Dynamic Changes in Chemical Constituents during Processing of Miang (Thai Fermented Tea Leaf) in Various Degree of Tea leaf Maturity

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***Abstract*—Miang (Thai fermented tea leaves) was made from wild tea leaves (*Camellia sinensis* var *assamica*) by traditional process with associated microorganism by natural. In this study we focus on to examine the chemical constituents as tea polyphenols, major catechins, free amino acids (FAAs) and organics acids belong with pH that dynamic changes during traditional Miang processing in various degree of tea leaf maturity (young and mature leaf). To indicate the level of fermentation which is related to Miang quality and provides the potential benefit for human health. This present study is the first report that the organic acids considered to be key components of Miang fermentation are acetic acid, citric acid, fumaric acid and lactic acid. The pH value in mature Miang reduced faster than young Miang, thus astringent Miang should be made by young tea leaves with optimal fermentation time 10-20 d, pH 5.33-5.34, sour Miang could be made by young or mature tea leave, completed fermentation time 30 d, pH 4.49-4.75. The predominant catechin composition in young and mature Miang for 30 d of fermentation are EGCG, C, EGC, EC, ECG and GCG. The highest caffeine content was in young Miang at 20 d (6.24%DW), and in mature Miang at 30 d of fermentation (6.23%DW). The predominant FAAs in young and mature Miang was theanine which was responsible for umami taste in Miang.**

***Index Terms*—Miang, fermented tea leaves, chemical constituents, organic acid, caffeine, processing, leaf maturity**

I. Introduction

Tea is the second most popular beverage in the world after water, its made from the dried leaves of evergreen shrub *Camellia sinensis* of which there are three main varieties*: C. sinensis* var *sinensis* (China), *C. sinensis* var *assamica* (Assam) and *C. sinensis* var *cambodiensis* (Cambodia) [1] that it was infused in boiling water. Many varieties of traditional fermented tea products, including dark tea, kombucha tea and pickled tea are making an attractive products. Among them, Miang is a kind of edible pickled tea, it can be consumed like snack or after meal. Miang made from Assam wild tea leaves and non-salted traditionally fermented, produced in the mountainous areas of northern Thailand, particularly hill tribes group, which is where it likely originated of Miang tea [2], [3]. After fermentation process of Miang, it has a unique pickled, sour and flowery flavor, astringent or sour taste depending on the duration of fermentation and aging of tea leaves, the golden yellow color of steamed tea leaves developed to dark yellow color and the pulp very soften when it completed fermentation [4]. Miang quality is classified by local people or Miang producer with their organoleptic evaluation, that it mainly divided into two kinds by the fermentation time and the taste of products as either astringent Miang or sour Miang [3] and sometimes divided by the raw material or the appearance of product as either young Miang or mature Miang. Accordingly, there has not been any exact criteria for the classification or establishing the characteristics quality standard of Miang product.

Nowadays, the popularity of many verities of tea products consumption has been increased with the reason that it contributes to human health benefits. However, Miang, edible or chewing pickled tea, has very less report that can revealed the effect of pickled tea consumption on human health benefits because of the limited information of chemical component in Miang were studied and the problem of Miang quality which do not have manufacturing standard and the quality control that always occur when the product quality is decided by organoleptic evaluation or fake responsible of human.

Pickled tea products are found in several countries in Asia. There are different names in each country, include: Suancha, Yancha and Liangpan-tea in China, Awa-bancha and Goishi-cha in Japan, Laphet in Myanmar and Miang in Thailand and Laos [5]. Recently reports show the high levels of gallic acid in pickled tea by anaerobic solid-state fermentation reached to the level in *Galla chinensis* as can be functional tea was reported [5], the resorcinol, a specific- antioxidant in Awa-ban tea, was identified as a novel antioxidant [6], the caffeine and the catechins contents in Miang tea produced in Thailand were analyzed [3]; [7] as well as the polyphenol content of Laphet was also analyzed [8]. Besides the aroma components of Miang examined [9]. Other investigations

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have focused on the identification of microbial strains that play an important role in the fermentation of pickled tea [2], [4], [10]-[12].

Therefore, in order to describe the Miang quality and reveal evidently of the functional components which will promote the health benefits of edible pickled tea. As well as, to apply the Miang standard production instead of the traditional process which it may be caused of problems related to the defective in Miang product. This study aimed to investigate changes in chemical constituents during Miang processing by measuring pH, total acidity, polyphenol, flavonoids, soluble sugar, catechin composition, free amino acids and organic acids that indicated the level of fermentation and provides potential of Miang as a source of functional food. Further investigation on the characteristics quality of Miang produced by difference degree of Tea leaf Maturity also will be discussed.

II. Materials and Methods

A. Preparation of Miang Samples

Tea leaves were obtain from Miang producer in Chiangmai province in the mountainous areas of northern Thailand. At the village, wild tea leaves (*Camellia sinensis* var. *assamica*) were plucked from the branches (June, 2015), starting at first leaf through sixth leaf by their degree of maturity, young and mature fresh tea leaves were wrapped together tightly individual bundles, all the bundles of tea leaves were placed in a wooden container, steamed with boiling water until the tea leaves became to yellowish in color, steamed tea leaves were spread out on the mat and cooled at room temperature (25 ℃) and separated to young leaves and mature leaves by the skill of Miang producer then they were wrapped tightly individual bundles, packed in plastic bags, kept in bamboo basket containers, pressed tightly and weighted down, covered with banana leaves and plastic sheets and were fermented at room temperature by natural way for 30 days.

B. Collection of Tea Leaves Samples

Tea leaves samples were collected for Miang processing by dividing into young and mature leaves from plucked tea leaves (fresh leaves), steamed leaves, and under the fermentation from the containers on days 0, 10 , 20 and 30 of fermentation period. Tea leaves samples of each stage in Miang processing were immediately taken for determination of pH and the titratable acidity content. About 50 g of young and mature leaves of plucked leaves (fresh leaves), steamed leaves and the fermented tea leaves (0d,10d, 20d and 30d) were dried by a rotary evaporator under reduce pressure and finally dried using vacuum-concentration at 55 ± 2 ℃. These dried tea leaves samples were prepared for analysis of the quality characteristics of Miang such as tea polyphenols, flavonoids, soluble sugars, caffeine, catechin composition (C, EC, EGC, EGCG, ECG and GCG), free amino acids and organic acids on the Key laboratory of Tea science of Ministry of Education & Hunan Provincial, Hunan agricultural university, Changsha, Hunan, China.

C. Determination of pH and the Titratable Acidity Content

Tea leaves samples (10g) were added into 90 ml distilled water and blending for 15 min at high speed in a stomacher, then the mixed homogenate was filtered through filter paper No.4 (125 mm Ø; Whatman TM, UK).The filtrate was used to measure the pH value in triplicated with a pH meter (Mettled Toledo, FE20-kit, UK). And a titratable acidity was determined by the method of NaOH titration.

D. Determination of Tea Major Chemical Components

The major chemical component of tea leaves samples, including tea polyphenols, flavonoids, and soluble sugars were determined by using a Uv-vis spectrometry [13], [14]

E. Determination of Caffeine, Catechin Composition, Free Amino Acids, and Organic Acids by HPLC

Dried tea leaves samples (3g) were extracted with 450 mL boiling ultrapure water in a bottle covered with a lid in a boiling water bath for 45 min with shaking (3-5 times), and the extracted solutions were filtrated by cotton gauze into 500 mL constant volume with ultrapure water. After cooling, the samples were filtered through filter paper (0.45 µm) for analysis of catechin composition, caffeine, free amino acids and organic acids by high performance liquid chromatography (HPLC).

The composition of catechin and caffeine in the extract was determined using HPLC (LC-2010AHT Shimadzu, Japan; column: Ecosil C18 (150 mm × 4.6 mm, 5 µm).The detection wavelength was 278 nm. The mobile phase (A) was ultrapure water, and the mobile phase (B) was N, N-dimethyl form amide: methanol: glacial acetic acid (40:2: 1.5). The operating column temperature was maintained at 30 °C. The injection volume was 10 µL, and the flow rate was 1.0 mL/min. For the detection, pure catechin and caffeine were used as standard [15]

Free amino acid compositions were determined using HPLC (LC-2010AHT Shimadzu, Japan; column: AccQ. Tag TM (150 mm × 3.9 mm, 5 µm).The detection wavelength was 248 nm. The mobile phase (A) was AccQ.TagTM Eluent A concentrate diluted 1:10 with ultrapure water while the mobile phase (B) was 60% (v/v)acetonitrile. The operating column temperature was maintained at 37 °C. The injection volume was 10 µL, and the flow rate was 1.0 mL/min. For the detection, amino acid standard solution concentrate diluted 1:10 with 0.1N of hydrochloric acid.

Organic acid components were determined using HPLC (Agilent, PDA detector; column: ACCHROM XAqua C18 (250 mm × 4.6 mm, 5 µm).The detection wavelength was 214 nm. The mobile phase (A) was 0.1% (v/v) phosphoric acid while the mobile phase (B) was acetonitrile. The operating column temperature was maintained at 20 °C. The injection volume was 10 µL, and the flow rate was 1.0 mL/min [16].

F. Statistical Analysis

All experiments were conducted independently in triplicate, and the results were represented as the mean values of three individual replicates ± the standard deviation (SD). One-way analysis of variance (ANOVA) and Duncan's multiple range tests were conducted to determine significant differences ($p < 0.05$) between the means.

III. Results and Discussions

A. Appearance of Miang Product

The product of Miang in a commercial shown completed fermentation that determined from appearance, texture of pulps, taste, unique of aroma which estimated by organoleptic evaluation and fermentation time that have not been certain evident support (Fig. 1). Generally, Miang product can be sale after fermented under anaerobic condition for 4–7 days, or 1 year for mature tea leaves [17].

Figure 1. Typically of Miang commercials; sour Miang and astringent Miang classified by organoleptic evaluation.

B. pH and Total Acidity

The pH value and titratable acidity are relative with the quality charecteristics of taste in fermented foods. The production of acid and lowering pH are obvious changes during fermentation that result in an increase in sourness [18]. As shown in Fig. 2, the changes in pH and acidity content in miang during processing. We found pH value had a slight decreased from 5.35 and 5.37 to 5.28 ($p<0.05$) in young and mature leaves respectively from the fresh tea leaves to the steamed tea leaves. Then, in this step the steamed tea leaves were prepared for fermentation by spreading on the mat until tea leaves cooled and they were made into individual bundles before packed in plastic bags and kept in the bamboo basket containers. As a result, the pH droped slightly ($p<0.05$) from 5.28 to 4.78 (young tea leaves) and 5.04 (mature tea leaves)due to the microorganisms exhibiting in the environment infected the steamed tea leaves [19], which might be occurred the initial fermentation stage by mixed microorganism in natural. In the begining of the fermentation period, the pH increased rapidly from 4.78 to 5.34 (young Miang) and 5.04 to 5.86 (mature Miang) from 0 d to 10 d ($p<0.05$). After this, the pH of the young Miang remained stable at around 5.33 until at 20d and finally decreased rapidly to 4.49 ($p<0.05$) by the end of fermentation at 30 d, whereas the pH of the mature Miang decreased from 5.86 to 4.83 ($p<0.05$) between 10 d and 20 d, and finally remained stable by 4.75 at 30d of fermentation period. These results are similar to those in pickle tea, in which the pH value was 4.67 remain stable from 30d to 60d [5] and also reported a pH of Miang between 4.1 and 4.6 which fermented for a week or many months [20]. Interestingly,in this study the pH value in young Miang was in the range 5.33-5.34 between 10d and 20 d in fermentation time, which should be classified as astringent Miang,but the pH in mature Miang was in the range 4.83-4.75 from 20d to 30d of fermentation period, it could be as sour Miang, however at the 30d both of young and mature Miang has closely pH value (4.49 and 4.75 as sour Miang).This study was in good agreement with previous study [3] that indicated the astringent Miang often produced from young tea leaves but it has not been reported the actual period of fermentation, thus we can suggest the optimal fermentation time for production astringent Miang and sour Miang. Whereas, compared with the pH of Kombucha teas, it decreased abruptly from 5.03 to 1.88 at 21d of the fermentation [21]. While, the changes in the titratable acidity content during Miang fermentaion were increased sharply both young and mature Miang. Notably, The titratble acidity content in mature Miang increased faster than in young Miang were observed. However, the titratble acidity of young Miang was recorded evidently to be 1.30% at 30 d, while in mature leaves Miang was recorded to be 1.47% to 1.40% and from 20 to 30 d, respectively (Fig. 2).

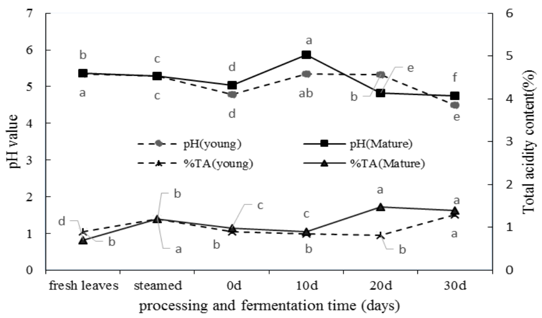

Figure 2. Changes in pH and total acidity content of miang during processing. Data are expressed as mean ± SD of n = 3 samples. The lowercase letters show significant differences at $p < 0.05$, and the different letters show significant differences.

C. Tea Polyphenols, Flavonoids and Soluble Sugar

Table I shows changes in the polyphenols content in young and mature tea leaves during Miang processing. During fermentation period 30 d, the result shown that tea polyphenols content in mature Miang fluctuate changes, while in young Miang the content of tea polyphenols increased slightly from 0d to 20 d, finally its dropped at 30 d of fermentation. Particularly, the highest content of tea polyphenol during fermentation were approximately 31% and 34% in young Miang and mature Miang, respectively. Compared with former studies reported that the polyphenolic fraction of tea represents 30 to 40% wt/wt of extract solids [22]. Tea polyphenols are responsible for the color and astringent and partially responsible for the flavor of tea beverage, the compounds are known antioxidants and are being studied as agent that might reduce risk factors associated with cancer and heart disease [22]. Table I also shows changes in the flavonoids content in young and mature tea leaves during Miang processing, the highest of flavonoids content was found as ~9.0% and ~11.5% in young and mature steamed tea leaves. However, during fermentation period 30 d, the flavonoids content in young Miang was a steady decrease until at the end of the fermentation, at 30 d, whereas the flavonoids content of mature Miang was a dramatic fluctuate for the following steamed tea leaves to the end of fermentation, at 30d. Notably, the amounts of flavonoid of young and mature Miang decreased and closely values at 30d of fermentation time, these results are in good agreement with the previous report of pickled tea that fermented by anaerobic solid-state in controlled condition [5] and similar to the changes in flavonoid content in Pu-erh tea during pile fermentation [23]. Decreasing in flavonoids content and changing of tea polyphenol content in Miang tea by natural fermentation might be only related to the extracellular enzymes derived from the microorganisms appearing in the fermentation process, because of the indigenous enzymes in fresh tea leaves are inactivated by streaming which is the heat treatment before they are started to the beginning of fermentation [19]. The results of changes in the soluble sugar content in young and mature tea leaves during Miang Processing are also shown in Table I. There were a similar trends changing between young and mature tea leaves during Miang processing, the highest of soluble sugar contents in young and mature steamed tea leaves by 32.86% and 29.82 % respectively, then they decreased sharply at 10 d of fermentation period, after this the contents of soluble sugar were a steady hold between 10 d and 30 d of fermentation with 13.73% and 12.87% in young and mature Miang, respectively. Decreasing trends of soluble sugar contents in Miang tea during fermentation were similar to that pickle tea which produced in controlled condition [5]. Additionally, we observed stream-heating in Miang processing having influence on the flavonoids and soluble sugar which were released and were easy using for microorganisms during fermentation process.

TABLE I. CHANGES IN THE MAJOR CHEMICAL COMPONENTS; TEA POLYPHENOL CONTENT, FLAVONOIDS CONTENT, AND SOLUBLE SUGAR CONTENT OF MIANG DURING PROCESSING

Samples	**Young leaf Miang**		
(%DW)	**Polyphenols**	**flavonoids**	**Soluble sugar**
Fresh	35.82±0.26a	9.36±0.17b	20.96±1.19c
Steamed	30.16±0.21cd	11.54±0.05a	32.87±0.42a
fermented 0d	31.04±0.54bc	5.17±0.02c	23.76±0.04b
fermented10d	31.39±0.16b	3.24±0.09d	13.96±0.52d
fermented20d	31.42±0.16b	3.07±0.04d	13.72±0.16d
fermented30d	29.57±0.35d	2.59±0.04e	13.52±0.17d
Samples	**Mature leaf Miang**		
(%DW)	**Polyphenols**	**flavonoids**	**Soluble sugar**
Fresh	39.56±0.04a	7.46±0.06c	22.63±0.86c
Steamed	31.54±0.14c	9.09±0.06b	29.83±0.76a
fermented 0d	34.28±0.25b	9.37±0.13a	24.96±0.62b
fermented10d	28.58±0.60d	2.18±0.03d	12.94±0.57d
fermented20d	28.03±0.35d	5.96±0.01c	13.41±0.58d
fermented30d	34.49±0.15b	1.69±0.213e	12.28±0.01d

Average value ± standard deviation. Different letters in the same column indicate that values are significantly different ($p<0.05$)

D. *Major Catechins and Caffeine*

Tea catechins are well known as a natural powerful of antioxidant, hence they has been claimed for promoting the health benefits in tea-products. Moreover, Tea catechins are also responsible for the bitterness and astringency of green tea [22], As the same in case of Miang tea, the catechin composition in Miang during processing was analyzed and identified by HPLC (Fig. 3), that we found six main catechins in both of young and mature Miang are: (+)-catechin (C), (-)-epicatechin (EC), (-)-epicatechin-3-gallate (ECG), (-)-epigallocatechin (EGC), (-)-epigallocatechin-3-gallate (GCG). Interestingly, the C, EC, ECG, EGC and GCG in both young and mature steamed tea leaves was higher than fresh tea leaves and fermented tea leaves during fermentation period, and we also observed the C in mature steamed leaves (11.68%) was higher than young steamed leaves (6.28%). During the fermentation period, the C declined gradually till at 30 d of final product which it contain 3.37% and 3.19% in young and mature Miang respectively. Additionally, In ester catechins, the EC and EGC contents decreased gradually during the period of fermentation ($p<0.05$), except at 20d that the EC increased both of young and mature fermented tea leaves. While in the non-ester catechins content, the EGCG, ECG and GCG contents fluctuated wildly ($p<0.05$) between 0 d and 30 d of fermentation period. Furthermore, we considered the levels of predominant catechins composition in the final product at 30 d of fermentation period, both young and mature Miang was in order EGCG > C > EGC > EC > ECG >GCG. These results might be possible to describe the taste of Miang from the amount of EGCG was responsible for bitter and astringent taste as well as the amount of C, EGC and EC were responsible for the bitter with sweet after taste [24]. Moreover, compared the amount of all catechins changing between young and mature Miang during fermentation (Fig. 3A and 3B), we observed mature Miang more fluctuated dramatically than young Miang, this result shows evidently that the maturity of tea leaf and fermentation period have very important influence on the taste quality of Miang, that it might be the cause of the steamed-heating step affect the tea polyphenols derived to the simply structure substances [3], which the mixed microorganisms in these fermented tea can be used as the carbon source and produced the active constituents such as EGCG, C, EGC and EC which is great effect on the taste quality of Miang.

Caffeine is an important substance that the consumer is awareness when drinking tea because of its potential stimulate the central nervous system [25], As shown in Fig. 3A and B, the caffeine content reduced in both of young and mature steamed tea leaves, but increased gradually during the fermentation, the highest caffeine content was 6.24 % (at 20 d, young Miang) and 6.23% (at 30 d, mature Miang) which was similar to the amount of caffeine in Pu-erh ripen tea 5.17% [26], and this trend was similar to the increase of caffeine content in the manufacturing of dark green tea treated leaves with mixed microorganisms for a period of time and revealed the molds (i.e. *Aspergillus niger*) ,during the fermentation ,it increased the caffeine content, but yeast fermentation decreased the caffeine content [27]. Previous studies also illustrated the action of fungi and bacteria had the influence on chemical composition of pickled tea fermentation [2], [17], [20], [28], [29].

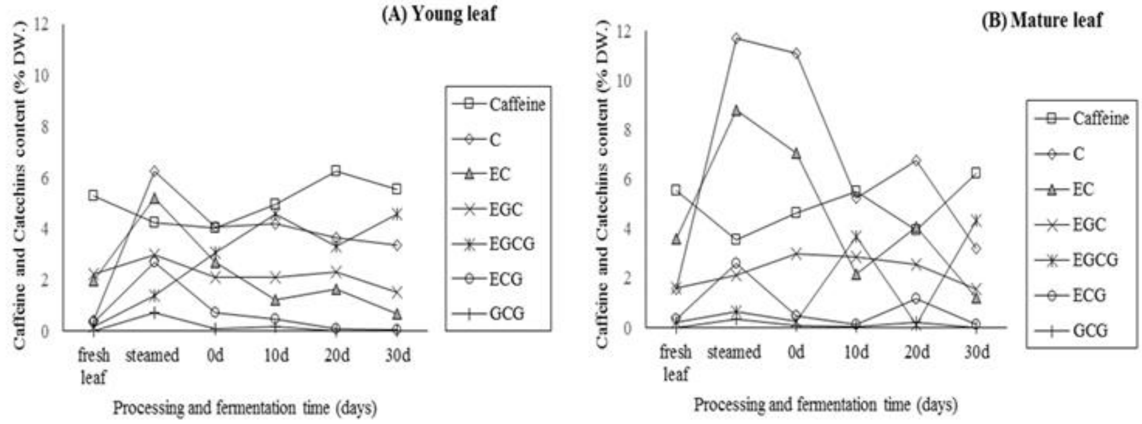

Figure 3. Changes in caffeine and the contents of major catechins during Miang processing of young leaf Miang (A) and mature leaf Miang (B)

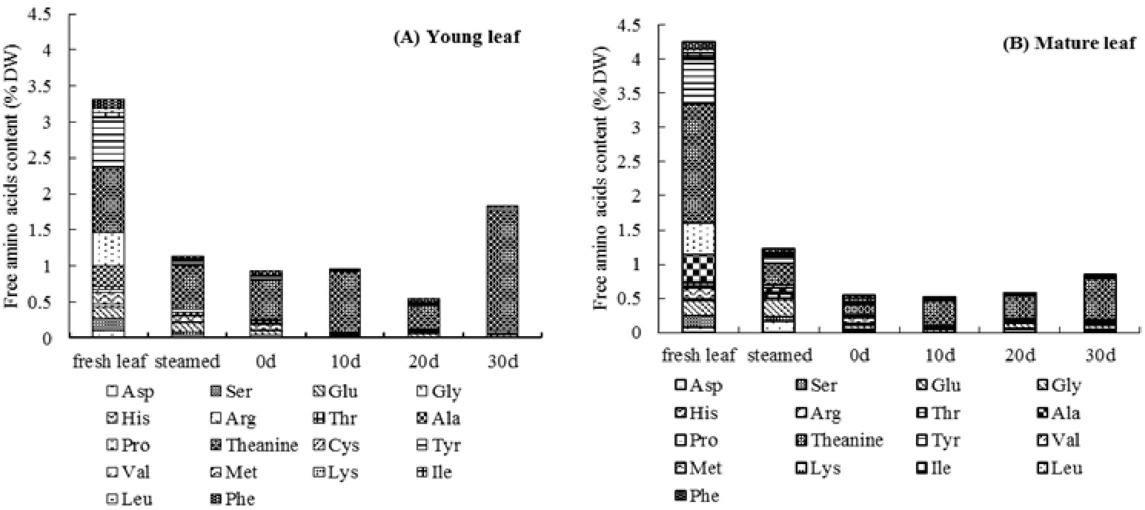

Figure 4. Changes in the contents of free amino acids during Miang processing of young leaf Miang (A) and mature leaf Miang (B).

E. Free Amino Acids

Amino acids is present in tea flush around 2-4% [30] and FAAs was considered to be important in the taste and aroma of tea product produced by traditional tea processing [5]. Fig. 5 shows changes in FAAs content in young and mature tea leaf during Miang processing, the amount of FAAs was highest in fresh tea leaves.

After this, total FAAs content reduced sharply that caused by steaming, with 1.13% and 1.22% in young and mature steamed tea leaves, respectively. During fermentation period, in young Miang (Fig. 5A), the FAAs contents fluctuated with significant ($p<0.05$) between 0d and 30d.While in mature Miang (Fig. 5B), the FAAs content lowest in the initial of fermentation time at 0d then its increased steadily from 0d to 30d. However, we found the amount of FAAs content recovered and highest at 30d of fermentation period both in young and mature Miang. Obviously, after tea leaves were fixed by steaming and initial of fermentation period, the total FAAs contents in Miang tea decreased that might be caused by some of the amino acids change into volatile substances [30] and the action of microbial fermentation process, this results which is in good agreement with a significant decrease of total FAAs in Pu-erh ripened tea during fermentation [31]. In the case of Miang, the theanine was the dominating amino acid in all stages of Miang processing. Interestingly, at 30d of the fermentation time, Miang contained the highest of theanine follow by Pro, Glu, His and Phe were 97.01% of the total amino acids in young Miang as well as the highest of the theanine follow by Glu, His, Asp and Phe were 88.88% of the total amino acid in mature Miang. Compared with the recent study these results are different from those pickled tea fermented by controlled condition [5] in which the amount of theanine was lower than the fermented tea for 0 d that might be because of the different raw material and processing condition. Amount of theanine are due to the umami taste of tea product [32] in which suggests it may be also related to the taste of Miang and the optimal fermentation period for 30d. Besides in former studies have also shown the theanine exhibited pharmacological and physiological effects a relaxation causing in human, thus it may become a functional additive to foods to make stressed people relax [30], [33].

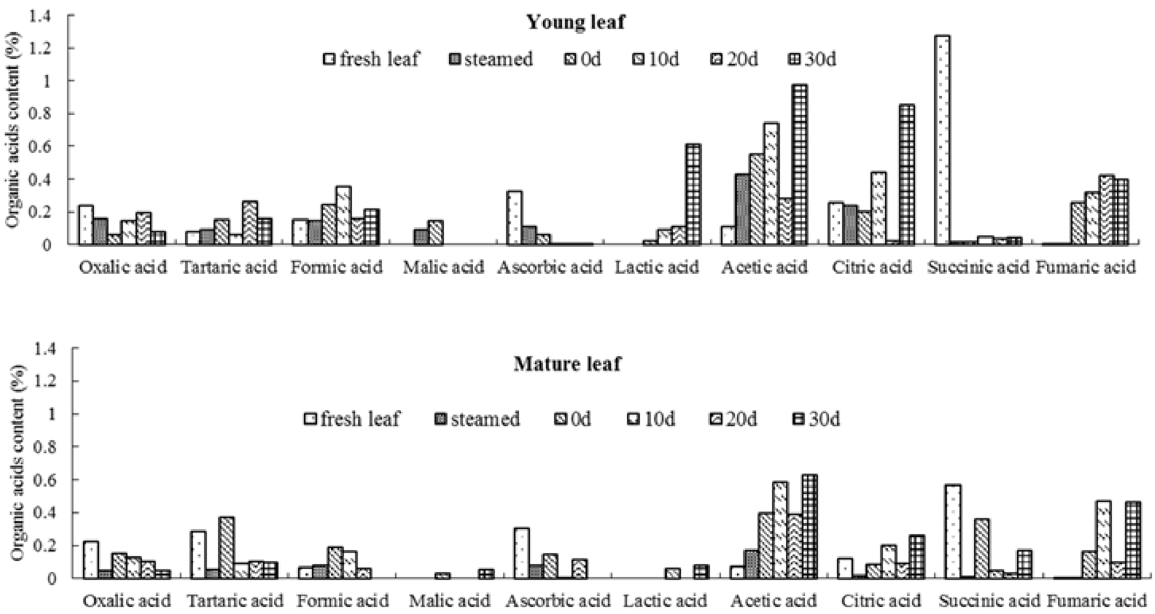

Figure 5. Changes in the contents of organic acids during Miang processing of young leaf Miang (A) and mature leaf Miang (B).

F. *Organic Acids*

Fresh tea flushes contain 0.5-2.0% total organic acids by weight. Organic acids are crucial constituents in tea leaves that has influence on the tea quality product [34]-[36]. Particularly, organic acids in kombucha tea are believed to be an active chemical component [37], similarly, this work focus on the changes in organic acids contents in young and mature tea leaves during Miang processing, as shown in Fig. 5A and B, we found 8 kinds of organic acids in young and mature fresh leaves Miang; oxalic acid, tartaric acid, formic acid, ascorbic acid, acetic acid, citric acid, succinic acid and fumaric acid. Amount of total organic acid contents in young fresh leaves (2.43%) was higher than mature fresh leaves (1.64%) were also evident observed. After this, tea leaves were heated by steaming, malic acid was detected only in young steamed tea leaves. During fermentation period, tea leaves were fermented by mixed microorganism in natural way, obviously the results indicated that the changes in organic acids varying with time which their can mainly classified in two groups; increasing organic acids and reducing organic acids. Included in the increasing organic acids were acetic acid, citric acid, fumaric acid and lactic acid, whereas the reducing organic acids were oxalic acid, tartaric acid, formic acid, ascorbic acid and succinic acid. Those of increasing organic acids, the level of acetic acid and citric acid increased sharply, except at 20 d, then their maximum level were recorded at 30 d of fermentation time by 0.97% and 0.62% of acetic acid and 0.85% and 0.25 % of citric acid in young and mature Miang respectively, for the level of fumaric acid increased steadily in young Miang and fluctuated dramatically in mature Miang, remarkably it was high level at 30 d of fermentation.

Whereas, the level of lactic acid was detected on the initial of fermentation time (0 d) and increased steadily from 0 d to 30 d in young Miang but only detected on 10 d and 30 d in mature Miang, the maximum level of lactic acid was 0.60 % in young Miang on 30 d of fermentation time, but it was very less in mature Miang. Generally, acetic acid, citric acid, and fumaric acid were found in fresh tea leaves and also were produced during food fermentation by natural microbial fermentation, several studies revealed that acetic acid bacteria (e.g. *Acetobacterium*) produce acetic acid, *Aspergillus niger* produce citric acid by using carbohydrates source of material, fumaric acid is by-product in L-lactic acid production by *Rhizopus oryzae*, Among of them, lactic acid is not naturally present in food, but is only produced during fermentation of food by lactic acid bacteria [38], this work is similar to kombucha tea which in symbiotic association of bacteria and fungus play an important rule for metabolic pathway that influence on the formation constituent of fermented tea-product [21], [37] accordingly it should be extremely studied in the near future. In this part, this study suggests that the organic acids illustrated to be an important key components in Miang tea were acetic acid, citric acid, fumaric acid and lactic acid. Some of these organic acids are beneficial to human health because they aid the activities of proteinase and amylase, and the growth of bifidobacterium in the intestine and stomach [30], [39].

IV. Conclusion

The changes in chemical constituents during traditional processing of Miang (Thai fermented tea leaves) which is prepared from various degree of tea leaf maturity (young and mature leaf) and fermentation by associated microorganism in natural way. During 30 d of fermentation, the pH value in mature Miang reduced faster than young Miang, at 20d of fermentation, after this their pH values were closely at 30d of fermentation. Thus, this study suggested that the production of astringent Miang should be made by young tea leaves with the optimal fermentation time for 10d-20d that pH value around 5.33-5.34, whereas sour Miang could be made by young or mature tea leave, the complete fermentation on 30d, pH value around 4.49-4.75. The flavonoids and soluble sugar were highest level when tea leaves was fixed by steam-heating, then decreased steadily during fermentation. The EC and EGC contents decreased gradually except at 20d ,while EGCG, ECG and GCG contents fluctuated wildly, however, the predominant catechins composition in both young and mature Miang on 30d of fermentation are EGCG, C, EGC, EC, ECG and GCG. The highest caffeine content was 6.24% and 6.23% in young Miang at 20d, and in mature Miang at 30 d of fermentation, respectively. The predominant of FAAs in young and mature Miang was theanine that might be related to umami taste in Miang. The present study also revealed that the organic acids considered to be an important key components in young and mature Miang tea were acetic acid, citric acid, fumaric acid and lactic acid.

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