# EFFECT OF EXTRACTION SOLVENTS AND DRYING CONDITIONS ON TOTAL POLYPHENOL CONTENT AND INDIVIDUAL PHENOLIC COMPOSITION OF ORANGE PEELS

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Received: 2 May 2025; Revised: 20 May 2025; Accepted: 31 May 2025

## ABSTRACT

Although citrus peels are regarded as a byproduct of the juice production industry, they contain a high amount of bioactive compounds. This study aimed to evaluate the effects of different drying conditions (hot air oven) and extraction solvents (water, ethanol, methanol) on polyphenol content (TPC), phenolic composition (HPLC) in extracts from orange peels. The results showed that the water and methanol-to-water ratio of 1:1 presented the highest values for TPC in their extracts. Whereas, an ethanol-to-water ratio of 1:1 was found to be the best extracting solvent to get a higher yield of phenolic compounds (naringin and hesperidin). By comparing the drying conditions, the sample dried using a hot-air oven at 80 °C was the best temperature to retain polyphenolic content.

*Keywords:* Orange peels, byproducts, polyphenols, antioxidant activity, naringin, hesperidin

## 1. INTRODUCTION

Orange (*Citrus sinensis*) is one of the most widely cultivated and consumed citrus fruits worldwide. It originates from southern China and northeastern India and thrives in tropical and subtropical climates. Today, orange cultivation spans more than 130 countries. According to the Food and Agriculture Organization (FAO), oranges are among the six primary fruits produced on a large scale in Vietnam [1]. The orange fruit is recognized for its nutritional and medicinal benefits. It contains essential nutrients such as vitamin C, vitamin B1, and vitamin A, along with bioactive compounds like flavonoids and phenolics [2]. These constituents offer a range of health benefits, including antioxidant, anti-inflammatory, hepatoprotective, and cardioprotective effects [3]. Orange-derived products are widely used in the food, cosmetics, and traditional medicine industries. Among these, orange juice is one of the most consumed beverages globally, known for its refreshing taste and high nutritional content. However, juice production generates significant quantities of wet solid waste such as orange peels, which can comprise 30–50% of the processed fruit mass [4].

Although orange peel is rich in essential oils and biologically active compounds, it is often discarded as waste. This not only leads to environmental pollution but also results in the underutilization of valuable natural resources. Consequently, finding efficient and economically viable solutions for utilizing orange peel waste is imperative. Among such solutions, the recovery of flavonoids from orange peels has emerged as a promising approach. Flavonoids, one of the main polyphenol groups, are of particular interest due to their antioxidant, antimicrobial, and therapeutic properties, with applications in functional foods, nutraceuticals, and pharmaceuticals [5].

Numerous studies have investigated the extraction of phenolic compounds from orange peels, primarily focusing on individual factors such as solvent type or extraction conditions [6-8]. However, there is a limitation of research that simultaneously considers the combined effects of both drying methods and solvent selection on the extraction efficiency and flavonoid composition. The objective of this study is to recover flavonoid compounds from orange peels as a means of valorizing agricultural waste and enhancing the economic value of citrus by-products. The research aims to investigate the impact of solvents and different drying methods and on the extraction efficiency and flavonoid composition of the peel extracts.

## 2. MATERIALS AND METHODS

### 2.1. Materials

Orange peels, obtained as a by-product of industrial juice production from a manufacturer in Ho Chi Minh City, Vietnam, were stored at -18 °C before further experiments.

Reagents and chemicals: Folin-Ciocalteu reagent, sodium carbonate ($Na_2CO_3$), HPLC-grade methanol, acetic acid, gallic acid, quercetin, rutin, hesperidin, hesperetin, naringin, naringenin, sinensetin, nobiletin, tangeretin were purchased from Merck and Sigma (Germany).

### 2.2. Sample Preparation and Extraction Process

The extraction procedure used in this research followed the method outlined by Huynh et al. [9]. Before extraction, the peels were dried at three different temperatures (50 °C, 65 °C, and 80 °C) using a convection oven until the moisture content was reduced to approximately 10%. The dried samples were then ground and sieved to obtain particle sizes ranging from 0.5 to 1 mm. Extraction was performed using different solvent systems under controlled conditions. The obtained extracts were subsequently analyzed for total polyphenol content (TPC) and HPLC quantification.

### 2.3. Analytical methods

Determination of total phenolic content (TPC): The total phenolic content (TPC) was determined by the modified Folin-Ciocalteu procedure, according to Singleton et al. [10] Briefly, 250 µL of extract was diluted with 250 µL of distilled water and then mixed well with 500 µL of Folin-Ciocalteu reagent. The mixture was first shaken and incubated for 6 minutes at room temperature before sodium carbonate 20% (w/v) was added. The mixture was incubated for 2 hours at room temperature in the absence of light. Finally, the absorbance was measured at 765 nm using the UV-Vis spectrophotometer (PhotoLab 6100, WTW, Germany). Total phenol content was calculated against a calibration standard curve of gallic acid (10-100 mg/L), and the TPC was expressed in mg GAE/g DW.

Quantification of phenolic compounds using RP-HPLC: The phenolic composition of the extracts was analyzed using a reverse-phase high-performance liquid chromatography (RP-HPLC) system. The RP-HPLC system (LC-10 AI, Shimadzu, Kyoto, Japan) was equipped with a reversed-phase C18 column (1.7 µm particle size, 2.1 mm × 50 mm; Waters, Massachusetts). Detection was performed at 280 nm using a diode array detector (DAD). The mobile phase consisted of solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in methanol). The gradient elution profile was as follows: 10% B (0–5 min), 20% B (6–15 min), 30% B (16–30 min), 50% B (31–45 min), and 90% B (46–85 min), with a constant flow rate of 0.5 mL/min. Phenolic compounds were identified based on their retention times

and UV-VIS spectra, with reference to standards such as quercetin, rutin, hesperidin, hesperetin, naringin, naringenin, sinensetin, nobiletin, and tangeretin. The concentrations of the identified compounds were determined based on their peak areas (mAU).

### 2.4. Statistical analysis

All data were expressed as the mean ± standard deviation (SD). Each experimental condition was tested in triplicate to ensure reliability. For datasets following a normal distribution, one-way analysis of variance (ANOVA) was used, followed by Tukey's post hoc test to assess differences among groups. In cases where data did not meet parametric assumptions, the Kruskal–Wallis test was employed. Statistical significance was determined at a threshold of $p < 0.05$. Data analysis in this study was performed using Minitab version 20 (Minitab, LLC, USA).

## 3. RESULTS AND DISCUSSION

### 3.1. The effect of drying temperature and solvent on total phenolic content (TPC)

Figure 1 presents the total phenolic content (TPC) extracted from samples using various solvents under different temperatures, including water, ethanol, methanol, ethanol/water (1:1), and methanol/water (1:1). The data indicate that both solvent type and extraction temperature significantly influence TPC.

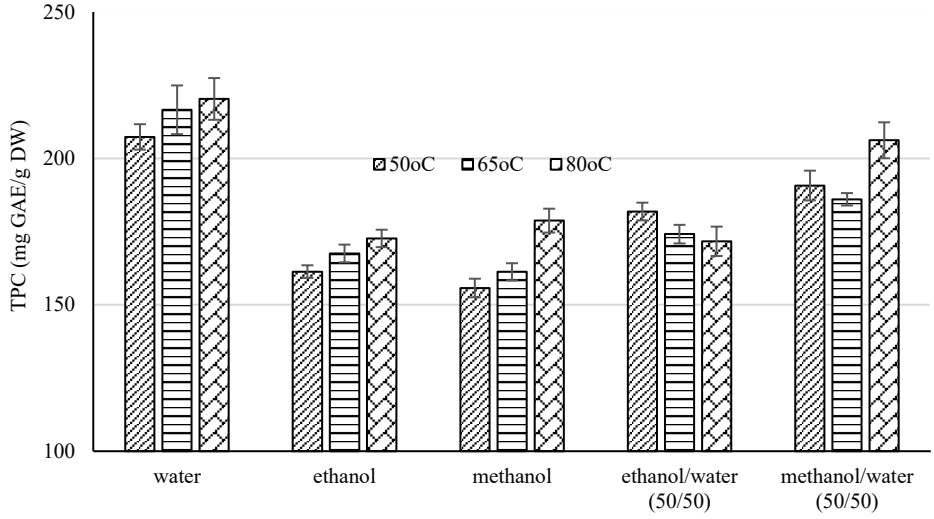


*Figure 1.* The effect of drying temperature and solvent on total phenolic content (TPC)

Among the solvents tested, water consistently exhibited high extraction efficiency at elevated temperatures. At 80 °C, water achieved a TPC value of 220.30 mg GAE/g, reflecting enhanced solubility and diffusion of phenolic compounds with increasing temperature. Ethanol and methanol also demonstrated moderate extraction efficiencies, with TPC values increasing as temperature rose. Methanol generally outperformed ethanol, particularly at 80 °C (178.74 mg GAE/g vs. 172.66 mg GAE/g), likely due to its higher polarity, which facilitates better solubilization of phenolic compounds [10].

The binary solvent systems revealed notable differences. The methanol/water (1:1) achieved the highest TPC yield among all combinations at 80 °C (206.17 mg GAE/g),

indicating a synergistic effect that enhances phenolic extraction [12]. In contrast, the ethanol/water (1:1) consistently showed lower TPC yields, with a peak of 181.86 mg GAE/g at 50 °C and declining values at higher temperatures.

In summary, both solvent polarity and extraction temperature play essential roles in optimizing phenolic recovery. Water at moderately high temperatures proved to be the most efficient single solvent, while methanol/water (1:1) at 80°C emerged as the most effective binary system for maximizing TPC yield. Based on these findings, the solvent with a water to methanol ratio of 1:1 at 80 °C was selected for the subsequent experiment to investigate the effect of extraction time

### 3.2. The effect of extraction time on the TPC

The effect of extraction time on the TPC was evaluated using a hydroalcoholic solvent system (MeOH/$H_2O$, 1:1) at 80 °C. Figure 2 shows a clear increase in polyphenol yield over time, especially during the first 36 hours of extraction. At the initial time point (0 hour), the TPC was 206.17 mg GAE/g. After 12 hours of extraction, the content increased to 218.33 mg GAE/g, followed by a more marked rise to 253.15 mg GAE/g at 36 hours. After that, although a slightly higher value of 255.64 mg GAE/g was recorded at 48 hours, statistical analysis revealed that the difference between 36 hours and 48 hours was not significant ($p > 0.05$).

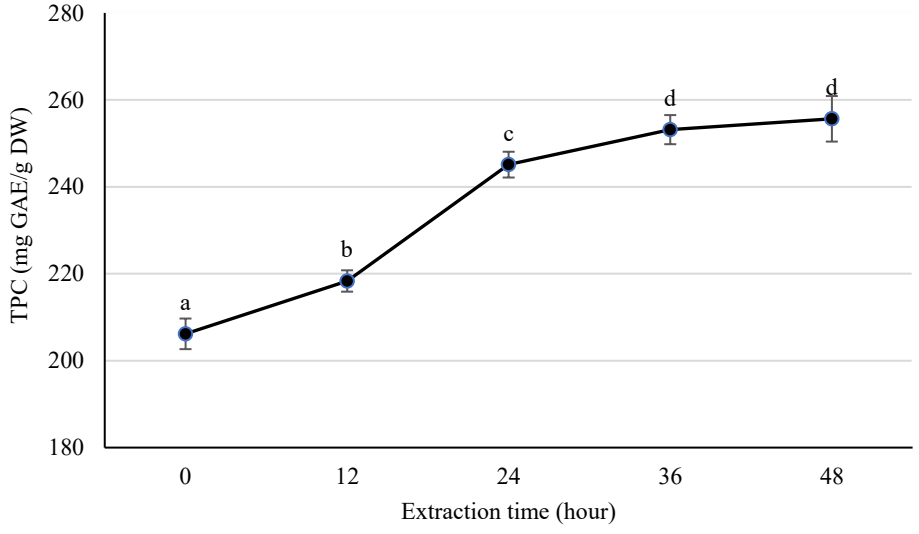


*Figure 2.* The effect of extraction time on the TPC (Different letters (a, b, c, d) indicate statistically significant differences with $p$-value <0.05)

This finding suggests that the majority of extractable polyphenols had already been released by 36 hours, and further extraction beyond this point yields diminishing returns. Prolonged extraction time may lead to unnecessary energy consumption without a substantial gain in polyphenol recovery. Therefore, from both a scientific and practical standpoint, 36 hours appears to be the optimal extraction duration under these conditions.

These findings in this study are consistent with previous studies that have shown the extraction of polyphenols to be highly time-dependent, with maximal yields often achieved within the first 24–36 hours of extraction under elevated temperatures [13].

### 3.3. Effect of extraction conditions on the individual composition of flavonoids in the extract

The identification of naringin and hesperidin was confirmed based on retention times compared between the sample and the reference standards and chromatographic profiles obtained from HPLC analysis (Figure 3). The quantitative data, illustrated in Figure 4, demonstrate the influence of extraction conditions, including time, solvent system, and drying temperature, on both the concentration (measured in mAU) and the relative composition of the flavonoid constituents (naringin and hesperidin) present in the extracts.

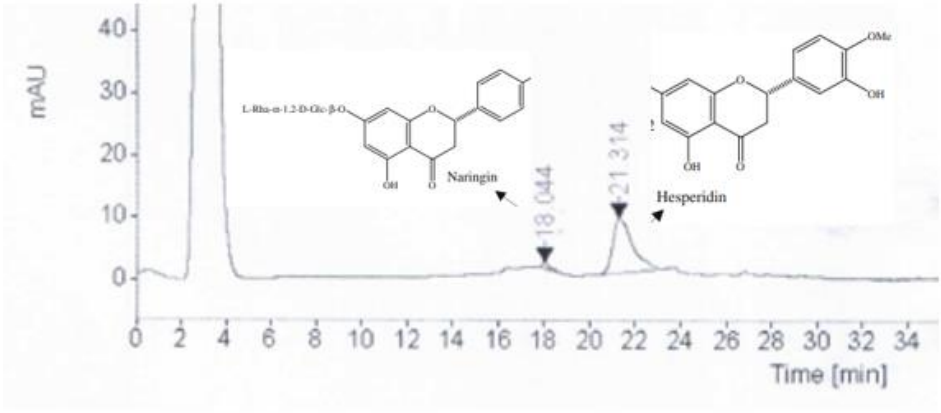


*Figure 3.* HPLC analysis of the individual composition of phenolic compounds in the extract of orange peels

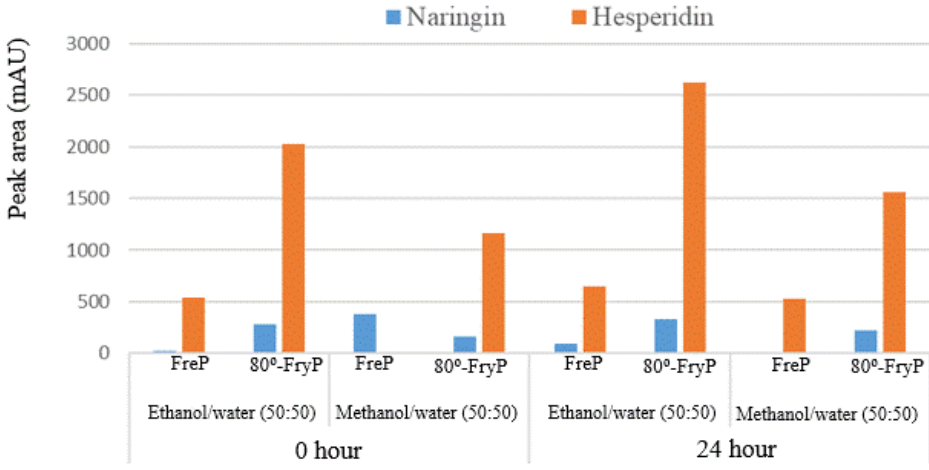


*Figure 4.* Effect of solvent system, drying temperature, and extraction time on the Content of Naringin and Hesperidin in the extract of orange peels (FreP: fresh orange peels; 80°-FryP: orange peels dried at 80 °C)

The results show that samples dried at 80 °C yielded higher concentrations of both naringin and hesperidin compared to fresh (undried) samples. Similarly, prolonged extraction times significantly enhanced flavonoid content; samples extracted for 24 hours exhibited greater levels of both compounds compared to those extracted for 0 hours.

Among the solvent systems tested, the ethanol/water mixture proved more effective than methanol/water in extracting flavonoids. Specifically, after 24 hours of extraction, the

concentrations of naringin and hesperidin in the ethanol/water extract were 331.0 mAU and 2616.5 mAU, respectively, representing increases of approximately 67% and 49% compared to the methanol/water system.

These differences may be explained by structural modifications to plant cells induced by the drying process, which likely increased cell wall porosity and, consequently, the surface area available for solvent interaction [14]. In addition, ethanol, having lower polarity than methanol, may better solubilize less polar flavonoid compounds such as naringin and hesperidin [15].

## 4. CONCLUSION

The study demonstrated that extraction conditions, including solvent type, drying temperature, and extraction time, significantly influence phenolic content. Ethanol/water proved to be a more effective solvent system than methanol/water for the extraction of flavonoids such as naringin and hesperidin, while water and methanol-to-water ratio of 1:1 presented the highest values for total polyphenol content (TPC) in their extracts. These findings support the use of moderate drying temperatures and ethanol-based solvents for optimizing the recovery of bioactive flavonoids from orange peels.

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## TÓM TẮT

### ẢNH HƯỞNG CỦA DUNG MÔI VÀ ĐIỀU KIỆN SẤY ĐẾN HÀM LƯỢNG POLYPHENOL TỔNG SỐ VÀ THÀNH PHẦN CẤU TỬ PHENOLICS TRONG VỎ CAM

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Mặc dù vỏ từ cam thường được xem là sản phẩm phụ của ngành công nghiệp sản xuất nước ép, chúng lại chứa hàm lượng cao các hợp chất có hoạt tính sinh học. Mục tiêu của nghiên cứu này là đánh giá ảnh hưởng của các điều kiện sấy khác nhau (sấy đối lưu) và các dung môi (nước, ethanol, methanol) đến hàm lượng polyphenol tổng số (TPC) và thành phần cấu tử phenolic (phân tích bằng HPLC) trong các dịch chiết từ vỏ cam. Kết quả cho thấy, dung môi nước và hỗn hợp methanol-nước theo tỉ lệ 1:1 cho các giá trị TPC cao nhất trong các mẫu chiết. Trong khi đó, hỗn hợp ethanol-nước theo tỉ lệ 1:1 được xác định là dung môi chiết xuất hiệu quả nhất để thu được hàm lượng cao các hợp chất phenolic, cụ thể như naringin và hesperidin. Khi so sánh các điều kiện sấy, mẫu được sấy bằng lò không khí nóng ở nhiệt độ 80 °C cho thấy khả năng giữ lại hàm lượng polyphenol tốt nhất.

*Từ khóa:* Vỏ cam, phế phụ phẩm, polyphenols, hoạt tính kháng oxy hóa, naringin, hesperidin.