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Modern Methods for Determining Total Polyphenol Content in Food

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Abstract. Polyphenolic compounds represent one of the most characteristic bioactive constituents in plants, exhibiting prominent biological functions including antioxidant, anti-inflammatory, anti-tumour, and cardiovascular protective properties. Their content frequently serves as a key indicator for food quality assessment and functional food development. With the rapid advancement of the nutritional health industry, the demand for precise detection of total polyphenols and their monomeric compounds in foods has significantly increased. Against this backdrop, this paper systematically reviews the principal modern analytical techniques currently employed in polyphenol detection, including the Folin–Ciocalteu colorimetric method, infrared spectroscopy, nuclear magnetic resonance spectroscopy (NMR/qNMR), and high-performance liquid chromatography (HPLC). A comparative analysis is conducted on the detection principles, sensitivity, matrix adaptability, and respective advantages and disadvantages of each method. The paper further explores the application prospects of multi-technique integration, data model optimisation, and green analytical strategies in polyphenol detection. By summarising existing research advances, this study aims to provide a comprehensive and systematic reference for quality control of functional foods, evaluation of active components, and selection of food analytical methods.

Keywords: Polyphenolic compounds, Modified Folin-Ciocalteu Assay, Infrared Spectroscopy, NMR, HPLC

1. Introduction

Polyphenolic compounds were first identified in the 19th century from plants such as tea leaves, grapes, and oak bark, representing a class of secondary metabolites occurring in nature. Research indicates that polyphenols not only influence plant colouration, flavour, and antioxidant properties but also exert diverse physiological effects on human health, including anti-inflammatory, cardiovascular protective, anti-tumour, antioxidant, and anti-ageing actions. As a representative polyphenolic compound, resveratrol significantly mitigates oxidative stress damage to the cardiovascular system through mechanisms such as activating the SIRT1 signalling pathway, enhancing mitochondrial function, and inhibiting low-density lipoprotein (LDL) oxidation [1].

Due to their rich biological activity and natural sources, polyphenolic compounds have become the focus of food science research in recent years, mainly due to the growing demand of consumers

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for "natural, healthy and sustainable" food. A large number of studies have shown that wild edible plants have excellent antioxidant properties and are usually rich in phenols and flavonoids. These substances can be used as functional ingredients for food preservation and nutrition enhancement, as well as natural antioxidants [2]. In addition to their nutritional and pharmacological value, these plant resources also meet the "clean label" standards and the principles of sustainable environmental development in the contemporary food industry.

Therefore, accurate determination of polyphenol content in food is crucial to assessing food quality, clarifying its functional characteristics, and supporting health claims. Traditional detection technologies (such as the Folin-phenol reagent method) are widely used because of their low cost and easy operation, but they also have the disadvantages of poor specificity and vulnerability to interference. In recent years, modern analysis methods such as near-infrared spectroscopy (NIR), capillary electrophoresis (CE), liquid chromatography-mass spectrometry (LC-MS) and high-performance liquid chromatography (HPLC) have gradually replaced traditional methods and become mainstream technologies for research and application due to their significant improvement in sensitivity and accuracy. This paper systematically reviews the research progress of modern analytical methods for determining the total polyphenol content in food. It focuses on traditional colorimetric methods (such as Folin-Ciocalteu methods) and modern instrument analysis techniques including near-infrared spectroscopy, high-performance liquid chromatography and nuclear magnetic resonance. The research deeply explores the detection principle, sensitivity, applicability and existing limitations of these methods in different food substrates. Through the systematic collation and comparative analysis of these methods, this study aims to provide a scientific and comprehensive reference framework for the selection and establishment of analytical methods for functional food development, natural product quality control and industrial production.

2. Detection methods for polyphenolic compounds

2.1. Modified Folin-Ciocalteu assay

Due to its easy operation and relatively low cost, the Folin-phenol reagent method is still a common traditional method for detecting polyphenols in food. However, this method often has problems such as obvious matrix interference, narrow concentration application range, and large instrument system error, especially when analyzing complex matrix food [3]. With the expansion of the food industry, the market demand for polyphenol-rich products (such as tea drinks and health food) is increasing, which requires more standardized and precise analysis methods.

In response to the limitations of Appendix A of the current national standard GB/T 21733-2008, including the narrow range of concentration and the large differences between laboratories due to the optical properties of the instrument, Gao Shucai and others have carried out a study to optimize the determination method of tea polyphenols in tea beverages [4]. The study systematically optimized the derivative conditions by using single-factor experiments. In the process of sample preparation, the purity of the sample was improved through steps such as ultrasonic degassing and milk breaking, lead acetate precipitation protein and sodium sulfate removal of excessive lead. Then the standard curve of gallic acid was drawn. The optimized method is compared with the national standard method, and the precision and accuracy of the method are verified through the standardization experiment. In the 10 mL solution system, the best derivation conditions were: 1.0 mL of benzotriol reagent and 1.5 mL of sodium carbonate solution. The reaction can be carried out in natural light for 60 minutes. The resulting calibration curve equation was $y = 0.100876x + 0.015891$, and the correlation coefficient $R^2 = 0.9989$. The linear range was 100–2000 mg/kg,

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covering almost all possible phenolic concentrations in commercially available tea drinks. The average recovery rate of this method was 106.9%, and the relative standard deviation was only 0.6% (n=6). Compared with the national standard method, this optimization method can effectively reduce matrix interference and instrument system error. For complex matrix samples (such as Shiti Dahongbao milk tea), this method can obtain more accurate results, and the absorbance value was always within the optimal measurement range (0.2–0.7) of the spectrophotometer.

In view of the relative lack of tea polyphenol determination methods in the field of health food, Shang Yan and others explored the applicability of the Folin-phenol reagent method in such products [5]. The study uses 70% methanol as the extraction solvent. The sample is centrifuged after soaking in a 70℃ water bath for 10 minutes. Take the supernatant and dilute it to a fixed volume many times for subsequent use. At the same time, the standard reserve solution of gallic acid and 10% Folin reagent solution were prepared. By adding the standard solution, Folin reagent and 7.5% sodium carbonate solution in turn, mix and leave for 60 minutes, and then determine the absorbance at 765 nm. From this, draw a standard curve and calculate the sample concentration. High, medium and low concentration parallel sample determination and standard recovery rate test were carried out. The results show that the relative standard deviation of the method to determine the parallel sample was always 1.0%, the standard recovery rate was between 95.79% and 99.72%, and the relative standard deviation was between 0.23% and 2.20%. All data meet the requirements of the GB/T 27404-2008 standard, indicating that the method has excellent repeatability and accuracy. This provides reliable technical support for the accurate determination of tea polyphenol content in health food.

After targeted optimization, the Folin-phenol reagent method has shown excellent performance in the detection of polyphenols in tea drinks and health foods. By adjusting the derivation conditions and optimizing the sample pretreatment procedure, this method effectively solves the interference problem caused by different food substrates and broadens the applicable concentration range. With its high accuracy and precision, this method has become an important technical means to determine the total polyphenol content in food, providing a reliable reference for the quality control and standard formulation of related foods.

2.2. Infrared spectroscopy

Infrared spectroscopy, as an efficient, non-destructive and rapid detection method, has shown great potential in the field of food polyphenol content analysis in recent years. Traditional detection methods such as the Folin-Ciocalteu method, despite their high accuracy, have problems including cumbersome operation, sample destruction and long time-consuming, making it difficult to meet the demand for efficient quality control in the modern food industry. Near-infrared spectroscopy has gradually become a research hotspot in polyphenol content analysis due to its advantages of rapidity, environmental friendliness and in-situ detection.

Xu Jinchai et al. developed a tea fresh leaf quality detection device integrating visible/short-wave near-infrared and long-wave near-infrared spectrometers, aiming to solve the problems of low efficiency and sample destruction existing in traditional methods for tea tree variety identification and tea polyphenol content detection [6]. In this study, spectral data in the 400~1050 nm and 1051~1650 nm bands were collected simultaneously. Data-level and feature-level fusion strategies were adopted, combined with preprocessing methods such as Savitzky-Golay smoothing, to construct various machine learning models including partial least squares discriminant analysis and one-dimensional convolutional neural network. In terms of tea polyphenol content prediction, the one-dimensional convolutional neural network model based on data-level fusion performed the best,

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with a coefficient of determination of the prediction set reaching 0.8020, a root mean square error of prediction of 0.6368%, and a residual predictive deviation of 2.2684. Compared with single-band modeling, multi-source data fusion effectively improved the generalization ability and prediction accuracy of the model. It not only significantly reduced the variable dimension, but also overcame the problem of incomplete information caused by the band limitation of a single sensor, providing reliable technical support for tea tree variety selection and fresh leaf quality monitoring.

Yang Chengen et al. proposed a strategy combining mid-infrared spectroscopy and chemometrics to address the problems of low efficiency and poor repeatability of traditional methods in the detection of polyphenol content in Aronia melanocarpa [7]. In this study, 750 Aronia melanocarpa samples from 15 different producing areas were collected. Spectral data in the range of 4000~400 cm^{-1} were collected using a Fourier transform infrared spectrometer, and the calibration set and validation set were divided in a ratio of 4:1 by the K-S algorithm. To improve model performance, the scheme compared various spectral preprocessing methods such as multiplicative scatter correction, standard normal variate transformation, smoothing, first derivative and second derivative, and selected characteristic wavelengths using the competitive adaptive reweighted sampling algorithm and successive projections algorithm. Finally, the optimized wavelength variables were input into models such as random forest regression, partial least squares regression, extreme learning machine and support vector regression for performance comparison. The results showed that the random forest regression model constructed based on 269 characteristic wavelengths selected by the competitive adaptive reweighted sampling algorithm performed the best, with a coefficient of determination of the validation set reaching 0.9743, a root mean square error of prediction of 0.1006, and a relative percent deviation as high as 6.2356. By effectively compressing the dimension of spectral data, this method significantly improved the prediction accuracy and stability of the model. It not only overcomes the limitations of traditional methods such as complex processes and long time consumption, but also provides reliable technical support for the rapid and non-destructive detection of polyphenol content in Aronia melanocarpa.

Jin Jiarui et al. took 144 black tea samples as research objects, systematically evaluated the applicability of near-infrared spectroscopy in the rapid detection of tea polyphenols, and focused on the influence of different sample forms (intact tea leaves and powdered tea leaves) on model performance [8]. In this study, a Fourier transform near-infrared spectrometer was used to collect spectra in the range of 4000~12500 cm-1. After comparing various preprocessing methods, it was found that standard normal variate transformation combined with first derivative and Savitzky-Golay smoothing could effectively eliminate baseline drift and noise interference. In partial least squares modeling, the correlation coefficient of the prediction set of the model built with intact tea leaf samples was 0.9541, and the root mean square error of prediction was 0.870%, which were better than 0.9243 and 0.972% of the powdered samples. This result indicated that intact tea leaf samples can better retain their original spectral characteristics and avoid additional variations introduced by crushing, thus confirming the feasibility of near-infrared spectroscopy for non-destructive detection of tea polyphenol content while maintaining sample integrity, and providing a practical method for on-site rapid evaluation of black tea quality [8].

In summary, near-infrared spectroscopy can significantly improve the accuracy and efficiency of tea polyphenol content detection through multi-band data fusion and optimized preprocessing methods. Future research can be further deepened in aspects such as expanding sample diversity, developing portable equipment and fusing more sensing information, so as to promote the application of this technology in a wider range of food matrices.

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2.3. Nuclear Magnetic Resonance spectroscopy (NMR)

Nuclear magnetic resonance spectroscopy (NMR) is a structural analysis technology based on nuclear spin characteristics, which has become an important tool for quantitative research on polyphenolic compounds in recent years. Unlike traditional analysis methods that rely on photoabsorption or chromatographic separation, NMR does not require standard products to establish calibration curves, nor does it depend on the ultraviolet absorption characteristics or chromatographic retention behavior of compounds. The quantitative results come directly from the integral area of the signal of the analyzed nucleus (such as ^{1}H or ^{13}C). Therefore, NMR can accurately quantify multiple components at the same time without destroying samples or complex preprocessing, and has high specificity, excellent reproducibility and low sensitivity to matrix interference. These characteristics give it a unique advantage in analyzing the total polyphenol content and monomer polyphenol structure in complex food systems, especially suitable for samples with scarce standard products, complex substrates or high degree of component coexistence.

In practical applications, polyphenol analysis based on quantitative nuclear magnetic resonance (qNMR) has become an important research direction in recent years. The relevant research adopts the external standard method or internal standard method to realize the absolute quantification of polyphenol compounds by measuring the integral area of the characteristic proton signal in the sample solution and combining the structural information. For example, Shi et al. accurately quantified the total phenol content and six major phenolic monomers (such as catechins and proanthocyanins) in cinnamon skin extracts by selecting high-resolution proton signals and using the external standard method. This shows that quantitative nuclear magnetic resonance (qNMR) has good linearity, method stability and high precision. This method overcomes the common problems of incomplete separation, peak overlap and lack of standard products in high-performance liquid chromatography (HPLC), and provides a feasible new way for the accurate quantification of polyphenol components in complex plant matrix [9].

In addition, with the improvement of the sensitivity of nuclear magnetic resonance instruments, the optimization of pulse sequences and the progress of multi-dimensional nuclear magnetic resonance technology, the application of qNMR in food analysis continues to expand. Research shows that nuclear magnetic resonance (NMR) can quickly analyze the main phenolic compounds in tea, polyphenol extracts and fruit and vegetable products. It distinguishes phenolic monomers with similar structures through parameters such as chemical shift, coupling constant and integral intensity, showing significant advantages in structural identification. Unlike infrared spectrum, NMR does not require spectral pretreatment or model calibration, nor is it affected by stability problems caused by peak overlap. Compared with high-performance liquid chromatography (HPLC), NMR eliminates tedious separation steps, making it more suitable for fast high-throughput sample screening.

In a word, NMR spectrum has the advantages of high specificity, no need for calibration curves, anti-matrix interference, and the ability to quantify multiple components at the same time. These characteristics make it an important supplementary technology for the quantitative research of polyphenols in food. With the further improvement of sensitivity (for example, the use of low-temperature probes and the improvement of the acquisition rate) and the optimization of quantitative methods, quantitative nuclear magnetic resonance (qNMR) is expected to become an indispensable quantitative "gold standard" in food polyphenol research. It is expected to play an increasingly key role in the development of functional food, quality control and component analysis of complex natural products.

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2.4. High-Performance Liquid Chromatography (HPLC)

High-performance liquid chromatography (HPLC) is the most critical analysis technology for detecting functional polyphenols in food at present. It has a significant advantage in separating polyphenol compounds in complex substrates with similar structure, similar polarity and low concentration. Compared with traditional colorimetric or infrared spectroscopy, HPLC has higher separation efficiency, sensitivity and reproducibility. Therefore, it has been widely used in the field of functional food, such as health food, tea drinks, fruit and vegetable products and plant extracts.

Xu Xiaoyun and others are committed to the efficient utilization of polyphenol resources in longan nuclear processing waste [10]. In response to the challenges of complex polyphenol composition and difficult separation and preparation, they systematically optimized the determination conditions of high-performance liquid chromatography (HPLC). In this study, Censial C18 chromatography column was used to systematically screen and determine the best analysis conditions: the mobile phase was methanol and water (volume ratio 10:90), the flow rate was 0.200 mL/min, and the detection wavelength was 254 nm. The sample was dissolved in methanol, filtered and analyzed, which effectively simplified the pretreatment steps. Method verification showed that the polyphenol content had a good linear relationship in the range of 0.5 to 5 μg/μL. The linear equation was y = 100738x, and the correlation coefficient R was 0.9998, which confirmed the quantitative reliability of the method. The optimized HPLC method not only had high precision and good reproducibility (relative standard deviation as low as 0), but also was easy to operate and stable. It provided practical analytical support for the quality control and in-depth development of polyphenol resources such as longan seeds.

Zhou Zhaojuan et al. developed an analysis method based on ultra-high-performance liquid chromatography-tandem triple quadrupole mass spectrometry (UPLC-MS/MS) in response to the problems of polyphenol detection in tobacco leaves - such as excessive analysis time, cumbersome extraction steps, and limited coverage of polyphenol types in industry standards [11]. The method took ACQUITY UPLC BEH Phenyl chromatography column as the core, and used methanol and 0.2% formic acid aqueous solution as the mobile phase for gradient elution. This method could simultaneously determine six types of polyphenols in negative ion mode - neophytic acid, chlorogenic acid, cryptochloric acid, scopolamine, rutin and sannafen. By optimizing mass spectrometry parameters and chromatographic conditions, the study significantly improved the peak shape and separation efficiency. Methodological evaluation showed that the standard recovery rate was between 90.3% and 108.2%, indicating that the method has excellent accuracy. Compared with the existing industry standards, this method not only expanded the scope of analysis to six compounds, but also greatly shortened the analysis time. High-performance liquid chromatography (HPLC) has the advantages of high sensitivity and easy operation. It is a reliable analysis tool for quickly evaluating and accurately controlling the quality of tobacco leaves.

HPLC is widely used in green tea extracts, grape seed extracts, cranberries, blueberries, cocoa products and traditional Chinese medicine health foods, and is used to identify key functional monomers, such as EGCG, ECG, anthocyanins, proanthocyanins and various phenolic acid components. Taking tea polyphenols as an example, HPLC can accurately distinguish a variety of catechin monomers, making it a key method for detecting product mallusion and evaluating the quality of extraction process. In foods rich in anthocyanins, HPLC-FLD further improves the sensitivity and specificity of monitoring due to its natural fluorescent properties. For foods rich in polyanthocyanins, such as grape seeds and cocoa, the application of HPLC-MS overcomes the limitations of traditional chromatography in the complete separation of polymers, thus enabling more systematic and reliable research on these complex polyphenol systems.

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Although high-performance liquid chromatography (HPLC) has significant advantages in the detection of food polyphenols, its application still has some limitations. For example, its pretreatment steps are relatively cumbersome and time-consuming, and consume a large amount of organic solvent, which is not fully consistent with the development trend of green analytical chemistry. In addition, polyphenolic compounds are sensitive to temperature, pH and oxidation conditions, and improper operation may lead to the degradation of certain components. In addition, some polymer anthocyanins are difficult to be completely separated by traditional chromatography and still need to be analyzed by mass spectrometry. Therefore, future research will pay more attention to reducing solvent consumption, shortening analysis time and improving separation.

3. Conclusion

As the most significant natural bioactive constituents in foodstuffs, the accurate determination of polyphenolic compounds holds considerable importance for food quality control, functional component evaluation, and providing scientific substantiation for health claims. A review of current research progress reveals that different detection techniques possess distinct characteristics in terms of their principles, sensitivity, and scope of application. The Folin–Ciocalteu method remains widely employed for total polyphenol determination due to its operational simplicity, though its limited specificity renders it susceptible to matrix interference. Infrared spectroscopy offers distinct advantages in rapid, non-destructive analysis, with chemometric optimisation significantly enhancing model stability and quantitative capability. Nuclear magnetic resonance (NMR/qNMR) is increasingly becoming a vital method for analysing complex systems and scenarios lacking standard reference materials, owing to its high specificity and absolute quantitative capability without requiring calibration curves. High-performance liquid chromatography (HPLC) remains the most mature and reliable technique for separating and quantitatively analysing monomeric polyphenols, holding an irreplaceable position particularly in complex health food matrices.

Overall, modern polyphenol detection technologies are rapidly advancing towards high sensitivity, eco-friendliness, multi-technique integration, and intelligent modelling. Future research will increasingly focus on simplifying pre-treatment steps, achieving high-throughput and precision in instrumental analysis, identifying and eliminating coexisting interferents, and applying machine learning to spectral and chromatographic data analysis. Concurrently, as the food industry continues its shift towards health-oriented and functional products, high-standard, multidimensional polyphenol detection systems will play an increasingly vital role in functional food development, natural product utilisation, and food safety monitoring. Establishing more comprehensive, stable, and versatile detection methodologies applicable to diverse food matrices will become a key direction for advancing food science analysis.

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