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Communication

Phenolic content and antioxidant properties of green chilli paste and its ingredients

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Abstract: Green chilli paste and its ingredients (chilli, red onion and garlic) from different stages of processing were analysed for total phenolic content and antioxidant properties, i.e. total antioxidant capacity, DPPH radical scavenging activity, and β-carotene bleaching activity. The effects of processing stage on total phenolic content and antioxidant properties of green chilli paste and its ingredients were discussed, along with the correlation between the total phenolic content and the antioxidant properties.

Keywords: green chilli paste, total phenolic content, antioxidant properties

Introduction

An antioxidant refers to any substance which, when present at low concentration compared to that of an oxidisable substrate, significantly delays or prevents oxidation of that substrate. Antioxidants are divided into two groups: natural enzymatic antioxidants and non-enzymatic ones. The natural enzymatic antioxidants, e.g. superoxide dismutases, catalases and enzymes, are located mostly in peroxisomes. Natural and synthetic non-enzymatic antioxidants consist of vitamin E and related antioxidants such as vitamin C, BHT, BHA, carotenoids, glutathione and derivatives, phenolic compounds, flavonoids and alkaloids [1-2]. Foods or food materials are an important source of antioxidant compounds for human consumption. Natural antioxidants present in the diet increase the resistance to oxidative damage. Fruits and vegetables are immensely valuable not only for their nutritional value but also for their potential health functionality against various degenerative diseases [3-4].

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Green chilli paste (GCP) is a traditional food from the northern part of Thailand. In a large local market it is not unusual that about 500 kg/day of GCP are sold. GCP is made from chilli, red onion and garlic. Chilli (*Capsicum annuum* Linn.) is a rich source of phenolics and a good source of flavonoids, which of late have aroused great interest owing to their antioxidant activities. Red onion (*Allium ascalonicum* Linn.) and garlic (*Allium sativum* Linn.) are widely used vegetables in diets around the world. The antioxidant activity of *Allium* plants has mainly been attributed to a variety of sulphur-containing compounds and their precursors [5-7].

Some GCP manufacturers have been developing and improving the quality of GCP to delay spoiling. Sterilisation methods of GCP have been improved [8-9]. Normally the cooking process and storage are the main causes of the loss of nutritional value and desired physical characteristics in food and beverages [10-13]. However, thermal processing, which inactivates microorganisms and enzymes, is also the most common method for extending the shelf life of a food product [14].

In this study, the total phenolic content and antioxidant properties of GCP are investigated along with its ingredients, i.e. chilli, red onion and garlic. GCP at different stages of processing and both fresh and heat-processed ingredients are similarly examined.

Materials and Methods

Chemicals

Chemicals were purchased from the following: β-carotene, quercetin and Folin-Ciocalteu reagent from Sigma-Aldrich (USA); 1,1-diphenyl-2-picrylhydrazyl (DPPH) and linoleic acid from Fluka (Switzerland); ammonium molybdate and Tween 20 from AJAX (Australia); acetonitrile from Fluka (USA); gallic acid from Merck (USA); and BHT from BDH (UK). Reference compounds were of HPLC grade while the rest including other common chemicals were of analytical grade.

GCP and its ingredients

Unprocessed GCP was prepared by blending together roasted and peeled ingredients, i.e. chilli, red onion and garlic, and then the resulting mixture was salted. Degassed GCP was prepared by steaming and agitating unprocessed GCP contained in a bottle at 90-100^{o}C for 5 minutes before tightly capping the bottle. Sterilised GCP was prepared by heating degassed GCP at 108^{o}C for 30 minutes at a reduced pressure of 5 psi before storage. Each heat-processed ingredient was prepared in a similar manner as sterilised GCP except no salt was added in the process. All samples for analysis were freeze-dried and ground in a mortar, then kept under nitrogen at -4 ^{o}C before experiment.

All prepared GCP and its ingredients mentioned above were kindly supplied by Chiang Mai Vanusnun Co., Ltd., Chiang Mai, Thailand.

Sample extraction

A sample extract was obtained by methanol extraction [6]. A ground sample (1.5 g) was extracted with methanol (10 ml) by stirring for 1 hour at room temperature followed by sonicating for 20 minutes in an ultrasonic bath. The mixture was then centrifuged at 3000 rpm for 20 minutes, the supernatant decanted, and the above extraction was repeated with an additional 10 ml of methanol. The combined supernatant from the two extractions was used for analysis as described below.

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Total phenolic content

The total phenolic content was measured spectrophotometrically following the procedure outlined by Siddhuraju [15]. The reaction mixture contained 50% Folin-Ciocalteu reagent (0.5 ml), 20% (w/v) sodium carbonate solution (2.5 ml), and gallic acid solution or sample extract (1.0 ml). The mixture was placed in the dark for 40 minutes and the absorbance was recorded at 750 nm against a blank with a spectrometer (Perkin-Elmer: Lamda 25 UV/VIS).

Preparation of the calibration curve for total phenolic content determination was carried out using gallic acid (2-10 μg/ml). The total phenolic content was expressed based on gallic acid equivalent (GAE).

Determination of total antioxidant capacity

The assay is based on the reduction of Mo(VI) to Mo(V) by the extract and subsequent formation of a green phosphate/Mo(V) complex at acidic pH. The procedure set out by Banergee et al. [16] was followed. Standard gallic acid solution or the extract (1 ml) was combined with 3.0 ml of the reagent solution containing 0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate. The mixture was incubated at 95^{o}C for 90 minutes. After cooling to room temperature, the absorbance of the solution was measured at 725 nm.

Preparation of the calibration curve for total antioxidant capacity was carried out using gallic acid (2.5-10 μg/ml). The total antioxidant capacity was expressed based on GAE.

DPPH radical scavenging activity

Free radical scavenging activity was determined using the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical. The extract (1 ml) was added to 3.0 ml of 0.004% methanolic solution of DPPH. After 30 minutes the absorbance (A) at 517 nm was measured. The per cent inhibition was calculated as $[1 - A_{extract} / A_{blank}] \times 100$. The relationship between per cent inhibition and sample concentration was plotted to determine the IC_{50} value [16].

β-Carotene bleaching activity

The determination of the antioxidant activity as the ability to delay the bleaching of β-carotene in a water/linoleic acid emulsion was performed according to Nsimba et al [17]. To prepare the β-carotene emulsion, 0.2 ml of β-carotene solution (0.2 mg/ml in chloroform) was transferred to a round-bottom flask containing linoleic acid (20 μl) and Tween 20 (200 μl). The mixture was evaporated at 40^{o}C for 10 minutes to remove the solvent and distilled water (100 ml) was immediately added. The β-carotene emulsion (5.0 ml) was transferred to a test tube containing the test sample (0.2 ml). The mixture was shaken and placed in a water bath at 50^{o}C for 2 hours before its absorbance was measured at 470 nm.

The percent inhibition was calculated as $[1 - (A_0 - A_t)/ (A^0_0 - A^t_0)] \times 100$), where A_0 and A^0_0 are the absorbance values measured at initial time of the incubation for sample and control respectively, and A_t and A^t_0 are the absorbance values of sample and control respectively at t minutes. The relation between per cent inhibition of β-carotene oxidation and sample concentration was plotted to determine the IC_{50} value.

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Results and Discussion

Total phenolic content

Thermal processing can cause both positive and negative changes in total phenolic content of GCP and its ingredients as shown in Table 1. Heat-processed chilli and garlic have slightly lower phenolic content than their fresh counterparts while heat-processed onion has an increase of total phenolic content. On the one hand, high temperature involved in the processing might have assisted in the decomposition of complex phenolic compounds thus releasing the free phenolics leading to an increase in total phenolic content [8, 18]. On the other hand, many other studies have shown that heating process has both negative and positive effects on total phenolic content of plant materials including fruits and vegetables, depending on the type of raw materials and the groups of compounds present [13-14, 18-21]. Li et al. [22] reported that heat treatment caused a reduction in total phenolic content during the processing of purple wheat bran. Zhang and Hamauzu [13] also reported that antioxidant components in broccoli are significantly lost during cooking.

From Table 1, the total phenolic content in degassed GCP, which has also been subjected to heat treatment, is not lowered compared to that in unprocessed GCP. Apparently, oxygen removal reduces the oxidation process and inactivates enzymatic reactions in GCP. Oms-Oliu et al. [12] have also found that a low oxygen level is ideal for maintaining vitamin C and phenolic content during storage.

As in the case of onion, the total phenolic content in sterilised GCP is significantly higher than untreated GCP, which is also probably due to the same reason, i.e. the release of free phenolic compounds from some more complex molecules upon sterilisation.

Table 1. Total phenolic content and total antioxidant capacity of GCP and its ingradients

Sample		Total phenolic content (mg GAE/ mg DPM)	Total antioxidant capacity (mg GAE/ mg DPM)
Chilli	Fresh	3.42±0.08	14.91±0.75
	Heat-processed	2.69±0.02	14.22±0.60
Red onion	Fresh	10.59±0.34	10.98±0.60
	Heat-processed	12.82±0.46	11.44±0.30
Garlic	Fresh	2.00±0.13	6.65±0.89
	Heat-processed	1.82±0.07	7.14±0.36
Unprocessed GCP		5.93±0.26	18.22±0.49
Degassed GCP		5.97±0.38	24.89±0.64
Sterilised GCP		7.88±0.38	18.78±0.55

Note: GAE = gallic acid equivalent; DPM = dry plant material;
Results are reported with ± SD.

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Antioxidant properties

The antioxidant properties were investigated using in vitro methods. The total antioxidant capacity is shown in Table 1. Fresh and heat-processed chilli apparently has a higher level of total antioxidant capacity than that of red onion or garlic. An earlier study also reported that chilli has a strong antioxidant activity [21]. Banerjee et al. [16] reported that the phenolics of green pepper (*Piper nigrum* L.) has higher DPPH radical scavenging capacity than the acetone extract of nutmeg mace (*Myristica fragrans*).

Heat processing is observed in this study to have pronounced effects on the three ingredients of GCP. In addition, removal of chilli skin prior to heat processing may result in the loss of some important compounds in chilli such as vitamin C, tocopherol, β-carotene and alkaloids, with consequent reduction of antioxidant activities of heat-processed chilli compared to the fresh one. However, they are enhanced in heat-processed red onion and garlic (Tables 1-2). In this case, it is most probable that heat processing has released such compounds as free aglycones or Maillard products that can reduce Mo(VI) to Mo(V) and also react as an electron donor or transfer a hydrogen atom to the DPPH radical, thus increasing the antioxidant properties.

From Tables 1-2, the antioxidant capacity and DPPH radical scavenging activity of both types of processed GCP apparently increase compared to unprocessed GCP. Earlier studies showed that sterilisation causes changes in the texture, colour and flavour of GCP [8, 23]. Sterilisation also produces a bitter taste in GCP. The main bitter compounds, identified as catechins, were shown to increase upon sterilisation [8]. This result supports the hypothesis that sterilisation releases some phenolic compounds and these products increase the antioxidant properties. Randhir et al. [18] found that thermal processing of sprouts and seedlings of wheat, buckwheat, corn and oat causes changes in their health-relevant functionality and suggested that these changes are due to modifications in the total phenolic content leading to a higher content with consequent increase in scavenging-linked antioxidant activity. However, the antioxidant capacity of sterilised GCP is seen to be lower than that of degassed GCP, which may be accounted for by the postulate that certain compounds with reducing property might have been adversely affected by the sterilisation process.

In attempting to correlate the total phenolic content with antioxidant properties, one can see that the correlation is rather poor. Among the three ingredients, fresh and heat-processed chilli is second in total phenolic content, yet it is strongest in both antioxidant properties. Red onion has the highest total phenolic content although it shows only medium levels in both antioxidant properties (Tables 1-2). In this regard, it is clear that the antioxidant properties may not only come from a phenolic group [6]; substances such as capsaicin and sulfur compounds may also be associated with the antioxidant properties of chilli and garlic [24]. Conversely, it is well known that not every phenolic substance is a good antioxidant. This may be evident from a result in Table 1, in which sterilised GCP, though highest in total phenolic content among the three GCPs, is not highest in total antioxidant capacity.

The β-carotene bleaching method is based on the loss of the yellow colour of β-carotene due to its reaction with radicals formed by linoleic acid oxidation in an emulsion. The rate of β-carotene bleaching can be slowed down in the presence of an antioxidant. However, it can be seen in this experiment that the different processing steps did not seem to affect the antioxidant property of GCP as

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determined by the β-carotene bleaching method. The IC_{50} remains relatively constant as shown in Table 2. Conforti et al. [25] reported that a high level of phenolic content and an acyclic diterpene alcohol (phytol) in green pepper fruit are responsible for the inhibition of lipid peroxidation, although this effect takes place only at the ripening stage of the fruit. Furthermore, although the correlation between total phenolic content and antioxidant activity by the DPPH test was found for small green and red pepper extracts, no correlation was found for β-carotene bleaching test and bovine brain peroxidation assay.

Table 2. DPPH radical scavenging and β-carotene bleaching activities of GCP and its ingredients

Sample		IC_{50} (mg/ml)	
		DPPH method	β-Carotene method
Chilli	Fresh	2.12±0.02	-
	Heat-processed	2.33±0.11	-
Red onion	Fresh	4.00±0.34	-
	Heat-processed	3.75±0.28	-
Garlic	Fresh	19.83±0.77	-
	Heat-processed	17.63±0.93	-
Unprocessed GCP		3.48±0.15	1.02±0.02
Degassed GCP		3.07±0.12	1.10±0.05
Sterilised GCP		2.67±0.08	1.07±0.03
Standard	BHT	8.31±0.18*	0.54±0.01*
	quercetin	0.31±0.01*	4.37±0.06*

Note: - = Not investigated; * = in μg/ml;
Results are reported with ± SD.

Conclusions

The total phenolic content and antioxidant properties of green chilli paste and its ingredients are observed to be affected on being processed. The overall effect of processing, though not apparently extensive or outstanding, nevertheless seems to be somewhat beneficial to both of the green chilli paste products (i.e. degassed and sterilised) compared to the unprocessed product.

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References

1. S. I. Baskin and H. Salem, "Oxidants, Antioxidants, and Free Radicals", Taylor and Francis, New York, **1997**.
2. B. Halliwell, R. Aeschbach, J. Loliger and O. I. Aruoma, "The characterization of antioxidants", *Food Chem.*, **1995**, *33*, 601-617.
3. N. Deepa, C. Kaur, B. George, B. Singh and H. C. Kapoor, "Antioxidant constituents in some sweet pepper (*Capsicum annuum* L.) genotypes during maturity", *LWT-Food Sci. Technol.*, **2007**, *40*, 121-129.
4. B. Dimitrios, "Sources of natural phenolic antioxidants", *Food Sci. Technol. Res.*, **2006**, *17*, 505-512.
5. F. Ferreres, M. I. Gil and F. A. Tomas-Barberan, "Anthocyanins and flavonoids from shredded red onion and changes during storage in perforated films", *Food Res. Int.,* **1996**, *29*, 389-395.
6. A. M. Nuutila, R. Puupponen-Pimia, M. Aarni and K. M. Oksman-Caldentey, "Comparison of antioxidant activities of onion and garlic extracts by inhibition of lipid peroxidation and radical scavenging activity", *Food Chem.*, **2003**, *81*, 485-493.
7. K. S. Yoo and L. M. Pike, "Determination of flavor precursor compound S-alk(en)yl-L-cysteine sulfoxides by an HPLC method and their distribution in *Allium* species", *Sci. Hortic.*, **1998**, *75*, 1-10.
8. K. Leelasakultum, "Study on bitter compounds in green chilli paste" (in Thai), Special Problem, **2004**, Department of Chemistry, Chiang Mai University, Thailand.
9. M. Wayseangjarean, "Development of green chilli paste in bottle" (in Thai), Research Report, **1999**, Department of Agro Industry, Chiang Mai University, Thailand.
10. G. N. Gayathri, K. Platel, J. Prakash and K. Srinivasan, "Influence of antioxidant spices on the retention of β-carotene in vegetables during domestic cooking processes", *Food Chem.*, **2004**, *84*, 35-43.
11. L. Greco, R. Riccio, S. Bergero, A. A. M. Del Re and M. Trevisan, "Total reducing capacity of fresh sweet peppers and five different Italian pepper recipes", *Food Chem.*, **2007**, *103*, 1127-1133.
12. G. Oms-Oliu, I. Odriozola-Serrano, R. Soliva-Fortuny and O. Martin-Belloso, "The role of peroxidase on the antioxidant potential of fresh-cut 'Piel de Sapo' melon packaged under different modified atmospheres", *Food Chem.*, **2008**, *106*, 1085-1092.
13. D. Zhang and Y. Hamauzu, "Phenolics, ascorbic acid, carotenoids and antioxidant activity of broccoli and their changes during conventional and microwave cooking", *Food Chem.*, **2004**, *88*, 503-509.
14. I. Odriozola-Serrano, R. Soliva-Fortuny and O. Martin-Belloso, "Changes of health-related compounds throughout cold storage of tomato juice stabilized by thermal or high intensity pulsed electric field treatments", *Innov. Food Sci. Emerg. Technol.*, **2007**, *9*, 272-279.
15. P. Siddhuraju, "Antioxidant activity of polyphenolic compounds extracted from defatted raw and dry heated *Tamarindus indica* seed coat", *LWT-Food Sci. Technol.*, **2007**, *40*, 982-990.
16. A. Banerjee, N. Dasgupta and B. De, "In vitro study of antioxidant activity of *Syzygium cumini* fruit", *Food Chem.*, **2005**, *90*, 727-733.

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17. R. Y. Nsimba, H. Kikuzaki and Y. Konishi, "Antioxidant activity of various extracts and fractions of *Chenopodium quinoa* and *Amaranthus* spp. seeds", *Food Chem.*, **2008**, *106*, 760-766.
18. R. Randhir, Y. N. Kwon and K. Shetty, "Effect of thermal processing on phenolics, antioxidant activity and health-relevant functionality of select grain sprouts and seedlings", *Innov. Food Sci. Emerg. Technol.*, **2007**, *9*, 355-364.
19. C. Ewald, S. Fjelkner-Modig, K. Johansson, I. Sjoholm and B. Akesson, "Effect of processing on major flavonoids in processed onions, green beans, and peas", *Food Chem.*, **1999**, *64*, 231-235.
20. K. Lombard, E. Peffley, E. Geoffriau, L. Thompson and A. Herring, "Quercetin in onion (*Allium cepa* L.) after heat-treatment simulating home preparation", *J. Food Compos. Anal.*, **2005**, *18*, 571-581.
21. N. Turkmen, F. Sari and Y. S. Velioglu, "The effect of cooking methods on total phenolics and antioxidant activity of selected green vegetables", *Food Chem.*, **2005**, *93*, 713-718.
22. W. Li, M. D. Pickard and T. Beta, "Effect of thermal processing on antioxidant properties of purple wheat bran", *Food Chem.*, **2007**, *104*, 1080-1086.
23. R. Pibunchaisit, "High pressure technique for improvement of shelf-life in green chilli paste" (in Thai), Research Report, **2006**, Department of Agro Industry, Chiang Mai University, Thailand.
24. K. Kogure, S. Goto, M. Nishimura, M. Yasumoto, K. Abe, C. Ohiwa, H. Sassa, T. Kusumi and H. Terada, "Mechanism of potent antiperoxidative effect of capsaicin", *Biochim. Biophys. Acta*, **2002**, *1573*, 84-92.
25. F. Conforti, G. A. Statti and F. Menichini, "Chemical and biological variability of hot pepper fruits (*Capsicum annuum* var. *acuminatum* L.) in relation to maturity stage", *Food Chem.*, **2007**, *102*, 1096-1104.