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DISCRIMINATION OF VIETNAMESE GREEN TEAS FROM DIFFERENT GEOGRAPHICAL ORIGINS BY AROMA PROFILE USING HS-SPME/GC–MS INTEGRATED WITH MULTIVARIABLE ANALYSIS

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Abstract. Volatile compounds (VOCs) from brewed green teas of different geographical origins in Viet Nam were analyzed by headspace solid phase micro-extraction coupled with gas chromatography–mass spectrometry (HS-SPME/GC–MS). Forty-six VOCs from 15 green tea brands were identified and quantified by GC-MS. The GC-MS profiles were subjected to multivariable analysis such as principal component analysis and cluster analysis. The results demonstrated that the developed method in this study can be used to discriminate Vietnamese green tea samples from different geographical origins with high reliability.

Keywords: Camellia sinenesis, volatile compounds, geographic origin, HS-SPME/GC–MS, discrimination.

Classification numbers: 1.1, 1.4.4.

1. INTRODUCTION

Camellia sinensis is a plant commercially grown for production of tea. There are different tea products which are made from the bud and young leaves of tea plant with different flavours such as green tea, black tea, white tea, oolong tea, etc. depending on the degrees of fermentation and processing parameters. Green tea is the most popular consumed beverage in Viet Nam and other Asian countries such as China, Japan, India, Taiwan. Various green teas are available to consumers that represent different processing methods, harvest times, plant varieties and growing regions, all of which may contribute to quality of tea [1]. Aroma, flavour and appearance are important aspects of the evaluation of green tea quality [2]. Many researchers have studied green tea for its aromatic volatile compounds to understand the aroma characteristics of green teas. In addition, studying aromatic volatile compounds from brewed green tea liquor can help researchers to understand the composition of tea brewed as consumers actually would consume it [3 - 5].

Consumers are now paying more attentions on the origins of teas because there is variation in tea quality that affect the tea prices on the market. Tea authenticity therefore plays more

important role in the tea markets and it is necessary to find a feasible method for the discrimination of tea from different origins [6]. Consumers can make their choice or preference of tea based on the perception of tastes, aroma and flavour. However, sensory evaluation is not always consistent and may give subjective results on tea quality and authenticity. Instead, chemical analysis according to tea geographical origins is crucial to quality control because it can give consistent and robust results. Recently, several methods for the discrimination of tea samples were developed by the employment of chemical analysis. Several instrumental methods were reported for the discrimination of teas with different geographical origins, such as high-performance liquid chromatography, capillary electrophoresis, near-infrared spectroscopy, atomic spectrometry, and electronic nose [7 - 8].

The objectives of this study were to use GC-MS paired with SPME to: (a) identify common volatile compounds in the brewed liquor of a wide range of green tea samples from various provinces, and (b) determine if green teas from different regions can be discriminated based on volatile composition in samples that are prepared for normal consumption.

2. MATERIALS AND METHODS

2.1. Materials

A total of fifteen different commercial brands of Vietnamese green teas were collected at processing factories located at 5 tea-producing provinces: Thai Nguyen (TN): 04 brands, Phu Tho (PT): 03 brands; Yen Bai (YE): 03 brands; Hoa Binh (HB): 02 brands Nghe An (NA): 03 brands. All collected samples were harvested in the same season. Different quality levels of teas were collected for this research. About 20 grams of each green tea sample and two samples of each brand were sealed in aluminum bag and stored in a cool and dry place until analyses.

2.2. Sample preparations

One gram of each green tea sample was infused with distilled water (5 ml) of 98 °C in a 10 ml glass septum vial by heating on a hot plate for 10 min which was similar to the normal condition of tea brew preparation. After equilibration, SPME fibre (Supelco, Bellefonte PA, USA) coated with 65 μm polydimethylsiloxane/divinylbenzene (PDMS/DVB) was rapidly inserted into the headspace of the vial. The absorption step was kept at 90 ^{o}C for 30 min. The PDMS/DVB fibre was preconditioned for 5 min in the injection port of the GC at 220 ^{o}C before each analysis. Sample analyses are carried out in duplicates [9].

2.3. GC-MS analysis

A Thermo trace GC Ultra gas chromatograph coupled with the DSQ II mass spectrometer was used to perform the aroma analysis on HP-5 capillary column (30 m × 0.25 mm × 0.25 μm) with purified helium as the carrier gas at a constant flow rate of 1 ml min^{-1}. After extraction, the fibre was desorbed in the injector port of the GC at 220 °C for 5 min. The oven temperature was held at 50 °C for 3 min and then increased to 190 °C at a rate of 5°C min^{-1} and held at 190^{o}C for 1 min, and then increased to 240 ^{o}C at a rate 20 ^{o}C min^{-1}, held at this temperature for 3 min. The ion source temperature was of 200 °C and the mass spectra were produced under the electron impact (EI) mode at 70 eV [10].

2.4. Data processing

After GC–MS analysis, each sample was represented by a GC–MS total ion chromatogram (TIC). Chromatograms of all tea samples were subjected to noise reduction prior to peak area integration, and then the peak areas of compounds in the chromatogram were integrated. Compounds were identified by comparing the mass spectrum with a standard mass spectrum in the Xcalibur software Mass Spectra Library (Thermo Technologies). Aligned peaks were finally selected and imported to form a data set. Identification of the selected peaks was made by searching NIST98 MS data library (a match quality of 80 % minimum was used as a criterion). Any known artificial peaks should be excluded from the data set. Principal component analysis (PCA) and Hierarchical cluster analysis (HCA) were performed in the XLSTAT 2014.5.03 with add-in software for the discrimination of tested tea samples.

3. RESULTS AND DISCUSSION

3.1. Volatile compounds in green teas from different geographical origins

Forty-six volatile compounds (VOCs) were identified from the 15 green tea samples which were collected in five production provinces in the north and central of Viet Nam. Table 1 and Table 2 provide the contents of VOCs which is presented in the form the average relative percentage peak areas and the information on potential aroma associated with those compounds from prior literature [9-12]. It can be seen that some VOCs were present at higher level in teas originated from specific province. The Thai Nguyen's samples had a higher level of 2-acetyl furan (with relative percentage peak area ranged from 1.69 % to 1.73 %), benzene acetaldehyde (1.47 % - 1.98 %), phenethyl aclcohol (2.01 % - 2.45 %), α-terpineol (4.08 % - 4.87 %), jasmone (1.35 % - 1.73 %), β-ionone, 5,6,7,7a-tetra 2(4)-benzofuranone (3.05 % - 4.53 %) and 4-methyl-2-propyl furan, and benzofuranone. On the other hand, the Phu Tho's teas had high content of nerolidol (5.63 % - 6.74 %) and linalool (1.97 % - 2.38 %), 2, 3-dihydrobenzofuran (2.97 - 3.26 %) and hexanal. The Hoa Binh's samples had the content of 1-pentanol and 2-acetyl pyrrole higher than all other samples. Linalool oxide I, β-pinene and dihydroactinidiolide were found with higher content in Yen Bai's samples. The Nghe An's samples had significant content of hexanal, benzaldehyde, D-limonene, and 1-penten-3-ol.

Many VOCs play an important role in creating the characteristics flavor to tea which are known the key odor active compounds [10]. The Thai Nguyen's tea samples were characterized by the higher level of ordor-active compounds such as α-terpineol, 2-acetyl furan, β-ionone, jasmone, 5,6,7,7a-tetra 2(4)-benzofuranone and 4-methyl-2-propyl furan which are known to impart lilac, balsamic, nutty, woody and jasmine aroma to teas [11]. The Phu Tho's samples contained linalool, 2, 3-dihydrobenzofuran, hexanal and especially high proportion of nerolidol which have been reported previously to have green, floral, woody and citrus aroma to tea [12] and Nghe An' samples had floral, fruity, and nutty aromas.

In this study dihydroactinidiolide presented at rather high content in tea samples from Thai Nguyen, Yen Bai and Hoa Binh provinces. This compound was reported to have nut-like flavour in previous study and is known to be an odor active compound in tea which impart typical tea flavour [11]. Nerolidol was found in the highest content compared to the other VOC profile of the 15 samples from 5 provinces, however this compound presented with significant level in the Phu Tho's samples (with the level of 5.63 - 7.27 %) and in Hoa Binh samples (6.14 - 6.86 %). This compound is known to bring floral aroma to tea.

Table 1. Volatile compounds detected in 7 green tea samples from Thai Nguyen and Phu Tho provinces by HS-SPME/GC-MS and their odor characteristics reported in the literature.

No	Compounds	Odor characteristics	Relative percentage areas						
			TN1	TN2	TN3	TN4	PT1	PT2	PT3
1	1-pentanol	sweet, balsamic	1.00	0.47	0.93	1.03	1.47	1.17	1.32
2	(*Z*)-2-penten-1-ol	fishy, grassy	0.40	0.92	0.97	0.95	1.03	1.43	1.68
3	(*Z*)-3-hexen-1-ol	sweet, green, fruity	1.42	1.34	1.27	1.28	1.04	1.37	1.54
4	1-penten-3-ol	butter, mild, green	1.45	1.99	2.19	1.48	1.65	1.45	1.98
5	hexanal	fruity	2.98	2.75	2.22	2.55	2.19	1.90	2.44
6	(*E*)-2-hexenal	sweet and fruity	2.23	3.25	2.34	2.29	3.63	3.75	4.29
7	α-furfural	almonds	2.10	2.26	2.06	2.13	2.39	2.60	2.44
8	3-furfuryl alcohol	faint burning odor	1.17	1.26	1.33	1.22	1.66	1.53	1.17
9	heptaldehyde	strong fruity odor	0.88	0.57	0.25	0.75	0.98	0.83	1.18
10	2-acetyl furan	balsamic, nutty	1.69	1.76	1.64	1.93	1.19	0.95	1.21
11	benzaldehyde	almond-like odor	0.74	0.97	1.09	0.89	0.97	0.70	1.20
12	2, 3-octandione	anis-like and brackish	0.95	0.69	0.46	0.47	0.82	0.62	0.88
13	β-pinene	woody-green pine-like	0.29	0.93	0.83	1.02	0.21	0.13	0.09
14	*D*-limonene	oranges	0.79	0.98	1.09	0.72	0.20	0.12	0.32
15	benzyl alcohol	light floral rose	2.14	2.72	2.01	2.34	2.47	2.71	2.57
16	benzene acetaldehyde	hyacinth, lilac	1.74	1.47	1.87	1.98	0.85	0.53	0.20
17	β-ocimene		1.31	1.16	1.06	1.22	1.74	1.43	1.84
18	2-acetyl pyrrole	herbal, nutty, anisic	0.17	0.21	0.15	0.42	1.47	1.15	1.35
19	linalool oxide I	floral, green	1.32	1.15	1.21	1.19	0.98	0.76	0.65
20	linalool	bergamot oil, lavender	1.59	1.79	1.58	1.95	2.20	1.97	2.38
21	3, 7-dimethylocta-1, 5, 7-trien-3-ol		1.79	1.39	1.90	1.21	2.42	2.03	2.53
22	phenethyl alcohol	rose	2.18	2.01	2.45	2.36	1.70	1.97	1.37
23	α-terpineol	lilac	4.33	4.08	4.70	4.87	3.71	4.33	3.13
24	safranal	slightly spicy	0.18	0.21	0.15	0.21	0.24	0.65	0.77
25	2, 3-dihydrobenzofuran	fenugreek	2.09	1.21	1.85	1.92	3.26	2.53	2.97
26	nerol	pleasant geranium	0.57	0.75	0.46	0.66	0.22	0.15	0.42
27	nonanoic acid	fatty, green	0.74	0.69	0.87	0.97	0.54	0.43	0.37
28	jasmone	jasmine	1.35	1.44	1.73	1.54	1.12	1.24	1.38
29	β-damascenone	rose	0.79	0.57	0.71	0.88	1.18	1.24	1.37
30	α-ionone	woody, violet	1.78	1.50	1.33	1.58	1.09	0.93	0.43
31	geranylacetone	sweet-floral, fruity	1.62	1.46	1.70	1.86	2.37	2.04	3.02
32	β-ionone	woody	5.88	5.35	5.65	5.26	4.05	4.65	4.36

33	n-pentadecane		0.66	0.37	0.24	0.84	1.04	0.94	0.79
34	dihydroactinidiolide	sweet creamy, nut-like	5.48	5.33	5.66	5.77	2.72	3.25	3.48
35	tetrahydro-2,2,5,5-tetramethyl furan		1.74	2.10	1.99	1.42	1.06	1.36	1.24
36	nerolidol	floral scent	4.75	4.36	4.57	4.47	6.74	7.27	5.63
37	2-hydroxy methyl ester benzoic acid		2.07	2.32	2.31	2.21	1.56	1.36	1.86
38	4-methyl-2-propyl furan		2.06	1.08	1.98	1.16	0.55	0.25	0.83
39	n-heptadecane		1.14	1.40	1.54	1.44	1.16	1.24	1.02
40	2, 6, 10, 14-tetramethylpentadecane		0.17	0.22	0.16	0.22	0.57	0.41	0.84
41	farnesylacetone	creamy, winey	0.40	0.66	0.33	0.92	0.82	1.23	1.02
42	methyl hexadecanoate		0.57	0.98	0.78	0.65	0.32	0.17	0.83
43	5,6,7,7a-tetra 2(4)-benzofuranone		4.53	3.59	3.01	3.45	1.13	1.43	1.66
44	hexadecanoic acid	fatty	1.37	1.56	1.40	1.75	1.24	1.04	1.44
45	3-butyl-1-oxide-pyridine		0.69	0.98	0.37	1.09	0.65	0.42	0.94
46	3-ethyl-2,5-dimethyl-pyrazine		1.87	1.57	1.99	2.19	1.04	1.38	1.26

Table 2. Volatile compounds detected in 8 green tea samples from Yen Bai, Hoa Binh and Nghe An provinces by HS-SPME/GC-MS and their odor characteristics reported in the literature.

No	Compounds	Odor characteristics	Relative percentage areas							
			YB1	YB2	YB3	HB1	HB2	NA1	NA2	NA3
1	1-pentanol	sweet, balsamic	1.22	1.16	1.20	2.19	1.71	1.46	1.57	1.33
2	(*Z*)-2-penten-1-ol	fishy, grassy	0.97	0.95	1.37	1.15	1.12	0.73	0.97	0.60
3	(*Z*)-3-Hexen-1-ol	sweet, green, fruity	1.67	1.35	1.49	1.22	1.38	1.05	1.15	0.95
4	1-penten-3-ol	butter, mild, green	2.51	2.28	2.42	2.53	2.79	3.88	2.75	3.19
5	hexanal	fruity	2.75	2.17	2.32	2.12	2.04	3.72	3.17	4.24
6	(*E*)-2-hexenal	sweet and fruity	2.84	2.52	2.65	2.65	2.45	2.12	2.15	2.03
7	α-furfural	almonds	1.20	0.97	1.10	2.27	2.17	1.27	1.32	1.42
8	3-furfuryl alcohol	faint burning odor	1.22	1.17	1.20	2.30	1.97	1.34	1.42	1.45
9	heptaldehyde	strong fruity odor	0.96	0.87	0.75	0.87	0.66	0.75	0.46	0.15
10	2-acetyl furan	balsamic, nutty	0.74	0.43	0.54	1.03	1.19	1.00	0.94	1.20
11	benzaldehyde	almond-like odor	1.01	1.12	1.21	1.10	0.95	1.32	1.41	1.53

12	2, 3-octandione	anis-like and brackish	0.36	0.24	0.33	0.99	0.59	0.98	0.82	0.58
13	β-pinene	woody-green pine-like	0.75	0.55	0.64	0.53	0.33	0.80	0.95	1.09
14	*D*-limonene	oranges	1.04	1.23	1.33	0.97	0.69	1.45	1.52	1.65
15	benzyl alcohol	light floral rose	1.47	1.35	1.67	1.29	1.41	1.27	1.15	1.04
16	benzeneacetaldehyde	hyacinth, lilac	2.27	2.15	2.37	0.69	0.93	2.74	2.52	2.45
17	β-ocimene		0.43	0.79	0.58	1.97	1.57	0.86	0.58	0.16
18	2-acetyl pyrrole	herbal, nutty, anisic	0.73	0.47	0.59	1.87	1.65	0.85	1.08	0.47
19	linalool oxide I	floral, green	1.76	1.65	1.97	0.59	0.88	1.46	1.37	1.15
20	linalool	bergamot oil, lavender	1.37	1.21	1.29	1.37	1.67	1.42	1.61	1.18
21	3, 7-dimethylocta-1, 5, 7-trien-3-ol		1.76	1.47	2.05	2.75	2.25	1.43	1.35	1.15
22	phenethyl alcohol	rose	2.26	2.79	2.48	1.47	1.90	2.21	2.15	2.42
23	α-terpineol	lilac	3.36	3.70	3.50	2.33	2.67	1.87	1.70	2.29
24	safranal	slightly spicy	0.88	0.78	0.98	0.98	0.38	0.60	0.98	0.96
25	2, 3-dihydrobenzofuran	fenugreek	2.33	2.82	2.46	4.09	3.73	3.04	2.08	2.90
26	nerol	pleasant geranium	0.70	0.46	0.65	0.74	0.36	0.69	0.99	0.17
27	nonanoic acid	fatty, green	0.77	0.64	0.56	0.98	0.76	0.80	0.96	0.38
28	jasmone	jasmine	1.76	1.55	1.64	1.38	1.18	1.15	1.05	1.47
29	β-damascenone	rose	0.77	0.99	0.86	1.55	1.87	1.23	1.20	1.32
30	α-ionone	woody, violet	0.78	0.48	0.75	1.33	1.44	0.98	0.85	0.30
31	geranylacetone	sweet-floral, fruity	1.62	1.87	1.72	2.91	2.55	2.28	2.19	2.58
32	β-ionone	woody	2.43	2.78	2.82	3.05	3.78	3.32	3.18	3.03
33	n-pentadecane		0.79	0.66	0.97	0.84	0.35	1.48	1.37	1.75
34	dihydroactinidiolide	sweet creamy, nut-like	7.25	6.65	6.47	8.05	6.54	4.17	4.65	4.72
35	tetrahydro-2,2,5,5-tetramethyl furan		1.36	1.15	1.21	1.51	1.67	1.75	1.45	2.07
36	nerolidol	floral scent	5.53	5.37	5.70	6.14	6.84	3.97	3.37	3.13
37	2-hydroxy methyl ester benzoic acid		2.85	2.14	2.44	0.38	0.84	1.64	1.36	1.86
38	4-methyl-2-propyl furan		0.75	0.48	0.95	0.89	0.75	0.97	0.68	0.60
39	n-heptadecane		1.77	1.56	1.67	0.96	0.98	1.14	1.27	1.35
40	2, 6, 10, 14-tetramethylpentadecane		0.44	0.32	0.63	0.20	0.10	0.97	0.73	0.20
41	farnesylacetone	creamy, winey	0.76	0.64	0.96	1.32	1.12	0.40	0.96	0.64
42	methyl hexadecanoate		0.78	0.99	0.85	0.75	0.54	0.84	0.43	0.08
43	5,6,7,7a-tetra 2(4)-benzofuranone		2.41	2.03	2.37	0.36	0.13	2.42	2.56	2.62
44	hexadecanoic acid	fatty	1.96	1.47	1.89	2.14	2.35	1.97	1.87	2.40
45	3-butyl-1-oxide-pyridine		1.46	1.21	1.31	0.68	0.84	1.85	1.51	2.85
46	3-ethyl-2,5-dimethyl-pyrazine		2.53	2.22	2.14	1.35	1.50	2.84	2.42	2.14

3.2. Principal component analysis

Principal components analysis (PCA) was conducted using the relative peak areas for the 46 compounds from 15 tea samples which are reported in Tabs. 1 and 2.

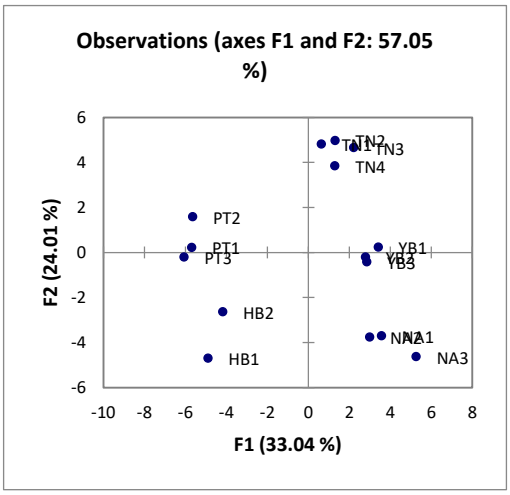

Figure 1. PCA biplot showing relationship between the green tea samples.

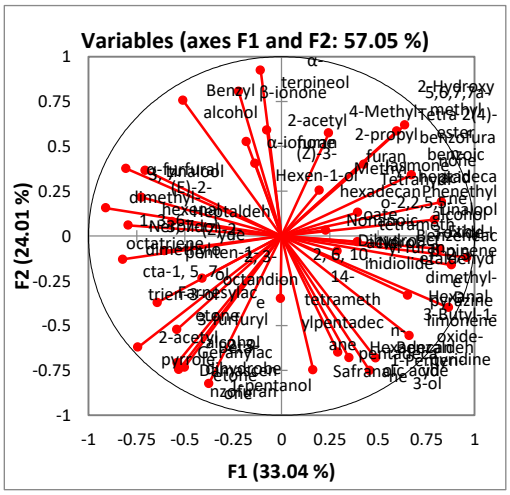

Figure 2. PCA biplot showing relationship variables of the volatile compounds.

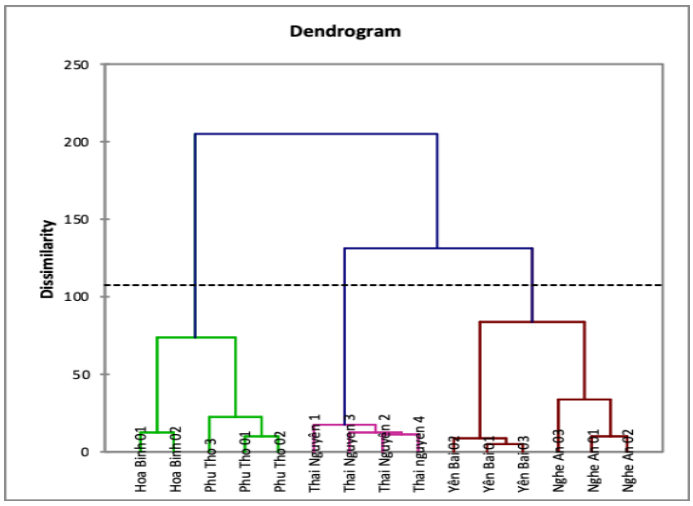

Figure 3. Clustering analyses of the relative peak areas of 15 green tea samples.

In this data set, principal components F1 and F2 explained 33.01 % and 24.03 % of the variation of the data, respectively. *D*-limonene, benzene acetaldehyde, 3, 7-dimethyl-1, 3, 6-octatriene, jasmone, and tetrahydro-2,2,5,5-tetramethyl furan were the main vectors for F1. F2 accounted for α-terpineol, β-ionone, α-terpineol, α-ionone and linalool. It is clear from the PCA map (Fig. 1) that tea samples from certain provinces are located on a closed location and can be separated from the samples of other provinces. From this result is can be seen green teas of certain geographical origin in this study was the differentiated based on volatile chemical composition.

3.3. Hierarchical clustering analysis (HCA)

Hierarchical clustering analysis (HCA) was also performed to discriminate between green tea samples of different categories. In our study, HCA was defined by Euclidean distances and Ward's linkage method using the whole data set. Here the cluster analysis was carried out based on the similarity on the peak intensities of each tea sample. As a result of the statistical analysis we could obtain clusters of the different tea samples according to the origin (Fig. 3). Three clusters included group I (Hoa Binh and Phu Tho), group II (Thai Nguyen), and group III (Yen Bai and Nghe An) could be generated at a dissimilarity of higher than 100 in the dendrogram, while cluster has dissimilarity at 50 could be assigned to Yen Bai teas vs Nghe An teas, and Hoa Binh vs Phu Tho teas, respectively (Fig. 3). This result is similar to the PCA showing the grouping of green teas from different province.

4. CONCLUSIONS

In this study, a comprehensive comparison of aroma profiles of green teas collected from different production areas was performed using HS-HPME/GC-MS. Common volatile compounds in typically brewed green tea were identified and the classification of green tea from different geographical origins was carried out by principal component analysis and hierarchical cluster analysis. Our results demonstrate that the developed method in this study can be used to discriminate the Vietnamese green tea samples from different geographical origins with high reliability.

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Authors contributions: HQT: Methodology, Investigation. CTTQ: Supervision. VHS: Data analysis. NTT: Formal analysis, Supervision, Funding acquisition.

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