



Regional Training Course on Efficient Screening Methods for Improved Nutritional
Quality in Mutant Populations
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Biochemical and Analytical Methods for Nutritional Quality Assessment

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CHAPTER 01

01 Research Background and Significance



Nutritional Quality Assessment

Definition:

The quantitative analysis of nutrients and their bioavailability in food using chemical, physical, and biological methods to evaluate nutritional value.

Assessment Dimensions:

Dimension	Content
Macronutrients	Proteins, Lipids, Carbohydrates
Micronutrients	Vitamins, Minerals
Bioactive Compounds	Polyphenols, Flavonoids, Dietary Fiber
Anti-nutritional Factors	Phytate, Tannins, Trypsin Inhibitors
Functional Properties	Digestibility, Bioavailability

Nutritional Quality Assessment

Core Objectives & Values:



Quantify Nutrients- Precisely measure macro and micronutrients content.



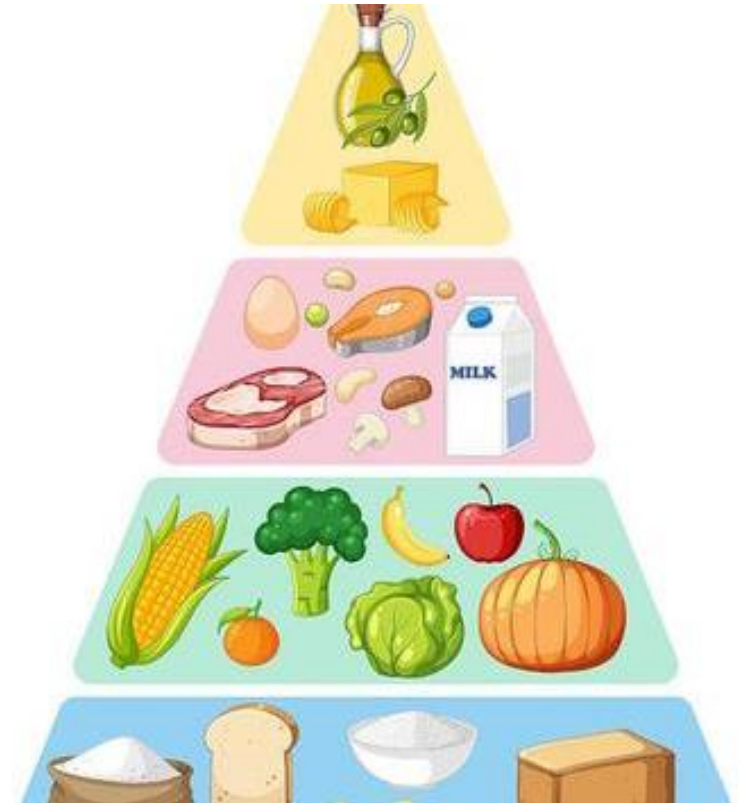
Evaluate Value- Assess protein quality, fat composition, and vitamin levels.



Detect Anti-nutrients- Identify phytic acid, tannins that may hinder absorption.



Ensure Safety- Screen for contaminants, additives, and allergens.



Healthy diet and nutritional pyramid

Significance of Nutritional Assessment



Prevent chronic Diseases

Identifies unhealthy dietary patterns that cause obesity, diabetes, and heart diseases, guiding healthier choices.



Safeguard Vulnerable Populations

Formulates specialized diets for infants, pregnant women, and seniors to meet their unique nutritional needs.



Drive Food Industry Innovation

Guides product R&D, formula optimization, and creation of fortified/functional foods for better public health.



Support Public Health Policy

Provides scientific data for national nutrition labeling laws, food standards, and dietary guideline updates.

Crop Diversity and Nutritional Assessment Challenges

The diversity of crop varieties: It includes grains, fruits and vegetables, legumes, oilseeds, and tubers, and the nutritional characteristics of each crop vary significantly.

Specificity of nutritional characteristics: Grains are rich in starch, beans contain high levels of protein, fruits and vegetables are rich in vitamins and polyphenols, and oil crops provide unsaturated fatty acids.

Challenges of the Evaluation System: The diversity poses a significant challenge in establishing a unified, precise and comprehensive nutritional assessment system covering all types.



谷物 (小麦)



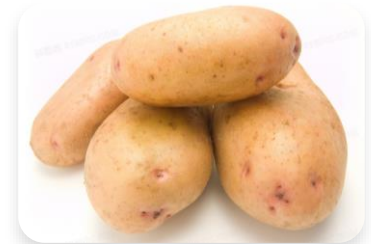
豆类 (大豆)



果蔬 (番茄)



油料 (油菜)



薯类 (马铃薯)



Building a scientific assessment system requires: 1. Establishing classification standards based on the characteristics of different crops; 2. Integrating multi-dimensional nutritional data; 3. Developing an assessment model that balances universality and specificity.

Technical value and core objective



↳ Technical value

Biochemical and analytical methods serve as the bridge connecting "food components" and "human health effects", and are the core tools for unraveling the complex interrelationship of "nutritional components - biological efficacy - consumption safety".

🎯 Core objective

Integrate existing technologies to achieve "**precise composition, standardized methods, and comprehensive evaluation**" under full crop coverage, providing scientific basis for crop breeding, food processing, and nutritional intervention.

CHAPTER 02

Technical system foundation

Technical system foundation



Spectral analysis technology



Chromatographic separation technology



Mass spectrometry detection technology



Biochemical and immunological assay techniques



Metabolomics technology



Comparison and Summary of Technical Platforms



Spectral analysis technology

A powerful tool for rapid screening and quantification

Typical Applications and Technical Features



Diagram of rapid field detection using handheld spectrometer

▣ Typical application scenarios

- **Functional component quantification:** Rapid determination of total phenols, flavonoids, vitamin C, etc. content
- **Grain quality analysis:** Measurement of starch and protein contents, assessment of grain quality
- **Safety and traceability:** Food authenticity identification, origin traceability, and rapid screening

⚙ Analysis of Technical Features

Advantages: Simple operation, fast analysis, low cost, supports non-destructive testing, suitable for on-site screening.

Limitations: The specificity is relatively weak. The complex matrix is prone to interference, making it difficult to distinguish structurally similar compounds.



二、Chromatographic separation technology

The gold standard for precise separation and quantification

Core platform and application fields



High Performance Liquid Chromatography (HPLC/UPLC)



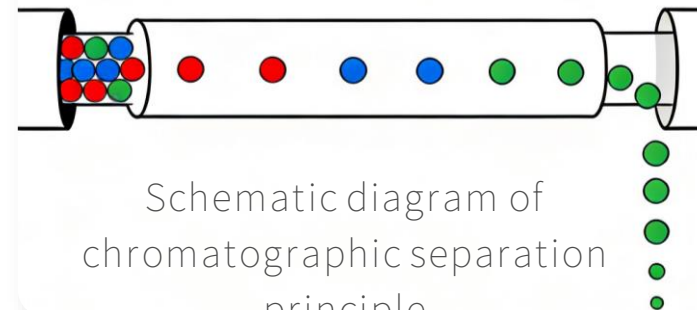
Gas chromatography (GC)



Ion chromatography (IC)

Separation principle

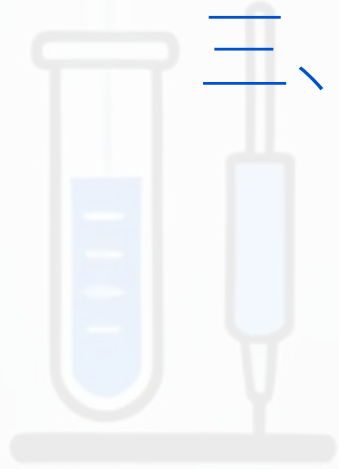
Based on the differences in the partition coefficients of various substances between the stationary phase and the mobile phase, a chromatographic column is utilized to achieve efficient separation and analysis of each component in a complex mixture.



Schematic diagram of chromatographic separation principle

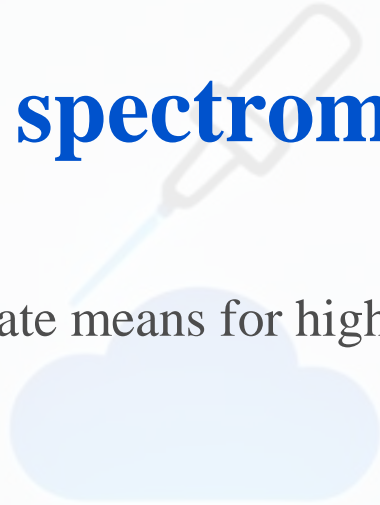


样品引入



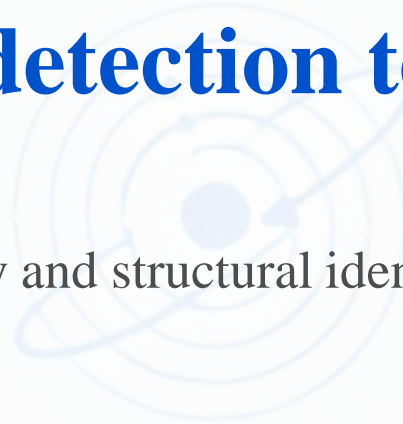
第一步：
样品引入

离子化



第二步：
离子化

质量分析器



第三步：
质量分析

检测器



第四步：
检测记录

三、Mass spectrometry detection technology

The ultimate means for high sensitivity and structural identification

Core platform and application scenarios



Core principle: Mass-to-charge ratio separation and detection

After the target substance is ionized, it is separated and detected based on its mass-to-charge ratio (m/z), thereby enabling precise qualitative and quantitative analysis of the target substance.



HPLC-tandem mass spectrometry analyzer



Core technology platform

- Liquid chromatography-tandem mass spectrometry (LC-MS/MS): Combining the separation ability of HPLC with the high sensitivity of MS, it is currently the most widely used mass spectrometry technology.
- Gas chromatography-mass spectrometry (GC-MS): Suitable for qualitative and quantitative analysis of volatile components.
- Inductively coupled plasma mass spectrometry (ICP-MS): Focused on elemental analysis, it can simultaneously determine dozens of metallic and non-metallic elements.



Main application scenarios

- High-throughput screening of trace vitamins and contaminants (mycotoxins, pesticide residues) in food.
- Structural identification and confirmation of functional components.
- Precise analysis of nutrients and their metabolites in biological samples.



四、 **Biochemical and immunological assay techniques**

Professional tools for functional activity and targeted detection

Principles and Application Directions



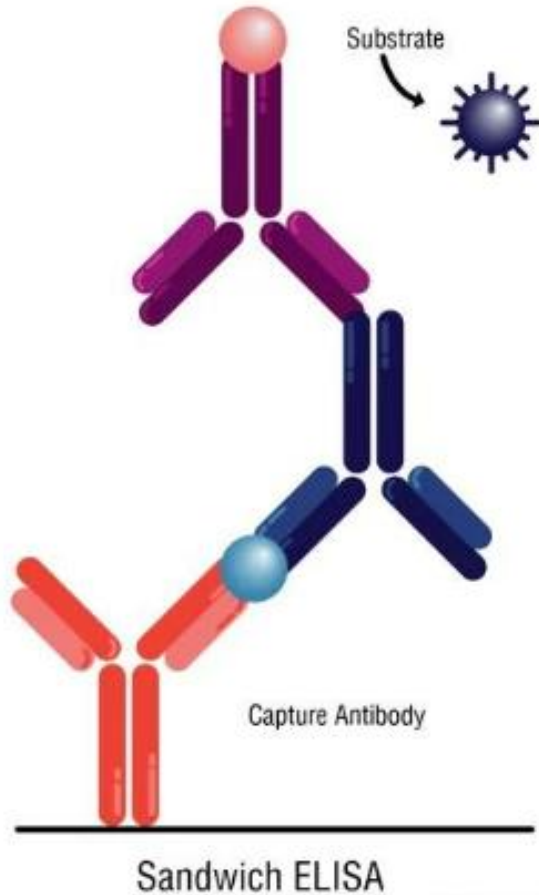
Core principle

Based on specific biochemical reactions (such as color changes, enzyme activity changes, etc.), the content of the target substance is quantitatively determined by detecting the reaction signals.

Main application directions

- ✓ Rapid determination of protein content (Bradford method)
- ✓ Antinutritional factor activity test (phytic acid/inhibitor)
- ✓ Antinutritional factor activity test (DPPH/ABTS experiment)
- ✓ Study on the intestinal absorption efficiency of nutrients

Core technology platform



- **Colorimetric/Enzymatic Analysis Method:** Quantitative analysis of target components and their activities through color development or enzymatic reactions.
- **Enzyme-Linked Immunosorbent Assay (ELISA):** Achieves high-sensitivity detection based on the specific binding of antigens and antibodies.
- **Bradford Protein Assay:** A classic rapid protein quantification method with simple operation.
- **Folin-Ciocalteu Method:** Widely used in food science for determining the total phenolic content in samples.
- **Cell Model (Caco-2):** Simulates the physiological environment of the human body to evaluate the bioavailability of nutrients.

The operation is relatively simple · The detection cost is low · Suitable for rapid and large-scale testing

PC2

1.0
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-0.5
-1.0
-1.5

五、**Metabolomics technology**

Frontier Approaches to Panoramic Analysis and Marker Discovery

Core platform and application value



Nuclear Magnetic Resonance Spectrometer
(NMR)

principle

Conduct a comprehensive qualitative and quantitative analysis of all small molecule metabolites within the organism, and construct a "metabolic fingerprint map" that reflects the physiological state of the organism.

Core technology platform

- **Nuclear Magnetic Resonance Spectroscopy (NMR):** unbiased and non-destructive, suitable for comprehensive analysis of metabolites.
- **Non-targeted Metabolomics (LC-MS/GC-MS):** high sensitivity, wide coverage, suitable for large-scale screening.

Multidimensional application value



Constructing metabolic fingerprinting profiles for crops or foods for the purpose of variety identification and origin tracing



Discover new functional metabolites and biomarkers to assist in precision nutrition research



Component analysis and marker discovery








Processing and storage quality assessment



Dietary and Metabolic Association Study

Key Technology Classification and Platform

technological type	Representative platform	core advantage	applicable scene
 spectrum technology	UV-Vis / FTIR	Fast, low-cost , non-destructive testing	Batch sample rapid screening and on-site testing
 chromatographic technique	HPLC / UPLC / GC	High separation efficiency and accurate quantitative results	Precise quantitative analysis of known nutritional components
 mass-spectrometric technique	LC-MS/MS / ICP-MS	High sensitivity , strong specificity, and structural resolution	Analysis of trace components, pollutants and complex matrices
 Biochemical/Immuno Technology	ELISA / Colorimetric method, enzyme inhibition method	Evaluation of functional activity and bioavailability	Antinutritional factors, biological activity, absorption studies
 Metabolomics technology	NMR / LC-MS	Strong capabilities in panoramic analysis and marker detection	Discovery of new functional components and the mechanism of processing influence

CHAPTER 03

03 Core Method for Nutritional Quality Assessment

Classification

01



Method for determining macronutrients

02



Method for determining micronutrients

03



Method for determining functional components

04



Method for determining anti-nutritional factors

05



Conclusion

Analysis of Basic Nutrient Content and Composition

Overview of Macronutrients

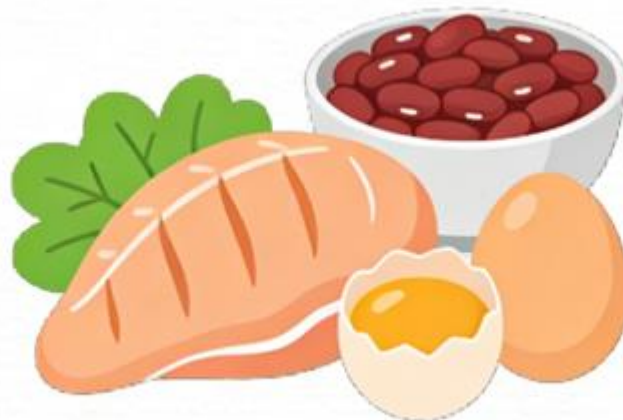
Definition: Macronutrients are nutrients that the human body requires in large quantities. Their main functions are to provide energy and form the body's tissues. These are the primary sources of energy in our diet

Three categories: **carbohydrates, proteins, and fats.**

carbohydrates



proteins



fats



Protein Analysis: Methods for Quantification



Classic: Kjeldahl Method

- **Principle:** The organic nitrogen in the sample is converted into inorganic nitrogen (ammonium sulfate) through a chemical reaction, and then the content of nitrogen is determined by titration, from which the content of protein is calculated.
- **advantage:** Official standard, accurate for pure proteins.
- **limitation:** Cannot distinguish Non-Protein Nitrogen (NPN).



Preferred: Sum of Amino Acids

- **Principle:** Hydrolyze protein into free amino acids, Separate and quantify each amino acid by chromatography(HPLC), Sum the mass of all amino acids to calculate the total protein content.
- **advantage:** Direct & accurate ("true protein"), FAO preferred.
- **limitation:** High cost & requires advanced instrumentation.

Key Insight: Amino Acid Analysis provides the "true protein" value, critical for quality control.

Fat Analysis: Total Content & Composition



Total Fat Content

- **Soxhlet Extraction:** The classic method for determining crude fat involves reflux extraction with ether or petroleum ether, followed by drying and weighing.
- **advantage:** Classic, accurate, Simple equipment; low cost
- **limitation:** Time-consuming



Fatty Acid Profiling

- **Principle:** Lipids are extracted then hydrolyzed & methylated to form Fatty Acid Methyl Esters (FAMES), FAMES are separated by GC / GC-MS to quantify individual fatty acids.
- **advantage:** Gold standard for lipid quality assessment, distinguish between beneficial (Omega-3) and harmful (trans) fatty acids.
- **limitation:** Requires derivatization, expensive instrumentation

Carbohydrate Analysis

▣ Carbohydrates: Subtraction Method and HPLC

- **Subtraction method:** Subtract the moisture, ash, protein and fat from the total weight to obtain the estimated total amount.
- **HPLC method:** Directly determine the precise contents of monosaccharides (such as sucrose, fructose, glucose) and disaccharides.

☞ Dietary fiber: Enzyme-weight method

Sequentially perform enzymatic hydrolysis using α -amylase, protease and glucosidase, then precipitate and weigh. The total dietary fiber, soluble and insoluble dietary fibers can be determined separately.



Soxhlet extractor and HPLC chromatograph

Micronutrient

Definition

Refers to nutrients that are required in very small quantities by the human body, but are indispensable for maintaining normal physiological functions, growth and development, and metabolism. These nutrients cannot be synthesized by the body or are synthesized in insufficient amounts, and must be obtained from food. They are in contrast to macronutrients such as carbohydrates, proteins, and fats.

Classification

I. Vitamins

Fat-soluble vitamins: Vitamin A, D, E, K

Water-soluble vitamins: B vitamins (B1, B2, B6, B12, niacin, folic acid, pantothenic acid, biotin), Vitamin C

II. Minerals

Macro-elements (daily requirement $\geq 100\text{mg}$): Calcium, Phosphorus, Potassium, Sodium, Chlorine, Magnesium, Sulfur

Micronutrients (daily requirement in milligrams/microgram level): Iron, Zinc, Selenium, Iodine, Copper, Manganese, Molybdenum, Chromium, Fluorine, etc.

Methods for Determination of Micronutrients



Vitamin Determination Techniques

HPLC: Separates & quantifies fat/water-soluble vitamins.

LC-MS/MS: Higher sensitivity for complex/low-content samples.



Mineral Element Analysis

AAS: Precise single-element determination.

ICP-OES: Rapid simultaneous multi-element analysis.

ICP-MS: Highest sensitivity for trace elements.



Atomic Absorption Spectrophotometer (AAS) Setup

Functional Ingredients

Definition

Substances in foods & natural products that, beyond basic nutrition, regulate physiological functions, improve health, prevent chronic diseases, and maintain homeostasis. They are the core active components of functional foods.

Classification



Plant-derived: Polyphenols, flavonoids, plant polysaccharides, saponins, alkaloids, terpenoids, phytosterols.



Animal-derived: Bioactive peptides, collagen, fish oil (Omega-3), Coenzyme Q10, chitin/chitosan.



Microorganism-derived: Probiotics, prebiotics, fungal polysaccharides, gamma-aminobutyric acid (GABA).



Other: Functional lipids, functional carbohydrates, functional mineral chelates.

Methods for Determination of Functional Ingredients



Total Phenols

Folin-Ciocalteu Method: Uses gallic acid as standard, the gold standard for phenol content.



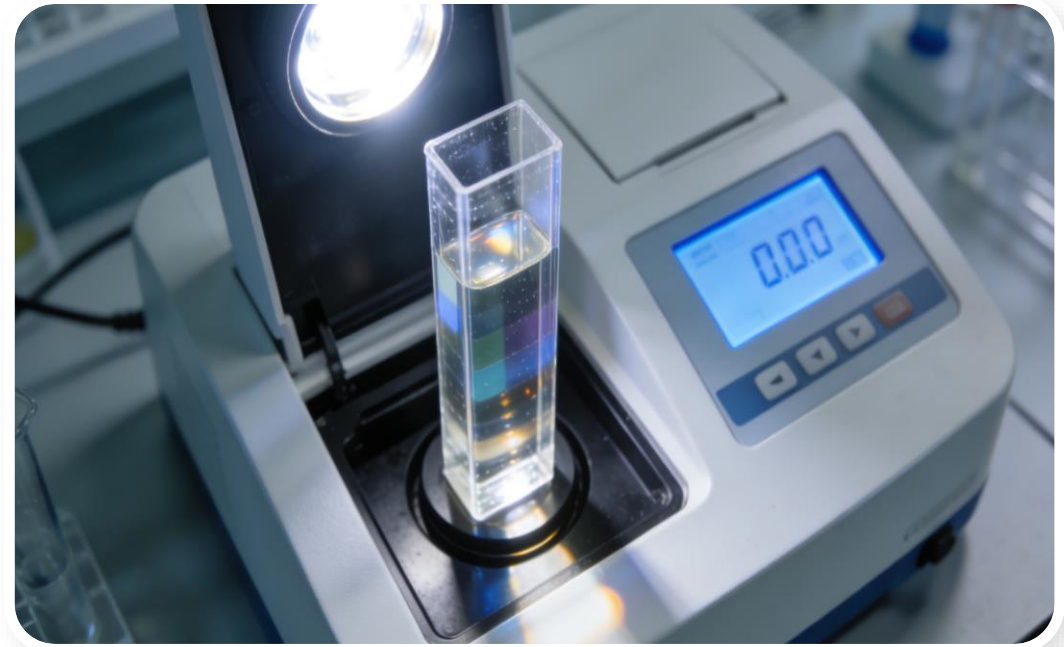
Total Flavonoids

Colorimetry(NaNO_2 - $\text{Al}(\text{NO})_3$ Method): Uses rutin as standard, widely used for flavonoid quantification.



Carotenoids

- **Spectrophotometry:** Simple solvent extraction & absorbance.
- **HPLC:** Separates & quantifies α/β -carotene, lutein.



Laboratory Analysis

Advanced spectrometers ensure accuracy and repeatability in detecting trace functional components.

Methods for Determination of Functional Ingredients



Anthocyanins | pH Differential Method

Utilizes color change with pH values. Calculates total content by measuring absorbance differences.



Polysaccharides | Phenol-Sulfuric Acid

Uses water extraction & alcohol precipitation, then measures total sugars to derive polysaccharide content.



Capsaicin | HPLC (National Standard)

Measures capsaicin & dihydrocapsaicin to calculate the total pungency (SHU) of chili peppers.



Anti-nutritional Factors Analysis

Definition

Naturally occurring plant defense compounds that interfere with nutrient digestion, absorption, and utilization, reducing nutritional efficiency or causing mild adverse physiological reactions.

Classification & Examples

- **Digestive Enzyme Inhibitors:** Trypsin inhibitors, Amylase inhibitors, Pepsin inhibitors
- **Mineral-binding Factors:** Phytic acid, Oxalic acid, Tannins
- **Utilization Interferents:** Phytohemagglutinins, Saponins
- **Others:** Anti-vitamin factors, Glucosinolates, Cyanogenic glycosides

Methods for Determination of Anti-nutritional Factors



Phytic Acid

Ion exchange column separation + spectrophotometry, or direct **HPLC**.



Tannin

Folin-Denis **colorimetric** method or vanillin-hydrochloric acid method.



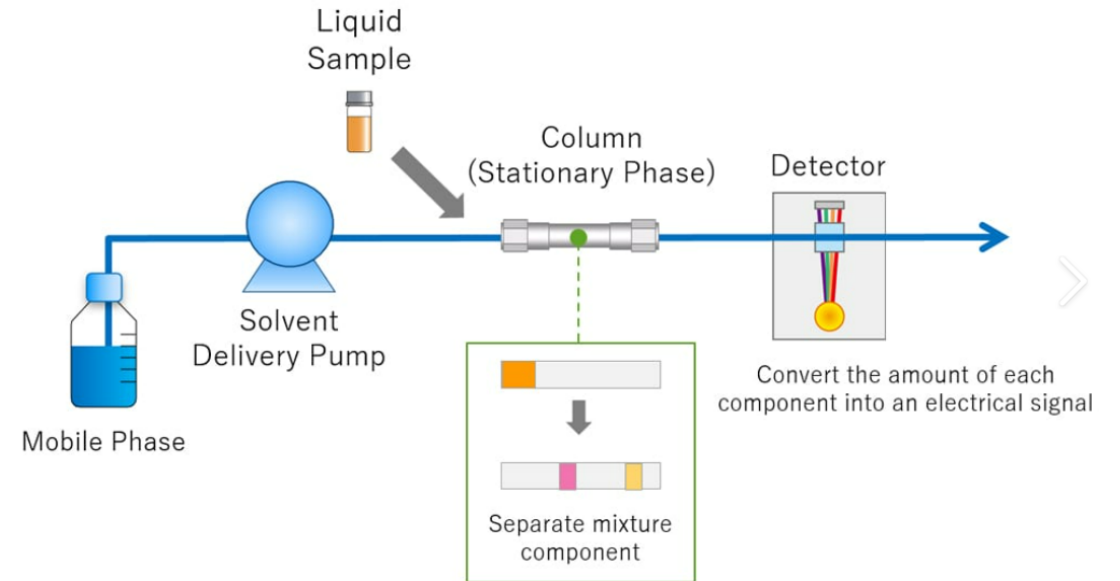
Trypsin Inhibitor

Enzyme activity inhibition assay measuring the degree of inhibition.



Nitrates / Nitrites

Griess reagent spectrophotometry or ion chromatography.



Working Principle of High-Performance Liquid Chromatography (HPLC)

Summary of Core Analytical Methods



Macronutrients

- Moisture: Gravimetric method (Drying)
- Protein: Kjeldahl method | Fat: Soxhlet extraction
- Carbs: Difference/HPLC | Fiber: Enzymatic-gravimetric



Micronutrients

- Vitamins: HPLC / LC-MS
- Minerals: AAS / ICP-OES / ICP-MS



Functional Ingredients

- Polyphenols: Colorimetric methods
- Carotenoids: HPLC
- Polysaccharides: Water extraction + Phenol-sulfuric acid



Anti-nutritional Factors

- Phytic Acid: Ion exchange / HPLC
- Tannins: Colorimetric | Nitrates: Spectrophotometry
- Inhibitors: Enzyme activity inhibition assay

CHAPTER 04

04 Challenges and Prospects

Current Core Challenges: Technologies and Methods

Severe interference from complex matrices

Food matrices are complex, micronutrients are easily masked, traditional methods involve cumbersome pretreatment, and the recovery rates are unstable.

Imbalance between detection efficiency and cost

National standard methods are time-consuming, costly, and low-throughput, making it difficult to meet the requirements of high-throughput screening.

Lack of bioavailability assessment

Most existing methods only measure total content, which does not reflect actual human absorption, and there is a large discrepancy between in vitro models and in vivo results.

New food evaluation gap

Cell-cultured meat, plant-based proteins and other novel foods lack suitable detection methods and evaluation systems.



Current Core Challenges: Data and Standards

Lack and fragmentation of baseline data.

The local food nutrition database is incomplete, results from different laboratories are incomparable, and there is a lack of a unified quality control and traceability system.

The evaluation system is single and static.

It focuses on content as the core, ignoring function, interaction and health effects, and lacks dynamic, multi-dimensional and personalized evaluation models.

Standards update lagging behind

Detection methods and limit standards cannot keep pace with technological and industrial development, and standards for emerging pollutants and functional components are lacking.



Current Core Challenges: Application and Transformation

Disconnection among detection, breeding, production and consumption

Breeding focuses on yield rather than nutrition, production lacks nutrition-oriented strategies, and there is information asymmetry among consumers.

These lead to the fact that high-quality products do not obtain premium prices, and there is insufficient collaboration among all links in the industrial chain.

Difficulties in the implementation of precision nutrition

Individual differences are substantial, yet low-cost and non-invasive personalized assessment tools are lacking.

Moreover, insufficient data privacy protection and inadequate clinical validation hinder the popularization of precision nutrition strategies.

High technical barriers for small and medium-sized enterprises (SMEs)

High-end instruments and testing services are costly, making it difficult for small and micro enterprises to afford equipment and technical investment, which restricts the overall progress of nutrition upgrading.

Current Core Challenges: Environment and Climate

Climate change leads to nutrient dilution

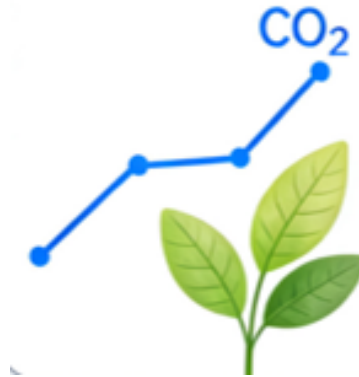
Elevated CO₂ levels and extreme weather have led to widespread reductions in protein, iron, zinc, vitamins and other nutrients in crops, exacerbating the risk of hidden hunger.

Nutrient degradation caused by modern agriculture

Continuous cropping, reliance on chemical fertilizers and excessive processing have resulted in substantial losses of vitamins, minerals and functional components in crops.

Insufficient model predictive capacity

Existing models focus on yield while neglecting nutrition. There is a lack of climate-nutrition-health coupling models, making it difficult to quantify future risks.



Forward Outlook: Innovation in Detection Technology

Integrated multi-technology platform

By integrating NIR, hyperspectral imaging, LC-MS/MS and AI interpretation, a one-stop, full-dimensional closed-loop detection system will be established.

Microfluidics and Chip Miniaturization

Using microfluidic chips and organoid models to accurately simulate the in vivo environment and efficiently evaluate bioavailability.

Nanotechnology and Sensing Innovation

The application of nanomagnetic separation and electrochemical array technology greatly improves sensitivity and enables the rapid detection of trace substances.

Non-invasive / Online / Portable

Real-time, non-invasive on-site dynamic monitoring can be achieved through devices such as swallowable capsules and portable spectrometers.

Future Outlook: Driven by Data and Intelligence



Nutrition Foundation Model & Omics

Integrate genomics, metabolomics, and microbiomics data. Leverage AI to train nutrition models for precise prediction and personalized health recommendations.



Digital Twin & In Silico Trials

Build a "crop-food-human" digital twin system to enable virtual screening and optimization, drastically reducing R&D costs and cycles.

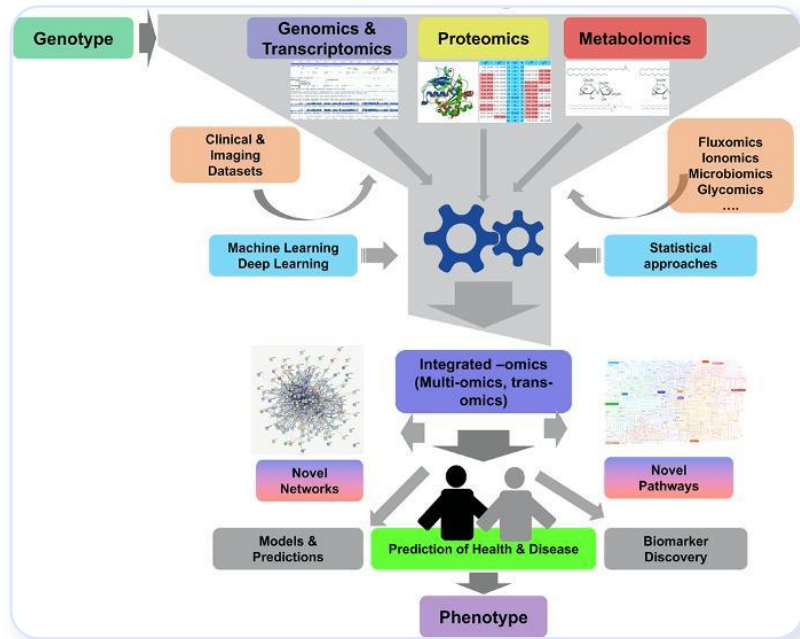


Blockchain + Nutrition Traceability

Utilize blockchain for full-chain traceability and verification of nutritional data, supporting "quality-based pricing" and consumer trust.



Frontier Technology Outlook: Multi-Omics Integration



- By deeply integrating metabolomics with genomics, proteomics and other technologies, this approach enables a comprehensive analysis of the molecular mechanisms underlying the formation of crop nutritional quality from multiple dimensions including genes, proteins and metabolites. It also enables the precise identification of new molecular markers related to nutrition.
- ✓ This technological breakthrough will shift the research paradigm from a simple "component analysis" to a more in-depth "mechanism analysis", providing more targeted theoretical basis and technical support for crop molecular breeding.

Future Outlook: Upgrading the Evaluation System



Multi-Dimensional Dynamic Evaluation Model

Evolving from a single "content" evaluation to a comprehensive system that includes content, bioavailability, functional activity, health effects, and environmental cost.



Climate-Smart Nutrition Assessment

Developing climate-crop-nutrition-health coupling models to predict the impacts of climate change and guide stress-resilient biofortification breeding.



Personalized Nutrition Assessment

Based on individual differences in genes, microbiota, and metabolism, enabling customized nutritional quality evaluation and intervention plans.

Future Outlook: Breakthroughs in Rapid Detection Technology

Tech Trend: From Lab to Field

Biosensors and Raman spectroscopy are rapidly iterating, breaking lab constraints to enable real-time, in-situ detection at "field" and "processing sites".

Typical App: Handheld Raman Spectrometer

Portable devices enable direct, non-destructive measurement of key indicators (e.g., Vitamin C) right in the field, greatly enhancing efficiency.



References

Analyte	Method	Reference Title
Crude protein	Kjeldahl method	Protein Determination in some Animal Products from Sulaymaniyah Markets Using Kjeldahl Procedure
Crude fat	Soxhlet extraction	Soxhlet Extraction to Measure the Influence Factors of U.S. Soybeans Crude Fat Content Analysis
Total fat	Soxhlet extraction	Optimization of Crude Fat Content Determination in Castor Seeds by Soxhlet Extraction
Total polyphenols	Folin-Ciocalteu	Total (poly)phenol analysis by the Folin-Ciocalteu assay in biological samples
Total phenols	Folin-Ciocalteu	Optimization of Folin-Ciocalteu Method for Determination of Total Phenols in Cherry Wine by RSM
Vitamins	HPLC	Eco-friendly RP-HPLC method for simultaneous determination of water/fat-soluble vitamins
B vitamins	HPLC	Determination of 9 B Vitamins in Health Foods by High Performance Liquid Chromatography
Fatty acids	GC-MS	GC-MS analysis of fatty acid profiles in edible oils: A comparative study
Dietary fiber	Enzymatic-gravimetric	GB 5009.88-2014 National Food Safety Standard: Determination of Dietary Fiber in Foods
Allergens, Mycotoxins	ELISA	Application of ELISA in Rapid Detection of Food Safety Hazards

Thanks!



Technology-based Breeding · Protecting the Future · Creating a New Era of Nutritional Health Together