Total phenolic compound content, antioxidant property and quality changes of the southern sour curry paste, keang-hleung, as affected of garcinia, *Garcinia atroviridis*, salt during storage

[1]Preeyaporn Promjiam, [1*]Sunisa Siripongvutikorn, [1]Santad Wichienchot and [2]Worapong Usawakesmanee

[1]Nutraceutical and Functional Food Research Center, Faculty of Agro-Industry, Prince of Songkla University, Songkhla 90112; [2] Department of Food Technology, Faculty of Agro-Industry, Prince of Songkla University, Songkhla 90112. *Corresponding Author: sunisa.s@psu.ac.th and sunisasiripongvutikorn@gmail.com

Abstract. Southern sour curry or Keang-hleung soup is traditional popular spicy-sour curry consumed not only in southern part of Thailand. The ingredients used in the paste are turmeric rhizome, garlic, shallot and chili which have been reported as a source of antimicrobial and antioxidant compounds. The total phenolic compound content and the antioxidant activities of Keang-hleung paste with and without garcinia were monitored. It was found the total phenolic compound content of the basic paste without the garcinia, garcinia Keang-hleung paste, garcinia- Keang-hleung paste without salt and the garcinia extract determined by the Folin-Ciocalteu method were 0.236±0.039, 0.245±0.009, 0.639±0.006 and 0.457±0.030 g GAE/100g dw respectively. The DPPH (2,2-diphenyl-1-picrylhydrazyl) activity of the basic paste, garcinia Keang-hleung paste, garcinia- Keang-hleung paste without salt and the garcinia extract were 0.658±0.010, 0.736±0.047, 0.818±0.147 and 0.018±0.001 g GAE/100g dw, while the ferric reducing power were 0.405±0.028, 0.590±0.030, 1.150±0.044 and 0.015±0.001 g GAE/100g dw, respectively. Total viable count (TVC) of all paste sample were in range of 10^2-10^3 log cfu/g. Yeast and mold counts of basic and garcinia Keang-hlueng paste were less than 30 cfu/g during storage. While, yeast and mold counts of garcinia Keang-hlueng paste without salt were less than 10^2 cfu/g during storage. Lactic acid bacteria counts of garcinia Keang-hlueng paste were less than 30 cfu/g during storage. While, lactic acid bacteria counts of the basic and garcinia Keang-hlueng paste without salt were less than 10^2 log cfu/g during storage. Moreover, *Staphylococcus aureus*, *Bacilluus cereus, Clostridium perfringens, Salmonella, Escherichia coli* and coliforms were not detected in all treatments throughout the storage period. All paste samples were accepted by panelist with higher border line score. However, addition of garcinia in paste tended to obtain higher score in all attributes compared with the basic paste.

Key words: Keang-hleung paste, southern sour curry, garcinia, antioxidant, shelf-life, Thailand.

Introduction

Free radicals are unstable highly reactive and energized molecules having unpaired electron such as superoxide, hydroxyl, peroxyl and alkoxyl. Outside the living cell, these compounds produced by sunlight, ultraviolet light, ionizing radiation, chemical reactions and metabolic processes however, they continuously produced in the human body and also controlled by endogenous enzyme (superoxide dismutase, glutathione peroxidase, catalase). An over production of these species, exposure to external oxidant substance or failure in the defense mechanisms, leads to damaging of valuable bio-molecules (DNA, lipids, proteins) which associated with and increased risk of cardiovascular disease, cancer and other chronic disease (Aruoma, 1998). In recent years human health relates to nutrition, fitness and beauty have exaggerated concerns over diet. Therefore, a new diet health paradigm is more emphasis on the positive aspects of diet.

The meaning of some Thai words such as "keang" means curry which is hot and spicy, while "som" means sour and "hleung" means yellow color as pigment derived from turmeric rhizome. Keang-Hleung or Southern sour curry soup is now popular not only in southern part of Thailand. It is also claimed as a healthy food because of low calories due to less fat but high propotion of vegetable. Since Southern sour curry normally contains many kinds of vegetables therefore it has high fiber which good for health. Moreover, the ingredients used in the paste are turmeric rhizome, garlic, shallot and chili which have been reported as a source of antimicrobial and antioxidant compounds (Ruby et al. 1995 ; Ahsan et al. 1999; Cousin et al. 2006; Jayaprakasha et al. 2006).

For cooking the sour curry soup, souring agent such as lime juice, tamarind juice, and garcinia fruit, or any sour fruit will be used if available. Hydroxy citric acid, an active compound found in garcinia fruit, a local fruit of the Southern part of Thailand, can help metabolize glucose and carbohydrates and reduce the accumulation of fat (Hayaizu et al. 2003; Soni et al. 2004). Currently garcinia powder or garcinia extract is used as weight controlling product. Therefore,

garcinia Keang-Hleung is planned to make for convenient product and may also serve some functional property. However, the addition of garcinia in the paste may alter some qualities, and then antioxidant property, consumer acceptability of the paste and the soup were investigated.

Materials and Methods

Material and reagent

Turmeric rhizomes (*Curcuma longa*), garlic (*Allium sativum*), dry finger chili (*Capsicum annuum*), shallot (*Allium ascalonicum*) and dried garcinia (*Garcinia atroviridis*) were purchased from a local market in Hat Yai, Thailand. All chemical, reagent, and media were of analytical grade and obtained from Sigma Chemical Co. (St. Louis, MQ, USA) and Merck (Darmstadt, Germany).

Keang-hleang paste preparation

All spices were sorted, trimmed and washed thoroughly to remove dust and dirt, then soaked in 150 ppm and 10 ppm of chlorine solution, respectively for 1 minute, then weighed according to recipe. They were then ground with blender (Moulinex, TYPE 276, France) to make a fine paste (20-40 mesh).

Extraction procedure

Both the paste and individual ingredient, 100 g (fresh weight) was extracted with 300 ml of distilled water then stirred with a magnetic stirrer 12 hr before being subjected to filter through cheesecloth and centrifuge at 6,000 rpm for 25 minutes. Thereafter, the supernatant was dried with freezed dryer and kept at 20 ºc until used.

Analyses

Total phenolic contents

The total phenolic content of each sample was determined using a Folin-Ciocalteu assay with slight modification (Kahkonen et al. 1999). Briefly, the reaction mixture contained 20 µl of extract, 100 µl of the Folin-Ciocalteu reagent and 80 µl of 7.5 g/100 ml sodium carbonate. After 30 min of reaction at ambient temperature, the absorbance at 765 nm was measured using Microplate Reader (PowerWaveX, Biotex, U.S.A.). A calibration curve was calculated using standard gallic acid (125, 100, 80, 60, 40, 20, 10 µg/ml, r^2 = 0.9993). The results were expressed on a dry weight basis (dw) as g gallic acid equivalents (GAE), per 100 g of dry sample.

Antioxidant properties

Free radical scavenging (DPPH•) assay

Free radical scavenging was determined using the free radical generator DPPH• (2,2-diphenyl-1-picrylhydrazyl) assay based on slight modification (Wu et al. 2003). An aliquot (150 µl) of the serially dilute extract samples was mixed with 150 µl of 0.15 mM/l DPPH• solution was added. The mixture was thoroughly mixed using a vortex and kept in the dark for 30 minutes. The absorbance, using a Microplate Reader (PowerWave, Biotex, U.S.A.), was measured later at 518 nm against a blank of ethanol without DPPH•. The results were express on a dry weight basis (dw) as g gallic acid equivalents (GAE), per 100 g of dry sample.

Ferric reducing/antioxidant power (FRAP) assay

A FRAP assay was performed using a modified method (Benzie & strain, 1996). Briefly a 15 µl of aliquot of properly diluted extract was mixed with 285 µl FRAP reagent and incubated at 37 ºc for 30 minute. The absorbance was then determined at 593 nm against a blank that was prepared using distilled water. FRAP was freshly prepare 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 (5, 10, 20, 25, 30 µg/ml, r^2= 0.9997). FRAP values were expressed on a dry weight basis (dw) as g gallic acid equivalents (GAE), per 100 g of dry sample.

Statistical Analysis

Data were subjected to analysis of variance, and mean comparison were made using Duncan's new multiple range test. Statistical analyses were carried out using the SPSS statistical software version 6 (SPSS, Inc., Chicago, IL).

Results and Discussion

Total phenolic content and antioxidant of Keang-hleung paste with and without garcinia

The amount of the total phenolic contents of the paste with and without garcinia was showed in Table 1. The result showed that addition of garcinia into the Keang-hleung paste increased total phenolic content. Since garcinia used in the paste was dried form which color was brown from Maillard reaction, therefore the brown pigment may interact with Folin reagent giving over estimation value.

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 (Hatano et al. 1998; Amarowicz et al. 2004) because of its stability (in radical form), simplicity and fast assay (Bozin et al. 2008). The DPPH free radical scavenging activities of the Keang-hleung paste with and without garcinia were presented in Table 1. The results showed that highest DPPH free radical scavenging activity was in the garcinia Keang-hleung paste without salt which was agreement with the total phenolic content. This was probably due to the high activity of the active compound of garcinia and/or the synergistic effect of various compounds used in the Keang-hleung paste. For example, Surh (2002) reported that capsaicin (trans-8-methyl-n-vanillyl-6-nonenamide) was identified as principle phenolic substance in chili. Kim et al (1997), also mentioned that a variety of sulfur-conpounds including allicin, diallyl disulfide and diallyl trisulfide appear to be main antioxidative compounds in garlic volatiles. Moreover, the active ingredients in shallot are a group of phenolic compounds such quercetin possesses antioxidant activity (Fattorusso et al. 2002). The main compound present in turmeric rhizomes are curcuminoid compounds (curcumin, demethoxycurcumin and bisdemethoxycurcumin), including curcumin, which is well known for its strong antioxidant activity (Miquel et al. 2002). The dried fruit rind of *Garcinia indica* (cv. Kokum) contains 2-3% garcinol, a polyisoprenylated benzophenone derivative (Krishnamurthy *et al*. 1981) having nearly three times greater DPPH scavenging activity than that of DL-α- tocopherol (Yamaguchi et al. 2000). However, Seah et al (2010) reported that the lowest free radical scavenging activities were found in garlic and chilli, while Keang-hlueng paste possessed the hightest DPPH free radical scavenging activity. This may due to the synergistic antioxidant activity. Shobana & Naidu (2000) reported that a spice mix (ginger, onion and garlic; onion and ginger; ginger and garlic) showed accumulative inhibition of lipid peroxidation meant that a synergistic property occurred compared with individual ones.

Ferric reducing/antioxidant power (FRAP)

The highest value of FRAP was found in the garcinia Keang-hlueng paste without salt (Table1), while the lowest value was in the basic keang-hleung paste. The antioxidant activities of phenolic compounds are mainly of redox properties, including free radical scavenging, hydrogen donating and singlet oxygen quenching (Mayachiew & Devahastin, 2008). Pulido et al. (2000) reported that the reducing capacity of polyphenols, as determined by the FRAP assay seemed to depend on the degree of hydroxylation and extent of conjugation of the phenolic compounds. Surprising, garicinia has highest phenolic content even lowest antioxidant properties when determined by DPPH radical scavenging and FRAP assays. It implied that blending of herbs/spices led to chemical reaction improved some antioxidant properties. Many researchers reported that a major phenolic compound in the garcinia plant is xanthone, a hydrophobic compound, which could be extracted with the hexane and chloroform solvent (Bennet & Lee, 1989; Minami et al. 1994; Joseph et al. 2005). This meant that using garcinia as an additional antioxidant was not successful as assumed because of the solvent system (Siripongvutikorn et al. 2009). However, in this present experiment showed higher value of total phenolic content, 0.457 $\pm$ 0.030 g gallic acid /100 g sample (dried wt) compared with 0.19 $\pm$ 0.01 g gallic acid /100 g sample (dried wt) reported by Siripongvutikorn et al. (2009). This discrepancy might be because of the differences in herb preparation, freshness, physiological characteristics, variety and the solvent used (Siripongvutikorn et al. 2009).

Table 1. Total phenolic contents and antioxidant properties of Keang-Hleang paste extraction. Each value is expressed as a mean ±SD (n=3) a-d means that with different letters within a column are significant different ($p<0.05$).

Sample	Total phenolic (g GAE/100g dw.)	DPPH value (g GAE/100g dw.)	FRAP value (g GAE/100g dw.)
Basic	0.236±0.039^{c}	0.658±0.010^{c}	0.405±0.028^{c}
Basic+garcinia with salt	0.245±0.009^{b}	0.736±0.047^{b}	0.590±0.030^{b}
Basic+garcinia without salt	0.639±0.006^{a}	0.818±0.147^{a}	1.150±0.044^{a}
Garcinia	0.457±0.030^{b}	0.018±0.001^{d}	0.015±0.001^{d}

Microbiological quality in basic and garcinia Keang-hleung paste during storage at 4±2 ºC.

The basic and garcinia Keang-hleung paste with and without salt stored at ambient temperature and 4 ºC monitored for TVC, yeasts and moulds, *staphylococcus aureus*, *Bacilluus cereus*, Lactic acid bacteria, *Clostridium perfringens, Salmonella, Escherichia coli* and coliforms were showed in Table 2. At the initial stage, TVC of all treatments were in range of 10^2-10^3 log cfu/g and increased as the storage time (Table 2). TVC of the basic and garcinia Keang-hleung paste without and with added 20% salt and kept at both could control TVC values were not more than 5×10^3 cfu/g within 4 months. However, using certain salt concentration as 20%, garcinia and chilled storage as hurdle seemed to pronounce more inhibitory effect Yeast and mold counts of the basic and garcinia Keang-hlueng paste with addition salt were under 30 cfu/g during storage. Without salt, yeast and mold counts of garcinia Keang-hlueng paste more increased and reached 10^2 cfu/g before declined to lower than 30 cfu/g at the end of storage. Lactic acid bacteria counts of the garcinia Keang-hlueng paste with added salt were less than 30 cfu/g during storage while, the bacteria counts of the basic and garcinia Keang-hlueng paste without salt were higher even were less than 10^2 log cfu/g during storage. Moreover, *Staphylococcus aureus*, *Bacillus cereus, Clostridium perfringens, Salmonella, Escherichia coli* and coliforms were not detected in all treatments throughout the storage period. This might be because of allicin function, weak acid derived from the ingredients particularly garcinia as previous described and good sanitation of procedure. Generally, washing step would have positive side as removing dust source of microorganism and negative side as added more moisture or A_w into sample therefore draining method was necessary for controlling negative result that need to be concerned.

Table 2. Microbiological quality in basic and garcinia Keang-hlueng paste with and without salt during storage at 4±2 ºC.

Treatment	Bacteria count (cfu/g)			
	Storage (weeks)	TVC	Lactic acid bacteria	Yeast and mold
Basic AT	0	1.89×10^3	3.10×10^2	<30
	2	4.70×10^3	9.70×10^2	<30
	4	4.30×10^3	6.20×10^2	<30
Basic 4ºC	2	8.70×10^2	3.20×10^2	<30
	4	1.28×10^3	4.80×10^2	<30
Garcinia Keang-hlueng paste AT	0	6.60×10^2	<30	<30
	2	4.70×10^2	<30	<30
	4	3.00×10^3	<30	<30
Garcinia Keang-hlueng paste4ºC	2	5.30×10^2	<30	<30
	4	6.90×10^2	<30	<30
Garcinia Keang-hlueng paste without salt AT	0	1.95×10^3	<30	<30
	2	4.70×10^2	3.50×10^2	5.20×10^2
	4	7.40×10^2	3.00×10^2	<30
Garcinia Keang-hlueng paste without salt 4ºC	2	5.30×10^2	3.20×10^2	4.80×10^2
	4	3.50×10^2	3.70×10^2	<30

AT=Ambient temperature.

Sensory evaluation of basic and garcinia keang hlueng paste

Sensory score of basic Keang-hlueng paste, garcinia keang-hlueng paste and garcinia keang-hlueng paste without salt (P1, P2 and P3) showed in Table 3. The results showed that there was no significantly difference between score of basic and garcinia keang-hlueng paste in terms of appearance, color, odor and overall liking. However, addition of garcinia in paste tended to increase sensory acceptability in all attributes compared with basic Keang-hlueng paste. This may due to bleaching affect of β-carotene (Anguelova & Warthesen, 2000) as function of hydroxyl acid leading to redness reducing but yellowness increasing which was a typical character of the paste. Moreover, the panelist also noted that the pate with added the garcinia was interesting not stale.

Table 3. Sensory score of basic and garcinia Keang-hlueng paste.

Treatment	Attribute			
	Appearance	Color	Flavor	Overall liking
P1	6.73 ± 1.00 [a]	7.08 ± 0.89 [a]	6.92 ± 0.93 [ab]	6.88 ± 0.86 [ab]
P2	6.85 ± 1.18 [a]	7.30 ± 1.01 [a]	7.42 ± 0.86 [a]	7.27 ± 0.96 [a]
P3	7.08 ± 0.97 [a]	7.11 ± 0.76 [a]	6.54 ± 1.10 [b]	6.76 ± 0.95 [b]

a-b means that with different letters within a column are significantly difference ($p<0.05$)
P 1 means Basic Keang-hlueng paste.
P 2 means Basic + garcinia with salt
P 3 means Basic + garcinia without salt

Conclutions

Making Keang-hleung paste with garcinia addition could improve the total phenolic content, free radical scavenging and ferric reducing antioxidant powers compared with the basic Keang-hleung paste. Using salt and garcinia help to prolong to the shelf-life of the paste. Moreover, addition of garcinia in the paste seemed to increase sensory acceptability in all attributes compared with basic Keang-hlueng paste.

Acknowledgments

The authors would like to thanks the National Research University Project of Thailand' s Office of the Higher Education Commission, Nutraceutical and Functional Food Research and Development Center (NFFRDC) and the Graduated School, Prince of Songkla University for the financial support.

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. Chemico-Biological Interactions, 121: 161-175.

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.

Anguelova, T.and Warthesen, J. 2000. Degradation of Lycopene, α-carotene, and β-carotene during lipid peroxidation. Food chemistry and Toxicology, 65: 71-75.

Aruoma, I. O. 1998. Free radicals, oxidative stress and antioxidants in human health and disease. Journal of the American Oil Chemists Society, 75: 199-212.

Bennet, G. J. and Lee, H. H. 1989. Xanthone from Guttiferae. Phytochemistry, 28: 967-999.

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.

Cousins, M., Adelberg, J., Chen, F. and Rieck, J. 2006. Antioxidant capacity of fresh and dried rhizomes from four clones of turmeric (*Curcuma longa* L.) grown in vitro. Industrial Crops and Products, 7-14.

Fattorusso, E., Iorizzi, M., Lanzott, V., and Taglialatela-Scafati, O. 2002. Chemical composition of shallot (*Allium ascalonicum* Hort.) Journal of Agricultural and food chemistry, 50: 5686-5690.

Hanato, 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.

Hayaizu, K., Ishii, Y., Kaneko, I., Shen, M., Okuhara, Y., Shigematsu, N., Tomi, H., Furuse, M., Yoshino, G. and Shimasaki, H. 2003. Effect of *garcinia cambogia* (Hydroxycitric acid) on visceral fat accumulation: adouble-blind, randomized, placebo-controlled trial. Current therapeutics research, 64: 551-567.

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

Joseph, G. S., Jayaprakasha, G. K., Selvi, A. T., Jena, B. S. and Sakariah, K. K. 2005. Antiaflatoxigenic and antioxidant activities of garcinia extracts. International Journal of food microbiology, 101: 153-160.

Kahkonen, M. P., Hopia, A. I., Vuorela, H. J., Rauha, J. P., Pihlaja, K., Kujala, T. S. and Heinonen, M. 1999. Anioxidant activity of plant extracts containing phenolic compounds. J. Agric. Food Chem, 47: 3954-3962.

Kim, S. M., Kubota, K. and Kobayashi, A. 1997. Antioxidative activity of sulfar-containing flavor compounds in garlic. Bioscience biotechnology and biochemistry, 61: 1482-1485.

Krishnamurthy, N., Lewis, Y. S. and Ravindranath, B. 1981. On the structures of garcinol, isogarcinol and camboginol. Tetrahedron Letters, 22: 793-796.

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

Miquel, J., Bernd, A., Sempere, J. M., Diaz-Alperi and Ramiraz A. 2002. The curcuma antioxidants: pharmacological effects and prospects for future clinical use. A review. Journal of Microbes and infection, 2: 125-129.

Minami, H., Kinoshita, M., Fukuyama, Y., Kodama, M., Yoshizawa, T., Sugiura, M., Nakagawa, K. and Tago, H. 1994. Antioxidant xanthone from *Garcinia subelliptica*. Phytochemistry, 36: 501-506.

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

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

Seah, R., Siripongvutikorn, S. and Usawakesmanee, W. 2010. Antioxidant and antibacterial properties in Keang-hleung paste and its ingredients. Asian journal of food and agro-industry, 3: 213-220.

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

Siripongvutikorn, S., Thongraung, c., Usawakesmanee, W., Buatoom, T. and Thammarutwasik, P. 2009. Development of instant garcinia (*Garcinia atroviridis*) Tum-Yum mix as a high acid seasoning. Journal of food processing and preservation, 33: 74-86.

Soni, M.G., Burdock, G.A., Preuss, H.G., Stohs, S.J. Ohia, S.E. and Bagchi, D. 2004. Safety assessment of (-)-hydroxycitric acid and Super Citrimaax, a novel calcium/potassium salt. Food Chemistry, 42: 1513-1529.

Surh, Y. J. 2002. Anti-tumor promoting potential of selected spice ingredients with antioxidative and anti-inflammatory activities: A short review. Food Chemistry. Toxicol, 40: 1091-1097.

Wu, H. C., Chen, H. M. and Shiau, C. Y. 2003. Free amino acid and peptide as related to antioxidant properties in protein hydrolysates of mackerel (*Scomber austriasicus*). Food research international, 36: 949-957.

Yamaguchi, F., Ariga, T., Yoshimura, Y. and Nakazawa, H. 2000. Antioxidative and anti-glycation activity of garcinol from *Garcinia indica* fruit rind. Journal of Agricultural and food chemistry, 48: 180-185.