Nat. Volatiles & Essent. Oils, 2020; 7(4): 26-33
DOI: 10.37929/nveo.780505

RESEARCH ARTICLE

Extraction process optimization and characterization of the Pomelo (*Citrus grandis* L.) peel essential oils grown in Tien Giang Province, Vietnam

Dao Tan Phat[1,2,3], Kha Chan Tuyen[4], Xuan Phong Huynh[6] and Tran Thanh Truc[5,*]

[1]Graduate University of Science and Technology, Vietnam Academy of Science and Technology, Ha Noi, VIETNAM
[2]Center of Excellence for Biochemistry and Natural Products, Nguyen Tat Thanh University, Ho Chi Minh City, VIETNAM
[3]NTT Hi-Tech Institute, Nguyen Tat Thanh University, Ho Chi Minh City, VIETNAM
[4]Faculty of Food Science and Technology, Nong Lam University, Ho Chi Minh City, VIETNAM
[5]College of Agriculture, Can Tho University, Can Tho City, VIETNAM
[6]Biotechnology Research and Development Institute, Can Tho City, VIETNAM

*Corresponding author. Email: tttruc@ctu.edu.vn

Submitted: 14.08.2020; Accepted: 19.12.2020

Abstract
Hydrodistillation was used to obtain the essential oil from pomelo (*Citrus grandis* L.) peels collected in Vietnam. The effects of extraction variables including size of material, temperature, extraction time, and water-to-material ratio on the yield of essential oil were investigated. In addition, to assess the quality of pomelo essential oil, the chemical composition was compared. The optimized conditions were as follows: material size of grind, temperature 120°C, water to material ratio 5 mL/g, and extraction time 105 min., respectively. The chemical composition of the pomelo oil was then determined by GC-MS, where 5 components were identified, of which, limonene was the highest (97.1%). This method can be considered as a green method of extraction method as it is less energy intensive process and offers high performance. The results are expected to aid in justification of hydrodistillation in industrial applications and in refining of extraction parameters.

Keywords: Pomelo peels, *Citrus grandis* L., hydro-distillation, GC-MS analysis

Introduction

Essential oils are widely applied in various industries such as cosmetics, food and pharmaceuticals and carry wide clinical implications (Dao et al., 2019a; Thuong Nhan et al., 2020; Thuy et al., 2019). To obtain essential oils from raw materials, a wide range of methods could be adopted. While old, traditional methods, such as hydrodistillation and expression, only require simple instruments to produce essential oils with adequate quality, newly developed extraction techniques (instant controlled pressure drop technique, supercritical fluids, liquefied gas technology and ultrasound extraction) allow for better solvent and energy saving and capability to afford products with better quality and sustainability (Chemat et al., 2020). However, newer technologies are often more complex and require costly instruments such as microwave irradiators, steam generators and vacuum pumps. Therefore, intensification of established processes plays a key role in improving essential oil-derived products and have been attracting scientific attention recently.

Essential oils isolated from pomelo (*Citrus grandis* L.) fruits are a widely adopted agent with a wide range of applications and have been known as alternative for antimicrobial and antioxidant agents (Mokbel & Suganuma, 2006). It has been shown that bioactivities of the essential oils are dependent on their chemical compositions (Uysal et al., 2011). Therefore, chemical composition of pomelo essential oil from different countries is also a subject of extensive research. One important component of pomelo essential oil is limonene, accounting for around 85 to 96% of total content while myrcene, sabinene, α-pinene and α-terpinene have been reported at less than 2.5% (Tsai et al., 2017; Shaw, 1979). Other minor components including nootkatone, octanal, octyl acetate, citronellyl acetate, citral and carvone have been found at trace levels in the essential oil (<1.0%) and have been designated as the main agents of scents (Ortuno et al., 1995; Sawamura & Kuriyama, 1988). Such variance in pomelo essential oil composition could be attributable to the variety of the pomelo plant, the plant part, used extraction technique and process parameters. To date, numerous extraction methods including microwave-assisted hydrodistillation and supercritical CO_2 extraction have been attempted to isolate essential oil bearing extracts from pomelo materials (Hien et al., 2018; Gyawali et al., 2012). However, such processes lack applicability in producing essential oil at commercial scale due to their high operational costs, complexity and limited scalability.

In this study, pomelo essential oils were extracted from peels of pomelo collected from Tien Giang province, Vietnam. Although this region is known as a major producer of pomelo, essential oil of the fruit originating from this region was not extensively studied. Hydrodistillation was selected as the extraction method in this study due to its ease of operation, low cost and the ability to perform exhaustive extraction from raw materials. The extraction parameters were also optimized with respect to maximal essential oil yield and the obtained product was characterized by gas chromatography-mass spectrometry (GC-MS) to determine the volatile composition. The results are expected to contribute to enhanced valorization of pomelo fruits and justify the use of hydrodistillation in larger scale manufacturing processes.

Materials and Methods

Materials and chemical

Fresh pomelo (*Citrus grandis* L.) fruits were selected from Tien Giang province (latitudes 10°25’13’’N and longitudes 106°17’49’’E) of Vietnam in March 2020, then washed and peeled manually. The peels were cut into pieces of approximately 2x2 cm^2 and kept in a plastic bag at low temperatures (4°C). Prior to distillation, the sample was exactly weighted, treated and used immediately to avoid the loss of essential oil. The water content of fresh pomelo peels was 73.76±0.19, which was determined following the Association of Official Analytical Chemists (AOAC) method 925.10.

Hydro-distillation process

Hydro-distillation technique was used to extract pomelo peel essential oil. The procedure is as follows. First, 100g of peels were placed in a 1L round-bottom flask with 500 mL of distilled, deionized water and connected to a Clevenger’s distillation unit. The essential oil was extracted by hydro-distillation, which was carried out for 120 min. The extraction duration that allows exhaustive oil extraction from peel tissues was justified by previous studies (Dao et al., 2019; Thuy et al., 2019). The obtained essential oil, which was collected in Clevenger’s receiver, was separated and anhydrous with Na_2SO_4. The extracted essential oil was stored in a glass amber bottle and refrigerated at 4°C until further analysis. Each extraction was performed three times under same conditions.

Yield of pomelo peels oil (Y) obtained for every run that was calculated as follow (1):

$$\mathrm{Y}\ (\%) = \frac{Volume\ of\ essential\ oil (ml)}{Amount\ of\ raw\ materials\ (g)} 100\% \tag{1}$$

Single factor investigation was used to survey the optimum extraction conditions of the hydrodistillation process. Four factors including material size, water-material ratio, temperature and extraction time were consequently allowed to vary. The yield of essential oil was selected as the outcome of the optimization. The survey is performed to estimate the best conditions for the extraction process, and it was repeated to avoid errors.

Gas chromatography–mass spectrometry analysis

A Gas Chromatography-Mass Spectrometry (GC-MS) is used to analyze the composition of the essential oils of all extraction methods. 25 µL sample of essential oil in 1.0 mL n-hexane. Name of the equipment: GC Agilent 6890N, MS 5973 inert with HP5-MS column, head column pressure 9.3psi. GC-MS system was performed hold under the following conditions: carrier gas He; flow rate 1.0 mL/min; split 1:100; injection volume 1.0 µL; injection temperature 250°C; oven temperature progress included an initial hold at 50 °C for 2 min, and a rise to 80°C at 2°C /min, and them to 150°C at 5°C /min, continue rising to 200 °C at 10 °C /min and rise to 300 °C at 20°C /min for 5 min.

Results and Discussion

Single factor investigation

Effects of four experimental parameters on the yield of pomelo essential oil are shown in Figure 1. Figure 1a refers to the change in obtained essential oil yield when changing the size of materials. Generally, the performance of pomelo essential oil extraction improved as the size of the material decreased. Specifically, the efficiency increased from 1% to 1.8% when changing the size from the original size to puree. This can be explained as follows. The smaller the material size, the faster the water diffuses into the essential oil sacs, promoting the release of essential oils under thermal exposure and in turn causing better extraction yield. On the contrary, the large peel sizes will yield lesser oil due to small surface area in contact with the solvent. Therefore, grind peels have been chosen in subsequent investigations (Dao et al., 2019b; Tran et al., 2019).

One of the main factors influencing the extraction process is the extraction temperature. It is observed from Figure 1b that when the temperature increases from 100 to 120°C, the yield of the extracted essential oil gradually increases from 1.5 to 1.8%. However, the yield of essential oil ceased to increase at 1.8% and tended to decrease when rising temperature to 130°C and to 140°C. This observation can be explained from the fact that the heat and the steam generated at 120°C exerts stronger rupture on the plant cells, therefore accentuating the pushing of essential oil to the surrounding media. This is consistent with result of Tran et al. [2018] where the efficiency of the process of extracting plants was found to be greatly affected by temperature. Therefore, for pomelo peel material, the temperature of 120°C is the optimal for hydrodistillation and beyond this temperature, the essential oil might degrade thus impairing extraction yield.

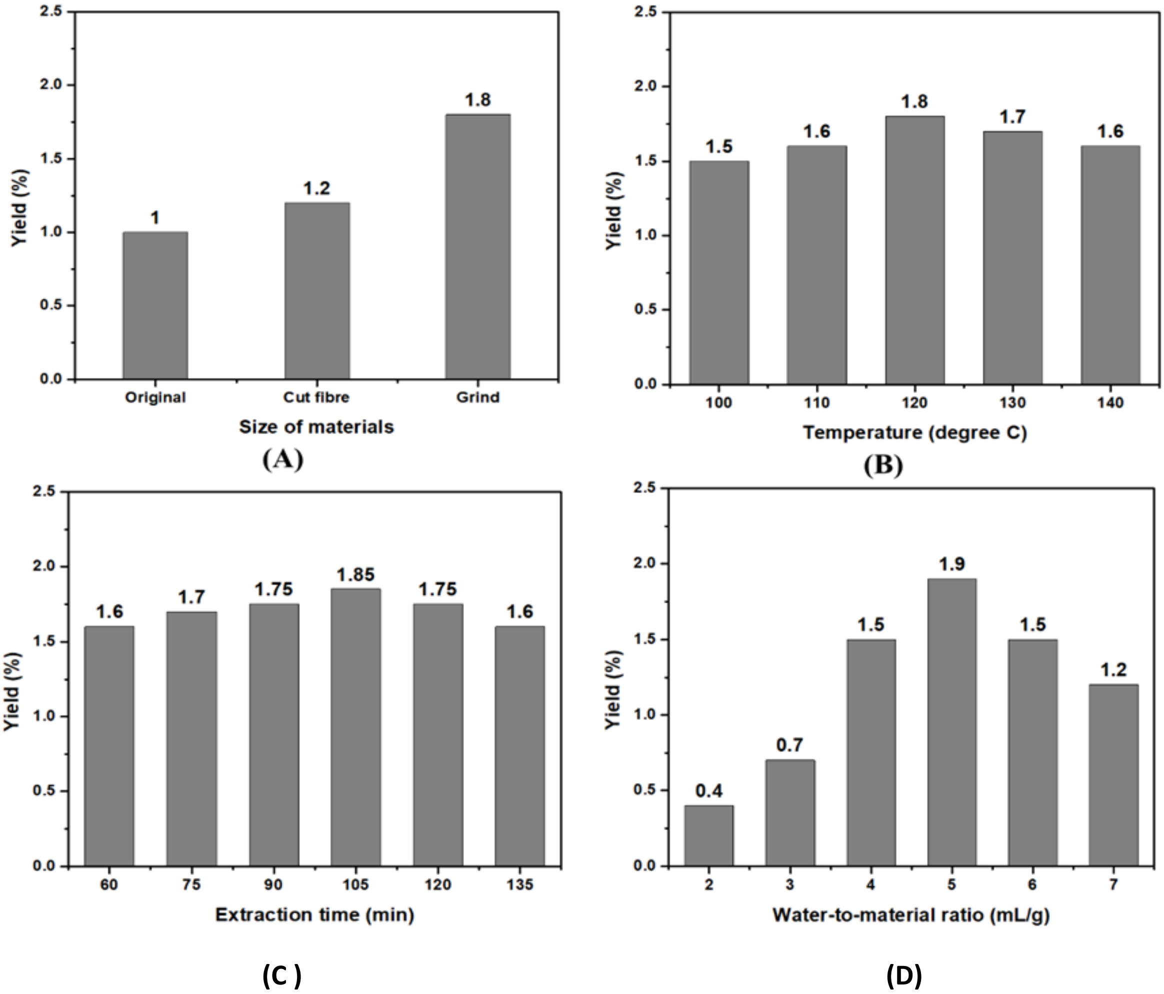
Similarly, the effect of extraction time on yield of pomelo essential oil can be seen in Figure 1c. At the first stage, extraction time and process yield were directly proportional to each other. At the 105-minute mark, yield reached its highest value at 1.85% and then decreased to 1.75% and to 1.6 % at 120 min and at 135 min, respectively. It is regarded that the longer the extraction time, the more oil is obtained. As distillation time extends to a certain limit, the amount of essential oil obtained reaches a state of saturation and is likely to be denatured due to prolonged heat exposure (Boukhatem et al., 2014). The determination of extraction time is related to energy consumption and production cost of the process. Therefore, 105 min is selected as appropriate duration to conduct the next experiment.

During distillation, at the boiling point, the essential oils in the plant cells diffuse out the surface of the material and are swept away by the steam. Simultaneously, water penetrates the material in the opposite direction and the oil continues to be swept into the water. The process occurs until the essential oil in the tissues completely escapes. Therefore, determining the ratio of water to raw materials is necessary for the

DOI: 10.37929/nveo.780505

extraction process to attain maximum yield. The effect of ratio water and material can be seen in Figure 1d. As the ratio changed, the process efficiency also changed, and the highest yield was achieved at 5: 1 mL/g. At a low water ratio of 2-3 mL/g, the low amount of collected essential oil is due to the insufficient amount of water required dissolve the colloidal wrappers surrounding the essential oil bag. This leaves a large quantity of residual essential oil in the material, impairing the efficiency of the process. On the other hand, at higher ratios such as 6-7:1 mL/g, the amount of essential oil that is obtained is also not high, possibly due to slow water evaporation rate and oil losses in the collection tube, effectively reducing the efficiency of the distillation process. In practical terms, large amounts of water also lead to prolonged distillation times and higher operation costs. The highest amount of essential oil obtained (1.9%) at the water-material ratio of 5:1 mL/g.

Figure 1. Effects of four experimental parameters on the yield of pomelo essential oil



(A) Size of the materials, (B) Temperature, (C) Extraction time, (D) Water-to-material ratio to the yield of the essential oil

The results of GC-MS

The compositions of essential oil from pomelo peels was determined by comparing the retention time and mass spectra of the compounds in the essential oil with the spectral data library. *Citrus* essential oils are usually characterized by the abundance of one or two main compounds. Essential oils from grapefruit which was reported to be strong antibacterial activity against the bacterial strains (Gram-negative and Gram-positive bacteria), yeast and mold tested by Chanthaphon et al. (2008). The mechanism of this

DOI: 10.37929/nveo.780505

bioactivity was elaborated by Lis-Balchin (1996) maintaining that limonene, α-pinene, and sabinene might disrupt membrane of microorganisms and inhibit ion transport processes and respiration.

In this study, around 100g of pomelo fruit peel yielded around 1.9 mL of essential oil using Clevenger's apparatus at optimal conditions. The composition of the volatile oil was analyzed by GC-MS (Table 1 and Figure 2). Five constituents were detected from peels, accounting for almost 100% essential oil content. limonene, α-pinene and β-myrcene were the main components in the essential oil. The most abundant component was limonene, a monoterpene hydrocarbon, at 97.1%. Other important constituents were β-myrcene (1.3%) and α-Pinene (0.7%). These results are similar with the other literature data for *Citrus* genus (Uysal et al., 2011; Chen & Viljoen, 2010).

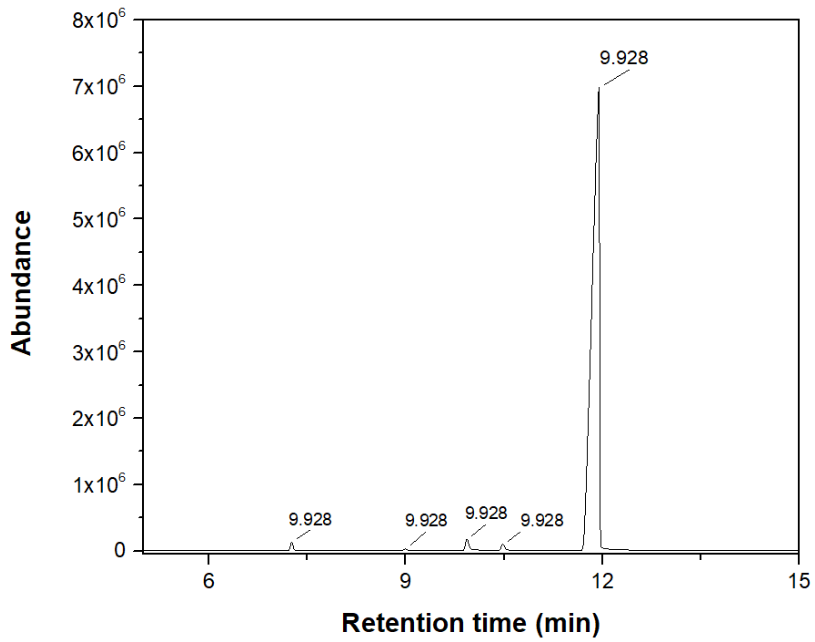
Limonene is a well-studied compound with diverse bioactivities and finds numerous uses in cosmetic, food and beverage industries. In agri-food applications, limonene is a common ingredient in pesticide, herbicide and anti-spoilage products. In medicinal field, limonene also finds clinical relevance due to its antioxidant activity. However, limonene instability and susceptibility against external conditions have called for development of preservation processes such as microencapsulation, coacervation and nano-emulsification and urged for further innovations in enhancing biocompatibility of resulting products (Ibanez et al., 2020; Mahato et al., 2019).

Citrus species have been well known as an abundance source for limonene, whose content has been reported to range from 30.1 to 75.5% (Patil et al., 2009; Kamal et al., 2011). The content of limonene in pomelo peel oil was also greatly varied depending on the origin of the material and the extraction technique. Our result indicates that hydrodistillation might be justifiable when it comes to extraction performance with regard to limonene content in the resulting pomelo peel oil. Khan et al. (2007) indicated that limonene in the essential oil hydrodistilled from Pakistani pomelo peels contributed to around 72.3% of total content. Meanwhile, essential oils obtained by pressing from Japanese and Kenyan peels showed limonene content of 87.1% and 94.8%, respectively (Sawamura et al., 2001; Njoroge et al., 2005). On the contrary, very low limonene content (3.7%) was determined in Korean pomelo volatiles extracted by supercritical fluid, which could be explained by more complex composition of supercritical extracts (Gyawali et al., 2012). These discrepancies suggest that both material origin and extraction technique could largely determine the composition of the essential oil products and that rationalization for extraction technique should be individually made for each purpose of use.

Table1. Chemical constituents of pomelo oil

Peak	**R.T min**	**Compounds**	**%**
1	7.261	α-Pinene	0.7
2	8.997	Sabinene	0.2
3	9.938	β-Myrcene	1.3
4	10.482	α-Phellandrene	0.7
5	11.936	Limonene	97.1

Figure 2. The GC-MS chromatogram of essential oil from pomelo peels



Conclusion

The extraction of essential oil from pomelo peels was attempted using hydro-distillation method. The process was optimized by varying several parameters including water-to-materials ratio, temperature, and extraction time. The optimum conditions for extraction process are as follows: temperature of 120°C, ratio of 5:1 mL/g, and extraction time of 105 minutes. Pomelo essential oil yield was 1.9% under these conditions. Besides, GC-MS analysis indicates that the composition of pomelo oil in Vietnam obtained using hydro-distillation method contains approximately 97% limonene. Therefore, hydro-distillation can be considered a green extraction method because it is a low energy process and does not require a subsequent solvent separation stage. Further studies should contemplate assessment of bioactivities of pomelo essential oil with respect to varying volatile compositions.

ACKNOWLEDGMENT

This research was funded by the Science and Technology Program of Ministry of Education and Training (Vietnam): "Application and Development of Advanced Technologies in Preservation and Processing of Aquatics and Agricultural Products in the Mekong Delta" (Code: CT2020.01.TCT-07).

REFERENCES

AOAC. (2000). Official Method of Analysis. 17th ed. Method 925.10. Association of Official Analytical Chemists, Rocville, MD, USA.

Boukhatem, M. N., Ferhat, M. A., Kameli, A., Saidi, F., & Kebir, H. T. (2014). Lemon grass (*Cymbopogon citratus*) essential oil as a potent anti-inflammatory and antifungal drugs. *Libyan Journal of Medicine, 9*(1), 25431.

Chanthaphon, S., Chanthachum, S., & Hongpattarakere, T. (2008). Antimicrobial activities of essential oils and crude extracts from tropical *Citrus* spp. against food-related microorganisms. *Songklanakarin Journal of Science & Technology, 30*.

Chemat, F., Abert Vian, M., Fabiano-Tixier, A.-S., Nutrizio, M., Režek Jambrak, A., Munekata, P. E. S., Lorenzo, J. M., Barba, F. J., Binello, A., & Cravotto, G. (2020). A review of sustainable and intensified techniques for extraction of food and natural products. *Green Chemistry, 22*(8), 2325–2353.

Chemat, F., Vian, M. A., Fabiano-Tixier, A. S., Nutrizio, M., Jambrak, A. R., Munekata, P. E. S., Lorenzo, J.M., Barba, F.J., Binello, A. & Cravotto, G. (2020). A review of sustainable and intensified techniques for extraction of food and natural products. *Green Chemistry, 22*(8), 2325-2353.

Chen, W., & Viljoen, A. M. (2010). Geraniol—a review of a commercially important fragrance material. *South African Journal of Botany, 76*(4), 643-651.

Dao, T. P., Nguyen, D. C., Nguyen, D. T., Tran, T. H., Nguyen, P. T. N., Le, N. T. H., Le, X.T., Nguyen, D.H., Vo, D.V.N. & Bach, L. G. (2019b). Extraction Process of Essential Oil from *Plectranthus amboinicus* Using Microwave-Assisted Hydrodistillation and Evaluation of It's Antibacterial Activity. *Asian Journal of Chemistry, 31*, 977-81.

Dao, T. P., Tran, T. H., Quyen Ngo, T. C., Kieu Linh, H. T., Yen Trung, L. N., Danh, V. T., Le Ngoc, T. T., Yen Pham, N. D., Quan, P. M., & Toan, T. Q. (2019). Extraction of essential oils from Vietnam's orange (*Citrus sinensis*) peels by hydrodistillation: Modeling and process optimization. *Asian Journal of Chemistry, 31*(12), 2827–2833.

Gyawali, R., Jeon, D. H., Moon, J., Kim, H., Song, Y. W., Hyun, H. B., Jeong, D., & Cho, S. K. (2012). Chemical composition and antiproliferative activity of supercritical extract of *Citrus grandis* (L.) Osbeck fruits from Korea. *Journal of Essential Oil Bearing Plants, 15*(6), 915–925.

Hien, T. T., Nhan, N. P. T., Trinh, N. D., Ho, V. T. T., & Bach, L. G. (2018). Optimizing the pomelo oils extraction process by microwave-assisted hydro-distillation using soft computing approaches. *Solid State Phenomena, 279*, 217–221.

Ibáñez, M. D., Sanchez-Ballester, N. M., & Blázquez, M. A. (2020). Encapsulated limonene: a pleasant lemon-like aroma with promising application in the agri-food industry. A Review. *Molecules, 25*(11), 2598.

Kamal, G. M., Anwar, F., Hussain, A. I., Sarri, N., & Ashraf, M. Y. (2011). Yield and chemical composition of *Citrus* essential oils as affected by drying pretreatment of peels. *International Food Research Journal, 18*(4), 1275.

Khan, M. N., Munawar, M. A., Munawar, S., Qureshi, A. K., & Rehman, S. (2007). Characterization of essential oil of local varieties of *Citrus grandis*. *Journal-Chemical Society of Pakistan, 29*(3), 272-274.

Lis-Balchin, M. (1996). Bioactivity of the enantiomers of limonene. *Medical Science Research, 24*, 309-310.

Mahato, N., Sinha, M., Sharma, K., Koteswararao, R., & Cho, M. H. (2019). Modern extraction and purification techniques for obtaining high purity food-grade bioactive compounds and value-added co-products from citrus wastes. *Foods, 8*(11), 523.

Mokbel, M. S., & Suganuma, T. (2006). Antioxidant and antimicrobial activities of the methanol extracts from pummelo (*Citrus grandis* Osbeck) fruit albedo tissues. *European Food Research and Technology, 224*(1), 39–47.

Njoroge, S. M., Koaze, H., Karanja, P. N., & Sawamura, M. (2005). Volatile constituents of redblush grapefruit (*Citrus paradisi*) and pummelo (*Citrus grandis*) peel essential oils from Kenya. *Journal of Agricultural and Food Chemistry, 53*(25), 9790-9794.

Ortuno, A., Garcia-Puig, D., Fuster, M. D., Perez, M. L., Sabater, F., Porras, I., Garcia-Lidon, A., & Del Rio, J. A. (1995). Flavanone and nootkatone levels in different varieties of grapefruit and pummelo. *Journal of Agricultural and Food Chemistry, 43*(1), 1–5.

Patil, J. R., Jayaprakasha, G. K., Murthy, K. C., Tichy, S. E., Chetti, M. B., & Patil, B. S. (2009). Apoptosis-mediated proliferation inhibition of human colon cancer cells by volatile principles of *Citrus aurantifolia*. *Food Chemistry, 114*(4), 1351-1358.

Sawamura, M., & Kuriyama, T. (1988). Quantitative determination of volatile constituents in the pummelo (*Citrus grandis* Osbeck forma *Tosa-buntan*). *Journal of Agricultural and Food Chemistry, 36*(3), 567–569.

Sawamura, M., Song, H. S., Choi, H. S., Sagawa, K., & Ukeda, H. (2001). Characteristic aroma components of Tosa-buntan (*Citrus grandis* Osbeck forma *Tosa*) fruit. *Food science and technology research, 7*(1), 45-49.

DOI: 10.37929/nveo.780505

Shaw, P. E. (1979). Review of quantitative analyses of citrus essential oils. *Journal of Agricultural and Food Chemistry, 27*(2), 246-257.

Thuong Nhan, N. P., Tan Thanh, V., Huynh Cang, M., Lam, T. D., Cam Huong, N., Hong Nhan, L. T., Thanh Truc, T., Tran, Q. T., & Bach, L. G. (2020). Microencapsulation of lemongrass (*Cymbopogon citratus*) essential oil *via* spray drying: Effects of feed emulsion parameters. *Processes, 8*(1), 40.

Thuy, D. T. T., Tuyen, T. T., Thuy, T. T. T., Minh, P. T. H., Tran, Q. T., Long, P. Q., Nguyen, D. C., Bach, L. G., & Chien, N. Q. (2019). Isolation process and compound identification of agarwood essential oils from *Aquilaria crassna* cultivated at three different locations in vietnam. *Processes, 7*(7), 432.

Tran, T. H., Ke Ha, L., Nguyen, D. C., Dao, T. P., Thi Hong Nhan, L., Nguyen, D. H., Nguyen, T. D., N. Vo, D.-V., Tran, Q. T., & Bach, L. G. (2019). The study on extraction process and analysis of components in essential oils of black pepper (*Piper nigrum* L.) seeds harvested in Gia Lai province, Vietnam. *Processes, 7*(2), 56.

Tran, T. H., Nguyen, H. H. H., Nguyen, D. C., Nguyen, T. Q., Tan, H., Nhan, L. T. H., Nguyen, D.H., Tran, L.D., Do, S.T. & Nguyen, T. D. (2018). Optimization of microwave-assisted extraction of essential oil from Vietnamese Basil (*Ocimum basilicum* L.) using response surface methodology. *Processes, 6*(11), 206.

Tsai, M.-L., Lin, C.-D., Khoo, K. A., Wang, M.-Y., Kuan, T.-K., Lin, W.-C., Zhang, Y.-N., & Wang, Y.-Y. (2017). Composition and bioactivity of essential oil from *Citrus grandis* (L.) Osbeck 'mato peiyu' leaf. *Molecules, 22*(12), 2154.

Uysal, B., Sozmen, F., Aktas, O., Oksal, B. S., & Kose, E. O. (2011). Essential oil composition and antibacterial activity of the grapefruit (*Citrus paradisi* L.) peel essential oils obtained by solvent-free microwave extraction: comparison with hydrodistillation. *International Journal of Food Science & Technology, 46*(7), 1455-1461.