

발효시간이 뽕잎차 구성성분에 미치는 효과

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Effect of Fermentation Time on the Chemical Composition of Mulberry (*Morus alba* L.) Leaf Teas

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ABSTRACT : *Morus alba* (Mulberry) leaves were exposed to fermentation for varying amounts of time: no fermentation (0 min, MANF), medial fermentation (10 h, MAMF), and full fermentation (24 h, MAFF). The chemical compositions of the teas were determined and compared with those of commercial *Camellia sinensis* teas. The results showed that mulberry leaf teas contained significantly higher amounts of ash and fat than *Camellia sinensis* tea. Compared with *Camellia sinensis* teas, all mulberry leaf teas contained significantly more total free amino acids (24.26–54.25 mg L-glutamic acid equivalent g⁻¹), but the concentration of caffeine was relatively low for mulberry leaf teas. High thiamine, riboflavin, and niacin contents were found in all mulberry leaf teas, but ascorbic acid and pyridoxine were found at higher levels in *Camellia sinensis* teas than in mulberry leaf teas. Color measurements demonstrated that mulberry leaf tea infusions generally had lower a* (greenness) and b* (yellowness) values than *Camellia sinensis* tea infusions. All infusions exhibited low turbidity levels (less than 10%). The contents of total phenols were measured as 71.8 and 74.9 mg 100 mL⁻¹ infusion in MANF and MAFF, respectively, but the MAMF tea infusion showed significantly lower total phenols (64.6 mg 100 mL⁻¹ infusion). The total flavonoid contents of mulberry leaf tea infusions were lower (8.9–20.6 mg 100 mL⁻¹ infusion) than those of *Camellia sinensis* teas and thus had lower antioxidant capacities (DPPH: 326.8–526.9 µM trolox equivalent g⁻¹ and FRAP: 364.6–387.6 µM trolox equivalent g⁻¹) than *Camellia sinensis* teas. The amounts of γ-aminobutyric acid (GABA) and rutin were higher in fermented mulberry leaf teas; the level of GABA increased with increasing fermentation time and the content was highest in MAFF, but rutin content was highest in MAMF.

Key Words : Mulberry Leaf, Fermentation, Tea Infusions, GABA (γ-aminobutylic acid)

INTRODUCTION

Mulberry (*Morus alba* L.) is cultivated in Korea, China, and Japan, and their leaves have long been used to feed silkworms (*Bombyx mori* L.). Mulberry leaves, barks, and branches have also long been used in Chinese medicine for healing various health-related problems (Brown, 1995). Mulberry leaf accounts for 64% of the output on the ground and has been used as a traditional medicine in Korea. Mulberry leaf exhibits various beneficial pharmacological effects, such as hypoglycemic

activity, antioxidant properties, ability to reduce blood pressure, anti-aging activity, immunity enhancement, and anti-cancer effects (Wang *et al.*, 2000; Kayo, 2001; Ou and Chen 2003). Different parts of the mulberry plant are used in herbal medicines for blood serum glucose reduction (Andallu and Varadacharyulu, 2002) and for cholesterol and lipid level reduction (Bang *et al.*, 2007; Chen and Li, 2007). The components include flavones, steroids, triterpenes, amino acids, vitamins, and trace minerals. Reports have also indicated that mulberry leaves contain proteins, carbohydrates, calcium, iron, ascorbic acid, β-carotene,

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vitamin B-1, folic acid, and vitamin D (Bose, 1989). It has recently been reported that the mechanism of action is related to antioxidant activity (Bang *et al.*, 2010). The presence of GABA (Bang *et al.*, 1998), rutin, quercetin, isoquercetin, and other flavonoids in mulberry leaves has also been reported (Zhishen *et al.*, 1999). Among the six N-containing sugars isolated, 2-O- α -D-galactopyranosyl-DNJ (GAL-DNJ) and fagomine have the most potent antihyperglycemic effects (Kimura *et al.*, 1995). Nine flavonoids have been isolated from mulberry leaves and tested for free radical-scavenging activity using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical and quercetin-3-O-h-D-glucopyranosyl-(1 \rightarrow 6)-h-D-glucopyranoside and quercetin were shown to be antioxidative (Kim *et al.*, 1999). Three phytoalexins, moracin C, moracin N, and chalcomoracin, possessing free radical-scavenging activity have also been reported in mulberry leaves (Sharma *et al.*, 2001). Consumption of mulberry leaves, as infusions or powdered juices, is widespread in Korea, and these nutraceutical foods offer high dietary intakes of beneficial compounds (Kim *et al.*, 2003). Mulberry leaf products have also recently become available in the market in the forms of tea, soft drinks, and other beverages.

However the development of herbal teas exclusively from mulberry leaves has been restricted, because they are normally prepared by simple processing and are usually supplemented and mixed with other herbal teas, to enhance flavor, taste, and health-promoting effects. The fermentation process is believed to improve the quality of herbal teas in terms of color, flavor, and taste (Du Toit and Joubert, 1998; Heck and Mejia, 2007). Because there is little information on the use of fermented leaves of mulberry teas in beverage production, the main objective of this research was to explore the feasibility of mulberry leaves as herbal teas produced by three types of fermentation: no fermentation (MANF), medial fermentation for 10 h (MAMF), and full fermentation for 24 h (MAFF). *Camellia sinensis* tea was used as a reference, using the same fermentation techniques as used for mulberry herbal teas. The composition, water-soluble vitamin content, sensory properties (color, turbidity), antioxidative characteristics, and contents of rutin and GABA were assayed and compared with those of *Camellia sinensis* teas, such as green tea (GT), oolong tea (OT), and black tea (BT).

MATERIALS AND METHODS

1. Materials

Fresh *Morus alba* leaves obtained from Yang-Pyeong

Agricultural Development & Technology Center, Yang-Pyeong, Gyeonggi-do, Korea in during June to August 2010. commercial *Camellia sinensis* teas were purchased from a local market in Jeju island in 2010.

Folin-Ciocalteu's phenol reagent, sodium carbonate, aluminium caffeine and iron (III) chloride were purchased from Merk (Darmstadt Germany). Gallic acid, DPPH(1,1-diphenyl-2-picrylhydrazyl) radical TPTZ(2,4,6-triphenyl-s-triazine), ninhydrin were purchased from Sigma-Aldrich(St. Louis, Mo, USA). All other chemicals used were analytical grade.

2. Fermentation processing of mulberry leaves

Mulberry leaves were washed under running tap water. The cleaned leaves were chopped into 2–3 cm, then was dried in a hot air oven at 230°C for 15 min until the moisture content was less than 6.5%. The dried leaves was quickly cooled down using motor fan and kept in an air tight container (MANF). The cleaned leaves were withered in locally obtained forced air oven at 30°C for 2 hour. The withered leaves were manually twisted and torn for 20 min. The crumble leaves were allowed to undergo medial fermentation for 10 hour (MAMF). Fermented leaves of mulberry was rolled for 20 min using rolling machine (DA 780, Donga Co. LTD, Korea), then pan-firing at 230°C for 60 min. For MAFF, a similar procedure was repeated, but the fermentation time was prolonged to 24 hour.

3. Tea infusion preparation

For all experiments, 1 g of tea leaves was weighted into a beaker. Hot distilled water (100 mL) was the added and allowed to infuse the leaves for 10 min. The infusions were filtered through Whatman filter paper No. 1 prior to analysis.

4. Proximate analysis

Determination of moisture, crude protein, crude fat, and crude ash contents were performed according to the AOAC, (1993). Moisture (method 14-004), crude protein (method 2.057), crude fat (method 7.056) and crude ash (method 14-006) were assayed.

5. Determination of total free amino acids

The total free amino acids were determined using the modified method described by Friedman *et al.* (1984). Tea infusion 1 mL, 0.5 mL of phosphate buffer solution and 0.51 mL of 2% ninhydrin solution containing 0.8 mg \cdot mL⁻¹ of thin chloride were placed in to 25 mL volumetric flask. The Mixture in the volumetric flask was

then heated in a boiling water bath for 15 min. The flask was quickly cooled down, and the volume was adjusted to 25 mL with distilled water. After the solution was left standing for 10 min, the resulting blue-purple products were read at 570 nm using a UV-VIS spectrophotometer (UV-1650; Shimadzu, Kyoto, Japan). Result were expressed as mg L-glutamic acid equivalent/100 mL infusion. The measurement was performed in triplicate.

6. Vitamin determination

Analysis was carried out using a Shimadzu HPLC with autosampler (SIL-20A) injection. The system comprises a binary pumps LC20AD, a SPD 20A UV detector, and degasser. The HPLC column used was a reversed-phase Zorbax SB-C18 (75 × 4.6 mm, 3.5 μ m) with guard column from Agilent. Data acquisition was done with Lab solution software. The mobile phase of (A) 0.05M KH_2PO_4 in water (pH 2.5) and (B) acetonitrile. The gradient elution was programmed as follows : 0.5 min, 0.6% B; 0.5~4 min, 6% B; 4~12 min, 30% B; 12~17 min 60% B, 17~19 min 60% B; 19~20 min 6% B at flow rate 1.0 mL · min⁻¹ with an injection volume 5 μ L, column effluent being monitored at 204 nm wavelength. Pure standards of thiamine, riboflavin, niacin, panthothenic acid, pyridoxine, biotin, folic acid and cyanocobalamine obtained from BDH Biochemicals (England), were used as external standards in the analysis. Different concentrations of the standards were used based on the range required to plot a suitable calibration curve. Tea Infusions samples were prepared by pipetting out 5 mL of the sample and making it up to 25 mL with water. In the same way prior to sample injection the solution was filtered through a membrane filter (0.45 μ m).

For ascorbic acid analysis, tea infusion (10 mL) extracted using an equal volume of 4.5% metaphosphoric acid solution Sample filtered through 0.45 μ m membrane filter in aliquots of 10 μ L for each tea infusion. A Shimadzu HPLC system (LC20AD, Shimadzu corporation, Kyoto, Japan) was used. The separation was performed with TSK-GEL ODS-120T (250 × 4.6 mm, 5 μ m) (TOSOH corporation, Tokyo, Japan) column. The mobile phase was 85% water was adjusted with metaphosphoric acid to pH2.5 and 15% methanol (HPLC grade) at flow rate 1.0 mL · min⁻¹ and detected 254 nm. Results were expressed as mg ascorbic acid/100 mL infusion.

7. Caffeine determination using HPLC

The tea infusion were diluted with water and filtered through a 0.45 μ m membrane filter and injected into Shimadzu HPLC

system (LC20AD, Shimadzu corporation, Kyoto, Japan). The separation was performed on a Hypersil ODS C18 column (250 × 4.6 mm, 5 μ m) (Thermo Scientific, Waltham, MA, USA) fitted with a Hypersil ODS guard column containing a mobil phase 25% methanol and 75% water at flow rate 1.0 mL · min⁻¹ and detected 276 nm. Results were expressed as mg caffeine/100 mL infusion.

8. Sensory quality of tea infusion

Quantitative colour measurements of the tea infusion were performed using a computerized spectrophotometer (Konica Minolta CM-3500d, Osaka, Japan) with illuminant D65 was used to measure CIE system. The calculation of L*, a*, b* for each color is based on CIE XYZ values (Perez-Magarino *et al.*, 2003). L* is the degree of lightness of the color. This refers to the relation between reflected and absorbed light. L* values equals to zero for black and 100 for white. a* (red-green) is the degree of redness (0 to 60) or greenness (0 to -60) and b* (yellowblue) is the degree of yellowness (0 to 60) or blueness (0 to -60). The turbidity of tea infusions was measured according to the method described by Morton and Murray (2001) using spectrophotometer (UV-1650; Shimadzu corporation, Kyoto, Japan) at 800 nm. The percent transmittance (T%) was recorded and 100-T% was used as a measure of turbidity.

9. Determination of total phenolic content (TPC)

The total phenolic content was determined using the Folin-Ciocalteu method. Approximately 0.1 mL of 50% Folin-Ciocalteu reagent was added into the 2.9 mL of tea infusions, and the mixture was allowed to stand at room temperature for 5 min. Later, 2 mL of 2% sodium carbonate solution (Na_2CO_3) was added into the mixture and incubated at room temperature for 30 min. The absorbance was measured at 750 nm using a spectrophotometer (UV-1650; Shimadzu corporation, Kyoto, Japan), and the results were expressed as gallic acid equivalents (GAE) in mg/100 mL infusion. The measurement was performed in triplicate

10. Determination of total flavonoid content (TFC)

The total flavonoid content was assayed using aluminum chloride colorimetric method. In brief 0.5 mL of the tea infusions was mixed with 1.5 mL of 95% EtOH, 0.1 mL of 10% aluminum chloride hexahydrate (AlCl_3), 0.1 mL of 1M potassium acetate (CH_3COOK) and 2.8 mL distilled water. After 40 min the absorbance was read at 415 nm. The result were expressed as mg

quercetin equivalents (QE)/100 ml infusion.

11. DPPH free radical scavenging activity

DPPH radical scavenging activity was evaluated by the method of Okada and Okada (1998) with a slight modification. The assay mixture contained 0.1 ml of 1.0 mM DPPH radical solution in ethanol, 0.8 ml of 99% ethanol and 0.1 ml of tea infusions were rapidly mixed and after 30 min the scavenging activity was measured spectrophotometrically by the decrease in absorbance at 517 nm. The results expressed μmol Trolox equivalents/100 ml infusion.

12. Ferric Reducing Antioxidant Power (FRAP) assay

The Ferric Reducing Antioxidant Power (FRAP) assay was estimated according to the method described by Benzie and Strain (1996) with slight modifications. Firstly, fresh FRAP reagent was prepared by mixing 2.5 ml of 10 mM TPTZ solution in 40 mM hydrochloric acid with 2.5 ml of a 20 mM FeCl_3 solution and 25 ml of a 0.3 M acetate buffer (pH 3.6). Later, 200 μl of the tea infusions, and 1.5 ml of the FRAP reagent were mixed. The absorbance was read after 4 min at 593 nm using a spectrophotometer. Trolox solution was used to create the calibration curves. The results were expressed as μmol Trolox equivalents/100 ml infusion.

13. Rutin and GABA analysis

One gram of the different type of teas sample was weighed and put into a bottle. Twenty milliliters of distilled water was added to the bottle. The sample was extracted in a water bath at 95°C for 3 hours with stirring to get water extract. The supernatant and the sediment were filtered. The extraction solution was dried by vacuum-evaporator. The dried extract was weighed to calculate yield, based on the weight of teas. HPLC (high performance liquid chromatography) was used for quantitative analysis. The separation was performed on AM303 (4.6 mm \times 250 mm, YMC) with mobile phase (A) 45% acetonitrile containing 2% acetic acid and (B) water containing 2% acetic acid. The gradient elution was programmed as follows : 0 min 50% A; 0–18min 100% A; 18min–22 min 50% A at flow rate 1 ml/min with injection volume 20 μl . For calibration curve of rutin and quercetin the typical concentrations of 5, 10, 20 and 40 $\mu\text{g/ml}$ were prepared. Each solution was injected and chromatogram was recorded. The peak areas of rutin and quercetin were calculated and respective calibration curves were plotted against concentration. ($R^2=0.9908405$)

Concentrations of GABA was determined by the AccQTag HPLC detection system (Waters, Japan). The sample was filtered by syringe filter (DISMIC-13HP, PTFE 0.45 μm , ADVANTEC, Toyo Roshi Kaisha Ltd., Japan). Once the filtrate had been diluted to a suitable concentration, fluorescence derivatization was performed by AccQTag method (Waters, Japan). A 50 μl aliquot of sample solution, 3500 μl of AccQ-Fluor borate buffer (Waters), and 100 μl of AccQ-Fluor reagent (Waters) were mixed, and the mixture was subsequently incubated for 10 min at 55°C. Separation of derivatized GABA was conducted on an AccQTag amino acid analysis column (3.9 \times 150 mm, Waters) by gradient elution at 37°C. Gradient elution system were used: A (10% AccQ-Tag Eluent A in water) and B (60% Acetonitrile in water), 0% of B concentration for initial time, 2% B concentration for 0.5 min, 7% B concentration for 15 min, 10% B concentration for 19 min, 33% B concentration for 32 min, 33% B concentration for 34 min and 100% B concentration for 34 min. The derivatized amino acids were detected by using UV detector (LC20AD, Shimadzu corporation, Kyoto, Japan) with wavelength 395 nm. The chromatography data produced was analyzed using Lab solution (Shimadzu Corporation, Kyoto, Japan). GABA standard liquor (Sigma) was prepared by diluting GABA with AccQ-Fluor Borate buffer to different strength (50, 100, 150 and 200 $\mu\text{g/ml}$) to obtain different chroma value ($R^2=0.9939757$).

14. Statistical analysis

Experimental data were analysed using statistical package for the Social Sciences (SPSS) 17.0. A ANOVA procedure followed by Duncan test was used to determined the significant difference ($P < 0.05$) between treatment means.

RESULTS AND DISCUSSION

Three types of mulberry leaf teas (MANF, MAMF, MAFF) were successfully prepared (Fig. 1). The moisture contents of the MANF, MAMF, and MAFF were 6.42, 5.92, and 5.24%, whereas the moisture contents of the GT, OT, and BT were 6.11, 6.32, and 7.12%, respectively. These results were similar to the previously reported moisture content of 6.37% in green tea (Park *et al.*, 2005). The moisture contents of OT and BT were higher than previously reported values of 4.3–4.9% and 6.4%, respectively (Lee *et al.*, 1998). Control of moisture content is an important factor in tea preservation, particularly for the inhibition of microbial growth. Owuor (2003) suggested that the moisture



Fig. 1. The three type of teas and tea infusions from leaves of mulberry by fermentation. (A) non-fermented tea (MANF); (B) medial fermented tea (MAMF); (C) full fermented tea (MAFF), (D) non-fermented tea infusion; (D) medial fermented tea infusion; (E) full fermented tea infusion.

Table 1. Chemical properties of fermented teas from mulberry leaf and *Camellia sinensis*.

Properties	Green tea (GT)	Oolong tea (OT)	Black tea (BT)	Mulberry		
				MANF	MAMF	MAFF
Moisture (%)	6.11±0.02 ^{bc}	6.32±0.08 ^b	7.12±0.06 ^a	6.42±0.11 ^b	5.92±0.18 ^c	5.24±0.25 ^{db}
Protein [†]	28.45±0.97 ^a	27.23±0.54 ^b	28.45±1.12 ^a	18.23±0.43 ^c	18.46±0.86 ^c	18.37±0.75 ^c
Fat [†]	0.16±0.02 ^f	0.28±0.01 ^e	0.40±0.05 ^d	1.25±0.07 ^a	0.78±0.04 ^b	0.56±0.06 ^c
Ash [†]	5.98±0.06 ^b	5.97±0.11 ^b	5.76±0.31 ^c	10.44±0.16 ^a	9.89±0.87 ^a	10.11±0.26 ^a
Total free amino acid [‡]	39.58±2.14 ^b	30.12±3.24 ^c	29.77±4.12 ^c	53.43±6.14 ^a	54.25±4.46 ^a	24.26±8.16 ^d
Caffeine [§]	26.14±2.18 ^b	14.26±2.21 ^c	28.66±1.68 ^a	0.03±0.01 ^f	0.19±0.02 ^e	0.28±0.05 ^d

[†]Values in dry weight basis.

[‡]Total free amino acid in mg L-glutamic acid equivalent /100 ml infusion.

[§]Caffeine content in mg/100 ml infusion, Value marked by different letters in each row are significantly different ($p < 0.05$). *Data are Mean±SD, n = 9

content of a tea product should be below 6.5%. However tea leaves with moisture contents of 2.5% or less may have a smoky taste (Owuor, 2003). All the teas analyzed were within the suggested limit, with the exception of BT.

The crude protein contents of mulberry leaf teas were significantly lower than those of *Camellia sinensis* teas; the protein content was approximately constant in all types of teas from mulberry and *Camellia sinensis*. This was consistent with the results of Tsai *et al.* (1990) who reported that the nitrogen content of the tea leaf was not affected by harvesting or fermentation processing. A similar result was found for ash contents, which remained unchanged in the three samples

(MANF, MAMF, MAFF). This may be due to the stability of minerals present in the ash because a short time period of 15 min was used for drying, although the drying temperature was 230°C. The crude fat content of MANF tea was significantly higher than that of MAMF or MAFF tea, and the fat contents of mulberry leaf teas generally were higher than those of *Camellia sinensis* teas. The fat content decreased with fermentation time, which could have been due to the release of volatile fat content from the mulberry leaves during fermentation. However, Ye and Bae (2010) reported different results; they compared the ash and lipid contents in mulberry leaf tea and fermented mulberry leaf tea. They concluded that crude lipids and ash from mulberry leaf tea

were higher than in fermented mulberry leaf tea. Generally, fat contents are decreased in tea processing as a matter of quality control for herbal teas. Herbal teas contain up to 4% lipids, which can affect the quality control of tea products because deterioration of lipids can occur if storage conditions are less than optimal (Jo *et al.*, 2006).

Free amino acid content is regarded as an important criterion in tea quality assurance and contributes to overall quality in terms of taste, flavor, and color (Yao *et al.*, 2006). In MANF and MAMF tea infusions, the total free amino acid released showed no significant change but was higher than that released from MAFF tea. A similar trend was found for free amino acid changes with fermentation in the three *Camellia sinensis* teas. The dramatic decrease in total amino acid content in MAFF may have been due to the breakdown of protein during fermentation for 24 h, as described by Yao *et al.* (2006). Caffeine concentrations were also low in the MANF, MAMF, and MAFF infusions, with values of 0.03, 0.19, and 0.28 mg 100 mL⁻¹ infusion, respectively. In contrast, the GT, OT, and BT infusions had caffeine contents of 26.14, 14.26, and 28.66 mg 100 mL⁻¹ infusion, respectively. This indicates that mulberry leaf tea has potential for use in the growing caffeine-free tea market. Recently, decaffeination has become popular to minimize the caffeine content in various products, including tea and coffee. The caffeine content in beverages should be minimized because of caffeine-related side effects, including anxiety, nausea, jitteriness, nervousness, changes in heart rate, increased blood pressure, and the risk of cardiovascular disease (Temple, 2009).

Vitamins in general play an important role in our health, although they constitute only a very small part of the food we eat each day (Olaniyi, 2000). Diets that do not contain adequate amounts of these vitamins ultimately result in deficiency diseases. The amount of water-soluble vitamin B and C present in the tea infusions was determined. "Vitamin B" consists of a

complex group of eight vitamins: B₁ (thiamine), B₂ (riboflavin), B₃ (niacin), B₅ (panthothenic acid), B₆ (pyridoxine), B₇ (biotin), B₉ (folic acid), and B₁₂ (cyanocobalamin). The amounts of thiamine in mulberry leaf teas were significantly higher than in GT, and thiamine was not detected in OT or BT (Table 2). Fermented mulberry leaf teas, MAMF and MAFF, had significantly higher amounts of thiamine than unfermented mulberry leaf tea (MANF). Mulberry leaf teas also contained significantly more riboflavin compared to *Camellia sinensis* teas. Niacin was the predominant B vitamin type present in teas from both mulberry and *Camellia sinensis* and was found to decrease with fermentation time. The pyridoxine content in both tea types increased with fermentation time. *Camellia sinensis* teas were shown to have higher pyridoxine contents than those found in MANF or MAMF. The pyridoxine amount in MAFF was 9.14 µg 100 mL⁻¹ infusion, similar to that in GT (9.78 µg 100 mL⁻¹ infusion). Biotin was found only in MAFF, and pantothenic acid, folic acid, and cyanocobalamin were not detected in any of the tea infusions examined (data not shown). The absence of folic acid may be due to its sensitivity to sunlight, air, light, and heat from the boiling water (Leskova *et al.*, 2006). Cyanocobalamin has been reported to be absent in black teas (Pasha and Reddy, 2005).

The amount of ascorbic acid in mulberry leaf teas was generally lower than in *Camellia sinensis* teas and ascorbic acid was found to decrease with degree of fermentation process, likely indicating that ascorbic acid was oxidized during fermentation. Similar results were also shown for *Camellia sinensis* teas, as has been reported previously (Chung and Shin, 2005; Choi and Choi, 2003).

Tea color and turbidity are important sensory qualities. The CIELAB and turbidity values for tea infusions from mulberry and *Camellia sinensis* are provided in Table 3. The L* value decreased (from 99.88 to 97.16) with the degree of fermentation

Table 2. Water soluble vitamins contents of fermented leaf teas form mulberry and *Camellia sinensis*.

Vitamins	Green tea (GT)	Oolong tea (OT)	Black tea (BT)	Mulberry		
				MANF	MAMF	MAFF
B ₁ thiamine [†]	1.25±0.00 ^c	ND	ND	10.26±1.11 ^b	25.52±2.21 ^a	25.21±3.25 ^{a*}
B ₂ riboflavin [†]	8.14±0.66 ^d	5.24±0.1 ^e	2.45±0.21 ^f	32.17±5.72 ^c	48.42±8.86 ^b	64.31±9.7 ^a
B ₃ niacin [†]	678.22±15.85 ^b	587.14±21.01 ^c	247.40±15.07 ^e	987.21±11.07 ^a	487.11±12.00 ^c	284.16±8.04 ^d
B ₆ pyridoxine [†]	9.78±0.11 ^c	15.74±0.41 ^b	23.84±1.09 ^a	3.11±0.54 ^e	5.18±0.77 ^d	9.14±1.23 ^c
B ₇ biotin [†]	ND	ND	ND	ND	ND	11.25±1.58
C Ascorbic acid [‡]	1.88±0.47 ^a	0.44±0.07 ^c	0.27±0.08 ^d	0.68±0.14 ^b	0.18±0.01 ^e	0.09±0.02 ^f

[†]Values in µg/100 mL infusions. [‡]Values in mg/100 mL infusion. ND: Not detected. *Value marked by different letters in each row are significantly different (p < 0.05). Data are Mean±SD, n = 3

Table 3. Color parameters and turbidity of tea infusions form mulberry and *Camellia sinensis*.

Properties	Green tea	Oolong tea	Black tea	Mulberry		
				MANF	MAMF	MAFF
L [†]	99.64±0.47 ^a	97.32±0.12 ^c	94.27±0.25 ^d	99.88±0.11 ^a	98.61±0.45 ^b	97.16±0.15 ^{c*}
a [†]	-0.48±0.05 ^b	-0.28±0.03 ^c	-2.84±0.03 ^a	-0.24±0.01 ^d	-0.25±0.01 ^d	-0.09±0.04 ^e
b [†]	3.17±0.19 ^c	5.12±0.23 ^b	16.32±0.54 ^a	1.45±0.05 ^a	2.24±0.11 ^d	2.57±0.65 ^{cd}
Turbidity [‡]	2.64±0.08 ^d	3.68±0.23 ^c	3.24±0.45 ^c	2.48±0.22 ^d	5.16±0.23 ^b	7.12±0.43 ^a

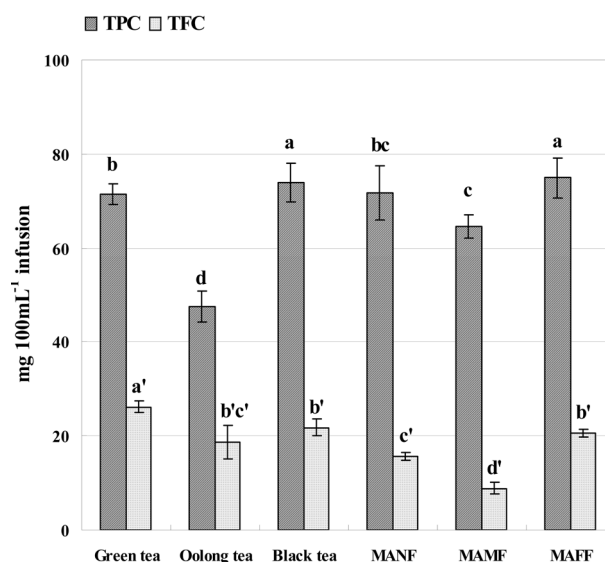
[†]L: [(0 = black, 100 white); +a : Redness -a : Greenness ; +b : Yellowness; -b : Blueness].

[‡]Turbidity in (100-T%). *Value marked by different letters in each row are significantly different (p < 0.05). Data are Mean±SD, n = 6

in mulberry leaf teas. A similar trend was found in *Camellia sinensis* teas. The L* values indicated that the infusions of both major tea types were bright and clear. The colors of the teas were yellow and the color of the tea infusion correlated with that of leaves. The depth of color of the tea infusions was higher in MANF and MAMF than in MAFF for greenness, but yellowness was higher in MAFF and MAMF than in MANF. The greenish value (-a) and yellowish value (+b) of *Camellia sinensis* teas were significantly higher than those in all the mulberry leaf tea infusions; the BT infusion had the highest greenish and yellowish values. This indicates that *Camellia sinensis* tea infusions were darker than mulberry leaf tea infusions. In mulberry teas, fermentation significantly improved the redness and yellowness of the infusion. Oxidation of catechins and epicatechins and their gallates, containing a benzotropolone nucleus, generate flavin products, such as flavin, theaflavin-3-gallate, theaflavin-3'-gallate, and theaflavin 3,3'-digallate. These compounds are of interest because of their unique presence in fermented leaf teas, such as black tea, and their contribution to the color of tea brews (Hong and Yang 2006). Claims have been made that the theaflavin contents correlate with the ratings of tea tasters and with the quality of tea, but this remains controversial.

The turbidity values of the three mulberry leaf teas were generally higher than those of the *Camellia sinensis* teas, except for MANF, in which turbidity was significantly lower than those of OT and BT and not significantly different from that of GT. In mulberry teas, turbidity increased with fermentation time, with the following trend: MANF < MAMF < MAFF. The results showed that turbidity values in both tea types were generally below 10%, indicating a low level of turbidity. MAFF exhibited a higher level (7.12%) of turbidity, suggesting that compounds linked with macromolecules come off the leaves during fermentation, probably allowing these compounds to diffuse readily and thus increasing the turbidity of the infusion.

Total phenol and total flavonoid contents of the tea infusions


Fig. 2. Total phenolic content (TPC) and Total flavonoid content (TFC) of teas in mulberry leaf and *Camellia sinensis*. TPC in mg gallic acid equivalent/100 mL infusion, TFC in mg quercetin equivalent/100 mL infusion. Values marked by different letters are significantly different (P < 0.05). Data are Mean±SD, n = 5.

from mulberry and *Camellia sinensis* were determined and are shown in Fig. 2. Yao *et al.* (2006) reported that phenolic compounds were the main quality parameters for tea; polyphenols are aromatic secondary metabolites widely found in herbs and associated with color, sensory qualities, and nutritional and antioxidant properties of foods and beverages (Gupta and Prakash, 2009). The amount of tea polyphenols has been regarded as a quality indicator of tea (Obanda *et al.*, 1997). All types of unfermented leaf teas (GT, MANF) and fully fermented leaf teas (BT, MAFF) had higher total polyphenol contents than the medial fermented leaf teas (OT, MAMF). There was no significant difference between the total phenol contents of unfermented (GT, MANF) and fully fermented teas (BT, MAFF). The catechins present in green tea are commonly called polyphenols. Green and black teas are processed differently

during manufacturing. Fresh green tea leaves, which are very rich in catechins, are not fermented; they are withered, and catechin oxidation by polyphenol oxidase is prevented by steaming or by panning (Graham, 1999), processes that essentially maintain the polyphenols in their monomeric forms. However, black tea leaves are subjected to crushing and a full fermentation process in which catechin derivatives are oxidized, resulting in the formation of polymeric compounds: theaflavins, theaflagillins, thearinsins, theacitrins, and thearubigins (Lee *et al.*, 2002). We suggest that there was no significant difference between the total phenol contents of unfermented and fully fermented teas because oligomer polyphenols were converted to secondary polymer compounds by oxidation by polyphenol oxidase during the fermentation process. Teas with high total phenol contents also exhibited high total flavonoid contents. Flavonoids, such as catechins and other polyphenols, have antioxidant activities (Dufresne and Farnworth, 2001). They act as antioxidants *in vitro* by sequestering metal ions and by scavenging reactive oxygen and nitrogen species (Frei and Higdon, 2003; Wiseman *et al.*, 1997).

Two antioxidant assays, a DPPH free radical-scavenging assay and a ferric reducing/antioxidant power (FRAP) assay, were used to determine the antioxidative activities of the tea infusions. All *Camellia sinensis* teas had higher FRAP values than did mulberry leaf teas, while GT had the highest DPPH radical-scavenging activity, followed by MAFF, OT, and BT, and then MAMF and MANF (Fig. 3).

The presence of rutin in green tea has been extensively reviewed and found to have the highest concentration among all flavonol glycosides in green tea (Stewart *et al.*, 2005; Markowicz Nastos *et al.*, 2007; Yen and Chen, 1995). GT had the highest rutin content (25.38 mg/gDW), followed by BT and OT; these contents were significantly higher than those in mulberry leaf teas (Table 4).

Rutin, which is the most predominant flavonol glycoside of quercetin in *Camellia sinensis*, was degraded by oxidation. Oxidative degradation of rutin and its aglycone quercetin has

been reported previously by Makris and Rossiter (2000). The flavonols show antioxidant activity as a result of their characteristic structure, such as the *ortho*-dihydroxy structure of the B ring (also known as the catechol structure), and the C ring contributes to the formation of a *para*-quinoic structure by donating protons. In the case of OT and GT, occurrence of oxidative degradation may be responsible for the lowered antioxidant capacity of green teas during the fermentation processing (Fig. 3).

In mulberry leaf teas, the rutin content of MAMF was higher than those of MANF or MAFF, and the FRAP activity of MAMF was similar to that of MANF, although the total flavonoid content of MAMF was lower than that of MANF (Fig. 3). These results showed that the high amount of rutin present in MAMF also contributed to the antioxidant activity. Rutin in buckwheat grit cake was degraded at a higher rate as treatment time and temperature (240 to 258 °C) increased in a study by Im

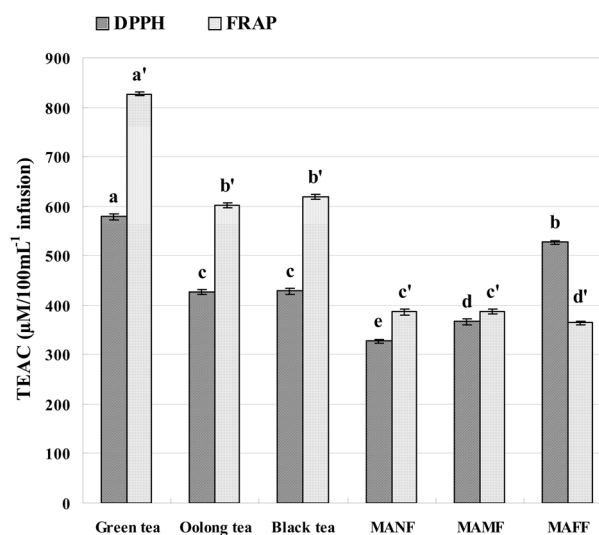


Fig. 3. Total antioxidant activity of teas from mulberry leaf and *Camellia sinensis*. TEAC (Trolox equivalent antioxidant capacity) DPPH (1,1-diphenyl-2-picrylhydrazyl free radical scavenging activity, FRAP (Ferric reducing antioxidant potential assay). Values marked by different letters are significantly different ($P < 0.05$). Data re Mean \pm SD, $n = 5$

Table 4. Rutin and GABA contents of Chemical properties of teas from mulberry leaf and *Camellia sinensis*.

Properties	Green tea (GT)	Oolong tea (OT)	Black tea (BT)	Mulberry		
				MANF	MAMF	MAFF
Rutin [†]	25.38 \pm 1.14 ^a	20.64 \pm 0.86 ^c	18.17 \pm 0.86 ^b	0.700 \pm 0.01 ^f	1.78 \pm 0.02 ^d	1.05 \pm 0.02 ^{e*}
GABA [†]	0.16 \pm 0.01 ^d	0.18 \pm 0.04 ^d	0.24 \pm 0.02 ^c	0.17 \pm 0.01 ^d	0.43 \pm 0.03 ^b	1.40 \pm 0.02 ^a

[†]Values in dry weight basis, mg/gDW. *Value marked by different letters in each row are significantly different ($p < 0.05$). Data are Mean \pm SD, $n = 6$

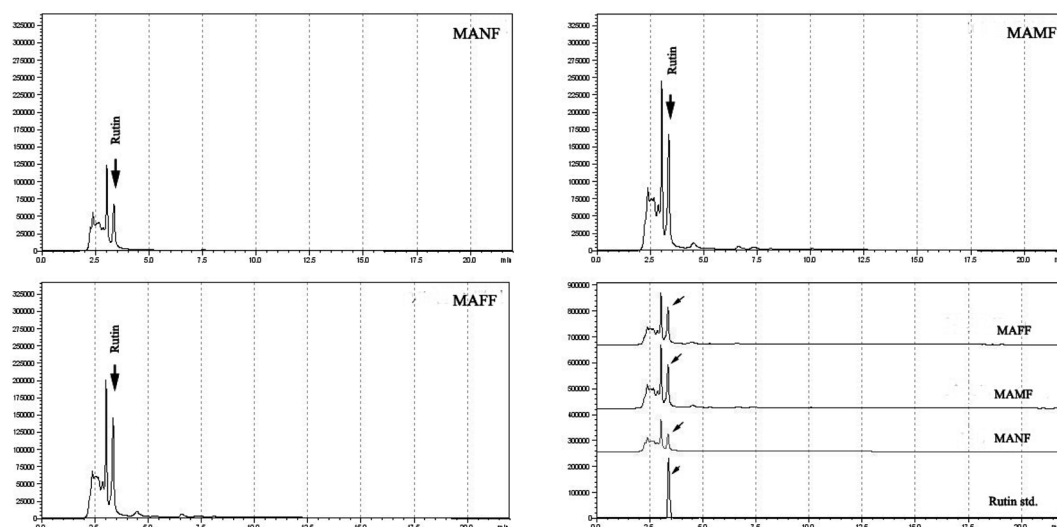


Fig. 4. Comparison of HPLC profiles of rutin (quercetin 3-O- β -D-rutinoside, RT 5.10 min) in types of tea from leaves of mulberry. MANF, not fermented tea; MAMF, medial fermented tea (10 hour); and full fermented tea (24 hour).

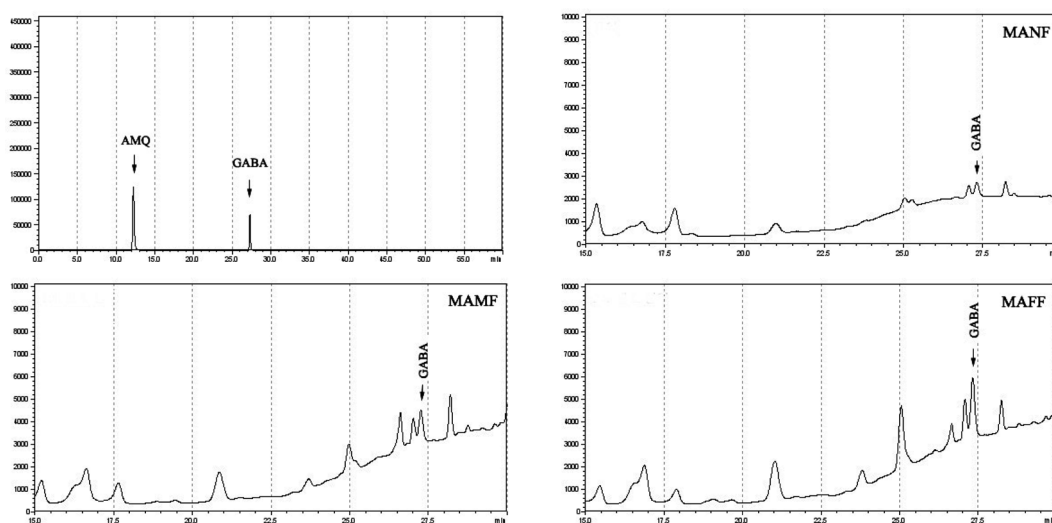


Fig. 5. Comparison of HPLC profiles of GABA (γ -aminobutylic acid, RT 27.30 min) in types of tea from leaves of mulberry. MANF, not fermented tea; MAMF, medial fermented tea (10 hour); and full fermented tea (24 hour).

et al. (2003). Additionally, those authors illustrated that heat treatment time contributed to changes in rutin concentration, indicating that mild heat treatment increased the stability of rutin. Thus, mild heat treatment, such as the 230°C used in this study, is recommended over pan firing in terms of phytochemical stability of all phenolic compounds in tea processing.

GABA is a non-protein amino acid that is well known as a major inhibitory neurotransmitter in the nervous system of animals (Jakobs *et al.*, 1993; Wong *et al.*, 2003). GABA is primarily produced from irreversible α -decarboxylation of L-glutamic acid, catalyzed by glutamic acid decarboxylase (GAD),

which has also been found in bacteria, plants, and animals (Ueno, 2000). Indeed, GABA occurs naturally in many kinds of foods at low levels, while in fermented food products, GABA levels are higher. The GABA content ranged from 0.17 to 1.40 mg/gDW in mulberry leaf teas. The GABA content of MAFF was about 5.8 times greater than that in BT. The present results also showed that the GABA contents of teas from medial (MAMF) and fully fermented (MAFF) mulberry leaves were significantly higher than that in unfermented tea (MANF), and that they increased 2.5- and 8.2-fold, respectively, over the value for fresh leaves after fermentation for 10 and 24 h (Table 4, Fig. 5).

In conclusion, the present study shows that fermented teas from mulberry leaves have potential as herbal teas because of their high GABA content, physicochemical properties, soluble vitamin content, and low caffeine in comparison with *Camellia sinensis* teas. Our results suggest that the fermentation process can improve the quality of tea and beverages derived from mulberry leaves and may increase the health benefits.

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