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

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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°C, 90°C and 100°C 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 (diferuloylmethane) 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 galanga*) is used for medical purposes, such as for carminative, stomachic, antispasmodic, antichlorotic 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).
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°C until used.

Heat treatment of the curry paste extract

Samples of the curry paste extract were heated at 80, 90 and 100°C 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, r² = 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 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°C 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).

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 50°C. Stock culture from both organisms was transferred to 5 ml of Brain heart infusion broth (BHI) and incubated at 37°C for 15 h. This culture was used for the antimicrobial assay.

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°C 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 FeCl₃ 6H₂O 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, r² = 0.997). FRAP values were expressed on a dry weight basis as mg GAE/100 g dry sample.
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 R²=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 (Allothman 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 (Table 1),
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 (Table 2). A reasonable correlation
between the mean values of total phenolic contents
and FRAP of R²=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

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

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).
Antibacterial activities

The bacterial loads of the garlic, galangal rhizome, turmeric rhizome, dried chili and the paste are $10^2$-$10^3$, $10^3$-$10^4$, $10^4$-$10^5$, $10^5$-$10^6$ and $10^6$-$10^7$ 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. aureus* and *B. cereus*. When a higher concentration of garlic extract was applied the bactericidal 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°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.
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 \textit{S. aureus} and \textit{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

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

<table>
<thead>
<tr>
<th>Spices</th>
<th>MIC (mg/ml)</th>
<th>MBC (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>\textit{S. aureus}</td>
<td>\textit{B. cereus}</td>
</tr>
<tr>
<td>Garlic</td>
<td>0.407 ± 0.003</td>
<td>0.409 ± 0.003</td>
</tr>
<tr>
<td>Turmeric</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Galangal</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Dry chili</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Turmeric-chili</td>
<td>1.306 ± 0.005</td>
<td>1.307 ± 0.005</td>
</tr>
</tbody>
</table>

paste

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

<table>
<thead>
<tr>
<th>Heating of Turmeric-chili paste</th>
<th>MIC (mg/ml)</th>
<th>MBC (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>\textit{S. aureus}</td>
<td>\textit{B. cereus}</td>
</tr>
<tr>
<td>Temp Time</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.308</td>
<td>2.616</td>
</tr>
<tr>
<td>80 °C 10</td>
<td>2.616</td>
<td>5.232</td>
</tr>
<tr>
<td>80 °C 20</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>80 °C 30</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>90 °C 10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>90 °C 20</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>90 °C 30</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>100 °C 10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>100 °C 20</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>100 °C 30</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

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

References


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