中国农业科学院油料作物研究所
OIL CROPS RESEARCH INSTITUTE, CHINESE ACADEMY OF AGRICUTURAL SCIENCES

The International Rapeseed Congress 2023

High coverage profiling of phenolic compounds in rapeseed based on metabolomics and its application

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Outline

- **Research Background**
- **Research Content and Methods**
- **Results and Discussions**
- **Conclusion and Perspective**

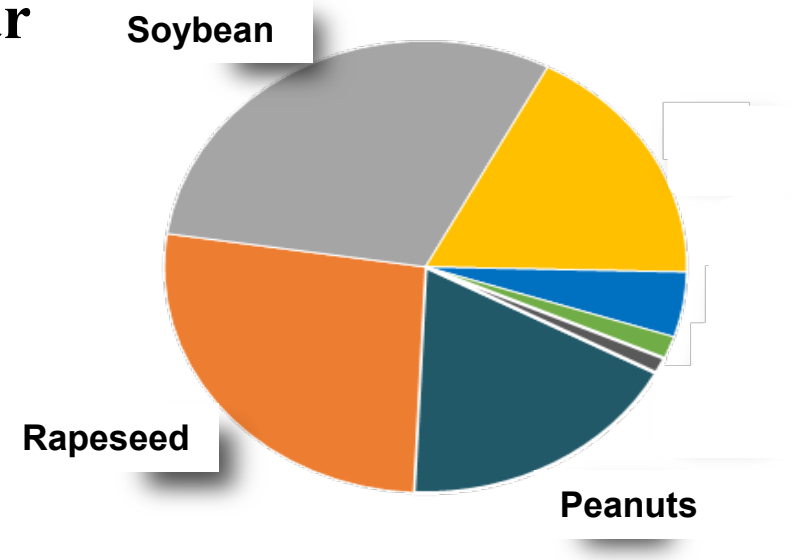
1. Research Background

Rapeseed: Oil Crops

Economic Crops

Output of ~13 million tons/year

Oil Crops planting area in China (10000 acres)

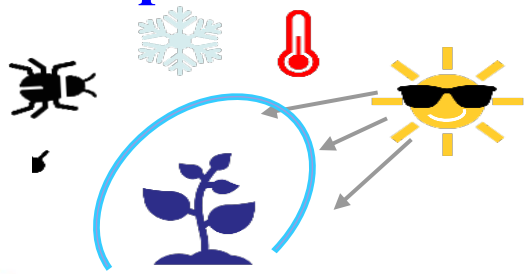


- **Lipids** (35%-50%)
- **Protein** (21%-30%)
- ……
- **Carbohydrate** (15%-18%)
- **Companions**

The role of phenolic compounds

Crop growth

Oil antioxidant



Adversity defense

1. Research Background

Rapeseed polyphenols are a naturally occurring secondary metabolite in rapeseed.

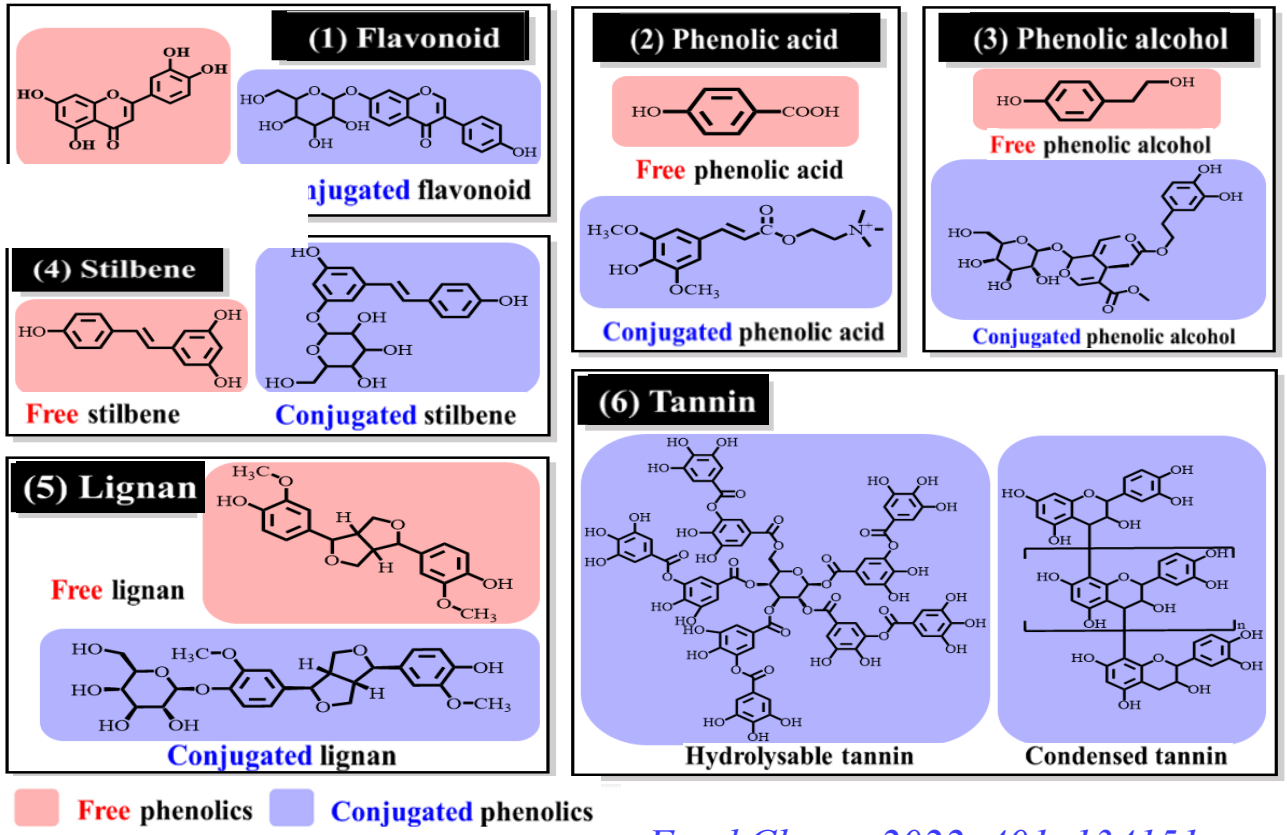
The polyphenol content in rapeseed is about 10-30 times that of other oilseeds.

Forms

- **Free phenol** — 60-262 mg/100 g
  - Sinapic
- **Esterified phenol** — 570-1520 mg/100 g
  - Sinapine
- **Insoluble bound phenol** — 0-105 mg/100 g

Exists in the seed coat and forms a network structure with proteins or cellulose.

Structures



Food Chem., 2022, 401, 134151.
Front. Nutr., 2022, 9, 1044871.

1. Research Background

Phenolic compounds in rapeseed have various physiological functions

Reducing fatty liver

Preventing ulcerative colitis

Therefore, a comprehensive and high coverage analysis of phenolic compounds in rapeseed is of great significance for the development of a nutritious and healthy rapeseed industry.

Protecting cardiovascular system

Alleviating Alzheimer's disease

Food Chem., 2019, 276, 768.
J. Agric. Food Chem., 2017, 65, 6886.
Rev. Endocr. Metab. Disord., 2021, 22, 367.

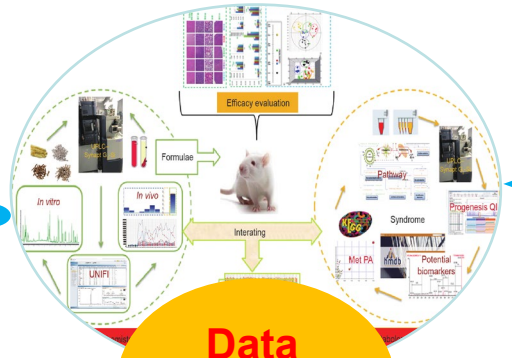
Process for analyzing phenolic compounds based on LC-MS

Sample collection

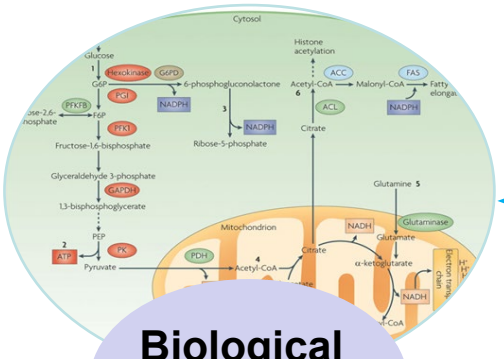
Metabolite extraction

Concentration

Data collection



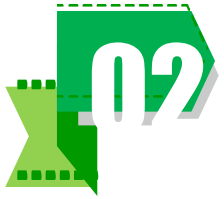
Data analysis



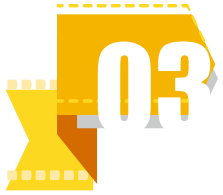
Biological interpretation

2. Research Content and Methods

Establishment of a high coverage targeted metabolomics method for profiling of phenolic compounds



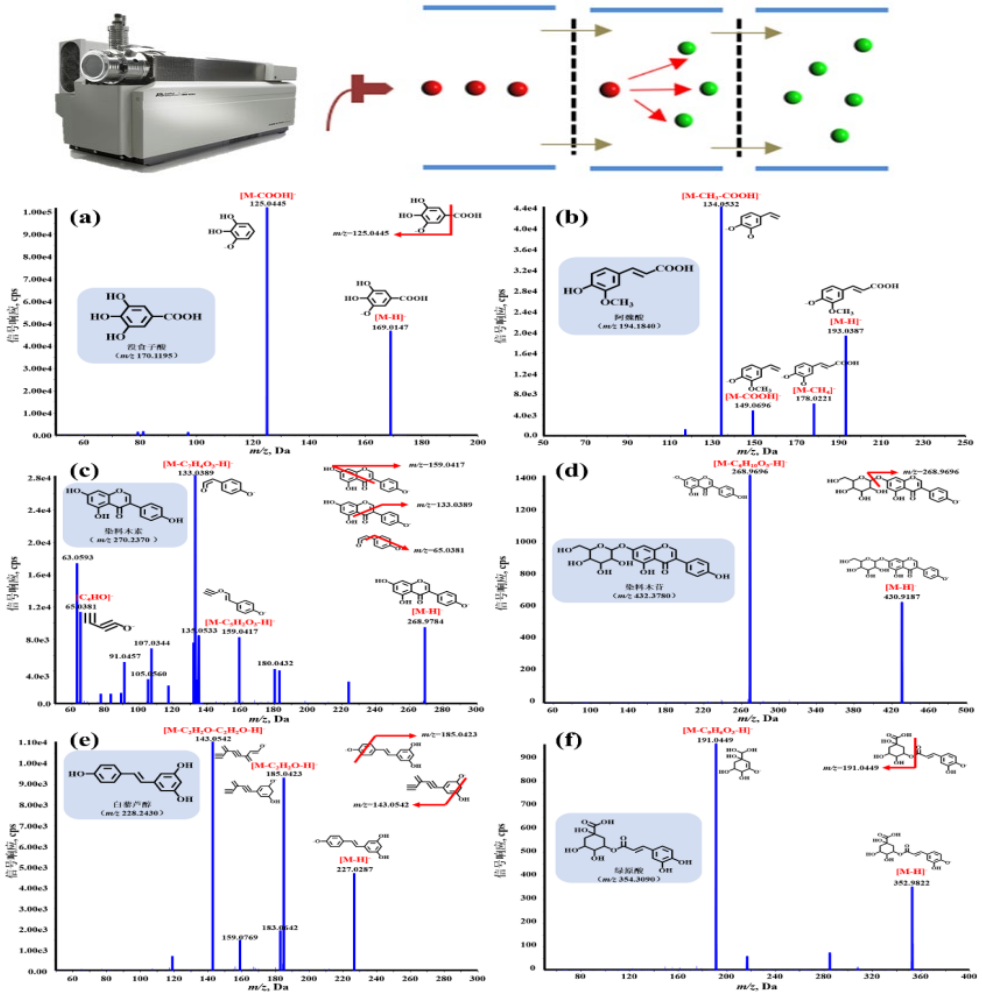
Analysis of the composition and spatial distribution of phenolic compounds in different varieties of rapeseed



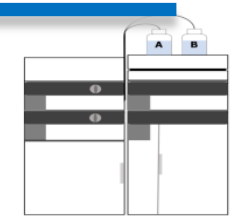
Comprehensive analysis of phenolic compounds in rapeseed using non-targeted metabolomics combined with molecular network technology

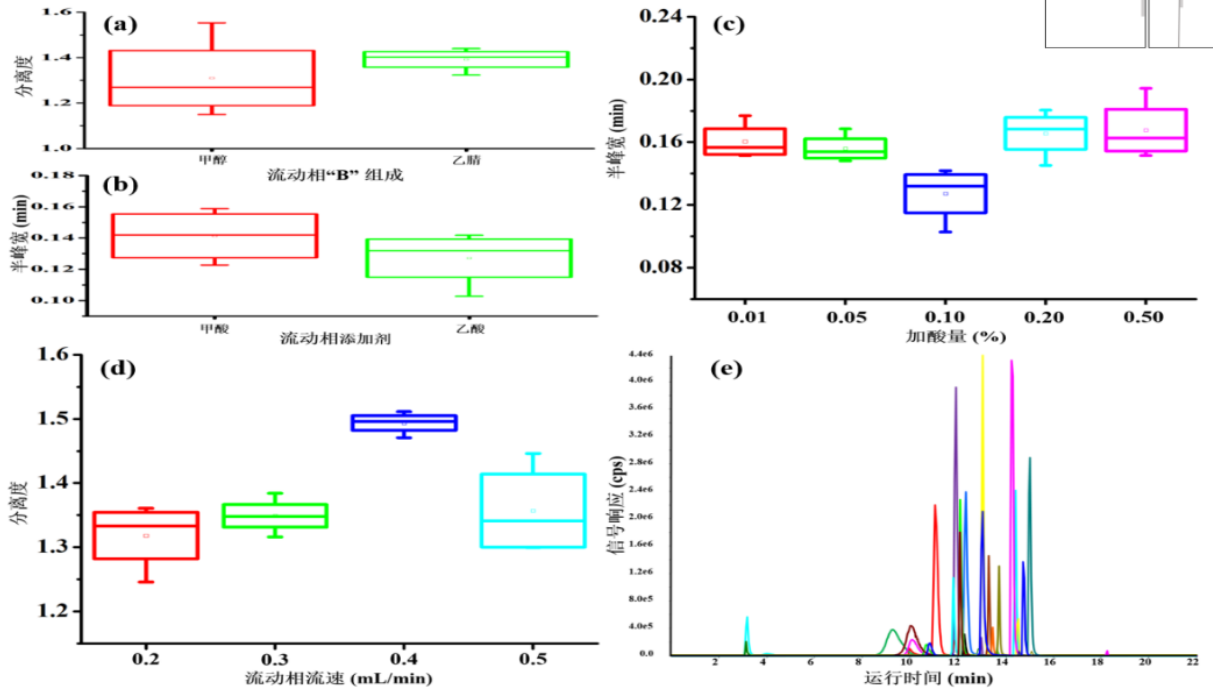
3.1 Establishment of a high coverage targeted metabolomics method

Optimize mass spectrometry conditions



Optimize chromatography conditions





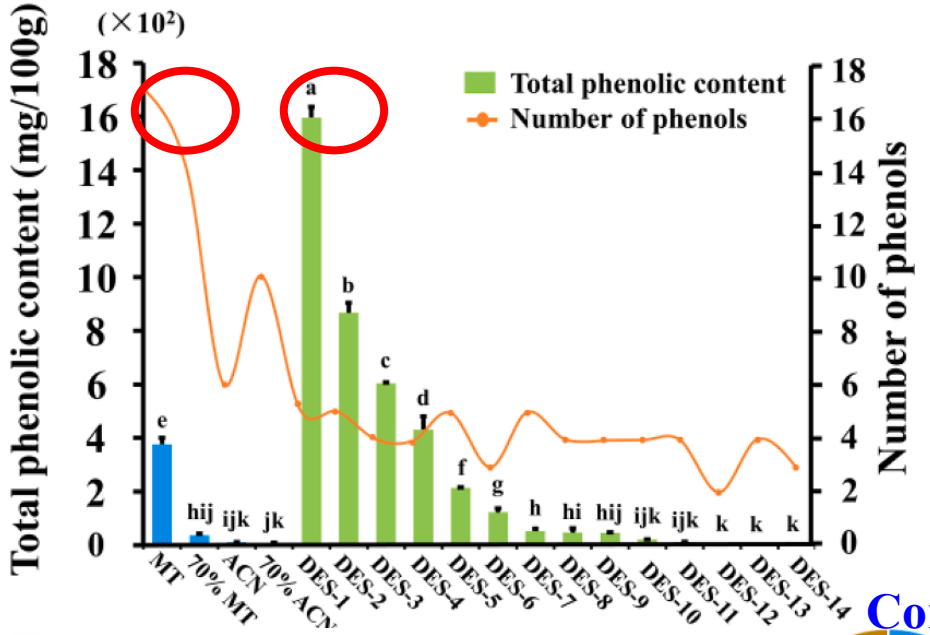
This targeted analysis method can achieve profiling of 44 phenolic compounds in rapeseed simultaneously.

The MS/MS information of phenolic compound standards was obtained through direct injection, and the corresponding MS parameters were optimized.

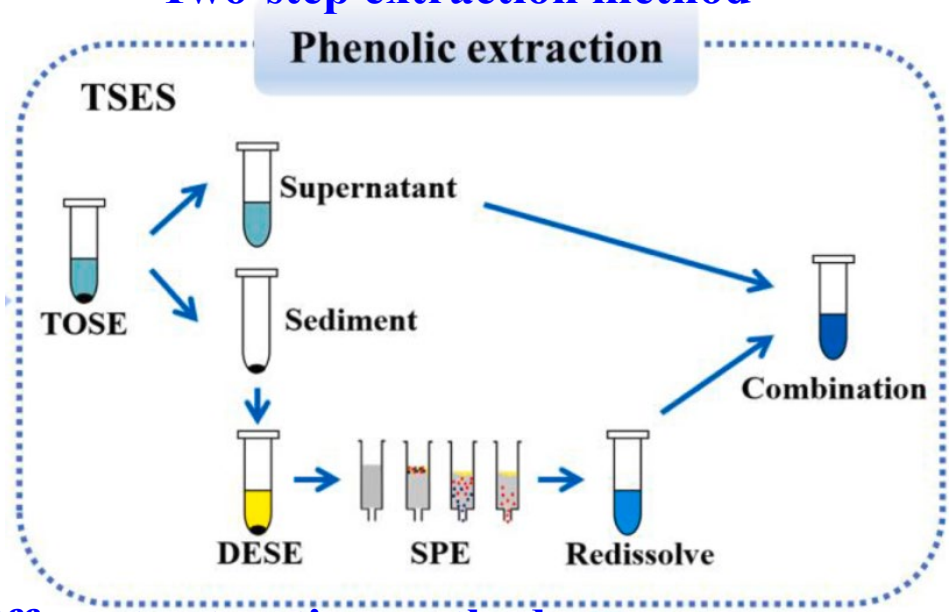
3.1 Establishment of a high coverage targeted metabolomics method

C

Deep eutectic solvent extraction

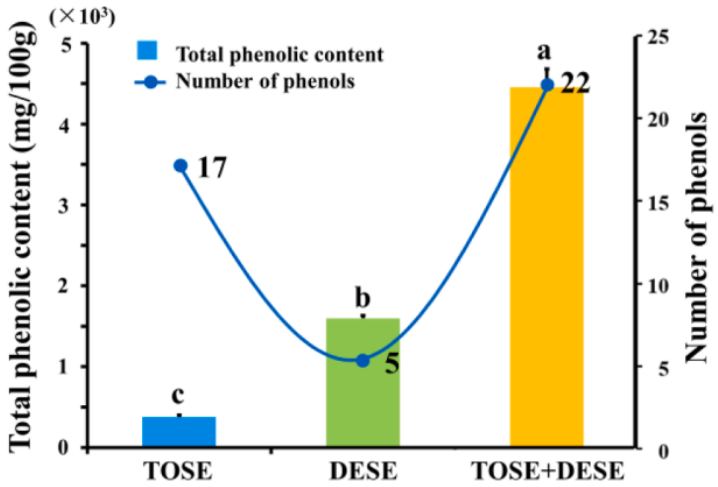


Two-step extraction method

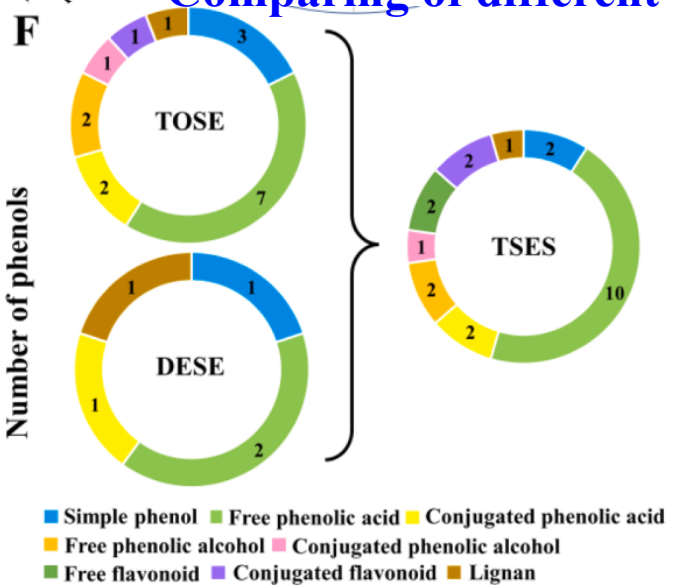


Comparing of different extraction methods

E

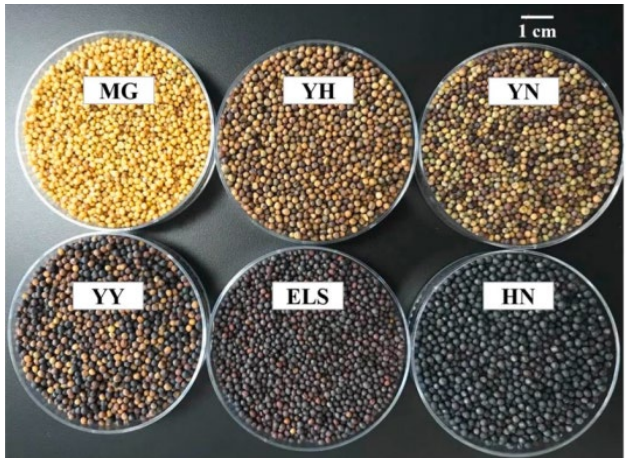


F

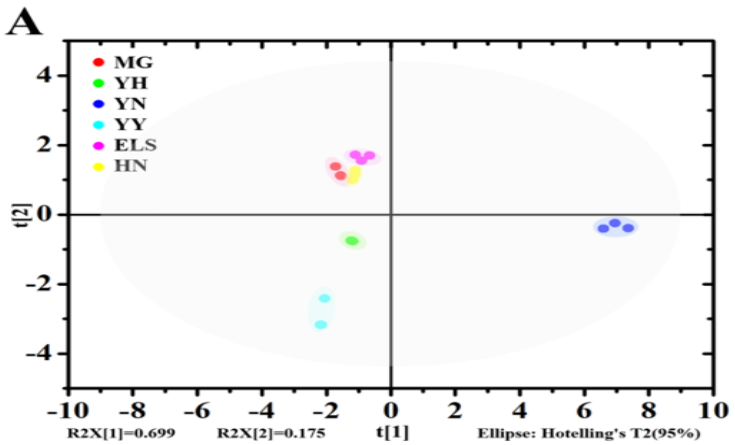


The two-step extraction method increased the total phenol content by nearly 10 times compared to the methanol extraction method, and the types of extracted phenols increased by 5.

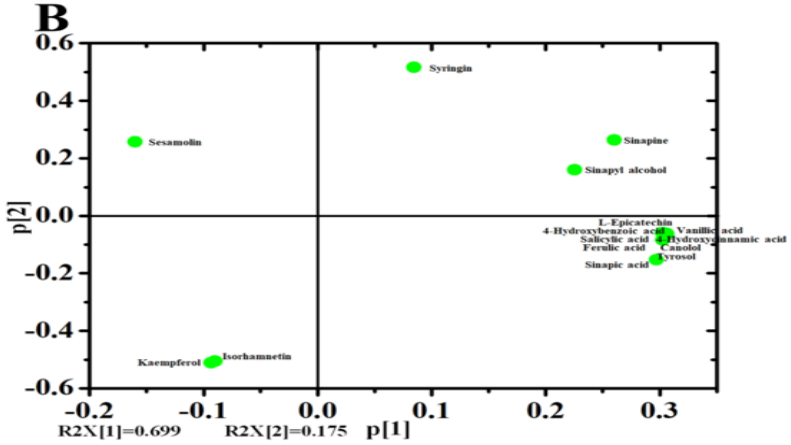
3.2 The composition and spatial distribution of phenolic in rapeseed



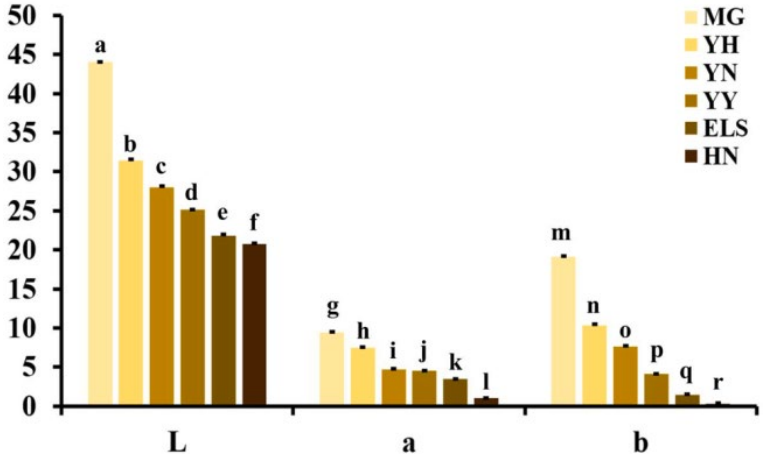
Six different varieties of rapeseed



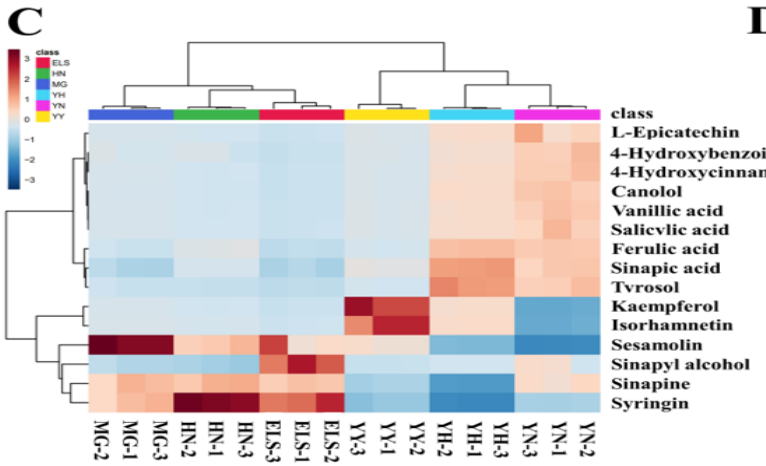
PCA score plot



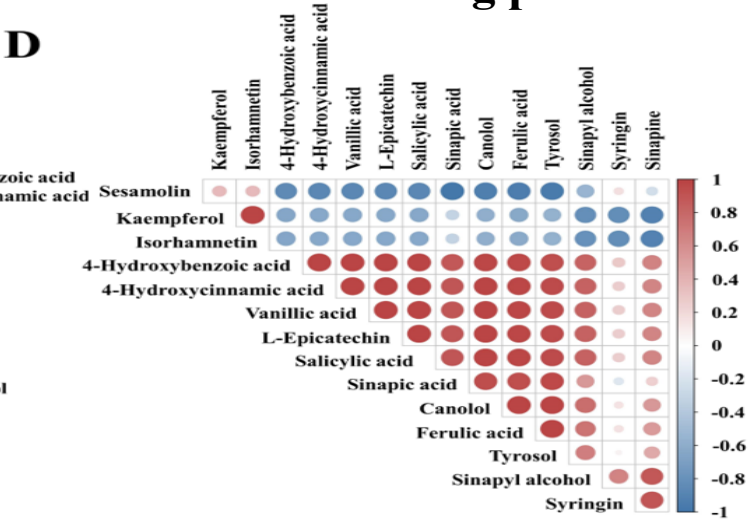
PCA loading plot



Colors of six varieties rapeseed



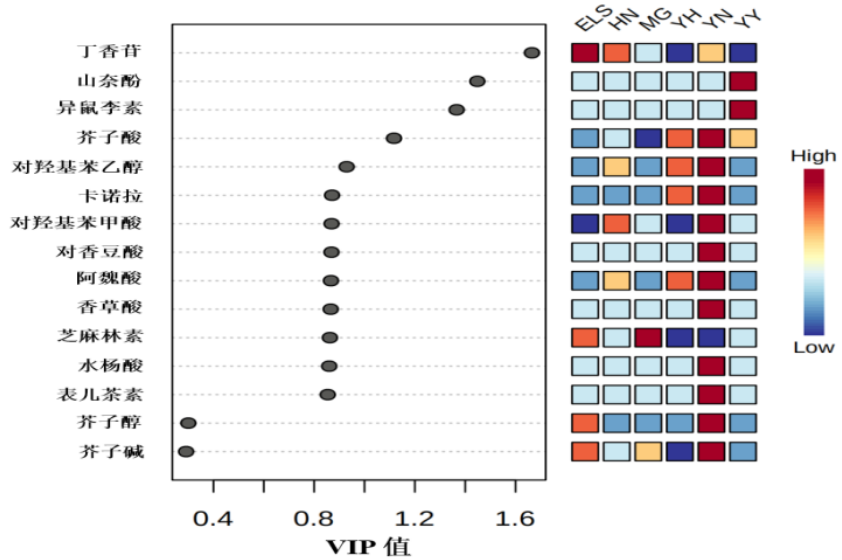
Hierarchical clustering heatmap



The correlation heatmap

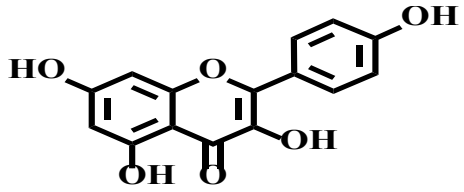
Significant differences in the composition and content of phenolic compounds in different rapeseed varieties, with Yunnan rapeseed having the greatest difference compared to other rapeseed varieties.

3.2 The composition and spatial distribution of phenolic in rapeseed

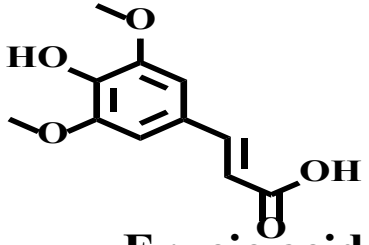


VIP score plot of phenolic compounds in rapeseeds

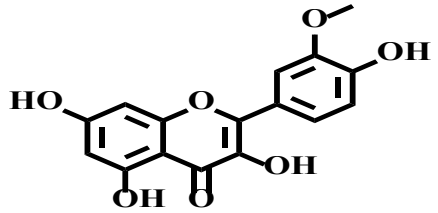
Syringin



Kaempferol

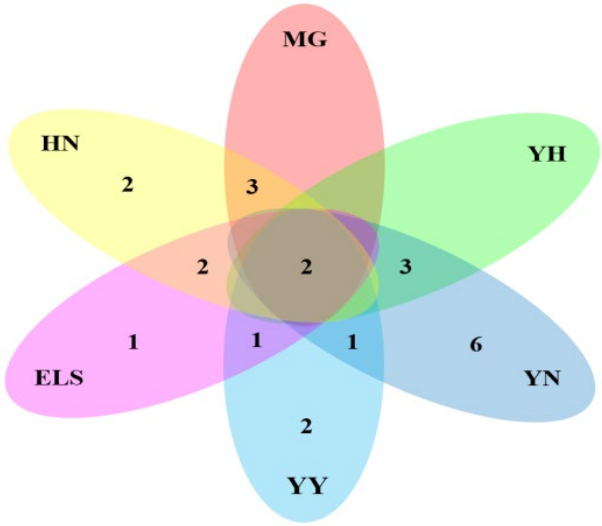


Erucic acid

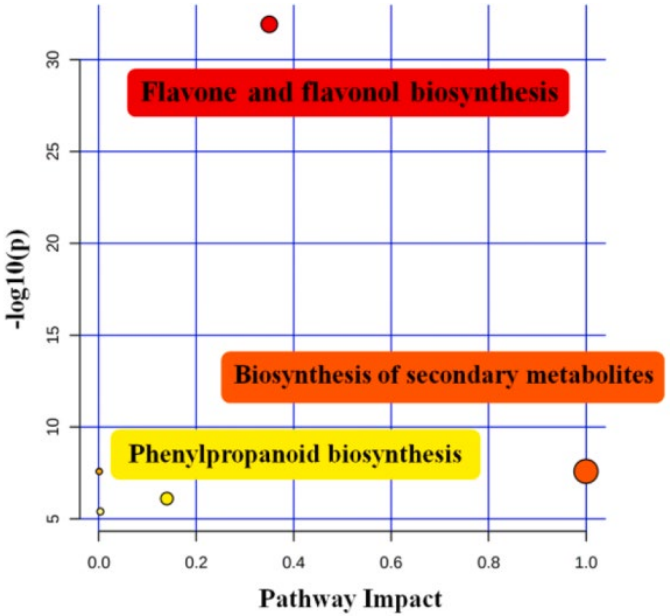


Isorhamnetin

Six rapeseed cultivars had only two common phenolic compounds, sinapic acid and sinapine



Venn diagram of phenolic compounds



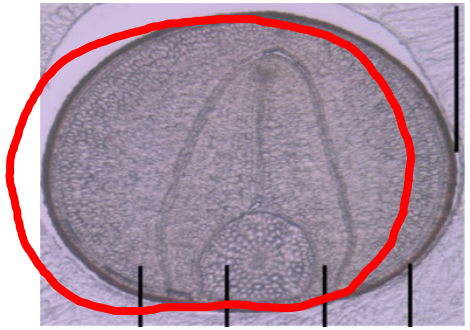
Metabolic pathway analysis

Metabolic pathways affecting differential metabolites include:

1. Flavone and flavonol biosynthesis pathway
2. Biosynthesis of secondary metabolites pathway
3. Phenylpropanoid biosynthesis pathway

3.2 The composition and spatial distribution of phenolic in rapeseed

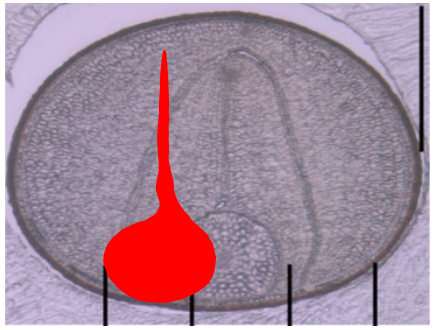
Seed coat



Seed coat

Significant different phenolic compounds in rapeseed seed coats: syringin, erucic acid, ferulic acid, canola, and para-coumaric acid.

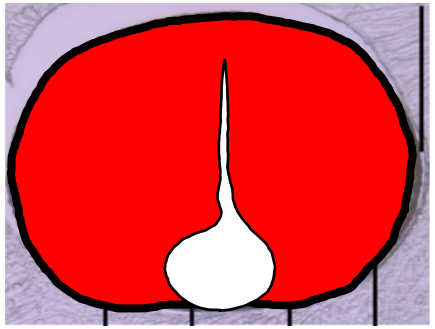
Seed embryo axis



Seed embryo axis

Significant different phenolic compounds in rapeseed embryo axis: isorhamnetin, kaempferol, vanillic acid, erucic acid, and ferulic acid.

Seed cotyledon

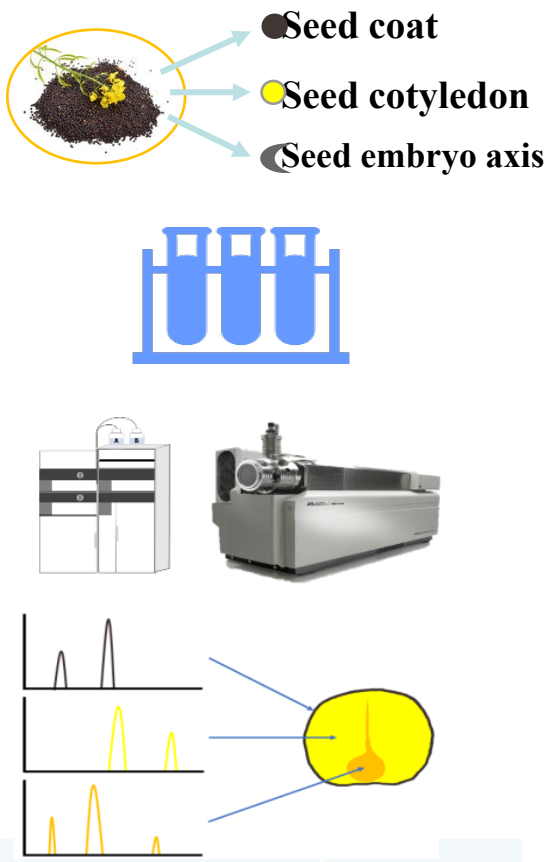


Seed cotyledon

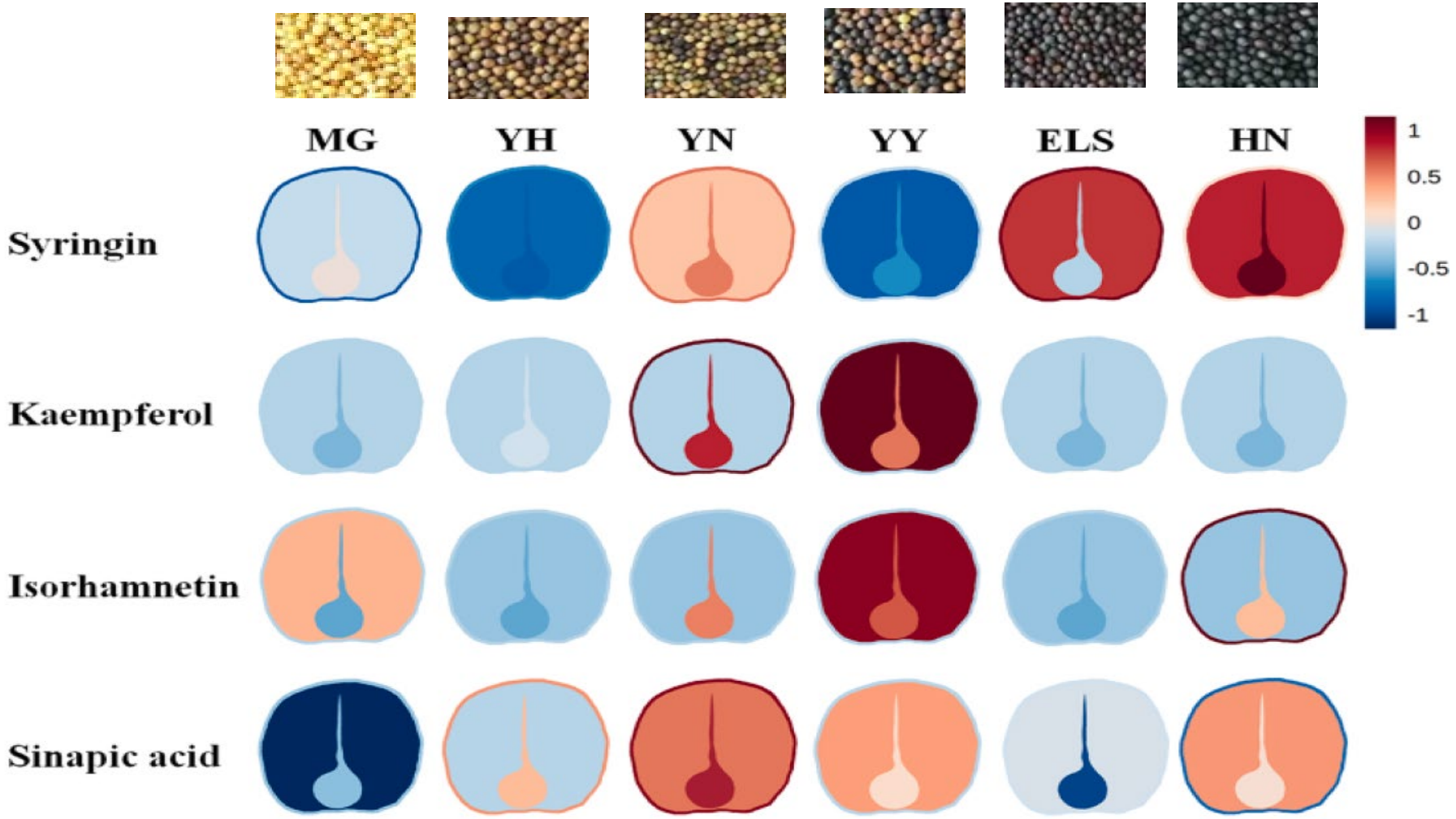
Significant different phenolic compounds in rapeseed cotyledons: syringin, sinapyl alcohol, kaempferol, and sinapic acid.

3.2 The composition and spatial distribution of phenolic in rapeseed

Spatial distribution of phenolic compounds in different rapeseeds



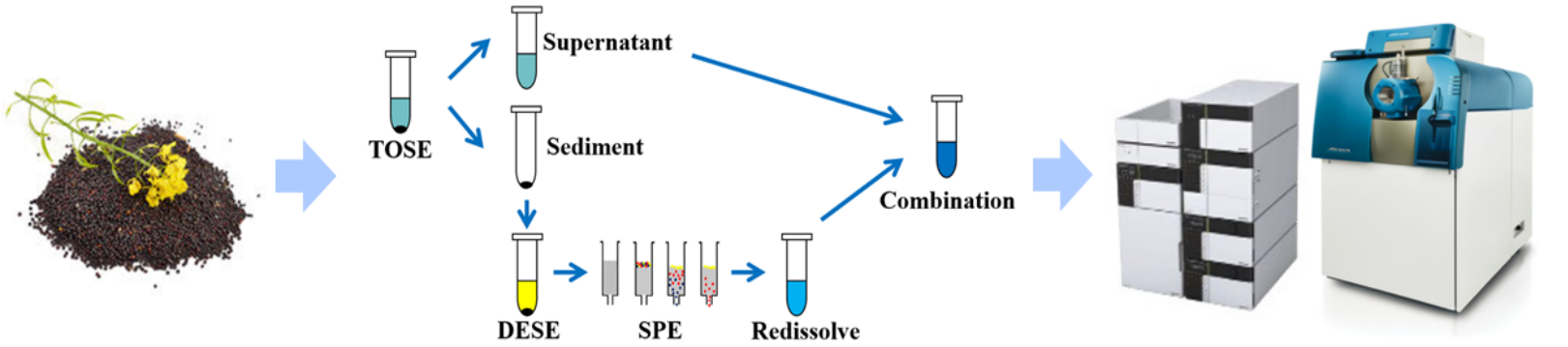
Seed embryo axis	1993±46
Seed coat	1741±43
Seed cotyledon	1511±39



This study can provide data support for the breeding of rapeseed varieties and the comprehensive utilization of phenolic compounds in rapeseed.

3.3 Comprehensive profiling of phenolic compounds in rapeseed

Flow chart of phenolic compounds in rapeseed using non-targeted metabolomics combined with molecular network technology

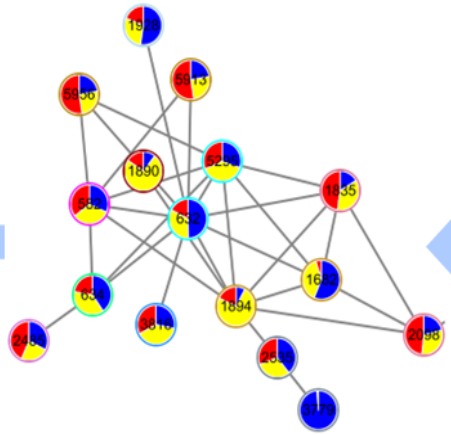


Rapeseed

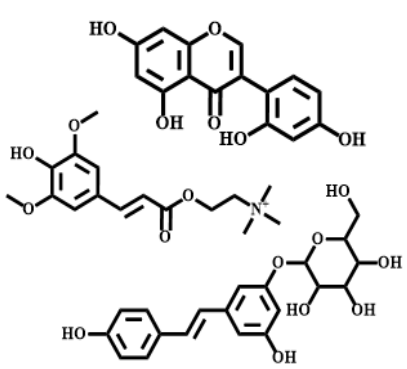
Two-step extraction strategy

UPLC-QTOF-MS/MS

Data processing



Molecular networking

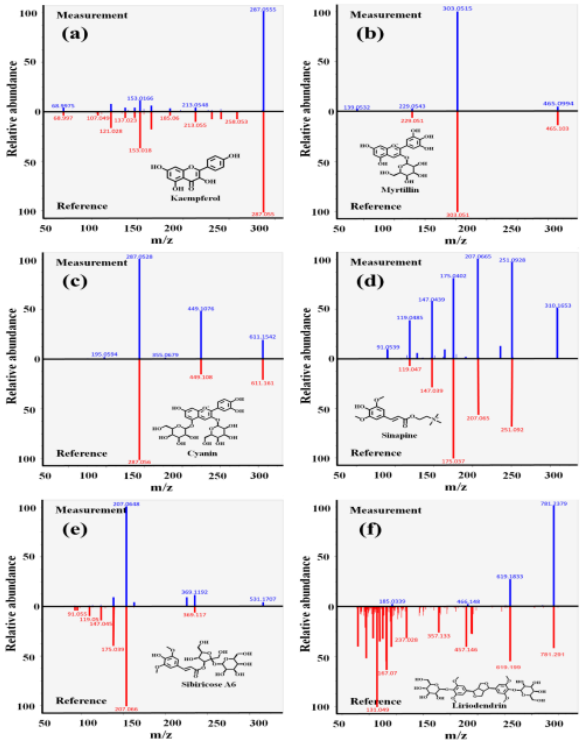


Structural annotation

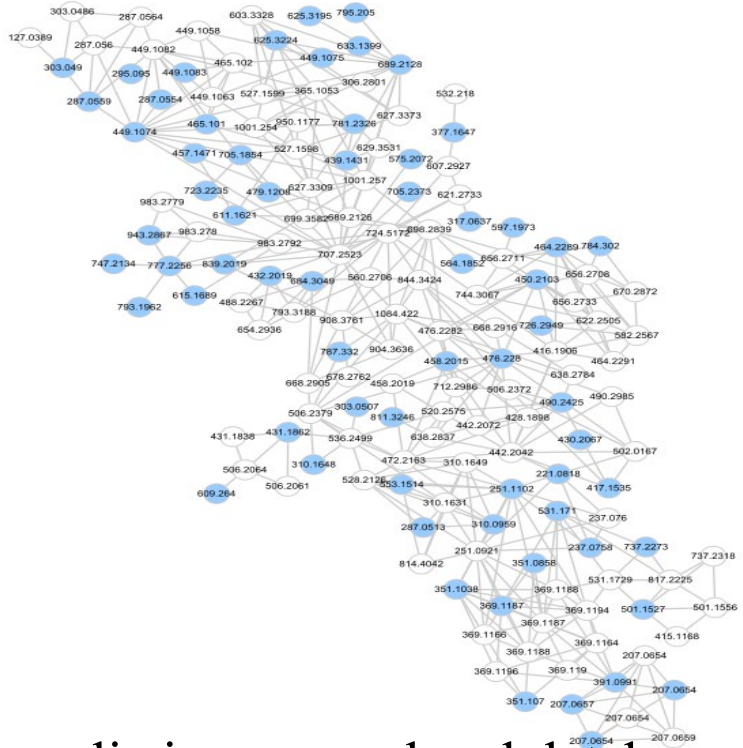
- The correlation data between different phenolic compounds was obtained by comparing the fragmentation patterns of phenolic compounds in the MS/MS spectra.
- The molecular network was visualized using Cytoscape software, and the molecular networking diagram of the correlation between phenolic compounds in rapeseed was obtained for further annotation of the structure of phenolic compounds in rapeseed.

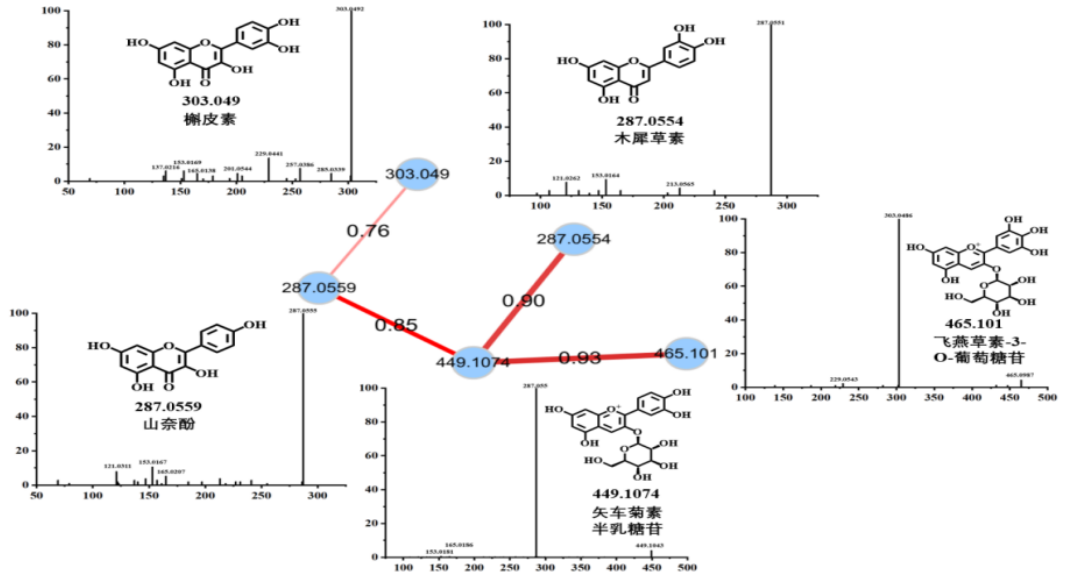
3.3 Comprehensive profiling of phenolic compounds in rapeseed

Profiling of phenolic compounds by MS/MS molecular networking



Matching diagram of phenolic in rapeseed and database





Molecular networking diagram of rapeseed

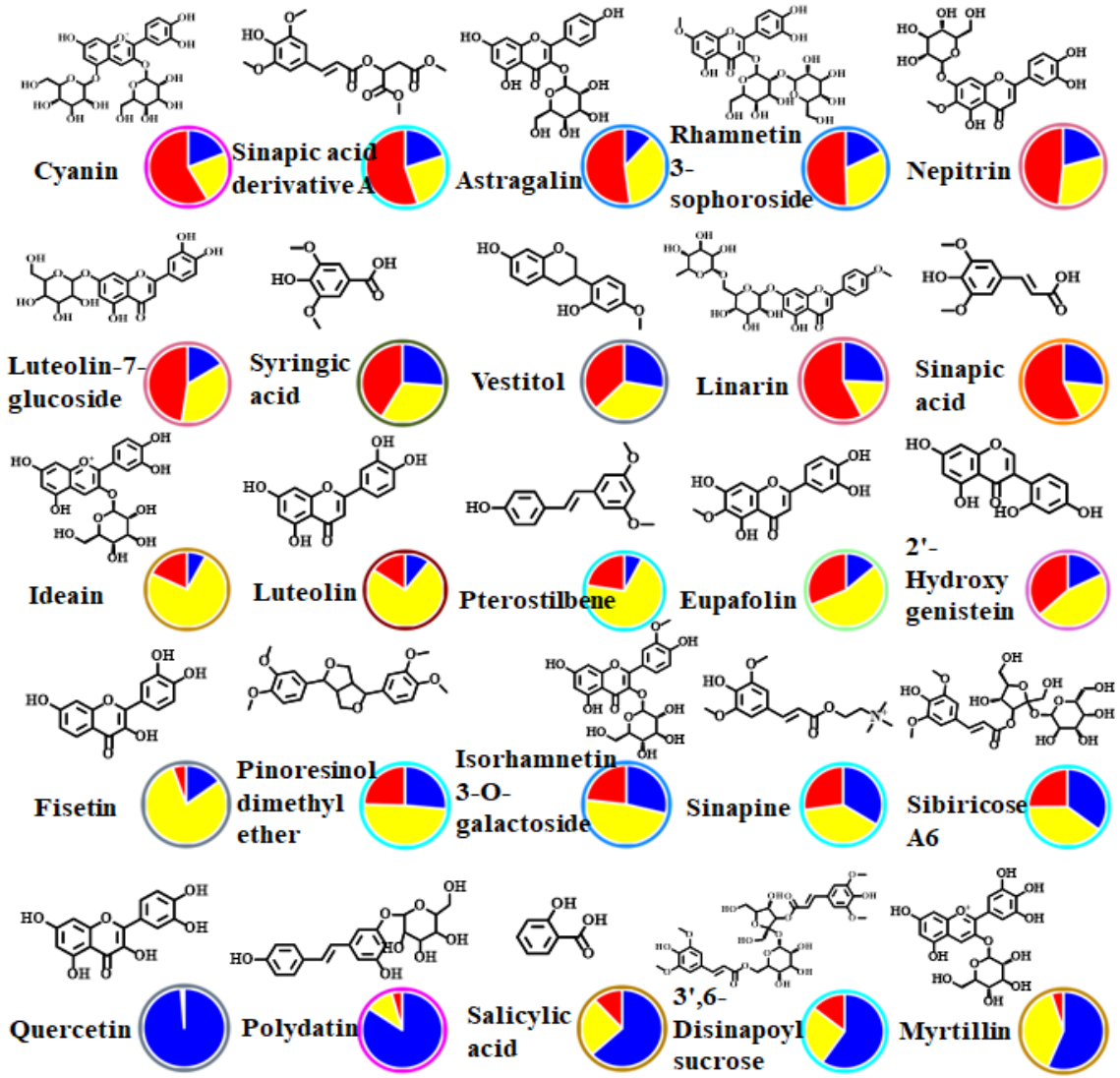
Based on the closer aggregation of compounds with similar structures in the molecular network, further structural annotation of phenolic compounds in rapeseed was carried out. 117 phenolic compounds in rapeseed were identified, covering 36 flavonoids, 23 coumarins, 12 phenolic acids, 10 lignins, 4 stilbenes, 4 diarylheptanes, 1 tannin, and some other phenolic compounds.

3.3 Comprehensive profiling of phenolic compounds in rapeseed

Comparison of phenolic compounds from different genotypes of rapeseed

- ***Brassica napus L.***
- ***Brassica rapa L.***
- ***Brassica juncea L.***

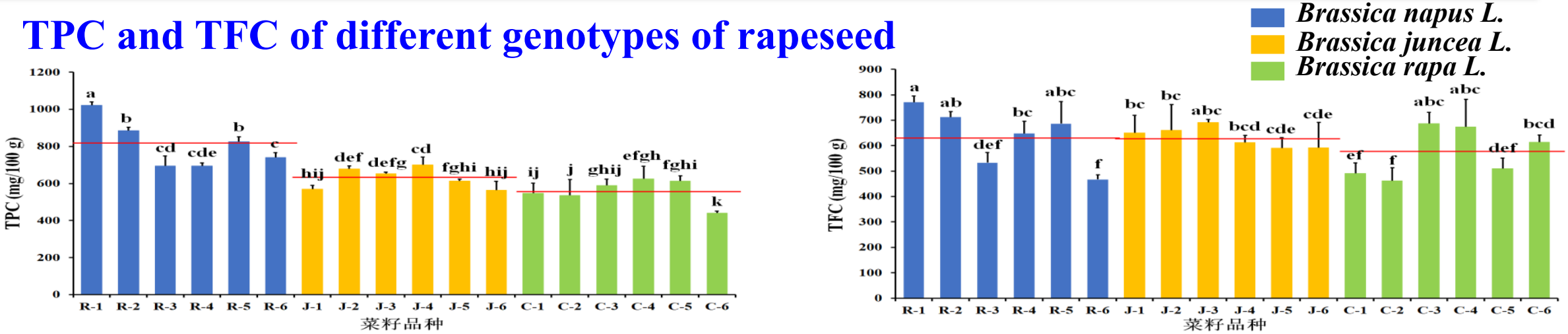
39 phenolic compounds such as anthocyanins have the highest content in *Brassica napus L.* rapeseed, 37 phenolic compounds such as luteolin have the highest content in *Brassica rapa L.* rapeseed, and 41 phenolic compounds such as quercetin have the highest content in *Brassica juncea L.* rapeseed.



Pie chart of phenolic compounds in rapeseeds of different genotypes

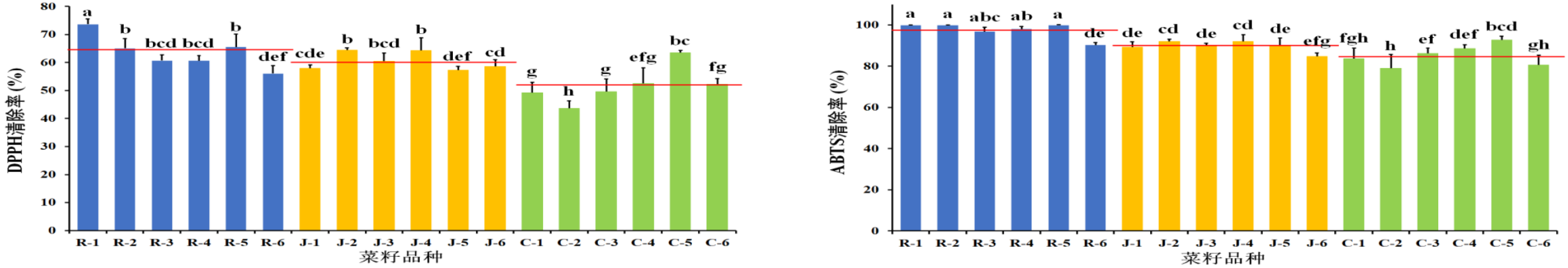
3.3 Comprehensive profiling of phenolic compounds in rapeseed

TPC and TFC of different genotypes of rapeseed



- TPC of *Brassica napus L.* rapeseed is significantly higher
- TFC of *Brassica napus L.* and *Brassica juncea L.* rapeseeds are generally higher

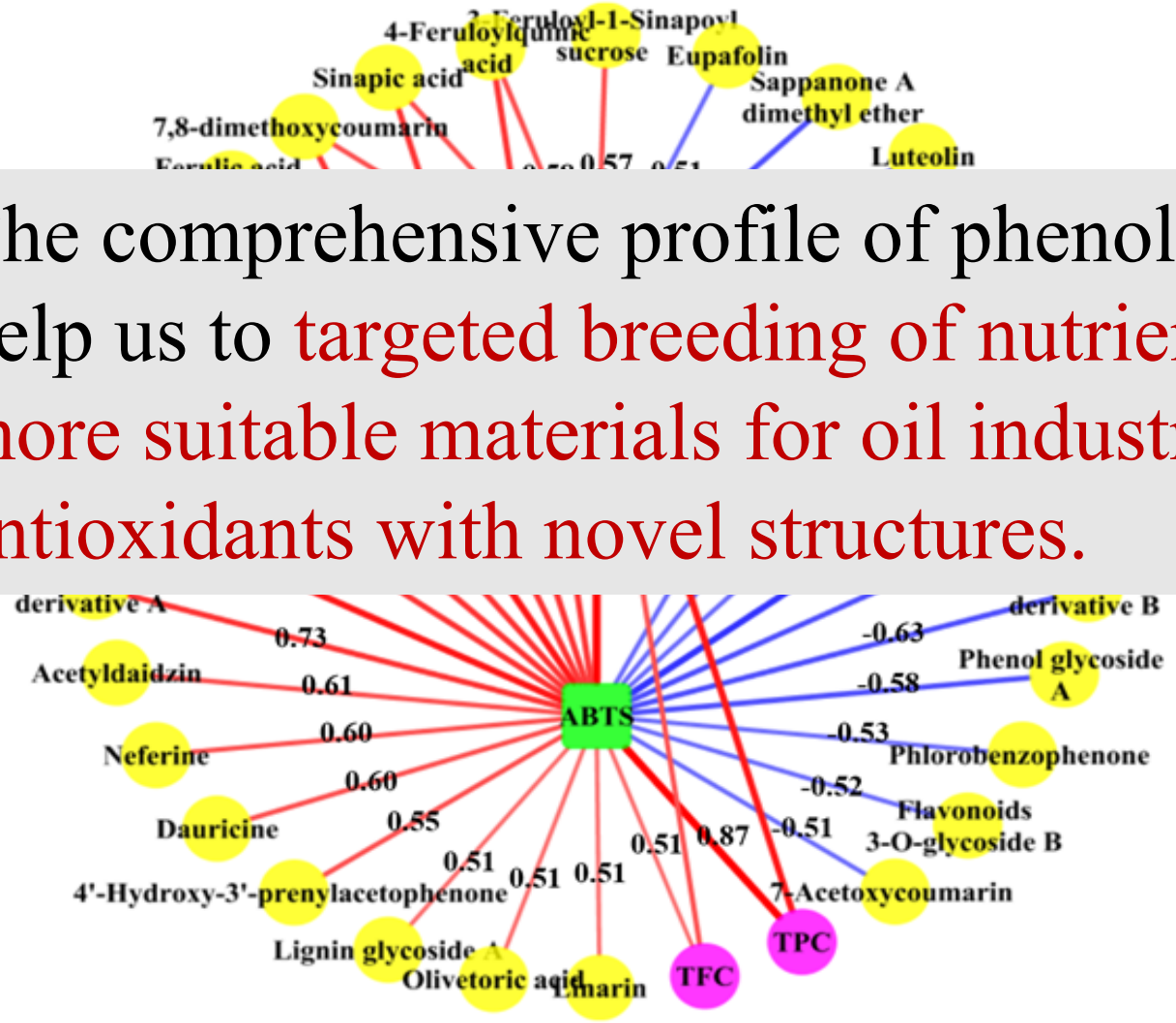
Antioxidant activity of different genotypes of rapeseed



- *Brassica napus L.* and *Brassica juncea L.* rapeseeds to scavenge DPPH free radicals is significantly stronger
- Brassica napus L. rapeseed has the strongest ability to scavenge ABTS cationic free radicals

3.3 Comprehensive profiling of phenolic compounds in rapeseed

Correlation analysis between antioxidant activity and phenolic compounds



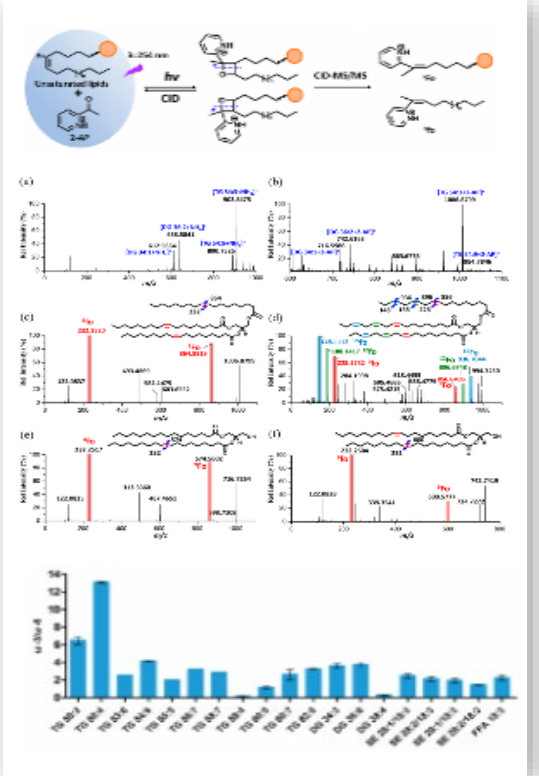
The comprehensive profile of phenolic compounds in rapeseed will help us to targeted breeding of nutrient-rich rapeseeds, selection of more suitable materials for oil industry, and discovery of natural antioxidants with novel structures.

- DPPH free radical and ABTS cationic free radical scavenge ability were
- Flavonoid 3-O-glycoside A, methyl chlorogenate, scoparone, p-coumaric acid derivative A, ferulic acid derivative A and sinapic acid derivative A have strong antioxidant activity.

Conclusion and Perspective

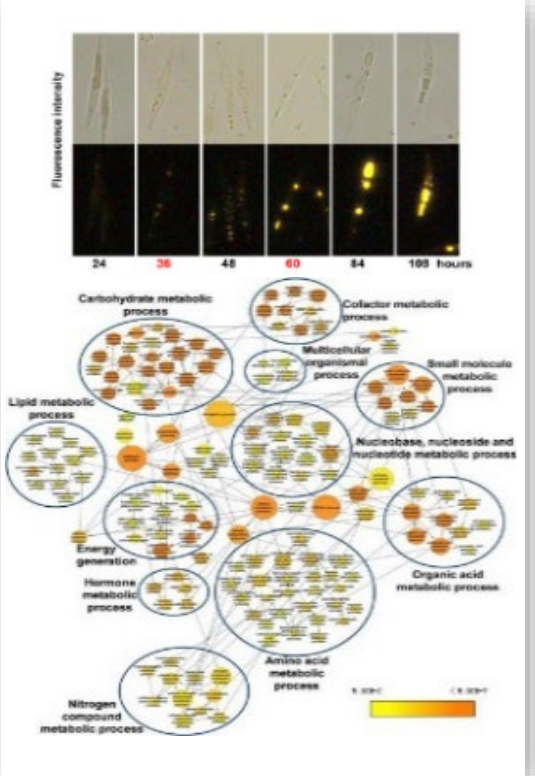
Liposome/metabolome analysis technology platform

Resource Discovery



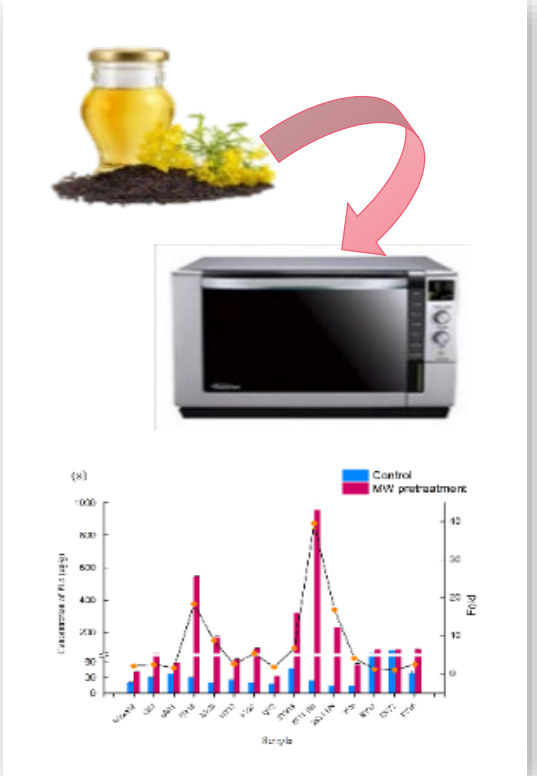
Fine structure analysis of lipids
J. Agric. Food Chem
2021, 69, 9012-9024

Metabolic Regulation



Clarify the metabolism
of biological lipids
Plant Cell
2014, 26, 3023

Quality Control



Lipid analysis during rapeseed
oil processing
Food Chemistry
2019, 285, 194

Nutrition Evaluation

Functional products regulate
lipid metabolism
Food & Function
2018, 9, 5027

Acknowledgments

National Natural Science Foundation of China （31571926, 31271879, 31171661, 21105119）

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Innovation Project of Chinese Academy of Agricultural Sciences

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Wuhan Science and Technology Planning Project（2019020701011468）

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Thank you!

Public Account: Oil Lipid Analysis Laboratory