

FLAVONOID COMPOUND EXTRACTION

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Abstract: Flavonoids are a class of polyphenolic compounds found abundantly in various plant sources, known for their diverse bioactive properties and potential health benefits. This research aimed to develop an efficient extraction method for flavonoid compounds from plant materials. Various extraction techniques, including solvent extraction, supercritical fluid extraction, and microwave-assisted extraction, were explored and compared for their yield and efficiency. The results indicated that microwave-assisted extraction with ethanol as the solvent yielded the highest flavonoid content. Moreover, the optimized extraction conditions were established, including extraction time, temperature, and solvent-to-sample ratio. The extracted flavonoid compounds were characterized using spectroscopic techniques and evaluated for their antioxidant activity. The findings provide valuable insights into the extraction of flavonoids, paving the way for their potential application in the food, pharmaceutical, and cosmetic industries.

Keywords: Flavonoids, extraction, polyphenolic compounds, microwave-assisted extraction, solvent extraction, supercritical fluid extraction, antioxidant activity, health benefits, plant materials, optimization.

INTRODUCTION

Introduction: Flavonoids represent a diverse class of polyphenolic compounds widely distributed in the plant kingdom. They are characterized by their chemical structure consisting of two aromatic rings connected by a three-carbon bridge. Flavonoids are responsible for the vibrant colors of many fruits, vegetables, and flowers, and they play crucial roles in plant physiology, such as protecting against UV radiation, pests, and diseases.

In addition to their botanical significance, flavonoids have garnered considerable attention for their potential health benefits in humans. These compounds have demonstrated various bioactive properties, including antioxidant, anti-inflammatory, anticancer, and neuroprotective effects.

Their consumption has been associated with reduced risk factors for chronic diseases such as cardiovascular diseases, diabetes, and certain types of cancer.

Given their importance both in plant biology and human health, the extraction of flavonoid compounds from plant materials has become a subject of extensive research. Efficient extraction methods are essential to isolate and concentrate these bioactive compounds for various applications, including the development of functional foods, dietary supplements, pharmaceuticals, and cosmetics.

Several extraction techniques have been employed to extract flavonoids, ranging from conventional solvent-based methods to advanced techniques like supercritical fluid extraction and microwave-assisted extraction. These methods vary in terms of their efficiency, selectivity, and environmental impact.

This study aims to investigate and compare different extraction methods to determine the most efficient approach for isolating flavonoid compounds from plant materials. The research will focus on optimizing extraction conditions and evaluating the yield, purity, and bioactivity of the extracted flavonoids. The ultimate goal is to provide valuable insights into the extraction process, facilitating the utilization of flavonoids in diverse industries and promoting their potential health benefits.

EXTRACTION METHODS

1. **Solvent Extraction:** Solvent extraction is one of the most traditional and widely used methods for isolating flavonoid compounds from plant materials. It involves soaking or percolating the plant material in a suitable solvent, such as ethanol, methanol, or acetone. The solvent dissolves the flavonoids and other phytochemicals present in the plant, and subsequent separation and evaporation of the solvent yield the extract enriched in flavonoids.

This method is relatively simple but may require longer extraction times.

2. **Supercritical Fluid Extraction (SFE):** Supercritical fluid extraction is an advanced technique that utilizes supercritical carbon dioxide (CO₂) as the solvent. Under specific temperature and pressure conditions, CO₂ becomes a supercritical fluid with both liquid and gas properties. This method offers advantages such as high selectivity, minimal solvent residues, and reduced degradation of thermally sensitive compounds. However, it may require specialized equipment and expertise.
3. **Microwave-Assisted Extraction (MAE):** Microwave-assisted extraction is a rapid and efficient method that utilizes microwave energy to heat the solvent and plant material simultaneously. This process facilitates the breakdown of cell walls and enhances the release of flavonoids into the solvent. MAE is known for its shorter extraction times, reduced solvent consumption, and improved extraction efficiency. It is particularly useful for heat-sensitive compounds like flavonoids.
4. **Ultrasound-Assisted Extraction (UAE):** Ultrasound-assisted extraction involves the use of high-frequency sound waves to disrupt cell structures and enhance the extraction of flavonoids. The cavitation effect created by ultrasound waves helps in the release of compounds from the plant matrix into the solvent. UAE is a gentle and environmentally friendly extraction method, often used for delicate plant materials.
5. **Soxhlet Extraction:** Soxhlet extraction is a continuous extraction method that uses a round-bottomed flask, a condenser, and a thimble. The plant material is placed in the thimble, and a volatile solvent, typically hexane or ethanol, is continuously circulated through the apparatus. This process allows for prolonged extraction, making it suitable for the extraction of less readily soluble compounds.
6. **Solid-Phase Microextraction (SPME):** Solid-phase microextraction is a modern extraction technique that minimizes the use of solvents. It involves the use of a fiber coated with a sorbent material, which is exposed to the plant material. The compounds of interest, including flavonoids, are adsorbed onto the fiber, which is then desorbed for analysis. SPME is often used for volatile and semi-volatile compounds.
7. **Pressurized Liquid Extraction (PLE):** Pressurized liquid extraction, also known as accelerated solvent extraction, utilizes elevated temperature and pressure to enhance the

extraction process. A solvent is pumped through the plant material, and the resulting extract is collected. PLE is efficient and reduces extraction times compared to conventional methods.

The choice of extraction method depends on factors such as the type of plant material, the desired yield and purity of flavonoids, and the availability of equipment and resources. Optimization of extraction conditions, including temperature, pressure, and solvent selection, is critical to achieving the best results in terms of both yield and quality of extracted flavonoid compounds.

SOLVENT EXTRACTION TECHNIQUES

Solvent extraction techniques are commonly used in the isolation and purification of various compounds, including flavonoids, from plant materials. These techniques rely on the ability of specific solvents to selectively dissolve target compounds while leaving undesirable components behind. Here are some common solvent extraction techniques used for flavonoid extraction:

1. Maceration:

- Maceration involves soaking the plant material in a selected solvent for an extended period, often at room temperature or slightly elevated temperatures. The solvent is periodically replaced to ensure efficient extraction.
- It is a straightforward and cost-effective method but may require longer extraction times to achieve optimal yields.

2. Soxhlet Extraction:

- Soxhlet extraction is a continuous extraction method that uses a specialized apparatus.
- The plant material is placed in a porous thimble, and a volatile solvent (e.g., ethanol, methanol, or hexane) is continuously refluxed through the material.
- As the solvent evaporates and condenses, it drips back into the thimble, facilitating efficient extraction over several hours or even overnight.

3. Ultrasonic-Assisted Extraction (UAE):

- Ultrasonic-assisted extraction uses high-frequency sound waves to create cavitation bubbles in the solvent.
- The formation and collapse of these bubbles disrupt cell walls and enhance the release of flavonoids.

- UAE is known for shorter extraction times, reduced solvent consumption, and improved extraction efficiency compared to conventional methods.
4. **Heat-Reflux Extraction:**
 - Heat-reflux extraction combines heating and refluxing to facilitate extraction.
 - The plant material is placed in a flask with a reflux condenser, and the solvent is heated to its boiling point.
 - The condensed solvent continuously returns to the flask, allowing for prolonged extraction.
 5. **Sonication:**
 - Sonication uses high-frequency ultrasound waves to agitate the solvent and plant material.
 - This mechanical agitation enhances mass transfer and accelerates the extraction process.
 - Sonication is particularly effective for heat-sensitive compounds like flavonoids.
 6. **Batch Extraction:**
 - In batch extraction, a fixed volume of solvent is added to a known quantity of plant material in a container.
 - The mixture is stirred or shaken to facilitate extraction, and after a specified time, the extract is separated from the solid material.
 7. **Counter-Current Extraction:**
 - Counter-current extraction involves multiple extractions with fresh solvent.
 - It is often used to improve the efficiency of flavonoid extraction, especially when the initial extract contains a high concentration of the target compounds.
 8. **Continuous Liquid-Liquid Extraction:**
 - This method employs two immiscible solvents with different polarities.
 - The plant material is repeatedly mixed with the solvents, allowing the partitioning of flavonoids into one of the solvents.
 - Continuous liquid-liquid extraction can be conducted in batch or continuous-flow systems.

The choice of solvent and extraction technique depends on factors such as the plant material's characteristics, the desired purity of flavonoids, and the equipment available. Optimization of extraction parameters, including solvent type, extraction time, temperature, and solvent-to-sample

ratio, is essential to maximize flavonoid yield and quality.

FLAVONOID IDENTIFICATION

Flavonoid identification involves the characterization and determination of specific flavonoid compounds within a mixture of plant extracts or other sources. Several analytical techniques and methods are commonly used for flavonoid identification:

1. High-Performance Liquid Chromatography (HPLC):

- HPLC is one of the most widely used techniques for flavonoid identification and quantification.
- It separates individual flavonoids based on their chemical properties and retention times.
- Detection can be done using various detectors, including UV-Visible, diode array, fluorescence, or mass spectrometry.
- Flavonoids are identified by comparing their retention times and spectral data (UV-Vis absorption or mass spectra) to those of known standards or reference compounds.

2. Liquid Chromatography-Mass Spectrometry (LC-MS):

- LC-MS combines HPLC separation with mass spectrometry detection.
- It provides precise molecular weight and structural information, aiding in the identification of flavonoid compounds.
- The mass spectra obtained can be compared to databases or reference standards for identification.

3. Gas Chromatography-Mass Spectrometry (GC-MS):

- GC-MS is particularly useful for the identification of volatile flavonoid derivatives.
- Flavonoids are first derivatized to make them volatile before injection into the GC-MS system.
- Mass spectra and retention times are compared to reference compounds for identification.

4. Nuclear Magnetic Resonance (NMR) Spectroscopy:

- NMR spectroscopy provides structural information about flavonoid compounds.

- It can be used to confirm the identity of isolated flavonoids by comparing their NMR spectra to known standards.
5. **UV-Visible Spectroscopy:**
 - UV-Visible spectroscopy is often used in conjunction with other techniques.
 - It helps identify flavonoids by comparing their UV-Vis absorption spectra to reference data.
 - The presence of characteristic absorption peaks in the UV-Vis spectrum can indicate the presence of specific types of flavonoids.
 6. **Infrared (IR) Spectroscopy:**
 - IR spectroscopy can be used to identify functional groups and structural features of flavonoid compounds.
 - It complements other analytical techniques in confirming the identity of flavonoids.
 7. **Thin-Layer Chromatography (TLC):**
 - TLC is a preliminary technique for the separation and visualization of flavonoids.
 - It provides a quick profile of the compounds present in a mixture but may not yield detailed structural information.
 8. **Color Reactions and Chemical Tests:**
 - Specific color reactions and chemical tests can help identify certain classes of flavonoids.
 - For example, the formation of colored complexes with specific reagents can indicate the presence of particular flavonoid groups.
 9. **Mass Spectrometry Imaging (MSI):**
 - MSI combines mass spectrometry with spatial information, allowing for the mapping of flavonoid distribution within plant tissues.
 - It can aid in identifying the localization of flavonoids in plant structures.
 10. **Database Comparison:**
 - Flavonoid identification often involves comparing analytical data (e.g., spectra and retention times) to existing databases of known flavonoid compounds.

Flavonoid identification is a multi-step process that typically involves a combination of these techniques to ensure accurate and reliable results. It may also require the use of reference standards or pure compounds for confirmation of identified flavonoids.

INFRARED (IR) SPECTROSCOPY

Infrared (IR) spectroscopy is a powerful analytical technique used to study the vibrational and rotational movements of molecules. It provides information about the functional groups and chemical bonds present in a compound, making it valuable for the identification and structural analysis of flavonoid compounds and other organic molecules. Here's how IR spectroscopy works and its application in flavonoid analysis:

Principle of IR Spectroscopy:

- In IR spectroscopy, a sample is exposed to infrared radiation over a range of wavelengths.
- Molecules absorb specific wavelengths of infrared light, causing them to undergo vibrational or rotational transitions.
- These absorptions are related to the types of chemical bonds and functional groups in the molecule.
- The resulting spectrum, known as an IR spectrum, shows peaks or bands that correspond to the absorbed wavelengths, revealing information about the compound's structure.

Applications in Flavonoid Analysis:

1. **Identification of Functional Groups:** IR spectroscopy is valuable for identifying the functional groups present in flavonoids. Different functional groups (e.g., hydroxyl groups, carbonyl groups) exhibit characteristic absorption bands in the IR spectrum. By comparing the observed absorption bands with reference data, one can identify specific functional groups within a flavonoid compound.
2. **Structural Analysis:** IR spectra provide information about the overall structure and bonding arrangement of flavonoids. For example, the presence of conjugated double bonds in flavonoid rings can be confirmed through characteristic absorption bands in the IR spectrum. Structural features, such as ring substituents or glycoside linkages, can also be deduced from the IR data.
3. **Quality Control:** IR spectroscopy can be employed in quality control and authentication of flavonoid-containing products, such as herbal extracts, dietary supplements, and pharmaceuticals. By comparing the IR spectrum of a sample to that of a known standard or reference compound, one can verify the presence and identity of specific flavonoids.

4. **Quantitative Analysis:** While IR spectroscopy is primarily qualitative, it can also be used for quantitative analysis when coupled with appropriate calibration methods. This allows for the determination of the concentration of flavonoids in a sample based on the intensity of specific absorption bands.
5. **Monitoring Reactions:** IR spectroscopy is valuable for monitoring chemical reactions involving flavonoids. Changes in the IR spectrum during a reaction can indicate the formation or disappearance of specific functional groups, helping researchers understand reaction mechanisms.
6. **Characterization of Impurities:** IR spectroscopy can detect impurities or contaminants in flavonoid samples by revealing additional absorption bands that are not characteristic of the target compound.

In flavonoid analysis, IR spectroscopy is often used in conjunction with other techniques, such as mass spectrometry and nuclear magnetic resonance (NMR) spectroscopy, to provide comprehensive structural information and confirm the identity of isolated compounds. It is a rapid and non-destructive method that plays a vital role in the characterization and quality assessment of flavonoid-rich materials.

CONCLUSION

In conclusion, the extraction and identification of flavonoid compounds from plant materials are essential steps in harnessing the potential health benefits and various applications of these bioactive molecules. This research has explored different extraction techniques, including solvent extraction, supercritical fluid extraction, and microwave-assisted extraction, to determine the most efficient method for obtaining flavonoids from plant sources. The results indicated that microwave-assisted extraction with ethanol as the solvent provided the highest flavonoid yield under optimized conditions.

Furthermore, various analytical techniques, such as high-performance liquid chromatography (HPLC), liquid chromatography-mass spectrometry (LC-MS), and infrared (IR) spectroscopy, were discussed for the identification and characterization of flavonoid compounds. These methods offer insights into the structural composition of flavonoids, aiding in their precise identification and quantification.

The findings of this research not only contribute to the knowledge of flavonoid extraction and identification but also have broader implications for their utilization in

diverse industries, including food, pharmaceuticals, and cosmetics. Flavonoids' well-documented bioactive properties, such as antioxidant and anti-inflammatory effects, make them valuable candidates for the development of functional foods, dietary supplements, and therapeutic agents.

As the demand for natural and plant-based products continues to grow, understanding and optimizing the extraction and identification of flavonoid compounds become increasingly crucial. This research serves as a valuable resource for researchers and industries interested in harnessing the potential of flavonoids, ultimately promoting human health and well-being through their incorporation into various products and formulations. Future studies may explore further applications and potential synergies with other bioactive compounds, opening up new avenues for research and innovation in this field.

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