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Phytochemical analysis of trifoliolate orange during fermentation by HPLC–DAD–ESI–MS/MS coupled with multivariate statistical analysis

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ABSTRACT

During the process of fermentation, the chemical compositions of trifoliolate orange (*Poncirus trifoliolate* (L.) Raf) changed greatly. To provide a completely phytochemical profile, high-performance liquid chromatography-diode array detector-hyphenated with tandem mass spectrometry (HPLC–DAD–ESI–MS/MS) has been successfully applied to screen and identify the unknown constituents of trifoliolate orange during fermentation, which make it available for the quality control of fermented products. Multivariate statistical analysis was performed to classify the trifoliolate oranges based on the status of fermentation. A total of 8 components were identified among the samples. Hierarchical Clustering Analysis (HCA) and Principal Component Analysis (PCA) demonstrated the fermented and unfermented trifoliolate oranges were obviously different, an effective and reliable Partial Least Square Discriminate Analysis (PLS-DA) technique was more suitable to provide accurate discrimination of test samples based their different chemical patterns. Furthermore, a permutation validated the reliability of PLS-DA and variable importance plot revealed that the characterized syringing, naringin, and poncirin showed the high ability to distinguish the trifoliolate oranges during fermentation. The present investigation could provide detailed information for the quality control and evaluation of trifoliolate oranges during the fermentation process.

KEYWORDS

trifoliolate orange, HPLC–DAD–ESI–MS/MS, dynamic changes, fermentation, multivariate statistical analysis

INTRODUCTION

Trifoliolate orange or *Poncirus trifoliolate* (L.) Raf is a semi-deciduous or deciduous shrub categorized in the family Rutaceae and the genus Poncirus, which has been widely planted in China and Korea for many years [1]. Trifoliolate orange is an important source of medicinal material and food in East Asia since it has high nutritional values and therapeutic benefits [2]. Numerous *in-vitro* and *in-vivo* studies demonstrate trifoliolate orange possess diverse biological activities, such as anti-inflammation, anti-tumor, antidiabetic, and anti-anaphylactic effects [3–6].

The fresh trifoliolate orange is not appropriate for direct consumption as it has very little pulp, many seeds, and a bitter taste. Consequently, it is commonly dried and crushed using in daily life. Recently, fermentation technology has been applied to the processing of trifoliolate orange to reduce its bitterness and enhance its nutritional value and biological activities. However, the complex fermentation process makes it hard to standardize and evaluate the quality of fermented trifoliolate oranges. It is a known fact that the change of chemical composition occurs in the process of fermentation, which leads to an increase in biological activity and nutritive values [7, 8]. Therefore, it is urgent to monitor changes in chemical

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compositions of trifoliate oranges occurring during the fermentation process for the quality control of fermented trifoliate oranges.

In this study, high-performance liquid chromatography-diode array detector-hyphenated with tandem mass spectrometry (HPLC–DAD–ESI–MS/MS) was performed to investigate the dynamic changes of trifoliate oranges during the different fermentation statuses. Multivariate statistical analyses, including Hierarchical Clustering Analysis (HCA), Principal Component Analysis (PCA), and Partial Least Square Discriminate Analysis (PLS–DA), were conducted to screen the characteristic components as chemical indicators for assessing the quality of fermented trifoliate oranges.

MATERIALS AND METHODS

Materials and chemicals

HPLC-grade acetonitrile and methanol were purchased from Burdick&Jackson (Muskegon, MI, USA); a buffer containing formic acid, acetic acid, and trifluoroacetic acid (MS grade) was bought from Sigma-Aldrich (St. Louis, Mo, USA). The reference compounds of citric acid, naringin, and hesperidin were achieved from Sigma-Aldrich Chemical Co. (St. Louis, Mo, USA). Trifoliate oranges were obtained from a traditional market in Jeju city and identified by Professor Young Ho Kim (College of Pharmacy, Chungnam National University). Distilled water was purified using a Milli-Q system (Sinhan, Seoul, Korea).

Fermentation of trifoliate oranges

The fresh trifoliate orange was crushed and added with water in a ratio of 1–3. Then, the obtained water-soluble solution was pasteurized for 30 min at 70 °C. After the solution was cooled to room temperature, which was added with the bacterial starter and incubated at 37° for 0 (F0), 5 (F5), 10 (F10), 20 (F20), and 30 hours (F30). This experiment was duplicated four times to validate its stability and reproducibility.

Sample and standard preparation

The fermented trifoliate orange solutions (10 mL) were added to 10 mL of methanol in a conical flask and sonicated at 45 °C for 30 min by a Mujigae ultrasonic machine (Seoul, Korea). The extracted solutions were then centrifugated for 5 min at 3,000 rpm and filtered through a 0.22 µm syringe filter before being injected into the HPLC system. All reference standards were prepared at the concentrations of 1.0 mg/mL in a methanol solution. The extract and standard solutions were then diluted 20-fold for LC–MS analysis. Finally, these solutions were stored at –20 °C until HPLC–DAD–ESI–MS/MS analysis.

Phytochemical analysis by HPLC–DAD–ESI–MS/MS

MS spectra were analyzed on a Shimadzu LCMS-8040 system (Kyoto, Japan) with an electrospray ionization (ESI)

source. ESI mass spectrometry was performed at the interface voltage of –3.5 kV for the negative mode and at 4.5 kV for the positive mode. The parameters of optimized ionization conditions are as follows: drying gas, 15 L/min; desolvation line temperature, 250 °C; heat block temperature, 350 °C; nebulizing gas, 3 L/min. LC–MS data were recorded in the range of 100–1,000 *m/z* with the full scan mode. The MS/MS analysis was automatically performed by a product ion survey scan at positive and negative modes. The detection wavelength range of DAD was set up at 190–400 nm. A Hecor C18 column (250 × 4.6 mm, 5 µm, RStech, Daejeon, Korea) was employed as the stationary phase. To optimize chromatographic separation, various HPLC parameters were verified, including the composition of mobile phase (acetonitrile–water and methanol–water containing different buffers, such as formic acid, acetic acid, and trifluoroacetic acid), the gradient program, and the flow rate of mobile phase (0.5, 0.7, 0.9, 1.0 and 1.2 mL/min). Finally, the best conditions were achieved with a chromatographic method with 0.1% formic acid, both in water (v/v) (solvent A) and in acetonitrile (solvent B), 0.1% formic acid, v/v, and an optimized gradient elution program from 7% to 40% of solvent B over 50 min with a flow rate of 1.0 mL/min.

Data processing and multivariate statistical analysis

Unsupervised PCA and HCA were utilized as the first step in data analysis. Based on the chemical variations existed in the fermented samples, these unsupervised methods enable the visualization of the hidden patterns by decreasing the dimensions of data and preserving as much as possible the original information present in data sets. PLS–DA method is a supervised method that can maximize the covariance between variables *X* and response variables *Y* to classify the samples [9]. The discriminant ability of each observed variable was decided by the variable influence on projection (VIP) scores. Variables with a VIP score >1 are deemed to be the potential marker compounds to distinguish different groups. HCA, PCA, and PLS–DA were performed using SIMCA 14.1 software (Umetric, Umea, Sweden).

RESULT AND DISCUSSION

Metabolomic identification of trifoliate orange during fermentation

HPLC–DAD–ESI–MS/MS was chosen to identify the major constituents of trifoliate orange. Some components were unambiguously identified by comparing them with standards. However, due to the lack of reference compounds, some of the peaks were tentatively assigned by comparing their elution order, UV spectra and MS spectra with the valuable references of components isolated and identified in the same plants or genus. The retention time (RT), UV, MS, and MS² spectral data were summarized in Table 1 and Supplementary Figs. S1–S11. The structures of characterized compounds were shown in Fig. 1.

Table 1. List of compounds detected in fermented and unfermented trifoliate orange by HPLC–DAD–ESI–MS/MS

Peak no.	RT (min)	$\lambda_{\max}$ (nm)	Precursor ion (m/z)	Product ion (m/z)	Molecular formula	Identification
1 ^a	3.45	197	190.85 $[M-H]^-$	111.00 $[M-H-2H_2O-CO_2]^-$	$C_6H_8O_7$	Citric acid
2	3.98	198	284.00 $[M+H]^+$	203	–	Unknown
3	7.64	220, 264	395.13 $[M+Na]^+$	233.02 $[M-Glc+Na]^+$	$C_{17}H_{24}O_9$	Syringin
4	13.45	221, 309	400.58 $[M+HCOOH-H]^+$	332.90	–	Unknown
5	13.85	283	337.29 $[M-H]^-$	225.45	–	Unknown
6	20.93	214, 285	765.20 $[M+Na]^+$	581.25 $[M-Glc+H]^+$; 435.11 $[M-Glc-Rha+H]^+$; 273.05 $[M-2Glc-Rha+H]^+$	$C_{33}H_{42}O_{19}$	Naringin 4'-glucoside
7	28.24	226, 282	603.10 $[M+Na]^+$	435.11 $[M-Rha+H]^+$; 273.06 $[M-Glc-Rha+H]^+$	$C_{27}H_{32}O_{14}$	Narirutin
8 ^a	29.89	227, 282	603.10 $[M+Na]^+$	435.11 $[M-Rha+H]^+$; 273.06 $[M-Glc-Rha+H]^+$	$C_{27}H_{32}O_{14}$	Naringin
9 ^a	32.05	224, 282	633.15 $[M+Na]^+$	449.10 $[M-Rha+H]^+$; 303.05 $[M-Glc-Rha+H]^+$	$C_{28}H_{34}O_{15}$	Hesperidin
10	39.42	227, 283	593.19 $[M-H]^-$	285.08 $[M-Glc-Rha-H]^-$	$C_{28}H_{34}O_{14}$	Neoponcirin
11	41.43	227, 281	617.20 $[M+Na]^+$	287.05 $[M-Glc-Rha+H]^+$	$C_{28}H_{34}O_{14}$	Poncirin

^a Compared with reference standards. Glc: -beta-D- glucopyranosyl; Rha: -alpha-L-rhamnopyranosyl.

Fig. 1. Chemical structures of identified compounds

In the positive and negative mode of the full scan, all compounds showed the pseudo molecular ion $[M+H]^+$ and/or adducted ions $[M+Na]^+$ in positive mode and exhibited $[M-H]^-$ and/or $[M+HCOOH-H]^-$ in negative mode. Thus, these ions were selected as parent ions for further identification of the components in the fermented and

unfermented trifoliate orange using the automatic product ion scan mode. Peak 1, which displayed positive molecular ions at m/z 214.95 ($[M+Na]^+$) and 407.05 ($[2M+Na]^+$), a negative molecular ion at m/z 190.85 ($[M-H]^-$), and fragment ion at m/z 111.00 ($[M-H-2H_2O-CO_2]^-$), was deduced to be citric acid (Supplementary Fig. S1). By comparing with standards, this peak was confidently assigned as citric acid. Similarly, peak 3 corresponding to the precursor ion at m/z 395.13 ($[M+Na]^+$), and product ion at m/z 233.02 by losing a glucopyranosyl group was determined as syringin (Supplementary Fig. S3) [10, 11]. From peak 6 to peak 8, all MS² spectra exhibited product ions at m/z 273, and the maximum absorption wavelength ($\lambda_{\max}$) was near 225 and 285 nm, which was attributed to protonated naringenin (Supplementary Figs. S6–S8). Hence, by comparison with available literature and standards, peak 6–8 were identified as naringin 4'-glucoside, narirutin, and naringin, respectively, which have been published in the previous studies of trifoliate orange [11–13]. Peak 9, which showed a $[M-H]^-$ at m/z 609.20 and a $[M+H]^+$ at m/z 633.15 that produced fragments at 449.10 and 303.05 because of losing a rhamnopyranosyl group and continued losing a glucopyranosyl group (Supplementary Fig. S9), respectively, was tentatively assigned as hesperidin, a bioactive component previously identified from trifoliate orange [14]. Peak 10 and 11 showed precursor ions $[M-H]^-$ at m/z 593.19 and $[M+Na]^+$ at m/z 585.20 (Supplementary Figs. S10 and S11). Meanwhile, during fragmentation, peak 10 and 11 yielded the protonated ion at m/z 286 by losing a rhamnopyranosyl and glucopyranosyl groups. Therefore, peak 9 and 10 were inferred as neoponcirin and poncirin, which were considered as the main components of anti-inflammatory activity in trifoliate oranges [15].

However, only depending on the data obtained from HPLC–DAD–ESI–MS/MS, it was unable to obtain a convincing structural identification of peak 2, 4 and 5.

Furthermore, peak 2 was solely exhibited in fermented trifoliate orange, which was suggested as a promising new compound that needs further analysis.

Chemometric analysis

Fig. 2 demonstrated that there were abundant differences in the chemical components of trifoliate oranges at different fermentation times. For example, peak 2 only appeared in the fermented trifoliate oranges with different content, while peak 3 was unable to be detected in the fermented trifoliate oranges. Therefore, multivariate statistical analyses, such as HCA, PCA, and PLS-DA, were implemented to visualize and characterize the difference of identified compounds in trifoliate orange during fermentation.

Hierarchical Clustering Analysis

HCA was a powerful multivariate statistical analysis method used to cluster samples into partitions or groups [16]. Thus, HCA was applied to evaluate affinities and differences of trifoliate oranges with different fermentation times. In this study, Ward's method is used as clustering algorithm and Euclidean distance is employed as the similarity measure to explain the scheme of clustering. Peak 8 (naringin) was preferred as a reference peak because it was the most abundant of all the 11 peaks. The relative peak areas of the 11 components of the 20 samples from different fermentation stages formed an 11×22 matrix. The result of HCA was present in Fig. 3a. It was obvious that 20 samples could be sorted into two clusters. Cluster-I was formed by the unfermented trifoliate orange samples. Trifoliate oranges fermented for 5, 10, 20, and 30 h were classified to cluster-II. Additionally, as shown in the HCA plot, the fermented trifoliate oranges with the fermentation time of 30 h was apparently different from that of other fermented samples. In other words, the qualities of the fermented trifoliate oranges with the fermentation time of 30 h were quite different from those of other fermented samples. This result is highly consistent with the visual comparison of their chromatograms (Fig. 2), suggesting their difference in the content and distribution of chemical constitutions. Nonetheless, how to more exactly to explain the difference between the individual samples in the same cluster was found to be a complication of HCA. From the point of view, the chemical pattern recognition approaches, including PCA and PLS-DA, should be taken into account for further evaluate sample clustering based on their fermentation degree.

Principal Component Analysis

To assess the discrimination ability of the identified constitutions and classify the samples from different fermentation status, PCA was performed using the same method of HCA for data preprocessing. As shown in Fig. 3b, the score plot of PCA showed the obvious difference between the fermented and unfermented samples as clustered by HCA. The first two principal components could explain 91.85% of the data variance. Therefore, the first two components could

Fig. 2. HPLC–DAD chromatograms at 280 nm of the detected compounds in trifoliate oranges during fermentation. Solvent A (water, 0.1% formic acid, v/v) and solvent B (acetonitrile, 0.1% formic acid, v/v) were carried out for gradient elution program from 7 to 40% of solvent B over 50 min with a flow rate of 1.0 mL/min. (Compound 1, 8 and 9 were compared with standards)

be employed as the marker compounds to discriminate the unfermented and fermented samples for the quality evaluation of trifoliate orange in the processing of industrial production. However, the overlaying clusters were also observed among the samples from the different fermentation processes of samples, such as the samples from the fermentation of 5 and 10 h were crossed and could not be classified, which demonstrated that the unsupervised PCA approach could only classify the fermented and unfermented samples. From the results of these samples, unsupervised PCA classification using basic data achieved from the 20 samples could not be able to provide accurate and reliable clustering by exact fermentation time. Given this situation, the supervised PLS-DA was further performed to discriminate different fermentation status of samples based on characteristic components in the samples.

Partial Least Square-Discriminate Analysis

To produce a high level of sample discrimination and group separation, a supervised PLS-DA method was executed. PLS-DA was usually used to discriminate two or more groups by exploring the variables (X matrix) that are associate with group membership (Y matrix). In this method, the axes are

Fig. 3. HCA and PCA of trifoliate orange samples at different fermentation times. (a) Cluster analysis of trifoliate orange samples at different fermentation times. (b) PCA score plot of trifoliate orange samples at different fermentation times

calculated to maximize the partition between groups and can be applied to verify partition that would otherwise be across three or more principal components. The performance of the PLS-DA model could be evaluated using the R^2 and Q^2 parameters, both of which change between 0 and 1, where 1 suggested an excellent fit. R^2X is another important parameter to assess the developed PLS-DA model, which indicating how much of the variation within a data set can be explained by the various components of the model. In this study, based on the common peaks of HPLC-PDA chromatogram, the presented score plot of PLS-DA demonstrated that the 20 samples could be classified into 5 groups (Fig. 4). The values of R^2X and R^2Y were 0.999 and 0.975 in the model of PLS-DA, respectively, which indicated that this established model was reliable and excellent for prediction and fitness [17, 18]. Furthermore, a permutation test was operated to evaluate the risk of overfitting of the established model, results revealed that this PLS-DA model was acceptable as the intercept values of R^2 and Q^2 were 0.224 and -0.613 , respectively (Fig. 5a). Therefore, the PLS-DA technique was more effective than PCA and HCA approach to provide an accurate classification of experimental samples based on the different constitutions of them, which also can be applied to evaluate the quality of trifoliate oranges from different fermentation statuses.

On the bases of the PLS-DA model, a loading plot was built up to present the ability of each component to distinguish samples. As illustrated in Fig. 5b, all the characterized peaks were ranked on the loading diagram according to their contribution value. Three characteristic compounds (syringing, naringin, and poncirin) showed considerable contribution to the classification of samples because of their high VIP values. At the same time, many *in-vivo*, and *in-vitro* studies have proved that syringing and naringin have antioxidant, antimicrobial, anti-inflammatory, enhancement of CaMKII (calcium/calmodulin-dependent protein kinase II) and antinociceptive activities [19–21]. Thus, these components have the potential to be selected as

Fig. 4. PLS-DA score plot of trifoliate orange samples at different fermentation times

Fig. 5. R^2 and Q^2 intercept values from 20 permutations (a) and variable importance plot of identified components in trifoliolate orange samples based on PLS-DA (b)

marker compounds to control the quality of trifoliolate oranges in the process of fermentation.

CONCLUSION

In this study, the major components of water-soluble juices of trifoliolate oranges during different fermentation times were analyzed using HPLC–DAD–ESI–MS/MS, which provided a better understanding of changes of major bioactive constituents in trifoliolate oranges during the fermentation process. The characteristic compounds of the trifoliolate orange samples were identified and validated by chemometric analysis. PCA and HCA obviously revealed the fermented and non-fermented samples were extremely dissimilar. A reliable and effective PLS-DA model was created to classify the trifoliolate orange samples based on the fermentation status. Besides, three marker compounds were discovered to be the most discriminant variables for the classification and quality evaluation of trifoliolate orange samples.

Conflict of interest: None of the authors has any conflict of interest to declare.

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SUPPLEMENTARY MATERIAL

Supplementary data to this article can be found online at <https://doi.org/10.1556/1326.2020.00818>.

REFERENCES

- Jang, Y.; Kim, E. K.; Shim, W. S. Phytotherapeutic effects of the fruits of *Poncirus trifoliata* (L.) Raf. on cancer, inflammation, and digestive dysfunction. *Phytother. Res.* **2018**, 32(4), 616–24.
- Nizamutdinova, I. T.; Jeong, J. J.; Xu, G. H.; Lee, S.-H.; Kang, S. S.; Kim, Y. S.; Chang, K. C.; Kim, H. J. Hesperidin, hesperidin methyl chalone and phellopterin from *Poncirus trifoliata* (Rutaceae) differentially regulate the expression of adhesion molecules in tumor necrosis factor- α -stimulated human umbilical vein endothelial cells. *Int. Immunopharmacol.* **2008**, 8(5), 670–8.
- Han, A.-R.; Kim, J.-B.; Lee, J.; Nam, J.-W.; Lee, I.-S.; Shim, C.-K.; Lee, K.-T.; Seo, E.-K. A new flavanone glycoside from the dried immature fruits of *Poncirus trifoliata*. *Chem. Pharm. Bull.* **2007**, 55(8), 1270–3.
- Shin, E. M.; Zhou, H. Y.; Xu, G. H.; Lee, S. H.; Merfort, I.; Kim, Y. S. Anti-inflammatory activity of hispidol A 25-methyl ether, a triterpenoid isolated from *Poncirus trifoliata* Fructus. *Eur. J. Pharmacol.* **2010**, 627(1–3), 318–24.
- Yi, J.-M.; Kim, M.-S.; Koo, H.-N.; Song, B.-K.; Yoo, Y.-H.; Kim, H.-M. *Poncirus trifoliata* fruit induces apoptosis in human promyelocytic leukemia cells. *Clin. Chim. Acta* **2004**, 340(1–2), 179–85.
- Lee, Y.; Kim, D.; Kim, S.; Shin, T.; Kim, H. Antianaphylactic activity of *Poncirus trifoliata* fruit extract. *J. Ethnopharmacol.* **1996**, 54(2–3), 77–84.
- Ejigui, J.; Savoie, L.; Marin, J.; Desrosiers, T. Beneficial changes and drawbacks of a traditional fermentation process on chemical composition and antinutritional factors of yellow maize (*Zea mays*). *J. Biol. Sci.* **2005**, 5(5), 590–6.
- Zhang, Z.; Lei, Z.; Yun, L.; Zhongzhi, L.; Chen, Y. Chemical composition and bioactivity changes in stale rice after fermentation with *Cordyceps sinensis*. *J. Biosci. Bioeng.* **2008**, 106(2), 188–93.
- Folch-Fortuny, A.; Prats-Montalbán, J. M.; Cubero, S.; Blasco, J.; Ferrer, A. VIS/NIR hyperspectral imaging and N-way PLS-DA models for detection of decay lesions in citrus fruits. *Chemometrics Intellig. Lab. Syst.* **2016**, 156, 241–8.
- Zhu, J.; Yi, X.; Huang, P.; Chen, S.; Wu, Y. Drug-protein binding of Danhong injection and the potential influence of drug combination with aspirin: Insight by ultrafiltration LC–MS and molecular modeling. *J. Pharm. Biomed. Anal.* **2017**, 134, 100–7.
- Tundis, R.; Bonesi, M.; Sicari, V.; Pellicanò, T.; Tenuta, M.; Leporini, M.; Menichini, F.; Loizzo, M. *Poncirus trifoliata* (L.) Raf.: Chemical composition, antioxidant properties and hypoglycaemic activity via the inhibition of α -amylase and α -glucosidase enzymes. *J. Funct. Foods* **2016**, 25, 477–85.
- Liu, S.; Hu, L.; Jiang, D.; Xi, W. Effect of post-harvest LED and UV light irradiation on the accumulation of flavonoids and limonoids in the segments of Newhall navel oranges (*Citrus sinensis* Osbeck). *Molecules* **2019**, 24(9), 1755.
- Shi, Z.; Li, T.; Liu, Y.; Cai, T.; Yao, W.; Jiang, J.; He, Y.; Shan, L. Hepatoprotective and Anti-oxidative effects of total flavonoids

- from Qu Zhi Qiao (Fruit of *Citrus Paradisi* cv. Changshanhuoyou) on nonalcoholic steatohepatitis in vivo and in vitro through Nrf2-ARE signaling pathway. *Front. Pharmacol.* **2020**, *11*, 483.
14. Hussain, S.; Curk, F.; Dhuique-Mayer, C.; Urban, L.; Ollitrault, P.; Luro, F.; Morillon, R. Autotetraploid trifoliate orange (*Poncirus trifoliata*) rootstocks do not impact clementine quality but reduce fruit yields and highly modify rootstock/scion physiology. *Sci Horti-Amsterdam* **2012**, *134*, 100–7.
15. Avula, B.; Joshi, V.; Weerasooriya, A.; Khan, I. Liquid chromatography for separation and quantitative determination of adrenergic amines and flavonoids from *Poncirus trifoliatus* Raf. fruits at different stages of growth. *Chromatographia* **2005**, *62*(7–8), 379–83.
16. Wang, H.; Chen, M.; Li, J.; Chen, N.; Chang, Y.; Dou, Z.; Zhang, Y.; Zhuang, P.; Yang, Z. Quality consistency evaluation of Kudiezi injection based on multivariate statistical analysis of the multidimensional chromatographic fingerprint. *J. Pharm. Biomed. Anal.* **2020**, *177*, 112868.
17. Bi, Q.; Liu, Q.; Han, S.; Zhang, L.; Xing, L.; Zhang, X.; Wang, Z.; Miao, Y.; Tan, N. An integrated multiple reaction monitoring strategy based on predicted precursor ions and characteristic product ions for global profiling Rubiaceae-type cyclopeptides in three Rubiaspecies. *J. Chromatogr. A* **2020**, 460902.
18. Ao, H.; Wang, J.; Chen, L.; Li, S.; Dai, C. Comparison of volatile oil between the fruits of *Amomum villosum* Lour. and *Amomum villosum* Lour. var. *xanthioides* TL Wu et Senjen based on GC-MS and chemometric techniques. *Molecules* **2019**, *24*(9), 1663.
19. Al-Saman, M. A.; Abdella, A.; Mazrou, K. E.; Tayel, A. A.; Irmak, S. Antimicrobial and antioxidant activities of different extracts of the peel of kumquat (*Citrus japonica* Thunb). *J. Food Meas. Charact.* **2019**, *13*(4), 3221–9.
20. Oh, Y.-C.; Jeong, Y. H.; Cho, W.-K.; Gu, M.-J.; Ma, J. Y. Inhibitory effects of palmultang on inflammatory mediator production related to suppression of NF- κ B and MAPK pathways and induction of HO-1 expression in macrophages. *Int. J. Mol. Sci.* **2014**, *15*(5), 8443–57.
21. Wang, D.-M.; Yang, Y.-J.; Zhang, L.; Zhang, X.; Guan, F.-F.; Zhang, L.-F. Naringin enhances CaMKII activity and improves long-term memory in a mouse model of Alzheimer's disease. *Int. J. Mol. Sci.* **2013**, *14*(3), 5576–86.