved in lipid synthesis, decrease platelet aggregation, prevent lipid peroxidation of oxidised erythrocytes and LDL,

increase antioxidant status, and inhibit angiotension-converting enzyme in the body [2-3]. It also prevents DNA damage in essential hypertension [4].

Evidence from several investigations suggests that the biological and medicinal functions, such as antimicrobial, hypolipidemic, antioxidant, and antithrombotic properties that have been attributed to garlic are related to a variety of sulphur-containing compounds, including volatiles such as allicin, non-volatile water-soluble sulphur compounds such as S-allyl cysteine, and lipid-soluble sulphur compounds such as diallyl sulphide and diallyl disulphide [5-9]. Allicin is formed when alliin, a sulphur-containing amino acid, comes into contact with the enzyme alliinase when raw garlic is chopped, crushed, or chewed. Allicin scavenges hydroxyl radicals ($OH^{\bullet}$), and prevents the lipid peroxidation of liver homogenate in a concentration-dependent manner [10]. The antioxidant activity in the liposome system of diallyl sulphide, diallyl disulphide, S-ethyl cysteine, and N-acetyl cysteine derived from garlic was demonstrated, but this activity was lost when the temperature reached 65 °C [11].

Fresh, dried and fried garlic are used as food ingredients or seasonings in Thai cuisine. During the cooking or heating process, non-enzymatic browning reactions including Maillard reaction, caramelisation, and chemical oxidation of phenols occur. The antioxidant activity of the products from Maillard reaction [12-14] and caramelisation [15] have been reported. On the other hand, the decrease in antioxidant capacity of boiled garlic at 100 °C has been found [16-17].

In this present work we report the determination of the antioxidant capacity of garlic during the drying and wet heating process at 70, 100, and 121 °C, which are representatives of three heating conditions, i.e. heating below boiling point of water or pasteurisation, heating at boiling point of water or sterilisation of high acid and acid food (pH < 4.5), and heating above boiling point of water or sterilisation of low acid food (pH > 4.5). The determination was done by three different methods, viz. ferric reducing antioxidant power (FRAP) assay, improved ABTS radical cation decolourization assay, and DPPH free radical scavenging assay. Total phenolic content and formation of browning pigments (absorbance at 420 nm) were also determined. The correlations of antioxidant capacity (%) with total phenolic content (%), and of antioxidant capacity (%) with absorbance at 420 nm were then analysed.

Materials and Methods

Chemicals

Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was purchased from Aldrich. TPTZ (2,4,6-tripyridyl-s-triazine) and DPPH (2,2-diphenyl-1-picrylhydrazyl) were purchased from Sigma. ABTS [2,2'-azinobis (3-ethylbenzothiazoline-6-sulphonic acid)], Folin-Ciocalteau phenol reagent, ferric chloride, ferrous sulphate, gallic acid, glacial acetic acid, hydrochloric acid, sodium acetate, potassium persulphate, sodium carbonate, and vitamin C were purchased from Fluka. All chemicals were of analytical grade.

Sample preparation

Fresh garlic was purchased from a local market and edible portion was homogenised with a blender. Sample tubes (25 x 150 cm) containing 2 g each of blended garlic were dried in a hot air oven at 70, 100, or 121 °C. For wet heating, 10 ml of deionised water was added to the blended garlic in each

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sample tube before heating in a water bath at 70 or 100 °C, or in an autoclave at 121 °C. A sample tube was collected 10 times during a maximum of 10 hours of heating period. Sample extraction method of Leong and Shui [18] was modified. Ten ml of deionised water were added to the collected dried sample tube. (For wet heating, deionised water had been added before heating.) The extraction was done by vortex mixing for 1 min. The mixture was then filtered through a Whatman filter paper no. 1. The filtrate was adjusted to 10 ml by deionised water and this extract was used for all assays. An extract of blended fresh garlic was prepared for comparison and the weight change during the drying process also was recorded.

Ferric reducing antioxidant power (FRAP) assay

FRAP, a method for measuring total reducing power of electron-donating substances, was applied according to Benzie and Strain [19]. 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 were mixed with 20 µl of extract sample. The absorbance at 593 nm was recorded after a 30-min incubation at 37 °C. FRAP values were obtained by comparing with standard curves created by Fe^{2+} (0 - 14 µg), Trolox (0 - 35 µg) and vitamin C (0 - 15 µg), and reported as mg Fe^{2+}, Trolox and vitamin C equivalent per gram of sample (dry weight).

ABTS radical cation decolourization assay

The method of Re et al. [20], 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 potassium 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 deionised water and 95 % ethanol (1 : 1) to an absorbance of 0.70 ($\pm$ 0.02) at 734 nm. Twenty microlitres of the extract were mixed with 6 ml of the diluted $ABTS^{\bullet+}$ solution. The decrease of absorbance was recorded at 1 min after mixing. Trolox (0 - 30 µg) and vitamin C (0 - 20 µg) were used as standards, and the results were reported as mg Trolox and vitamin C equivalent per gram of sample (dry weight).

DPPH free radical scavenging activity

The method of Brand-Williams et al. [21], based on the reduction of DPPH radical solution in the presence of hydrogen donating antioxidants, was used with some modification. DPPH radical solution (0.8 mM) in 95% ethanol was prepared. One thousand microlitres of the extract were diluted to 5.4 ml using deionised water and 95 % ethanol (1 : 1) before 0.6 ml DPPH radical solution was added and the mixture shaken vigorously. The decrease of absorbance was recorded at 1 min after mixing. Trolox (0 - 50 µg) and vitamin C (0 - 40 µg) were used as standards, and the results were reported as mg Trolox and vitamin C equivalent per gram of sample (dry weight).

Total phenolic content (TPC)

The Folin-Ciocalteau micro method of Waterhouse [22] was used. Sixty microlitres of the extract were diluted with deionised water to 4.8 ml, and 300 µl of Folin-Ciocalteau reagent were added and the mixture shaken. After 8 min, 900 µl of 20% sodium carbonate were added and mixed. The solution

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was left 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 were reported as mg gallic acid equivalent per gram of sample (dry weight).

Formation of browning pigments

Formation of browning pigments was determined by the official method [23]. The absorbance of extracts was measured at 420 nm.

Calculation and statistical analysis

The values of FRAP, ABTS, DPPH, and TPC (mg standard equivalent per gram of sample (dry weight)) were calculated using the equation below:

$$\text{Values of FRAP, ABTS, DPPH, and TPC (mg standard equivalent per gram of sample (dry weight))} = \frac{[(SA - BA) / (Slope)]\,[10 / U]}{[2]\,[1-MC][1{,}000]}$$

where: 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 deionised water for blank)

Slope = Slope of standard curve

[10 / U] = Total volume of extract (10 ml) / Used volume of extract (ml)

[2] = Weight of used sample (g)

MC = % Moisture content / 100

[1,000] = Factor for changing μg to mg

Changes of antioxidant capacity, TPC, and weight (%) were calculated using the equation below:

$$\text{Changes of antioxidant capacity, TPC, and weight (\%)} = \frac{\text{Value of collected sample} \times 100}{\text{Value of blended fresh garlic}}$$

Each experiment was performed in triplicate and was conducted on separate purchased samples (triple measurements for each purchased sample). The bivariate correlations between changes of antioxidant capacity and total phenolic content, and between changes of antioxidant capacity and absorbance at 420 nm were analysed.

Results and Discussion

In this experiment, water was used as extracting solvent since about 97% of garlic components are water-soluble [6], and some previous work also confirmed that water is a good solvent for measuring antioxidant capacity and TPC of garlic extract [16,24], although a better result with hexane has been obtained by Leelarungrayub et al. [25]. Antioxidant capacity and TPC of aqueous fresh garlic extract are shown in Table 1. Values of antioxidant capacity and TPC of aqueous garlic extract have been

reported by several workers but they are difficult to compare because of the differences in sample sources, sample preparations, methodology details, standards used, among others. For example, Gorinstein et al. [16] reported ABTS = 26.1 $\pm$ 2.4 µmol Trolox equivalent per gram of sample (dry weight), and TPC = 11.42 $\pm$ 1.45 mg gallic acid equivalent per gram of sample (dry weight). Jastrzebski et al. [17] reported FRAP = 3,400 $\pm$ 270 mmol Trolox equivalent per 100 grams of sample (fresh weight), DPPH = 69 $\pm$ 5.1 % inhibition, and TPC = 49.3 $\pm$ 3.1 mg gallic acid equivalent per 100 grams of sample (fresh weight). Wangcharoen and Morasuk [24] reported FRAP, ABTS and DPPH = 0.14 $\pm$ 0.04, 1.06 $\pm$ 0.11 and 0.16 $\pm$ 0.03 mg vitamin C equivalent per gram of sample (fresh weight) respectively, and TPC = 0.41 $\pm$ 0.10 gallic acid equivalent per gram of sample (fresh weight). Leelarungrayub et al. [25] found ABTS = 1462 $\pm$ 22 mg extract equivalent to 1 mg of Trolox, and TPC ≈ 3,800 mg gallic acid equivalent per kg of dry weight of extract. Holvorsen et al. [26] found FRAP = 0.21 – 0.24 mmol $FeSO_4.7H_2O$ equivalent per 100 grams of fresh weight of edible portion, and Nuutila et al. [27] found DPPH = 62.1, 60.9, and 43 % inhibition, and TPC = 75 $\pm$ 8.8, 115 $\pm$ 12.9, and 95 $\pm$ 7.8 mg gallic acid equivalent per kg of freeze-dried samples of Finnish organic garlic, Finnish garlic, and Hungarian garlic, respectively.

Table 1. Antioxidant capacity by various methods and total phenolic content (TPC) of aqueous extract of fresh garlic samples (mg standard equivalent per gram of sample (dry weight))

Standard	FRAP	ABTS	DPPH	TPC
Fe^{2+}	0.56 $\pm$ 0.15			
Trolox	1.13 $\pm$ 0.31	5.17 $\pm$ 0.54	0.57 $\pm$ 0.08	
Vitamin C	0.44 $\pm$ 0.12	3.41 $\pm$ 0.35	0.48 $\pm$ 0.07	
Gallic acid				1.29 $\pm$ 0.19

Changes of antioxidant capacity (%), TPC (%), weight (%), and 420 nm absorbance of the aqueous extract of garlic samples during heating process are shown in Figures 1 and 2. In the case of drying (Figure 1), the antioxidant capacity values and TPC tended to decrease with drying time when garlic samples were dried at 70 °C, except the DPPH value, which seemed to increase at 10 hours. The weight of garlic samples rapidly decreased in the first three hours and was steady after 4 hours, whilst the absorbance at 420 nm evidently decreased in the first and the second hour and gradually increased after the third hour. At 100 and 121 °C, all antioxidant capacity values and TPC decreased and reached the lowest point at 2.5 and 1.5 hours respectively, before increasing when the drying time was further increased. The weight of garlic extracts rapidly decreased in the first hour and was constant after that

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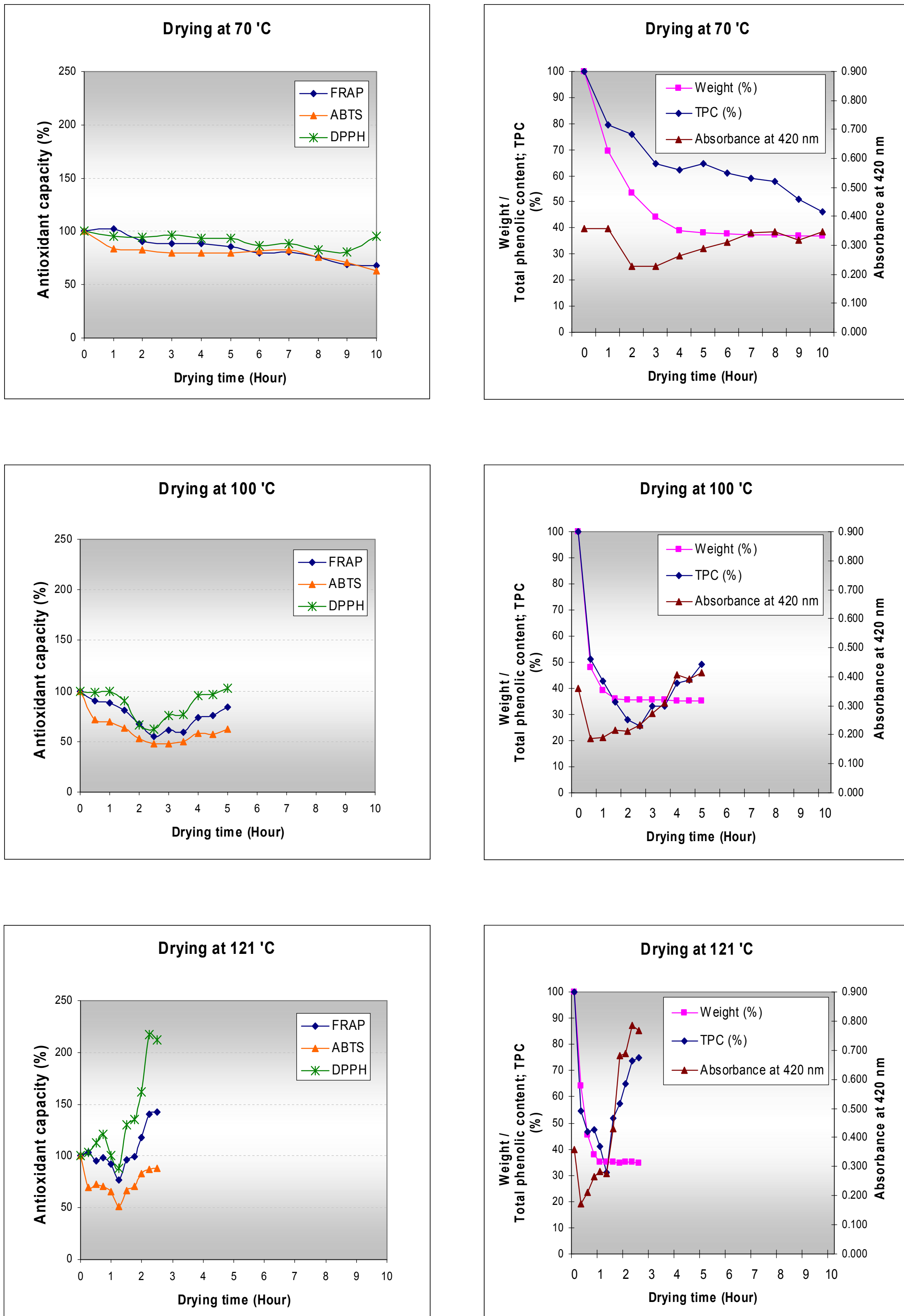

Figure 1. Antioxidant capacity, total phenolic content, and absorbance at 420 nm of aqueous extracts of garlic samples, together with weight of garlic samples during drying process at 70, 100, and 121 °C

time, whilst the absorbance at 420 nm decreased to a minimum at about 0.5 hour before it evidently increased after that.

In the case of wet heating (Figure 2) at 70 °C, FRAP and ABTS values tended to decrease for 70 min before slightly increasing after that time, whilst DPPH value slowly increased until about 30 min, after which it slowly decreased and started to increase again after 70 min. At 100 °C, ABTS value steadily decreased, whilst FRAP and DPPH values seemed to increase for about 20 min before decreasing steadily for FRAP and with fluctuation for DPPH. This result agrees with that of Gorinstein et al. [16], who reported 13 – 17 % decrease of ABTS assay for boiled garlic at 100 °C for 20 min, and also agrees with that of Jastrzebski et al. [17], who showed a significant decrease of FRAP and DPPH assay of boiled garlic at 100 °C for 40 and 60 min. On the other hand, at 121 °C of wet heating, DPPH value increased rapidly, whilst FRAP and ABTS values also did albeit less so and only after an initial decrease.

The decrease of antioxidant capacity of garlic extract upon heating was most probably due to the decomposition of some phenolic compounds and also some sulphur-containing compounds such as diallyl sulphide, diallyl disulphide, S-ethyl cysteine, and N-acetyl cysteine, which would be lost when the temperature reached 65 °C [11]. However, when browning pigments were formed, the antioxidant capacity of the heated brown garlic was regained as a result of certain compounds, including phenolic compounds, being created during the browning reactions [12-15]. The browner garlic, with higher value of absorbance at 420 nm, expressed higher antioxidant capacity, but at the end of the heating period at 121 °C, the antioxidant capacity started to decrease (Figures 1 and 2). This shows that the antioxidant capacity of the heated brown garlic would be dropped if it was heated for a long time so that its colour was too dark.

From the results of bivariate correlation, the correlation coefficient (a number between -1 and 1 which measures the degree to which two variables are linearly related) of changes of antioxidant capacity (%) with TPC showed that ABTS values were higher correlated with TPC in all cases of drying process, followed by FRAP and DPPH values respectively. In the case of wet heating process, FRAP values seemed to be higher correlated with TPC than ABTS and DPPH values respectively. The bivariate correlation of changes of antioxidant capacity (%) with absorbance at 420 nm showed that FRAP and DPPH values were highly correlated with browning pigment formation in drying and wet heating process at 121 °C (Table 2). These differences might be due to the fact that ABTS and DPPH method involve free radicals reacting with phenolic and browning pigment compounds, while for FRAP assay, it is a method for measuring total reducing power of electron donating substances, which is not directly related to free radical reactions and not as specific as ABTS and DPPH assay.

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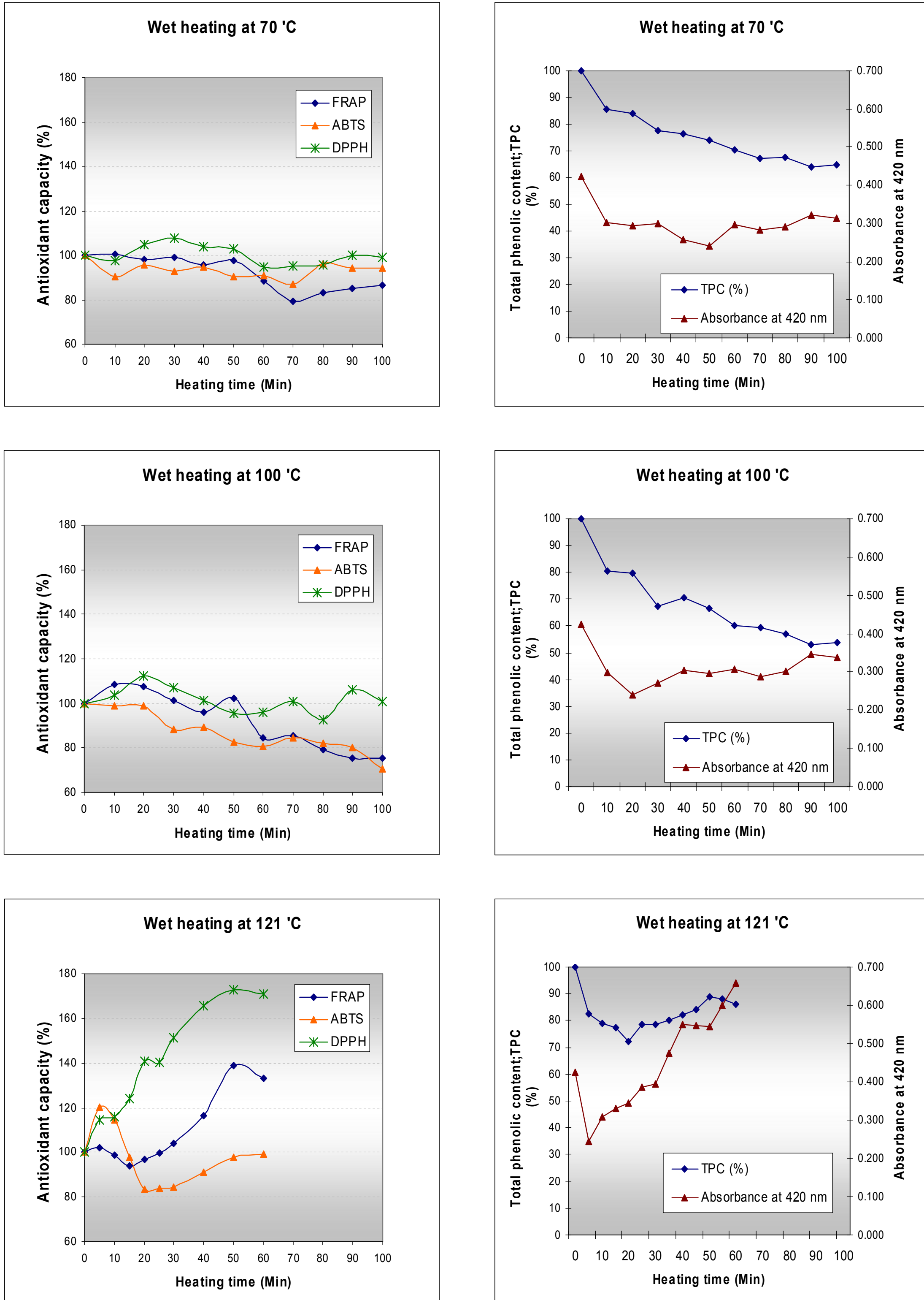

Figure 2. Antioxidant capacity, total phenolic content, and absorbance at 420 nm of aqueous extracts of garlic samples during wet heating process at 70, 100, and 121 °C

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Table 2. Correlation coefficients of changes of antioxidant capacity with total phenolic content (TPC) and with absorbance at 420 nm of aqueous extract of garlic samples during heating processes

Drying process		FRAP	ABTS	DPPH
70 °C	Total phenolic content (TPC)	0.891	0.919	0.636
100 °C		0.799	0.941	0.616
121 °C		0.597	0.961	0.436
70 °C	Absorbance at 420 nm	-0.114	0.050	-0.151
100 °C		0.090	0.100	0.377
121 °C		0.757	0.419	0.834
Wet heating process				
70 °C	Total phenolic content (TPC)	0.801	0.489	0.313
100 °C		0.768	0.893	0.243
121 °C		0.374	0.271	-0.153
70 °C	Absorbance at 420 nm	0.156	0.637	-0.131
100 °C		-0.275	-0.017	-0.315
121 °C		0.847	-0.306	0.774

Conclusions

This work has shown that the antioxidant capacity of garlic is decreased by both drying and wet heating. However, if browning pigments are developed from non-enzymatic browning reactions, the brown garlic will regain its antioxidant capacity, which may increase to an appreciably higher level than the starting value depending on the degree of browning. This expresses the high antioxidant capacity of heated brown garlic, which is normally used as an ingredient in many Thai food recipes.

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