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Research Article

Assessment of active ingredients in pickled tea

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Abstract

Miang (pickled tea) is a favourite light-meal for some people in certain areas of Thailand, particularly the north, and it has been found to play a role in various native cultures. In order to develop Lampang miang products for a wider market, relationships between processing conditions and quality of miang were studied. Apart from substances affecting miang flavour and appearance, phenolic compounds [EGCG (epigallo-catechin-gallate), EGC (epigallo-cathechin) and cathechin] and caffeine were reported as the most important functional substances in fermented tea products and also having significant impact on the product's final quality. Assam and Oolong tea leaves; fresh, steamed and fermented Assam tea leaves (miang); from Pa Miang Village, Jae Son Sub-district, Jae Hom District, Lampang Province were examined to determine EGCG, EGC and caffeine contents using high performance liquid chromatography (HPLC), together with total phenolic compound determination using spectrophotometry. Small amounts of cathechin (1.34-8.71mg/g) were found in all miang samples while relatively high EGCG contents (range from 18.50 to 37.24 mg/g) were found to vary among treatments with total phenolic compounds around 26.24 – 48.76 mg/g. A higher EGCG and EGC content was presented in miang produced from younger fresh (50.53 mg/g) and steamed tea leaves (94.78 mg/g), compared with older fresh ones (48.12 mg/g). Both higher phenolic compounds and caffeine were found in Assam tea leaves compared with Oolong leaves. The miang produced from steamed tea leaves was found to contain more caffeine than from fresh leaves. It could be concluded that EGCG is the obvious phenolic compound in miang products and the conditions that increase the phenolic compound contents appear to affect the caffeine contents in the same manner.

Keywords: fermented tea, miang, *Camellia sinensis*, EGCG, HPLC, phenolic compounds, caffeine, Thailand.

Introduction

Miang or fermented Assam tea leaf is considered a lifestyle staple of northern people in Thailand, particularly hilltribes, which is where it likely originated. Miang traditionally consists of steamed tea leaves with salt, oil, garlic, pig fat and dried fish, all rolled into a ball [1], or the fermented leaf alone may be consumed. It can be taken in the form of snack, a light food for welcoming house guests, or used in various northern ceremonies [2]. Miang gardens are usually incorporated amongst highland forests, hillsides and slopes, as well as higher areas with plentiful water where it flourishes. Miang cultivation is achieved by seed culture and seedlings are usually planted in the intervening space between two large trees. Miang culture thus has a relatively benign effect on the environment. There is no need to apply fertilizer and maintenance involves occasional trimming and weed removal. The villagers will collect miang 4-5 times a year by picking the miang leaf and tying it into small bunches before steaming to make it cooked, prior to fermentation which may be for 2-3 months or longer. It can be consumed in the form of sour miang, astringent miang or cooked miang with various flavours as desired. It is particularly favoured by hilltribes who embark on extensive foot journeys throughout the mountain ranges as it seems to provide energy and prevents the mouth from becoming dry at these high elevations [3].

Phenolic compounds, in particular catechin, are a group of main astringent and bitter substances in tea and contain obvious antioxidant properties [4]. The phenolic compounds affect miang flavour in the same manner. Furthermore, in miang processing, the tea leaves have to be steamed before fermentation (fresh, or partially steamed leaves will spoil before the transition into miang).

The assumption is that the steaming process makes some compounds in tea leaf change and become amenable for particular fermenting microbes to grow, while other potentially harmful microbes cannot grow, even if the process is conducted under non-sterile conditions [5, 6]. Thus, the phenolic compounds should be the first choice in microbial selection due to their broad microbial inhibition properties. For this reason, the phenolic compounds need to be studied quantitatively in order to determine the potential of using their levels as an indicator of miang quality.

Caffeine is an alkaloid that can stimulate the nervous system and heart. It makes us vigorous, fresh and removes sleepy condition. Caffeine is found in tea, coffee, soft drinks and food containing cocoa products. The amount that exhibits nervous system stimulation ranges from 50 to 200 milligrams and any amount over than 200 milligrams will likely cause headache, stress and hypertension, while consumption in excess of 1,000 milligrams will cause toxicity called “caffeinism” characterized by overactive, fidgeting, fast heart beat, nausea, loss of appetite and more frequent urination behaviour. These symptoms readily occur in those persons who rarely take caffeine, while those who take it regularly may build tolerance. Dry tea leaf contains about 1.4 - 3.5% caffeine, 1 – 30% tannin and organic substances make up the remainder. One cup of tea, made by a teaspoon of dry tea leaf, contains about 0.1 gram caffeine [7]. The caffeine can react with the nervous system and cause addiction. Thus, tea products or additives used as foodstuff constituents should contain the least amount of caffeine. One suggested standard is that caffeine should not be consumed more than 50 milligrams per drink or not more than 200 milligrams per day [8]. While the standards for caffeine content are yet to be clearly defined, study of the caffeine amount in miang is necessary to provide information to prevent miang consumers from harm through excess consumption [9]. The quantitative studies of various functional chemicals in miang were conducted in order to obtain knowledge for

miang quality development while recognition was given to the need for conservation of native northern people's lifestyle at the same time [2, 3].

Definition

- Miang (Pickled Tea) means a type of food product made from steamed Assam tea leaf that may undergo lactic acid fermentation once.
- Miang Som (Northern term which scholars often refer to as "Miang Prew") means miang made by steamed tea leaf, left overnight, and then fermented until it develops a sour taste before it is ready for consumption.
- Astringent Miang means the miang produced from young tea leaves. It is then tied and steamed before consumption, or it can be fermented immediately after steaming in order to be kept longer without becoming sour.
- Miang plant means the Assam tea (*Camellia sinensis*) found in the Northern Thailand.
- Miang Kum refers to a snack using fresh leaves of *Piper sermentosum* with added condiments. It is unrelated to true miang.

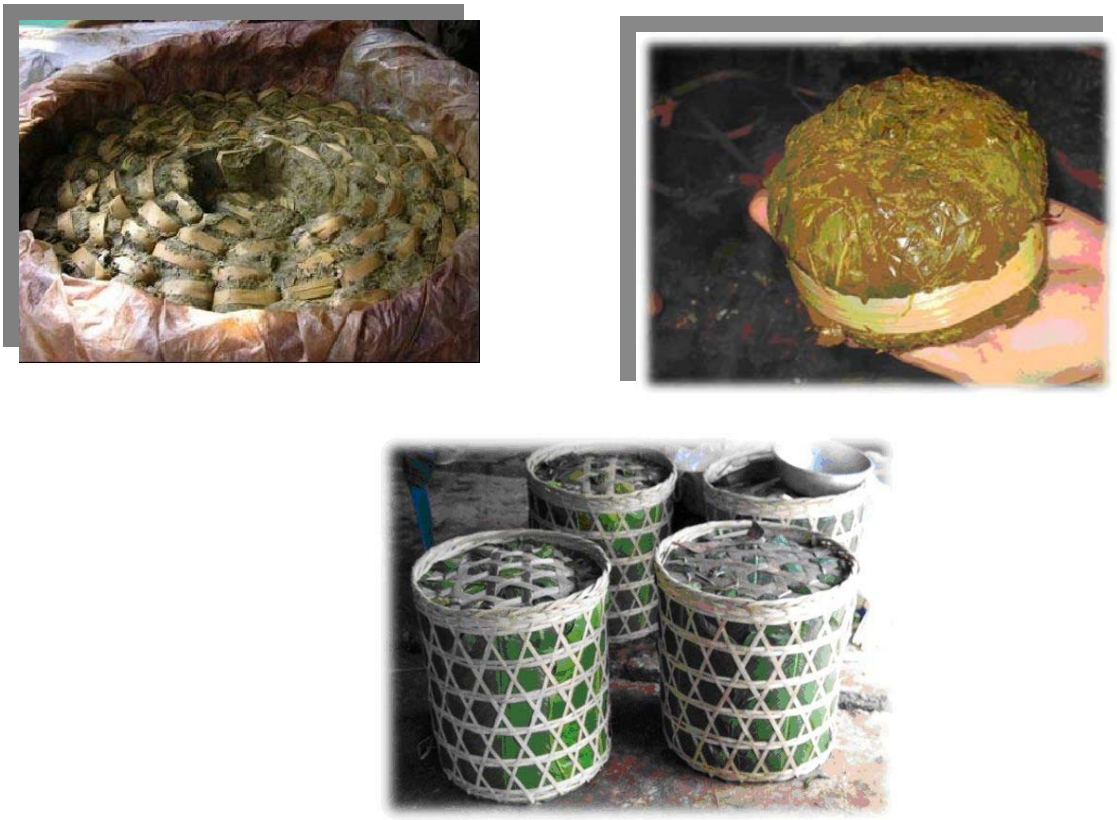

Figure 1. Commercial miang from northern Thailand.

Materials and Methods

Miang samples were collected from cultivating areas including Ban Pa Pan, Sankampang District, Chiang Mai; Ban Pa Miang, Ban Mae Jam and Ban Mae Keae, Muangpan District, Lampang (the quality of the selected samples were based on standards applied by the private sector purchasers in the miang business) and were stored in a refrigerator prior to analysis.

Samples were extracted for phenolic compounds for analysis as follows:
The miang samples were ground and soaked in ethanol for 15 minutes. They were then filtered with a membrane filter (pore size 0.45 micron) and examined for phenolic compounds such as epigallocatechin (EGC), epigallo catechin gallate (EGCG) and catechin using HPLC analyser. Three replications were conducted for each analysis. Retention time from each peak was compared to standard known concentration reagents for certain compound quantities.
Ground miang sample extractions for caffeine determination were carried out as above, but 0.1% phosphoric acid solution + acetonitrile (9:1) was used as the solvent instead of ethanol.

Results

It was found from the preliminary analysis that all miang samples contain too small amounts of catechin to be detected. It was thus decided to analyze fresh and non-fermented steamed tea leaves (or astringent miang) together with comparing the amount of certain substances in fresh tea leaves from other sources in order to determine whether the steaming and fermenting processes affected to the phenolic compound contents or not. The experiments also covered the substances like epigallocatechin (EGC) and epigallocatechin gallate (EGCG) which belong to the tannin group and take responsibility for the astringent-bitter taste, which contribute to the main tea and miang flavours [10]. These substances also possess antioxidant properties, especially EGCG which the best one of this group.

The results of the analysis are shown in Table 1. It was found that the phenolic compound contents in miang from each area were different according to local people's preferences or market trends that relied on miang flavour. In the other words, the market favoured miang with high phenolic compound content and younger miang which was made from the fully-bloomed 1-2 tip leaves only. This is the most desirable miang for the commercial market because it has good flavour and appearance, together with the highest content of phenolic compounds. The lesser favoured ones were the miang from Ban Pa Miang, Ban Pa Pan and Pan Mae Ka, respectively. However, the miang from Mae Jam contained relatively the same amount of phenolic compounds as the miang from Pa Pan, but the popularity was different. It was possible that the popularity depended on marketing trends rather than flavour. Nevertheless, the phenolic compounds content was easily changed by various factors such as amount of water, nutrient elements, temperature, light, destruction from insects and other pests including individual genetic factors. Even a single plant can exhibit a different amount of interesting substances when harvested during different periods [11]. Thus, it can be said that the environmental factors have more effect on phenolic compound contents than genetic factors, resulting in tea leaves from different areas which are varied in terms of genetic and environmental factors containing different amounts and patterns of phenolic compounds.

For the effect of steaming, it was found that steamed miang contained higher total phenolic compounds (both EGCG and EGC) compared with non-steamed samples. This might be due to the phenolic compounds in fresh leaves being all in polymerized (undetectable) form and they may be decomposed by heat into single molecules. Due to catechin (the smallest molecule

monitored) being absent in the miang samples, it can be deduced that the fermenting microbes may use this substance as their carbon source because of its simple structure and is easy to hydrolyze.

Table 1. Essential chemical constituents in miang leaf from various areas.

Sample	Amount (Unit : mg/g Miang leaf)				
	EGCG	EGC	catechin	total phenolic	caffeine
sour miang (Pa Miang)	37.24	11.52	UDL	48.76	2.76
sour miang (Mae Jam)	18.86	14.36	UDL	33.22	1.37
sour miang (Mae Ka)	19.59	6.65	UDL	26.24	1.42
sour miang (Pa Pan)	18.50	14.35	UDL	32.85	1.34
young miang (Pa Miang)	35.04	15.49	UDL	50.53	2.59
fresh miang leaves	43.46	4.66	UDL	48.12	6.21
astringent miang	76.90	17.88	UDL	94.78	8.71
dried fresh miang leaves 1	49.10	4.80	0.25	56.45	1.66
dried fresh miang leaves 2	6.50	UDL	UDL	6.50	2.07
dried fresh miang leaves 3	62.8	9.50	0.36	75.96	1.55
Oolong tip No. 12	3.86	UDL	UDL	3.86	-
Oolong tip No. 17	0.34	UDL	UDL	0.34	-

Note: UDL means the amount was too low to detect (< 10 ppm)

In terms of caffeine content, the sour miang and younger miang from Pa Miang contained the highest caffeine content which was showed a similar trend to all the other miang samples except the sample from Mae Ka which still had a higher caffeine content than the miang from Mae Jam and Pa Pan, but total phenolic compound content, in particular EGC, was slightly lower.

In examining the steaming process, the steamed miang and astringent miang contained higher caffeine than fresh tea leaf (8.71 and 6.21 units, respectively). This may be due to the caffeine normally accumulated in cell sacs being dissolved when the cell walls were broken down by heat. After the miang was fermented, lower levels of caffeine remained in the product. After being dried with a microwave until crisp products were obtained, 3 tea leaf samples also demonstrated lower caffeine content but higher amounts of phenolic compounds were found (microwave contributes a vibration wave that generates heat and causes water to evaporate from products).

Considering the amount of caffeine the body gets through the consumption of tea (50 mg per time), miang consumption should not exceed 35 g (dry leaf) per time and should not be over 140 g per day. However, although tea products have been restricted to not exceed 50 mg per time, miang is different in that it is consumed as a form of wet solid (whole leaf) which contains more concentrated substances including the caffeine. Furthermore, a normal amount of miang must contain more substances than a cup of tea (a cup of tea is made from 1 teaspoon of tea leaf), so this cannot be used as standard for miang because the caffeine contents will surely be over the suggested limited amount for all samples. This is main reason why miang product standards should be established on the basis of adequate research data.

Conclusions

From the results of the analysis it was found that both miang and Assam tea leaf samples contained catechin at such low levels it could not be detected, whereas some samples had a relatively high level of EGCG content. The levels varied due to location, processing and storage methods.

The caffeine content in all samples ranged from 1 to 3 mg per g of dry leaf which was regarded as safe according to recommended amounts which can be consumed without being harmful to health (200 mg). It was found that a regular portion of standard miang may contain up to 66 g, while sour miang may reach 200 g.

It is suggested that further analysis and sampling of miang should be extended to larger population sizes, with caffeine being the only substance monitored. The parameters may then be compared to other foodstuffs such as cocoa in order to establish the proper caffeine standard for miang which is more reliable and is determined on the basis of adequate research data.

The relationship amongst various active substances (phenolic compounds, caffeine and other leaf constituents; for example; starch, sugar, nitrogen and organic acids) and cultivation or harvesting factors (area, soil, air, age of leaf) including processing conditions (fermentation time, predominant microbes) should be further studied in order to obtain additional information for further improving and optimizing the quality of miang.

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