

Advancements in the singlemer separation of polymethoxyflavones in citrus peels

Junfeng Shen^{a*}, Haiqing Zhu^b, Yixing Zhu^b, Wenyu Wang^b, Jiamin Wang^b
and Alexander Gossiau^c

^aCollege of Biology and Agricultural Resources, Huanggang Normal University, Huanggang 438000, China

^bCollege of Food Science and Pharmacy, Xinjiang Agricultural University, Urumqi 830052, China

^cDepartment of Science, City University of New York, BMCC, 199 Chambers Street, New York, NY 10007, USA

*Corresponding author: Junfeng Shen, College of Biology and Agricultural Resources, Huanggang Normal University, Huanggang 438000, China. E-mail: Dummauthorname@xxx.com

DOI: 10.26599/JFB.2025.95029401

Received: March 12, 2025; Revised received & accepted: March 26, 2025

Citation: Shen, J., Zhu, H., Zhu, Y., Wang, W., Wang, J., and Gossiau, A. (2025). Advancements in the singlemer separation of polymethoxyflavones in citrus peels. J. Food Bioact. 000: 000–000.

Abstract

Polymethoxyflavones (PMFs) including 5,6,7,4'-tetramethoxyflavone (TMF), tangeretin, sinensetin, nobiletin, and 3,5,6,7,8,3',4'-heptamethoxyflavone (HMF), possess multiple significant health-promoting activities. They have drawn increasing attention in recent years. However, the supply of pure singlemers of these PMFs, even the major content of tangeretin, nobiletin, HMF and TMF has been a limiting factor for their *in vivo* study and human testing, due to the difficulties in large scale preparation. Due to the close structural and configurational similarities of these PMFs in plants such as citrus peels, the purification to obtain single PMFs for in-depth efficacy evaluation has been an enormous challenge, preventing further large scale and broad study for their potential excellent biological properties. In this review, we summarized the up to date reported purification methods chiefly from citrus peel extract using various chromatography techniques to obtain singlemers of these PMFs. These methods including normal phase and reversed phase liquid chromatography, macroporous gel separation, counter current chromatography, and supercritical fluid chromatography as well as the combination of those isolation techniques. The aim of this review is to provide key references to establish an efficient and scalable separation method in acquiring single polymethoxyflavone isomers which will enable further investigations on the health-promoting and medicinal properties of these PMFs and associated mechanisms.

Keywords: Separation; Polymethoxyflavones; Citrus peels; Nobiletin; Tangeretin; Counter current chromatography; Supercritical fluid chromatography.

1. Introduction

A special class of citrus flavonoids represents the polymethoxyflavones (PMFs) as illustrated in Figure 1, comprising tangeretin (5,6,7,8,4'-pentamethoxyflavone), nobiletin (5,6,7,8,3',4'-hexamethoxyflavone), 3,5,6,7, 3',4'-heptamethoxyflavone (HMF), 5,6,7,4'-tetramethoxyflavone (TMF or scutellarein tetramethyl ether), 3,5,6,7,3',4'-hexamethoxyflavone, sinensetin (5,6,7,3',4'-pentamethoxyflavone), and 5-demethylnobiletin (5-hydroxy-6,7,8,3',4'-

pentamethoxyflavone) as major components (Lin et al., 2012; Toledo et al., 2024). Due to their effective biological activities, PMFs have gained increasing interest in recent years and have been demonstrated to possess important biological properties, such as antioxidant by scavenging free radicals, inhibiting lipid peroxidation, and enhancing the activity of endogenous antioxidant enzymes (Gozde et al., 2025; Murakami et al., 2000; Wang et al., 2018), anti-inflammatory effects by modulating key signaling pathways involved in inflammation, attenuating the expression of COX-2, suppressing

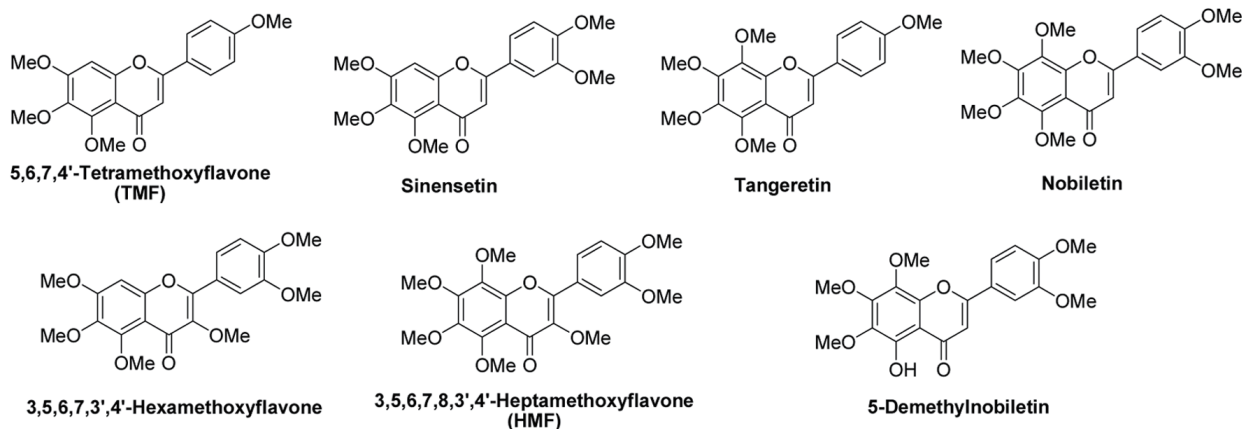


Figure 1. Structures of common Polymethoxyflavones from citrus peels.

the NF- κ B and MAPK pathways, and inhibiting the expression of pro-inflammatory cytokines such as TNF- α and IL-6 (Fontana et al., 2023; Lin et al., 2013; Xu et al., 2025). Cardioprotective effects are exerted by reducing oxidative stress, inflammation, and lipid accumulation, key factors contributing to atherosclerosis and other cardiovascular diseases (Du et al., 2024; Ramunno et al., 2024; Whitman et al., 2005). Anti-cancer activities are mediated through multiple mechanisms, including the inhibition of the PI3K/Akt/mTOR signaling pathway, activation of the p53 pathway, and suppression of the epithelial-mesenchymal transition (Aidiel et al., 2025; Rawson, Ho, Li, 2014; You et al., 2024). The neuroprotective effects of PMFs were majorly attributed to its antioxidant and anti-inflammatory properties that help protect neurons from oxidative damage and neuroinflammation (Alivirdiloo et al., 2024; Siima et al., 2020). An optimized enzyme/acid-catalyzed hybrid hydrolysis process was used to effectively enrich PMFs from citrus fruits. These PMFs were administered to mice and demonstrated the ability to mitigate metabolic syndrome in gut microbiota indicating that the presence of gut bacteria is crucial for these effects to manifest (Sun et al., 2025).

Nobiletin is a major polymethoxyflavone predominantly found in citrus fruits, particularly in the peels of tangerines and bitter oranges. It has been studied for its wide array of biological activities, including antioxidant, inhibitory effects of inflammation, anticancer, neuroprotective and metabolic benefits. Nobiletin has been known for its strong antioxidant properties, scavenging free radicals and reducing oxidative stress. This action plays a significant role in protecting cells from oxidative damage, contributing to the prevention of aging and several chronic diseases (Wang et al., 2018; Yang et al., 2024). For instance, nobiletin inhibited cisplatin-induced ototoxicity by suppressing apoptosis and oxidative stress, thus protected against cisplatin-induced nephrotoxicity and neurotoxicity (Song et al., 2024). Nobiletin has also shown potent anti-inflammatory effects by modulating various signaling pathways including NF- κ B and MAPK pathways, such as the inhibition of NF- κ B/NLRP3 inflammasome activation to ameliorate sodium urate-induced gouty arthritis in mice (Liu, Chu, Bai and Yang, 2025; Pandith et al., 2013; Yang et al., 2024). Neuroprotective effects of nobiletin has been demonstrated in multiple studies, particularly in preclinical models of neurodegenerative diseases such as dementia and Parkinson's diseases (Nakajima and Ohizumi, 2019). It protected neurons from oxidative stress, regulated neuro-inflammatory pathways, and improved cognitive function (Tsiloni et al., 2025). Nobiletin reduced the oxidative stress, inflammation and lipid accumulation, i.e. three of the key contributors to cardio-

vascular diseases, hence it could prevent atherosclerosis and other cardiovascular conditions (Lin et al., 2013; Ramunno et al., 2024). Moreover, nobiletin has been shown to improve insulin sensitivity and regulate glucose metabolism by enhancing glucose uptake and lipid metabolism thus reducing hepatic glucose production (Yuan et al., 2022). Also, nobiletin has demonstrated efficacy against a wide variety of cancers, including breast, colon, lung and liver cancer (Cheng et al., 2016; Gutierrez-Venegas and Rosas-Martinez, 2025; Murakami et al., 2000). The action mechanisms of nobiletin include promoting apoptosis, inhibiting tumor cell proliferation, and suppressing metastasis. Since nobiletin has been shown to modulate multiple molecular targets such as PI3K/Akt, MAPK, and apoptosis-regulating proteins it offers a multi-target approach to cancer therapy (Ashrafizadeh et al., 2020).

Furthermore, it has been found that nobiletin shows potential as a beneficial compound to maintain or restore proper circadian rhythm function. Nobiletin could modulate the molecular mechanisms that regulate the body's internal clock, particularly targeting key proteins involved in circadian rhythm regulation, such as CLOCK and BMAL1 (He et al., 2016). Therefore, nobiletin could enhance the function of the molecular oscillator and help the synchronization of circadian rhythms, leading to improved sleep-wake cycles and better metabolic function. Due to the remodeling of circadian metabolic gene expression, nobiletin has shown the protective effects against metabolic syndrome, a cluster of conditions including obesity, insulin resistance, and cardiovascular disease, by promoting healthier metabolic processes (He et al., 2016). Nobiletin improved mitochondrial function and increased ATP production, which is particularly beneficial in tissues with high energy demands, such as the brain and muscles. Hence, by modulating the circadian rhythm and enhancing cellular bioenergetics, nobiletin could promote healthy aging and preventing age-related diseases (He et al., 2016; Mileykovskaya et al., 2020).

However, the *in vivo* efficacy and safety investigation of single polymethoxyflavones, such as nobiletin, tangeretin, HMF and TMF, has not been broadly performed due to the limited availability of pure form singlemers. The majority of *in vivo* studies that have been performed thus far were a mixture of polymethoxyflavones extracted from citrus plants. To better understand its efficacy and safety profile, single form PMFs should be thoroughly evaluated. An efficacy or safety study in animals may easily consume grams of phytochemicals. Therefore, it is necessary and urgent to develop an efficient method for large scale separation PMF singlemers. In this review, we have summarized the most recent purifi-

cation methods of single PMFs, mandatory to provide adequate research material for future scientific investigations on levels of in vitro, in vivo as well as clinical trials.

2. Extraction methods of citrus polymethoxyflavones

Extraction of PMFs could be derived from essential oils of citrus peels or directly from the peels. Essential oils from citrus peels such as oranges or tangerines, were either obtained from direct distillation or steam distillation, or extracted out with sub- or super-critical fluid chromatography (Rifna et al., 2023). The essential oils are used in flavor, fragrances, cosmetic, beverage, and other applications in food industry (Suresh et al., 2020). Their resin-like residue after distillation or extraction are rich in PMFs, sometimes as high as 80% of PMF content (Yoshizakia et al., 2017), which may be used for direct chromatography separation of PMF singlemers.

Traditional extraction methods of PMFs from citrus peels are generally applied by solid-liquid extraction with aqueous ethanol and heating conditions (Ke et al., 2018; Tong et al., 2018). Resulted crude citrus peel extract after solvent removal is then directly analyzed for PMF contents (Ke et al., 2018) and loaded onto a silica gel column for fractionation to obtain different groups of PMF mixture for further separation. Also, PMFs could be efficiently enriched by macroporous adsorptive resin prior to chromatographic separation (Li, Zhao, Zhou, 2018). Moreover, the conventional extraction of citrus peel PMFs are often modified or assisted by other technologies, such as the ultrasound-assisted method (Garcia-Castello et al., 2015), microwave-assisted method (Rifna et al., 2023), pressurized extraction (Castro-Vázquez et al., 2021), or the combination of two or more technologies (Rifna et al., 2023).

Supercritical fluids (SCF), such as carbon dioxide (CO₂), are often used nowadays to efficiently extract plant bioactives (Oba et al., 2017; Uwineza and Waskiewicz, 2020). SCF technology has been vastly used in extracting essential oils, flavonoids, alkaloids, and terpenes among others, to avoid the use of harmful solvents or high temperatures. Comparing to conventional extraction methods, SCF extraction has higher selectivity, faster extraction times, and the ability to preserve the integrity of sensitive compounds. The potential of SFE extraction as an efficient, eco-friendly method is tremendous with ongoing advancements in commercially viable for large-scale applications extracting bioactive compounds from plant materials (Uwineza and Waskiewicz, 2020). Often an alcohol modifier is added to SCF-CO₂ for most efficient extraction of various polarities of compounds. Generally, ethanol, methanol or isopropyl alcohol is used with different percentages depending on the resolution of interested analytes. For instance, SFC was used to efficiently extract three PMFs, tangeretin, nobiletin and HMF, from *Citri reticulatae* for high speed counter-current chromatography (HSCCC) separation of the three single PMFs (Long et al., 2019).

Subcritical fluids (Sub-CF) substances that are in a state where they are below their critical temperature and critical pressure in extraction are also often used in extracting phytochemicals, including butane, water and others.

When water is heated to temperatures between 100°C and 374°C (below its critical point), it becomes subcritical. Subcritical water is employed to extract essential oils and plant oils from plant materials. Subcritical fluid extraction is an environmentally friendly alternative to traditional extraction methods like solvent or steam distillation (Diaz-Reinoso et al., 2023). The extraction of PMFs with Sub-CF water yielded 89% of extraction rate of PMFs at 160 °C (Kim and Lim, 2020). There are also other advanced extraction techniques including ultrasound-assisted extraction using ultrasound waves to

enhance the extraction efficiency of PMFs from citrus peels. Microwave-assisted extraction employs microwave energy to accelerate the extraction process. Enzyme-assisted extraction uses enzymes to break down plant cell walls, facilitating the release of PMFs.

3. Separation of PMF singlemers

The development of rapid and efficient separation methods for PMFs is essential for their large-scale production and application of PMF singlemers. The separation methods used to separate PMFs and their pros and cons are described in the following sections.

3.1. Normal phase chromatography

Normal phase silica gel chromatography is the most used separation technique for several key advantageous factors: economical cost, timesaving, convenience and efficiency. A rapid flash chromatography method was developed using a silica gel column predominantly. The extract is concentrated, impregnated with silica gel, and subjected to flash chromatography using a gradient solvent system of hexane and ethyl acetate or methanol and methylene chloride or other suitable organic solvent systems. However, due to the structural proximity and similarity, PMFs cannot be separated by normal phase flash chromatography.

3.2. Separation using high performance liquid chromatography (HPLC)

Since normal phase silica gel chromatography is incapable of isolating a single polymethoxyflavone from another in general, reversed phase liquid chromatography was then employed to separate polymethoxyflavones in some cases, but the separation result is very inefficient and cannot isolate sufficient singlemers of PMFs for biological screening and evaluation. Multiple pre-purification processes including steps of targeted extraction and silica gel chromatography are usually performed prior to the loading onto an HPLC system equipped with C18 reversed-phase columns. After steps of macroporous resin treatment of tangerine peel extract, the concentration of PMFs increased from 31.7 mg/g to 594.6 mg/g. Then a preparative HPLC-MS equipped with a C18 column (1.9x25 cm) was followed for further separation of singlemers of PMFs. The injection volume was 0.65 mL of PMF-enriched solution at concentration of 100 mg/mL, resulting in separation of singlemers of nobiletin, tangeretin, sinensetin, TMF and 5-demethylnobiletin with purities of 95% or above (Li et al., 2018). After fractionation of sweet orange peel extracts with flash silica gel chromatography and ethyl acetate and hexanes as eluting solvents, a fraction mainly containing nobiletin and TMF was loaded on to an HPLC system equipped with a chiral preparative column under UV detection (Li et al., 2006). Within 45 min of the HPLC separation, 2.48 g of nobiletin and 0.85 g of TMF were yielded with 99% purity or above, demonstrating an efficient and scalable separation method (Figure 2a). However, both reversed-phase and chiral phase preparative HPLC separation required extensive pre-treatment of PMF-containing extracts (Li et al., 2018; Li et al., 2006).

3.3. Separation with supercritical fluid chromatography (SFC)

As compared to conventional separation methods – normal phase

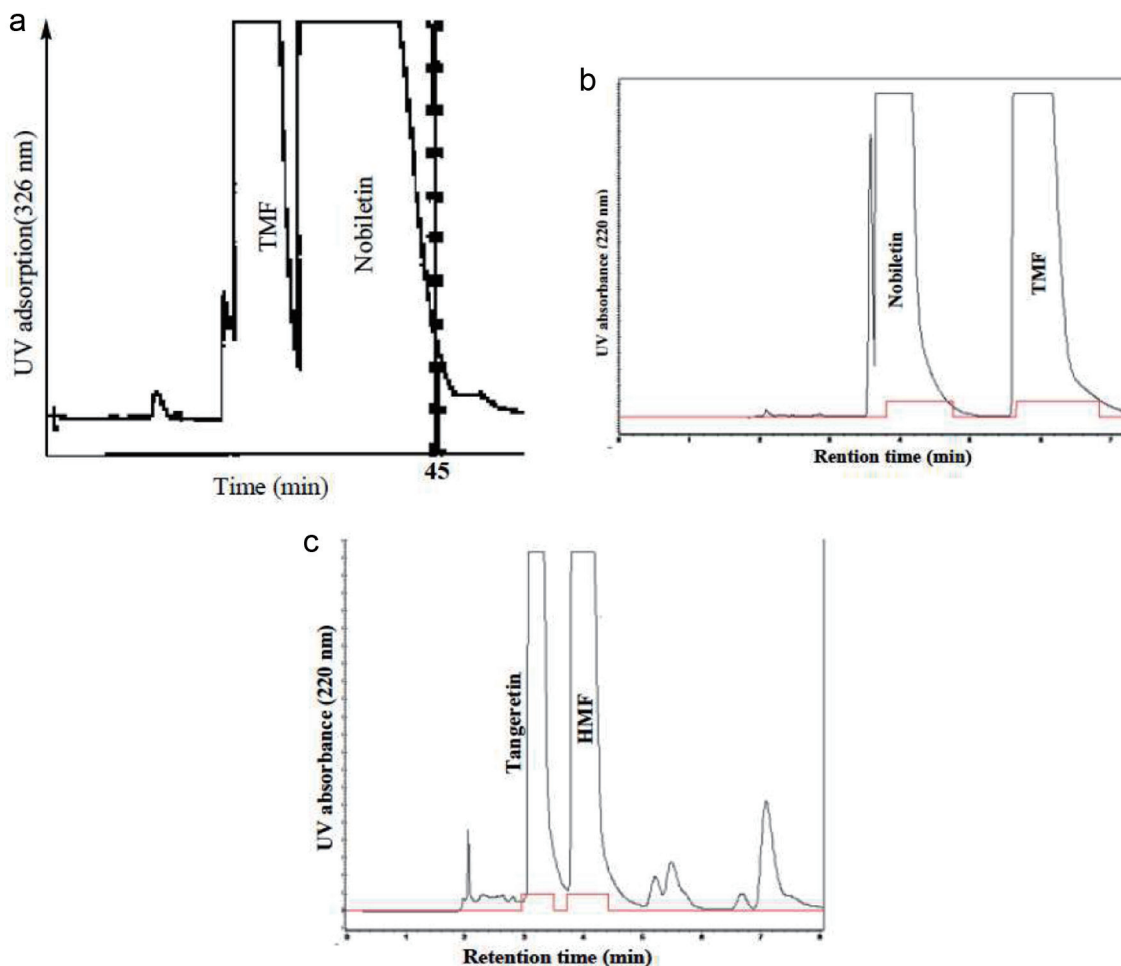


Figure 2. Separation of PMFs. (a) HPLC method with a chiral column (Li et al., 2006), (b) and (c) SFC method (Li et al., 2007).

silica gel chromatography and reversed-phase HPLC, SFC have higher column efficiencies, lower solvent usage, easier post-purification solvent removal, and overall lower operational costs. Thus, SFC has been gaining more and more popularity in the separation of nutraceuticals and pharmaceuticals, which, particularly in the past two decades, has gained an increasing amount of attention.

Preparative SFC separation was used in PMFs to separate nobiletin from TMF, and HMF from tangeretin (Li et al., 2007). In this isolation of pure PMFs, total of four single PMFs were yielded in short period of time and high purity. Prior to the SFC separation, flash column was employed to divide sweet orange peel extracts into six groups of PMFs. One fraction majorly consisted of nobiletin and TMF, and another contained predominantly tangeretin and HMF. The two groups were separated on an analytical SFC and the method was then transferred to a preparative SFC, which was performed on Berger MultiGram II Supercritical Fluid Chromatography system, consisted of an automatic liquid injection system with a 5 mL injection loop, a thermal control module to control column temperature, and Knauer variable wavelength UV detector with high pressure flow cell for SFC detection set at 220 nm. A preparative Chiralpak AD column (3 × 25 cm) was employed for SFC purification (Li et al., 2007). The baseline separation between nobiletin and TMF was a 7-min isocratic run, and an 8-min SFC program for separating tangeretin and HMF, but next injection was

at 6.5 min due to the isocratic solvent system (Figure 2b and c). The isocratic solvent system allows stack injection for maximum time saving. The purity of the four isolated PMFs was above 99%.

3.4. Counter-current chromatographic separation

Countercurrent chromatography (CCC) is a liquid-liquid separation technique that has the same separation mechanism as separation funnel. It relies on the partitioning of compounds between two immiscible liquid phases. The stationary phase of CCC is formed by a liquid phase that is continuously flowing in one direction, while the mobile phase (another liquid phase) flows in its opposite direction. This countercurrent movement separates complex mixtures based on the differential solubility of compounds in the two phases. The CCC technique is widely used in various fields, such as analytical chemistry, biochemistry, and pharmacology, for isolating and purifying natural products, pharmaceuticals, and bioactive compounds. Advantages of CCC include high resolution, free solid stationary phases, and advanced handling of large amount of samples.

High speed CCC (HSCCC) has been practically used in the separation of PMFs to isolate nobiletin (26 mg), tangeretin (35 mg), HMF (6 mg) and 5-demethylnobiletin (11 mg) from 150 mg crude

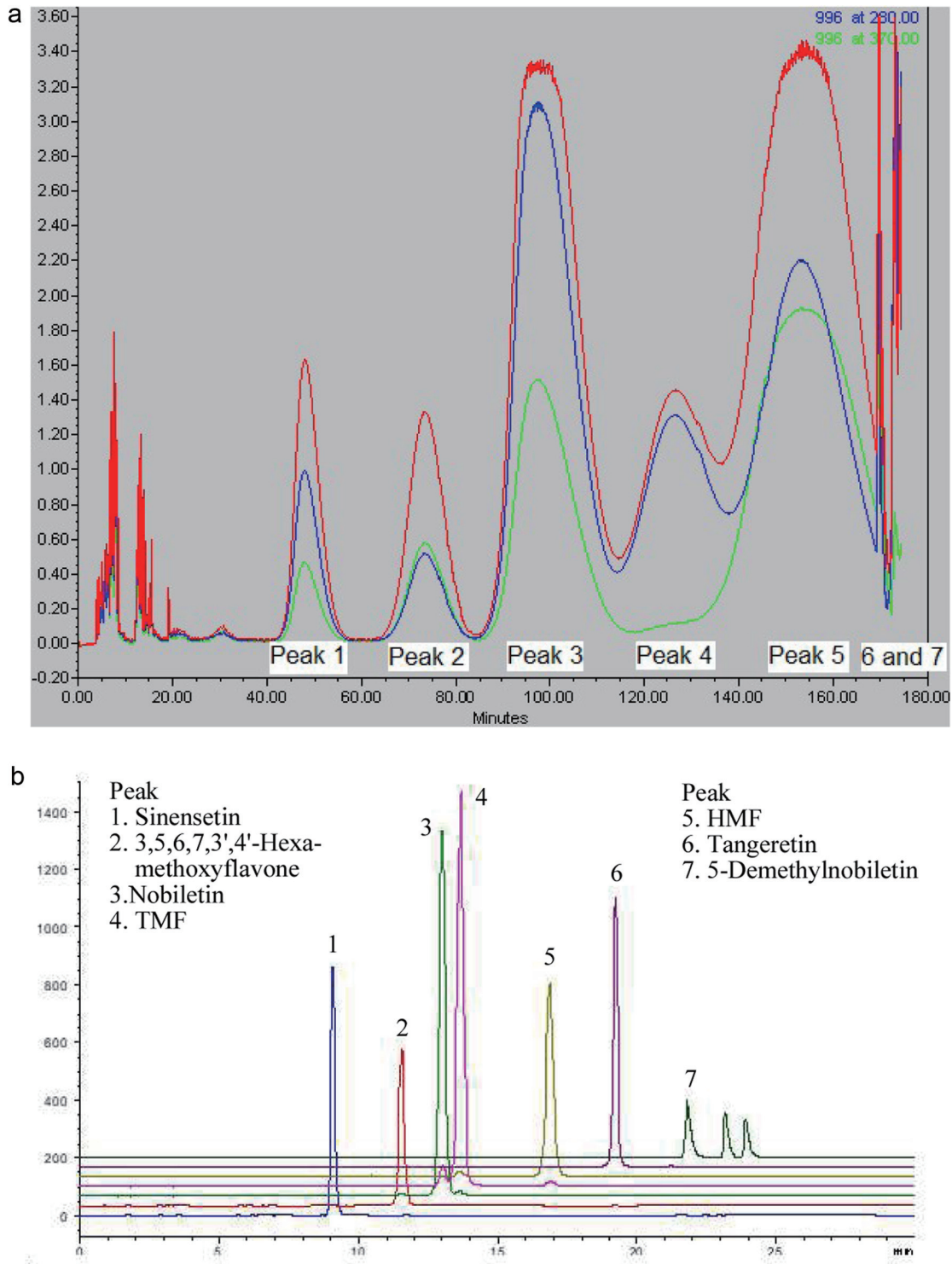


Figure 3. HPLC separation of PMFs from sweet orange peel extracts. (a) HPLC separation spectrum and (b) analytical HPLC spectra of PMFs after HPLC separation (Xu et al., 2019).

tangerine peel extract in one-cycle of HSCCC separation (Wang et al., 2005). Similarly, nobiletin (6.4 mg), tangeretin (20.2 mg) and 5-demethylnobiletin (2.8 mg) were successfully separated from *Citrus reticulata* peel by HSCCC (Liu et al., 2012). In another

HSCCC purification of nobiletin, tangeretin and 5-demethylnobiletin, the crude extract of *Citrus reticulata* cv. *Suavissima* fruits was first pre-treated with a solid-phase-extraction (SPE) column to enrich the PMF content, and then the enriched PMF mixture

was loaded onto an HSCCC system. In one cycle of purification, 16.39 mg of nobiletin, 15.51 mg of tangeretin and 3.21 mg of 5-demethylnobiletin were separated with purity of 99.87, 99.76 and 98.75%, respectively (Wang et al., 2019). An efficient approach for SCF-CO₂ extracting and HSCCC purifying polymethoxyflavones from *Citri reticulatae* has demonstrated that the combined method effectively isolated pure (purity ≥98%) nobiletin (194.5 mg), tangeretin (107.3 mg) and HMF (128.7 mg) in seven hours with an injection of 10 mL (20 mL loop) for each purification cycle of HSCCC (Long et al., 2019).

High performance counter current chromatography (HPLCCC) is a more advanced CCC version than HSCCC, used for the separation and purification of compounds also based on their differential distribution between two immiscible liquid phases. With higher rotation speed and higher pressure, HPLCCC has demonstrated higher speed separation, higher resolution, and more efficient and powerful separation than HSCCC. Due to the high resolution and speed, HPLCCC can separate more complex mixtures and also in large-scale amount as compared to HSCCC. In the application of separating PMFs from sweet orange peel extracts, six pure single-mers of PMFs (Figure 3a), namely, nobiletin (17.1 mg), tangeretin (3.3 mg), HMF (12.1 mg), TMF (2.6 mg), sinensetin (1.4 mg), and 3,5,6,7, 3',4'-hexamethoxyflavone (1.3 mg), were isolated with a semi-preparative HPLCCC equipped with a small loop (10 mL) in one cycle (Xu et al., 2019). The purity of the single PMFs was greater than 96.6% (Figure 3b). Also, a mixture of 5-demethylated PMFs, majorly 5-demethylnobiletin in the the seventh fraction collected, which provided easier purification of 5-demethylnobiletin (Peak 7 in Figure 3b) and other 5-demethyl-PMFs (Xu et al., 2019). Based on this result, a scale-up separation to acquire large amount of pure PMF single-mers would be achieved with a larger loop in the preparative HPLCCC setting.

4. Summary

Polymethoxylated flavonoids naturally exist in many plants, particularly rich in citrus peels. They possess multiple biofunctionalities including antioxidant properties, inhibition of chronic inflammation, cardioprotective and neuropreventive effects, modulation of metabolic syndrome and anticancer effects. However, the evaluation of their efficacy and safety profiles encounters enormous difficulties because of the availability of pure single-mers of these PMFs. In reviewing the most recent separation techniques of PMFs from citrus peel extracts, this review summarizes the extraction, enrichment and pretreatment of citrus peel extracts by means of traditional methods, microwave- or ultrasound-assisted extraction, silica gel flash column fractionation, solid phase extraction for enrichment, supercritical or subcritical fluid extraction and other technologies. Efficient separation methods of PMFs include high speed- and high performance-counter current chromatography (HSCCC and HPLCCC) and supercritical fluid chromatography (SFC-CO₂).

References

- Aidiel, M., Abdul Mutalib, M., Ramasamy, R., Nik Ramli, N.N., Tang, S.G.H., and Adam, S.H. (2025). Mechanistic insights into the anticancer potential of methoxyflavones analogs: A review. *Molecules* 30: 346.
- Alivirdiloo, V., Hajiabbasi, M., Gargari, M.K., Gargari, H.K., Ghazi, F., Mohammadi, M., Rahimi, F., Mobed, A., and Mehra, A. (2024). Neuroprotective role of nobiletin against amyloid- β (A β) aggregation in Parkinson and Alzheimer disease as neurodegenerative diseases of brain. *Med. Chem. Res.* 33(7): 1055–1063.
- Ashrafizadeh, M., Zarrabi, A., Saberifar, S., Hashemi, F., Hushmandi, K., Hashemi, F., Moghadam, E.R., Mohammadinejad, R., Najafi, M., and Garg, M. (2020). Nobiletin in cancer therapy: how this plant derived-natural compound targets various oncogene and onco-suppressor pathways. *Biomedicines*. 8: 110.
- Castro-Vázquez, L., Lozano, M.V., Rodríguez-Robledo, V., González-Fuentes, J., Marcos, P., Villaseca, N., Arroyo-Jiménez, M.M., and Santander-Ortega, M.J. (2021). Pressurized extraction as an opportunity to recover antioxidants from orange peels: heat treatment and nanoe-mulsion design for modulating oxidative stress. *Molecules* 26: 5928.
- Cheng, H.-L., Hsieh, M.-J., Yang, J.-S., Lin, C.-W., Lue, K.-H., Lu, K.-H., and Yang, S.-F. (2016). Nobiletin inhibits human osteosarcoma cells metastasis by blocking ERK and JNK-mediated MMPs expression. *Oncotarget* 7(23): 35208.
- Díaz-Reinoso, B., Rivas, S., Rivas, J., and Domínguez, H. (2023). Subcritical water extraction of essential oils and plant oils. *Sustainable Chem. Pharm.* 36: 101332.
- Du, Y., Su, J., Yan, M., Wang, Q., Wang, T., Gao, S., Tian, Y., Wang, Y., Chen, S., Lv, G., and Yu, J. (2024). Polymethoxyflavones in citrus extract has a beneficial effect on hypercholesterolemia rats by promoting liver cholesterol metabolism. *J. Ethnopharmacol.* 322(2024): 117644.
- Fontana, G., Bruno, M., Sottile, F., and Badalamenti, N. (2023). The chemistry and the anti-inflammatory activity of polymethoxyflavonoids from *Citrus* genus. *Antioxidants* 12: 23.
- García-Castello, E.M., Rodríguez-Lopez, A.D., Mayor, L., Ballesteros, R., Conidi, C., and Cassano, A. (2015). Optimization of conventional and ultrasound assisted extraction of flavonoids from grapefruit (*Citrus paradisi* L.) solid wastes. *LWT-Food Sci. Tech.* 64: 1114–1122.
- Gözde, A.U., Hamit, U., Çoban, T.A., Özkaraca, M., Mendil, A.S., and Aygörmez, S. (2025). Nobiletin reduces 5-FU-induced lung injury with antioxidative, anti-inflammatory and anti-apoptotic activities. *Nauyn Schmiedebergs Arch. Pharmacol.*
- Gutierrez-Venegas, G., and Rosas-Martinez, M. (2025). Nobiletin and 5-demethylnobiletin ameliorate hypopharyngeal squamous cell carcinoma by suppressing TGF- β -mediated epithelial-mesenchymal transition. *Oncol. Lett.* 29: 176.
- He, B., Nohara, K., Park, N., Park, Y.-S., Guillory, B., Zhao, Z., Garcia, J.M., Koike, N., Lee, C.C., Takahashi, J.S., Yoo, S.-H., and Chen, Z. (2016). The small molecule nobiletin targets the molecular oscillator to enhance circadian rhythms and protect against metabolic syndrome. *Cell Metab.* 23: 610–621.
- Ke, Z., Zhao, Z., Zhao, Y., Xu, X., Li, Y., Tan, S., Huang, C., and Zhou, Z. (2018). PMFs-rich Citrus extract prevents the development of non-alcoholic fatty liver disease in C57BL/6J mice induced by a high-fat diet. *J. Funct. Foods.* 47: 28–39.
- Kim, D.-S., and Lim, S.-B. (2020). Kinetic study of subcritical water extraction of flavonoids from citrus unshiu Peel. *Sep. Purif. Technol.* 250: 117259.
- Li, S., Lambros, T., Wang, Z., Goodnow, R., and Ho, C.-T. (2007). An efficient and scalable method in isolation of polymethoxyflavones from orange peel extract by supercritical fluid chromatography. *J. Chromatogr. B.* 846: 291–297.
- Li, S., Yu, H., and Ho, C.-T. (2006). Nobiletin: efficient and large quantity isolation from orange peel extract. *Biomed. Chromatogr.* 20: 133–138.
- Li, Z., Zhao, Z., and Zhou, Z. (2018). Simultaneous separation and purification of five polymethoxylated flavones from “Dahongpao” tangerine (*Citrus tangerina* Tanaka) using macroporous adsorptive resins combined with prep-HPLC. *Molecules* 23: 2660.
- Lin, N., Sato, T., Takayama, Y., Mimaki, Y., Sashida, Y., Yano, M., and Ito, A. (2013). Novel anti-inflammatory actions of nobiletin, a citrus polymethoxy flavonoid, on human synovial fibroblasts and mouse macrophages. *Biochem. Pharmacol.* 65: 2065–2071.
- Lin, Y.-S., Li, S., Ho, C.-T., and Lo, C.-Y. (2012). Simultaneous analysis of six polymethoxyflavones and six 5-hydroxypolymethoxyflavones by high performance liquid chromatography combined with linear ion trap mass spectrometry. *J. Agric. Food Chem.* 60: 12082–12087.
- Liu, L., Xu, X., Cheng, D., Yao, X., and Pan, S. (2012). Preparative separation of polymethoxylated flavones from Ponkan (*Citrus reticulata* Blanco cv. Ponkan) peel by high-speed counter-current chromatography and their antifungal activities against *Aspergillus niger*. *Eur. Food Res.*

- Technol. 235: 631–635.
- Liu, Z., Chu, A., Bai, Z., and Yang, C. (2025). Nobiletin ameliorates monosodium urate-induced gouty arthritis in mice by enhancing AMPK/mTOR-mediated autophagy to inhibit NF- κ B/NLRP3 inflammasome activation. *Immunol. Lett.* 274: 106982.
- Long, T., Lv, X., Xu, Y., Yang, G., Xu, L.-Y., and Li, S. (2019). Supercritical fluid CO₂ extraction of three polymethoxyflavones from *Citri reticulatae pericarpium* and subsequent preparative separation by continuous high-speed counter-current chromatography. *J. Chromatogr. B* 1124: 284–289.
- Mileykovskaya, M., Seung-Hee Yoo, S.-H., Dowhan, W., and Chen, Z. (2020). Nobiletin: targeting the circadian network to promote bioenergetics and healthy aging. *Biochemistry (Mosc.)* 85(12): 1554–1559.
- Murakami, A., Nakamura, Y., Torikai, K., Tanaka, T., Koshiba, T., Koshimizu, K., Kuwahara, S., Takahashi, Y., Ogawa, K., Yano, M., Tokuda, H., Nishino, H., Mimaki, Y., Sashida, Y., Kitanaka, S., and Ohigashi, H. (2000). Inhibitory effect of citrus nobiletin on phorbol ester-induced skin inflammation, oxidative stress, and tumor promotion in mice. *Cancer Res.* 60(18): 5059–5066.
- Nakajima, A., and Ohizumi, Y. (2019). Potential Benefits of Nobiletin, A Citrus Flavonoid against Alzheimer's Disease and Parkinson's Disease. *Int. J. Mol. Sci.* 20: 3380.
- Oba, C., Ota, M., Noura, K., Fujiwara, H., Takito, J., Sato, Y., Ohizumi, Y., and Inomata, H. (2017). Extraction of nobiletin from Citrus Unshiu peels by supercritical fluid and its CRE-mediated transcriptional activity. *Phytomedicine* 27: 33–38.
- Pandith, H., Zhang, X., Thongpraditchote, S., Wongkrajang, Y., Gritsanapan, W., and Baek, S.J. (2013). Effect of Siam weed extract and its bioactive component scutellarein tetramethyl ether on anti-inflammatory activity through NF- κ B pathway. *J. Ethnopharmacol.* 147: 434–441.
- Ramunno, A., Vitale, R.M., Amodeo, P., Crescenzi, C., Panti, A., Fiorenzani, P., De Luca, M., Spizzirri, U.G., Restuccia, D., Aiello, F., and Fusi, F. (2024). bioguided identification of polymethoxyflavones as novel vascular Ca_v1.2 channel blockers from citrus peel. *Molecules* 29: 5693.
- Rawson, N.E., Ho, C.-T., and Li, S. (2014). Efficacious anti-cancer property of flavonoids from citrus peels. *Food Sci. Hum. Wellness.* 3: 104–109.
- Rifna, E.J., Misra, N.N., and Dwivedi, M. (2023). Recent advances in extraction technologies for recovery of bioactive compounds derived from fruit and vegetable waste peels: A review. *Crit. Rev. Food Sci. Nutr.* 63(6): 719–752.
- Siima, A.A., Stephano, F., Munissi, J.J.E., and Nyandoro, S.S. (2020). Ameliorative effects of flavonoids and polyketides on the rotenone induced *Drosophila* model of Parkinson's disease. *Neurotoxicology* 81: 209–215.
- Song, W., Zhang, L., Cui, X., Wang, R., Ma, J., Xu, Y., Jin, Y., Wang, D., and Lu, Z. (2024). Nobiletin alleviates cisplatin-induced ototoxicity via activating autophagy and inhibiting NRF2/GPX4-mediated ferroptosis. *Sci. Rep.* 14: 7889.
- Sun, M., Liang, J., Peng, Y., Qin, L., Ma, D., Cai, X., Ran, L., Wang, Y., Wang, H., Yang, C., Liu, X., and Deng, Z. (2025). Enrichment of polymethoxyflavones from citrus fruits using an optimized enzyme/acid-catalyzed hybrid hydrolysis process and its influence on mice gut microbiota. *Food Funct.* 16(2): 510–523.
- Suresh, A., Velusamy, S., Ayyasamy, S., and Rathinasamy, M. (2020). Techniques for essential oil extraction from kaffir lime and its application in health care products—A review. *Flavour Fragrance J.* 36: 5–21.
- Toledo, R., Tomas-Navarro, M., Yuste, J.E., Crupi, P., and Vallejo, F. (2024). An update on citrus polymethoxyflavones: chemistry, metabolic fate, and relevant bioactivities. *Eur. Food Res. Tech.* 250: 2179–2192.
- Tong, C., Peng, M., Tong, R., Ma, R., Guo, K., and Shi, S. (2018). Use of an online extraction liquid chromatography quadrupole-time-of-flight tandem mass spectrometry method for the characterization of polyphenols in Citrus paradisi cv. Changshanhuayu peel. *J. Chromatogr. A.* 1533: 87–93.
- Tsilioni, I., Kempuraj, D., and Theoharides, T.C. (2025). Nobiletin and Eriodictyol Suppress Release of IL-1 β , CXCL8, IL-6, and MMP-9 from LPS, SARS-CoV-2 Spike Protein, and Ochratoxin A-Stimulated Human. *Microgliia. Int. J. Mol. Sci.* 26: 636.
- Uwineza, P.A., and Waskiewicz, A. (2020). Recent Advances in Supercritical Fluid Extraction of Natural Bioactive Compounds from Natural Plant Materials. *Molecules* 25: 3847.
- Wang, M., Meng, D., Zhang, P., Wang, X., Du, G., Brennan, C., Li, S., Ho, C.-T., and Zhao, H. (2018). Antioxidant protection of nobiletin, 5-demethylnobiletin, tangeretin, and 5-demethyltangeretin from citrus peel in *Saccharomyces cerevisiae*. *J. Agric. Food Chem.* 66: 3155–3160.
- Wang, X., Li, F., Zhang, H., Geng, Y., Yuan, J., and Jiang, T. (2005). Preparative isolation and purification of polymethoxylated flavones from tangerine peel using high-speed counter-current chromatography. *J. Chromatogr. A.* 1090: 188–192.
- Wang, Y., Zhang, W., Li, S., Cao, J., and Sun, C. (2019). Three polymethoxyflavones purified from ougan (*Citrus reticulata* Cv. Suavissima) inhibited LPS-induced NO elevation in the neuroglia BV-2 cell line via the JAK2/STAT3 pathway. *Nutrients* 11: 791.
- Whitman, S.C., Kurowska, E.M., Manthey, J.A., and Daugherty, A. (2005). Nobiletin, a citrus flavonoid isolated from tangerines, selectively inhibits class A scavenger receptor-mediated metabolism of acetylated LDL by mouse macrophages. *Atherosclerosis* 178: 25–32.
- Xu, Y., Lv, X., Yang, G., Zhan, J., Li, M., Long, T., Ho, C.-T., and Li, S. (2019). Simultaneous separation of six pure polymethoxyflavones from sweet orange peel extract by high performance counter current chromatography. *Food Chem.* 292: 160–165.
- Xu, Z., Wang, K., Hu, H., Hu, Y., Huang, J., and Luo, Z. (2025). Sinensetin attenuates LPS-induced acute pulmonary inflammation in mice and RAW264.7 cells by modulating NF- κ B p65-mediated immune resistance and STAT3-mediated tissue resilience. *Inter. Immunopharmacol.* 148: 114101.
- Yang, J., Xia, X., Du, M., Cheng, S., Zhu, B., and Xu, X. (2024). highly effective nobiletin-MPN in yeast microcapsules for targeted modulation of oxidative stress, NLRP3 inflammasome activation, and immune responses in ulcerative colitis. *J. Agric. Food Chem.* 72: 13054–13068.
- Yoshizakia, N., Hashizumea, R., and Masaki, H. (2017). A polymethoxyflavone mixture extracted from orange peels, mainly containing nobiletin, 3,3',4',5,6,7,8-heptamethoxyflavone and tangeretin, suppresses melanogenesis through the acidification of cell organelles, including melanosomes. *J. Dermatological. Sci.* 88: 78–84.
- You, Q., Li, L., Ding, H., and Liu, Y. (2024). Proteomics-based network pharmacology and molecular docking reveal the potential mechanisms of 5,6,7,4'-tetramethoxyflavone against HeLa cancer cells. *Heliyon* 10(20): e38951.
- Yuan, S., Ye, Z., Li, Y., Zou, J., Wu, M., Wang, K., Liao, W., and Shen, J. (2022). Hypoglycemic Effect of Nobiletin via Regulation of Islet β -Cell Mitophagy and Gut Microbiota Homeostasis in Streptozocin-Challenged Mice. *J. Agric. Food Chem.* 70: 5805–5818.