International Food Research Journal 18: 397-404 (2011)

Stability of antioxidant and antibacterial properties in heated turmeric-chili paste and its ingredients

[1] Romson, S., [2,*] Sunisa, S. and [2]Worapong, U.

[1,2]*Food Technology Department , Agro-Industry Faculty, Prince of Songkla University, Hat Yai, Songkhla, 90112, Thailand*

Abstract: Turmeric-chili or yellow curry paste is a traditional condiment used in hot and sour curry soup consumed in Thailand and Southeast Asia. The effect of heat treatment on total phenolic content, antioxidant and antibacterial activities of the curry paste was investigated and results showed that the total phenolic content of the curry paste extract increased after heating at 80^0C, 90^0C and 100^0C for 10, 20 and 30 min. The 2.2-diphenyl-1-picrylhydrazyl activity (DPPH) and ferric reducing/antioxidant power (FRAP) values of the heated curry paste extract were higher compared with unheated paste extract. The minimal inhibitory concentration (MIC) values of the heated paste extract on *Staphylococcus aureus* and *Bacillus cereus* were 2.616 and 5.232 mg/ml, respectively, and the minimal bactericidal concentration (MBC) values of the heated paste extract on *S. aureus* and *B. cereus* were 2.616 and 10.464 mg/ml, respectively. It was concluded that curry paste could retain its antioxidant activity even after heating therefore it could be valuable as a functional food.

Keywords: Turmeric-chili paste, antioxidation, antibacterial, pathogenic bacteria, curry

Introduction

In recent years, interest in plant-derived food additives has grown and gained popularity because they are assumed to be functional food. Therefore, high quality and nutritious food products, having long shelf-life without chemical preservatives are the most sought after (Oonmetta-aree *et al*., 2005). Traditional Thai food has a very distinctive characteristic because of the special combinations of herbs and spices used in its preparation (Chaisawadi *et al*., 2005). Thai native herbs are becoming more widely used on a commercial scale in the food industry, mainly for their flavoring properties (Voravuthikunchai *et al*., 2006).

Turmeric-chili or yellow curry, hot and sour soup is one of Thailand's traditional foods popularly consumed in Southeast Asia because of its low calorie and unique taste. In general, the ingredients used in the paste are turmeric rhizome, garlic and chili; however, galangal rhizome may also be added in some regions. Some of these ingredients are considered natural preservatives because their active compounds consist of free radical scavengers such as curcumin (difeuryloylmethane) from turmeric rhizome (Ruby *et al*., 1995; Ahsan *et al*., 1999; Jayaprakasha *et al.,* 2006; Cousins *et al*., 2006). The principle antimicrobial compound, allicin, of garlic (*Allium sativum*), exhibits antibacterial activity against a wide range of Gram-negative and Gram-positive bacteria (Jonkers *et al*., 1999; Ankri and Mirelman, 1999; Curtis *et al*., 2004; Bakri and Douglas, 2005). In Thailand, galangal (*Alpinia galangal*) is used for medical purposes, such as for carminative, stomachic, antispasmodic, antichloristic and antibacterial drugs (Mayachiew and Devahastin, 2008), and as an antioxidation agent (Ruby *et al*., 1995; Juntachote and Berghofer, 2005; Zaeoung *et al.,* 2005; Jayaprakasha *et al*., 2006; Kumar *et al.,* 2006; Mayachiew and Devahastin, 2008). Capsaicin is a compound in chili that could inhibit the growth of *Helicobacter pylori* (Jones *et al*., 1997). Preparation of turmeric-chili paste involves heating, but the effect of heating on the bioactive properties of the herbs and spices in the product, such as turmeric and chili, has not been reported. Therefore, the objective of this study was to determine the effect of thermal treatment on the antioxidant and antimicrobial activities of the curry paste.

Materials and Methods

Materials and reagents

Turmeric rhizomes (*Curcuma longa*), garlic (*Allium sativum*), chili (*Capsicum frutescense*) and galangal rhizomes (*Alpinia galanga*) were purchased from a local market in Hat-Yai, Thailand. All chemicals, reagents and media were of analytical grade obtained from Sigma Chemical Co. (St. Louis, MQ, USA) and Merck (Darmstadt, Germany).

*Corresponding author.
Email: *sunisa.s@psu.ac.th*
Tel: +6674286320-1, Fax: + 6674212889

The curry paste and its ingredients

For the preparation of the curry paste, all spices were sorted, trimmed and washed thoroughly to remove dust and dirt, and weighed according to the recipe. They were then ground with a blender (Moulinex, TYPE 276, France) to make a fine paste. A 100 g sample of the paste was extracted with 300 ml of distilled water twice and the combined extract was filtered through a filter paper (φ125 mm, Cat. no. 1001 125 (Whatman Schleicher and Schuell, England) and kept at 4^0C until used.

Heat treatment of the curry paste extract

Samples of the curry paste extract were heated at 80, 90 and 100^0C for 10, 20 and 30 min in a water bath (Adihan Lab tech Co., Ltd, Korea) and allowed to cool to room temperature. Unheated curry paste extract was used as control. The control and heated samples were subjected to various tests to determine the total phenolic content, and the antioxidant and antibacterial activities.

Total phenolic content

Total phenolic content of heated curry paste extract was determined by using Folin–Ciocalteu assay with slight modification (Zhou and Yu, 2006). The reaction mixture contained 1 ml of the extract, 0.5 ml of the Folin–Ciocalteu reagent, 1 ml of 10 g/100 ml sodium carbonate and 7.5 ml of distilled water. After 45 min of reaction at ambient temperature, the absorbance at 765 nm was measured using a UV-visible spectrophotometer (Jasco V-530, Japan Servo Co., Ltd.). A calibration curve was prepared using standard gallic acid (0.016, 0.008, 0.004, 0.002 and 0.001 mg/ml, r^2= 0.995). The results were expressed on a dry weight basis (dw) as mg gallic acid equivalents (GAE) per 100 g of dry sample.

Antioxidant activities

Free radical scavenging (DPPH.) assay

Free radical scavenging was determined by using the free radical generator DPPH (2,2-diphenyl-1-picrylhydrazyl) assay based on slight modification (Yen and Hsieh, 1997). An aliquot (1 ml) of the serially diluted extract samples was thoroughly mixed to which 1 ml of 500 µM DPPH solution was added. The mixture was thoroughly mixed using a vortex and kept in the dark for 30 min. The absorbance, using a spectrophotometer, was then measured at 518 nm against a blank of ethanol without DPPH. The results were expressed on a dry weight basis as mg GAE/100 g dry sample.

Ferric reducing/antioxidant power (FRAP) assay

A FRAP assay was performed using a modified method (Benzie and Strain, 1996). Briefly, a 150 µl aliquot of properly diluted extract was thoroughly mixed with 2850 µl FRAP reagent and incubated at 37^0C for 4 min. The absorbance was then determined at 593 nm against a blank that was prepared using distilled water. FRAP was freshly prepared by mixing 2.5 ml of a 10 mM 2,4,6-tris (1-pyridyl)-5-triazine (TPTZ) solution in 40 mM HCl with 2.5 ml of 20 mM $FeCl_3$ $6H_2O$ and 25 ml of 0.3 M acetate buffer at a pH of 3.6. A calibration curve was prepared using different concentrations of gallic acid (0.016, 0.008, 0.004, 0.002 and 0.001 mg/ml, r^2 = 0.997). FRAP values were expressed on a dry weight basis as mg GAE/100 g dry sample.

Preparation of microorganisms

Staphylococcus aureus and *Bacillus cereus* (ATCC 1778) were obtained from Department of Medical Sciences, Ministry of Public Health, Thailand. Each organism was maintained in Nutrient Agar (NA) at 5^0C. Stock culture from both organisms was transferred to 5 ml of Brain heart infusion broth (BHI) and incubated at 37^0C for 15 h. This culture was used for the antimicrobial assay.

Antibacterial activities

Minimum inhibitory concentration (MIC)

One loopful of *S. aureus* and *B. cereus* was individually cultured into 5 ml of BHI and incubated at 37^0C for 15 h. Each bacterium was diluted with 0.85% sterile sodium chloride to achieve a final concentration of approximately 106 cfu/ml. One-ml of the bacterial suspension at 106 cfu/ml was transferred into 1 ml of Muller Hinton Broth (MHB) then 1 ml of a series of two-fold dilutions of each extract was added. The MIC of the extracts was regarded as the lowest concentration of extracts that did not permit any turbidity of the tested microorganism (Lorian, 1995; Lennette *et al*., 1991).

Minimum bactericidal concentration (MBC)

All the tubes used in the MIC studies that did not show any turbidity with the bacteria and the last tubes with turbidity were determined for MBC. An aliquot of the suspension (0.1 ml) was spread onto Muller Hinton Agar (MHA) and incubated at 35^0C for 15 h. The MBC was the lowest concentration in which the initial inoculums were killed at the least one log cycle or more (Lorian, 1995; Lennette *et al*., 1991).

Statistical analysis

Data were subjected to analysis of variance, and mean comparisons were made using Duncan's new multiple range test. Statistical analyses were carried out using the SPSS statistical software (SPSS, Inc., Chicago, IL).

Results and Discussion

Total phenolic content and antioxidant activity of the curry paste and its ingredients

Phenolic substances have been shown to be responsible for the antioxidant activity of plant materials (Rice-Evans *et al*., 1996). Table 1 shows the total phenolic content of the curry paste and its ingredients before heating. The highest level of phenolic content of any of the individual spices or herbs was found in chili, while the lowest content was found in galangal. Surprisingly, when the ingredients were made into the curry paste, the total phenolic content increased. This may be due to some chemical changes by enzymatic browning reaction as quinones and melanins and new compounds or active agent derived from other enzymatic system such as furulic acid during the blending process used in the paste making. Additionally, after heating the total phenolic content of the curry paste extract tended to increase when heating temperature and heating time increased (Table 2). An increase of total phenolic content in some plants after heating may be due to the disruption of the plant cell wall, and therefore bound polyphenolic and flavonoid compounds may be released more easily than those in fresh plants (Peleg *et al*., 1991). This is similar to the finding of Choi *et al*. (2006) who reported that concentration of free polyphenolic and flavonoid compounds in heated Shiitake mushrooms was significantly higher than in raw mushrooms. Guihua *et al*. (2007) also found that the heating process improve phenolic content due to the cleaving of bound form (i.e. esterified and glycosylated), thus leading to the increase of free forms. Another probable reason for an increase of phenolic content in the heated sample is the decrease/ inhibition of enzymatic oxidation involving in the antioxidant compounds in the raw plant material (Dewanto *et al*., 2002; Nicoli *et al*., 1999).

Antioxidant activities

DPPH free radical scavenging

DPPH is a free radical compound that has been widely used to determine the free radical scavenging capacity of various samples (Amarowicz *et al*., 2004; Hatano *et al*., 1988) because of its stability (in radical form), simplicity and fast assay (Bozin *et al*., 2008). The DPPH free radical scavenging activities of the curry paste and its ingredients before heating are presented in Table 1. The results showed that the curry paste had the highest in free radical scavenging activities and total phenolic content compared with other samples. This was probably due to the high activity of the active compound and/or the synergistic effect of curcuminoid compounds, such as curcumin, demethoxycurcumin and bisdemethoxycurcumin in turmeric rhizomes. The bioactives in turmeric rhizomes are a group of phenolic compounds, including curcumin, which is a strong antioxidant (Miquel *et al*., 2002). Jayaprakasha *et al.* (2006) reported that the other two curcuminoids were also effective antioxidants. Ruby *et al*. (1995) noted that curcuminoid compounds found in turmeric rhizomes were potent scavengers of hydroxyl radicals. Darrick *et al*. (2001) also reported that bisdemethoxycurcumin and demethoxycurcumin were good in trapping the DPPH radical. While the lowest free radical scavenging activities were found in garlic and chili. It was found that the curry paste possessed the highest DPPH free radical-scavenging activity. This may be due to synergistic antioxidant activity of the various ingredients in the paste. Shobana and Naidu (2000) reported that spice mix (ginger, onion and garlic; onion and ginger; ginger and garlic) showed accumulative inhibition of lipid peroxidation. They exhibited a synergistic property when compared with individual ones.

The effect of heating on DPPH radical activity in the curry paste is presented in Table 2. The results showed a slight increase in the scavenging activity when the heating temperature and heating time increased. An interesting observation in this study was that increased heating would gradually improve antioxidant activity ($p<0.05$). It is possible that complex compounds, curcumin, may convert or degrade to simple compound, furulic acid (one molecule of curcumin can be derived to two molecule of furulic acid) that must be further investigated. The high correlation between the mean values of total phenolic content and DPPH activity was observed as $R^2=0.986$. This indicated that the compounds present in the curry paste were heat resistant and highly efficacious in reducing DPPH radicals. This is similar to the findings of Maria Lúcia *et al*. (2002) who reported that curcuminoids were heat stable. In addition, formation of new active compounds (Dewanto *et al*., 2002; Kim *et al*., 2002) or a release of bound antioxidants during the heating process (Shobana and Naidu, 2000) may occur.

Many researchers found that heat treatment could

either reduce or retain the DPPH scavenging activity. However, the good correlation between the total phenol analysis and the antioxidative assays had been previously reported (Zheng and Wang, 2001). From the present study it can be suggested that greater total phenolic content can be translated into increased DPPH activity. This may be due to curcuminoid, a major active agent in turmeric, which is a strong H^+ donor (Pulla and Lokesh, 1992) and thermally stable (Maria *et al*., 2002).This confirmed that foods containing turmeric rhizomes could provide health benefits through its antioxidant property, even when thermally processed.

Ferric reducing/antioxidant power (FRAP)

The FRAP assay measures the antioxidant effect of any substance in the reaction medium as its reducing ability (Siddhuraju and Becker, 2007) and is commonly used to study the antioxidant capacity of plant materials (Alothman *et al*., 2009). Betoncelj *et al*. (2007) found a strong relationship between antioxidant capacity evaluated by the FRAP assay and the phenolic content of honey. The antioxidant potential of curry paste and its ingredients was estimated from their ability to reduce TPTZ-Fe(III) complex to TPTZ-Fe(II) complex. The highest value of FRAP was found in turmeric and chili (Table1), while the lowest value was in garlic. Making curry paste with these ingredients did not improve FRAP activity of the paste compared with turmeric or chili individually.

The probable reason for the lower FRAP value of the paste could be due to the presence of compounds not reactive towards FRAP. This may be explained from the basic concept that antioxidants are reducing agents because of their ability to donate a single electron or hydrogen atom for reduction. However, not all reducing agents are antioxidants (Dini *et al*., 2008).The antioxidant activities of phenolic compounds are mainly due to their redox properties, including free radical scavenging, hydrogen donating and singlet oxygen quenching (Mayachiew and Devahastin, 2008). Pulido *et al*. (2000) reported on the reducing capacity of polyphenols, as determined by a FRAP assay. This seems to depend on the degree of hydroxylation and extent of conjugation of the phenolic compounds. However, the FRAP test cannot detect compounds which act by radical quenching (H transfer), particularly thiols and proteins (Cao *et al*., 1997). This implies that mixing of herbs or spices may or may not improve their antioxidant properties, depending on the chemical reactions that take place.

The FRAP of heated curry paste was significantly higher compared with that of the unheated paste, the control sample (Table2). A reasonable correlation between the mean values of total phenolic contents and FRAP of R^2=0.552 was observed. This indicated that compounds present in the curry paste provided some reducing ability. It implies that heat treatment could improve both DPPH radical scavenging and FRAP activities.

Table 1. Total phenolic content and antioxidative properties of the extracts of turmeric-chili paste and its ingredients

Sample	Total phenolic contents (mg GAE/100g)	Scavenging radical (mg GAE/100g)	FRAP (mg GAE/100g)
Garlic	0.30 ± 0.02 [a]	0.38 ± 0.07 [a]	0.03 ± 0.01 [a]
Galangal	0.20 ± 0.01 [b]	0.65 ± 0.41 [b]	0.05 ± 0.01 [b]
Turmeric	0.30 ± 0.06 [a]	1.01 ± 0.34 [c]	0.07 ± 0.02 [c]
Chili	0.60 ± 0.06 [c]	0.28 ± 0.65 [a]	0.07 ± 0.02 [c]
Turmeric-chili paste	0.80 ± 0.02 [d]	1.77 ± 0.38 [d]	0.05 ± 0.01 [b]

Each value is expressed as a mean + SD (n=3)
a-e means that with different letters within a column are significantly different ($p<0.05$).

Table 2. Effect of heat on total phenolic content and antioxidative properties on the extract of turmeric-chili paste

Heating of Turmeric-chili paste		Total phenolic contents (mg GAE/100g)	Scavenging radical (mg GAE/100g)	FRAP (mg GAE/100g)
Temp	**Time (min)**			
Control	0	0.81 ± 0.02^{a}	1.75 ± 0.05^{a}	0.063 ± 0.005^{a}
80 ^{0}C	10	0.82 ± 0.02^{ab}	1.77 ± 0.11^{ab}	0.062 ± 0.004^{a}
	20	0.83 ± 0.04^{ab}	1.79 ± 0.03^{ab}	0.065 ± 0.002^{a}
	30	0.85 ± 0.04^{ab}	1.81 ± 0.05^{abc}	0.079 ± 0.004^{a}
90 ^{0}C	10	0.87 ± 0.04^{abc}	1.82 ± 0.06^{abc}	0.080 ± 0.006^{b}
	20	0.89 ± 0.07^{abc}	1.85 ± 0.05^{abcd}	0.078 ± 0.003^{b}
	30	0.89 ± 0.04^{abc}	1.87 ± 0.07^{bcde}	0.078 ± 0.003^{b}
100 ^{0}C	10	0.93 ± 0.02^{bc}	1.92 ± 0.07^{cde}	0.079 ± 0.007^{b}
	20	0.97 ± 0.07^{cd}	1.95 ± 0.02^{de}	0.077 ± 0.002^{b}
	30	1.06 ± 0.13^{d}	1.98 ± 0.06^{e}	0.079 ± 0.005^{b}

Each value is expressed as a mean + SD (n=3)
a-e means that different letters within a column are significantly different ($p<0.05$).

Antibacterial activities

The bacterial loads of the garlic, galangal rhizome, turmeric rhizome, dried chili and the paste are 10^{2}-10^{3}, 10^{4}-10^{5}, 10^{4}-10^{5},10^{6}-10^{7}, and 10^{4}-10^{5} cfu/g, respectively. The results show that dried chili was heavily contaminated with bacteria, while garlic had low bacterial loads. Spices and natural agricultural seasoning materials are commonly contaminated with microorganisms including bacteria, mold and yeasts (Alemela *et al*., 2002). However, the number and type of the microorganisms may vary with harvesting, storage, transport and packaging. When these ingredients were made into curry paste, the bacterial population was reduced to around 2 log cycles (i.e. showing the MIC of 2.616 mg/ml for *S. aureus* and 5.232 mg/ml for *B. cereus*) when compared with dried chili (Table 3). This is due to the antibacterial property of allicin in garlic, which is similar to the findings of Siripongvutikorn *et al*. (2005). The results further show that only garlic could provide inhibitory activity on both *S. sureus* and *B. cereus*. When a higher concentration of garlic extract was applied the bacteriocidal activity occurred (i.e. showing the MBC of 2.616 mg/ml for *S. aureus* and 10.464 mg/ml for *B. cereus*) as shown in Table 3. The antimicrobial effect of garlic apparently results from the interaction of sulphur compounds, like allicin, with sulphur (thiol) groups of microbial enzymes such as trypsin and other proteases. This leads to an inhibition of microbial growth (Jonkers *et al*., 1999; Bakri and Douglas, 2005).

Some researchers have found that capsaicin, the main active compound for pungency or heat sensation, has an antimicrobial property against *Helicobacter pylori* (Jones *et al*., 1997). However, no antimicrobial effect of dried chili was found in this study. This may be because the capsaicin compound has less water solubility (Santamaria *et al*., 2002). Some researchers have also found that ethanolic extract of galangal, and turmeric rhizomes have an antimicrobial activity (Oonmetta-aree *et al*., 2006; Khattak *et al*., 2005). This implies that most compounds in galangal and turmeric which have antimicrobial capacity cannot be dissolved in water or the polar phase. It is also possible that the concentration and/or purity of the active compounds are not high enough to inhibit the test bacteria (Siripongvutikorn *et al*., 2005). When the ingredients were made into curry paste, it showed an even less antimicrobial effect than garlic. This suggests that the allicin from garlic was diluted by other ingredients in the paste.

Table 4 shows the effect of heat treatment on MIC and MBC of the curry paste. The antibacterial activity was evident only when heated to 80^{0}C for 10 min. It may be due to the heat sensibility of allicin in garlic, which is the main antibacterial component of the curry paste. However, there is some different in MIC and MBC value of non–heated curry paste in Table 3 and 4, this may be affected by using different lot of ingredient. Therefore, it implies that active compound contents in the raw material may vary with season, harvesting, transport and handling etc.

Table 3. Antibacterial activities of the extracts of turmeric-chili paste and its ingredients

Spices	MIC (mg/ml)		MBC (mg/ml)	
	S. aureus	***B. cereus***	***S. aureus***	***B. cereus***
Garlic	0.407 ± 0.003	0.409 ± 0.003	3.259 ± 0.001	0.815 ± 0.001
Turmeric	0	0	0	0
Galangal	0	0	0	0
Dry chili	0	0	0	0
Turmeric-chili paste	1.306 ± 0.005	1.307± 0.005	5.224 ± 0.004	2.612 ± 0.004

Each value is expressed as a mean + SD (n=3)

Table 4. Effect of heat treatment on antibacterial activities on the extract of turmeric-chili paste

Heating of Turmeric-chili paste		MIC (mg/ml)		MBC (mg/ml)	
Temp	**Time**	***S. aureus***	***B. cereus***	***S. aureus***	***B. cereus***
Control	0	1.308	2.616	1.308	5.232
80 ^{0}C	10	2.616	5.232	2.616	10.464
	20	0	0	0	0
	30	0	0	0	0
90 ^{0}C	10	0	0	0	0
	20	0	0	0	0
	30	0	0	0	0
100 ^{0}C	10	0	0	0	0
	20	0	0	0	0
	30	0	0	0	0

Each value is expressed as a mean + SD (n=3)

Conclusion

The total phenolic content and DPPH scavenging activity of turmeric-chili paste were higher than those of individual spices or herbs used to make it and they increased slightly after heating. Fresh garlic plays the key role in the paste on its ability to inhibit both *S. aureus* and *B. cereus*. However, the antibacterial activity of the paste was less when the heating temperature and time were increased. The curry paste retained its antioxidant activity even after cooking; therefore, it should be able to withstand the canning process, which would make it a valuable functional food that provides health benefits.

Acknowledgments

Financial support was provided by the Graduate School and the Nutraceutical and Functional Food Research and Development Center, Prince of Songkla University. We are grateful to Professor Dr. Buncha Ooraikul from Alberta University, Canada for his supervising and helpful comment during manuscript preparation.

References

Ahsan, H., Parveen, N., Nizam, U. K. and Hadi S. M. 1999. Pro-oxidant, anti-oxidant and cleavage activities on DNA of curcumin and its derivatives demethoxycurcumin and bisdemethoxycurcumin. Chemio.-Biological Interacttions 121: 161–175.

Alemela, L., Nieto-Sna Doval, J. M. and Lopez, F. J. A. 2002. Microbial inactivation of paprika by a high temperature short-x time treatment. Influence on color properties. Journal of Agriculture and Food Chemistry

50: 1435–1440.

Alothman, M., Hat, R. and Karim, A. A. 2009. Antioxidant capacity and phenolic content of selected tropical fruits from Malaysia, extracted with different solvents. Food Chemistry 115: 785–788.

Amarowicz, R., Pegg, R. B., Rahimi-Moghaddam, P., Barl, B. and Weil, J. A. 2004. Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. Food Chemistry 84: 551-562.

Ankri, S. and Mirelman, D. 1999. Antimicrobial properties of allicin from garlic. A review. Microbes and Infection 1: 125–129.

Bakri, I. M. and Douglas, C. W. I. 2005. Inhibitory effect of garlic extract on oral bacteria. Archives of Oral Biology 50: 645-51.

Bentoncelj, J., Dobersek, U., Jamnik, M. and Golob, T. 2007. Evaluation of the phenolic content, antioxidant activity and color of Slovenian honey. Food Chemistry 105: 822-828.

Benzie, I. F. F. and Strain, J. J. 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power" The FRAP assay. Analytical Biochemistry 239: 70-76.

Bozin, B., Dukic, N. M., Samojlic, I., Goran, A. and Igic, R. 2008. Phenolics as antioxidants in garlic (*Allium sativum* L., Alliaceae). Food Chemistry 111: 925–929.

Cao, G., Sofic, E., and Prior, R. L. 1997. Antioxidant and prooxidant behavior of flavonoids: Structure-activity relationships. Free Radical Biology and Medicine 22: 749–760.

Chaisawadi, S., Thongbute, D., Methawiriyasilp, W., Pitakworarat, N., Chaisawadi, A., Jaturongrasamee, K., Khemkhaw, J. and Tanuthumchare, W. 2005. Preliminary study of antimicrobial activities on medicinal herbs of Thai food ingredients. Bioprospecting and Ethnopharmacology 1: 111-114.

Choi, Y., Lee, S. M., Chun, J., Lee, H. B. and Lee, J. 2006. Influence of heat treatment on the antioxidant activities and polyphenolic compounds of Shiitate (*Lentinus edodes*) mushroom. Food Chemistry 99: 381-387.

Cousins, M., Adelberg, J., Chen, F. and Rieck, J. 2007. Antioxidant capacity of fresh and dried rhizomes from four clones of turmeric (*Curcuma longa* L.) grown in vitro. Industrial Crops and Products 25: 129-135.

Curtis, H., Noll, U., Judith, S. R. and Alan, J. S. 2004. Broad-spectrum activity of the volatile phytoanticipin allicin in extracts of garlic (*Allium sativum* L.) against plant pathogenic bacteria, fungi and Oomycetes. Physiological and Molecular Plant Pathology 65: 79–89.

Darrick, S. H. L. Kim, S. Y. and Kim, J. Y. 2001. Curcuminoids from *Curcuma longa* Linn. (Zingiberaceae) that protect PC12 rat pheochromocytoma and normal human umbilical vein endothelial cells from βA (1-42) insult. Neuroscience Letters 303: 57–61.

Dewanto, V., Wu, X., Adom, K. K. and Liu, R. H. 2002. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. Journal of Agriculture and Food Chemistry 50: 3010-3014.

Dini, I., Tenore, G.C. and Dini, A. 2008. Chemical composition, nutritional value and antioxidant properties of *Allium caepa* L. Var. *tropeana* (red onion) seeds. Food Chemistry 107: 613–621.

Guihua, X., Xingquian, Y., Jiachu, C. and Donghong, L. 2007. Effect of heat treatment on the phenolic compounds and antioxidant capacity of citrus peel extract. Journal of Agriculture and Food Chemistry 55: 330-335.

Hatano, T., Kagawa, H., Yasuhara, T. and Okuda, T. 1988. Two new flavonoids and other constituents in licorice root: their relative astringency and radical scavenging effects. Chemical and Pharmaceutical. Bulletin 36: 2090-2097.

Jayaprakasha, G. K., Rao, L. J. and Sakariah, K. K. 2006. Antioxidant activities of curcumin, demethoxycurcumin and bisdemethoxycurcumin. Food Chemistry 98: 720–724.

Jones, N. L., Shabib, S. and Sherman, P. M. 1997. Capsaicin as an inhibitory the growth of gastric pathogen *Helicobacter pylori.* FEMS Microbiology Letters 146: 227–233.

Jonkers, D., Van Den Broek, E., Van Dooren, I., Thijs, C., Dorant, E., Hageman, G. and Stobberingh, E. 1999. Antibacterial effect of garlic and omeprazole on *Helicobacter pylori.* Journal of Antimicrobial Chemotherapy 43: 837-839.

Juntachote, T. and Berghofer, E. 2005. Antioxidative properties and stability of ethanolic extracts of holy basil and galangal. Food Chemistry 92: 193–202.

Kim, W. Y., Kim, J. M., Han, S. B., Lee, S. K., Kim, N. D. and Park, M. K. 2002. Steaming of ginseng at high temperature enhances biological activity. Journal of Natural Products 63: 1702-1704.

Khattak, S., Saeed, U. R., Shah, H. U., Ahmad, W. and

Ahmad, M. 2005. Biological effects of indigenous medicinal plants *Curcuma longa* and *Alpinia galangal.* Fitoterapia 76: 254-257.

Kumar, G. S., Nayaka, H., Dharmesh, S. M. and Salimath, P.V. 2006. Free and bound phenolic antioxidants in amla (*Emblica officinal*) and turmeric (*Curcuma longa*). Journal of Food Composition and Analysis 19: 446–452.

Lenntte, T.H., Barilows, A., Hausler, W.J. and Shadany, H.J. 1991. Manual of Clinical Microbiology (5th ed.). American Society for Microbiology. Washington DC.

Lorian, V. 1995. Antibiotics in laboratory medicine, In Disk Susceptibility Test. J.F. Acar and F.W. Goldstein, (Eds.). Williams and Wilkins Awaverly. London.

Maria Lucia, A. B., Roberto, G. J. and Maria Beatriz, A. 2002. Influence of post harvest processing conditions on yield and quality of ground turmeric (*Curcuma longa* L.). Journal of Biology and Technology 45: 423-429.

Mayachiew, P. and Devahastin, S. 2008. Antimicrobial and antioxidant activities of Indian gooseberry and galangal extracts. Lebensmittel-Wissenschaft und Technologie 41: 1153-1159.

Miquel, J., Bernd, A., Sempere, J. M., Diaz-Alperi, J. and Ramiraz, A. 2002. The curcuma antioxidants: pharmacological effects and prospects future clinical use. A review. Archives of Gerontology and Geriatrics 34: 37–46.

Nicoli, M. C., Anes, M., Parpinel, M. T. and Franceschi, S. 1999. Influence of processing on the antioxidant properties of fruits and vegetables. International journal of Food Science and Technology 10: 94-100.

Oonmetta-Aree, J., Tomoko, S., Gasaluck, P. and Eumkeb, G. 2006. Antimicrobial properties and action of galangal (*Alpinia galanga* Linn.) on *Staphylococcus aureus*. Lebensmittel-Wissenschaft und Technologie 39: 1214–1220.

Peleg, H., Naim, M., Rouseff, R. L. and Zehavi, U. 1991. Distribution of bound and free phenolic acids in oranges (*Citrus sinensis*) and grape fruit (*Citrus papradisi*). Journal of the Science of Food and Agriculture 57: 417-426.

Pulido, R., Bravo, L. and Calixo, F. S. 2000. Antioxidant activity of dietary polyphenols as determined by as modified ferric reducing/antioxidant power assay. Journal of Agriculture and Food Chemistry 4: 3396-3402.

Pulla Reddy, A. Ch. and Lokesh, B. R. 1992. Studies on spice principles as antioxidants in the inhibition of lipid peroxidation of rat liver microsomes. Molecular and Cellular Biochemistry 111: 117-124.

Rice-Evans, C.A, Miller, N.J. and Paganga, G. 1996. Structure–antioxidant activity relationships of flavonoids and phenolic acids. Free Radical Biology and Medicine 20: 933–956.

Ruby, A. J., Kuttan, G., Baru, K. D., Rajasekharan, K. N. and Kuttan, R. 1995. Anti-tumour and antioxidant activity of natural curcuminoids. Cancer Letter 94: 79-83.

Santamaria, R. I., Reyes-Duarte, M. D., Barzana, E., Fernanda, D., Mota, M. and Lopez-Munguia, A. 2000. Selective enzyme mediated extraction of capsaicinoids and carotenoids from chilli Guajiillo Puya (*Capsicum annuum* L.) using ethanol as solvent. Journal of Agriculture and Food Chemistry 48: 3063–3067.

Shobana, S. and Naidu, K. A. 2000. Antioxidant activity of selected Indian spices. Prostaglandins, Leukotrienes and Essential. Fatty Acids 62: 107–110.

Siddhuraju, P and Becker, K. 2007. The antioxidant and free radical scavenging activities of processed cowpea (*Vigna unguiculata* L. Walp.) seed extracts. Food Chemistry 101: 10–19.

Siripongvutikorn, S., Thammarutwasik, P. and Hung, Y. W. 2005. Antibacterial and antioxidant effect of Thai seasoning, Tom -Yum. Lebensmittel-Wissenschaft und Technologie 38: 347-352.

Voravuthikunchai, S. P., Limsuwan S., Supapol, O. and Subhadhirasakul, S. 2006. Antibacterial activity of extracts from family Zingiberaceae against food borne pathogens. Journal of Food Safety 26: 325-334.

Yen, G. C. and Hsieh, G. L. 1997. Antioxidant effects on dopamine and relate compound. Bioscience. Biotechnology. and Biochemistry 61: 1646-1649.

Zaeoung, S., Plubrukarn, A. and Keawpradub, N. 2005. Cytotoxic and free radical scavenging activities of Zingiberaceous rhizomes Songklanakarin. Journal of Science Technology 27: 799-812.

Zheng, W. and Wang, S. 2001. Antioxidant activity and phenolic composition in selected herbs. Journal of Agriculture and Food Chemistry 49: 5165–5170.

Zhou, K. and Yu, L. 2006. Total phenolic contents and antioxidant properties of commonly consumed vegetables grown in Colorado. Lebensmittel-Wissenschaft und Technologie 39: 1155–1162.