การตรวจสอบคุณภาพแบบรวดเร็วของชาใบหม่อนระหว่างการเก็บรักษาโดยจมูกอิเล็กทรอนิกส์และลิ้นอิเล็กทรอนิกส์

นางสาวอัญชลี เรืองเดช

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
สาขาวิชาเทคโนโลยีทางอาหาร ภาควิชาเทคโนโลยีทางอาหาร
คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2560

RAPID QUALITY DETERMINATION OF MULBERRY LEAF TEA DURING STORAGE USING ELECTRONIC NOSE AND ELECTRONIC TONGUE

Miss Anchalee Ruengdech

A Dissertation Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy Program in Food Technology
Department of Food Technology
Faculty of Science
Chulalongkorn University
Academic Year 2017

Thesis Title	RAPID QUALITY DETERMINATION OF MULBERRY LEAF TEA DURING STORAGE USING ELECTRONIC NOSE AND ELECTRONIC TONGUE
By	Miss Anchalee Ruengdech
Field of Study	Food Technology
Thesis Advisor	Associate Professor Ubonrat Siripatrawan, Ph.D.

Accepted by the Faculty of Science, Chulalongkorn University in Partial Fulfillment of the Requirements for the Doctoral Degree

.....Dean of the Faculty of Science
(Professor Polkit Sangvanich, Ph.D.)

THESIS COMMITTEE

.....Chairman
(Assistant Professor Kiattisak Duangmal, Ph.D.)

.....Thesis Advisor
(Associate Professor Ubonrat Siripatrawan, Ph.D.)

.....Examiner
(Associate Professor Jirarat Anuntagool, Ph.D.)

.....Examiner
(Assistant Professor Varapha Kongpensook, Ph.D.)

.....Examiner
(Assistant Professor Romanee Sanguandeekul, Ph.D.)

.....External Examiner
(Assistant Professor Arpathsra Sangnark, Ph.D.)

อัญชลี เรืองเดช : การตรวจสอบคุณภาพแบบรวดเร็วของชาใบหม่อนระหว่างการเก็บรักษาโดยจมูกอิเล็กทรอนิกส์และลิ้นอิเล็กทรอนิกส์ (RAPID QUALITY DETERMINATION OF MULBERRY LEAF TEA DURING STORAGE USING ELECTRONIC NOSE AND ELECTRONIC TONGUE) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: รศ. ดร.อุบลรัตน์ สิริภัทราวรรณ, หน้า.

งานวิจัยนี้มีวัตถุประสงค์เพื่อพัฒนาวิธีการตรวจสอบอย่างรวดเร็วของลักษณะทางคุณภาพ (quality attributes; ได้แก่ สมบัติทางกายภาพ สารระเหย และลักษณะทางประสาทสัมผัส) และสารพฤกษเคมี (phytochemicals) ของชาใบหม่อนโดยใช้จมูกอิเล็กทรอนิกส์ (electronic nose) และลิ้นอิเล็กทรอนิกส์ (electronic tongue) การทดลองแบ่งออกเป็น 3 ส่วน ได้แก่ (1) ศึกษาผลของพันธุ์หม่อนต่อลักษณะทางคุณภาพและพฤกษเคมีของชาใบหม่อน (2) ศึกษาผลของบรรจุภัณฑ์และเวลาในการเก็บรักษาต่อลักษณะทางคุณภาพและสารพฤกษเคมีของชาใบหม่อน และ (3) ศึกษาการใช้ลิ้นอิเล็กทรอนิกส์ร่วมกับวิธีเคโมเมตริกซ์ (chemometrics) ทำนายปริมาณสารสารพฤกษเคมีและฤทธิ์การต้านออกซิเดชันของชาใบหม่อน

การศึกษาผลของพันธุ์หม่อนต่อคุณภาพของชาใบหม่อนทดลองโดยเตรียมผลิตภัณฑ์ชาใบหม่อนจากใบหม่อน 3 พันธุ์ ได้แก่ บุรีรัมย์ 60 คุณไพ และสกลนคร วิเคราะห์ total henolic content (TPC), total flavonoids content (TFC) ทดสอบฤทธิ์การต้านออกซิเดชันด้วยวิธี FRAP และ DPPH วิเคราะห์สารระเหยโดยใช้ SPME-GC/MS และจมูกอิเล็กทรอนิกส์ และทดสอบลักษณะทางประสาทสัมผัสด้วยวิธีพรรณนาเชิงปริมาณ โดยพิจารณาลักษณะ สีเขียว สีน้ำตาล กลิ่นเขียว กลิ่นใบไม้แห้ง และรสฝาด ผลการทดลองพบว่าชาใบหม่อนต่างพันธุ์มีลักษณะทางคุณภาพและสารพฤกษเคมีแตกต่างกันอย่างมีนัยสำคัญ ($p<0.05$) ชาใบหม่อนพันธุ์คุณไพมีค่า TPC (37.78 ± 0.34 mg GAE /g) TFC (29.73 ± 0.33 mg rutin /g) FRAP (11.07 ± 0.16 mM Trolox / 100 g) และ DPPH (ร้อยละ 55.51 ± 1.33) สูงกว่าชาใบหม่อนพันธุ์บุรีรัมย์และสกลนครตามลำดับ สารระเหยที่พบในชาใบหม่อนเป็นสารประกอบอัลดีไฮด์ คีโตน แอลกอฮอล์ เอสเตอร์ และกรด ผลการทดสอบทางประสาทสัมผัสพบว่า ชาใบหม่อนพันธุ์สกลนครมีค่าคะแนนสีเขียวสูงที่สุด (7.63 ± 0.51) แต่มีค่าคะแนนสีน้ำตาลต่ำที่สุด (4.27 ± 0.52) ชาใบหม่อนพันธุ์บุรีรัมย์ 60 มีคะแนนกลิ่นเขียว (7.81 ± 0.67) และกลิ่นใบไม้แห้ง (8.89 ± 0.59) มากกว่าชาใบหม่อนอีก 2 พันธุ์ การวิเคราะห์ตัวอย่างด้วยจมูกอิเล็กทรอนิกส์ร่วมกับการวิเคราะห์ด้วยเทคนิค principal components analysis (PCA) พบว่า PC1 และ PC2 สามารถอธิบายความแปรปรวนได้ร้อยละ 94 แสดงให้เห็นว่าจมูกอิเล็กทรอนิกส์สามารถจำแนกกลุ่มชาใบหม่อนจากองค์ประกอบของสารระเหยที่แตกต่างกันได้อย่างชัดเจน

การศึกษาผลของบรรจุภัณฑ์และเวลาในการเก็บรักษาต่อคุณภาพของใบหม่อนทดลองโดยเตรียมผลิตภัณฑ์ชาใบหม่อนพันธุ์บุรีรัมย์ 60 บรรจุลงในถุงอลูมิเนียมเคลือบพลาสติก (low-density polyethylene laminated aluminum (AL)) และโพลีโพรพิลีน (polypropylene (PP)) เก็บรักษาที่อุณหภูมิ 30 องศาเซลเซียส (ความชื้นสัมพัทธ์ร้อยละ 75) เป็นเวลา 18 เดือน สุ่มตัวอย่างทุก 3 เดือนเพื่อวิเคราะห์ปริมาณน้ำอิสระ (a_w), สี (L *, a *, b *),TPC, TFC, FRAP, และ DPPH สารระเหยง่าย และลักษณะทางประสาทสัมผัส ผลการทดลองพบว่าบรรจุภัณฑ์และเวลาในการเก็บรักษาส่งผลต่อการเปลี่ยนแปลงลักษณะทางคุณภาพและสารเคมีพฤกษเคมีของชาใบหม่อนอย่างมีนัยสำคัญ ($p<0.05$) ในระหว่างการเก็บรักษาตัวอย่างที่บรรจุในถุง PP มีค่า a_w และ a* เพิ่มขึ้น แต่มีค่า L *, b *, TPC, TFC, FRAP และ DPPH ลดลงอย่างมีนัยสำคัญ ($p<0.05$) คุณลักษณะด้านคุณภาพของตัวอย่างที่บรรจุในถุง AL ไม่แตกต่างกันอย่างมีนัยสำคัญ เมื่อเก็บรักษาเป็นเวลา 18 เดือน ตัวอย่างที่บรรจุในถุง PP สูญเสีย TPC, TFC, FRAP และ DPPH ร้อยละ 28.84, 34.39, 33.31 และ 30.34 ตามลำดับ สารระเหยที่เกิดจากปฏิกิริยาออกซิเดชันของไขมัน เช่น hexane และ 4-oxo-2-nonenal มีปริมาณเพิ่มขึ้นอย่างมีนัยสำคัญ ($p<0.05$) ผลการทดสอบทางประสาทสัมผัสพบว่าชาใบหม่อนที่บรรจุในถุง PP มีสีน้ำตาลเพิ่มขึ้น แต่มีสีเขียว กลิ่นเขียว และกลิ่นใบไหม้แห้งลดลงอย่างมีนัยสำคัญ ($p<0.05$) การวิเคราะห์ด้วยจมูกอิเล็กทรอนิกส์ร่วมกับเทคนิค PCA พบว่าจมูกอิเล็กทรอนิกส์มีประสิทธิภาพสูงในจำแนกกลุ่มชาใบหม่อนตามความแตกต่างของสารระเหยที่เป็นองค์ประกอบในตัวอย่าง โดย PC1 และ PC2 สามารถอธิบายความแปรปรวนได้ถึงร้อยละ 98

จากการศึกษาการใช้เครื่องลิ้นอิเล็กทรอนิกส์ทำนายปริมาณสารพฤกษเคมีและฤทธิ์การต้านออกซิเดชันของชาใบหม่อนในระหว่างเก็บรักษา โดยสร้างสมการเทียบมาตรฐานด้วยเทคนิค partial least square (PLS) regression เพื่อหาความสัมพันธ์ระหว่างปริมาณพฤกษเคมี และค่าตอบสนองที่ได้จากลิ้นอิเล็กทรอนิกส์ (e-tongue responses) พบว่าสมการ PLS มีประสิทธิภาพดีในการทำนายค่า TPC, TFC, DPPH และ FRAP ของ ชาใบหม่อน มีค่าสัมประสิทธิ์การกำหนดค่าสูง ($r^2>0.9$) และมีค่าผิดพลาดในการทำนายต่ำ จากผลที่ได้จากงานวิจัยแสดงให้เห็นว่าการใช้จมูกอิเล็กทรอนิกส์และลิ้นอิเล็กทรอนิกส์เป็นวิธีการที่รวดเร็ว ไม่ทำลายตัวอย่าง และเชื่อถือได้ในการตรวจติดตามลักษณะทางคุณภาพและสารพฤกษเคมีของชาใบหม่อน

ภาควิชา เทคโนโลยีทางอาหาร ลายมือชื่อนิสิต .....

สาขาวิชา เทคโนโลยีทางอาหาร ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

ปีการศึกษา 2560

5572845723 : MAJOR FOOD TECHNOLOGY
KEYWORDS: RAPID METHOD / MULBERRY LEAF TEA / PHYTOCHEMICALS / ELECTRONIC NOSE / ELECTRONIC TONGUE

ANCHALEE RUENGDECH: RAPID QUALITY DETERMINATION OF MULBERRY LEAF TEA DURING STORAGE USING ELECTRONIC NOSE AND ELECTRONIC TONGUE. ADVISOR: ASSOC. PROF. UBONRAT SIRIPATRAWAN, Ph.D., pp.

This study was aimed to develop rapid methods based on electronic nose (e-nose) and electronic tongue (e-tongue) to determine the quality attributes and phytochemicals of mulberry leaf tea. This study was divided into three parts: (1) to determine the effect of cultivar on the quality attributes and phytochemicals of mulberry leaf tea, (2) to evaluate the effect of packaging and storage time on quality attributes and phytochemicals of mulberry leaf tea, and (3) to employ e-tongue combined with chemometrics for prediction of phytochemicals and antioxidant activity of mulberry leaf tea.

Mulberry leaf tea prepared from three different mulberry cultivars including Burirum 60 (BR), Khunphai (KP), and Sakonnakhon (SK) were compared in terms of total phenolic content (TPC), total flavonoids content (TFC), antioxidant activity (FRAP and DPPH), volatile components, and sensory attributes. Volatile components of the samples were analyzed using SPME-GC/MS and e-nose. Quantitative descriptive analysis was performed to evaluate sensory attributes (e.g. greenness, brownness, green odor, dried-leaf odor, and astringency). The results showed that cultivar significantly affected ($p<0.05$) the quality attributes and phytochemicals of the mulberry leaf tea. KP cultivar had the highest values of TPC, TFC, FRAP and DPPH. The distinct patterns of volatile components including aldehyde, ketone, alcohol, ester, and acid as a factor of different mulberry cultivars were observed. The results of sensory analysis showed that color and odor attributes were significantly different ($p<0.05$) among three mulberry cultivars. SK cultivar had the highest greenness score (7.63±0.51) but the lowest brownness score (4.27±0.52). The highest score of green odor (7.81±0.67) and dried-leaf odor (8.89±0.59) were observed in BR cultivar. Principal components analysis (PCA) was used to analyze the e-nose response data and with 2 components (PC1 and PC2) could explain 94% of the total variance.

In order to investigate the effects of packaging and storage time on the quality of mulberry leaf tea, the sample prepared from mulberry leaves of Burirum 60 cultivar was packaged in a linear low-density polyethylene laminated aluminum (AL) bag and polypropylene (PP) bag and stored at 30±1°C (75% RH). The changes in tea qualities including water activity (a_w), color (L*, a*, b*), phytochemicals (TPC and TFC), volatile components, and sensory attributes were determined at three-month interval for 18 months. The results showed that packaging material and storage time significantly ($p<0.05$) affected quality attributes and phytochemicals of the mulberry leaf tea. As the storage time increased, a_w and a* values of the samples in PP increased but L*, b*, TPC, TFC, FRAP and DPPH values decreased, whereas the quality attributes of mulberry leaf tea were not significantly different throughout the storage. After 18 months of storage, TPC, TFC, FRAP and DPPH values of the samples in PP lost by 28.84, 34.39, 33.31, and 30.34%, respectively. The intensity of volatile compounds produced from lipid oxidation such as hexane and 4-oxo-2-nonenal of the mulberry tea in PP significantly ($p<0.05$) increased. The greenness, green odor, and dried-leaf odor scores of mulberry leaf tea in PP significantly ($p<0.05$) decreased while the brownness scores significantly ($p<0.05$) increased. PCA was performed to discriminate the quality of the samples by e-nose response data and the first two PCs (PC1 vs. PC2) could explain 98% of the total variance. The results suggested that e-nose is able to discriminate quality of the samples as a result of different storage periods.

In this research, e-tongue was used to monitor phytochemicals and antioxidant activity of mulberry leaf tea during storage. Partial least squares (PLS) regression was applied to predict phytochemical values from the e-tongue response data. The PLS models for TPC, TFC, FRAP and DPPH achieved good predictability with high values of coefficient of determination ($r^2>0.9$) and gave low mean square error of prediction. The results of this study indicated that e-nose and e-tongue have proved to be a non-destructive, rapid, and reliable method for quality discrimination of the mulberry leaf tea and have potential as an alternative to the conventional analyses of quality attributes and phytochemicals of the mulberry leaf tea.

Department:	Food Technology	Student's Signature	----------
Field of Study:	Food Technology	Advisor's Signature	----------
Academic Year:	2017		

ACKNOWLEDGEMENTS

I would like to express my sincere appreciation to my thesis advisor, Assoc. Prof. Ubonrat Siripatrawan who always gave me valuable advice, assistance, and encouragement throughout research work.

I am deeply grateful to my foreign tutors, Assoc. Prof. Susanna Buratti who work at Department of Food, Environmental and Nutritional Sciences (DeFENS), Università deli Studi di Milano, Italy for her technical support for the e-nose and e-tongue experiments, valuable advices, and constructive comments.

I would sincerely like to thank my committee members, Asst. Prof. Kiattisak Duangmal, Assoc. Prof. Jirarat Anuntagool, Asst. Prof. Romanee Sanguandeekul, Asst. Prof. Varapha Kongpensook, and Asst. Prof. Arpathsra Sangnark, who suggest me to complete my thesis content.

I am grateful to all laboratory technicians in the Department of Food Technology, Chulalongkorn University for their help and technical support.

I would like to thank Thailand Research Fund (TRF) and Chulalongkorn University for supporting a scholarship coming from the Royal Golden Jubilee (RGJ) Ph.D. Program and the 90th Anniversary of Chulalongkorn University Fund (Ratchadaphiseksomphot Endowment Fund), respectively. I also would like to thank the Queen Sirikit Sericulture Center, Saraburi, Thailand for providing the mulberry leaves.

Finally, I am especially grateful to my family and friends for their support and encouragement throughout my study.

CONTENTS

Page

Page

Page

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Mulberry (*Morus alba* L.) leaf tea has been claimed to be antioxidative, antimicrobial, cholesterol-lowering, glucose-lowering, and anti-hyperglycemic because it contains high active phenolic compounds such as rutin, quercetin, kaempferol, and chlorogenic acid which have strong radical scavenging and anti-inflammatory activity (Sánchez-Salcedo et al., 2015; Shoaib Zafar et al., 2013). Due to their potential health benefits, consumption of mulberry leaf tea is popularly increasing worldwide especially in Asian countries (Thabti et al., 2012).

The quality attributes defined by the physical properties (moisture content, water activity, and color), volatile components, and sensory attributes (appearance, odor, and taste) are the key factors to assess the mulberry leaf tea quality (Charalambous, 1992). While phytochemicals related to phenolic composition are indicators of the health benefits of mulberry leaf tea (Katsube et al., 2009). However, the quality attributes and phytochemicals of mulberry leaf tea vary in relation to cultivars, processing, packaging, and condition of storage. Current studies are mainly focused on the influence of cultivar on phenolic composition and content of mulberry leaf tea (Lee and Choi, 2012; Zou et al., 2012). Nevertheless, scientific information on aroma constituents and quality attributes of herbal tea from mulberry leaves of different cultivars are scarce. Information on the volatile compounds and quality attributes of mulberry leaf tea is beneficial to the herbal tea industry for quality control of raw material and end products.

Since the changes in flavor and aroma profile of herbal tea during storage result in the greatly decline in its quality and consumer acceptance, several studies have examined the effect of storage time on herbal tea quality. Harnnurak and Riebroy (2014) reported that the flavor and aroma of mulberry leaf tea stored at 30°C attenuated with increasing storage time. The off-flavor of green tea stored at 20°C increased with increasing storage time (Lee and Chambers, 2010). The intensity of floral note of Pouchung tea stored at 25°C significantly decreased whereas the intensity of stale and rancidity notes greatly increased with increasing storage time (Charalambous, 1992). During storage, not only the volatile components but also phytochemicals of tea and herbal tea obviously changed with period time. Friedman et al. (2009) reported that phenolic compounds (e.g. catechin) of green tea stored at 30°C decreased by one-third at 6 months of storage. Similarly, Naithani et al. (2006) stated that antioxidant activities of Indian herbal teas declined 57.7 - 92.1% at the end of 15 months storage. Dengliang (1998) investigated the relations between variation of polyphenols and quality of green tea during storage. The result showed that the 25% of polyphenol content decrease caused obvious deterioration on of tea quality to be without drinking value.

There are many reports indicated that the proper selection of packaging material could maintain the freshness and product quality, retard product deterioration, and extend shelf-life of tea and herbal tea during storage. Lee and Chambers (2010) reported that packaging materials significantly ($p \leq 0.05$) affected changes in aroma and volatile components of green tea during storage. Harnnurak & Riebroy (2014) reported that the alumimium foil bag could protect the quality attributes of mulberry leaf tea during storage better than paper bag and sachet, respectively. In addition, the study reported that the packaging material (nylon, polypropylene, and aluminum foil) significantly

affected physical, chemical, and sensory properties of mulberry during storage (Siripatrawan et al., 2008).

Monitoring of the changes in quality attributes and phytochemicals of mulberry leaf tea during storage is needed to ensure the quality and efficiency of its antioxidant activity. Generally, the quality attributes of tea and herbal tea has been assessed by human panel tasting. However, the result of sensory test is often less impartiality because it can be affected by subjective factors such as emotion, exhaustion, and physiological conditions (Liang et al., 2003). Furthermore, the sensory test is time-consuming and expensive. Although several techniques such as spectrophotometry, high-performance liquid chromatography, and capillary electrophoresis are available for analysis of phytochemicals of food, they are complicated, costly, and time-consuming (Caridi et al., 2007; Naczk and Shahidi, 2004; Siripatrawan et al., 2013). Due to the drawbacks of the conventional methods, the development of subjective, timely, and cost-effective methods for determination of volatile compounds and sensory attributes of herbal teas is of great demand for the tea industry.

Electronic nose (e-nose) and electronic tongue (e-tongue) have been applied successfully in the food field because they are non-destructive, fast, and reliable technology. E-nose system is the application of the sensor array which is sensitively to the volatile compounds The sensor responses are related to the amount of volatile compounds that contact with the corresponding sensor surface (Siripatrawan and Harte, 2015). E-nose has been efficiently used for discrimination of different herbal teas (Jin et al., 2015), for the detection of the optimum fermentation time in black tea manufacturing process (Bhattacharyya et al., 2007), and also for the discrimination of the quality changes in green tea during storage (Mirasoli et al., 2014).

E-tongue, also known as taste sensor, can be used to measures and compare tastes of food (Escuder-Gilabert and Peris, 2010). E-tongue is an instrument comprising an array of electrode sensors which response to chemical substances on the basis of their taste. When the detecting (working) sensors interact with chemical substances in a sample, voltammetric or potentiometric signals are generated and then converted to taste values (Vlasov et al., 2005). As a fast, reliable, and chemical-free technique, e-tongue is useful not only for identification or classification but also for quantitative analysis. E-tongue coupled with chemometrics has been applied to measure the concentration of phenolic compounds in beer, wine, tea, and olive oil (Apetrei and Apetrei, 2013; Ghosh et al., 2012; González-Calabuig and del Valle, 2018; Polshin et al., 2010).

To date, several researches have been undertaken on the quality attributes and health benefit of mulberry leaf tea. However, the effect of cultivars, packaging and storage time on qualities attributes and phytochemicals of mulberry leaf tea are still limited. Moreover, no attempt has been reported to use a combination of e-nose and e-tongue for monitoring quality attributes and phytochemicals of mulberry leaf tea during storage.

The hypothesis of this research is e-nose and e-tongue have potential as a rapid method for determination of quality attributes and phytochemicals of mulberry leaf tea. Therefore, this research aimed:

1. To study the effects of cultivars on quality attributes (water activity, color, volatile compounds, and sensory attributes), phytochemicals (total phenolic and flavonoids content), and antioxidant activity of mulberry leaf tea.

2. To study the effects of packaging material and storage time on quality attributes, phytochemicals, and antioxidant activity of mulberry leaf tea by using e-nose.

3. To develop and validate the predictive models between phytochemicals and e-tongue responses data of mulberry leaf tea during storage.

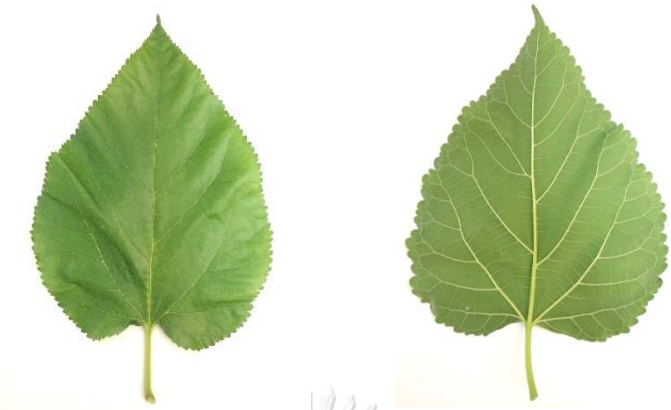
CHAPTER II

LITERATURE REVIEWS

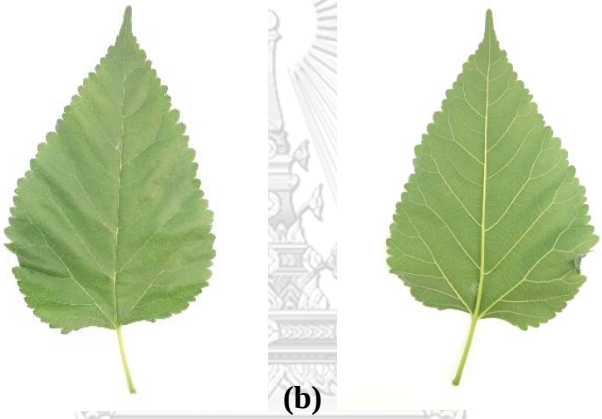
2.1 White mulberry leaves

White mulberry (Morus alba L.) is a plant in the Moraceae family widespread in the Middle East, southern Europe, and northern Africa (Shoaib Zafar et al., 2013). In Thailand, there are seven cultivars widely cultivated in north and north-eastern including Nakhonratchasima 60, Burirum 60, Sisaket 33, Sakonnakhon, Momnoi, Khunpai, and Burirum 51 (The Queen Sirikit Department of Sericulture, 2009). Morphology of white mulberry is shown in Fig. 2.1. The mulberry tree is a deciduous tree growing to 30 m tall and 1.8 m wide. The mulberry leaves are thin, glossy, light green color and serrated on the margin. The mulberry fruits are a multiple fruit, approximately 2–3 cm long. Immature fruits are white, green, or pale yellow and turn dark purple when fully ripe (Özgen et al., 2009; Vogrinčič et al., 2010).

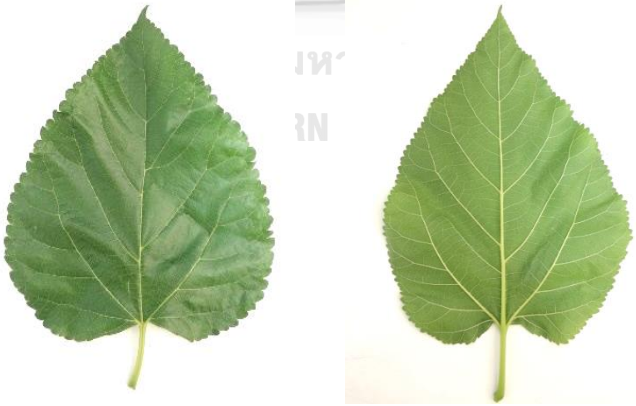
The nutritional compositions of mulberry leaves vary depending on the genotypic characteristics, cultivation, and factors of environmental. On a dry-weight basis, white mulberry leaves contain protein from 15.31 to 30.91%, from 2.09 to 4.93% for fat, from 9.70 to 29.64% for carbohydrate, and from 14.59 to 17.24% for ash content. The leaves contain ascorbic acid (100 to 200 mg/100 g), beta-carotene (8,438.00 to 13,125.00 µg/100 g), iron (19.00–35.72 mg/100 g), zinc (0.72–3.65 mg/100 g), and calcium (786.66–2,226.66 mg/100 g) (Srivastava et al., 2006). Moreover, the mulberry leaves contain high active phenolic compounds which are beneficial for human health (Lee & Choi, 2012).



(a)



(b)



(c)

Fig. 2.1 Front side and back side of three cultivars of mulberry leaves including Burirum 60 (a), Khunphai (b), and Sakhonnakorn (c).

Source: The Queen Sirikit Department of Sericulture (2009)

Mulberry leaves can used in many ways such as used in a fabric, food, and pharmacy industries (Venkatesh Kumar and Chauhan, 2008). Mulberry leaves are generally used as a food source of silkworms for silk production because of their high nutritional value (Shoaib Zafar et al., 2013). They have been used as a medicinal plant in many countries, particularly in eastern countries such as China, Korea and Japan (Kim et al., 2003). Moreover, mulberry leaves of white mulberries are used as food supplements which are commonly consumed as mulberry leaf tea, leaf powder, and leaf extract.

2.2 Mulberry leaf tea demands raised in the international market

Mulberry leaf tea means herbal tea produced by cutting or chopping fresh mulberry leaves, steaming or scalding with hot water, kneading the leaves to be contused, roasting, and drying with hot air oven (The National Bureau of Agricultural Commodity and Food Standards (ACFS), 2009). Mulberry leaf tea is commonly formed of dry pieces or dry powder. General characteristics, mulberry tea infusion has light green or light green with slightly brownish color, natural flavor, astringent taste, and no bitter taste (ACFS, 2009).

Nowadays, consumption of mulberry leaves as herbal tea is popularly increasing worldwide especially in Asian countries because of their benefit for health. Mulberry resources are rich in China. The report indicated that the market price of the mulberry leaves was up 30% at the end of 2013. It is expected that the consumption of mulberry leaves as herbal tea product on the Chinese market is still bullish in the future (Geneham Pharmaceutical Co., 2015). Mulberry leaf tea powdered juice are widely

consumed in Korea and Japan and demand have been increasing every year (Sánchez-Salcedo et al., 2015). In Thailand, Mulberry leaf tea has been paid attention to by both domestic and international consumers, considering in increasing growth rate of the domestic market as well as the rising trend of export volume and value (ACFS, 2009). Moreover, mulberry leaf tea and beverage sale increased year by year in the United States market (Geneham Pharmaceutical Co., 2015). Although demand for mulberry leaf tea is greatest in Asia market, there is a small and growing market in Western Europe, mainly in Germany and France (The Centre for the Promotion of Imports from developing countries (CBI), 2016). Now, the mulberry leaf tea and leaf extract in the United States and the European market had annual sales of millions of dollars (Andrew, 2015).

Mulberry leaf tea can be produced on a household level or industrial factory according to The Queen Sirikit Department of Sericulture (2010). The procedure of mulberry leaf tea production is following:

2.2.1 Household production

(1) Carefully clean and chop the leaves into small size (0.5-1.0) x (3.0-4.0) cm^2

(2) Steam the sliced leaves for 1-2 min

(3) Knead the leaves with hand and roast and in a pan for 20-25 min

(4) Dry the roasted leaves in hot air at 80°C for 1 h to reduce moisture not over 7%

(5) Store mulberry leaf tea in sealed packaging to prevent moisture, sunlight, and cigarette beetles

2.2.2 Industrial production

(1) Clean and chop the leaves along their width about 1-2 cm

(2) Stream the chopped leaves at higher than 95°C for 40-50s

(3) Roast for the first time at 80-90°C about 35 min

(4) Knead the leaves with machine for 5-10 min

(5) Reroast for the second time at 65-68°C about 35 min

(6) Remove the large leaf stalks

(7) Reroast for the third time at 100°C for 15-20 min

(8) Dry the leaves in a hot air oven at 80-90C about 25 min

(9) Pack in bags which prevent moisture, sunlight, and cigarette beetles

2.3 Phytochemicals of mulberry leaves and tea

Mulberry leaves are a rich source of phytochemicals including phenolic acids and flavonoids such as rutin, isoquercitrin, quercetin, astragalin, kaempferol, and chlorogenic acid which are well known for having various health benefits (Sánchez-Salcedo et al., 2015; Venkatesh Kumar and Chauhan, 2008).

Chlorogenic acid (5-O-caffeoylquinic acid) is a phenolic compound typically classified into one of either flavonoids and phenolic acids categories. It is formed by the esterification of caffeic acid and the aliphatic alcohol (–) quinic acid (1L-1(OH)-3,4/5-tetrahydroxycyclo-hexane carboxylic acid) (Fig. 2.2). Chlorogenic acid is present in many plants where it has many health-promoting properties, relating to the antioxidant, anti-inflammatory, anti-carcinogenic, anti-obesity, and antihypertensive

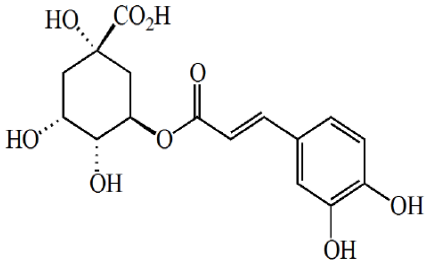
activities. (Plazas et al., 2013). Chlorogenic acid is the most abundant phenolic compound in mulberry leaves (150±60 - 965±71 mg/100 g dried sample) varied from the cultivar, harvest-time, and cultivation environment (Lee & Choi, 2012; Zou et al., 2012).

Rutin (quercetin-3-rutinosid) is a flavonol glycoside that is naturally synthesized in plants and used for protection against ultraviolet radiation and diseases (Vogrinčič et al., 2010). Rutin extracted from the mulberry leaves play a role in various health benefits, including antioxidative, hypolipidemic, antihyperglycemic, and antiatherogenic effects (Kim et al., 2017).

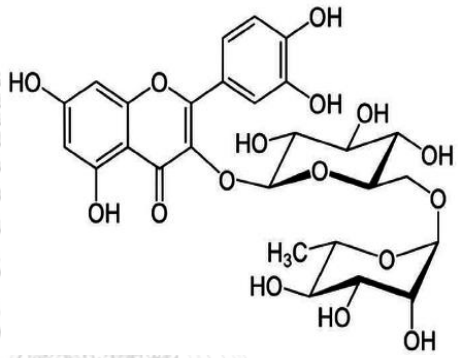
Quercetin (3,30,40,5,7-pentahydroxyflavone) belongs to the flavonoid compounds which contains several hydroxyl groups bound to the aromatic ring. Quercetin acted as antioxidants, anti-inflammatory, and anticancer activity. Sun et al. (2015) reported that quercetin glycoside found in mulberry leaves (quercetin 3-(6)-malonylglucoside) exhibited anti-obesity effects, reducing hepatic oxidative stress and enhancing lipid metabolism. However, quercetin is unstable when exposed to atmospheric oxygen, which causes degradation oxidation (Sokolová et al., 2012).

Moreover, white mulberry leaves contain a high melatonin (N-acetyl-5-methoxytryptamine) which involved in the regulation of circadian rhythm (sleep-wake cycle) and the alleviation of insomnia (Pothinuch and Tongchitpakdee, 2011).

(a) Chlorogenic acid



(b) Rutin



(c) Quercetin

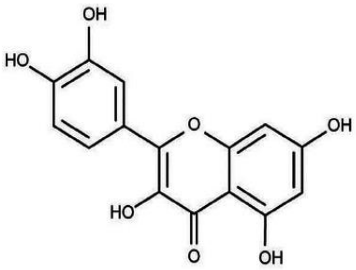


Fig. 2.2 The structure of the major phenolic compounds in mulberry leaves.

Source: Ashokkumar et al. (2013)

2.4 Volatile compounds of mulberry leaves and tea

Tea quality is defined by color, flavor, and aroma. While phenolic compounds are responsible for tea color and taste, volatile compounds are fundamental for tea odor and aroma. The volatile components and concentration of mulberry leaves and tea are influenced by overall differences in cultivar or variety, culturing systems, harvesting seasons, processing as well as storage conditions (Xue et al., 2012).

Mulberry leaves were found to potently attract silkworms because of their volatiles compounds. The main volatiles of mulberry leaf comprise hexanal, 1,5-hexadien-3-ol, 2-hexenal, hexyl acetate, 3-hexenyl acetate, 2-hexenyl acetate, 2-hexen-1-o, 1-hexanol, 3-hexen-1-ol, linalool, *cis*-jasmone, 2-phenylethyl and alcohol. Tanaka et al. (2009) found that *cis*-jasmone is a apotent attractant for silkworms.

Tea process causes remarkable changes of tea volatile composition in mulberry leaf tea. Phoonan et al. (2014) found that the 13 principal volatiles emitted from mulberry leaf tea was composed of five ketones (α-ionone, β-ionone, dihydroactinidiolide, neophytadiene, and 6,10,14-trimethyl-2-pentadecanone), four esters (methyl palmitate, methyl linoleate, methyl linolenate, and bis-(2-ethylhexyl)-hexanedioate), two alcohols (isophytol and phytol), and two unidentified compounds.

Xue et al. (2012), who studied the effects of harvest-time (summer, autumn, and winter) on volatile components of mulberry leaf tea, stated that the most predominant volatile compounds in mulberry leaf tea were aldehydes. A total of 39 aroma compounds were identified in mulberry leaf tea mainly including benzaldehyde, 1-octen-3-ol, 6-methyl-5-hepten-2-one, (E,E)-2,4-heptadienal, 2-ethyl-1-hexanol, nonanal, decanal, 2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde, 1-methoxy-4-(1-propenyl)-benzene, 6,10-dimethyl-5,9-undecadien-2-one, β-ionone, heptadecan, and so

on. The components including α-farnesene, β-ocimene, (Z)-3-hexen-1-ol, benzoate, indole, cedrol, naphthalene and its derivative, phytol, and hexanal revealed a significant seasonal difference in mulberry leaf green tea.

2.5 Food packaging

The important roles of food packaging are to contain the food, prevent the food quality, and prolong the shelf-life. The packaging can not only protect the qualities and benefits of the product but also catch the attention of the customers. The effects of packaging on quality of food products have widely been reported. Packing food products in the proper protective packaging can retard deterioration and prolong shelf life. The commercial mulberry leaf tea package in a vary of packaging material such as paper, plastic film, aluminium foil, and metallic film. However, two materials widely used to package the mulberry leaf tea are plastic films and aluminium.

2.5.1 Plastic film

Plastic films are material which made by condensation polymerization or addition polymerization of monomer units and through the process made into very thin film. The advantages of plastics are chemically resistant, heat sealable, easy to print, and can be variably formed, filled, and sealed in the same processing line. In addition, plastics are a wide range of physical and optical properties, lightweight, and inexpensive (Marsh & Bugusu, 2007). However, the disadvantages of plastics are variable permeability to moisture, gases, light, and oils. Polypropylene (PP) is one of an important plastic film used for food packaging.

Oriented polypropylene has a good optical property and a high tensile strength and puncture resistance. PP has moderate permeability to gases and a high barrier to water vapor (Allahvaisi, 2012). It is generally used to pack snack foods, herbal tea, and dried foods.

2.5.2 Aluminium

Aluminium is a silvery white metal derived from bauxite ore which is the combination with oxygen as alumina. Aluminium is excellent barrier to vopor, gases, oil, and light. It is commonly used for food packaging in the form of aluminum foil and laminated alumimium film.

2.5.2.1 Aluminium foil

Aluminium foil is a packaging material made by trundling aluminium metal into a thin sheet. The advantages of aluminium foil is lightweight, innert to acid, and excellent barrier to moisture, gases, light, and microorganisms.

2.5.2.2 Laminate alumimium film

In order to improve barrier properties, laminated aluminium was developed. Laminated aluminium is the binding of aluminum foil to plastic film. Laminated aluminium has a great chemical, moisture, and oxygen barrier. Combination of materials on the foil makes it opaque thus, it has a high prevention of products from light. More its advantages are seal abilities, well-formed, and maintain the quality of products with long-term preservation. Since laminated is quite expensive, it is typically used to package high value foods such as dried soups, coffee, tea, herbs, and spices (Marsh and Bugusu, 2007).

(a)

(b)

Fig. 2.3 Packaging; polypropylene bag (a) and laminated alumimiun bag (b).

Source: Shifang Packaging Material Co., Ltd. (2018)

2.6 Electronic nose

Electronic nose (e-nose) is a device for detecting odors or flavors that comprises 3 major parts including (1) a sample delivery system, (2) a detection system, and (3) a computing system. The sample delivery system enables the generation of the volatile compounds of a sample. The delivery system consists of an array of heterogeneous electrochemical gas sensors and pattern recognition systems. The general principle of sensor measurement is the changes in electric resistance when the sensor is exposed to volatile matters. Three different sensor types have been developed including metal oxides, conducting polymers composites, and intrinsically conducting polymers. E-nose with the metal oxide sensors have been widely used as a non-critical technique for food odor analysis that could confirm with sensory evaluation. E-nose are rapid, simple, and non-destructive technologies applied successfully food, cosmetic, packaging, and pharmaceutical industrials in a wide variety of applications for both qualitative and quantitative analyses. E-nose has been used to classify the different types of tea and herbal tea (Buratti et al., 2013; Zakaria et al., 2014), to detect the optimum fermentation time in black tea manufacturing process (Bhattacharyya et al., 2007), and also to discriminate the quality changes in green tea during storage (Mirasoli et al., 2014). In this study, two types of e-nose (Fox 4000 and Portable e-nose (PEN2)) were used to classify cultivars of mulberry leaf tea and discriminate the quality changes in green tea during storage.

2.6.1 Fox 4000

Fox 4000 (Alpha M.O.S., Toulouse, France) comprising 3 unit including measuring unit, AlphaSoft software, and autosampler is shown in Fig. 2.4. The FOX measuring unit comprises an injection port, 3 sensor chambers with 18 sensors (3 x 6

sensors) including LY2/LG, LY2/G, LY2/AA, LY2/Gh, LY2/gCTI, LY2/gCT, T30/1, P10/1, P10/2, P40/1, T70/21, PA/2, P30/1, P40/2, P30/2, T40/2, T40/1, and TA/2 sensors, and a number of electronic cards.

The AlphaSoft software of e-nose including;

(1) controls the autosampler

(2) acquires the sensor signals,

(3) performs statistical data processing and provides analyzed product quality information,

(4) monitors system operation through periodical diagnostics

(5) provides automatic scheduling for system maintenance.

An autosampler comprises a sample tray for 10 or 20 mL vials, a heated syringe for headspace sampling and injection, and a 6-position headspace generation oven.

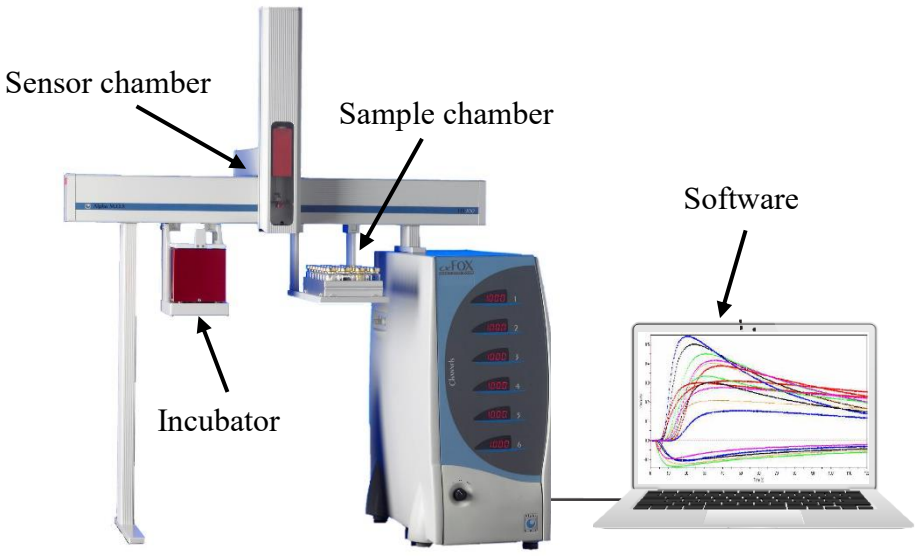


Fig. 2.4 E-nose unit: Fox 4000 (Alpha M.O.S., Toulouse, France)

Source: Alpha MOS (2017)

2.6.2 Portable e-nose (PEN2)

Portable e-nose (PEN2) (Win Master Airsense (WMA) Analytics Inc., Germany) based on a metal-oxide gas sensor array is shown in Fig. 2.5. By using a specific dilution technique the system is protected from overloading of substances. This extends the life time of the sensors and shortens the cycle times. The portable e-nose consists of a sensor array with 10 metal oxide sensors and the including sampling system. A notebook computer has been connected to it for data acquisition and analysis using software WinMuster PEN. Algorithm for analyses includes Euclid, Correlation, Mahalanobis, DFA, PCA, LDA, and PLS.

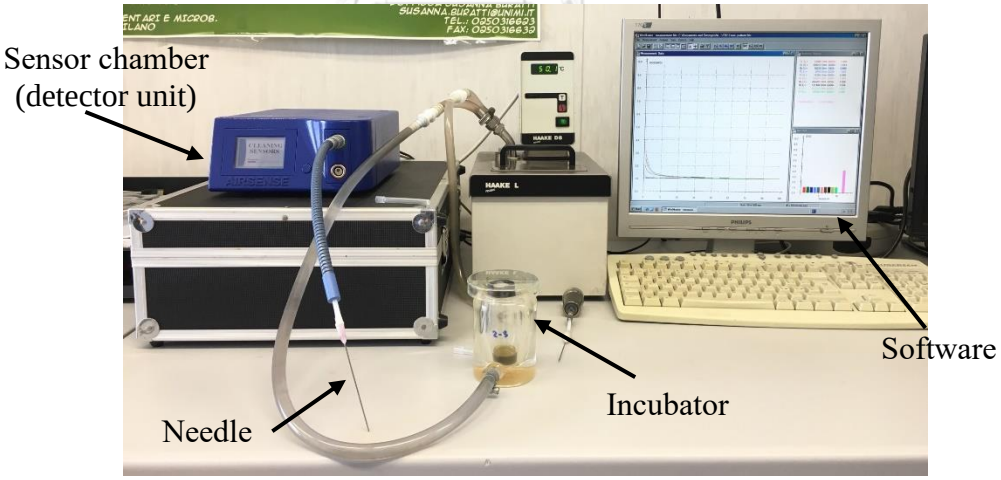


Fig. 2.5 E-nose unit: Portable e-nose (PEN2) (Win Muster Airsense (WMA) Analytics Inc., Germany)

2.7 Electronic tongue

The electronic tongue (e-tongue) is a measure and compares tastes instrument comprising a set of chemical sensors which are sensitive to the substances contributed to food taste. The sensor arrays obtained information from chemical substances and send it to a pattern recognition system. (Podrażka et al., 2017).

Figure 3 shows a diagram of the taste sensor system with the sensor acting as the detecting electrode and the Ag/AgCl electrode acting as the reference electrode. The mixing of 3.33 M KCl and saturated AgCl was used as the inner solution for the sensors and the reference electrode (Tahara and Toko, 2013). Types of taste sensors generated divided into five categories sourness, saltiness, bitterness, sweetness, and umami following the basic tastes. Each sensor is composed of different lipid and polymer membrane responding to specific taste characteristic. The principle of the e-tongue measurement is based on the ability of tasty substances to change the potential of detecting sensors through electrostatic interaction. The e-tongue responses were then used to calculate the relative value (R_v) and the absorption value (CPA_v). E-tongue responses were then transformed to taste values (taste information) base on The Weber-Fechner law (Kobayashi et al., 2010). Flowchart from measurement to evaluation using taste sensors is shown in Fig. 2.6. E-tongue has been successfully used to qualify different types and grade level of tea (Buratti et al., 2013; Chen et al., 2008). Moreover, e-tongue has been applied for prediction and quantification of the phenolic compounds in wine and beer (Polshin et al., 2010; Rudnitskaya et al., 2010).

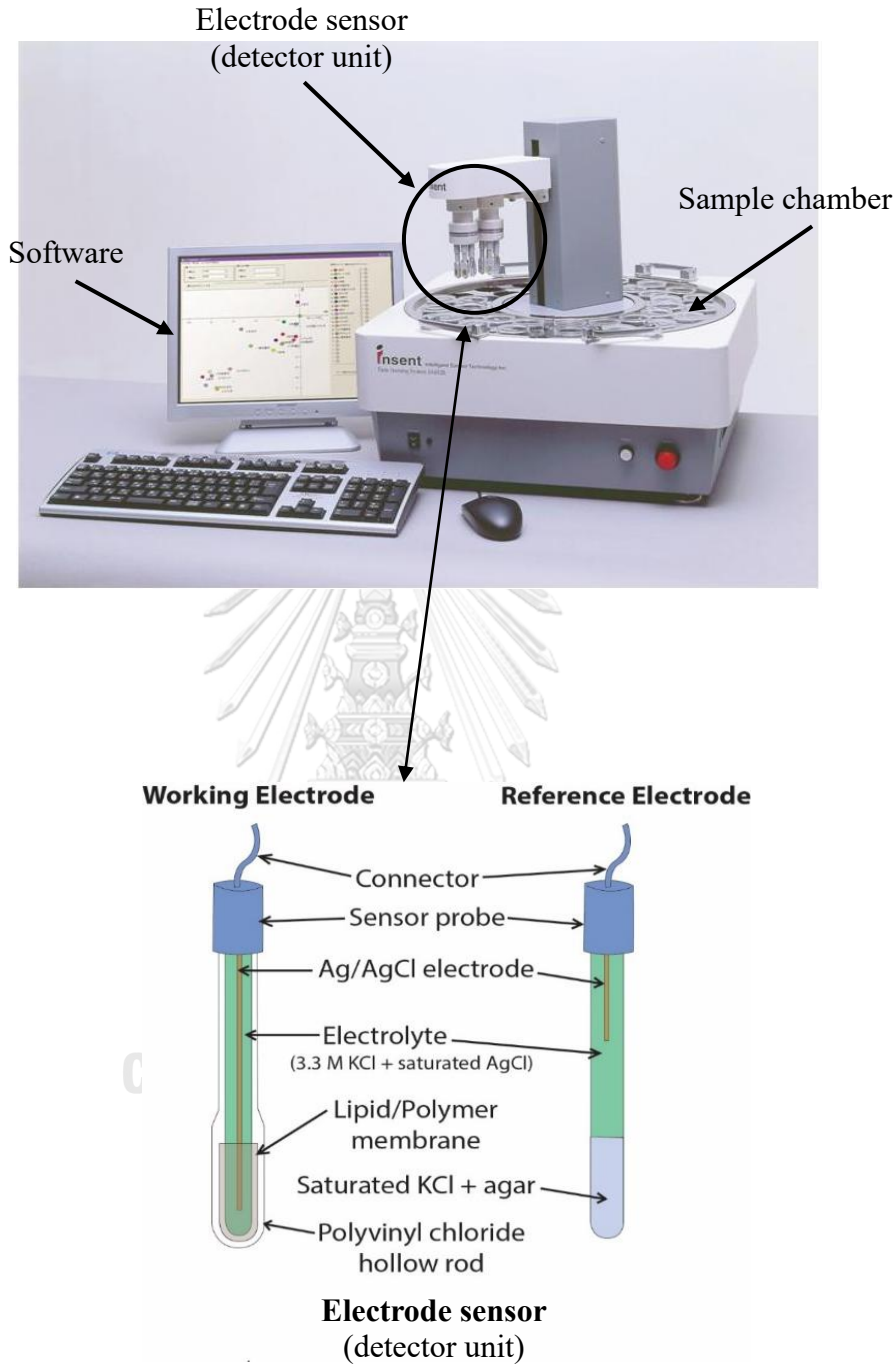


Fig. 2.6 Electronic tongue unit: Taste-Sensing System SA 402B

Source: Intelligent Sensor Technology Co. Ltd. (2017)

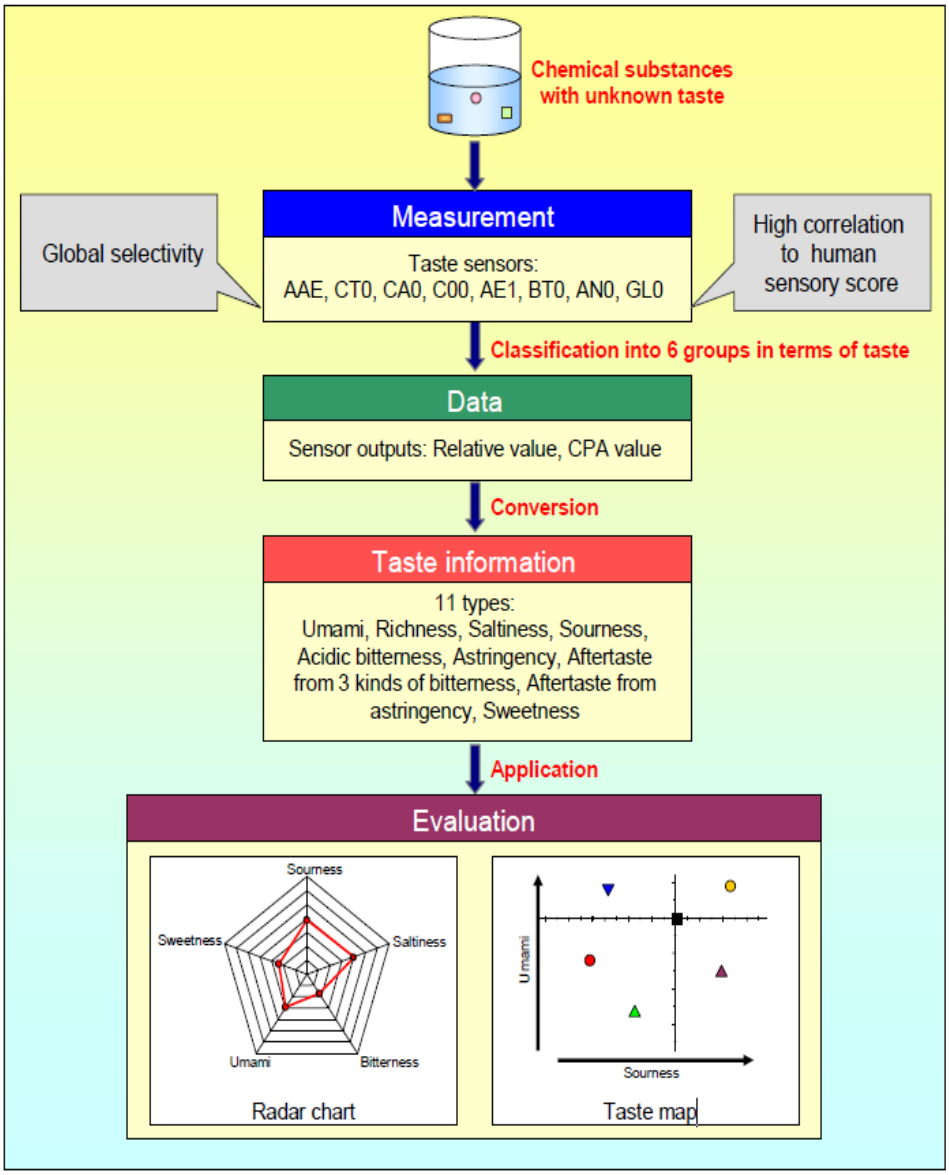


Fig. 2.7 Flowchart from measurement to evaluation using taste sensors.

Source: Kobayashi *et al*. (2010).

2.8 Chemometrics

Chemometrics is the science of extracting information from chemical systems by data-driven means. Chemometrics is used to solve both descriptive and predictive problems in experimental sciences. For the applications of description, properties of chemical systems are modeled with the purpose of learning the relationships the system. For the applications of prediction, properties of chemical systems are modeled with the purpose of predicting the new properties of the interest. Chemometric techniques include multivariate calibration, classification, pattern recognition, clustering, multivariate curve resolution, and other techniques. Multivariate analysis had variable techniques to using analysis such as principal components analysis (PCA) and partial least-squares (PLS).

2.8.1 Principal component analysis (PCA)

PCA is a mathematical procedure that transforms a large number of correlated variables into a small number of uncorrelated variables which is called principal components. The first principal component (PC1) accounts for as much of the variance in the data as possible, and each succeeding component accounts for as much of the remaining variance as possible. PCA is similar to another multivariate analysis which is called factor analysis. They are frequently complicated and many scientists do not understand the difference between the two methods (Miller and Miller, 2018).

2.8.2 Partial Least Squares (PLS) regression

PLS is a technique that combines vector on the principal components regression (PCR) ellipse upon which multiple regression (MLR) has the longest

projection. PLS regression can analyze data with many, noisy, collinear, and even incomplete both X and Y variables (Wold et al., 2001). PLS regression has the pleasant property that the precision of the model parameters improves with the increasing number of relevant variables and observations. The goal of PLS is to predict a set of dependent variables (Y) from a set of independent variables (X) or predictors. This prediction is accomplished by extracting from the data set of orthogonal factors which is called latent variables.

CHAPTER III

MATERIALS AND METHODS

3.1 Influence of cultivar on the quality of mulberry leaf tea

3.1.1 Preparation of mulberry leaf tea

Mulberry (*Morus alba L.*) leaves of three different cultivars including Burirum 60 (BR), Khunphai (KP), and Sakonnakhon (SK) were harvested in November, 2015 from Queen Sirikit Sericulture Center, Saraburi, Thailand. Mulberry leaf tea samples were prepared following the procedures of the household level production described by The Queen Sirikit Department of Sericulture (QSDS) (2010). Mulberry leaves were washed, drained, and cut into small piece (approximately 1x4 cm^2). Five hundred grams of sliced mulberry leaves were steamed in a steamer pot for 90 s, withered in the air for 20 min and then roasted at 55-60°C for 25 min in a pan. The leaves were dried in the hot air dryer at 80°C for 1 hour. The dried leaves were powdered using an electric grinder, passed through a 60-mesh sieve, and stored in an aluminum bag at -20°C under vacuum condition.

3.1.2 Determination of physical properties

3.1.2.1 Moisture content (MC) and water activity (a_w)

Moisture content of mulberry leaf tea determined following AOAC NO. 950.46. (AOAC, 2000). The measurement was carried out in triplicate and then mean and standard deviation (SD) of data were reported.

Water activity of the samples was determined using an Aqualab water activity meter (AquaLab Series 3 TE, Decagon Devices, Inc., USA). Mean and SD of data were obtained from triplicate.

3.1.2.2 Color

The color of samples was evaluated by a chroma meters (Konica Minolta Chroma Meters CR-400, Japan**).** CIELAB color (L*, a*, b*) method was performed and values for L* (lightness), a* (redness), and b* (yellowness) were recorded. Mean and SD of data were obtained from triplicate.

3.1.3 Determination of phytochemicals and antioxidant activity

3.1.3.1 Crude extraction

Extraction of phenolic compounds was conducted following the method of Katsube et al. (2009). Briefly, two grams of mulberry tea powder were extracted with 20 mL of 60% (v/v) ethanol solution at 40°C for 1 h in an ultrasonic bath (Elmasonic E70H, Elma-Hans Schmidbauer Gmbh & Co. KG, Germany) set at 37 kHz frequency. The mixture was centrifuged at 8500 rpm for 10 min (Hettich Universal 32 R, Hettich Zentrifugen, Germany). The supernatant was removed and the residue was then resuspended in 10 mL of 60% (v/v) ethanol solution. After repeating, the two extracts were combined and diluted with 1:3 (v/v) 60% ethanol solution before chemical determination.

3.1.3.2 Determination of total phenolic content (TPC)

TPC of mulberry leaf tea was analyzed using the Folin-ciocalteu method according to Naczk & Shahidi (2004). Briefly, 0.05 mL of diluted extract was mixed with 7.9 mL distilled water and 0.1 mL of Folin-Ciocalteu reagent. The mixture

was vortexed vigorously, and after 6 min added with 1 mL of 20% (w/v) sodium carbonate solution. After standing in the dark for 30 min, its absorbance was measured at 760 nm using a visible spectrophotometer (Genesys 20 4001/4, Thermo Fisher Scientific, USA). The measurement was performed in triplicate. Gallic acid solutions (50-800 mg/L) were used as standards for the calibration curve. The TPC was expressed as mg of gallic acid equivalents (GAE) per g of dried matter of mulberry leaf tea.

3.1.3.3 Determination of total flavonoids content (TFC)

TFC was determined using the colorimetric method described by Kaisoon et al. (2011). Briefly, 0.6 mL of diluted extract was mixed with 2.25 mL of distilled water and 0.5 mL of 5% sodium nitrite solution and stored for 6 min. Then, 0.3 mL of a 10% aluminum chloride solution was added and allowed to incubate for other 5 min. The mixture was added with 1.0 mL of 1 mol/L sodium chloride and the absorbance was measured immediately at 510 nm using a visible spectrophotometer. Rutin solutions (50-700 mg/L) were used as standard for the calibration curve. TFC was expressed as mg of rutin equivalents per g of dried matter of mulberry leaf tea.

3.1.3.4 Phenolic compounds

Phenolic compounds were determined using HPLC described by Katube *et al*. (2009). Phenolic compounds including chlorogenic acid, rutin, and quercetin 3-(6-malonylglucoside) in mulberry leaf tea were analyzed sing an Agilent 1200 series HPLC (Agilent Technologies Inc., Germany) equipped with a Zorbax SB-C18 column (250 mm × 4.6 mm i.d., 5 μm) and an Agilent photodiode array detector. Acetonitrile/0.1% formic acid (20:80 v/v) used as mobile phase was pumped at 1 mL/min. The column was kept at 30 °C, and the injection volume was 20 μL. Chlorogenic acid was monitored at 280 nm and rutin and quercetin 3-(6-

malonylglucoside) were measured at 370 nm. Their concentrations were calculated based on the standard curves constructed with authentic standards expressed as mg per 100 g dried sample.

3.1.3.5 Determination of Ferric reducing/antioxidant power (FRAP)

FRAP assay was done according to the method of Thaipong et al. (2006). The RAP reagent was prepared by mixing 0.3 M acetate buffer (pH=3.6), 0.01 M 2,4,6-tripyridyl-s-triazine (TPTZ) in 0.04 M hydrochloric acid solution, and 0.02 M Iron (III) chloride hexahydrate ($FeCl3\bullet6H_2O$) solution in ratio 10:1:1 by volume. After incubation at 37°C for 30 min, 2.58 mL of reagent were added to 0.15 mL of diluted extract. The solution was mixed and allowed to react in the dark for 30 min, then the absorbance was measured at 593 nm. Trolox solutions (50-600 μM) was used as standard for the calibration curve. The antioxidant activity was expressed as mM of Trolox per 100 of dry-matter of mulberry leaf tea.

3.1.3.6 Determination of DPPH radical scavenging activity (DPPH)

DPPH assay was performed according to the method of Shekhar & Anju (2014) with some modifications. One hundred microliters of diluted extract were mixed with 0.9 mL of 0.01 mM DPPH radical in methanol. The mixture was incubated in the dark for 30 min and the absorbance was measured at 517 nm. The antioxidant activity was calculated as percentage of DPPH radical scavenging activity using Eq. (1).

$$\text{DPPH scavenging activity (\% inhibition)} = 100\ x\ \frac{Ac-As}{Ac} \qquad (1)$$

Where *As* is the absorbance of the mixture of the sample. *Ac* is the absorbance of the mixture in which 0.1 mL distilled water was used instead of sample.

3.1.4 Determination of volatile compounds

The infusion used for determination of volatile compounds was prepared by adding 2 grams of mulberry leaf tea in 100 mL of hot water at 95°C. The sample was stirred with magnetic bar for 10 min and through filter paper Whatman® No.4 using a vacuum pump filter **(**Rocker 300A, Rocker Scientific Co., Ltd, Taiwan).

Volatile compounds of mulberry leaf infusion were collected by the solid phase microextraction (SPME) technique. Briefly, 10 milliliters of tea infusion were pipetted into a 20 ml glass vial (Chromselection, Italy) and 3.0 g of sodium chloride was added to reduce the solubility of hydrophobic compounds in the aqueous phase. This is called salting out which can assist in the extraction of organic compounds dissolved in water (Baldwin, 1996). The vial was then tightly closed with aluminum crimp cap attached with a PTFE silicone septum (Chromselection, Italy) using hand crimper (Kebby Industries Inc, USA). After 10 min of sample equilibration at 40°C, the 50/30 μm Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) SPME fiber (Stableflex; Supelco. Inc., USA) was exposed to the headspace of the sample vial for 30 min and then immediately inserted into the gas chromatograph (GC) (PerkinElmer AutoSystem XL GC, USA) injection port with splitless mode at 250°C for 5 min.

The GC column was Stabilwax®-MS capillary column (30 m x 0.25 mm ID, 0.25 μm film thickness; Restek) was employed for GC-MS analysis. Helium was used as carrier gas with flow rate at 1 mL/min. The GC oven temperature gradient was 50°C (held for 5 min) initially, increased to 125°C (held for 3 min) at 3°C min^{-1}, then ramped to 180°C (held for 3 min) at 2°C min^{-1}, and finally increased to 230°C (held for 5 min) at 6°C min^{-1}. The transfer line temperature was 280°C. Mass spectrometer

(MS) (PerkinElmer Turbomass MS, USA) was coupled to GC. The ion source temperature was 230°C and the MS was scanned at 70 eV over 40 to 500 mass range. Compounds were tentatively identified by using the NIST database (National Institute of Standards and Technology (NIST), 2011) with the R-match more than 80%.

3.1.5 Descriptive sensory analysis

The descriptive analysis was performed to evaluate the sensory attributes of tea infusion. Nine graduate students (2 males and 7 females, 23-35 years old) of the Department of Food Technology at Chulalongkorn University were panelists. The panelists had to take the sensory evaluation class before training. The panelists were trained for defining and scoring of the sensory attributes of the mulberry tea including color (greenness and brownness), aroma (green odor and dried leaf odor), and taste (astringency) for 60 h before testing. The references used to identify for each sensory characteristic were shown in the Appendix (Fig.C.1).

For sample evaluation, two grams of mulberry tea powder were packed in sachet bag and infused in 100 mL of hot water at 95°C for 5 min. Forty-five milliliters of samples labeled with random three-digit codes at 60°C were served. The panelists were asked to quantify the sample attributes (greenness, brownness, green odor, dried leaf odor, and astringency) on a numerical scale from 0 to 15 with 1.0 increment where 0 means weak and 15 means strong as described by Meilgaard, Civille, & Carr (1999). Drinking water and unsalted crackers were used to cleanse panelists' palate before each sensory. Three test samples were monadically served in random order to the panel. However, each panelist received the same samples in every serving. Each sample was individually evaluated in duplicate over the evaluation session held on different days.

The evaluation was conducted separately in the laboratory under normal lighting conditions at 25±1ºC

3.1.6 Electronic nose analysis

The electronic nose Fox 4000 (Alpha M.O.S., Toulouse, France) comprising a 64-position autosampler, the FOX measuring unit, and the AlphaSoft software was employed. The FOX measuring unit contains an injection port, 3 sensor chambers with 18 metallic oxide sensors, and a number of electronic cards. Each sensor responses to the specific compound including LY2/LG (fluoride, chloride, oxynitride, and sulfide), LY2/G (ammonia, amines, carbon, and oxygen compounds), LY2/AA (alcohol, acetone, and ammonia), LY2/Gh (ammonia and amine compounds), LY2/gCTI (hydrogen sulfide), LY2/gCT (propane and butane), T30/1 (polar compound and hydrogen chloride), P10/1 (nonpolar compound: hydrocarbon, ammonia, and chlorine), P10/2 (nonpolar compound: methane and ethane), P40/1 (fluorine and chlorine), T70/21 (toluene, xylene, and carbon monoxide), PA/2 (ethanol, ammonia, and amine compounds), P30/1 (hydrocarbons, ammonia, and ethanol), P40/2 (chlorine, hydrogen sulfide, and fluoride), P30/2 (hydrogen sulfide, and ketone), T40/2 (chlorine), T40/1 (fluorine), and TA/2 (ethanol).

E-nose analysis was performed following the method of Buratti et al. (2013) with slight modifications. The infusion used for determination of e-nose analysis were prepared by adding 2 grams of mulberry leaf tea in 100 mL of hot water at 95°C. The sample was stirred with magnetic bar for 10 min and through filter paper Whatman® No.4 using a vacuum pump filter **(**Rocker 300A, Rocker Scientific Co., Ltd, Taiwan). Three milliliters of mulberry tea infusion were placed in a 20 mL Pyrex vial fitted with a pierceable aluminum crimp cap attached with a PTFE silicone septum and

allowed to agitate at 60±1°C for 10 min on the HS100 autosampler. The headspace sample (500 µl) was then automatically injected into the sensor chamber using a gastight syringe. The measurement phase lasted for 120 s at a rate of 1 acquisition every 1s. The sensor response was indicated as resistivity (Ohm). After the measurement was completed, filtered air was conveyed over the sensors to clean the circuit and return sensors to their baseline. The response data were transformed into relative resistance changes (R_{max}) according to Eq. (2).

$$\Delta R_{max} = \frac{(R_{max} - R_0)}{R_0} \quad (2)$$

where R_{max} is the electrical resistance value taken at the apex of the response peak of each sensor and R_0 is the initial baseline resistance of the sensors.

Data were made up of 108 samples from 3 groups (BR, KP, and SK). Each group was randomly collected 36 replicates. Each sample was analyzed using 18 metal oxide sensors. The sensor responses of all samples were arranged in a matrix (108 samples×18 measurement variables).

Principal components analysis (PCA) was carried out to analyzed e-nose sensor response data. PCA, unsupervised multivariate method, has been used to visualize the hidden trends in a data matrix M composed of n cases defined by m variables. The first principal component (PC1) existed the largest possible amount of information was the horizontal axis and the second principal component (PC2) was the vertical axis. The various mulberry cultivars plotted in the new space of PCs (score plot) presented trending and described the differences or similarities between various mulberry cultivars and original variables (sensors) plotted in the new space of PCs (loadings plot) indicating which sensors contribute the most for such differences or similarities.

3.2 Influence of packaging and storage time on the quality of mulberry leaf tea

3.2.1 Tea preparation and storage condition

Herbal mulberry tea prepared from mulberry leaves of Burirum 60 cultivar was obtained from Thai Silk Products Co., Ltd., Nakhon Ratchasima, Thailand. Mulberry leaf tea samples were produced following the procedures of the industrial production described by QSDS (2010). Before stored, the mulberry tea was ground into powder with a grinding machine (Lita Brand NSB-6, Product of Lita Hatsuyuki, Thailand) and sieved through 60-mesh stainless-steel sieve shaker (vibratory sieve shaker type vibro Nr. 67468, RETSCH®, Germany). One hundred grams of mulberry tea powder were packed into two different types of packaging materials, including linear low-density polyethylene laminated aluminium (AL) bag (10x15 cm^2; 0.0924 mm in thickness) and polypropylene (PP) bag (8x15 cm^2; 0.0774 mm in thickness). The water vapor transmission rate (WVTR) and oxygen transmission rate (OTR) of polypropylene bag were 15.19 $g.m^2day^{-1}$ and 635.24 $cc.m^2day^{-1}$, respectively. Both WVTR and OTR of LLDPE-laminated aluminium bag were approximately zero. The samples were then sealed with an impulse sealer (PHS-Semi Auto Impulse Sealer, Glory Pack, Japan) under atmospheric condition. The samples were stored at 30±1°C in a controlled humidity chamber at 75% RH which represents average relative humidity of Thailand for 18 months. The samples were randomly retrieved at three-month interval to monitor quality attributes and phytochemicals.

3.2.2 Determination of physical properties

Changes in physical properties including MC, a_w, and color of mulberry leaf tea during storage were determined following 3.1.2.

3.2.3 Determination of phytochemicals and antioxidant activity

Changes in phytochemicals (TPC, TFC, and phenolic compounds) and antioxidant (FRAP and DPPH) activity of mulberry leaf tea during storage were determined following 3.1.3.

3.2.4 Determination of volatile compounds

Changes in volatile profiles of mulberry leaf tea during storage were determined following 3.1.4.

3.2.5 Descriptive sensory analysis

Changes in sensory attributes of mulberry leaf tea during storage were determined following 3.1.5.

3.2.6 E-nose analysis

The Portable e-nose (PEN2) provided by Win Muster Airsense (WMA) Analytics Inc. (Schwerin, Germany) was employed. E-nose comprising a sampling apparatus, a detector unit, and a software for pattern recognition (Win Muster v.1.6). The detector unit consists of 10 Metal Oxide Semiconductor (MOS) type chemical sensors including W1C (aromatic compounds), W5S (nitrogen oxides), W3C (ammonia and aromatic compounds), W6S (mainly hydrogen), W5C (alkanes and aromatic compounds), W1S (methane > 10 ppm), W1W (sulfur compounds), W2S (alcohol), W2W (aromatics compounds and sulfur organic compounds), and W3S (methane > 100 ppm).

E-nose analysis was carried out following the method of Buratti et al. (2013) with slight modifications. Two grams of mulberry leaf tea in 100 mL of hot water at 95°C. The sample was stirred with magnetic bar for 10 min and through filter

paper Whatman® No.4 using a vacuum pump filter **(**Rocker 300A, Rocker Scientific Co., Ltd, Taiwan). Three milliliters of mulberry leaf infusion were placed in 20 mL Pyrex vial sealed with a pierceable Silicone/Teflon cap and incubated at 50±1°C for 10 minutes. The sampling and venting needles were then inserted through a Teflon septum. The headspace was pump into the sensor chamber for 1 min with a constant rate of 400 ml/min. The collected signal data interval was 1 s. The response data were transformed into relative resistance changes (R_{max}). After the measurement was completed, filtered air was conveyed over the sensors for 6 min to clean the circuit and return sensors to their baseline. The next analysis was then allowed to measure. A 0.2% of ethanol was used as a standard solution for the sensor drift evaluation.

Data were set into 2 individual groups including samples packaged in AL bag and PP bag. Each group, data made up of 42 samples from 7 sub-groups (sample storage at 0, 3, 6, 9, 12, 15, and 18 months). Each sample was analyzed using 10 metal oxide sensors. The sensor responses of all samples were arranged in a matrix per group (42 samples×10 measurement variables).

PCA was used to reducing the number of variables and disposing of the redundant information of e-nose sensor response data. Before analyzed, the data were set the mean values to zero and scale on the basis of one standard deviation. The first principal component (PC1) existed the largest possible amount of information was the horizontal axis and the second principal component (PC2) was the vertical axis. The samples stored various time plotted in the new space of PCs (score plot) presented trending and described the differences or similarities between various storage time and original variables (sensors) plotted in the new space of PCs (loadings plot) indicating which sensors contribute the most for such differences or similarities.

3.3 Rapid determination of phytochemicals and antioxidant activity of mulberry leaf tea using e-tongue

3.3.1 Tea preparation and storage conditions

Mulberry leaf tea samples used in this part were the same of the samples of part 3.2. The followings were the procedure of sample preparation: one hundred grams of mulberry tea powder were packed into a 8x15 cm^2 polypropylene bag and sealed with an impulse sealer **(**PHS - Semi Auto Impulse Sealer**,** Glory Pack, Japan) under atmospheric condition. In order to dramatic changes in phytochemicals and antioxidant activity of mulberry leaf tea, the samples were stored at the accelerated condition. The samples were stored at various temperatures of 30, 40, 50, and 60°C in a controlled humidity chamber at 75% RH for 12 months. To determine phytochemicals and antioxidant activity values, the samples were randomly retrieved at three-month interval for measurements of TPC, TFC, FRAP, and DPPH using chemical analytical method (following 3.1.2) and e-tongue.

3.3.2 E-tongue analysis

E-tongue analysis was performed on 204 samples following the method of Buratti et al. (2013) with slight modifications. Two grams of mulberry tea powder were brewed in 100 mL distilled water (95°C) for 10 min and filtered through filter paper Whatman® No.1 using a vacuum pump filter (Rocker 300A, Rocker Scientific Co., Ltd, Taiwan). The mulberry leaf tea infusion was analyzed using an e-tongue (Taste-Sensing System SA 402B, Intelligent Sensor Technology Co. Ltd., Japan) with 3 working sensors including saltiness sensor (CT0), astringency sensor (AE1), and bitterness sensor (C00). Each sensor is composed of different lipid and polymer

membrane responding to specific taste characteristic. CT0 sensor made of tetradodecylammonium bromide, 1-hexadecanol, and dioctyl phenylphosphonte membrane respond to the saltiness (NaCl and KCl). AE1 sensor comprising tetradodecylammonium bromide and dioctyl phenylphosphonte membrane is sensitive to the astringency and aftertaste of astringency (aftertaste-A). C00 sensor responding to bitterness and aftertaste of bitterness (aftertaste-B) is composed of tetradodecylammonium bromide and 2-nitrophenyl octyl ether membrane (Kobayashi *et al*., 2010). The principle of the e-tongue measurement is based on the ability of tasty substances to change the potential of detecting sensors through electrostatic interaction. The experimental procedure was as follows: first, the sensor array was dipped into the reference solution (the mixture of 30 mmol/L potassium chloride and 0.3 mmol/L tartaric acid) for 30s, the electric potential of reference solution (Vr) was recorded. Secondly, the sensor array was immersed in the mulberry tea infusion for 30s to obtain its electrical potential (Vs). Thirdly, the sensor array was rinsed in the fresh reference solution for 6s and dipped into the reference solution again for 30s, after which the electrical potential (Vr′) was measured. Finally, the sensor array was immersed in the cleaning solution for 90s and then for 240s with the reference solution. The Vr, Vs, and Vr′ value from the e-tongue response were then used to calculate the relative value (R_v) and the absorption value (CPA_v) using Eq. (3) and Eq. (4), respectively.

$$R_v = Vs\text{-}Vr \quad (3)$$

$$CPA_v = Vr'\text{-}Vr \quad (4)$$

When the R_v is a measure of difference in electrical potential of sample and reference solution and the CPA_v is a measure of change of membrane potential caused by adsorption of the substances to the lipid membrane.

E-tongue responses were transformed to taste values (taste information) including astringency, bitterness, aftertaste-A, and aftertaste-B using Eq. (5) to Eq. (8), respectively base on the *Weber–Fechner law (*Kobayashi *et al*., 2010).

$$\text{Astringency} = -0.1575 \times R_{v(AE1)} + 0.1575 \times R_{v(CT0)} \quad (5)$$

$$\text{Bitterness} = -0.140 \times R_{v(C00)} + 0.084 \times R_{v(CT0)} \quad (6)$$

$$\text{Aftertaste-A} = -0.252 \times CPA_{v(AE1)} \quad (7)$$

$$\text{Aftertaste-B} = -0.210 \times CPA_{v(C00)} \quad (8)$$

Where $R_{v(CT0)}$, $R_{v(AE1)}$, and $R_{v(C00)}$ are the R_v of CT0, AE1, and C00 sensors, respectively. $CPA_{v(AE1)}$ and $CPA_{v(C00)}$ are the CPA_v of AE1 and C00 sensors, respectively.

3.3.3 PLS regression analysis

To predict TPC, TFC, FRAP, and DPPH of mulberry leaf tea, partial least squares (PLS) regression was used for developing the prediction models. It may be best to analyze the data without the outliers. After outlier analysis, data were 187 samples. 130 samples (70%) were used to build the calibration and the remaining 57 samples (30%) were for validation (prediction). Taste values (astringency, bitterness, aftertaste-A, and aftertaste-B) obtained from e-tongue analysis were used as the variable X while chemical analytical data (TPC, TFC, FRAP, and DPPH) were used as the variable Y. PLS models were developed to correlate the e-tongue response and the chemical analytical values of interest by full cross-validation (leave one out validation) method, the optimal number of PLS factor of the calibration models was then selected. The advantage of full cross-validation is it does not waste data involved in the model development and it is suited to a small sample set (Kobayashi et al., 2010). The performance of the prediction models was evaluated using coefficient of determination

(r^2), root mean square error of prediction (RMSEP), and standard error of prediction (SEP). Coefficient of determination is the proportion of the variance in the dependent variable is used to describe how well the data points fit the calibration model. RMSEP (Eq. (9)) measures the accuracy of prediction SEP (Eq. (10)) measures the precision of the prediction. Ideal models are expected when they show low RMSEP and SEP as well as a high coefficient of determination (Shetty & Gislum, 2011).

$$RMSEP = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(y_i - \hat{y}_i)^2} \quad (9)$$

$$SEP = \sqrt{\frac{\sum_{i=1}^{N}(y_i - \hat{y}_i)^2 - \frac{\left(\sum_{i=1}^{N}(y_i - \hat{y}_i)\right)^2}{N}}{N-1}} \quad (10)$$

Where N is the number of samples; i indicates the samples from 1 to N; y_i and $\hat{y}_i$ indicate the actual value and the predicted value, respectively.

The correlation between e-tongue responses (taste values) and chemical analytical data was computed with the Unscrambler® program.

3.4 Statistical analysis

One-way analysis of variance (ANOVA) was performed for data analysis using IBM SPSS Statistics V22.0 (International Business Machines (IBM) Corporation, United States). The completely randomized design was used for physical properties, phytochemicals, and volatile compounds analysis. The randomized complete block design was used for sensory evaluation where panelists were arranged as blocks. The comparison among means was determined according to Duncan's new multiple range test at 95% confidence level.

E-nose and e-tongue response data were analyzed by PCA and PLS regression, respectively, using the Unscrambler® X version 10.3 software package (CAMO Software, Norway).

CHAPTER IV

RESULTS AND DISCUSSIONS

4.1 Influence of cultivar on the quality of mulberry leaf tea

4.1.1 Physical properties

4.1.1.1 Moisture content (MC) and water activity (a_w)

Herbal tea is produced from fresh leaves of plants through the physical and chemical reactions, resulting in variations of their MC. The MC not only affects physicochemical properties of herbal tea but also determines their shelf life, therefore measurement of MC is an important task for producing high-quality herbal tea. The MC values of three different cultivars of mulberry leaf tea including Burirum 60 (BR), Khunphai (KP), and Sakonnakhon (SK) are ranged from 5.86±0.17 to 6.25±0.12%, with the highest in SK and the lowest in KP (Table 4.1). These results are in agreement with those of Li et al. (2012) who reported a wide MC variation from 4.24±0.09 to 6.90±0.17%.of the excellent grade of Chinese green tea. The MC less than 10% was claimed to be in an acceptable safety margin for the storage of tea and herbal tea (Davidson et al., 2005). However, assessment of acceptable levels should focus on the potential growth of mycotoxigenic molds which based on a_w of food.

The a_w is a measure of the amount of freely available water within foods desired for microbial growth. Therefore, foods with low a_w can prevent the growth of microorganisms. As shown in Table 4.1 the a_w values of three different mulberry cultivars, ranging from 0.341±0.002 to 0.347±0.003 were not significantly different. According to general principles for safe storage of foods (Troller, 1980),

pathogenic and spoilage bacteria do not grow in food with a water activity of less than 0.85. Yeasts and molds are more tolerant and usually no growth occurs at a_w<0.65 (Troller and Christian, 2012). Due to low a_w, all cultivar mulberry leaf tea samples may have long shelf life because at a_w lower than, foods are most stable with respect to lipid oxidation, non-enzymatic browning, enzyme activity, and microbial growth (Davidson et al., 2005).

4.1.1.2 Color

Color parameters of three different cultivar mulberry leaf tea including L* coordinate (lightness indicator), a* value (green color (negative) or red color (positive) indicator) and b* value (blue color (negative) of yellow color (positive) indicator) were compared. L*, a*, and b* values of 3 different mulberry cultivars and infusion are shown in Table 4.1. Cultivars significantly ($p \leq 0.05$) affected only color of mulberry leaf infusion. The L*, a* and b* values of infusion of SK was lower than those of infusion of BR and SK. The infusion of BR and SK were not significant different in color parameters.

Fig. 4.1 shows the images of mulberry leaves and mulberry tea infusions of three different mulberry cultivars. The configurations of three cultivar mulberry leaves were dark green, waxy, and glossy appearance, and serrated on the margin. SK cultivar has bigger and thicker leaves than those of BR and KP, respectively. The appearance of infusion of BR and KP showed similar in color which differed from the infusion of SK. The infusion of BR and KP were rather brownish yellow whereas that of SK was rather greenish yellow.

Table 4.1 Moisture content, water activity, and color of three different cultivars of mulberry leaf tea.

Samples	Moisture content (%)	Water activity	Color of mulberry leaf tea powder			Color of tea infusion		
			L*	a*	b*	L*	a*	b*
BR	6.14±0.14^{ab}	0.345±0.006^{a}	32.67±0.38^{a}	-4.77±0.21^{ab}	11.43±0.40^{a}	72.65±0.67^{a}	-1.66±0.31^{a}	15.87±0.37^{a}
KP	5.86±0.17^{b}	0.341±0.002^{a}	32.33±0.38^{a}	-4.56±0.36^{a}	12.02±0.36^{a}	73.36±0.45^{a}	-1.47±0.29^{a}	16.51±0.21^{a}
SK	6.25±0.12^{a}	0.347±0.003^{a}	32.40±0.52^{a}	-5.28±0.17^{b}	11.57±0.53^{a}	69.01±0.52^{b}	-2.52±0.25^{b}	13.97±0.24^{b}

Means ± SD of 3 replications.

Different letters in the same column(a-c) represent significant difference between means ($p \leq 0.05$).

Fig. 4.1 Infusion of three different cultivars of mulberry leaf tea including BR, KP, and SK.

4.1.2 Phytochemicals and antioxidant activity

4.1.2.1 Total phenolic content (TPC) and total flavonoids content (TFC)

Phytochemicals and antioxidant activity of three different cultivar mulberry leaf tea are shown in Table 4.2. Foline-Ciocalteu phenol reagent is used to obtain a crude estimate of the quantity of phenolic compounds presenting in the mulberry leaf tea. The TPC in three mulberry cultivars ranged from 29.81±0.58mg GAE /g dried sample for SK to 37.78±0.34 mg GAE /g dried sample for KP. These results were in agreement with those reported by Samransakul (2001), who evaluated the TPC of dried leaves of four mulberry cultivars (e.g. Nakhonratchsima 60, Burirum 60, Khunpai, and Noi) grown in Thailand, the TPC ranged from 5.72±0.43 mg GAE/g dried sample to 59.00±0.26 mg GAE/g dried sample.

The TFC of all mulberry leaf tea samples were measured spectrometrically and found to be significantly different ($p \leq 0.05$). The TFC of the samples arranged in descending order were 29.73±0.33, 26.81±0.12, and 23.05±0.25 mg rutin/g dried sample for KP, BR, and SK, respectively (Table 4.2).

4.1.2.2 Phenolic compounds

Phenolic compounds played a role in an antioxidant activity such as chlorogenic acid, rutin, kaempferol, and quercetin are abundant in mulberry leaf tea. HPLC was carried out to determine the specific phenolic compounds in mulberry leaves. The three phenolic compounds including chlorogenic acid, rutin, and quercetin in mulberry leaf were extracted using 60% (v/v) ethanol solution by comparing retention times of each standard polyphenol. The intensities of these phenolic compounds of mulberry leaf tea were found to be different as presented in Table 4.2. The results suggested that cultivar affects the quantity of phenolic compounds of mulberry leaf tea.

Chlorogenic acid being phenolic compound from the hydroxycinnamic acid group has been reported to exert antioxidant and anti-diabetic activity (Hunyadi et al., 2012). Chlorogenic acid was detected within 2.25 min of the operation time. Concentration of chlorogenic acid of BR, KP, and SK were 264±28, 186±30, and 169±33 mg/100 g dried sample, respectively. The results showed that chlorogenic acid was the most abundant compound of mulberry leaf tea. This result was in agreement with previous reports on Korean (328±19 - 965±71 mg/100 g dried sample), Chinese (150±60 - 230±40 mg/100 g dried sample), and Spanish (529±24 - 718±183 mg/100 g dried sample) mulberry leaves (Lee and Choi, 2012; Sánchez-Salcedo et al., 2015; Zou et al., 2012)

Rutin is the main components of mulberry leaves with the most effective function in controlling the concentration of fat in serum (Sun et al., 2001). Rutin was detected at 370 nm within 3.7 min of operation. Levels of rutin of BR, KP,

and SK were 55.43±8.4, 119.52±3.9, and 57.97±6.7 mg/100 g dried sample, respectively.

Quercetin is characterized as of a flavonol-type structure with the absorption of the spectrum at 370 nm. Quercetin is claimed to be a strong radical scavenging and has anti-inflammatory activities (Sánchez-Salcedo et al., 2015). Concentration of rutin of BR, KP, and SK were 0.47±0.02, 0.66±0.04, 0.35±0.03 mg/100 g dried sample, respectively.

4.1.2.3 Antioxidant activity

Antioxidant activity carried out by Ferric reducing/antioxidant power (FRAP) and 2,2-Diphenyl-1-picrylhydrazyl radical scavenging activity (DPPH) assays. FRAP and DPPH of three cultivars of mulberry leaf tea are shown in Table 4.2.

FRAP assay measures the reducing ability of antioxidants against oxidative effects of reactive oxygen species. Total antioxidant power may be referred analogously to total reducing power. FRAP of SK, BR, and KP were 7.90±0.29, 9.72±0.10, 11.07±0.16 mM Trolox/100 g dried sample, respectively.

DPPH assay provides information on the reactivity of phenolic compounds with a free radical. The efficiency of antioxidants is associated with their ability to donate electrons to quench the free radical. KP cultivar exhibited the highest DPPH of 55.51±1.33%, followed by BR (47.19±1.23%) and SK (36.65±1.29%). All three mulberry cultivars showed significantly different antioxidant activity at a significance level of $p \leq 0.05$. It is noteworthy that phenolic compounds play a key role mulberry leaf antioxidant activity. Therefore, the different antioxidant activity found among three mulberry cultivars may be attributed to different types and concentration of certain phenolic compounds.

It is known that that cultivars can affect phytochemical production of mulberry leaves which related with the concentration of their phenolic compounds and antioxidant activity. KP cultivar has the highest concentration of phenolic compounds and antioxidant activity, followed by BR and SK, respectively.

4.1.3 Volatile compounds

GC chromatograms of volatile profiles in mulberry leaf tea from three cultivars are shown in Fig. 4.2. Distinct patterns of volatile profiles attributed to different volatile compounds and their intensity were evident. In this work, 4-methyl-2-pentanone (RT = 4.43 min) was used as internal standard to determine the relative intensity of volatile compounds. Volatile compounds (e.g. aldehyde, alcohol, acid, ester, and others) identified in tea samples are shown in Table 4.3. The results showed that 54, 47, and 46 volatile compounds were identified in BR, KP and SK, respectively. Similar 43 volatile compounds were found in all three cultivars whereas 2-decen-1-ol, 2-pentene,2,4-dimethyl, 2,2-dimethylpropionic acid,decyl ester, 2-isobutyl-3-methylpyrazine, and 4-tridecene were found only in BR tea. As shown in Table 4.3, BR tea had significantly ($p \leq 0.05$) higher intensity of butanal, 2-methyl, pyrazine,2,5-dimethyl, 5-hepten-2-one, 6-methyl, pyrazine,2-ethyl-5-methyl, pyrazine,3-ethyl-2,5-dimethyl, (E,E)2,4-heptadienal,1,4-hexadiene,3-ethyl, 3,5-octadien-2-one, 1-octanol, pyrazine,2,5-dimethyl-3-(3-methylbuthyl), and pentanoic acid, 2,2,4-trimethy-3-hydroxy, and isobutyl ester than KP and SK. The GC/MS results suggested that different mulberry cultivars affected volatile compound found in the tea. The difference in volatile compounds and their intensity among three different cultivars are expected to have an impact on their sensory attributes.

Table 4.2 Phytochemicals and specific phenolic compounds of three different cultivars of mulberry leaf tea.

Samples	TPC (mg GAE/g dried sample)	TFC (mg rutin/g dried sample)	FRAP (mM Trolox/100 g dried sample)	DPPH (%Inhibition)	Chlorogenic acid (mg /100 g dried sample)	Rutin (mg /100 g dried sample)	Quercetin (mg /100 g dried sample)
BR	36.84 ± 0.54^{a}	26.81 ± 0.12^{b}	9.72 ± 0.10^{b}	47.19 ± 1.23^{b}	264 ± 28^{a}	55.43 ± 8.4^{b}	0.47 ± 0.02^{b}
KP	37.78 ± 0.34^{a}	29.73 ± 0.33^{a}	11.07 ± 0.16^{a}	55.51 ± 1.33^{a}	186 ± 30^{b}	119.52 ± 3.9^{a}	0.66 ± 0.04^{a}
SK	29.81 ± 0.58^{b}	23.05 ± 0.25^{c}	7.90 ± 0.29^{c}	36.65 ± 1.29^{c}	169 ± 33^{b}	57.97 ± 6.7^{b}	0.35 ± 0.03^{c}

Means ± SD of 3 replications.

Different letters in the same column(a-c) represent significant difference between means ($p\leq0.05$).

Volatile compounds including benzaldehyde, 1-octen-3-ol, 6-methyl-5-hepten-2-one, (E,E)-2,4-heptadienal, nonanal, decanal, 2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde, and 6,10- dimethyl-5,9-undecadien-2-one found in tea from 3 mulberry cultivars also found in mulberry tea produced in China as report by Liu *et al*. (2012). Hexanal and 2-hexenal which have been reported to be responsible for green and grassy odors in Japanese fresh mulberry leaves (Tanaka et al., 2009) also present in the mulberry leaf tea in our study. 2(4H)-benzofuranone,5,6,7,7a-tetrahydro-4,4,7a-trimethyl or dihydroactinidiolide (RT= 31.2 min) contributed to sweet, herbaceous, and tea-like odor found in all 3 mulberry cultivars has been reported as an important aroma constituent of tea and herbal tea (Chen et al., 2013; Shimoda et al., 1995).

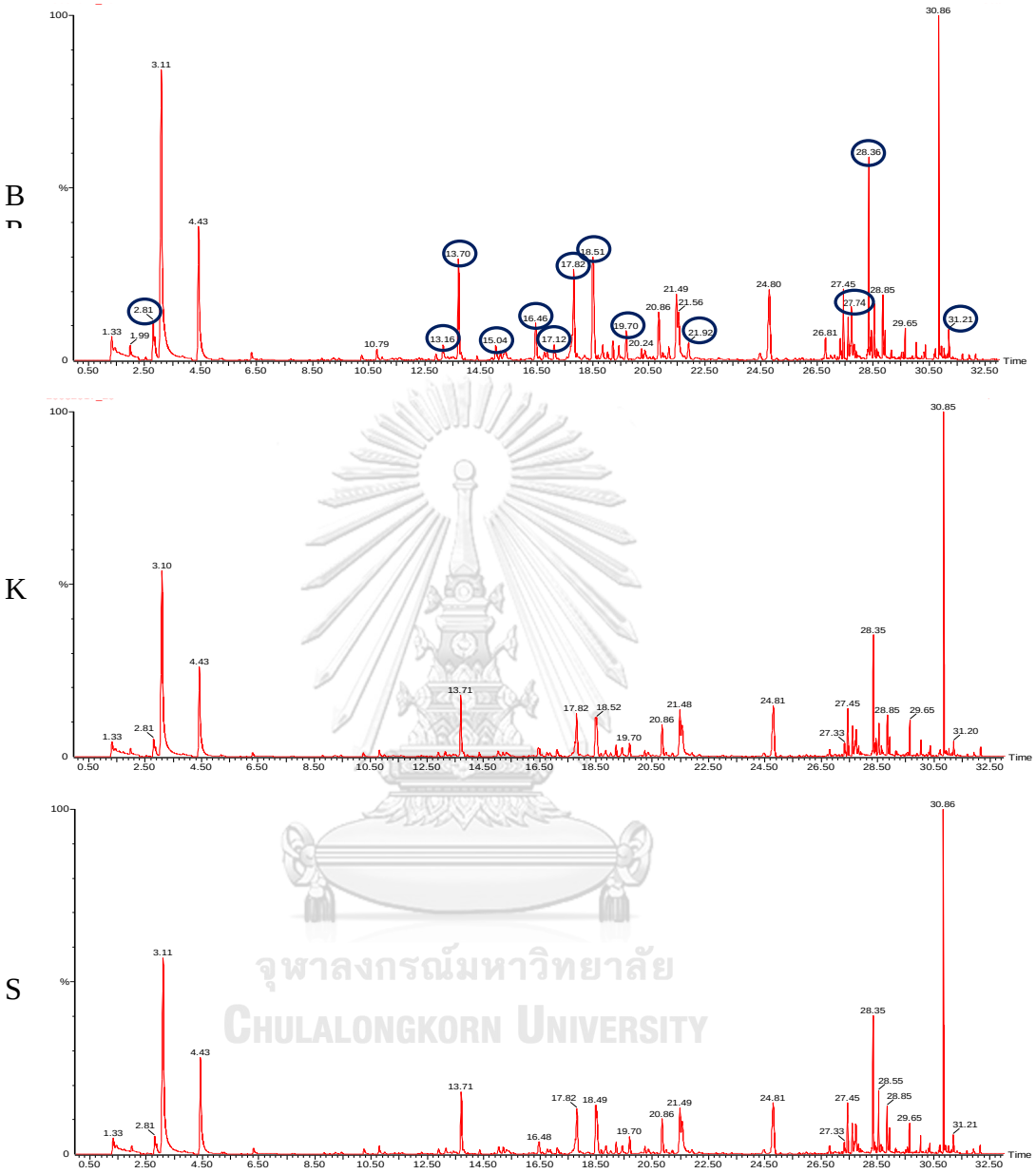


Fig. 4.2 GC chromatograms of volatile compounds in various tea prepared from three different cultivars including BR, KP, and SK.

Table 4.3 Relative content of volatile compounds of three different cultivar mulberry leaf tea obtained from SPME-GC/MS.

RT (min)	Volatile compound	Relative Content[a]		
		BR	KP	SK
2.81	Butanal, 2-methyl	0.311 ± 0.039*	0.195 ± 0.022	0.175 ± 0.001
6.32	Hexanal	0.060 ± 0.008	0.050 ± 0.005	0.062 ± 0.004
10.26	2-Hexenal	0.036 ± 0.002	0.038 ± 0.005	0.041 ± 0.003
10.79	1-Dodecene	0.080 ± 0.020	0.057 ± 0.014	0.053 ± 0.005
10.98	*cis*-4-Heptenal	0.016 ± 0.002	nd	0.012 ± 0.000
12.90	Cyclohexanone, 2,2,6-trimethyl	0.037 ± 0.005	0.035 ± 0.004	0.038 ± 0.001
13.16	Pyrazine, 2,5-dimethyl	0.109 ± 0.012*	0.049 ± 0.006	0.052 ± 0.001
13.70	5-Hepten-2-one, 6-methyl	0.508 ± 0.027*	0.455 ± 0.011	0.407 ± 0.005
14.37	Unknown	0.022 ± 0.008	0.023 ± 0.003	nd
14.89	Unknown	0.015 ± 0.001	nd	nd
15.04	Pyrazine, 2-ethyl-5-methyl	0.084 ± 0.008*	0.051 ± 0.006	0.054 ± 0.001
15.20	Nonanal	0.052 ± 0.012	0.029 ± 0.003	0.037 ± 0.012
15.32	2-Cyclohexen-1-one, 3,5,5-trimethyl	0.116 ± 0.014	0.058 ± 0.028	0.073 ± 0.003
16.46	Pyrazine, 3-ethyl-2,5-dimethyl	0.264 ± 0.030*	0.119 ± 0.016	0.116 ± 0.007
16.60	2-Decenal	0.020 ± 0.004	0.012 ± 0.002	0.013 ± 0.001
16.77	2-Octen-1-ol	0.050 ± 0.006	0.034 ± 0.005	0.039 ± 0.001
16.87	Unknown	0.050 ± 0.005*	0.033 ± 0.004	0.033 ± 0.003
17.12	(E,E) 2,4-Heptadienal	0.098 ± 0.009*	0.063 ± 0.009	0.054 ± 0.002
17.82	1,4-Hexadiene, 3-ethyl	0.753 ± 0.074*	0.475 ± 0.028	0.475 ± 0.009
17.94	2-Decen-1-ol	0.026 ± 0.005	nd	nd
18.21	Unknown	0.039 ± 0.003	0.022 ± 0.003	0.025 ± 0.001
18.51	3,5-Octadien-2-one	0.913 ± 0.066*	0.554 ± 0.082	0.567 ± 0.016
18.85	Unknown	0.104 ± 0.011	0.066 ± 0.008	0.076 ± 0.001
19.02	2-Pentene, 2,4-dimethyl	0.027 ± 0.005	nd	nd
19.22	1,6-Octadien-3-ol, 3,7-dimethyl, 2-aminobezoate	0.087 ± 0.009	0.072 ± 0.009	0.070 ± 0.001
19.70	1-Octanol	0.092 ± 0.020*	0.060 ± 0.009	0.066 ± 0.001
20.24	6-Methyl-3,5-heptadiene-2-one	0.050 ± 0.004	0.039 ± 0.005	0.039 ± 0.002

Values indicate mean ± SD from 3 replicates.

* indicates significant ($p \leq 0.05$) differences in relative intensity of volatile compounds among different mulberry

Table 4.3 Relative content of volatile compounds of three different cultivar mulberry leaf tea obtained from SPME-GC/MS (continued).

RT (min)	Volatile compound	Relative Content[a]		
		BR	KP	SK
20.36	Cyclohexanol, 2,4-dimethyl	0.061 ± 0.006	0.040 ± 0.005	0.041 ± 0.001
20.52	2,2-Dimethylpropionic acie, decyl ester	0.012 ± 0.001	nd	nd
20.65	2-Isobutyl-3-methylpyrazine	0.016 ± 0.001	nd	nd
20.86	1-Cyclohexene-1-carboxaldehyde, 2,6,6-trimethyl	0.303 ± 0.033	0.269 ± 0.030	0.267 ± 0.003
21.00	Unknown	0.041 ± 0.004	0.028 ± 0.003	0.025 ± 0.001
21.22	Pyrazine, 2,6-Dimethyl-3 (2-methyl-1-butyl)	0.073 ± 0.007	0.028 ± 0.003	0.025 ± 0.001
21.49	Cyclohexanol, 5-methyl-2-(1-methyl)	0.424 ± 0.041	0.407 ± 0.069	0.367 ± 0.008
21.56	1,3-Cyclohexadiene-1-carboxaldehyde, 2,6,6-trimethyl	0.374 ± 0.044	0.411 ± 0.035	0.348 ± 0.005
21.92	Pyrazine, 2,5-dimethyl-3-(3-methylbuthyl)	0.106 ± 0.010*	0.026 ± 0.007	0.032 ± 0.002
24.47	Unknown	0.056 ± 0.005	0.043 ± 0.006	0.046 ± 0.001
24.81	Benzaldehyde, 2,4-dimethyl	0.647 ± 0.066	0.629 ± 0.093	0.611 ± 0.008
26.81	Unknown	0.089 ± 0.012	0.045 ± 0.006	0.066 ± 0.019
27.33	3-Buten-2-one, 4-(2,6,6-trimethyl-2-cyclohexene-1-yl	0.064 ± 0.007	0.058 ± 0.007	0.046 ± 0.001
27.45	5,9-Undecadien-2-one, 6,10-dimethyl	0.209 ± 0.025	0.198 ± 0.024	0.197 ± 0.003
27.61	Propanoic acid, 2-methyl-3-hydroxy-2,4,4-trimethylpentyl ester	0.127 ± 0.015	0.126 ± 0.020	0.113 ± 0.004
27.74	Pentanoic acid, 2,2,4-trimethyl-3 -hydroxy, isobutyl ester	0.254 ± 0.025*	0.161 ± 0.018	0.186 ± 0.008
27.83	Cyclopropanemethanol, 2-methyl-2-(4-methyl-3-pentenyl)	0.042 ± 0.004	0.037 ± 0.005	0.033 ± 0.002
27.90	7-Methylene-9-oxabicyclo(6.1.0) one-2-ene	0.021 ± 0.002	nd	0.014 ± 0.001

Values indicate mean ± SD from 3 replicates.

* indicates significant ($p \leq 0.05$) differences in relative intensity of volatile compounds among different mulberry cultivars.

nd means not detected

Table 4.3 Relative content of volatile compounds of three different cultivar mulberry leaf tea obtained from SPME-GC/MS (continued).

RT (min)	Volatile compound	Relative Content[a]		
		BR	KP	SK
28.36	3-Buten-2-one, 4-(2,6,6-trimethyl-1-cyclohexene-1-yl	0.532 ± 0.029*	0.418 ± 0.049	0.442 ± 0.014
28.41	Benzeneacetaldehyde, a-(2-methylpropylidene)	0.043 ± 0.004	0.036 ± 0.007	nd
28.45	Unknown	0.066 ± 0.007	0.056 ± 0.006	nd
28.55	Cyclohexane, 1-methyl-2,4-bis (1-methylethenyl)	0.141 ± 0.011	0.118 ± 0.014	0.183 ± 0.014
28.64	4-Tridecene	0.027 ± 0.002	nd	nd
28.85	3,4,4-trimethyl-3-(3-oxo-but-1-enyl)-bicyclo(4.1.0)heptan-2-one	0.173 ± 0.017	0.143 ± 0.023	0.149 ± 0.008
28.93	4-(2,6,6-trimethylcyclohexa-1,3-dienyl)but-3-en-2-one	0.055 ± 0.002	0.051 ± 0.007	0.059 ± 0.005
30.04	Unknown	nd	0.032 ± 0.006	0.020 ± 0.003
30.86	Phenol, 2,4-bis (1,1-dimethyl ethyl)	0.683 ± 0.064	1.081 ± 0.045	0.922 ± 0.047
31.21	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl	0.078 ± 0.007*	0.056 ± 0.008	0.059 ± 0.006

Values indicate mean ± SD from 3 replicates.

* indicates significant ($p \leq 0.05$) differences in relative intensity of volatile compounds among different mulberry cultivars.

nd means not detected

4.1.4 Sensory attributes

In order to evaluate the differences in sensory characteristics of mulberry leaf tea from three different cultivars quantitative descriptive sensory was performed. Spider plots reflecting the differences in the sensory attributes including greenness, brownness, green odor, dried leaf odor, and astringency is shown in Fig. 4.3. Significant differences ($p \leq 0.05$) were observed for sensory attributes among three different mulberry leaf tea, with one exception for astringency. The color characteristics of tea infusions were described by greenness and brownness. The average greenness score of SK tea was significantly ($p \leq 0.05$) higher than those of BR and KP with 7.63±0.51, 5.27±0.42, and 4.78±0.64, respectively. The brownness score of SK tea showed significantly ($p \leq 0.05$) less than those of BR and KP. BR and KP were not significantly different in both greenness and brownness attributes. The results showed that SK could be separated from the others by using their appearance whereas BR and KP cannot be separated by this attribute

Considering the odor attributes, BR had green odor and dried-leaf odor scores significantly ($p \leq 0.05$) higher than both KP and SK. Mean scores of green odor and dried-leaf odor were not significantly different ($p \leq 0.05$) between KP (6.62±0.54) and SK (6.08±0.44).

The sensory scores are in accordance with the GC/MS results. The results from GC/MS showed that BR had more volatile compounds than the others. Moreover, the intensity of 13 volatiles in BR was higher than those of KP and SK. As the result of volatile components and their intensity, the green odor and dried-leaf odor scores obtained from sensory test of BR were higher than the others. KP and SK had

most similar pattern and intensity of volatile compounds and thus, their green and dried-leaf odor scores were similar.

All three cultivars of mulberry leaf tea showed similar score of astringent taste. SK had different color from BR and KP whereas BR can be separated from the others basing on the odor attributes. The result suggested that color and odor attributes of mulberry leaf tea depend on the cultivar.

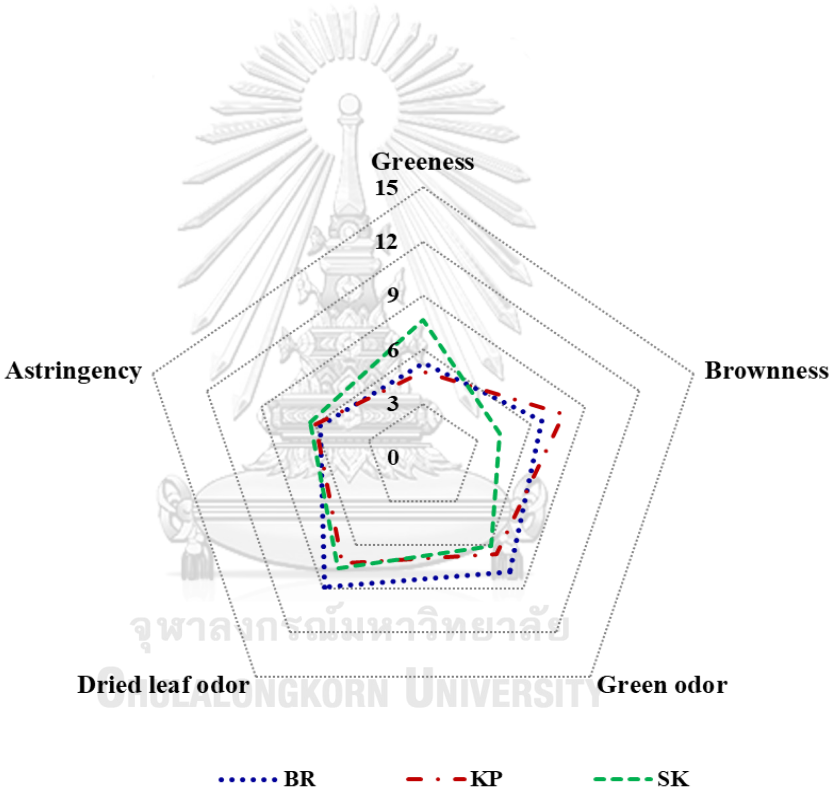


Fig. 4.3 Spider plots reflecting the differences in the sensory attributes of mulberry leaf tea

4.1.5 Volatile profiles from e-nose

The radar graph of sensor response intensity of BR, KP, and SK in Fig. 4.4 showed that BR was discernible from KP and SK, whereas KP and SK were somewhat overlapped. The detection principle of a metal oxide semiconductor (MOS) sensor is generally based on the gas–surface interaction, resulting in a change to the resistance of the sensor. That is the higher the concentration of volatile compounds, the greater the intensity of the sensor responses. (Cui et al., 2015). As the radar graph, it could be state that mulberry tea of different cultivar contains different concentrations of certain volatile ingredients. The results of GC-MS analysis revealed that BR had a higher intensity of 13 volatile compounds than KP and SK so as the P40/1, PA/2, P30/1, and P30/2 sensor response of BR were higher than those of KP and SK. Cui et al. (2015) reported that P40/1 and P30/1 are sensitive to aromatic compounds and organic solvents, respectively. Both of P30/2 and PA/2 are sensitive to alcohol compounds. Comparing the results of e-nose analysis with of GC, P40/1 may be sensitive to pyrazine,2,5-dimethyl, pyrazine,2-ethyl-5-methyl, pyrazine,3-ethyl-2,5-dimethyl, pyrazine,2-ethyl-3,5-dimethyl, and dihydroactinidiolide which found in BR higher than those in KP and SK.

PCA was applied to determine if there were differences in the aroma-active volatile patterns from the three cultivars mulberry leaf tea. Data were made up of 108 samples from 3 groups according to cultivars. Data were made up of 108 samples from 3 groups according to cultivars (BR, KP, and SK). Each group consist of 36 response data. When the data matrix was submitted to PCA, PC scores and PC loading were calculated. The score plot shows the relationship between objects (3$\boldsymbol{x}$36 samples).

The loading plot displays the relationship between the descriptor or measurement variables (18 sensors).

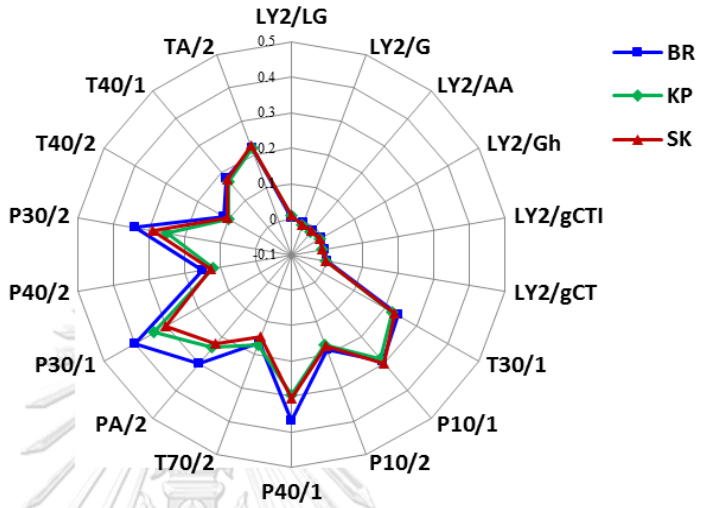


Fig. 4.4 The radar graph for 18 MOS sensors response intensity of e-nose for BR, KP, and SK

Fig. 4.5 shows PCA score plot of three different cultivar mulberry leaf tea on PC1 and PC2 using the e-nose responses data set. The PC1 account for a major fraction (89%) of the total variance of the data. The PC2 axis is orthogonal to the first eigenvector and accounts for 5% of the variation not accounted for by the first PC. These two PCs together represent 94% of the total variance. The results showed that BR located on the negative region of PC1 clearly isolated from the other cultivars. However, KP and SK located on the positive region of PC1 overlapped partially. This is probably because the volatile patterns of KP and SK were similar as evident on GC results.

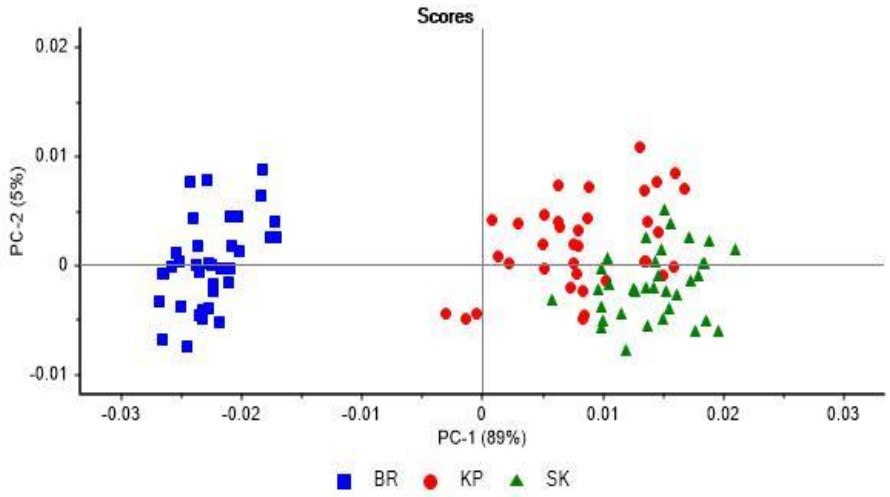


Fig. 4.5 PCA score plot of three different cultivar mulberry leaf tea on PC1 and PC2 using the e-nose responses data set.

Fig. 4.6 shows the loading plot with 32 variables, which established the relative importance of each e-nose sensor, and the relationships between the sensors and the samples. Sensors negatively correlated to PC1 were LY2/gCT, TA2, LY2/gCTI, and LY2/Gh, whereas the other sensors positively correlated to PC1. All sensors had a far Euclidean distance from the origin and were considered as important for discrimination. Among all mulberry leaf tea, LY2/gCT, TA2, LY2/gCTI, and LY2/Gh were associated with KP and SK whereas the other sensors were associated with BR. The sensor including P40/1, PA/2, P30/1, and P30/2 located in the negative region of PC1 and associated with BR. As the results of radar graph revealed that sensor response intensity was considered as important for the discrimination the samples. As the sensor response intensity correlated with concentration of volatile compounds of mulberry leaf tea revealed in GC results, volatile intensity was important factor to classify 3 different cultivars of mulberry leaf tea.

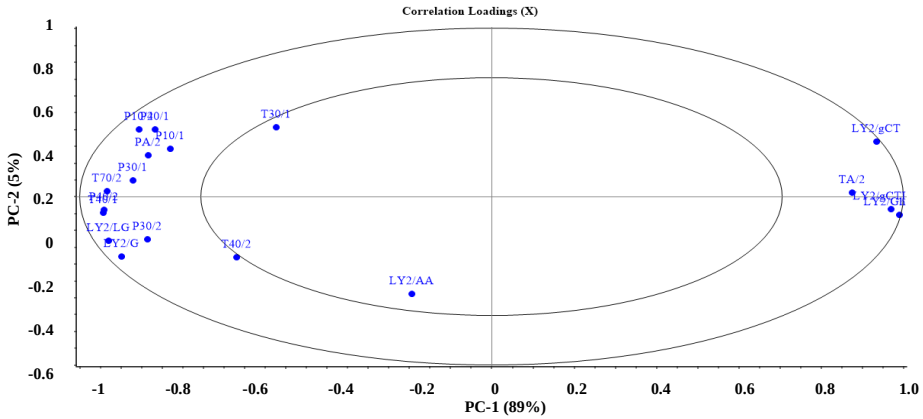


Fig. 4.6 PCA loading plot of three different cultivar mulberry leaf tea on PC1 and PC2 using the e-nose responses data set.

The results demonstrated that the quality attributes and phytochemicals of mulberry leaf tea could be influenced by cultivar. KP cultivar is interesting in terms of health benefits because it has the highest content of phenolic compounds and antioxidant activity. SK cultivar stands out in green color. Since BR cultivar has more volatile component and concentration than the others, BR cultivar is predominant in odor attributes. It is well known that aroma and flavor of tea and herbal tea are the factors influencing consumer acceptance (Zheng et al., 2016). Thus, BR cultivar is recommended for commercial tea production. E-nose could use to discriminate BR cultivar from the others according to their volatile profiles. Therefore, it is highly feasible for the application of e-nose as a rapid and non-destructive tool to screen the raw materials and control the product quality during processing and storage.

4.2 Influence of packaging and storage time on the quality of mulberry leaf tea

4.2.1 Physical properties

4.2.1.1 Moisture content and water activity

Fig. 4.7 shows the effect of packaging and storage time on the MC and a_w of mulberry leaf tea during storage at 30°C for 18 months. The results demonstrated that packaging material and storage time have significant impact on the physical properties of mulberry leaf tea. The MC and a_w of the sample in PP significantly ($p \leq 0.05$) increased with increasing storage time whereas those of the sample in AL were hardly changed throughout the storage. The MC of the mulberry leaf tea in PP dramatically increased from 3.70±0.08% to 6.36±0.21% during the first 3 months of storage, after that moisture content slightly increased till the end of storage (8.74±0.33%). The MC of the mulberry leaf tea in AL was quite stable throughout the storage, ranging from 3.70±0.08% to 3.80±0.28%.

The a_w of the mulberry leaf tea in PP increased from 0.226±0.003 to 0.587±0.007 at 18 months of storage. The a_w of the sample in PP rapidly increased within 9 months of storage but slightly changed after 12 months of storage. The a_w of the sample in AL was in an average of 0.28±0.004. The increase in MC and a_w of the sample in PP in comparison to the sample in AL was due to the lower water vapor transmission of PP (15.19 $g.m^2day^{-1}$) in comparison to AL (less than 0.01 $g.m^2day^{-1}$) (Piringer and Baner, 2008).

When mulberry leaf tea was stored in a relatively higher humidity environment, tea can absorb moisture, resulting in an increase in the a_w.

However, the a_w of both the samples in AL and PP were lower than 0.65 throughout the storage and thus no microbial growth was observed.

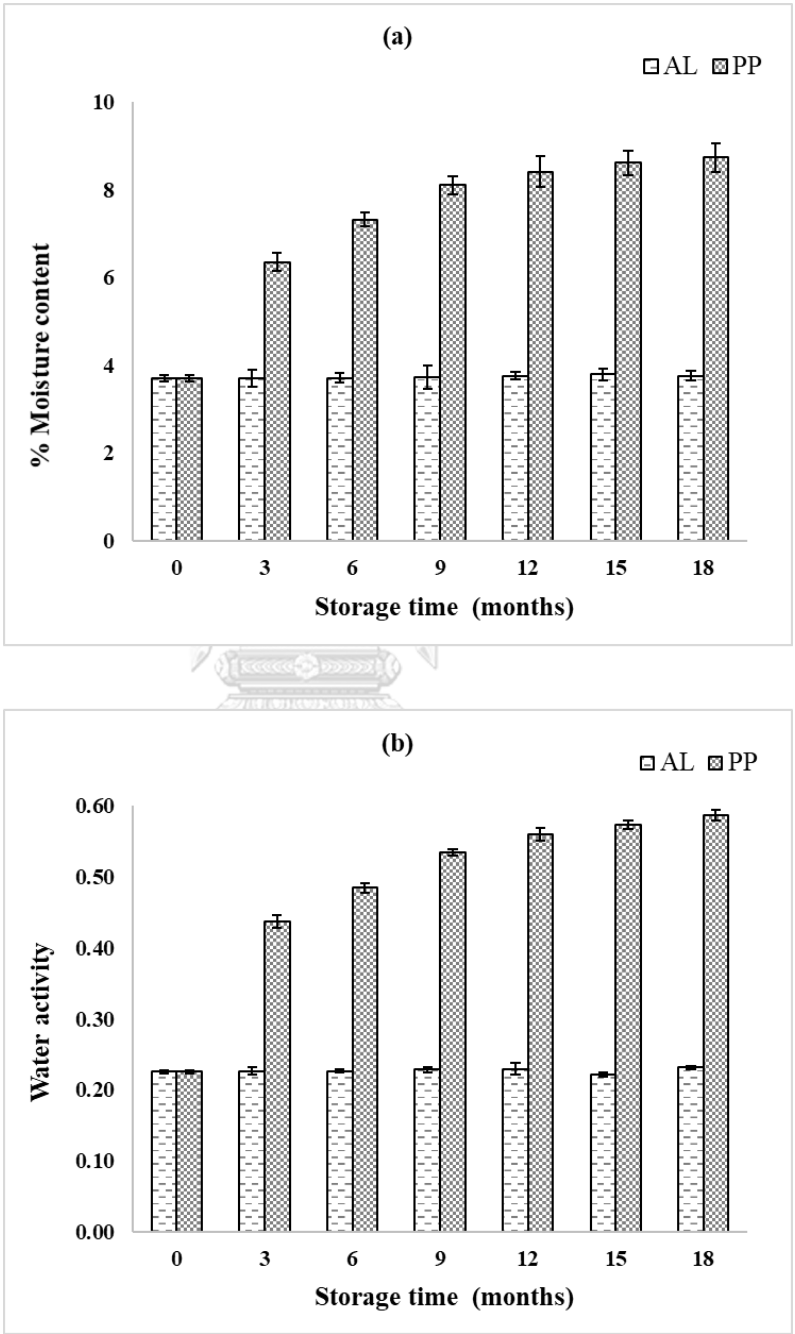


Fig. 4.7 Moisture content and water activity of mulberry leaf tea during storage at 30±1°C, 75% RH for 18 months; moisture content (a) and water activity (b).

4.2.1.2 Color of mulberry leaf tea powder and infusion

Fig. 4.8 shows the Changes in color parameters (L*, a*, and b*) of mulberry leaf tea powder and infusion. The results showed that both packaging material and storage time affected color of mulberry leaf tea and infusion. The changes in color parameters of mulberry leaf tea were consistent with those of mulberry leaf infusion. L* and b* of the samples decreased, whereas a* increased with increasing storage time. The changes the color parameters of PP were greater than those of AL. Considering PP, L* values of mulberry leaf tea powder and infusion significantly ($p \leq 0.05$) decreased from 33.28±0.16 to 31.01±0.14 and from 71.75±0.33 to 66.57±0.42, respectively. The a* values of mulberry leaf tea powder and infusion significantly ($p \leq 0.05$) increased from -4.84±0.04 to -2.04±0.10 and from -2.99±0.06 to -0.94±0.08, respectively. The b* values of mulberry leaf tea powder and infusion significantly ($p \leq 0.05$) decreased from 12.61±0.20 to 8.61±0.27 and from 17.51±0.25 to 13.51±0.32, respectively. Considering AL, L*, b* and a* values of mulberry leaf tea and infusion were not significantly different from the initial sample up to 12 months of storage, after that they were significantly different($p \leq 0.05$) from the initial sample. The changes in color of mulberry leaf tea and infusion may cause by oxidation of phenolic compounds and the chlorophyll degradation (Schmalko et al., 2005). Oxidation of phenolic compounds caused the formation of oligomeric theaflavins and polymeric thearubigins, thus the green color of tea turns into light brown, and deep brown in the course of their oxidation (Kosińska & Andlauer, 2014).

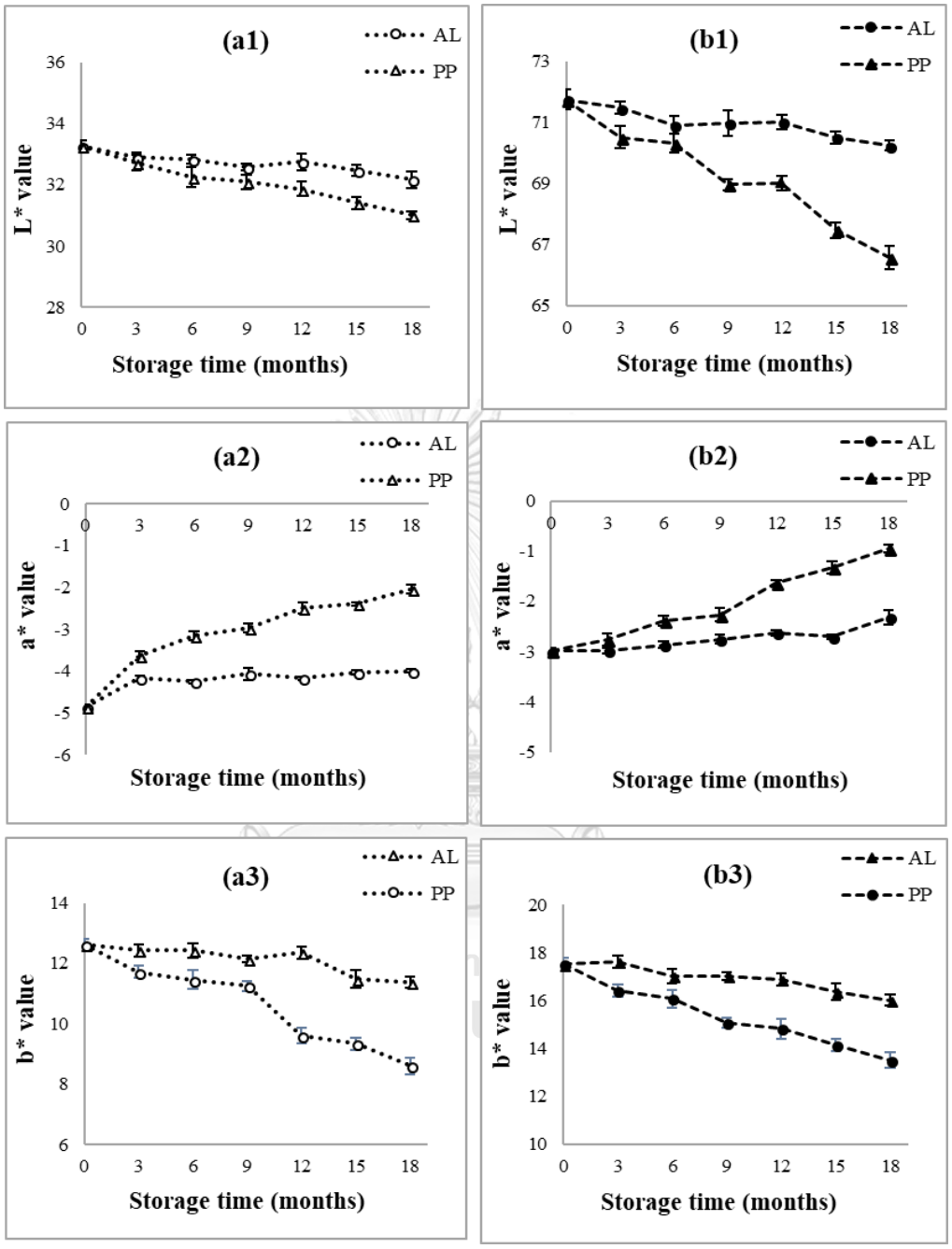


Fig. 4.8 L*, a*, and b* color values of mulberry leaf tea powder (a1-3) and infusion (b1-3) during storage at 30±1°C, 75% RH.

4.2.2 Phytochemicals and antioxidant activity

4.2.2.1 Total phenolic content and total flavonoids content

The changes in TPC and TFC of mulberry leaf tea during storage at 30±1°C in a controlled humidity chamber at 75% RH for 18 months are shown in Fig. 4.9. The results showed that packaging material and storage time significantly ($p \leq 0.05$) influenced the changes in TPC and TFC of mulberry leaf tea.

The TPC of the mulberry leaf tea at time zero was 39.73±0.93 mg GAE/g dried sample. After 18 months of storage, the TPC of AL and PP packaged samples was 34.75±0.91 and 28.27±1.16 mg GAE/g dried sample, respectively. The results indicated that the TPC of AL and PP lost by 12.53% and 28.84%, respectively, throughout the storage.

Flavonoids, the major group of natural phenolic compounds act as antioxidants in plants (Sulaiman and Balachandran, 2012). The results showed that packaging material and storage time also significantly affected the decline of TFC of mulberry leaf tea. The TFC of both AL and PP significantly ($p \leq 0.05$) decreased with increasing storage time. After 18 months of storage, TFC of AL and PP decreased from 29.95±0.68 mg rutin/g dried sample to 24.85±0.85 and 19.65±0.98 mg rutin/g dried sample, respectively. The TFC of AL and PP lost by 17.03% and 34.39%, respectively, throughout the storage.

The decrease of TPC and TFC was caused by the oxidative degradation of phenolic compounds when exposed to atmospheric oxygen and UV-light (Sokolová et al., 2012). The oxidative degradation process is associated with the

presence of subsequent chemical reactions such as hydroxylation or dimerization (Daskalaki et al., 2009). The decrease in TPC and TFC of the mulberry leaf tea in PP during storage were higher than those of the samples in AL because of the different barrier properties of packaging material. Since PP has a moderate permeability to gases and a high transparent (Allahvaisi et al., 2010), the oxidation of phenolic compounds in mulberry leaf tea occurred rapidly. Whereas AL is more effective barrier to the effects of air, temperature, moisture, and chemical attack and thus it can prevent the oxidation of phenolic compounds in mulberry leaf tea (Marsh & Bugusu, 2007).

Phenolic compounds are major phytochemicals contained in mulberry leaf tea. These substances not only play a role in health beneficial properties but also sensorial properties of food (Hufnagel and Hofmann, 2008). However, phenolic compounds are sensitive to oxygen, moisture, heat, and light which activate oxidative degradation (Wang and Lin, 2000). The loss of phenolic compounds in mulberry leaf tea during storage resulted in the serious decline in its qualities and health benefits. Therefore, to retard the rate of oxidation of phenolic compounds, mulberry leaf tea should be stored in proper packaging like laminated alumimium bag.

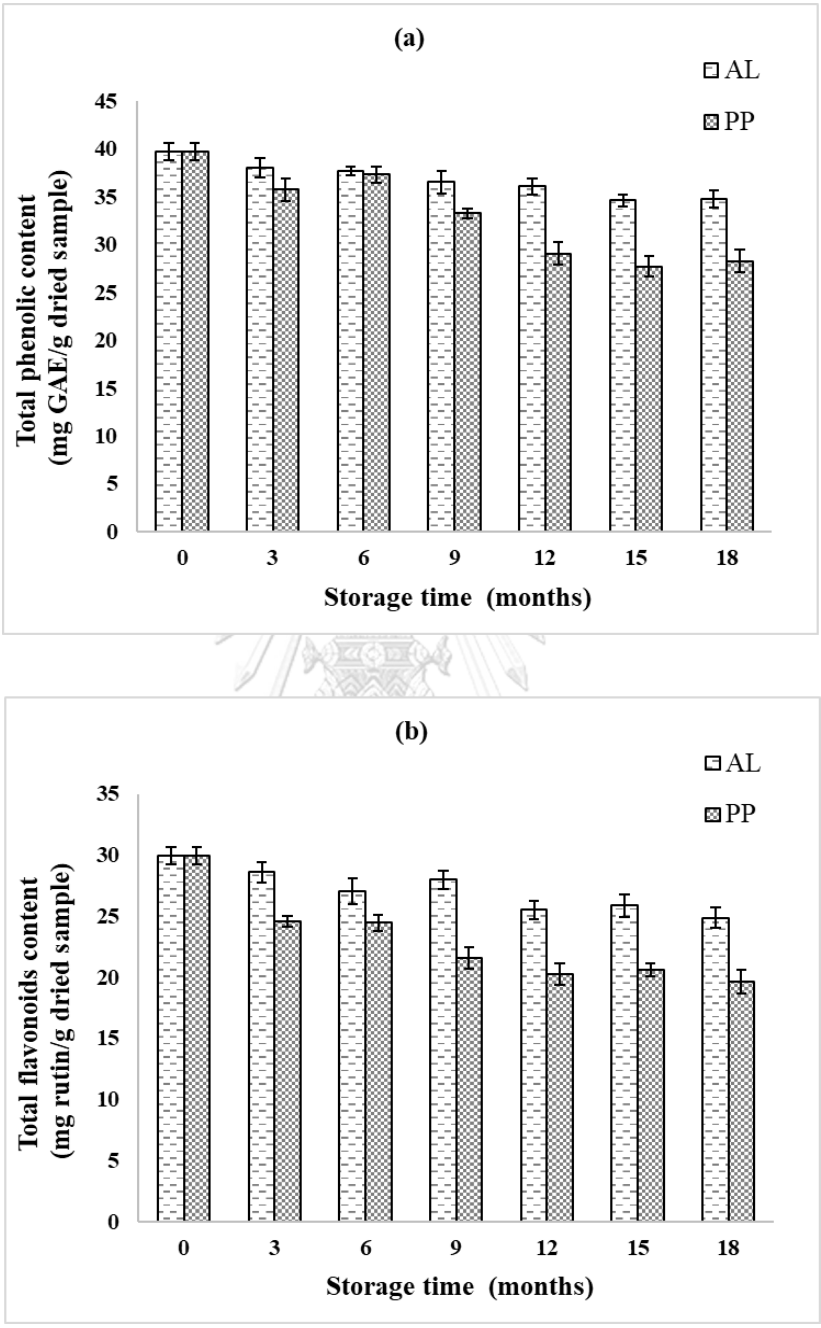


Fig. 4.9 Phytochemicals of mulberry leaf tea during storage at 30±1°C, 75% RH for 18 months; TPC (a) and TFC (b).

4.2.2.2 Phenolic compounds

To elucidate the effects of packaging material and storage time on the phenolic compounds of mulberry leaf tea, three major phenolics, including chlorogenic acid, rutin, and quecetin, were quantified using HPLC method. Changes in chlorogenic acid, rutin, and quercetin of mulberry leaf tea during storage at 30±1°C for 18 months are shown in Fig. 4.10. The results showed that all phenolic compounds significantly ($p \leq 0.05$) decreased with increasing storage time. Chlorogenic acid content of the PP decreased significantly ($p \leq 0.05$) at 3 months of storage time with 20.80% loss and the end of storage the loss of chlorogenic acid was 33.63%. Throughout the storage time, the content of chlorogenic acid in AL was not significantly different.

The storage time affected both rutin content and quercetin content of mulberry leaf tea in AL and PP. The content of rutin and quercetin of the sample in AL gradually decreased with increasing storage time. The content of rutin of mulberry leaf tea in AL and PP decreased from 76.65±2.4 mg/ to 64.98±5.1 and 50.87±4.9 mg/ 100 g dried sample, respectively. After 18 months of storage, quercetin content decreased from 0.42±0.019 to 0.35±0.029 and 0.27±0.032 mg/ 100 g for AL and PP packaged samples, respectively.

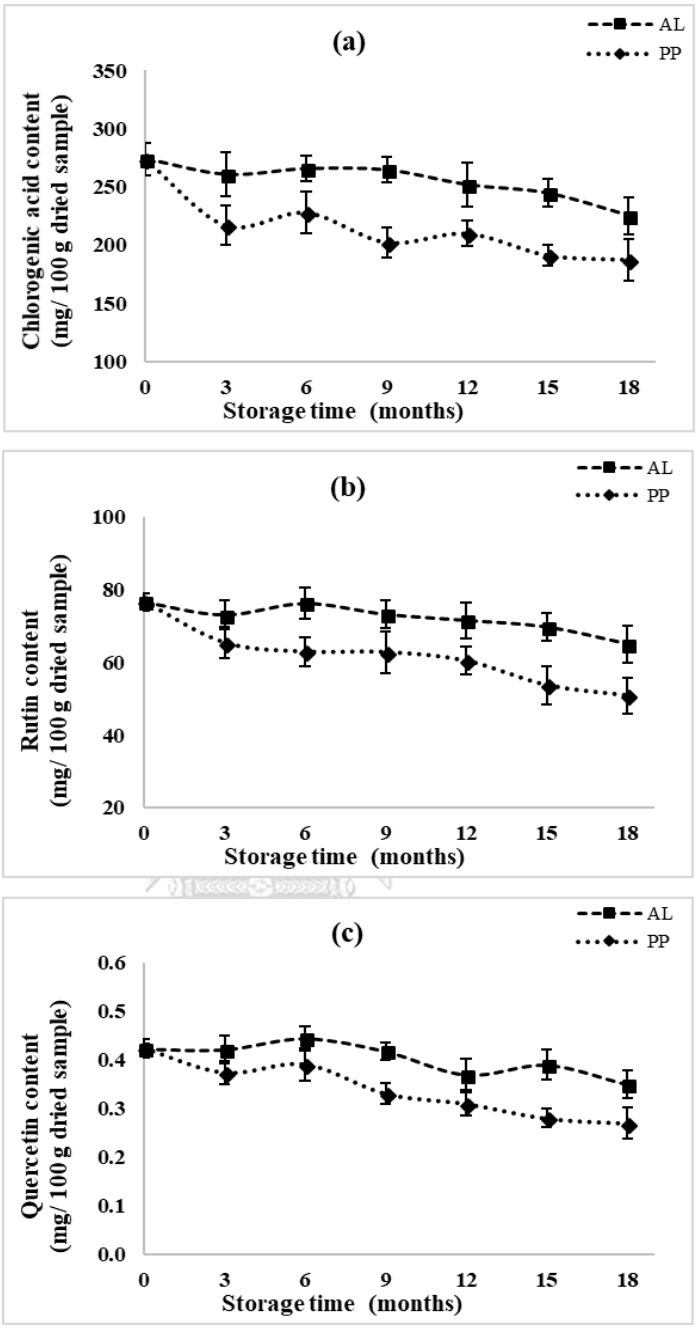


Fig. 4.10 Phenolic compounds of mulberry leaf tea during storage at 30±1°C, 75% RH for 18 months; chlorogenic acid (a), rutin (b), and quercetin (c).

4.2.2.3 Antioxidant activity

FRAP and DPPH assays were conducted to evaluate the effect of packaging material and storage time on antioxidant activity of mulberry leaf tea. The changes in FRAP and DPPH of the samples in AL and PP are shown in Fig. 4.11.

The results showed that FRAP of the samples significantly decreased ($p \leq 0.05$) with increasing storage time. At the beginning storage time, the FRAP of mulberry leaf tea was 13.39±0.55 mM Trolox/100 g dried sample. Afterward, the FRAP decreased as a function of time and packaging material The FRAP of the samples in AL and PP lost by 15.01% and 33.31%, respectively throughout the storage.

DPPH of the packaged mulberry tea expressed as % inhibition decreased from 52.57±1.52 to 46.38±1.25 and 36.62±1.53% for the samples in AL and PP, respectively at 18 months of storage.

The decrease of FRAP and DPPH of the mulberry leaf tea during storage directly related to the loss of its phenolic compounds. It has been reported that the antioxidant activity of plants was linearly positive correlated with its phenolic content (Turumtay et al., 2014). Our results are in agreement with those of Chen et al. (2013) who stated that TPC and TFC of raspberries correlated well with its antioxidant activity, indicating raspberries with higher content of phenolic and flavonoid compounds showed higher antioxidant activity.

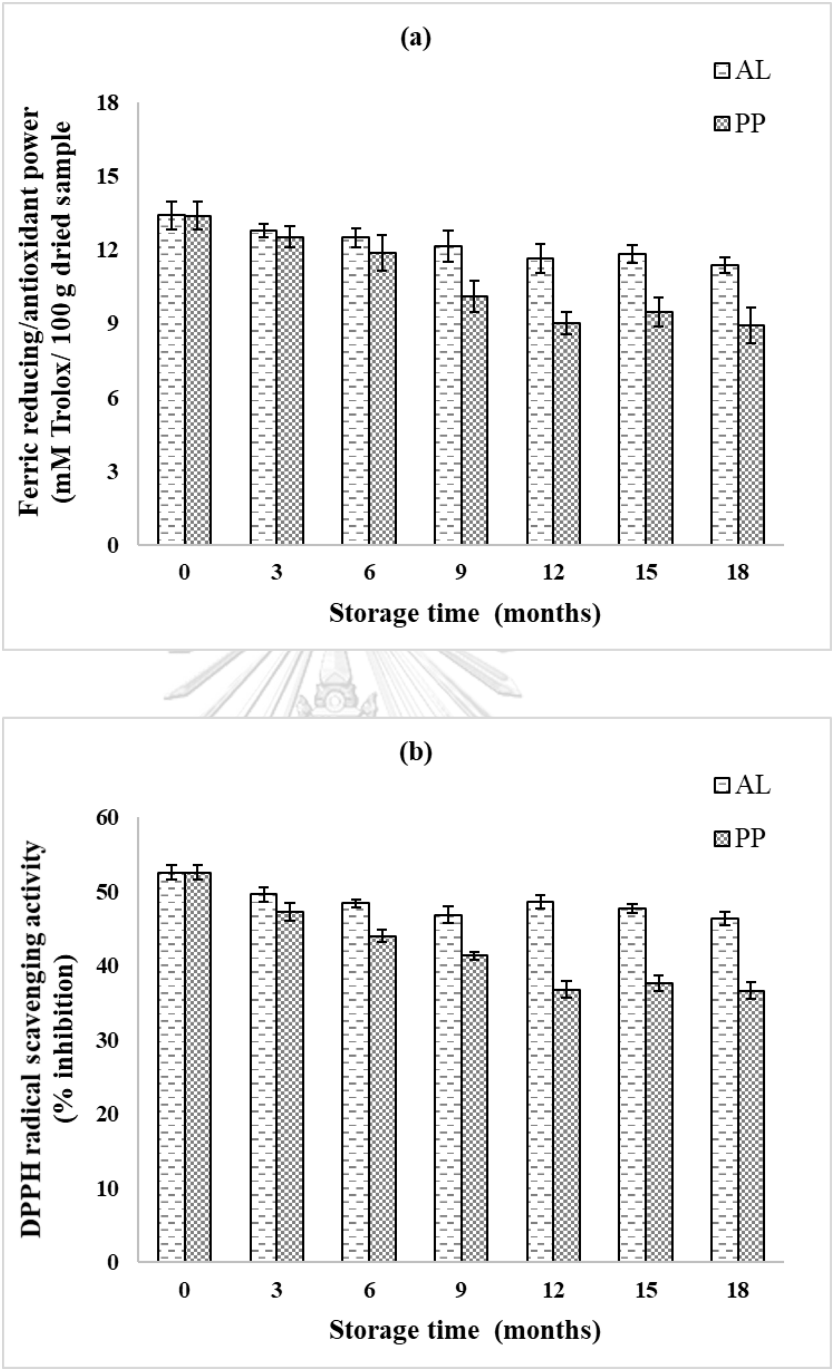


Fig. 4. 11 Antioxidant activity of mulberry leaf tea during storage at 30±1°C, 75% RH for 18 months; FRAP (a) and DPPPH (b).

4.2.3 Volatile compounds

The effects of packaging materials and storage time on volatile components of mulberry leaf tea stored at 30±1°C were determined using SPME-GC/MS. GC chromatograms of volatile profiles of mulberry leaf tea in AL and PP stored for 0, 6, 12, and 18 months are shown in Fig. 4.12 and Fig. 4.13, respectively. Volatile compounds were identified by comparison of their mass spectra of authentic reference compound with those of the NIST (version 11.0) database. Fifty-three volatile components screened in the initial sample (T0), the sample in AL stored for 18 months (AL18), and the sample in PP stored for 18 months (PP18) are shown in Table 4.4. The results showed that storage time did not affect volatile components of the mulberry leaf tea in AL. The average concentration of all volatile components of the samples in AL did not significantly change over the whole storage period. However, for samples in the PP, changes in concentration of twelve volatiles (butanal-2-methyl, hexanal, 5-hepten-2-one,6-methyl, 6-methyl-3,5-heptadiene-2-one, 3,5-octadien-2-one heptan-2-one,, benzaldehyde,3,4-dimethyl, 3-buten-2-one, 4(2,6,6-trimethyl-1-cyclohexen-1-yl), 2(4H)-benzofuranone,5,6,7,7a-tetradydro-4,4,7a-trimethyl, 5,9-undecadien-2-one, 6,10-dimethyl, and 1,6-octadien-3-ol,3,7-dimethyl,2 aminobenzoate) with storage time were observed (Fig. 4.14). Butanal-2-methyl and benzaldehyde,3,4-dimethyl found in mulberry leaf tea in PP gradually decreased with increasing storage time, whereas the remaining 10 compounds increased throughout the storage. Changes in the concentration of some volatiles associated with oxidation reaction of components in mulberry leaf tea. Butanal-2-methyl, which is a branched-chain aldehyde, is generally perceived as malty or chocolate-like odor in food products. A decrease of butanal-2-methyl of mulberry leaf tea in PP during storage may be attribute to the conversion of

branched-chain aldehyde compounds to alcohol or acid compounds via reduction or oxidation, respectively (Smit et al., 2009). Hexanal generated from lipid oxidation contributes to off-flavors such as stale, cardboard, and fishy in foods. Hexanal content is directly related to the deterioration of food quality during storage (Maarse, 2017). A 4-oxo-2-nonenal is a lipid peroxidation product derived from oxidized omega-6 polyunsaturated fatty acids such as arachidonic acid and linoleic acid. It has been widely used as a marker of lipid peroxidation (Lee and Blair, 2000). It actively modifies 2'deoxyguanosine, further implicating lipid peroxidation in mutagenesis and carcinogenesis which lead to various heritable diseases and cancer (Näsström et al., 2011).

Shoaib Zafar *et al.* (2013) reported that the fresh mulberry leaves contain fat in a range of 0.64 to 1.51 g/100 g dried sample. While in dried mulberry leaves, fat is in a range of 2.09 to 4.93 g/100g dried sample. During storage, UV-light, oxygen, and moisture can all induce lipid oxidation causing off-flavor development in mulberry leaf tea. Since AL has higher UV-light, oxygen, and moisture barrier properties than PP, it can prevent lipid oxidation better than PP bag. This result is in agreement with the studied of Kaack and Christensen (2008) who reported that AL bag had more efficiency to protect the quality of tea processed from flowers of black elder (*Sambucus nigra* L.) than plastic film.

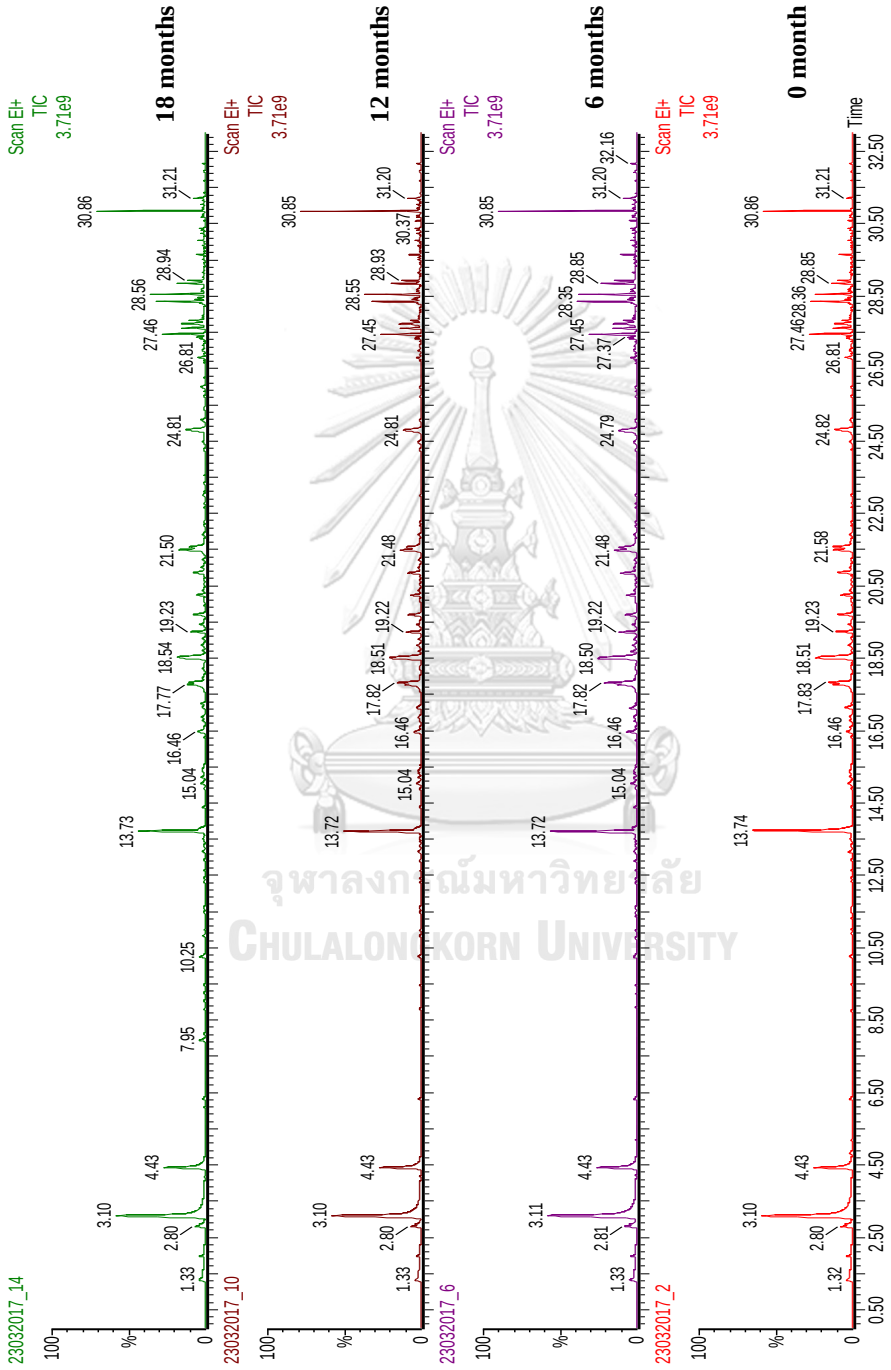


Fig. 4. 12 GC chromatogram of volatile compounds of mulberry leaf tea in AL bag during storage at 30±1°C, 75% RH for 0, 6, 12, and 18 months.

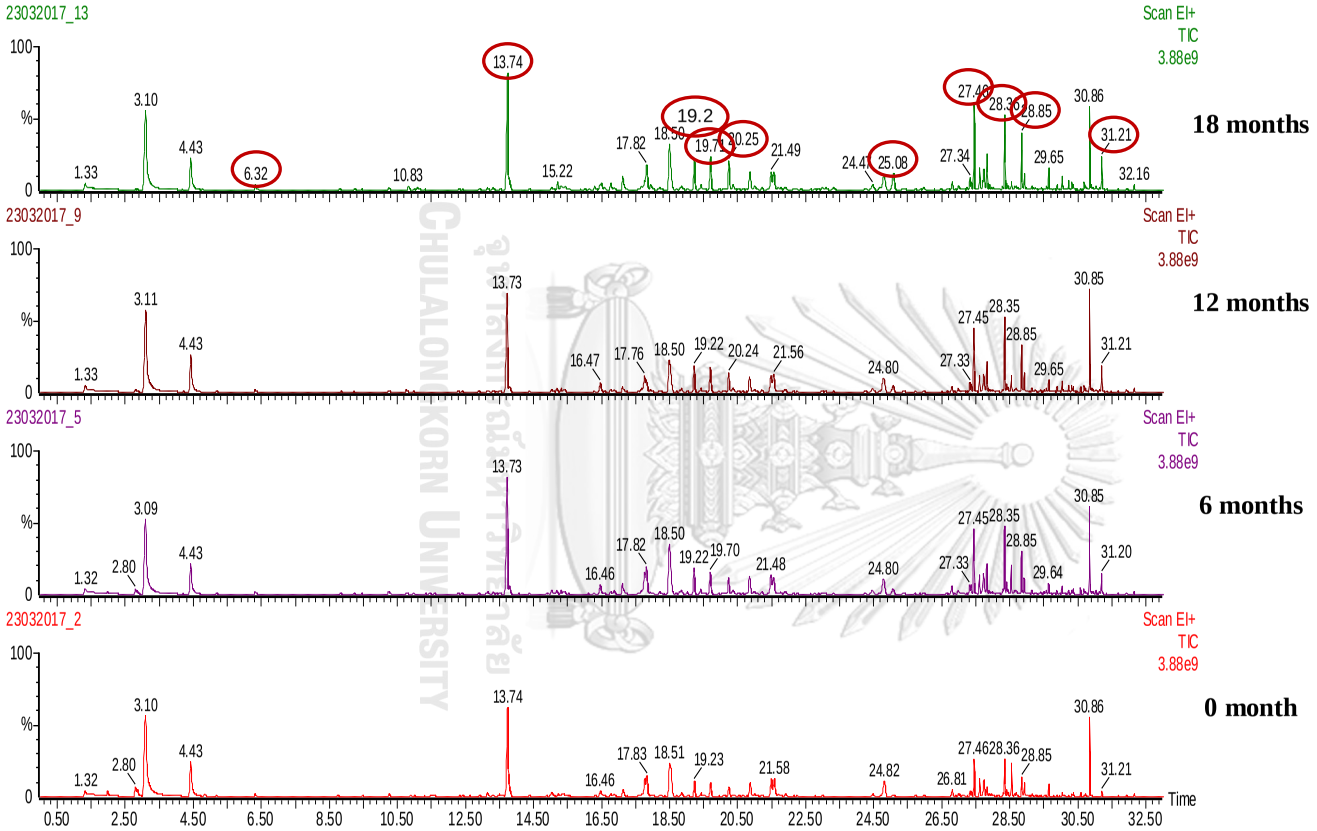


Fig. 4.13 GC chromatogram of volatile compounds of mulberry leaf tea in PP bag during storage at 30±1°C, 75% RH for 0, 6, 12, and 18 months.
The peaks of 10 volatile compounds that were circled presenting that the concentration of these volatiles significantly ($p<0.05$) increased during storage

Table 4.4 Relative content of volatile compounds of mulberry leaf tea stored at 30±1°C, 75% RH for 18 months.

TR (min)	Volatile compounds	Relative content		
		T0	AL18	PP18
1.98	Propanal-2-methyl	0.393 ± 0.019	0.202 ± 0.026	0.174 ± 0.031
2.80	butanal, 2-methyl	0.294 ± 0.033	0.232 ± 0.014	0.020 ± 0.012
6.32	hexanal	0.069 ± 0.019	0.068 ± 0.012	0.178 ± 0.013
10.26	2-hexenal	0.091 ± 0.006	0.125 ± 0.018	0.080 ± 0.016
11.10	Pyridine, 2,3-dimethyl-	0.038 ± 0.006	0.042 ± 0.016	0.037 ± 0.017
12.33	octanal	0.027 ± 0.018	0.234 ± 0.016	0.039 ± 0.019
12.93	Cyclohexanone, 2,2,6-trimethyl-	0.037 ± 0.015	0.035 ± 0.016	0.038 ± 0.018
13.15	Pyrazine, 2,5-dimethyl	0.093 ± 0.006	0.062 ± 0.019	0.041 ± 0.016
13.30	2-Penten-1-ol	0.035 ± 0.007	0.027 ± 0.029	0.044 ± 0.030
13.74	5-hepten-2-one, 6-methyl	1.855 ± 0.380	1.023 ± 0.217	3.785 ± 0.308
15.04	pyrazine, 2-ethyl-5-methyl	0.147 ± 0.007	0.091 ± 0.016	0.061 ± 0.020
15.23	Nonanal	0.083 ± 0.021	0.060 ± 0.016	0.133 ± 0.015
16.31	cyclopropanemethanol-2-methyl-2,4-methyl-3-pentanyl	0.023 ± 0.008	0.023 ± 0.004	0.047 ± 0.011
16.46	Pyrazine, 3-ethyl-2,5-dimethyl	0.178 ± 0.034	0.174 ± 0.012	0.255 ± 0.015
16.78	2-Octen-1-ol	0.055 ± 0.013	0.069 ± 0.008	0.138 ± 0.080
17.14	2,4-heptadiene	0.210 ± 0.011	0.065 ± 0.004	0.326 ± 0.008
17.56	1-hexanol, 2-methyl	0.477 ± 0.010	0.352 ± 0.007	0.387 ± 0.028
17.83	2,4-heptadienal	0.607 ± 0.083	0.287 ± 0.051	0.776 ± 0.014
18.51	3,5-heptadien-2-one	1.615 ± 0.014	0.783 ± 0.010	1.363 ± 0.009
19.23	1,6-octadien-3-ol, 3,7-dimethyl, 2 aminobenzoate	0.278 ± 0.031	0.211 ± 0.028	0.699 ± 0.039
19.43	1-octanol	0.094 ± 0.075	0.104 ± 0.008	0.124 ± 0.015
19.71	3,5-octadien-2-one	0.210 ± 0.041	0.299 ± 0.030	0.898 ± 0.034

Values indicate mean ± SD from 3 replicates.

Table 4.4 Relative content of volatile compounds of mulberry leaf tea stored at 30±1°C, 75% RH for 18 months (continued).

TR (min)	Volatile compounds	Relative content		
		T0	AL18	PP18
20.25	6-methyl-3,5-heptadiene-2-one	0.157 ± 0.029	0.137 ± 0.037	0.027 ± 0.048
20.37	Cyclohexanol, 2,4-dimethyl	0.059 ± 0.018	0.080 ± 0.004	0.110 ± 0.006
20.88	1-cyclohexene-1-caboxaldehyde-2,6,6-trimethyl	0.377 ± 0.029	0.254 ± 0.020	0.407 ± 0.008
21.23	2,6-Dimethyl-3(2-methyl-1-butyl)pyrazine	0.051 ± 0.024	0.074 ± 0.006	0.041 ± 0.015
21.50	cyclohexanol, 5-methyl-2-(1-methyethyl)	0.498 ± 0.013	0.482 ± 0.010	0.393 ± 0.006
21.59	1,3-cyclohexadien-1-caboxaldehyde, 2,6,6-trimethyl	0.585 ± 0.015	0.378 ± 0.004	0.444 ± 0.006
21.93	Pyrazine, 2,5-dimethyl-3-(3-methylbuthyl)	0.070 ± 0.010	0.073 ± 0.068	0.076 ± 0.010
24.48	benzaldehyde, 3,4-dimethyl	0.358 ± 0.043	0.042 ± 0.038	0.094 ± 0.031
25.07	4-oxo-2-Nonenal	0.063 ± 0.018	0.105 ± 0.021	0.694 ± 0.045
27.00	4-(2,4-dimethylcyclohex-3-enyl)but-3-en-2-one	0.061 ± 0.013	0.049 ± 0.010	0.091 ± 0.009
27.38	3-buten-2-one, 4(2,6,6-trimethyl-1-cyclohexen-1-yl)	0.389 ± 0.067	0.394 ± 0.081	1.191 ± 0.070
27.46	5,9-undecadien-2-one, 6,10-dimethyl	0.410 ± 0.080	0.391 ± 0.055	1.447 ± 0.081
27.62	propanoic acid, 2-methyl, 3-hydroxy-2,4,4-trimethyl pentyl ester	0.233 ± 0.019	0.193 ± 0.011	0.252 ± 0.006
27.75	pentanoix acid, 2,2,4-trimethyl-3-hydroxy-, isobutyl ester	0.344 ± 0.014	0.304 ± 0.015	0.416 ± 0.007

Values indicate mean ± SD from 3 replicates.

Table 4.4 Relative content of volatile compounds of mulberry leaf tea stored at 30±1°C, 75% RH for 18 months (continued).

TR (min)	Volatile compounds	Relative content		
		T0	AL18	PP18
28.36	Benzeneacetaldehyde, a-(2-methylpropylidene)	0.429 ± 0.009	0.343 ± 0.011	0.476 ± 0.010
28.46	Cyclohexane, 1-methyl-2,4-bis(1-methylethenyl)	0.040 ± 0.008	0.048 ± 0.010	0.041 ± 0.047
28.85	Heptan-2-one	0.224 ± 0.026	0.242 ± 0.031	0.798 ± 0.051
28.90	Oxacyclotetradeca-4, 1,1-diyne	0.015 ± 0.026	0.012 ± 0.028	0.015 ± 0.014
28.94	4-(2,6,6-trimethylcyclohex-3-enyl)but-3-en-2-one	0.131 ± 0.003	0.106 ± 0.005	0.140 ± 0.025
29.03	Diphenyl ether	0.006 ± 0.002	0.019 ± 0.004	0.003 ± 0.001
29.16	Falcarinol	0.022 ± 0.009	0.023 ± 0.011	0.036 ± 0.008
29.43	1,5-Decadiyne	0.011 ± 0.007	0.011 ± 0.009	0.020 ± 0.010
29.52	5-Methyl-2-phenyl-2-hexanal	0.020 ± 0.002	0.017 ± 0.004	0.021 ± 0.013
29.91	3,5,9-Undecatrien-2-one, 6,10-dimethyl	0.019 ± 0.008	0.018 ± 0.012	0.045 ± 0.007
30.23	2-Methyl-5-octyn-4-ol	0.014 ± 0.006	0.038 ± 0.005	0.076 ± 0.071
30.33	Megastigmatrienone	0.022 ± 0.011	0.030 ± 0.037	0.079 ± 0.018
30.37	thymol	0.036 ± 0.008	0.038 ± 0.008	0.052 ± 0.012
30.59	phenol, 2-(1,1-dimethylethyl)-5-methyl)	0.038 ± 0.033	0.010 ± 0.010	0.037 ± 0.031
30.86	phenol, 2,4-bis (1,1-dimethylethyl)	0.897 ± 0.021	0.624 ± 0.045	0.746 ± 0.021
31.21	2(4H)-benzofuranone, 5,6,7,7a-tetradydro-4,4,7a-trimethyl	0.095 ± 0.013	0.100 ± 0.017	0.420 ± 0.023
32.16	1,2-Benzene dicarboxylic acid, bris(2-methylpropyl) ester	0.043 ± 0.010	0.042 ± 0.014	0.053 ± 0.019

Values indicate mean ± SD from 3 replicates.

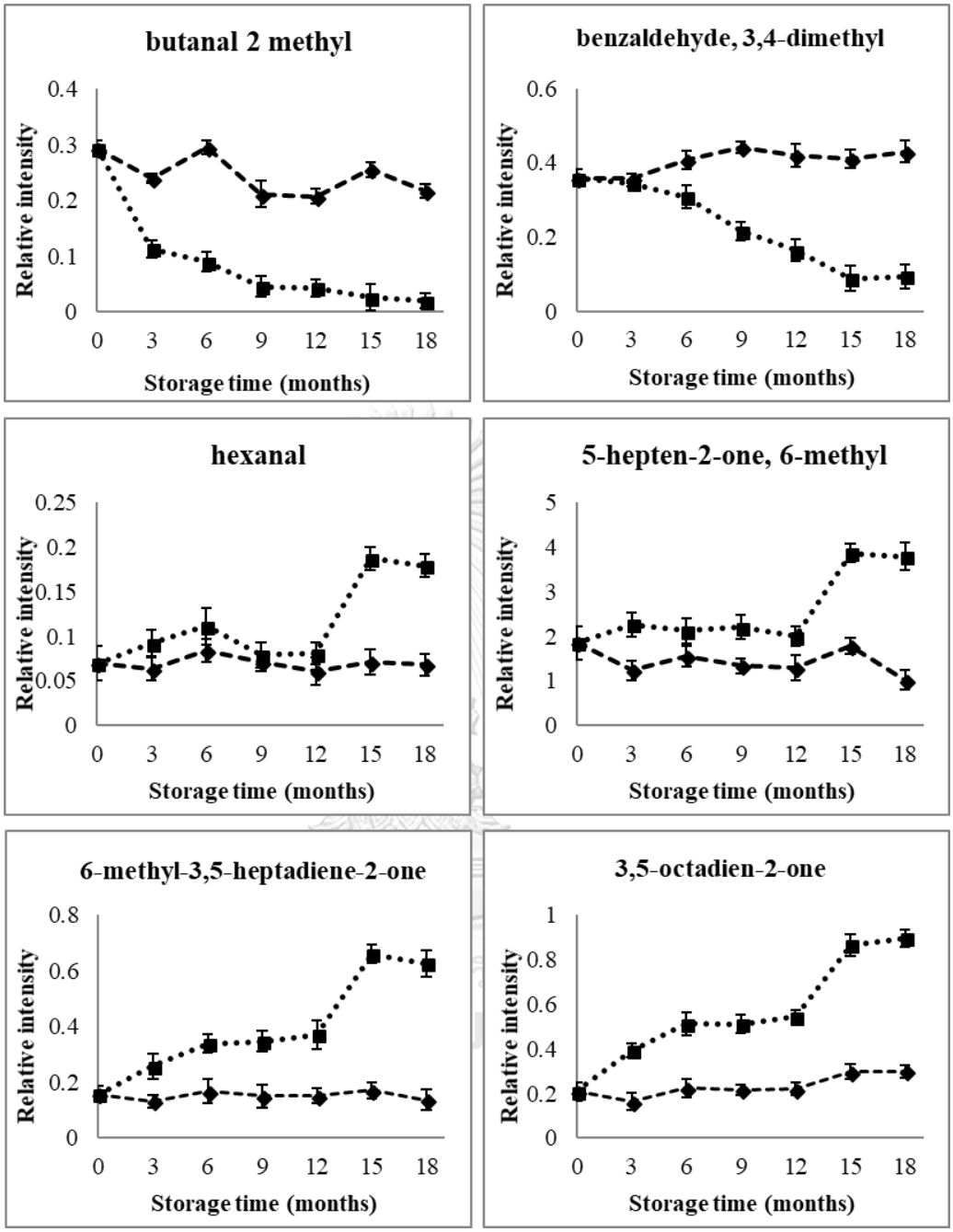


Fig. 4.14 Volatile compounds of mulberry leaf tea during storage at 30±1°C, 75% RH for 18 months; AL (--◆--) and PP (····■···).

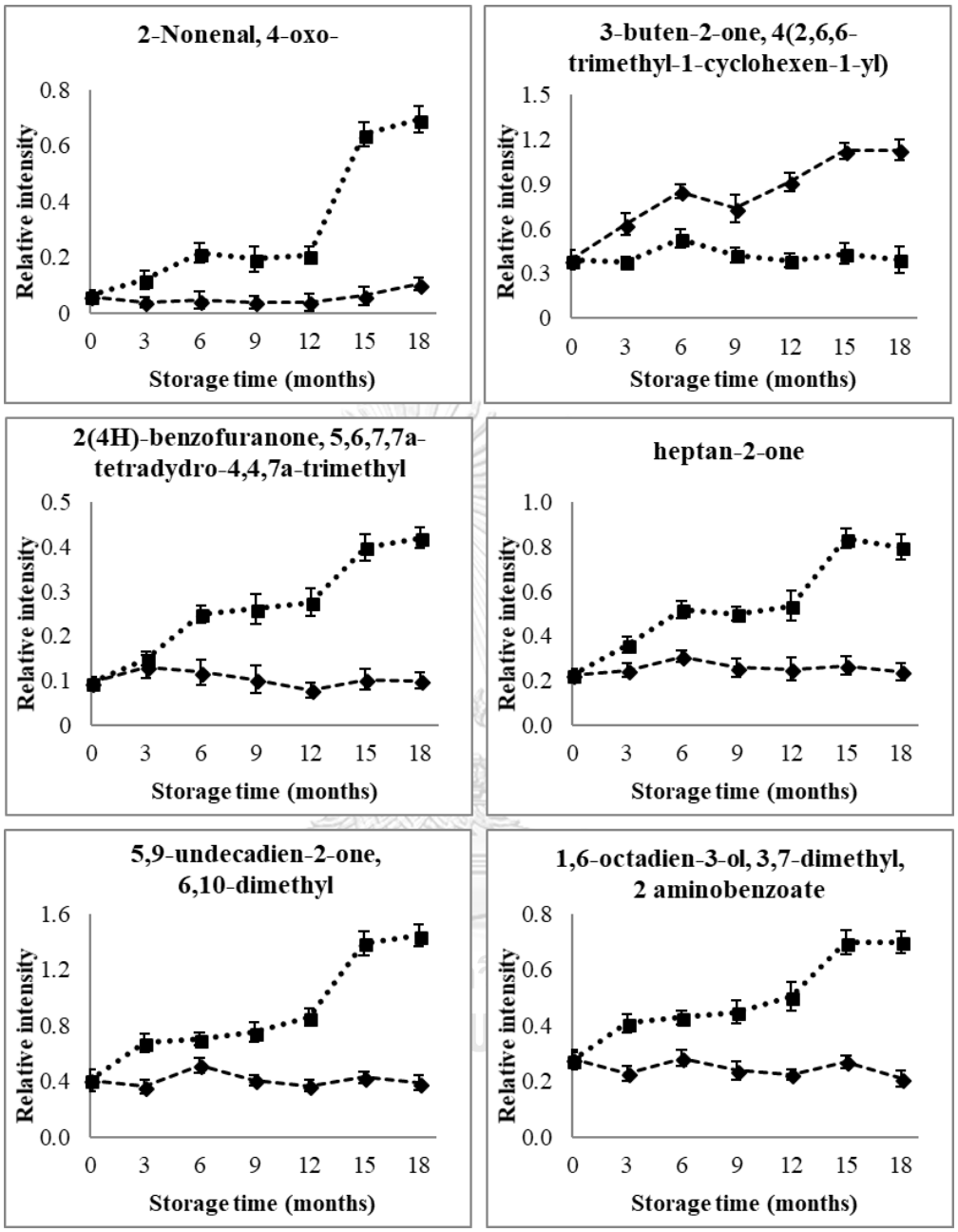


Fig. 4.14 Volatile compounds of mulberry leaf tea during storage at 30±1°C, 75% RH for 18 months; AL (--◆--) and PP (·····■·····) (continued).

4.2.4 Sensory attributes

The effects of packaging and storage time on sensory attributes of mulberry leaf tea were evaluated using quantitative descriptive analysis. Five attributes developed by panels including greenness, brownness, green odor, dried-leaf odor, and astringency were used to describe the characteristics of mulberry leaf tea in AL and PP. Control was initial mulberry leaf tea which packaged in AL bag under vacuum condition and kept at -20°C. The average scores of sensory attributes of mulberry leaf tea in AL and PP compared with those of the control are shown in Fig. 4.15.

Greenness and Brownness were used to describe color characteristics of the samples during storage. The average scores of greenness (6.93±0.65) and brownness (9.56±0.87) of the control were quite stable throughout the storage. During storage, color attributes (greenness and brownness) of mulberry leaf tea in AL were not significantly different. while the average greenness scores of the samples in PP significantly ($p \leq 0.05$) decreased from 7.22±0.57 to 5.06±0.76 at 18 months of storage. Whereas the average brownness scores of the samples in PP significantly ($p \leq 0.05$) increased from 9.36±0.63 to 11.53±0.49 at the end of storage. The greenness and brownness of PP packed samples became significantly ($p \leq 0.05$) difference from those of the control after 2 months of storage. The decrease of the greenness of mulberry leaf tea in PP may be due to the chlorophyll degradation associated with a_w level. Schmalko et al. (2005) reported that for $a_w > 0.52$, the chlorophyll degradation rate increased with a_w level, while below this value the influence of a_w on chlorophyll degradation was minor. An increase in brownness of mulberry leaf tea in PP may be caused by the Maillard reaction which is a chemical reaction between amino acids and reducing

sugars and give brown pigments (Martins et al., 2000). Phenolic oxidation degradation caused the green color of tea turns into brown (Kosińska & Andlauer, 2014).

Green odor and dried-leaf odor attributes were used to describe the changes in aroma characteristics of the samples during storage. The average scores of green odor and dried-leaf odor of the control throughout the storage time were 9.50±0.48 and 10.18±0.65, respectively. Similar to color attribute, the storage time did not affect the aroma characteristic of mulberry leaf tea in AL. The average scores of green odor and dried-leaf of AL throughout the storage time were 9.15±0.67 and 10.15±0.61, respectively. Whereas the decrease in green odor and dried-leaf odor took place in PP. After 18 months of storage, the average green odor scores of PP decreased from 9.23±0.76 to 7.69±0.65 while the average dried-leaf odor scores decreased from 10.22±0.89 to 8.66±0.76. This result agrees with the previous study which reported that the dried-leaf odor of mulberry leaf tea packaged in paper and sachet bags decreased with increasing storage time. However, this change was not found in the samples packaged in aluminum foil bag (Harnnurak and Riebroy, 2014). Due to its poor gas barrier package, mulberry leaf tea in PP may partially lose some volatiles during storage, resulting in the decrease of the perceived odor.

Astringency was used to describe taste characteristic of mulberry leaf tea. The results showed that packaging material and storage time did not affect the astringent taste of mulberry leaf tea. The average astringency scores of the control, the samples in AL, and the samples in PP were not significantly ($p \leq 0.05$) different throughout the storage time.

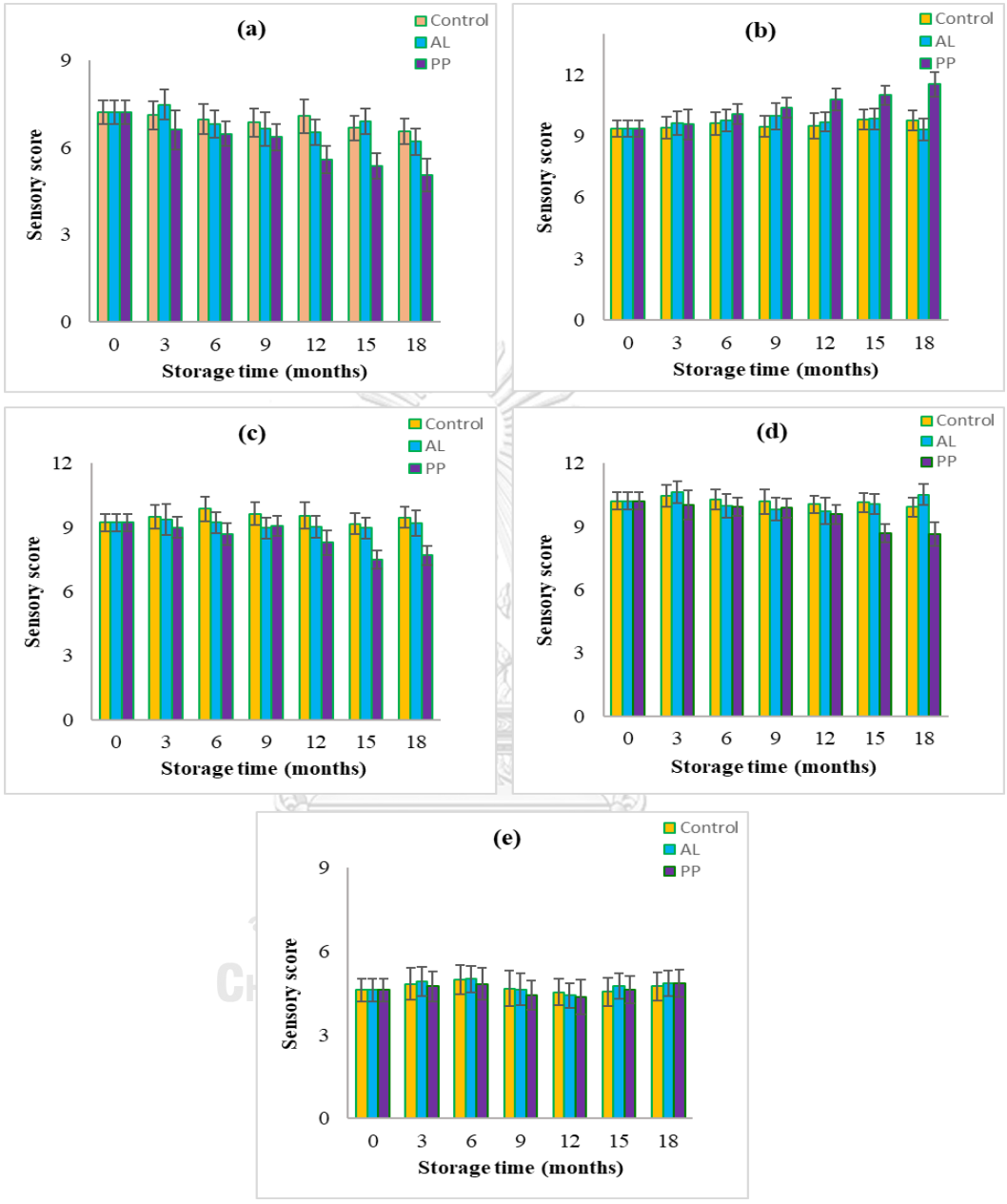


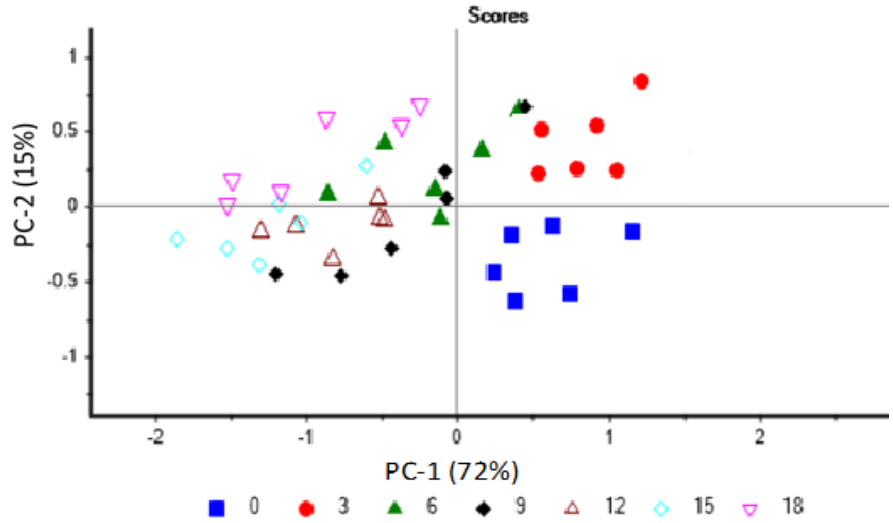
Fig. 4.15 Sensory attributes of mulberry leaf tea during storage at 30±1°C, 75% RH for 18 months; greenness (a), brownness (b), green odor (c), dried-leaf odor (e), and astringency (d).

4.2.5 Volatile profiles from e-nose

Fig. 4.16 shows PCA score plot and loading plot of mulberry leaf tea packaged in AL bag and stored at 30±1C for 18 months using the e-nose responses data set. The PC1 account for a major fraction (72%) of the total variance of the data. The PC2 axis is orthogonal to the first eigenvector and accounts for 15% of the variation. These two PCs together represent 87% of the total variance. The results showed that initial sample and sample stored at 3 months located on the positive region of PC1 overlapped partially with the samples stored at 6 months. The samples stored at 6, 9, 12, 15, and 18 months partially-overlapped located on the negative region of PC1. The result indicated that volatile compounds of the sample in AL were not significantly different during storage. PCA results are in accordance with the GC/MS and sensory evaluation. The results from GC/MS showed that volatile profiles of mulberry leaf tea in AL were not significantly different throughout the storage. The sensory test did not show significantly different in odor attributes as well.

The loading plot established the relative importance of each e-nose sensor, and the relationships between the sensors and the samples. Sensors negatively correlated to PC1 were W1C, W3C, and W5C whereas the other sensors positively correlated to PC1. From the loading plot, W1C, W3C, and W5C were associated with the samples stored form 6 months to 18 months of storage.

(a)



(b)

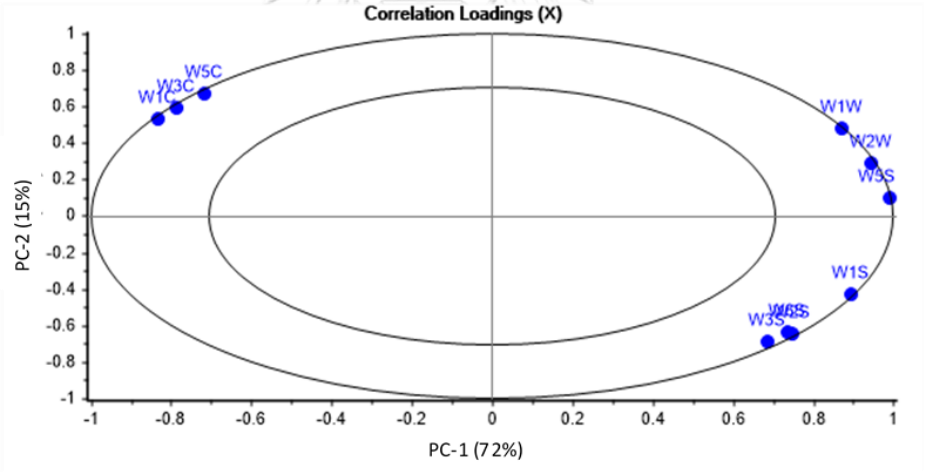


Fig. 4.16 PCA score plot (a) and loading plot (b) on PC1 and PC2 of mulberry leaf tea in AL bag and stored at 30±1°C, 75% RH for 18 months using e-nose responses data set.

Fig. 4.17 shows PCA score plot and loading plot of mulberry leaf tea packaged in PP bag and stored at 30±°C for 18 months using the e-nose responses data set. The result showed that the PCA result allowed for good discrimination among samples, with the PC1 and PC2 can explain 98% of the total variance. The samples stored at 0, 3, 6, 9, and 12 months located on the positive region of PC1 overlapped partially, while the samples stored at 15 and 18 months located on the negative region of PC1. From PCA results, mulberry leaf tea could divide into 2 groups; one group is the samples store at 0 to 12 months and the other is the samples stored at 15 to 18 months. The results of PCA are in accordance with the results of GC/MS and sensory evaluation. The results from GC/MS showed that 10 volatiles found in mulberry leaf tea in PP of which their intensities gradually increased during 12 months of storage and greatly increased after 12 months. The sensory results showed the average scores of green odor and dried-leaf odor of mulberry leaf tea in PP were significantly ($p \leq 0.05$) different when the sample stored after 12 months.

The loading plot showed that W1C, W3C, and W5C sensors negatively correlated to PC1 were associated with the mulberry leaf tea stored from 15 to 18 months. Whereas the other sensors positively correlated to PC1 were associated with the samples stored from 0 to 12 months

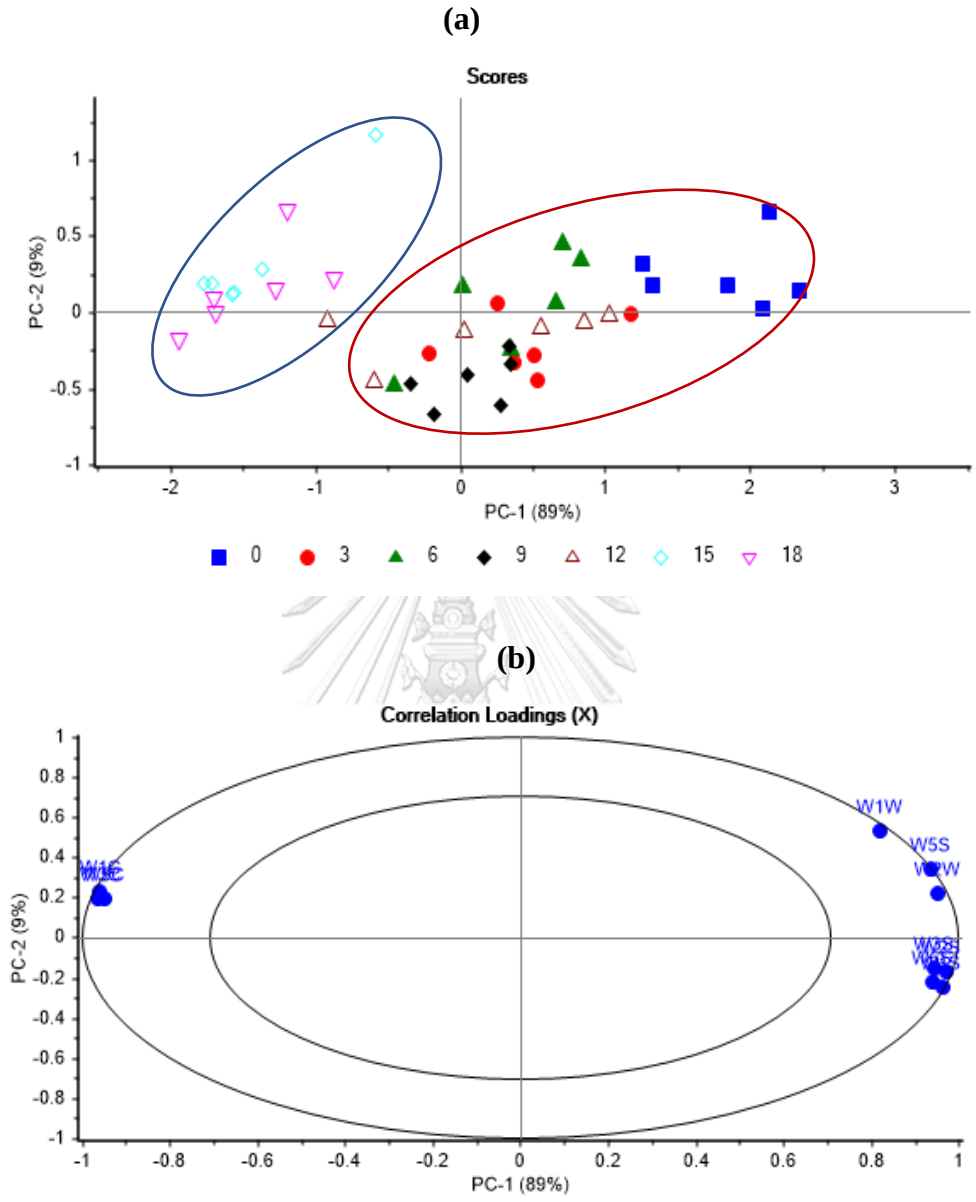


Fig. 4.17 PCA score plot (a) and loading plot (b) on PC1 and PC2 of mulberry leaf tea in PP bag and stored at 30±1°C, 75% RH for 18 months using e-nose responses data set.

The results of this part demonstrated that packaging material and storage time affected the quality attributes and phytochemicals of mulberry leaf tea. AL bag showed high-effective barrier properties with the quality attributes mulberry leaf tea were not significantly different throughout the storage. Due to a low effective barrier property of PP, the quality attributes and phytochemicals of mulberry leaf tea significantly ($p \leq 0.005$) difference during storage. The increase in brown color of the samples in PP was caused by phenolic oxidation and Maillard reaction. Moreover, oxidation of phenolic compounds resulted in the decrease in phytochemicals and antioxidant activity of the samples. The concentration of some volatiles significantly ($p \leq 0.05$) increase resulting from lipid oxidation. In order to protect the product quality during storage, AL bag is suggested as the packaging of mulberry leaf tea. E-nose is able clearly and rapidly discriminate quality of the samples as a result of different storage periods.

Recently, electronic noses are being widely used by some companies as a quality control instrument because it is rapid, non-destructive, and high sensitive method and its results highly correlate with human sensory panels for many applications (Loutfi et al., 2015; Wilson & Baietto, 2009). However, e-nose applications limit to their full potential includes loss of sensitivity in the presence of water vapor or high concentrations of a single component like alcohol, sensor drift and the inability to provide absolute calibration, necessity to do considerable method development work for each specific application, and lack of being able to obtain quantitative data for aroma differences (Harper, 2001; Zohora et al., 2013).

4.3 Rapid determination of phytochemicals and antioxidant activity of mulberry leaf tea using e-tongue

4.3.1 Phytochemicals and antioxidant activity

Storage time and temperature significantly ($p \leq 0.05$) influenced the changes in TPC and TFC of mulberry leaf tea. TPC and TFC were found in the range of 18.25-39.89 mg GAE/g dried sample and 11.09-29.95 mg rutin/g dried sample, respectively. The antioxidant activity of mulberry leaf tea was assessed by FRAP assays presented in the range of 5.66-13.84 mM trolox/100 g dried sample. The value obtained by DPPH assays, being expressed as % inhibition, were 22.72-53.96 %. The statistic values of TPC, TFC, FRAP, and DPPH of mulberry leaf tea were shown in table 4.5.

Table 4.5 Range, mean, and standard error (SE) of TPC, TFC, FRAP, and DPPH obtained by chemical analysis of mulberry leaf tea and used for e-tongue calibration and validation.

Parameters	Calibration set (n = 130)			Validation set (n = 57)		
	Range	Mean	SE	Range	Mean	SE
Total phenolic (mg GAE/g dried sample)	18.25-39.89	31.76	5.74	20.01-39.73	32.03	4.92
Total flavonoid (mg rutin/g dried sample)	11.09-29.95	21.66	5.29	12.37-29.64	21.75	4.72
FRAP (mM trolox/100 g dried sample)	5.66-13.84	10.34	2.21	6.18-13.34	10.40	1.96
DPPH (% inhibition)	22.72-53.96	40.31	8.03	26.57-52.57	40.54	6.83

4.3.2 E-tongue responses

E-tongue responses can be converted to taste value which is information on taste quality defined by multiplying the R_v or CPA_v by the conversion factor of each taste sensors suggested by (Kobayashi et al., 2010) who followed the Weber–Fechner law. As the sensors respond negatively to astringent and bitter substances, the conversion factor shown in Eq. (4) to Eq. (7) are negative number. The taste value obtained from e-tongue helps distinguish the difference in both taste characteristic and taste intensity between samples. Therefore, e-tongue can be used to evaluate the sensorial quality of food instead of sensory tests (Escuder-Gilabert and Peris, 2010).

Taste values of mulberry leaf tea obtained from e-tongue analysis included astringency, bitterness, aftertaste-A and aftertaste-B. Astringency value was related to the R_v of AE1 and CT0 sensors. Bitterness value was associated with the R_v of C00 and CT0 sensors. Aftertaste-A and aftertaste-B indicated the astringent and bitter aftertaste, respectively, remain in mouth after the expectoration (Yau and Huang, 2000). Aftertaste-A and aftertaste-B values associated with the CPA_v of AE1 and C00 sensors, respectively. Kobayashi et al. (2010) reported that AE1 sensor responded selectively to astringent substances such as gallic acid, caffeic acid, tannic acid, chlorogenic acid, and epigallocatechin gallate. The CPA_v of AE1 had high correlation with astringency score obtained from sensory test, indicating that AE1 sensor can be used to evaluate the astringency and aftertaste-A values. The C00 sensor responding to bitter substances such as tannic acid, quercetin, catechin, and theaflavin had been widely used to determine bitterness and aftertaste-B of food (Rudnitskaya et al., 2010; Wang et al., 2014). The R_v of CT0 sensor also used to calculate for the elimination of the astringency and bitterness values provided by potassium (K^+) salt which could

contribute to unpleasant side tastes such as bitter, chemical, and me tallic (Sinopoli and Lawless, 2012).

Fig. 4.18 shows the relationships between TPC of mulberry leaf tea and taste values obtained from e-tongue analysis. All taste values increased with total phenolic content. The astringency and aftertaste-A were high positive correlated (correlation coefficient (r) > 0.9) with TPC. The bitterness and aftertaste-B values showed positive correlated (r > 0.8) with TPC as well. The results indicated that astringent and bitter substances contributing to taste profile of mulberry leaf tea associated with the phenolic compounds in the mulberry leaf tea. The major phenolic compounds in mulberry leaf tea are flavonoids glycosides (phenolic compounds with sugar moieties) especially quercetin glycosides which have been perceived as astringent in food such as wine and tea (Hufnagel and Hofmann, 2008; Scharbert et al., 2004). In addition to being the astringent substances, flavonoids glycosides play a role in bitterness as well but they affect the perceived bitterness less than astringency (Stark and Hofmann, 2005).

Fig. 4.19 shows the relationship between taste values (astringency and bitterness) provided by e-tongue analysis and TFC, FRAP, and DPPH. The astringency and bitterness showed high positive correlated (r > 0.8) with TFC, FRAP, and DPPH. The high correlation (r > 0.8) between aftertaste values (aftertaste-A and aftertaste-B) and TFC, FRAP, and DPPH was observed as well (data not shown). Due to a high relationship between taste values and TPC, TFC, FRAP, and DPPH, e-tongue responses could be used to predict the concentration of phytochemicals and antioxidation activity of mulberry leaf tea. The results suggested that with e-tongue analysis not only the taste

of the mulberry tea in terms of astringency, bitterness, and aftertaste of astringency and bitterness but also the phytochemicals content and antioxidant activity can be obtained.

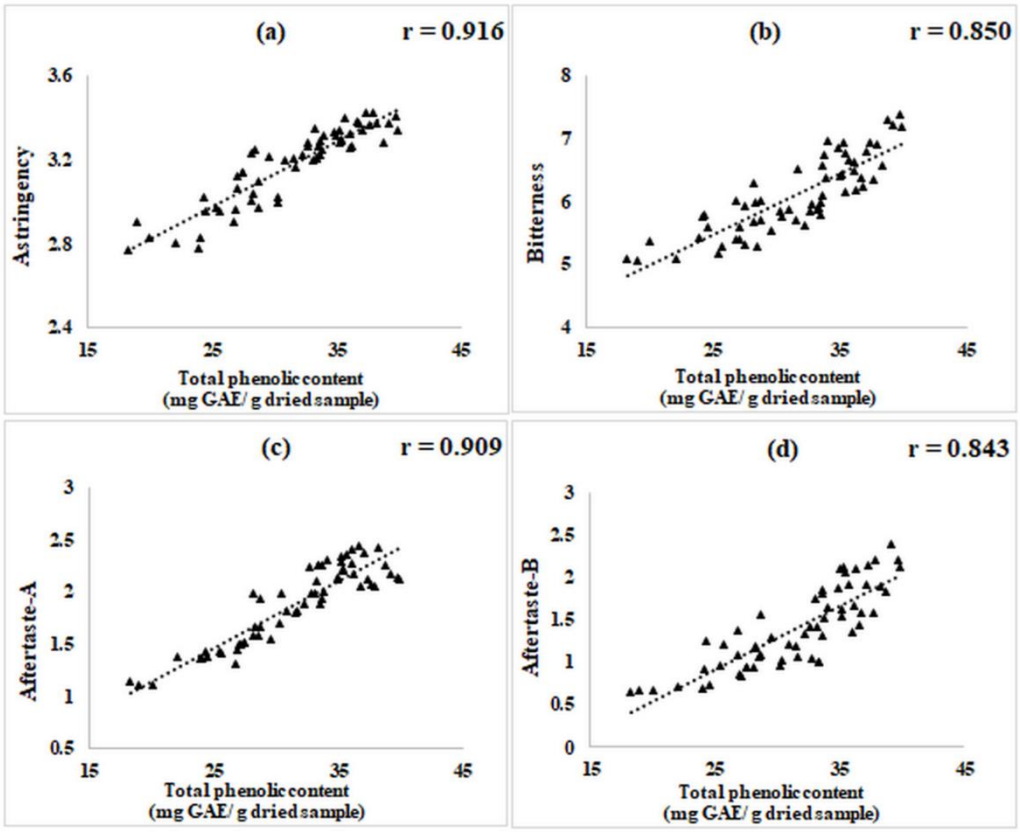


Fig. 4.18 The relationship between total phenolic content of mulberry leaf tea and taste values obtained from e-tongue analysis including astringency (a), bitterness (b), aftertaste-A(c), and aftertaste-B (d).

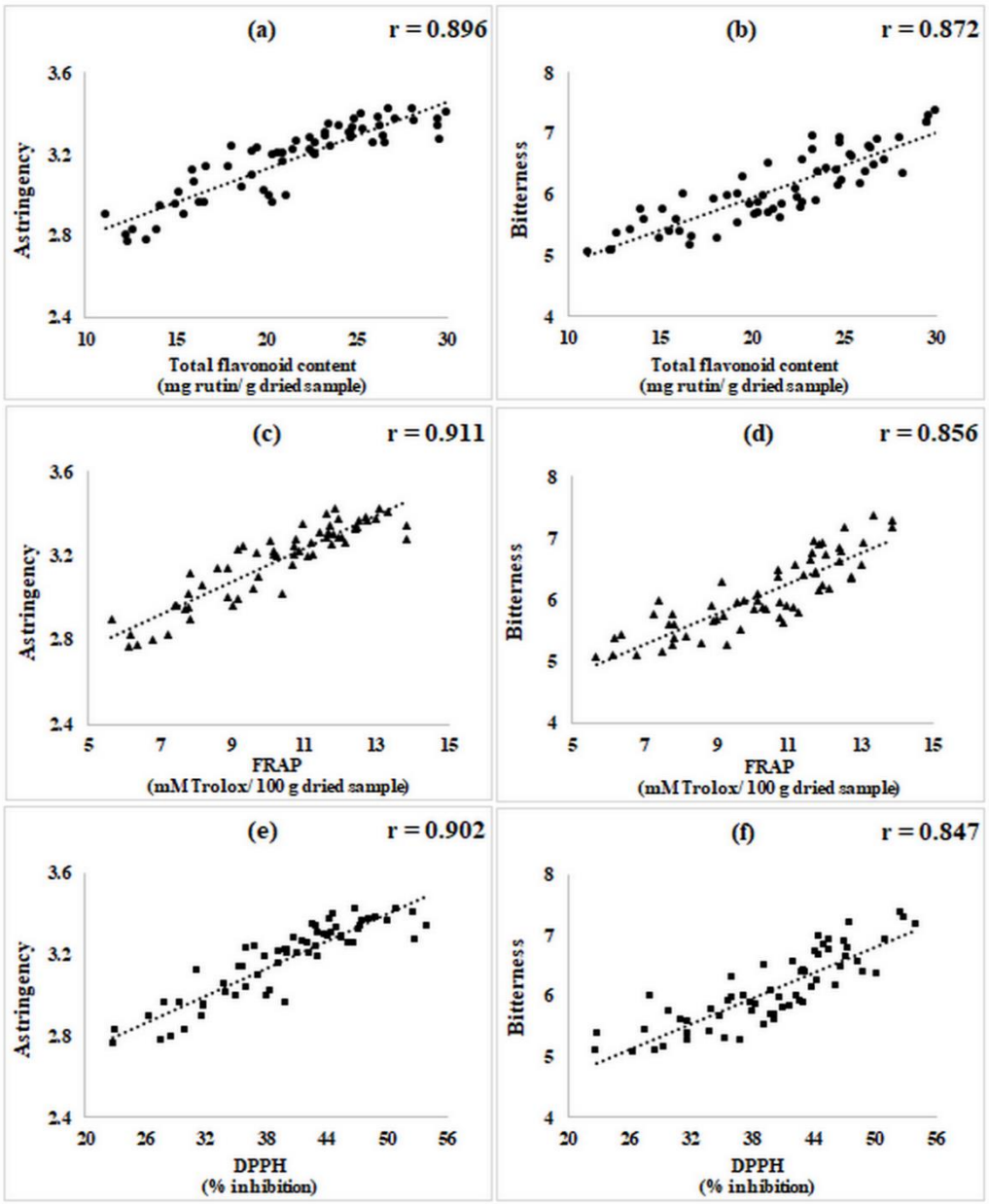


Fig. 4.19 The relationship between TFC and astringency (a), TFC and bitterness (b), FRAP and astringency (c), FRAP and bitterness (d), DPPH and astringency (e), and DPPH and bitterness (f).

4.3.3 Prediction of phenolic compounds

PLS regression was used to quantitatively correlate e-tongue response data to the phytochemicals and antioxidant activity data (TPC, TFC, FRAP, and DPPH) of mulberry leaf tea. For PLS regression analysis, taste values obtained from e-tongue analysis including astringency, bitterness, aftertaste-A, and aftertaste-B (predictor variables) were used for PLS modeling of TPC, TFC, FRAP, and DPPH values (response variables). The optimum number of factors of prediction models was selected where the root mean square error of cross-validation (RMSECV) was minimized. The obtained PLS models were then validated using an unknown validation set to avoid overfitting. Fig. 4.20 present the plots of the reference versus the predicted values of the TPC, TFC, FRAP, and DPPH using the validation data set. The PLS models gave a high value of the coefficient of determination ($r^2 > 0.9$) for the prediction models of TPC, TFC, FRAP, and DPPH. The results indicated that more than 90% of the total variation in each response y-variable (TPC, TFC, FRAP, and DPPH) were explained by the predictors (e-tongue response data). The parameters used to evaluate the performance of the PLS models include r^2, RMSEP, and SEP are shown in table 4.6. The RMSEP value of TPC, TFC, FRAP, and DPPH prediction models were 1.088 mg GAE/g dried sample, 0.927 mg rutin/g dried sample, 0.544 mM Trolox/100 g dried sample, and 1.66%, respectively. Considering the SEP value 1.087A mg GAE/g dried sample, 0.928 mg rutin/g dried sample, 0.534 mM Trolox/100 g dried sample, and 1.67% were obtain from of TPC, TFC, FRAP, and DPPH prediction models, respectively. Since high r^2 and low RMSEV and SEP were achieved, it was suggested that good predictions were obtained when using the PLS regression. The results obtained in this work demonstrated that e-tongue was successfully used as a quick and

chemical-free method for quantification of phenolic compounds and antioxidant activity of mulberry leaf tea.

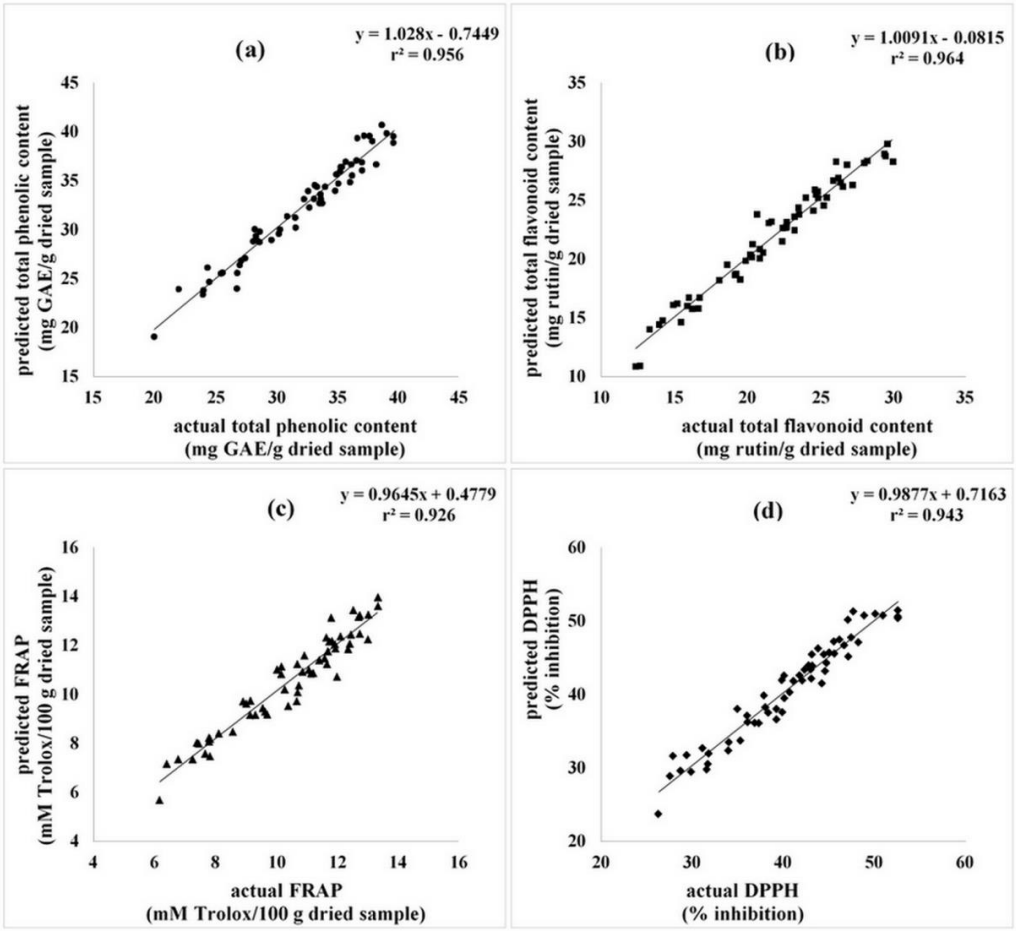


Fig. 4.20 The relationship between predicted values obtained from e-tongue analysis and the actual values; TPC (a), TFC (b), FRAP (c), and DPPH (d).

Table 4.6 Calibration and validation statistics for TPC, TFC, FRAP, and DPPH of mulberry leaf tea.

Parameters	Factor	Calibration		Validation		
		r^2	RMSECV	r^2	RMSEP	SEP
Total phenolic content (mg GAE/ g dried sample)	3	0.970	0.989	0.956	1.088	1.087
Total flavonoid content (mg rutin/ g dried sample)	3	0.960	1.053	0.964	0.927	0.928
FRAP (mM trolox/100 g dried sample)	3	0.935	0.544	0.926	0.544	0.534
DPPH (% inhibition)	3	0.949	1.800	0.943	1.661	1.671

CHAPTER V

CONCLUSIONS

The results obtained in this work demonstrated that cultivar significantly ($p \leq 0.05$) affected quality attributes and phytochemicals of mulberry leaf tea. BR and KP infusion were similar in color which was different from SK infusion. KP cultivar had the highest phytochemicals and antioxidant activity values, followed by BR and SK cultivars. Mulberry leaf tea of BR cultivar was classified from the others according to volatile profiles using e-nose.

The results showed that packaging material and storage time had an influence on quality attributes and phytochemicals of mulberry leaf tea. Mulberry leaf tea packed both in AL and PP bags showed significant ($p \leq 0.05$) decrease in phytochemicals and antioxidant activity with increasing storage time. Volatile profiles of the PP packed samples were changed during storage. As this reason, e-nose could classify the quality of mulberry leaf tea as a result of different storage time. At the end of the storage, the quality of mulberry leaf tea packed in AL bag exhibited greater stability than those in PP bag.

E-tongue is successfully used to predict phytochemicals and antioxidant activity of mulberry leaf tea during storage. The PLS models provided a good capability of predicting with high values of r^2 for TPC (r^2 = 0.956), TFC (r^2 = 0.964), FRAP (r^2 = 0.926), and DPPH (r^2 = 0.943) and gave low RMSEP and SEP values.

This research provides valuable information that can be a guideline for designing the appropriate package of mulberry leaf tea in order to maintain its quality during storage. Since e-nose and e-tongue has various advantages over conventional analytical

methods as it is non-destructive, fast and low cost, it could be used as a suitable tool for monitoring quality attributes and phytochemicals evolution during storage thus ensuring the quality and health benefit of mulberry leaf tea.

Recommendations for future research

Further studies could:

As this study aims to determine the effect of cultivar, packaging material, and storage time on the qualities of mulberry leaf tea, the shelf life of the samples was not assessed. However, the importance of shelf life of foods is to help consumers informed use of foods and make safe. Thus, the evaluation of shelf life of mulberry leaf tea by using e-nose and e-tongue should be investigated.

Moreover, electronic eye (e-eye) emulates the human vision. It is based on computer vision systems or colorimetric techniques which has been applied in many industries (e.g. food, pharmaceutical, and cosmetic) for quality control, process monitoring, and shelf life assessment. Consequently, the applicability of e-nose, e-tongue, and e-eye for determination of the quality attributes and phytochemicals of mulberry leaf tea is interesting.

REFERENCES

Allahvaisi, S. (2012). Polypropylene in the industry of food packaging. *In* "Polypropylene" (F. Dogan, ed.), pp. 1-22. InTech, Rijeka.

Allahvaisi, S., Pourmirza, A., and Safaralizade, M. (2010). The study on polymers permeability for foodstuffs packaging by some serious species of stored pest insects and phosphine gas. *Journal of Agricultural Science and Technology* **6**, 747-759.

Alpha MOS (2011). Electronic nose [Online]. Available From: https://www.alpha-mos.com/smell-analysis#electronic-nose [2017, July 12].

Apetrei, I. M., and Apetrei, C. (2013). Voltammetric e-tongue for the quantification of total polyphenol content in olive oils. *Food Research International* **54**, 2075-2082.

Ashokkumar, K., Selvaraj, K., and KM, S. D. (2013). Reverse phase-high performance liquid chromatography-diode array detector (RP-HPLC-DAD) analysis of flavonoids profile from curry leaf (Murraya koenigii. L). *Journal of Medicinal Plants Research* **7**, 3393-3399.

Bhattacharyya, N., Seth, S., Tudu, B., Tamuly, P., Jana, A., Ghosh, D., Bandyopadhyay, R., and Bhuyan, M. (2007). Monitoring of black tea fermentation process using electronic nose. *Journal of Food Engineering* **80**, 1146-1156.

Buratti, S., Casiraghi, A., Minghetti, P., and Giovanelli, G. (2013). The joint use of electronic nose and electronic tongue for the evaluation of the sensorial properties of green and black tea infusions as related to their chemical composition. *Food and Nutrition Sciences* **4**, 605-615.

Caridi, D., Trenerry, V. C., Rochfort, S., Duong, S., Laugher, D., and Jones, R. (2007). Profiling and quantifying quercetin glucosides in onion (*Allium cepa* L.) varieties using capillary zone electrophoresis and high performance liquid chromatography. *Food Chemistry* **105**, 691-699.

Charalambous, G. (1992). "Off-flavors in Foods and Beverages," Elsevier Science Publishers, Amsterdam.

Chen, L., Xin, X., Zhang, H., and Yuan, Q. (2013). Phytochemical properties and antioxidant capacities of commercial raspberry varieties. *Journal of Functional Foods* **5**, 508-515.

Chen, Q., Zhao, J., and Vittayapadung, S. (2008). Identification of the green tea grade level using electronic tongue and pattern recognition. *Food Research International* **41**, 500-504.

Chen, Y.-J., Kuo, P.-C., Yang, M.-L., Li, F.-Y., and Tzen, J. T. (2013b). Effects of baking and aging on the changes of phenolic and volatile compounds in the preparation of old Tieguanyin oolong teas. *Food Research International* **53**, 732-743.

Cui, S., Wang, J., Yang, L., Wu, J., and Wang, X. (2015). Qualitative and quantitative analysis on aroma characteristics of ginseng at different ages using E-nose and GC–MS combined with chemometrics. *Journal of Pharmaceutical and Biomedical Analysis* **102**, 64-77.

Daskalaki, D., Kefi, G., Kotsiou, K., and Tasioula-Margari, M. (2009). Evaluation of phenolic compounds degradation in virgin olive oil during storage and heating. *Journal Of Food & Nutrition Research* **48**, 31-41.

Davidson, P. M., Sofos, J. N., and Branen, A. L. (2005). "Antimicrobials in food," 3rd/Ed. CRC press, Florida.

Dengliang, W. (1998). Relations between Variation of Polyphenols and Organoleptic Quality of Green Tea during Storage. *Journal of Tea Science* **1**, 1-9.

Escuder-Gilabert, L., and Peris, M. (2010). Highlights in recent applications of electronic tongues in food analysis. *Analytica Chimica Acta* **665**, 15-25.

Friedman, M., Levin, C. E., Choi, S. H., Lee, S. U., and Kozukue, N. (2009). Changes in the Composition of Raw Tea Leaves from the Korean Yabukida Plant during High-Temperature Processing to Pan-Fried Kamairi-Cha Green Tea. *Journal of Food Science* **74**, 406-412.

Geneham Pharmaceutical Co., L. (2015). The Mulberry Leaf Extracts Demands Raised in the International Market. [Online].

Ghosh, A., Tudu, B., Tamuly, P., Bhattacharyya, N., and Bandyopadhyay, R. (2012). Prediction of theaflavin and thearubigin content in black tea using a voltammetric electronic tongue. *Chemometrics and Intelligent Laboratory Systems* **116**, 57-66.

Gomez, A. H., Wang, J., Hu, G., and Pereira, A. G. (2008). Monitoring storage shelf life of tomato using electronic nose technique. *Journal of Food Engineering* **85**, 625-631.

González-Calabuig, A., and del Valle, M. (2018). Voltammetric electronic tongue to identify Brett character in wines. On-site quantification of its ethylphenol metabolites. *Talanta* **179**, 70-74.

Harnnurak, A., and Riebroy, S. (2014). Sensory characteristics, color and 1-deoxynojirimycin of mulberry leaf tea during storage in different packaging. *In* "the Summer Program in Sensory Evaluation, SPISE2014", Ho Chi Minh City, Vietnam.

Hufnagel, J. C., and Hofmann, T. (2008). Orosensory-directed identification of astringent mouthfeel and bitter-tasting compounds in red wine. *Journal of Agricultural and Food Chemistry* **56**, 1376-1386.

Hunyadi, A., Martins, A., Hsieh, T.-J., Seres, A., and Zupkó, I. (2012). Chlorogenic acid and rutin play a major role in the in vivo anti-diabetic activity of Morus alba leaf extract on type II diabetic rats. *PLoS One* **7**, 506-519.

Intelligent Sensor Technology Co. Ltd. (2014). Electronic tongue [Online].

Jin, J., Deng, S., Ying, X., Ye, X., Lu, T., and Hui, G. (2015). Study of herbal tea beverage discrimination method using electronic nose. *Journal of Food Measurement and Characterization* **9**, 52-60.

Kaack, K., and Christensen, L. P. (2008). Effect of packing materials and storage time on volatile compounds in tea processed from flowers of black elder (*Sambucus nigra* L.). *European Food Research and Technology* **227**, 1259-1273.

Kaisoon, O., Siriamornpun, S., Weerapreeyakul, N., and Meeso, N. (2011). Phenolic compounds and antioxidant activities of edible flowers from Thailand. *Journal of functional foods* **3**, 88-99.

Katsube, T., Tsurunaga, Y., Sugiyama, M., Furuno, T., and Yamasaki, Y. (2009). Effect of air-drying temperature on antioxidant capacity and stability of

polyphenolic compounds in mulberry (Morus alba L.) leaves. *Food Chemistry* **113**, 964-969.

Kim, H. B., Kim, J. B., Ju, W. T., Kim, S. L., and Lim, J. D. (2017). Development of powder with increased rutin content from mulberry leaves for the application of food materials. *International Journal of Industrial Entomology* **35**, 77-82.

Kim, J. W., Kim, S. U., Lee, H. S., Kim, I., Ahn, M. Y., and Ryu, K. S. (2003). Determination of 1-deoxynojirimycin in Morus alba L. leaves by derivatization with 9-fluorenylmethyl chloroformate followed by reversed-phase high-performance liquid chromatography. *Journal of Chromatography A* **1002**, 93-99.

Kobayashi, Y., Habara, M., Ikezazki, H., Chen, R., Naito, Y., and Toko, K. (2010). Advanced taste sensors based on artificial lipids with global selectivity to basic taste qualities and high correlation to sensory scores. *Sensors* **10**, 3411-3443.

Lee, J., and Chambers, D. H. (2010). Flavors of green tea change little during storage. *Journal of Sensory Studies* **25**, 512-520.

Lee, S. H., and Blair, I. A. (2000). Characterization of 4-oxo-2-nonenal as a novel product of lipid peroxidation. *Chemical Research in Toxicology* **13**, 698-702.

Lee, W. J., and Choi, S. W. (2012). Quantitative changes of polyphenolic compounds in mulberry (*Morus alba* L.) leaves in relation to varieties, harvest period, and heat processing. *Preventive Nutrition and Food Science* **17**, 280-285.

Li, X., Xie, C., He, Y., Qiu, Z., and Zhang, Y. (2012). Characterizing the moisture content of tea with diffuse reflectance spectroscopy using wavelet transform and multivariate analysis. *Sensors* **12**, 9847-9861.

Liang, Y., Lu, J., Zhang, L., Wu, S., and Wu, Y. (2003). Estimation of black tea quality by analysis of chemical composition and colour difference of tea infusions. *Food Chemistry* **80**, 283-290.

Liu, X., Liu, F., Huang, X.-z., and Yang, J. (2012). Analysis of Aroma Compounds in Mulberry Leaf Green Tea by SPME-GC-MS. *Food Science* **13**, 238-242.

Maarse, H. (2017). "Volatile compounds in foods and beverages," MARCEL DEKKER, New York.

Marsh, K., and Bugusu, B. (2007). Food packaging—roles, materials, and environmental issues. *Journal of Food Science* **72**, 39-55.

Martins, S. I., Jongen, W. M., and Van Boekel, M. A. (2000). A review of Maillard reaction in food and implications to kinetic modelling. *Trends in Food Science & Technology* **11**, 364-373.

Meilgaard, M. C., Carr, B. T., and Civille, G. V. (1999). "Sensory evaluation techniques," CRC press.

Miller, J., and Miller, J. C. (2018). "Statistics and chemometrics for analytical chemistry," Pearson Education Limited, London.

Mirasoli, M., Gotti, R., Di Fusco, M., Leoni, A., Colliva, C., and Roda, A. (2014). Electronic nose and chiral-capillary electrophoresis in evaluation of the quality changes in commercial green tea leaves during a long-term storage. *Talanta* **129**, 32-38.

Naczk, M., and Shahidi, F. (2004). Extraction and analysis of phenolics in food. *Journal of Chromatography A* **1054**, 95-111.

Naithani, V., Nair, S., and Kakkar, P. (2006). Decline in antioxidant capacity of Indian herbal teas during storage and its relation to phenolic content. *Food Research International* **39**, 176-181.

Näsström, T., Fagerqvist, T., Barbu, M., Karlsson, M., Nikolajeff, F., Kasrayan, A., Ekberg, M., Lannfelt, L., Ingelsson, M., and Bergström, J. (2011). The lipid peroxidation products 4-oxo-2-nonenal and 4-hydroxy-2-nonenal promote the formation of α-synuclein oligomers with distinct biochemical, morphological, and functional properties. *Free Radical Biology and Medicine* **50**, 428-437.

National Institute of Standards and Technology (NIST) (2011). The NIST/EPA/NIH Mass Spectral Database. NIST, Washington DC.

Özgen, M., Serçe, S., and Kaya, C. (2009). Phytochemical and antioxidant properties of anthocyanin-rich Morus nigra and Morus rubra fruits. *Scientia Horticulturae* **119**, 275-279.

Phoonan, W., Deowanish, S., and Chavasiri, W. (2014). Food attractant from mulberry leaf tea and its main volatile compounds for the biocontrol of Lasioderma serricorne F.(Coleoptera: Anobiidae). *Journal of stored products research* **59**, 299-305.

Piringer, O. G., and Baner, A. L. (2008). "Plastic packaging materials for food: barrier function, mass transport, quality assurance, and legislation," Wiley New Jersey.

Plazas, M., Andújar, I., Vilanova, S., Hurtado, M., Gramazio, P., Herraiz, F. J., and Prohens, J. (2013). Breeding for chlorogenic acid content in eggplant: interest and prospects. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* **41**, 26.

Podrażka, M., Bączyńska, E., Kundys, M., Jeleń, P. S., and Witkowska Nery, E. (2017). Electronic Tongue—A Tool for All Tastes. *Biosensors* **8**, 3.

Polshin, E., Rudnitskaya, A., Kirsanov, D., Legin, A., Saison, D., Delvaux, F., Delvaux, F. R., Nicolaï, B. M., and Lammertyn, J. (2010). Electronic tongue as a screening tool for rapid analysis of beer. *Talanta* **81**, 88-94.

Pothinuch, P., and Tongchitpakdee, S. (2011). Melatonin contents in mulberry (Morus spp.) leaves: effects of sample preparation, cultivar, leaf age and tea processing. *Food chemistry* **128**, 415-419.

Qin, Z., Pang, X., Chen, D., Cheng, H., Hu, X., and Wu, J. (2013). Evaluation of Chinese tea by the electronic nose and gas chromatography–mass spectrometry: Correlation with sensory properties and classification according to grade level. *Food research international* **53**, 864-874.

Rudnitskaya, A., Rocha, S., Legin, A., Pereira, V., and Marques, J. C. (2010). Evaluation of the feasibility of the electronic tongue as a rapid analytical tool for wine age prediction and quantification of the organic acids and phenolic compounds. The case-study of Madeira wine. *Analytica Chimica Acta* **662**, 82-89.

Samransakul, R. (2001). Total polyphenol content and antioxidant activity of mulberry beaves and mulberry tea from some sources in Thailand. Master's thesis, Chulalongkorn university, Faculty of Phrmaceutical Sciences.

Sánchez-Salcedo, E. M., Mena, P., García-Viguera, C., Hernández, F., and Martínez, J. J. (2015). (Poly) phenolic compounds and antioxidant activity of white (*Morus alba*) and black (*Morus nigra*) mulberry leaves: Their potential for

new products rich in phytochemicals. *Journal of Functional Foods* **18**, 1039-1046.

Scharbert, S., Holzmann, N., and Hofmann, T. (2004). Identification of the astringent taste compounds in black tea infusions by combining instrumental analysis and human bioresponse. *Journal of agricultural and food chemistry* **52**, 3498-3508.

Schmalko, M. E., Scipioni, P. G., and Ferreyra, D. J. (2005). Effect of water activity and temperature in color and chlorophylls changes in yerba mate leaves. *International Journal of Food Properties* **8**, 313-322.

Shekhar, T. C., and Anju, G. (2014). Antioxidant activity by DPPH radical scavenging method of Ageratum conyzoides Linn. leaves. *American Journal of Ethnomedicine* **1**, 244-9.

Shifang Packaging Material Co., L. (2015). Products [Online]. Available From: http://th.simplefine.com.cn/aluminum-foil-bags/ [2017, July 12].

Shimoda, M., Shigematsu, H., Shiratsuchi, H., and Osajima, Y. (1995). Comparison of volatile compounds among different grades of green tea and their relations to odor attributes. *Journal of Agricultural and Food Chemistry* **43**, 1621-1625.

Shoaib Zafar, M., Muhammad, F., Javed, I., Akhtar, M., Khaliq, T., Aslam, B., Waheed, A., Yasmin, R., and Zafar, H. (2013). White Mulberry (Morus alba): A Brief Phytochemical and Pharmacological Evaluations Account. *International Journal of Agriculture & Biology* **15**.

Sinopoli, D. A., and Lawless, H. T. (2012). Taste Properties of Potassium Chloride Alone and in Mixtures with Sodium Chloride Using a Check-All-That-Apply Method. *Journal of food science* **77**.

Siripatrawan, U., and Harte, B. R. (2015). Data visualization of *Salmonella* Typhimurium contamination in packaged fresh alfalfa sprouts using a Kohonen network. *Talanta* **136**, 128-135.

Siripatrawan, U., Pongamphai, S., Douglas, S., and Harte, B. R. (2008). Antioxidant polyphenols in packaged mulberry (morus alba l.) leaves during storage. *In*, "Proceedings of the Society for Industrial Microbiology (SIM) Annual Meeting and Exhibition 2008", California, USA.

Siripatrawan, U., Vitchayakitti, W., and Sanguandeekul, R. (2013). Antioxidant and antimicrobial properties of Thai propolis extracted using ethanol aqueous solution. *International Journal of Food Science & Technology* **48**, 22-27.

Smit, B. A., Engels, W. J., and Smit, G. (2009). Branched chain aldehydes: production and breakdown pathways and relevance for flavour in foods. *Applied Microbiology and Biotechnology* **81**, 987-999.

Sokolová, R., Ramešová, Š., Degano, I., Hromadová, M., Gál, M., and Žabka, J. (2012). The oxidation of natural flavonoid quercetin. *Chemical Communications* **48**, 3433-3435.

Srivastava, S., Kapoor, R., Thathola, A., and Srivastava, R. P. (2006). Nutritional quality of leaves of some genotypes of mulberry (*Morus alba*). *International Journal of Food Sciences and Nutrition* **57**, 305-313.

Stark, T., and Hofmann, T. (2005). Isolation, structure determination, synthesis, and sensory activity of N-phenylpropenoyl-L-amino acids from cocoa (*Theobroma cacao*). *Journal of Agricultural and Food Chemistry* **53**, 5419-5428.

Sulaiman, C., and Balachandran, I. (2012). Total phenolics and total flavonoids in selected Indian medicinal plants. *Indian Journal of Pharmaceutical Sciences* **74**, 258.

Sun, L., Meng, L., Chen, J., Ma, J., Hu, R., and Jia, D. (2001). Determination of rutin and quercetin in mulberry leaves by high performance capillary electrophoresis. *Chinese Journal of Chromatography* **19**, 395-397.

Sun, X., Yamasaki, M., Katsube, T., and Shiwaku, K. (2015). Effects of quercetin derivatives from mulberry leaves: Improved gene expression related hepatic lipid and glucose metabolism in short-term high-fat fed mice. *Nutrition Research and Practice* **9**, 137-143.

Tahara, Y., and Toko, K. (2013). Electronic tongues–a review. *IEEE Sensors Journal* **13**, 3001-3011.

Tanaka, K., Uda, Y., Ono, Y., Nakagawa, T., Suwa, M., Yamaoka, R., and Touhara, K. (2009). Highly selective tuning of a silkworm olfactory receptor to a key mulberry leaf volatile. *Current Biology* **19**, 881-890.

Thabti, I., Elfalleh, W., Hannachi, H., Ferchichi, A., and Campos, M. D. G. (2012). Identification and quantification of phenolic acids and flavonol glycosides in Tunisian Morus species by HPLC-DAD and HPLC–MS. *Journal of Functional Foods* **4**, 367-374.

Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L., and Byrne, D. H. (2006). Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. *Journal of Food Composition and Analysis* **19**, 669-675.

The Centre for the Promotion of Imports from developing countries (CBI) (2016). Exporting mulberry fruits and leaves to Europe. [Online].

The Queen Sirikit Department of Sericulture (2009). varieties [Online]. Available From: https://www.qsds.go.th/osrd_new/inside_page.php?pageid=6 [2017, June 24].

The Queen Sirikit Department of Sericulture (2010). Mulberry leaf tea for commercial production [Brochure]. Bangkok.

Troller, J. A. (1980). Influence of water activity on microorganisms in foods. *Food Technology* **34**, 76-82.

Troller, J. A., and Christian, J. H. B. (2012). "Water activity and food," ACADEMIC PRESS, New York.

Turumtay, E. A., İslamoğlu, F., Çavuş, D., Şahin, H., Turumtay, H., and Vanholme, B. (2014). Correlation between phenolic compounds and antioxidant activity of Anzer tea (Thymus praecox Opiz subsp. caucasicus var. caucasicus). *Industrial Crops and Products* **52**, 687-694.

Venkatesh Kumar, R., and Chauhan, S. (2008). Mulberry: life enhancer. *Journal of Medicinal Plants Research* **2**, 271-278.

Vlasov, Y., Legin, A., Rudnitskaya, A., Di Natale, C., and D'amico, A. (2005). Nonspecific sensor arrays ("electronic tongue") for chemical analysis of liquids (IUPAC Technical Report). *Pure and Applied Chemistry* **77**, 1965-1983.

Vogrinčič, M., Timoracka, M., Melichacova, S., Vollmannova, A., and Kreft, I. (2010). Degradation of rutin and polyphenols during the preparation of tartary buckwheat bread. *Journal of Agricultural and Food Chemistry* **58**, 4883-4887.

Wang, K., Chen, Q., Lin, Y., Li, S., Lin, H., Huang, J., and Zhonghua, L. (2014). Comparison of phenolic compounds and taste of Chinese black tea. *Food Science and Technology Research* **20**, 639-646.

Wang, S. Y., and Lin, H.-S. (2000). Antioxidant activity in fruits and leaves of blackberry, raspberry, and strawberry varies with cultivar and developmental stage. *Journal of Agricultural and Food Chemistry* **48**, 140-146.

Wold, S., Sjöström, M., and Eriksson, L. (2001). PLS-regression: a basic tool of chemometrics. *Chemometrics and intelligent laboratory systems* **58**, 109-130.

Yau, N., and Huang, Y. (2000). The effect of membrane-processed water on sensory properties of Oolong tea drinks. *Food Quality and Preference* **11**, 331-339.

Zakaria, N. Z. I., Masnan, M. J., Zakaria, A., and Shakaff, A. Y. M. (2014). A bio-inspired herbal tea flavour assessment technique. *Sensors* **14**, 12233-12255.

Zheng, X.-Q., Li, Q.-S., Xiang, L.-P., and Liang, Y.-R. (2016). Recent advances in volatiles of teas. *Molecules* **21**, 338.

Zou, Y., Liao, S., Shen, W., Liu, F., Tang, C., Chen, C.-Y. O., and Sun, Y. (2012). Phenolics and antioxidant activity of mulberry leaves depend on cultivar and harvest month in Southern China. *International Journal of Molecular Sciences* **13**, 16544-16553.

APPENDIX

APPENDIX A

Standard curves

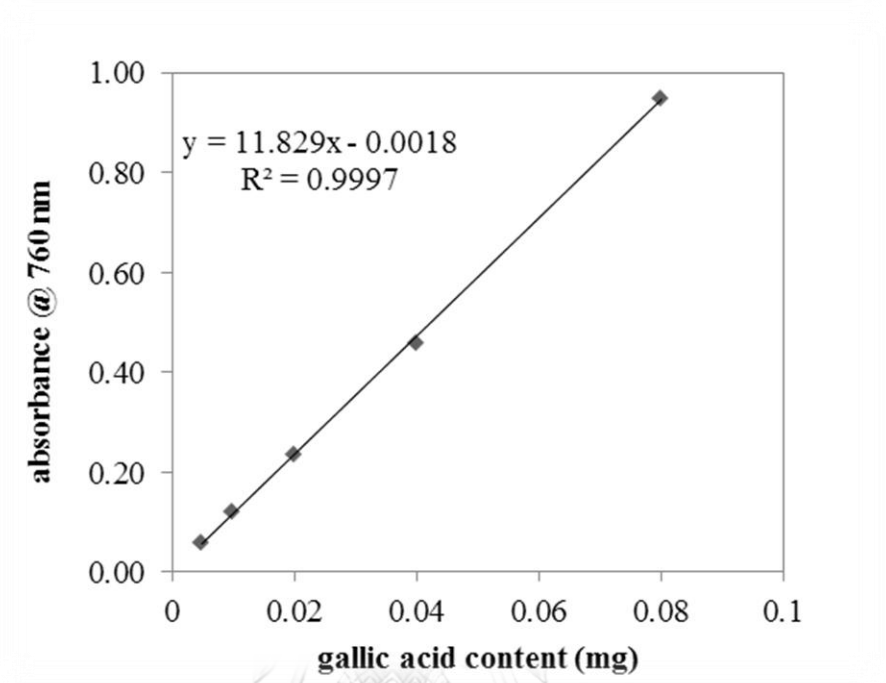


Fig. A.1 Gallic acid standard curves for total phenolic compounds**.**

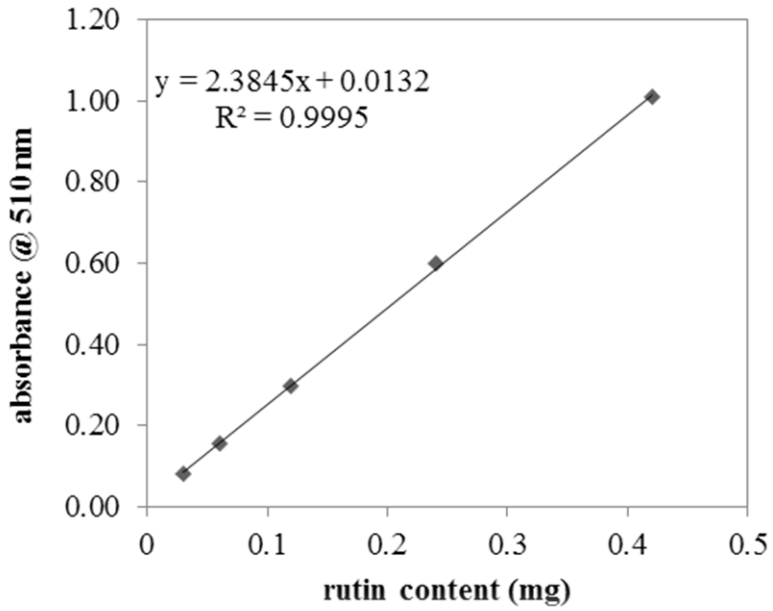


Fig. A.2 Rutin content standard curves for total flavonoid**.**

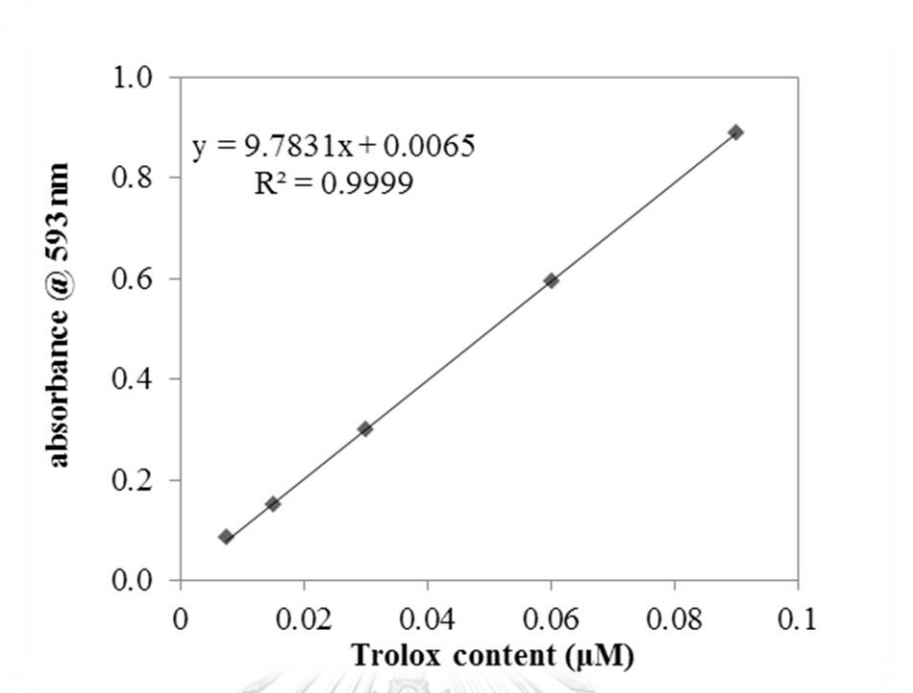


Fig. A.3 Trolox content standard curves for Ferric reducing antioxidant power (FRAP)**.**

APPENDIX B

HPLC chromatogram

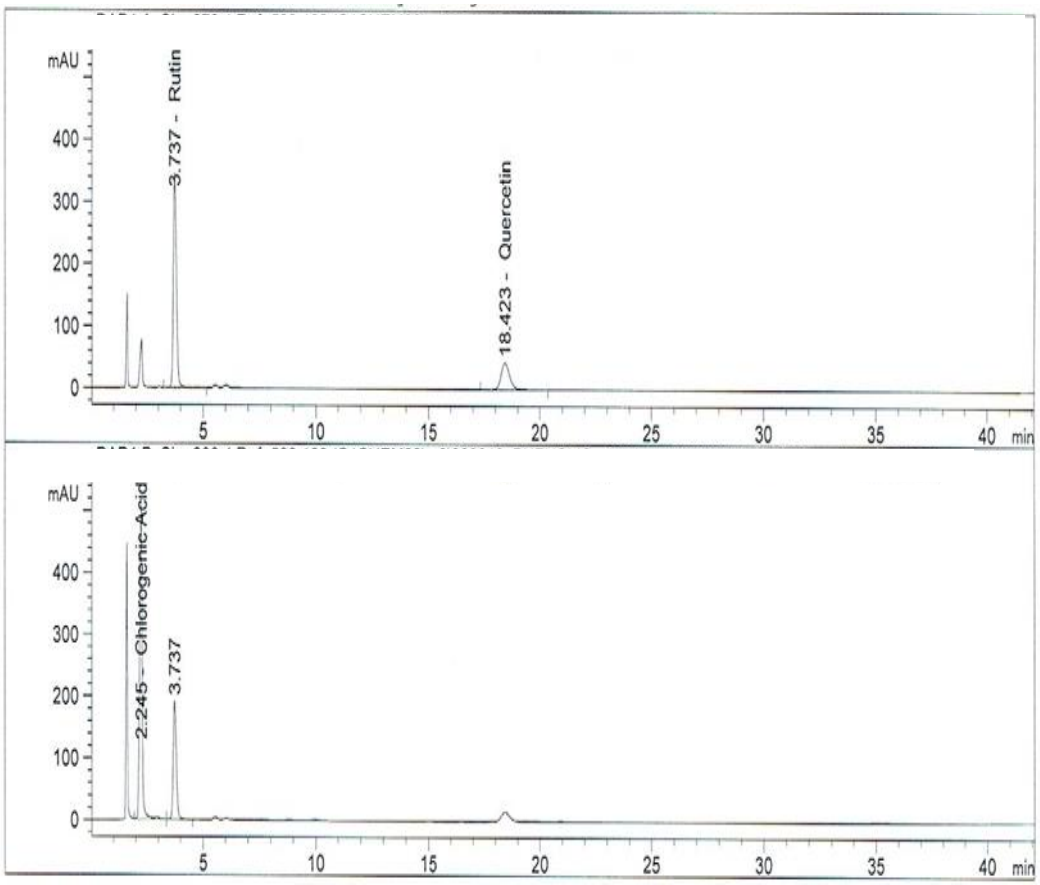
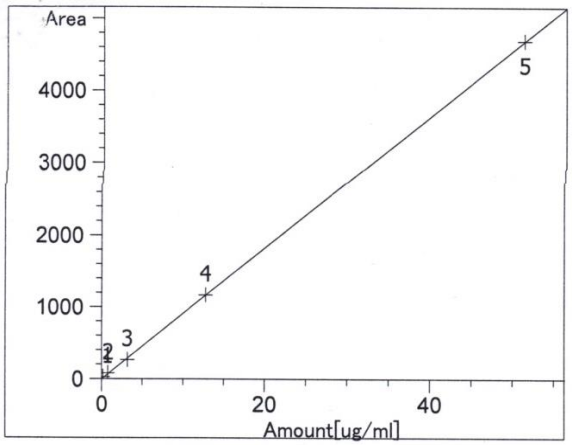
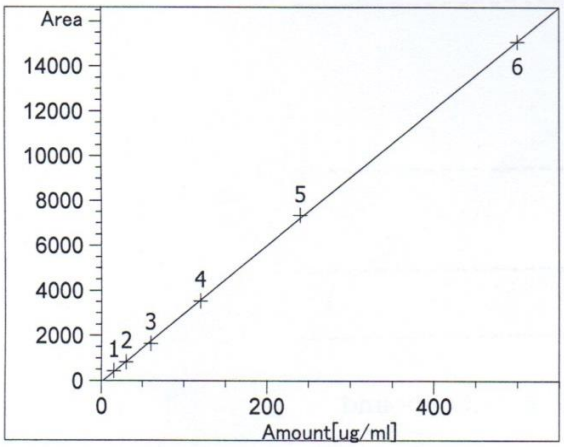


Fig. B.1 HPLC chromatogram of phenolic compounds including chlorogenic acid, rutin, and quercetin.



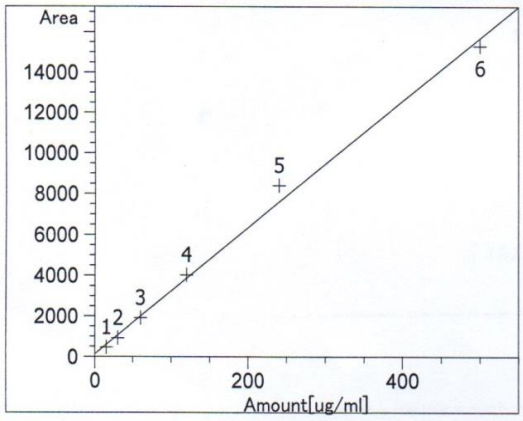
Quercetin at exp. RT: 18.661
DAD1 A, Sig=370,4 Ref=500,100
Correlation: 0.99998
Residual Std. Dev.: 14.07317
Formula: y = mx + b
m: 91.68023
b: -5.16912
x: Amount[ug/ml]
y: Area

Fig. B.2 Calibration curves of chlorogenic acid**.**



Rutin at exp. RT: 3.820
DAD1 A, Sig=370,4 Ref=500,100
Correlation: 0.99989
Residual Std. Dev.: 87.73389
Formula: y = mx + b
m: 30.36732
b: -65.55105
x: Amount[ug/ml]
y: Area

Fig. B.3 Calibration curves of rutin**.**



Chlorogenic Acid at exp. RT: 2.254
DAD1 B, Sig=280,4 Ref=500,100
Correlation: 0.99767
Residual Std. Dev.: 419.14019
Formula: y = mx + b
m: 31.14566
b: 139.90074
x: Amount[ug/ml]
y: Area

Fig. B.4 Calibration curves of quercetin**.**

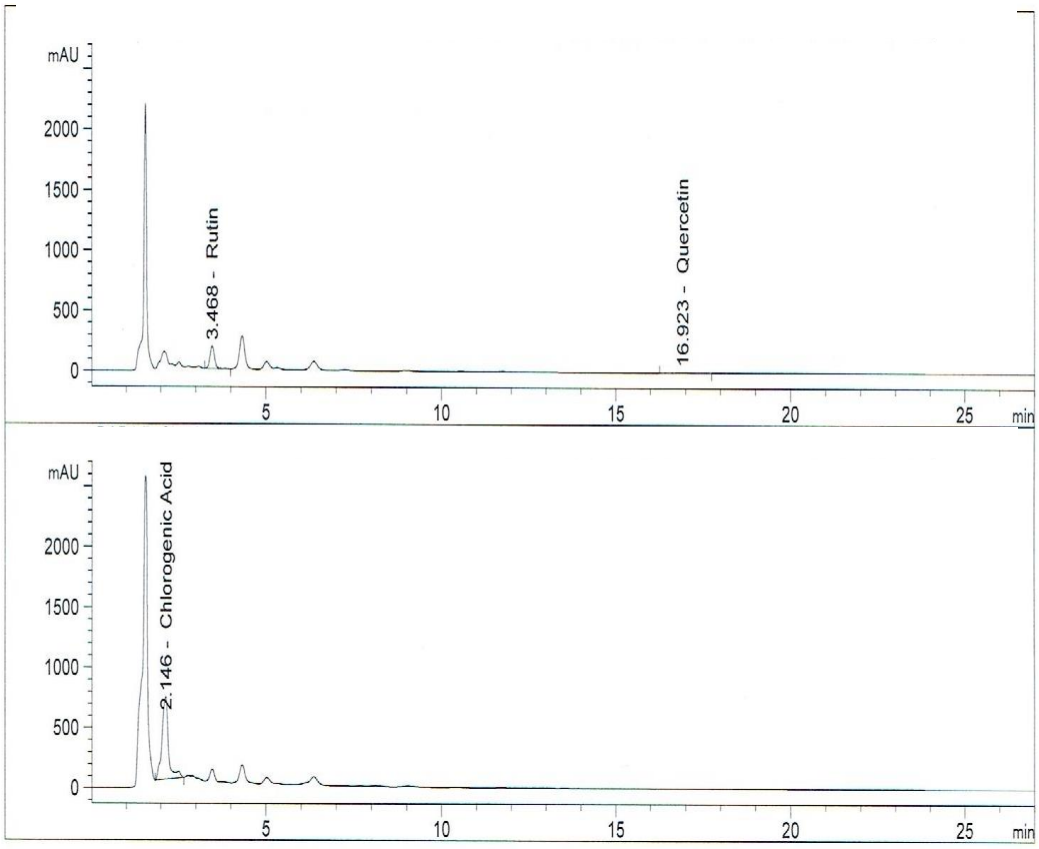


Fig. B.5 HPLC chromatogram of the initial mulberry leaf tea**.**

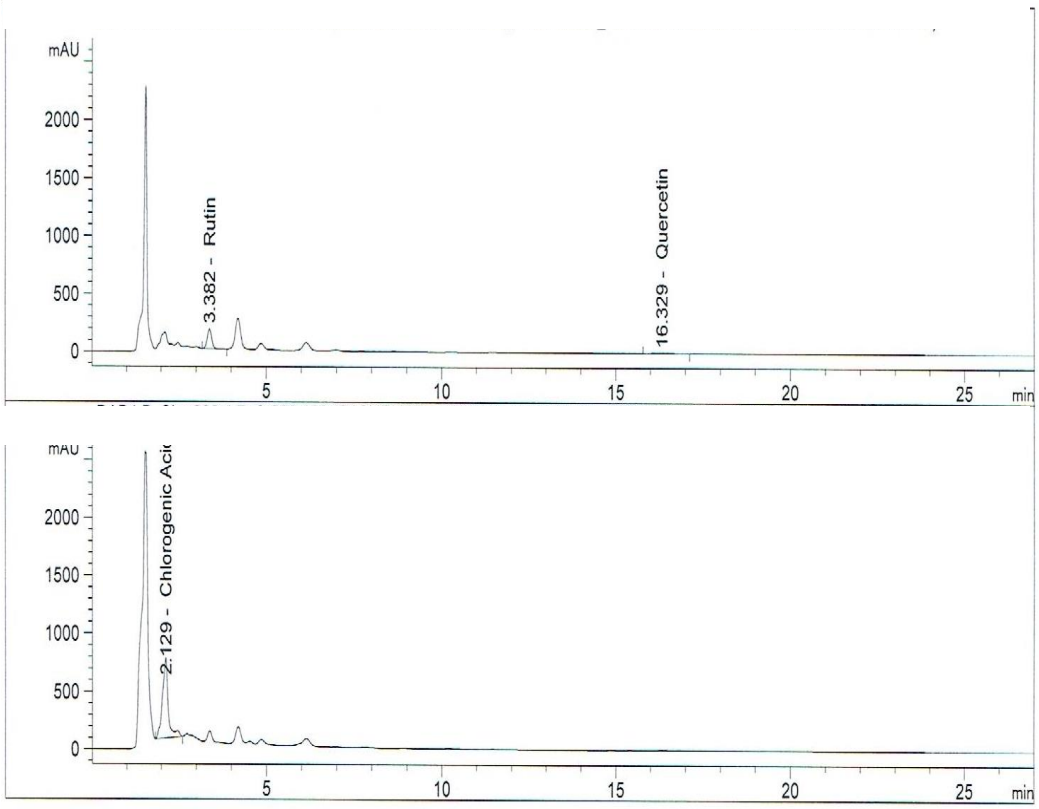


Fig. B.6 HPLC chromatogram of leaf tea package in AL bag stored at 30±1 °C (75%RH) for 18 months.

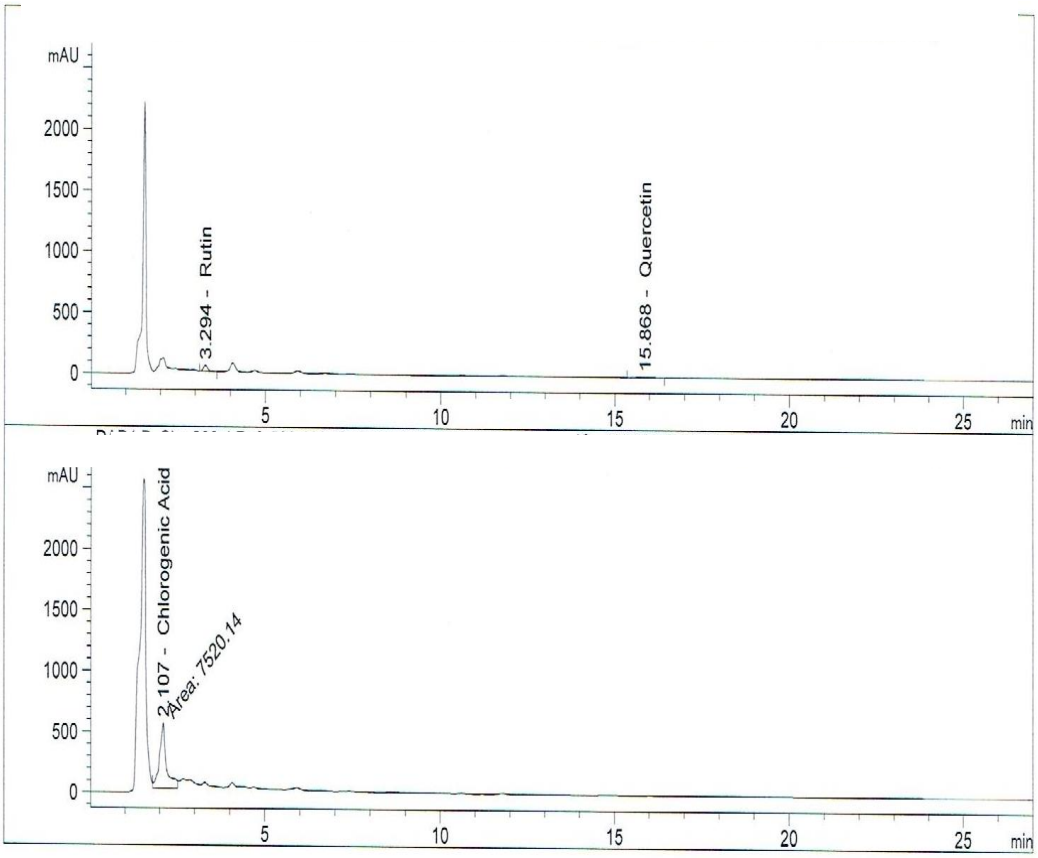


Fig. B.7 HPLC chromatogram of leaf tea package in PP bag stored at 30±1°, (75%RH) for 18 months.

APPENDIX C

Sensory evaluation

Table C.1 Definitions and references for sensory attributes

Sensory attribute	Definition	Reference
Greenness	Color associated with greenish yellow	Munsell book
Brownness	Color associated with brownish yellow	Munsell book
Dried-leaf odor	Aromatics associated with dried leaves	- Scale = 10; Dried Oolong tea from Green tea Co. Ltd., Thailand
Green odor	Aromatics associated with green, grassy	- Scale = 10; Cut fresh mulberry leaves frozen with liquid N and stored at -20°C.
Astringency	Feeling which shrivels the tongue and associated with phenolic compounds	- Scale = 0; Distilled water - Scale = 12; Oolong tea (1 g) from Green tea Co. Ltd., Thailand, infused with 500 ml boiled water for 2 min

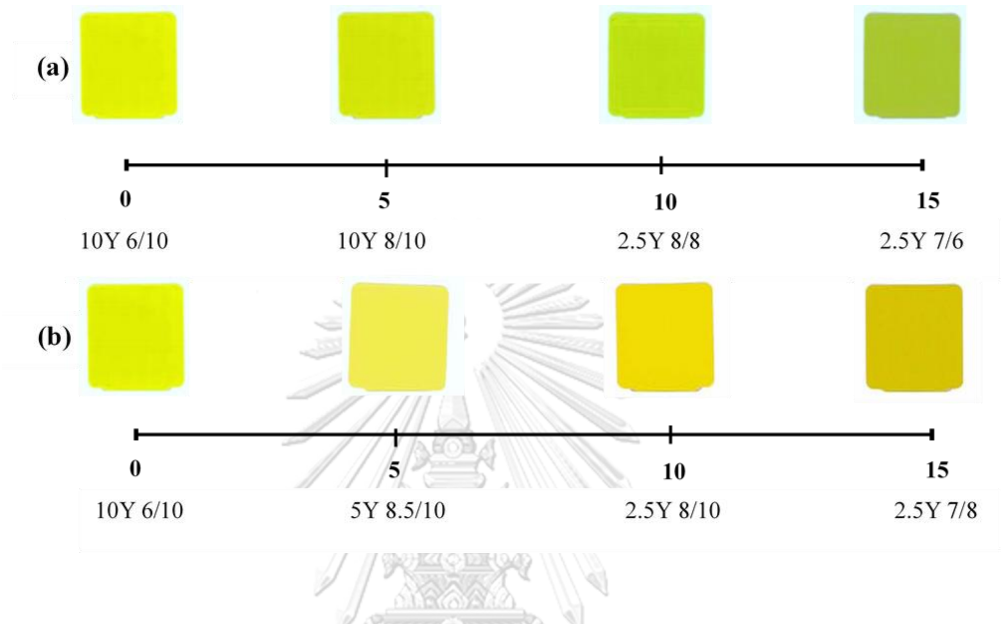


Fig. C.1 The references of color for greenness (a) and brownness (b) characteristic.

Sensory testing form for mulberry leaf tea

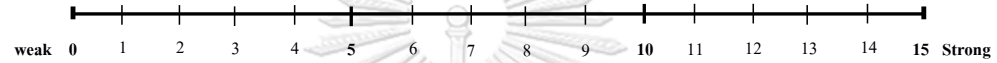
No……..Name:…...…….…….…….....…….Date:...……Age:.....

Please use a vertical line to indicate the intensity of following attributes. (R = intensity of references)

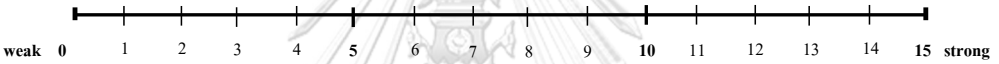
Sample: …….

APPEARANCE

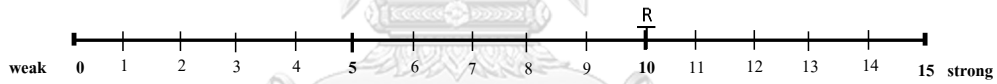
1. Greenness



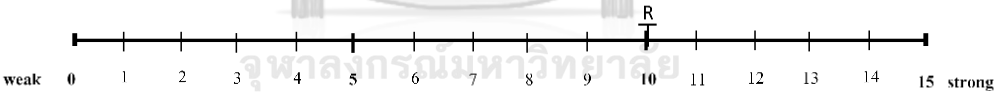
2. Brownness



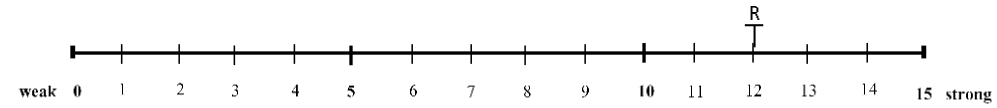
3. Dried-leaf odor



4. Green odor



5. Astringency



Suggestion

……..

……

……

Fig. C.2 Data sheet used to evaluate the intensity of each attribute of mulberry leaf tea

APPENDIX D

Electronic nose and Electronic tongue responses

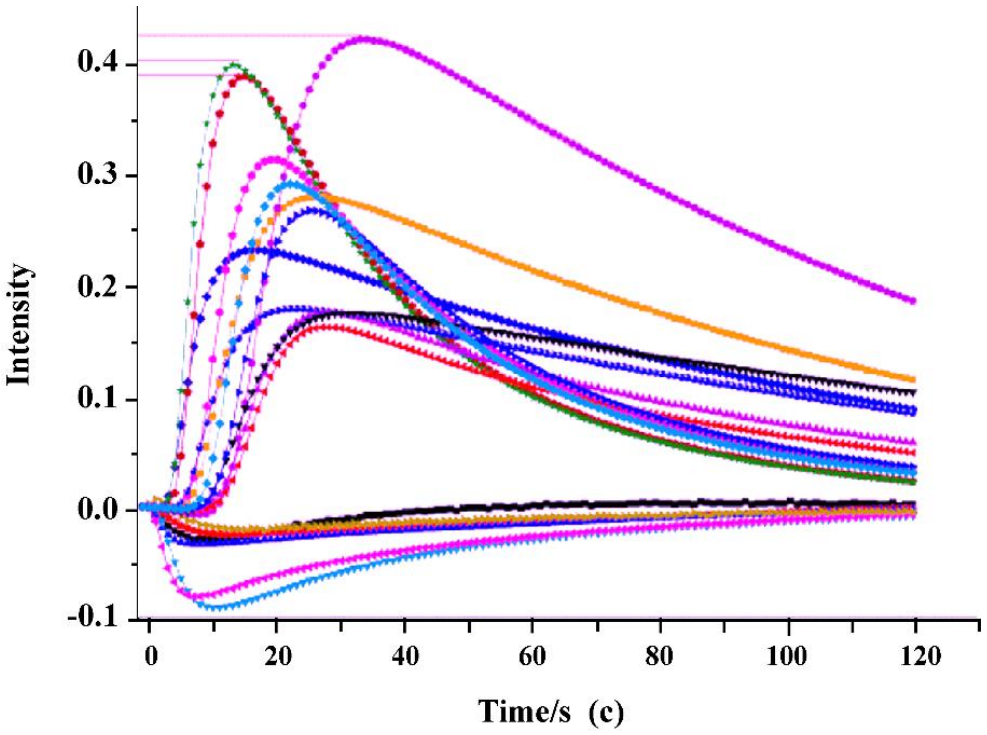


Fig. D.1 E-nose responses of mulberry leaf infusion were provided by the Fox 4000.

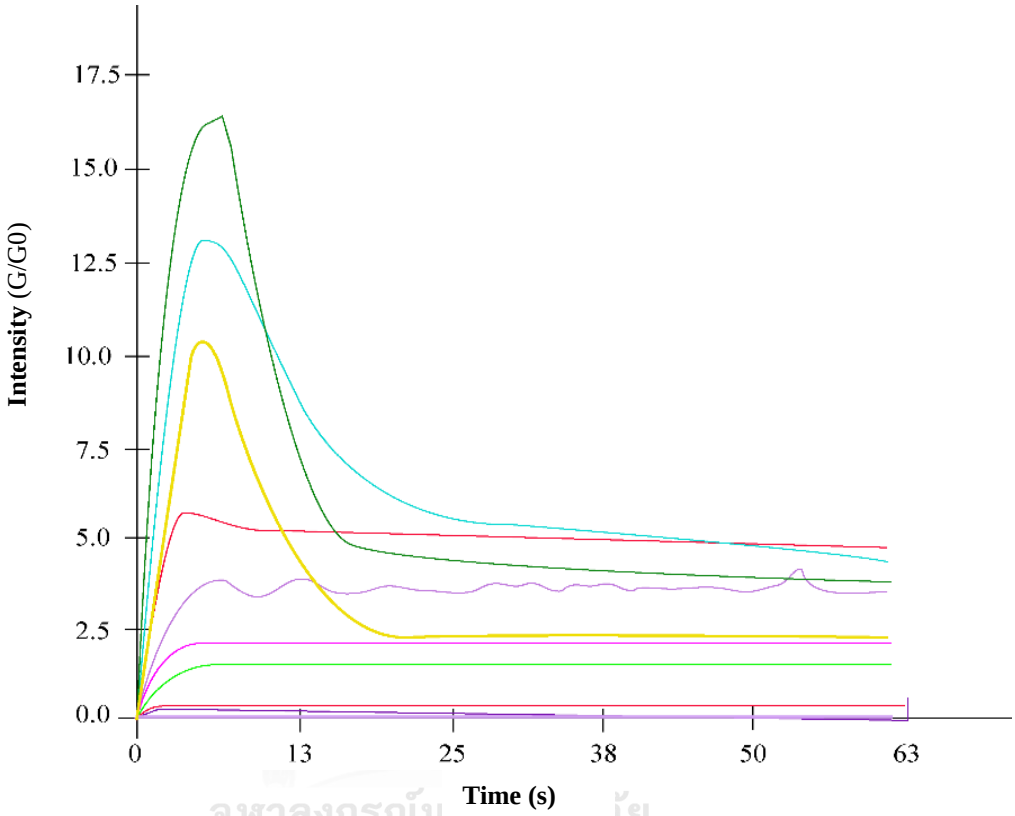


Fig. D.2 E-nose responses of mulberry leaf infusion were provided by the portable PEN2.

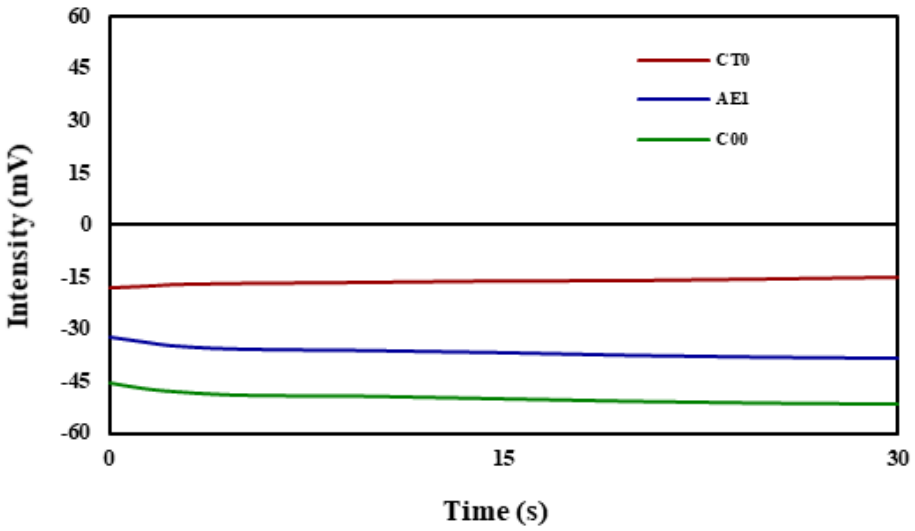


Fig. D.3 E-tongue responses (electrical potential) of mulberry leaf infusion were provided by the CT0, AE1, and C00 sensors.

APPENDIX E

GC-Chromatogram

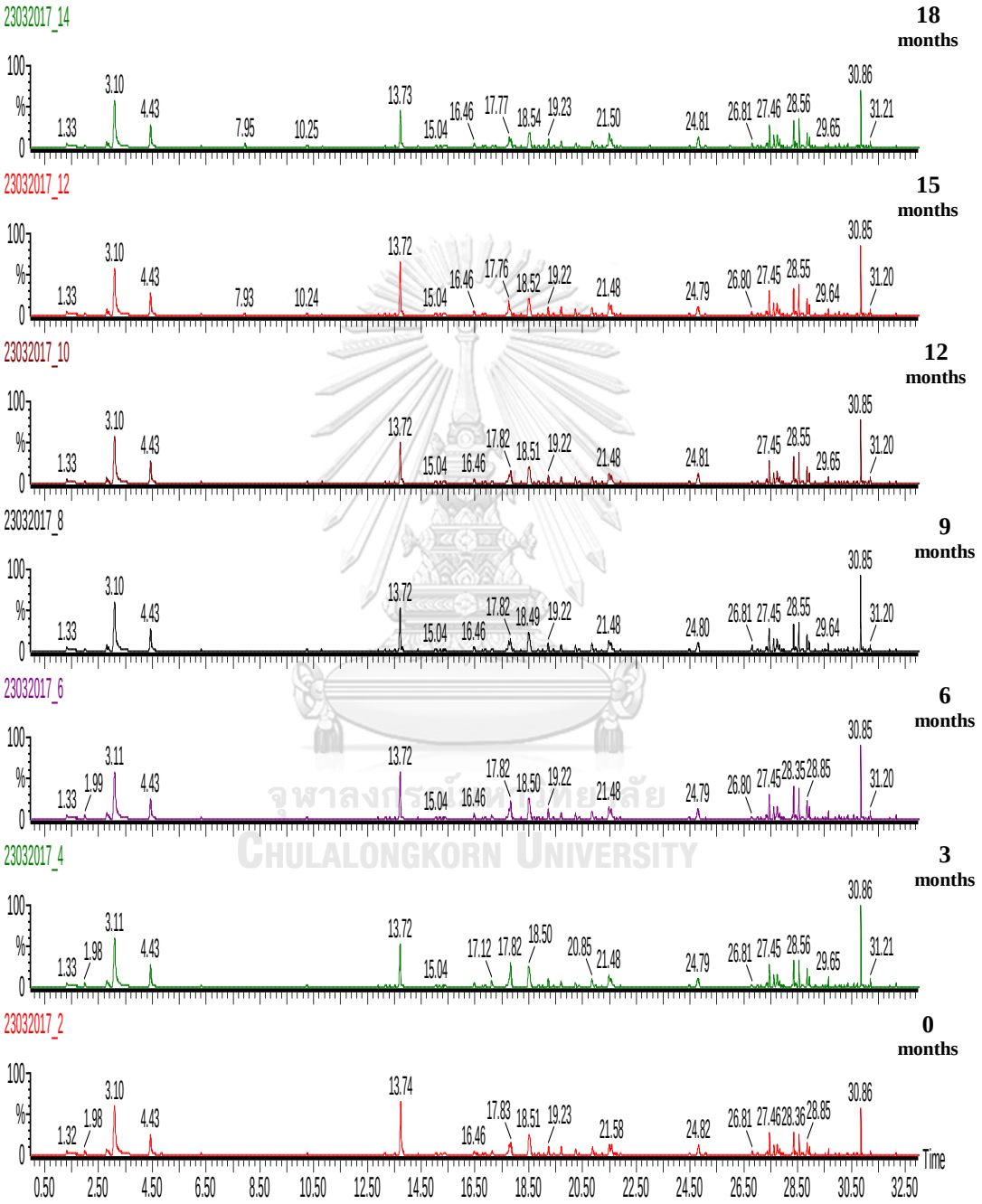


Fig. E.1 GC chromatogram of volatile compounds of mulberry leaf tea in AL bag during storage at 30±1°C, 75% RH for 0, 6, 9, 12, 15 and 18 months.

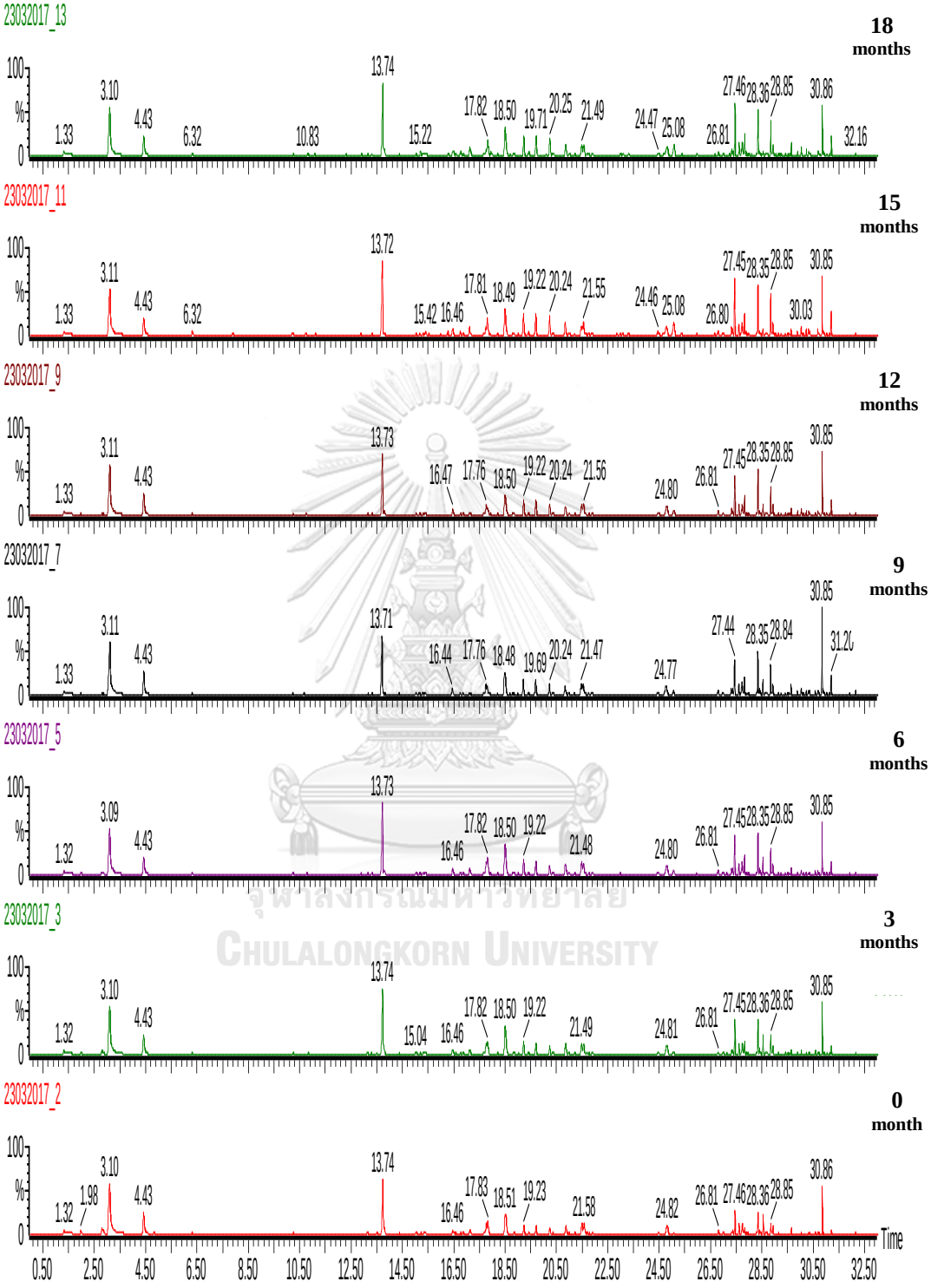


Fig. E.2 GC chromatogram of volatile compounds of mulberry leaf tea in PP bag during storage at 30±1°C, 75% RH for 0, 6, 9, 12, 15 and 18 months.

APPENDIX F

Moisture content (AOAC 2000) analysis

1. The empty dish and lid was dried at 105C for 1 h.
2. The empty dish and lid was cooled in the desiccator and weighed.
3. The sample was weighed and put in the dried dish.
4. The sample was dried at 105C for 6 h.
5. The sample was weighed and redried until the weight was stable.

Calculation

$$\text{Moisture (\%)} = \frac{(\text{Wu} - \text{Wd})}{\text{Wu}} \times 100$$

where: Wu = weight (g) of undried sample

Wd = weight (g) of dried sample

VITA

Miss Anchalee Ruengdech was born on March 12, 1981 in Chainat, Thailand. She obtained her B.Sc. (Food Technology) and M.Sc. (Food Technology) from Development of Food Technology, Silpakorn University in 2004 and 2008, respectively. She has worked as a food quality control supervisor at Kasemchai farm Group Co., Ltd, Nakhon Pathom, Thailand (2004-2005), as a food quality control supervisor at Dairy Plus Co., Ltd, Nakhon Sawan, Thailand (2005-2006), and as a lecturer at Department of Food Science and Technology, Faculty of Home Economics Technology, Rajamangala University of Technology Krungtep, Bangkok, Thailand (2008-2012). In 2012, she desired to study in the Ph.D. program at the Department of Food Technology, Chulalongkorn University.