

## ENHANCED RECOVERY OF SUGARS, PHENOLICS, AND TERPENES FROM *CITRUS PARADISI* CV. SHAMBER (GRAPE FRUIT) JUICE USING ADVANCED EXTRACTION TECHNOLOGIES

Waseem Ahmed<sup>1</sup>, Laiba Mumtaz<sup>2</sup>, Rafa Azmat<sup>3</sup>, Abdul Qayyum<sup>4</sup>, Mohamed Fawzy Ramadan<sup>5</sup>

### Address(es):

<sup>1</sup> Department of Horticulture, The University of Haripur, Pakistan.

<sup>2</sup> Department of Botany, The University of Haripur, Pakistan.

<sup>3</sup> Department of Chemistry, University of Karachi, Pakistan.

<sup>4</sup> Department of Agronomy, The University of Haripur, Pakistan.

<sup>5</sup> Department of Clinical Nutrition, Faculty of Applied Medical Sciences, Umm Al-Qura University, Makkah, Saudi Arabia.

\*Corresponding author: [Waseemuaf12@gmail.com](mailto:Waseemuaf12@gmail.com)

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### ABSTRACT

This study aims to optimize the extraction of valuable bioactive compounds, namely sugars, phenolics, and terpenes from *Citrus paradisi* cv. Shamber (grapefruit) juice that holds significant potential for applications in the food, pharmaceutical, and cosmetic industries. A comparative evaluation of various solvents (ethyl acetate, chloroform, water, n-hexane, and ascorbic acid solutions) combined with advanced extraction methods, including exhaustive extraction (EE), liquid-liquid micro-extraction (LLME), and supercritical fluid extraction (SFE) was conducted to identify the most efficient approach for maximizing yield and purity of these compounds. Results demonstrated that n-hexane paired with SFE provided the highest recovery of total sugars (7.2 g), fructose (1.9 g), glucose (1.9 g), and sucrose (3.9 g). In terms of phenolics, n-hexane yielded the highest concentration of hydroxycinnamic acids (2.9 mg/100 g), while ethyl acetate was superior for extracting quercetin, d-limonene, monoterpenes, and perillyl alcohol. Cold extraction with water resulted in comparatively lower bioactive content. Additionally, fresh juice and peel contained 0.37 mL and 0.59 mL of essential oil, respectively, with oils rich in aromatic compounds such as 3-hexanol, 2,3-heptanedione, cyclohexanol, d-limonene, and 1-(3-ethyloxiranyl)-ethanone. The batch-scale steam explosion technique negatively impacted oil yield. This systematic assessment identifies the most effective solvents and extraction methods for the quantification of essential compounds. It concludes that the optimal combination is n-hexane paired with SFE, which effectively enhances the extraction of valuable phytochemicals from grapefruit juice and supports sustainable and efficient phytochemical recovery.

**Keywords:** Citrus; Phytochemicals; Extraction; Oils; Sugar profiling; Terpenes; Phenolic compounds; Liquid-liquid micro-extraction

### INTRODUCTION

The Citrus genus, belonging to the Rutaceae family, includes a diverse group of fruit-bearing species that are widely cultivated across tropical and subtropical regions (Ahmed *et al.*, 2021). These fruits hold significant economic importance worldwide due to their sensory appeal, nutritional benefits, and contributions to global agriculture and food industries. Grapefruit (*Citrus paradisi*), in particular, has garnered increasing attention for its rich phytochemical composition, which includes a variety of sugars, phenolic compounds, and terpenes (Ahmed and Azmat, 2019; Arad, 2022; Ahmed *et al.*, 2023; Gilani *et al.*, 2023). These bioactive compounds are not only responsible for the fruit's characteristic flavor and aroma but also exhibit considerable health-promoting properties, such as antioxidant, anti-inflammatory, and cardioprotective effects. The cultivar *Shambers* of *C. paradisi* is especially noted for its potent nutritional and therapeutic potential, making it a valuable subject for phytochemical research (Khalid *et al.*, 2020; Ahmed *et al.*, 2023). Recent shifts in climate patterns have impacted citrus cultivation, affecting fruit yield and the concentration of bioactive compounds, which are sensitive to environmental factors. Consequently, there is a growing interest in maximizing the recovery of these compounds to maintain and enhance the nutritional and therapeutic qualities of citrus-derived products. Efficient extraction of these compounds is vital for their application in functional foods, nutraceuticals, and pharmaceutical formulations (Zhang *et al.*, 2022). The lost, altered, or destroyed active constituents during the preparation of extracts from plant samples determine how effective the extraction is and its impact on such commodities (Pszczolińska *et al.*, 2022; Haider *et al.*, 2022). Extraction is essential in analyzing various fruits, vegetables, and medicinal plants for desirable bioactive chemicals and their quantification process (Ali *et al.*, 2022; Al-Radadi *et al.*, 2022). Bioactive compounds are usually lost during extract preparation (Kultys *et al.*, 2022). The world focuses on green chemistry, aiming to develop non-toxic extraction procedures (Morin-Crini *et al.*, 2022; Lizundia *et al.*, 2022). Supercritical fluid extraction (SFE) is used in several fields, including environmental sciences, pharmaceuticals, natural products, and food science (Kupnik *et al.*, 2022). Rodrigues *et al.* (2022) investigated the supercritical gas-

assisted melting atomization process. The result was the best when extraction was performed at 1000 bar and 40°C. In addition, supercritical CO<sub>2</sub> was used successfully in many extraction fields, including nanonization (Zhang *et al.*, 2022) and micronization (Salehi *et al.*, 2022). Moreover, supercritical CO<sub>2</sub> extraction was performed at room temperature, and CO<sub>2</sub> was removed from the product in the extraction process (Sodeifian *et al.*, 2022). Ahmed *et al.* (2021) reported that the quantity of extracted bioactive phenolic acids depends on various solvents. A polar solvent, such as ethyl acetate or methanol, is used in hydrophilic extraction or combines dichloromethane/methanol to extract high lipophilic compounds to eliminate chlorophyll from tissues. In contrast, n-hexane extraction is employed in some circumstances (Rodríguez-González *et al.*, 2022). Green chemicals are trending, non-toxic, and highly used with increasing health concerns (Kashyap *et al.*, 2022).

Extraction of bioactive compounds from fruit matrices is inherently challenging due to the chemical complexity and diversity of target molecules. Sugars, phenolics, and terpenes differ markedly in polarity, stability, and solubility, requiring tailored extraction approaches to ensure high yield and preservation of bioactivity. Traditional extraction methods often involve the use of organic solvents and harsh conditions that can degrade sensitive compounds or introduce toxic residues, limiting their industrial and clinical applications (Granone and Hegel, 2022). The problem in successfully recovering bioactive chemicals like sugars, phenolic, and terpenes from *Shambers* juice extracts is establishing a strong extraction method that minimizes the degradation and loss of these important compounds throughout the extraction process. Due to temperature, duration, and solvent selection, current extraction procedures may provide poor yields, changed chemical profiles, or destruction of sensitive bioactive components. As a result, it is critical to identify and optimize a suitable solvent system and extraction procedure that maximizes the recovery of these bioactive chemicals while maintaining their purity. This study seeks to discover an optimal extraction procedure that combines efficiency, selectivity, and preservation, eventually adding to the nutritional and functional value of *Shambers* juice extracts. Green extraction technologies have gained momentum in recent years to overcome these limitations. Among them, supercritical fluid extraction (SFE) using carbon dioxide

stands out as a sustainable and efficient technique. SFE offers several advantages, including tunable solvent properties, minimal thermal degradation, and elimination of harmful solvent residues, making it ideal for recovering a broad spectrum of bioactive compounds. Despite these advantages, the application of SFE and other advanced extraction methods for the simultaneous recovery of sugars, phenolics, and terpenes from grapefruit juice remains underexplored. Moreover, the choice of solvent plays a critical role in extraction efficiency and selectivity. Solvents such as ethyl acetate, n-hexane, chloroform, water, and ascorbic acid solutions vary widely in polarity and extraction capabilities. A systematic comparison of these solvents combined with different extraction techniques exhaustive extraction, liquid-liquid microextraction, and SFE is necessary to identify optimal conditions that maximize yield and maintain compound integrity.

This study addresses a critical research question regarding the limitations of solvent-based extraction methods, which often result in lower quantification of compounds. It investigates which solvents and extraction methods are most effective in recovering sugars, phenolic acids, and terpenes from *Citrus paradisi* cv. Shambur juice while preserving their purity and biological activity. By conducting a comprehensive evaluation and optimization of extraction techniques, this research aims to advance the sustainable recovery of valuable phytochemicals from grapefruit juice. The outcomes will provide useful insights into efficient and environmentally friendly extraction protocols, supporting the development of high-quality grapefruit extracts for use in health-promoting food products, nutraceuticals, and therapeutic formulations. Additionally, this work contributes to the broader field of phytochemistry by highlighting the importance of integrating green technologies with traditional extraction approaches to enhance the recovery of multi-class bioactive compounds.

## MATERIAL AND METHODS

### Fruit Sampling and Preparation

Mature fruits of *Citrus paradisi* cv. Shambur were collected from the Citrus Nursery at the University of Agriculture, Faisalabad, in December 2021, following the standardized harvesting protocol (Ahmed et al., 2018b). Using sterilized fruit clippers, five randomly selected fruits per tree (500–700 g each) were harvested. The fruits were immediately transported under cooled conditions to the Pomology Laboratory. Upon arrival, fruits were washed, peeled, and juiced using a sanitized mechanical extractor. The juice was aliquoted and stored at  $-20^{\circ}\text{C}$  until further analysis to preserve phytochemical stability.

### Chemicals and Reagents

All solvents used were of analytical grade unless otherwise specified:

- **Solvents for extraction:** n-hexane, chloroform, ethyl acetate, dichloromethane, and carbon tetrachloride (Merck, Germany).
- **Solvents for polar compound extraction:** Methanol, ethanol, acetone, acetonitrile (Tedia, USA).
- **Aqueous phase:** Milli-Q deionized water (Millipore, USA).
- **Other reagents:** Ascorbic acid (Sigma-Aldrich), ammonium acetate, and reagents for HPLC mobile phases.

### Solvent Optimization

#### Optimization of extraction

The extraction methods were optimized with potential solvents for accuracy and efficiency. Different solvents (i.e., ethyl acetate, chloroform, n-hexane, water, and ascorbic acid solutions) were used at 10, 20, 60, and 70% concentrations. These solvents measure the ability of a solvent to dissolve or extract a target compound. The ascorbic acid (10 g of pure solvent) and volume of distilled water were adjusted to adjust the pH of the solution using a buffer solution. The prepared solution was stored in a dark and cool place to prevent the oxidation of ascorbic acid, and then the samples were subjected to HPLC. The peak regions, standard curves, and descriptive statistics were studied according to the methods of Williams et al. (2010). Supercritical Fluid Extraction (SFE)

According to Masturah et al. (2007), the extraction was performed with minor changes. The extracting studies were conducted utilizing a supercritical fluid extractor (MV-10 ASFE System, Waters, Malaysia) consisting of two motor-driven piston compressors, one for liquid  $\text{CO}_2$  and the other for the modifier. Before being piped to the extractor,  $\text{CO}_2$  was chilled to  $2^{\circ}\text{C}$  in a chiller (MV10; Waters Malaysia) to keep it in the liquid phase. A supercritical  $\text{CO}_2$  vessel was filled with 10 mL of citrus juice. SFE was performed with  $\text{CO}_2$  as the primary solvent at  $60^{\circ}\text{C}$  and 200 bar pressure, adding 50% (v/v) n-hexane. A static extraction was used for the first 23 minutes, followed by a 60-minute dynamic extraction with a 3 mL/min solvent flow rate. After 25 minutes, the isolated fractions were collected from the sample vessel. The material was first extracted and then methylated. The identification of compounds was performed using HPLC-DAD.

### Extraction Procedures

Each method was standardized with respect to juice volume, solvent-to-sample ratio, and time. All extractions were conducted in triplicate.

#### Supercritical Fluid Extraction (SFE)

Adapted from Masturah et al. (2007), 10 mL juice was extracted using a Waters MV-10 ASFE system with 50% n-hexane as a co-solvent.  $\text{CO}_2$  was pumped at 200 bar and  $60^{\circ}\text{C}$ . After 23 minutes of static phase, dynamic extraction was carried out for 60 minutes at 3 mL/min. Extracts were methylated before HPLC-DAD analysis.

#### Dispersive Liquid-Liquid Microextraction (DLLME)

According to Cheng et al. (2010), 5 mL of juice was mixed with 0.75 mL acetonitrile and 50  $\mu\text{L}$  carbon tetrachloride. After vortexing, centrifugation (3000 rpm, 5 min) was performed. Supernatants were analyzed using Agilent 1100 HPLC-DAD.

#### Cold Extraction Method (CEM)

Juice (5 mL) was incubated in 600 mL n-hexane under aluminum foil and shaken at 150 rpm for 30 min, followed by 72-hour resting. The extracts were filtered (Whatman No. 1) and stored at  $4^{\circ}\text{C}$  prior to analysis.

#### Microwave-Assisted Extraction (MAE)

Following Oroian et al. (2020), 10 mL juice was mixed with 70% ethanol. The mixture was heated in a microwave oven (200 W, 10 min), cooled, filtered, and stored for analysis.

#### Ultrasonic Extraction (UEM)

Based on Rodrigues et al. (2017), 5 mL juice was mixed with 50 mL solvent and sonicated at 170 W/24 kHz in an Ultrasonic bath for 20 min. Post centrifugation, extracts were filtered and subjected to polarity-based separation.

#### Dispersive Liquid-Liquid Micro-extraction (DLLME)

DLLME was carried out under the following conditions: Met a water sample of 5 mL, which was used for diethofencarb and pyrimethanil (Cheng et al., 2010). The instrument used was Agilent 1100 HPLC. A 10-mL test tube was used to hold 5 mL of juice sample. A solution of 0.75 mL dispersive solvent (acetonitrile) and 50 mL of extraction solvent (carbon tetrachloride) was injected into the 5 mL sample through a syringe. After a minor shaking, water/acetonitrile/charcoal solution appeared in the test tube. Carbon tetrachloride droplets were considered to be maintained in the liquid during the study of water samples. The sample was centrifuged at 3000 rpm for 5 min. The components were identified using the HPLC-DAD.

### Analytical Methods

#### Sugar Analysis

HPLC was reported as a method for sugar profiling in grapefruit juice. The total sugar profile of fructose, glucose, and sucrose was studied (Filip et al., 2016). We extracted sugars from 60% of methanol and then passed them through a C-18 column. The filtrated samples were taken in 0.46-mm-disk nylon, and then quantification and separation were carried out on an ammonium-bonded amino column with a mobile phase composed of 20:80 (v/v)  $\text{H}_2\text{O}$  and  $\text{CH}_3\text{CN}$ . Evaporative light scattering detector (ELSD) Sedex 60 It and an LCNET II/ADC interface and LCNET II/ADC interface were used. Acetonitrile (77%) and 23% HPLC grade water were degassed and ultra-sonicated as a mobile phase. The temperature was held at  $20^{\circ}\text{C}$ , and the pressure was 13.2 kPa for 1 min (Rodríguez-Alcalá et al., 2010). Sugars (glucose, fructose, sucrose) were extracted with 60% methanol, filtered (0.45  $\mu\text{m}$ ), and injected into a UHPLC system using a bonded amino column. ELSD detection was performed under isocratic conditions ( $\text{H}_2\text{O}$ : ACN = 20:80 v/v,  $20^{\circ}\text{C}$ , 13.2 kPa).

#### Phenolic and Terpene Identification by HPLC-DAD

UHPLC systems employ greater pressures than regular HPLC, allowing for the use of smaller particles in column packing. This results in improved resolution, quicker analysis, and increased sensitivity. Many UHPLC systems provide DAD detection. High-performance fluids evaluate individual phenolic acids and terpenes. The reserved phase was performed in an isocratic state with column C18. The moving phase of acetic acid and methanol (70:40, v/v) was used. The volume injected was 6 mL, and the range was 290 nm. In the operating time of bioactive compounds, the flow rate was 2.0 mL/min. The peaks and concentrations were determined

using elution conditions to compare the peak regions with reference substances (Pascale et al., 2018).

### Oil Extraction and Quantification

#### Oil Content in the Juice and Peel of Citrus

The oil content of fresh grapefruit juice and fruit peel was determined in fresh form by applying the bench-scale batch steam explosion method. The fresh juice and peel were obtained by slicing a grapefruit and juicing it with a Sunkist Juice Extractor (Model # 8-RA07 or 8-RC00, Ontario, CA, USA).

#### Bench Scale Batch Steam Explosion

The method was described by Dorado et al. (2019) and applied with minor modifications. In a batch bench steam explosion reactor, 300 mL of juice and 400 g of fruit peel were introduced into the pipe system. The samples were steam treated for 4 min and 6 min at 130°C and 150°C, at steam pressures of 205 and 415 kPa, respectively. A manual pressure regulator was given the pressure, and the steam-processed samples were pulverized and discharged in a stainless-steel catch container. The samples were then stored in plastic bags at -20 °C until analyzed.

#### Oil Analysis

Fresh or steam-exploded juice (30 mL) and peel (30 g) were blended with deionized water in a blender (Omni International Homogenizer, Model GLH-01, Omni International, Marietta, GA) or Waring Commercial Blender (Model # 7011S). The sample was then analyzed for oil content. Oil content was measured through the Scott bromate titration method (Dorado et al., 2021). A standardized d-limonene mixture was formed by adding d-limonene (100 µL) with isopropanol (100 mL). The standard d-limonene solution was diluted in deionized water (25 mL) and isopropanol (15 mL). The solution was refluxed for about 30 minutes as the alcohol part was extracted. Ten mL of HCl (50%) and 2-3 drops of methyl orange indicator were introduced to the alcohol part. It was then determined by titrating with a 0.0247N bromate-bromide solution until a colorless solution was achieved. A mathematical formula was used to calculate the concern values. The method was used for two reasons. One factor was the preparation of d-limonene with a standard solution.

**0.001 mL of d-limonene in 10 mL of standard solution/ mL of titrant used = Factor 1**

The titrated 0.0347 N of N bromide solution was the solution with a colorless endpoint. The d-limonene was measured in Equation 2. This was then titrated with a 0.0247 N bromate-bromide solution to a colorless endpoint. The amount of d-limonene was calculated using Equation 2:

**[(Factor from Equation 1 x mL of titrant used)/g of sample] x 100= % V/W (2)**

#### GC-MS Profiling

Peel oil (3 mL) was extracted with n-hexane, dried over sodium sulfate, and analyzed using Shimadzu QP 2010 Plus GC-MS with NIST spectral libraries.

#### 6. Statistical Analysis

The data were analyzed using a factorial design with separated variables. Duncan's multiple-range test was employed in this study. Each sample had three replicates (Williams et al., 2010), and the physical and maturity were calculated using GraphPad Prism 5 and non-linear regression. Software 1.7 (Sciex City Foster, USA) was used for the flavonoids and phenolic acids. Control data acquisition peaks were analyzed and confirmed using the program (Multi-Quant 2.1.1.2, Foster, CA, USA).

## RESULTS AND DISCUSSION

### Sugar Profile of *Citrus paradisi* Juice

The sugar content in *Citrus paradisi* juice was strongly influenced by the choice of extraction solvent and method. Analysis via HPLC-DAD (Figure 1) revealed that among the solvents tested, **n-hexane combined with supercritical fluid extraction (SFE)** yielded the highest total sugar concentration of 7.81 g per sample. This included fructose (2.10 g), glucose (1.9 g), and sucrose (3.6 g). The superior performance of n-hexane with SFE likely reflects the solvent's ability to penetrate the juice matrix under supercritical conditions, enhancing sugar solubilization and recovery. Other extraction techniques, such as cold extraction (CE), microwave-assisted extraction (MAE), exhaustive extraction (EE), and liquid-liquid micro-extraction (LLME), also influence sugar yields. Notably, LLME showed promising results with a total sugar content of 7.20 g, slightly lower than SFE but with comparable fructose (1.9 g), glucose (1.9 g), and a slightly

higher sucrose (3.9 g). These findings highlight LLME as a potential alternative for efficient sugar recovery when SFE is not feasible.

When ranking solvents by extraction efficiency for sugars, the order was **n-hexane > ascorbic acid solution > water > chloroform > ethyl acetate**. This trend suggests that less polar solvents better facilitate sugar extraction under the tested conditions, possibly by enhancing the disruption of juice matrices or solubilizing sugar molecules more effectively.

#### Extraction methods ranked in effectiveness as follows:

**SFE > LLME > EE > CE > MAE**. SFE's superiority stems from its unique combination of temperature and pressure that improves solvent diffusivity and penetration, optimizing sugar extraction. These results emphasize that choosing an appropriate solvent-method combination is critical for maximizing sugar recovery in grapefruit juice processing.

#### Extraction and Identification of Phenolic Acids

Phenolic acids contribute significantly to the antioxidant capacity of grapefruit juice. Their extraction was also solvent- and method-dependent (Table 1). Hydroxycinnamic acids reached their highest concentration (2.9 mg/100 g) with n-hexane, while ethyl acetate resulted in minimal recovery (0.13 mg/100 g). This again confirms the advantage of non-polar solvents for extracting these bioactives. Interestingly, ferulic acid exhibited an opposite pattern: the highest level (2.4 mg/100 g) was recovered using ascorbic acid solution, a polar solvent, while n-hexane yielded the lowest concentration (0.7 mg/100 g). This difference highlights the diverse polarity of phenolic compounds, indicating the necessity of solvent selection tailored to target molecules.

Among extraction methods, **SFE consistently yielded higher phenolic acid concentrations**, including ferulic and hydroxycinnamic acids, than other methods. Cold extraction (CE) resulted in the lowest recovery, likely due to its limited ability to disrupt cellular structures and extract bound phenolics.

The solvent efficiency order paralleled that of sugar extraction: n-hexane > ascorbic acid solution > water > chloroform > ethyl acetate, and the extraction method efficiency ranked: **SFE > LLME > EE > MAE > CE**.

These findings underscore SFE's potential for efficient, selective extraction of phenolic antioxidants from grapefruit juice, which has implications for producing functional beverages and nutraceuticals.

#### Terpene Extraction and Identification

Terpenes, which are responsible for many of the grapefruit's characteristic aromas and health benefits, were analyzed next (Table 2). N-hexane combined with SFE extracted the highest levels of key terpene compounds, such as quercetin, d-limonene, monoterpenes, and perillyl alcohol. The high recovery is attributed to n-hexane's non-polar nature, which effectively solubilizes hydrophobic terpenes. Conversely, ethyl acetate showed the lowest terpene extraction, indicating its limited suitability for these compounds. Extraction methods followed a similar trend, with **SFE outperforming other techniques**, reinforcing its status as a preferred method for terpene isolation, the chromatogram shown in Figure 3.

These results suggest that optimized SFE extraction with n-hexane can maximize the recovery of valuable terpene compounds, which are beneficial for flavor and therapeutic applications.

#### Oil Content in Juice and Peel

The oil content analysis revealed that grapefruit peel contains significantly more oil (0.59 mL) compared to fresh juice (0.37 mL) (Table 3). This difference aligns with previous reports (Dorado et al., 2021) and underscores the peel's role as a rich source of essential oils. However, when steam explosion treatment was applied, both juice and peel samples showed dramatic oil losses: approximately **95% reduction in juice and 75% reduction in peel oil content** (Figure 2). These results highlight the susceptibility of grapefruit oils to thermal degradation and evaporation during harsh treatments like steam explosion. Consequently, gentle extraction methods such as SFE or LLME are recommended for preserving oil quality and quantity, particularly for applications requiring high-value essential oils.

#### Aromatic Compounds from *Citrus paradisi* Peel

GC-MS analysis was employed to characterize the aromatic profile of grapefruit peel oils. Using [insert extraction method, e.g., steam distillation or solvent extraction] followed by analysis with an Agilent 6890 GC coupled to a 5973 MS, five major aromatic compounds were identified (Table 4). The peel oil composition was dominated by **d-limonene (>90%)**, consistent with previous studies showing its importance as the primary citrus aroma compound. Other key aroma compounds detected included 3-hexanol, 2,3-heptanedione, cyclohexanol, and 1-(3-ethoxyiranyl)-ethanone. Additionally, sesquiterpene hydrocarbons, alcohols, esters, aldehydes, and minor components like vitamin E, decanal,

cubebol, myrcene, and neral dodecanal were identified, as shown in **Figure 3**. These results confirm the complex and rich volatile profile of grapefruit peel oil, which contributes to its unique fragrance and potential health benefits. The dominance of d-limonene suggests that extraction methods preserving this compound are essential for maintaining peel oil quality.

**DISCUSSION**

The present study analyzed *Citrus paradisi* cv. shamber juice as affected by different solvents and extraction methods, revealing that proper extraction processes and solvent applications are necessary to obtain pure and high amounts of bioactive compounds with lower losses. **Thota et al. (2022)** and **Zuo et al. (2022)** used appropriate solvents and extraction processes linked with chromatography techniques for isolating pure natural compounds from complicated mixtures of raw materials.

**Sugar Profile of Citrus paradisi Juice**

Our results clearly show that both the choice of solvent and extraction method significantly affect the sugar content extracted from grapefruit juice. Notably, supercritical fluid extraction (SFE) using n-hexane achieved the highest yields of total sugars, including fructose, glucose, and sucrose. This suggests that SFE, with its unique combination of high diffusivity and solvent power under supercritical conditions, is highly effective at breaking down cellular structures and releasing sugars. The results proved that total sugar, fructose, glucose, and sucrose concentrations varied as affected by solvent extraction (**Figure 1**). Similar results were documented by **Pereira et al. (2018)** and **Yousef et al. (2012)**, who observed high solvent-based efficacy of the sugar contents. In the case of n-hexane and ethyl acetate, the sugars are completely dissolved in solvents and measured, where all individual sugars increased in SFE methods (**Antonelli et al., 2022**), which may be the result of the starch as well as the hemicellulose hydrolysis process. Liquid-liquid micro-extraction (LLME) also showed strong sugar recovery, suggesting it could be a practical alternative to SFE, especially in labs or industries where SFE equipment is unavailable or too costly. The ranking of solvents with n-hexane outperforming polar solvents like ethyl acetate highlights how solvent polarity influences sugar extraction, likely because non-polar solvents under SFE conditions better disrupt cell membranes and solubilize sugar molecules bound within the matrix.

**Phenolic Acids Extraction and Identification**

Phenolic acids, important for their antioxidant activity, were extracted most efficiently by n-hexane when paired with SFE, particularly for hydroxycinnamic acids. Interestingly, ferulic acid recovered better using ascorbic acid solutions, which may be linked to its chemical affinity or better stabilization of this compound during extraction. Plant secondary metabolites, phenolic acids, are potent free radical scavengers with various medical and biological functions in animals and plants (**Ghasemi et al., 2009**). The properties of solvent extraction were based on solvent-to-solvent ratio and temperature and are critical factors in the efficacy of the extraction process of essential compounds. The solvent base efficacy was higher in SFE due to the extraction process, which affected the equilibrium of the respect solute inside the compounds (**Thakur et al., 2022**). The low polarity of CO<sub>2</sub> makes it ideal for extracting non-polar natural products such as lipids and volatile oils. A modifier may be added to CO<sub>2</sub> to enhance its solvating traits. Some solvents are polar and non-polar, while n-hexane is semi-polar and recovers higher flavonoids, phenolics, and terpene compounds (**Addi et al., 2022**). Terpenes are a significant class of phytochemicals in grapefruit juice that contribute to its taste and flavor. Impacting solvent-based efficiency was active due to the polarity and nature of the solvent. It forms stable free radicals, which cannot initiate or propagate other oxidation. The poor performance of ethyl acetate indicates its limited ability to extract phenolic compounds from grapefruit under

our conditions. This reinforces the importance of matching solvent characteristics to target compounds. Our results confirm SFE’s versatility—not only in sugar extraction but also in isolating bioactive phenolics, which could enhance the nutritional and functional value of grapefruit-derived products.

**Terpene Extraction and Identification**

Our terpene analysis further supported SFE combined with n-hexane as the most effective method, recovering higher levels of key bioactive terpenes such as d-limonene and quercetin. This is consistent with the lipophilic nature of terpenes and demonstrates how SFE efficiently extracts these volatile, non-polar compounds (**Addi et al., 2022**). The lower extraction efficiency with ethyl acetate across sugars, phenolics, and terpenes suggests it is not well-suited for comprehensive citrus extraction. This insight is valuable for guiding solvent selection in future citrus bioactive compound extractions.

**Oil Content in Juice and Peel**

The measured oil content confirmed that grapefruit peel is a richer source of essential oils than juice, supporting previous findings. The substantial loss of oil after steam explosion treatment highlights the sensitivity of volatile oils to thermal and pressure stresses. These results emphasize that while steam explosion might be useful for other purposes, it is not ideal for preserving essential oils in citrus. Instead, gentler methods such as SFE or LLME should be preferred to maintain oil integrity. Citrus essential oils are rich in bio-functional chemicals with antioxidant, antibacterial, anti-inflammatory, and cytoprotective activities (**Pattanaik et al., 2022; Hashim et al., 2022; Granato et al., 2022**). The whole grapefruit has been used for the extraction of oil. Grapefruit peel oil is an important product from a juice processing plant, with white and pink oil fetching 65 and 43 USD per kg, respectively (**Ultra International B.V. 2019**). **Kutlu et al. (2022)** found a small amount of oil content (0.008-0.075%) in citrus juice. Valencia and Hamlin sweet orange fruit could yield up to 0.4% and 0.7% (w/w) peel oil (**Bouaita et al., 2022; Julacha, 2022**). Heat explosion effectively volatilized up to 98% of the available peel oil in white GFPR and up to 68% in red GFPR (**Dorado et al., 2021b**).

**Aromatic Compounds in C. paradisi Peel**

GC-MS analysis identified d-limonene as the dominant aromatic compound, representing over 90% of the peel oil composition. Its high abundance and consistency underline its importance as a quality marker for grapefruit oil. The presence of diverse secondary compounds such as cubebol, vitamin E, and neral contributes to the peel oil’s complex aroma and bioactivity. These findings highlight the need for extraction techniques that preserve the delicate balance of these compounds to maximize flavor and therapeutic potential.

**Limitations and Future Directions**

Our study provides important insights into solvent and method efficiencies, but it has limitations. For example, we did not evaluate the effects of extraction temperature or pressure variations within SFE, which could further optimize yields. Additionally, the stability of extracted compounds during storage was not assessed. The peel of *C. paradisi* is a rich source of aromatic compounds. The aromatic compounds contain >99% hydrocarbons, followed by pinene and terpinene (**Thangaleela et al., 2022**). Hydrocarbons are present in a lower amount, approximately <5%, contributing to fruit quality. These compounds have a scented aroma and medicinal importance (**Badalamenti et al., 2022**). Future work could explore optimizing SFE parameters for specific compounds, scaling the process for industrial applications, and investigating the biological activities of extracts obtained by different methods to link extraction efficiency with functional benefits better.

**Table 1** Levels of phenolic acids (mg/100g) in *Citrus × paradisi* cv. Shamber as affected by different solvents and extraction methods for their efficiency

	Solvent					Method of extraction				
	Ethyl acetate	Chloroform	Aqueous	Hexane	Ascorbic acid	Cold extraction (CE)	Microwave-assisted extraction (MAE)	Exhaustive extraction (EE)	Liquid-liquid micro-extraction (LLME)	Supercritical Fluid Extraction (SFE)
<b>Limonene</b>	61.5e	69.2d	71.10c	82.0a	76.12b	70.0 e	72.0 d	75.0 c	80.3 b	88.2 a
<b>Quercetin</b>	1.4de	1.5d	1.7b	3.2a	1.9c	1.5 e	1.9d	2.1 c	2.5b	2.9a
<b>Perillyl alcohol</b>	1.6e	1.7 d	1.4c	3.9a	1.7 b	1.1 e	1.4 d	1.8 c	2.4b	2.3 a
<b>Monoterpenes</b>	1.6e	1.9d	2.1c	3.1 a	2.7b	1.7 e	2.1 d	2.3 c	2.4 b	2.9 a

Different letters within the column indicate significant differences between solvents and extraction methods (p < 0.05).

**Table 2** Levels of terpenes in *Citrus × paradisi* cv. Shamber juice as affected by different solvents and extraction methods

Phenolic compound (mg/100g)	Solvent					Method of extraction				
	Ethyl acetate	Chloroform	Aqueous	Hexane	Ascorbic acid	Cold extraction (CE)	Microwave-assisted extraction (MAE)	Exhaustive extraction (EE)	Liquid-liquid micro-extraction (LLME)	Supercritical Fluid Extraction (SFE)
Hydroxycinnamic acids	0.13d	1.5c	1.5b	2.9a	1.2b	1.1 e	1.2d	1.5c	1.7 b	2.1 a
Ferulic acid	1.1 e	1.3d	1.5 c	3.7 a	2.4 b	1.21 of	1.14 d	1.3c	1.6b	1.9 a
Protocatechuic acid	1.3d	1.4c	1.15c	2.6a	1.5b	2.1e	2.2 d	2.5c	2.6.b	2.7 a
Neochlorogenic acid	1.2e	1.3d	1.4c	2.6a	1.1 b	1.2 e	1.3 d	1.4 c	1.5 b	1.6 a
Protocatechuic acid	1.3 e	1.4 d	1.5 c	3.2 a	1.6 b	1.2 ef	1.5 d	1.7 c	1.8 b	2.1 a
Neochlorogenic acid	1.3d	1.4c	1.15c	2.6a	1.5b	2.1e	2.2 d	2.5c	2.6.b	2.7 a

Data are mean ± standard deviation of triplicate determinations.

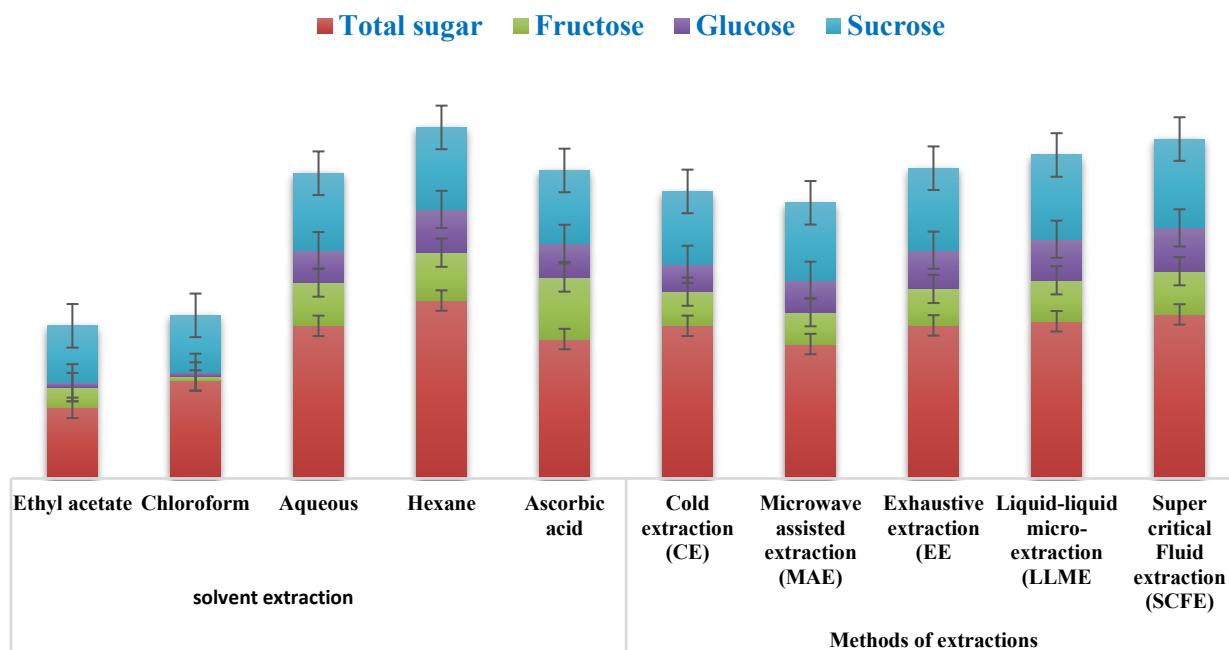
**Table 3** Juice oil content and peel oil content of fresh and steam-exploded juice and peel

Samples	Fresh sample (wet)	Bench scale batch explosion (dry)
Juice oil	0.37 ±0.011	0.015±0.015
Peel oil	0.59±0.013	0.13±0.016

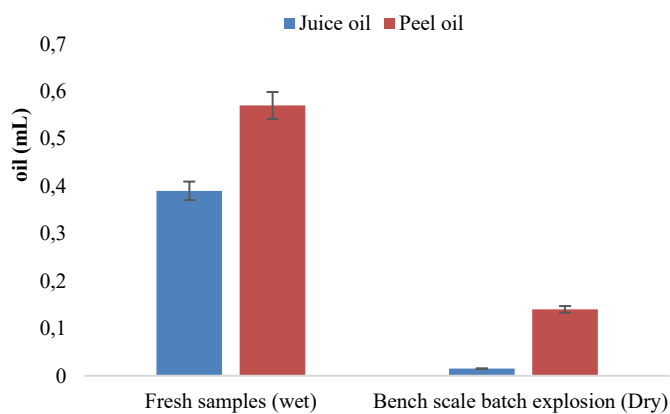
Data are mean ± standard deviation of triplicate determinations.

**Table 4** Percentage area of aromatic compounds in *Citrus × paradisi* cv. shamber peel oil

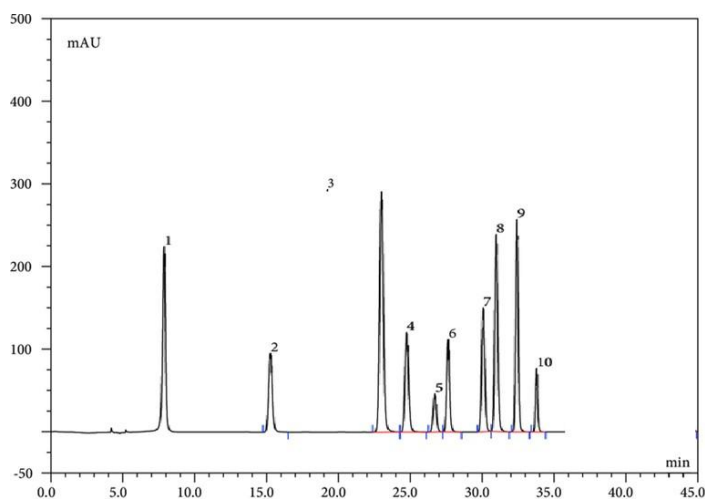
Compounds	Area (%)	Time (min)
Limonene	16.12 ±0.012	13.11±0.012
3-Hexanol	0.72±0.012	4.5±0.012
2,3-Heptanedione	0.69±0.012	9.8±0.012
Cyclohexanol	0.22±0.012	5.5±0.012
1-(3-Ethylxiranyl)-ethanone	17.02±0.012	11.9±0.012



**Figure 1** Levels of total and individual sugars in *Citrus × paradisi* cv. shamber as affected by different solvents and extraction methods



**Figure 2** Fresh and steam exploded oil content from *C. paradisi* juice and peel. Data are mean ± standard deviation of triplicate determinations.



**Figure 3** Chromatogram of terpenes (compounds 1-5) and aromatic compounds (compounds 6-10) in *Citrus × paradisi* cv. Shamber juice

**CONCLUSION**

This study highlights the critical role of solvents and extraction methods in accurately quantifying bioactive compounds from *Citrus paradisi* juice. Our results show that n-hexane and ascorbic acid solutions are the most effective solvents for extracting sugars, phenolic compounds, and terpenes, while ethyl acetate demonstrated significantly lower efficiency. Among the extraction methods tested, supercritical fluid extraction (SFE) proved superior, especially for sugar profiling via HPLC-DAD, yielding higher contents of fructose, glucose, phenolics, and terpenes compared to other methods such as cold extraction, microwave-assisted extraction, exhaustive extraction, and liquid-liquid micro-extraction. The findings have important implications for both analytical chemistry and industrial applications. Efficient extraction methods like SFE combined with suitable solvents can enhance the quality and accuracy of bioactive compound measurements, which is essential for the food, pharmaceutical, and nutraceutical industries aiming to maximize health benefits and product quality.

Furthermore, the relatively simple and rapid nature of these methods, which require no additional purification or derivatization steps, supports their practical adoption. However, the study has some limitations. Optimization of extraction parameters for large-scale industrial applications and assessment of compound stability during storage were beyond this work's scope and should be addressed in future studies. Additionally, the effects of solvent residues on product safety need further evaluation.

For future research, we recommend exploring greener and more sustainable solvents to reduce environmental impact, as well as investigating the bioavailability and functional efficacy of the extracted compounds in vivo. Expanding this research to include other citrus varieties and integrating advanced extraction techniques could further improve the recovery and applicability of bioactive substances. In conclusion, our work provides valuable insights into solvent and method selection for bioactive compound extraction from grapefruit, offering a foundation for improving analytical procedures and promoting the development of high-quality citrus-based products.

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#### Declarations

**Conflict of interest:** The authors declare no competing interests.

**Compliance with ethics requirements:** This study does not contain any studies with human participants or animals performed by any authors.

**Availability of data and material:** The datasets generated during the current study are made available by the corresponding author upon reasonable request.

**Code availability:** Not applicable

#### REFERENCES

- Addi, M., Elbouzidi, A., Abid, M., Tungmunthum, D., Elamrani, A., & Hano, C. (2022). An overview of bioactive flavonoids from *Citrus* fruits. *Applied Sciences*, *12*(1), 29. <https://doi.org/10.3390/app12010029>
- Ahmed, W., Azmat, R., Qayyum, A., Bibi, Y., Ali, S., Ahmed, R., & Ramadan, M. F. (2023). Application of HPLC, LC-Q-TOF-MS, UF-HPLC and LC-MS in the comparative analysis of flavonoids and sterols in *Citrus paradisi* cv. Shamber (grapefruit). *Rendiconti Lincei. Scienze Fisiche e Naturali*. Advance online publication. <https://doi.org/10.1007/s12210-023-01188-8>
- Ahmed, W., Azmat, R., Mehmood, A., Ahmed, R., Liaquat, M., Khan, S. U., Qayyum, A., & Khan, S. M. (2021). Comparison of storability and seasonal changes on new flavonoids, polyphenolic acids and terpenes compounds of *Citrus paradisi* (grapefruit) cv. Shamber through advanced methods. *Food Measurement and Characterization*, *15*, 2915–2921. <https://doi.org/10.1007/s11694-021-00815-y>
- Akbari, B., Baghaei-Yazdi, N., Bahmaie, M., & Mahdavi Abhari, F. (2022). The role of plant-derived natural antioxidants in reduction of oxidative stress. *BioFactors*. *BioFactors*, *48*(3):611–633. <https://doi.org/10.1002/biof.1831>
- Ali, A., Riaz, S., Sameen, A., Naumovski, N., Iqbal, M. W., Rehman, A., Mehany, T., Zeng, X. A., & Manzoor, M. F. (2022). The disposition of bioactive compounds from fruit waste, their extraction, and analysis using novel technologies: A review. *Processes*, *10*, 2014; <https://doi.org/10.3390/pr10102014>
- Al-Radadi, N. S. (2022). Laboratory scale medicinal plants mediated green synthesis of biocompatible nanomaterials and their versatile biomedical applications. *Saudi Journal of Biological Sciences*, *29*(5), 3848–3870. <https://doi.org/10.1016/j.sjbs.2022.02.042>
- Antonelli, M., Holčápek, M., & Wolrab, D. (2022). Ultrahigh-performance supercritical fluid chromatography-mass spectrometry for the qualitative analysis of metabolites covering a large polarity range. *Journal of Chromatography A*, *1665*, 462832. <https://doi.org/10.1016/j.chroma.2022.462832>
- Badalamenti, N., Bruno, M., Schicchi, R., Geraci, A., Leporini, M., Gervasi, L., Tundis, R., & Loizzo, M. R. (2022). Chemical compositions and antioxidant activities of essential oils, and their combinations, obtained from flavedo by-product of seven cultivars of *Citrus aurantium* L. *Molecules*, *27*(5), 1580. <https://doi.org/10.3390/molecules27051580>
- Bouaita, R., Derbal, K., Panico, A., Iasimone, F., Pontoni, L., Fabbicino, M., & Pirozzi, F. (2022). Methane production from anaerobic co-digestion of orange peel waste and organic fraction of municipal solid waste in batch and semi-continuous

- reactors. *Biomass and Bioenergy*, *160*, 106421. <https://doi.org/10.1016/j.biombioe.2022.106421>
- Deepak, P. (2022). Flavonoids: A functional food with anticancer properties. In *Handbook of Research on Natural Products and Their Bioactive Compounds as Cancer Therapeutics* (pp. 319–351). IGI Global. <https://doi.org/10.4018/978-1-7998-9258-8.ch015>
- Gilani, F., Raftani Amiri, Z., Esmailzadeh Kenari, R., et al. (2023). Investigation of extraction yield, chemical composition, bioactive compounds, antioxidant and antimicrobial characteristics of citron (*Citrus medica* L.) peel essential oils produced by hydrodistillation and supercritical carbon dioxide. *Food Measurement*, *17*, 4332–4344. <https://doi.org/10.1007/s11694-023-01956-y>
- Granato, D., Carochi, M., Barros, L., Zabetakis, I., Mocan, A., Tsoupras, A., Cruz, A. G., & Pimentel, T. C. (2022). Implementation of sustainable development goals in the dairy sector: Perspectives on the use of agro-industrial side-streams to design functional foods. *Trends in Food Science & Technology*. <https://doi.org/10.1016/j.tifs.2022.04.009>
- Granone, L. I., Hegel, P. E., & Pereda, S. (2022). Citrus fruit processing by pressure intensified technologies: A review. *The Journal of Supercritical Fluids*, *105646*. <https://doi.org/10.1016/j.supflu.2022.105646>
- Haider, M. W., Nafees, M., Valipour, M., Iqbal, R., Ali, S., Asad, H. U., Faried, H. N., Aslam, M., Iqbal, J., & Shahzad, M. A. (2022). Postharvest *Eucalyptus* leaf extract application extends the sustainable supply of strawberries by retaining physicochemical quality during cold storage. *Sustainability*, *14*(22), 14822. <https://doi.org/10.3390/su142214822>
- Hashim, M., Hamid, Z., Gul, Z., & Akbar, A. (2022). Functional, nutritional and medicinal potential of banana peel. *Pure and Applied Biology*, *12*(1), 470–490. <http://dx.doi.org/10.19045/bspab.2023.120049>
- Jena, G., & Philip, J. (2022). A review on recent advances in graphene oxide-based composite coatings for anticorrosion applications. *Progress in Organic Coatings*, *173*, 107208. <https://doi.org/10.1016/j.porgcoat.2022.107208>
- Julaeha, E., Nurzaman, M., Wahyudi, T., Nurjanah, S., Permadi, N., & Anshori, J. A. (2022). The development of the antibacterial microcapsules of citrus essential oil for the cosmetotextile application: A review. *Molecules*, *27*(22), 8090. <https://doi.org/10.3390/molecules27228090>
- Kashyap, P. K., Singh, S., Singh, M. K., Gupta, A., Tandon, S., Shanker, K., Verma, R. K., & Verma, R. S. (2022). An efficient process for the extraction of lutein and chemical characterization of other organic volatiles from *Tagetes erecta* L. *Food Chemistry*, *396*, 133647. <https://doi.org/10.1016/j.foodchem.2022.133647>
- Khalid, M. F., Shafiqat, W., Khan, R. I., Abbas, M., Ahmed, T., Ahmad, S., & Hussain, S. (2022). Citrus responses and tolerance against temperature stress. In *Citrus Production* (pp. 149–155). CRC Press. <https://doi.org/10.1201/9781003119852-10>
- Kultys, E., & Kurek, M. A. (2022). Green extraction of carotenoids from fruit and vegetable byproducts: A review. *Molecules*, *27*(2), 518. <https://doi.org/10.3390/molecules27020518>
- Kupnik, K., Leitgeb, M., Primožič, M., Postružnik, V., Kotnik, P., Kučuk, N., Knez, Ž., & Marevc, M. K. (2022). Supercritical fluid and conventional extractions of high value-added compounds from pomegranate peels waste: Production, quantification and antimicrobial activity of bioactive constituents. *Plants*, *11*(7), 928. <https://doi.org/10.3390/plants11070928>
- Kutlu, N., Pandiselvam, R., Kamiloglu, A., Saka, I., Sruthi, N. U., Kothakota, A., Socol, C. T., & Maerescu, C. M. (2022). Impact of ultrasonication applications on color profile of foods. *Ultrasonics Sonochemistry*, *89*, 106109. <https://doi.org/10.1016/j.ultsonch.2022.106109>
- Lizundia, E., Luzzi, F., & Puglia, D. (2022). Organic waste valorisation towards circular and sustainable biocomposites. *Green Chemistry*. <https://doi.org/10.1039/D2GC01668K>
- Morin-Crini, N., Lichtfouse, E., Fourmentin, M., Ribeiro, A. R. L., Noutsopoulos, C., Mapelli, F., Fenyvesi, É., Vieira, M. G. A., Picos-Corrales, L. A., Moreno-Piraján, J. C., & Giraldo, L. (2022). Removal of emerging contaminants from wastewater using advanced treatments: A review. *Environmental Chemistry Letters*. <https://doi.org/10.1007/s10311-021-01335-y>
- Pattanaik, K., Verma, A., & Sharma, D. (2022). Chapter 2: Potential uses and health benefits of citrus oil and its benefits. In *Isolation, Characterization, and Therapeutic Applications of Natural Bioactive Compounds* (p. 23). <https://doi.org/10.4018/978-1-6684-3540-4>
- Pszczolińska, K., & Kociólek, B. (2022). The pesticide residue analysis in commodities with high content of chlorophyll based on the quick, easy, cheap, effective, rugged, and safe method: A review. *Journal of Separation Science*, *45*(1), 149–165. <https://doi.org/10.1002/jssc.202100304>
- Rodrigues, V. H., Portugal, I., & Silva, C. M. (2023). Experimental optimization of the supercritical fluid extraction of triterpenoids from *Acacia dealbata* Link leaves. *Separation and Purification Technology*, *306*, 122637. <https://doi.org/10.1016/j.seppur.2022.122637>
- Rodríguez-González, I., Díaz-Reinoso, B., & Domínguez, H. (2022). Intensification strategies for the extraction of polyunsaturated fatty acids and other lipophilic fractions from seaweeds. *Food and Bioprocess Technology*. <https://doi.org/10.1007/s11947-021-02757-1>

- Salehi, H., Karimi, M., & Raofie, F. (2022). Micronization of thebaine extracted from *Papaver bracteatum* Lindl. using supercritical fluid technology. *Journal of AOAC International*, 105(2), 593–602. <https://doi.org/10.1093/jaoacint/qsab188>
- Seker, G., & Akbas, M. Y. (2023). Evaluation of bioactivities of *Pistacia vera* L. hull extracts as a potential antimicrobial and antioxidant natural source. *Food Science and Technology International*. <https://doi.org/10.1177/10820132231193478>
- Siano, F., Picariello, G., Castaldo, D., Cautela, D., Caruso, T., & Vasca, E. (2023). Monitoring antioxidants by coulometry: Quantitative assessment of the strikingly high antioxidant capacity of bergamot (*Citrus bergamia* R.) by-products. *Talanta*, 251, 123765. <https://doi.org/10.1016/j.talanta.2022.123765>
- Sodeifian, G., & Usefi, M. M. B. (2022). Solubility, extraction, and nanoparticles production in supercritical carbon dioxide: A mini-review. *ChemBioEng Reviews*. <https://doi.org/10.1002/cben.202200020>
- Thakur, R., Mehta, N., Singh, A., Joshi, K., Gautam, A., & Bithel, N. (2022). Techniques for the isolation of plant-based bioactive compounds. In *Isolation, Characterization, and Therapeutic Applications of Natural Bioactive Compounds* (pp. 280–296). IGI Global. <https://doi.org/10.4018/978-1-6684-3540-4.ch014>
- Thangaleela, S., Sivamaruthi, B. S., Kesika, P., Bharathi, M., Kunaviktikul, W., Klunklin, A., Chanthapoon, C., & Chaiyasut, C. (2022). Essential oils, phytoncides, aromachology, and aromatherapy: A review. *Applied Sciences*, 12(9), 4495. <https://doi.org/10.3390/app12094495>
- Thota, J. R., Maddula, R. K., Pandeti, S., & Yerra, N. V. (2022). Advances in extraction and analysis of natural products. In *Chemistry of Natural Products: Phytochemistry and Pharmacognosy of Medicinal Plants* (p. 203).
- Zhang, J., Zhang, Y., Liu, X., Xu, X., Li, Y., & Zhang, T. (2022). Supercritical fluid chromatography tandem mass spectrometry employed with evaporation-free liquid-liquid extraction for the rapid analysis of cinnarizine in rat plasma. *Journal of Separation Science*, 45(4), 968–975. <https://doi.org/10.1002/jssc.202100825>
- Zuo, J., Ma, P., Geng, S., Kong, Y., Li, X., Fan, Z., Zhang, Y., Dong, A., & Zhou, Q. (2022). Optimization of the extraction process of flavonoids from *Trollius ledebouri* with natural deep eutectic solvents. *Journal of Separation Science*, 45(3), 717–727. <https://doi.org/10.1002/jssc.202100802>