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Aroma volatile profiles of flavored cashew tea with licorice root addition

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Abstract

The flavor volatile compounds of dried red cashew flowers, cashew leaf-buds, cashew leaves and licorice roots as well as flavored herb teas were analyzed by gas chromatography-mass spectrophotometry (GC-MS). It was found that terpenes, including α-pinene, α-phellandrene and β-phellandrene were the main volatiles in the cashew leaf-buds and leaves. The major volatile compounds of the red cashew flowers were 3-butyn-1-ol, acetic acid, benzyl alcohol and β-caryophyllene. The contents of 2-propanol,1,1'-oxybis and pentanedioic acid of the tray-dried flowers were higher than in the freeze-dried flowers, whereas (Z)-3-hexan-1-ol, ethanone, benzyl nitrile and benzyl tiglate were presented at highest concentrations in the freeze-dried flowers. 3-Butyn-1-ol was the main component in dried licorice roots. Flavored cashew tea formula A (a mixture of 70% freeze-dried cashew flowers, 12.5% cashew leaf-buds, 12.5% cashew leaves and 5% licorice roots) contained total volatile contents higher than tea formula B (a mixture of 70% tray-dried cashew flowers, 12.5% cashew leaf-buds, 12.5% cashew leaves and 5% licorice roots). However, 2-propen-1-ol, 3-phenyl was not found in tea formula A, while benzyl tiglate was not detected in tea formula B. Tea formula A contained more volatiles in the alcohol, ketone and ester classes than tea formula B. The classification of two formula flavored herb teas was presented. There are some differences in the signal response of each sensor for two grade flavored herb teas. Two grades of flavored herb tea were discriminated using electronic tongue (e-tongue). The results of discrimination function analysis (DFA) and principal component analysis (PCA) displayed that the grades of tea samples were discriminated.

Introduction

Cashew (*Anacardium occidentale* L.) commercially grows in the North-Eastern, Eastern and Southern regions of Thailand. There are many importance food products prepared from the cashew apple, nut and trunk. Some parts of cashew tree including leaf-buds, leaves and flowers are considered to have aphrodisiac properties and used as herbal products (Ferrao, 1993; Estrella, 1995; Grenand *et al*., 2004). Licorice (*Glycyrrhiza uralensis* Fisch.) is the most important species of the genus Glycyrrhiza in China. The root and stolen have been employed as components in many traditional medicinal herb prescriptions as well as flavoring and sweetening agents (Fukai *et al.*, 2002) and it also applied in confectionary, beverages and medicine as a healthy food source.

Herbal tea is one of the most widely consumed drinks due to its flavor, healthy, dietetic and therapeutic benefits (Xiao and Wang, 2009). Flavored cashew tea is a new product, which prepares from the red cashew (leaf-buds, leaves and flowers) and licorice roots. The development of flavored cashew tea can contribute to an increase of an essentially economical value of cashew products. Aroma volatile compounds of this product affect the overall consumer acceptance. Maia *et al.* (2000) identified the flavor compounds in the essential oil from the leave of various cashew varieties in Brazil, and found that the major volatiles were (E)-β-ocimene (28.8%), α-copaene (13.6%) and δ-cadinene (9.1%). They also reported that the essential oil from the cashew flowers contained 32 volatiles, with the main compounds identified as β-caryophyllene (26.0%), methyl salicylate (12.8%) and benzyl tiglate (11.3%). Xu *et al.* (2009) identified 108 volatiles in licorice oil including caproic acid (30.6%), hexadecanoic acid (13.55%), ethyl hexanoate (3.99%), ethyl linoleate (3.93%), 11-hexadecenal (2.84%), 3-methyl-cyclopentanol (2.09%), 2-pentyl-furan (1.82%) and 1-hexanol (1.76%).

Currently, the analytical instruments have

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been developed for discovery and quantification of the taste and aroma compounds in food products (Linforth, 2000). Analytical techniques have been used for qualitative and quantitative analysis and these techniques have been applied to the sensors data (Rodriguez-Mendez *et al*., 2004). An electronic tongue (e-tongue) is a sensor device that detects liquid with sensors array, it can discriminate the concentration of compounds in a complex liquid and widely applications in many solutions (Legin *et al*., 1999; Vlasov *et al*., 2000). The e-tongue based on voltammetry combined with recognition techniques pattern, it has been applied to the evaluation and employed for the discrimination of different teas (Xiao and Wang, 2009) due to capable of discriminating of micro-system (Lvova *et al.*, 2003).

E-tongue data obtained in this study were processed by multivariate analysis methods including, principal component analysis (PCA) and discrimination function analysis (DFA). PCA is a useful and common statistical method for finding patterns in data of high dimensions. It helps to reduce the number of dimensions without much loss of data. This method was used for treatment of the sensors array output as a means to visualize different groups (Xiao and Wang, 2009). DFA is used to determine which variables discriminate between two or more naturally occurring groups. This method can be used to predict membership in groups based on measured variables.

In this study, we have analyzed the aroma volatiles of dried red cashew leaf-buds, cashew leaves, cashew flowers and licorice roots as well as flavored herb teas using a gas chromatography-mass spectrometer (GC-MS) and an e-tongue analytical instrument.

Materials and Methods

Sample preparation

The fresh cashew flowers, leaf-buds and leaves as well as licorice roots were collected from the Natural Resources Faculty, Rajamangala University of Technology Isan, Sakon Nakhon campus, Sakon Nakhon, Thailand. The ingredients were washed with running tap water before drying. The cashew flowers were dehydrated using a tray dryer (Mammert, Germany) at 50°C for 36 h or using a freeze dryer (alpha 1-2 LD plus, Germany) at -50°C for 12 h or overnight. Other ingredients were also dried using a tray dryer with the same condition. The dried herbal plants were powdered using a National blender model MX-20G (250 W, National, Thailand) at low speed for 2 min (Chunthanom *et al.*, 2013).

Flavored cashew tea was made into 2 formulas; formula A comprised 70% freeze-dried cashew flowers, 12.5% cashew leaf-buds, 12.5% cashew leaves and 5% licorice roots, and formula B comprised 70% tray-dried cashew flowers, 12.5% cashew leaf-buds, 12.5% cashew leaves and 5% licorice roots (Chunthanom *et al.*, 2013). A 5 g of each tea was extracted in 100 ml boiled deionize water for 5 min before analysis.

Identification of aroma volatile compounds

Sample analysis by GC-MS

Volatile compounds of all ingredients and flavored cashew teas were identified using a Shimadzu GCMS-QP2010 (Shimadzu Cooperation Analytical & Measuring Instruments Division Kyoto, Japan). A 10 ml clear glass vial containing 0.5 g of each dried sample or 1 ml of flavored cashew tea extract placed in the AOC-5000 auto injector (Shimadzu) with syringe volume 2.5 ml for headspace injection.

GC-MS conditions

GC-MS analysis was performed using a Shimadzu GC 2010 series equipped with a split/splitless injector, coupled to a GCMS-QP2010 MS, at an MS ionization voltage of 70 eV. Data acquisition was performed by a GC-MS solution software (Shimadzu). The separation was achieved using a Restek Rtx-5ms fused 5% diphenyl/95% dimethyl polysiloxane capillary column, 30 m x 0.25 mm i.d., 0.25 µm film thickness (Superchrom, Italy). GC oven temperature was programmed from 40°C to 250°C at a rate of 5°C/min. The injector port temperature was 250°C. Helium was used as carrier gas. The injection mode was split. The MS scan condition of source temperature and interface temperature was 200°C. The identification was based on comparison of the GC retention time and mass spectra, the data were collected and analyzed with a Shimadzu computing system.

E-tongue and data acquisition

Experiments were performed using an α-Astree II electronic tongue (Alpha M.O.S company, France) which included an array of seven different liquid cross-selective sensors (ZZ, BA, BB, CA, GA, HA, JB), a 16-position autosampler and associated interface electronic module. Each sensor was composed of an organic coating sensitive to the species in the samples and a transducer, which allows the response of the membrane to be converted into signals for analysis. The sensor response was the voltage difference between the sensor and the Ag/AgCl reference electrode. Therefore, an integral signal for each sample was comprised of a vector with 7 individual sensor determinations (Xiao and

Wang, 2009).

A 5 g of flavored cashew tea was infused with 100 ml boiled deionize water for 5 min and then the solids were removed. The infusion was quickly cooled down to 25 ± 2°C and 80 ml of each extract was used in the measurement. The measurement procedure was controlled by a computer program. The measurement phase lasted for 120 s, which was long enough for the sensors to give stable values. The interval for data collection was 1 s. A computer recorded the response of the e-tongue every second. When the measurement was completed, the acquired data was properly saved for use. In this research, the signal of each sensor at second 120 was used in the analysis. The sensors were cleaned with deionized water after a sample testing and were calibrated before testing a different grade sample with 0.01 M HCl.

Results and Discussion

Volatile aroma compounds of dried herbal plants

The volatile components of the dried red cashew leaf-buds, cashew leaves, cashew flowers and licorice roots are shown in Table 1. Individual compounds were identified by comparison of the mass spectrum and data system libraries. The 38 and 21 volatiles identified in dried red cashews and dried licorice roots, respectively. The results showed that terpenes were the most prominent compounds in the leaf-buds, leaves and flowers of the red cashew, whereas alcohols were the main compounds in licorice roots. Among 38 identified terpenes in the cashew leaf-buds and leaves, α-pinene (14.31-17.64%), α-phellandrene (5.06-10.09%) and β-phellandrene (51.51-56.22%) were the main volatiles. The major compounds of the cashew flowers were quite different in composition from that of the leaf-buds and leaves. The major compounds of the red cashew flowers dried by tray-dryer and freeze-dryer were 3-butyn-1-ol (3.01-15.95%), acetic acid (17.33-18.00%), benzyl alcohol (14.54-15.19%) and β-caryophyllene (12.72-17.72%). Maia *et al.* (2000) reported that main volatile compounds of red cashew leaves were (E)-β-ocimene (28.8%), α-copaene (13.6%) and δ-cadinene (9.1%) and the volatiles of red cashew flowers were β-caryophyllene (26.0%), methyl salicylate (12.8%) and benzyl tiglate (11.3%). They also reported that terpenes in cashew tree contributed to the composition of the leaves and flowers.

In this study, the content of α-pinene, β-pinene and β-phellandrene in the leaf-buds and leaves were higher than in the others. Acetic acid, benzaldehyde, benzyl alcohol, benzyl nitrile, methyl salicylate, α-copaene, β-caryophyllene, benzyl tiglate, 1,2-benzenedicarboxylic acid-dimethyl ester and β-cadinene were presented at higher content in flowers than others.

There is no published information about the effect of drying method on the volatile components of cashew flowers. The contents of 2-propanol,1,1'-oxybis and pentanedioic acid in the flowers dried by tray-drying were higher than in the freeze-dried sample, whereas (Z)-3-hexan-1-ol, ethanone, benzyl nitrile and benzyl tiglate were presented at lower concentration than in the freeze-dried flowers. It was interesting to note that some volatiles were lost during tray-drying, whereas others were generated. Wongfhun *et al.* (2010) revealed that a decrease of volatile compounds from the products could be caused by thermal degradation or by evaporation. On the contrary, the increase of some flavor volatiles might be a result of heat activation of flavor precursors or the release of aroma volatiles bound to cell membranes or macromolecules (Apichartsrangkoon *et al.*, 2009). In overall, freeze-drying caused more volatiles in alcohol, ketone and ester classes to be retained than tray-drying. This indicates that non-thermal processing could maintain the flavors better than thermal treatment. The detection of high amounts of alcohols, ketones and esters is a good marker that processing has been applied to the sample (Wongfhun *et al.*, 2010).

Licorice roots contained 21 compounds, the main volatile was 3-butyn-1-ol (67.11%). Xu *et al.* (2009) reported that the main compounds in licorice oil were caproic acid (30.6%), hexadecanoic acid (13.55%), ethyl hexanomate (3.99%), ethyl linoleate (3.93%), 11-hexadecenal (2.84%), 3-methyl-cyclopentanol (2.09%), 2-pentyl-furan (1.82%) and 1-hexanol (1.76%). From this study, the contents of 3-butyn-1-ol, β-myrcene, 2,2-dimethylcyclopropylbenzene and benzoic acid in licorice roots were higher than in red cashew leaf-buds, leaves and flowers.

Volatile aroma compounds of flavored cashew teas

Various volatile compounds of flavored cashew teas are shown in Table 2. Flavored cashew tea formula A displayed a higher content of a number of volatiles than tea formula B including, 3-butyn-1-ol, (Z)-3-hexan-1-ol, benzaldehyde, ethanone, benzyl alcohol, 1-propanol,2,2'-oxybis, cyclohexyl pentanoate, trans-linalool oxide, phenyl ethyl alcohol, acetic acid, phenyl methyl ester, methyl salicylate, β-caryophyllene, 1,2-benzenedicarboxylic acid, dimethyl ester, β-cadinene, diethyl phthalate and 2-propenoic acid/3-mehoxyphenyl. 2-Propen-1-ol, 3-phenyl was not found in tea formula A, while benzyl tiglate was not detected in tea formula B. The

Table 1. Peak area of volatile compounds identified in dried herbal plants

Volatile compounds (pA x s)*	RT**	Cashew leaf-buds	Cashew leaves	Cashew flowers		Licorice
				Freeze-dried	Tray-dried	
3-Butyn-1-ol	1.274	2,982.85	nd	4,023.73	425.14	7,868.66
Acetic acid	1.533	336.80	nd	3,468.08	2,296.41	nd
Pentanal	1.973	trace	200.65	nd	nd	nd
2-Buten-1-ol, 3-methyl	3.190	trace	trace	670.64	253.78	nd
1-Hexanol	3.599	trace	227.97	trace	61.70	161.20
Butanoic acid, 3-methyl	4.272	321.09	trace	trace	58.86	133.08
Hexanal	4.625	trace	83.69	nd	nd	nd
(*Z*)-3-Hexan-1-ol	4.688	trace	252.12	199.63	trace	nd
α-Pinene	6.489	6,200.71	4,649.74	209.71	171.27	27.05
Benzaldehyde	7.204	nd	nd	285.99	254.28	nd
2-β-Pinene	7.632	85.05	trace	nd	nd	nd
β-Pinene	8.029	138.96	305.37	nd	nd	nd
Cyclotetrasiloxane, octamethyl	8.239	trace	299.21	nd	nd	nd
α-Phellandrene	8.386	4,687.90	1,362.60	nd	nd	nd
α-Terpinene	8.730	186.30	trace	nd	nd	nd
2-Propanol, 1,1'-oxybis	8.918	nd	nd	trace	344.49	285.08
Ethanone	8.941	1,193.22	722.95	526.11	trace	nd
β-Phellandrene	9.078	24,031.22	14,831.49	nd	nd	nd
Benzyl alcohol	9.232	184.79	142.56	3,071.13	1,860.77	259.11
1-Propanol, 2,2'-oxybis	9.425	179.42	122.75	99.15	196.56	98.78
Cyclohexyl pentanoate	9.548	nd	nd	nd	nd	113.42
Phenylethyl alcohol	11.506	399.06	304.38	795.45	628.35	341.99
Benzyl nitrile	12.225	nd	nd	420.67	111.96	nd
Acetic acid, phenyl methyl ester	12.982	353.11	432.31	453.19	404.32	404.25
α-Terpineol	13.737	nd	nd	nd	nd	18.21
Methyl salicylate	13.840	nd	nd	432.75	544.49	nd
β-Myrcene	15.510	46.61	trace	nd	nd	66.34
2-Propen-1-ol,3-phenyl	16.913	nd	nd	trace	45.73	nd
α-Copaene	18.789	647.21	259.16	1,146.63	727.73	42.47
β-Caryophyllene	19.930	273.45	149.77	2,574.12	2,275.24	219.63
1,2-Benzenedicarboxylic acid, dimethyl ester	20.790	103.92	151.45	295.65	271.43	77.35
2-Propenoic acid,3-phenyl-, ethyl ester	21.042	trace	54.42	trace	34.49	85.56
δ-Cadinene	21.348	trace	trace	135.19	214.68	nd
α-Cubebene	21.470	trace	233.25	trace	trace	nd
Pentanedioic acid	22.173	133.40	117.58	trace	88.41	119.98
Benzyl tiglate	21.875	nd	nd	323.86	trace	nd
β-Cadinene	22.487	165.23	trace	336.25	236.26	nd
Diethyl phthalate	24.155	1,520.51	1,306.14	1,258.59	1,164.55	1,101.52
2,2-Dimethylcyclopropylbenzene	26.448	109.38	trace	nd	nd	103.93
2-Propenoic acid, 3-mehoxyphenyl	27.717	262.35	227.07	192.21	193.68	198.72
Benzoic acid	28.402	nd	nd	nd	nd	54.62

*Approximate area (pA x s x 10^4) in head space from 0.5 g of sample is estimated.
**RT is retention time.
Compounds identified below 10 x 10^3 (pA x s) are reported as trace and nd is not detected.
Components are the means of triplication.

Table 2. Peak area of volatile compounds identified in flavored cashew teas

Volatile compounds (pA x s)*	RT**	Tea formula A	Tea formula B
3-Butyn-1-ol	1.274	420.01	388.43
Acetic acid	1.533	174.77	trace
Pentanal	1.973	trace	trace
2-Buten-1-ol, 3-methyl	3.190	trace	trace
1-Hexanol	3.599	7.99	18.59
Butanoic acid, 3-methyl	4.272	trace	trace
Hexanal	4.625	trace	trace
(*Z*)-3-Hexan-1-ol	4.688	4.88	trace
α-Pinene	6.489	481.25	329.75
Benzaldehyde	7.204	12.68	10.79
2-β-Pinene	7.632	trace	5.31
β-Pinene	8.029	9.49	28.61
Cyclotetrasiloxane, octa methyl	8.239	trace	trace
α-Phellandrene	8.386	87.68	272.35
α-Terpinene	8.730	trace	trace
2-Propanol,1,1'-oxybis	8.918	trace	trace
Ethanone	8.941	67.59	62.14
β-Phellandrene	9.078	trace	trace
Benzyl alcohol	9.232	135.66	86.41
1-Propanol, 2,2'-oxybis	9.425	trace	9.41
Cyclohexyl pentanoate	9.548	11.68	trace
trans-Linalool oxide	10.335	39.89	13.93
Phenylethyl alcohol	11.506	54.84	46.87
Benzyl nitrile	12.225	16.30	trace
Acetic acid,phenyl methyl ester	12.982	36.94	32.65
α-Terpineol	13.737	trace	trace
Methyl salicylate	13.840	26.80	31.60
β-Myrcene	15.510	trace	trace
2-Propen-1-ol, 3-phenyl	16.913	nd	trace
α-Copaene	18.789	74.83	31.85
β-Caryophyllene	19.930	184.70	165.44
1,2-Benzenedicarboxylic acid, dimethyl ester	20.790	23.28	11.54
2-Propenoic acid,3-phenyl-, ethyl ester	21.042	trace	trace
δ-Cadinene	21.348	trace	trace
α-Cubebene	21.470	trace	trace
Benzyl tiglate	21.875	trace	nd
Pentanedinoic acid	22.173	trace	trace
β-Cadinene	22.487	23.72	21.54
Diethyl phthalate	24.155	105.26	104.26
2, 2-Dimethylcyclopropylbenzene	26.448	trace	trace
2-Propenoic acid, 3-mehoxyphenyl	27.717	17.53	18.99
Benzoic acid	28.402	trace	trace

Flavored cashew tea formula A was prepared from freeze-dried cashew flower 70%, cashew leaf-buds 12.5%, cashew leaves 12.5% and licorice roots 5%; formula B was prepared from tray-dried cashew flower 70%, cashew leaf-buds 12.5%, cashew leaves 12.5% and licorice roots 5%.
*Approximate area (pA x s x 10^4) in head space from 0.5 g of sample is estimated.
**RT is retention time.
Compounds identified below 10 x 10^3 (pA x s) are reported as trace and nd is not detected.
Components are the means of triplication.

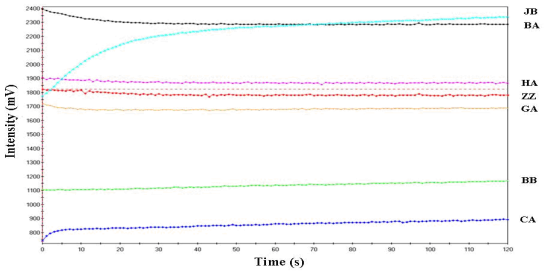

Figure 1. Response of e-tongue to favored cashew tea formula A

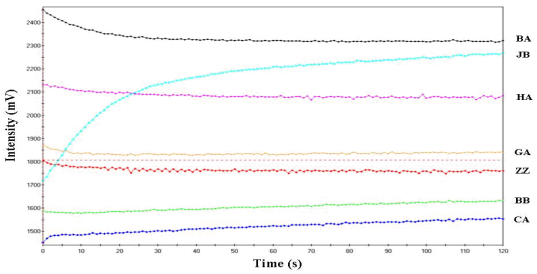

Figure 2. Response of e-tongue to favored cashew tea formula B

content of (Z)-3-hexan-1-ol, 1-propanol,2,2'-oxybis, cyclohexyl pentanoate and benzyl nitrile detected was less than 0.01% in tea formula B. Tea formula A contained more volatiles in the alcohol, ketone and ester classes than tea formula B, with a trend for freeze-dried cashew flowers to retain more volatiles than tray-dried cashew flowers.

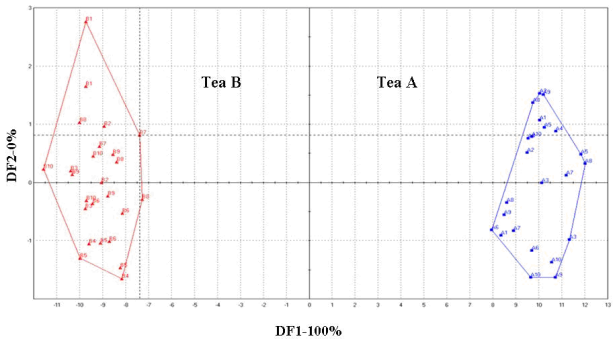

Figure 3. DFA plots of e-tongue data for favored cashew teas

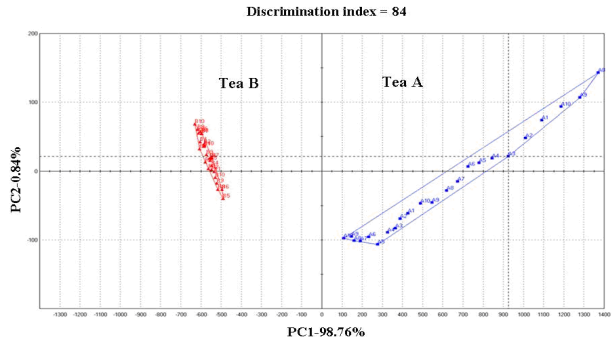

Figure 4. PCA plots of e-tongue data for favored cashew teas

Response of e-tongue and classification of flavored cashew teas

The typical signal of the seven sensors in response to cashew tea formula A is shown in Figure 1. The Y-axis represented the signal intensity, which was the voltage difference between the sensor and the reference electrode while the X-axis represented the measurement time. The sensors responded to cashew tea formula A in the sequence of JB>BA>HA>ZZ>GA>BB>CA and all sensors became stable after 100 seconds. The sensors responded to cashew tea formula B in the sequence of BA>JB>BB>GA>ZZ>BA>CA as shown in Figure 2. There are some differences in the average response values (mean of 20 samples) of each sensor between the two grades of cashew tea, which correspond to the variations in the taste of cashew tea.

DFA was performed to assess the adequacy of cashew tea's grade classification of 40 samples. DFA was used for determine which variables discriminate between the two groups. This method can be used to predict membership in groups based on measured variables. It was found that the samples were obviously separated into two clusters (Figure 3). PCA reduces the dimension of the data to some principal components and enables the extraction of the differences between samples in term of the main variables. The data for 40 samples were analyzed by PCA. The loading plot showed the relationship between the variables and facilitated an observation of the contributions of the variables to the weightings of each PC value (Figure 4). PC1 was mainly contributed by most sensors as shown in the high values for cashew tea formula A and B in PC1 compared to PC2. The discrimination index was 84. The DFA and PCA analysis of the sensor signals for cashew tea samples showed similar results in the classification which was due to the characteristics in the quality among different grades or tastes of cashew tea.

Normally, e-tongue analysis shows better ability to discriminate between the samples than sensory evaluation. The e-tongue is a very promising tool because the e-tongue can be thought of as a model for both olfaction and taste and it can be used for the detection of all types of dissolved compounds including volatile compounds which give odors after evaporation (Legin *et al.*, 2002).

Conclusion

Terpenes including α-pinene, α-phellandrene and β-phellandrene were the major class of volatile components as presented in the red cashew leaf-buds and leaves. The major compounds of the cashew flower were 3-butyn-1-ol, acetic acid, benzyl alcohol and β-caryophyllene. 3-Butyn-1-ol was the main component in dried licorice roots. The flavored cashew tea formula A contained more volatiles in the alcohol, ketone and ester classes than tea formula B. This study supplied new information on the volatile compounds of these new beverages for consumers. An attempt was made to discriminate between two grades of flavored cashew tea using an e-tongue. Two formulas of tea were discriminated by DFA and PCA based on the e-tongue sensor responses.

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