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REVIEW ARTICLE

Unveiling metabolic changes in stored rice seeds: A metabolomics approach

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Abstract

Rice is a vital staple crop that not only served as a primary food source but also contributed significantly to the national economy. Its propagation through seeds was therefore crucial for ensuring sustainable agricultural productivity. However, seed viability declined over time due to physiological and biochemical changes during storage, leading to deterioration and reduced quality. Understanding these metabolic changes was essential for preserving seed longevity. Metabolomics emerged as a robust analytical approach for profiling a wide range of rice seed metabolites and identifying biomarkers linked to seed ageing. Techniques like mass spectrometry (MS) and nuclear magnetic resonance (NMR) allowed for high-resolution, comprehensive analysis. Metabolomics enabled early detection of seed ageing with high accuracy and supported strategies that extended rice seed viability. This enhanced seed quality management and reduced post-harvest losses effectively. This review highlighted the metabolite composition of rice seeds, factors influencing changes during storage, key metabolomics methodologies and their applications in improving seed quality, longevity and future breeding programs.

Keywords: GC-MS; metabolic profiling; quality deterioration; seed ageing; seed viability

Introduction

Rice, (*Oryza sativa* L.), was the staple food for more than half of the world's population out of which 50 % of the rice produced and consumed comes from India and China. Every year, about 480 million metric tonnes of milled rice were produced (1). In addition, it provided employment to nearly 200 million households and served as a source of income in the underdeveloped nations (Food and Agriculture Organization. June 29, 2013). The post-harvest storage of rice seeds was a critical aspect of agricultural practice, influencing their quality, nutritional content and market value. Ensuring the quality and nutritional integrity of rice seeds throughout the post-harvest storage process was crucial for maintaining food security and meeting the dietary needs of populations worldwide. Post-harvest storage conditions had profoundly impacted the quality, shelf life and nutritional content of rice seeds, making it imperative to understand the biochemical changes that occurred during storage.

Seed longevity was influenced by the storage environmental conditions such as the temperature, equilibrium relative humidity and oxygen pressure (2). Many physiological and biochemical changes occurred during seed deterioration. The most accepted indicators of seed deterioration included reduced germination rates and decreased vigour. Research also identified various physiological, cellular, biochemical and metabolic changes that occur during seed storage. These included lipid peroxidation, enzyme inactivation, cellular membrane disruption, reduced energy production, alterations in protein synthesis and degradation of DNA and RNA and disruption in redox homeostasis. Among these, alterations in the metabolite composition of rice seeds played a pivotal role in determining their overall quality and nutritional value. Metabolomics, an advanced analytical approach, emerged as a powerful tool for studying these dynamic metabolic changes in rice seeds during storage. Thus, enhancing seed storability by preserving seed vigour during storage was crucial for rice production (3).

Metabolomics, a relatively new discipline within high-throughput functional genomics, enabled global analysis and identification of the accumulation of metabolites or small molecules within a cell at a given time. Consequently, metabolomics, which provided a comprehensive, unbiased, high-throughput examination of complex metabolite mixtures in target organisms, was employed in various seed studies. Metabolomics involved a complex field of analytical chemistry and bioinformatics in which advanced techniques to determine the levels of a wide range of metabolites were employed in a series of procedures, including sample extraction and preparation, metabolite detection using

analytical instruments and data processing and mining by means of bioinformatics techniques (4). The ultimate goal of plant metabolomics was quantification of the metabolome in plants (5). Metabolomics was divided into targeted and untargeted approaches. It utilized high-resolution separation techniques in conjunction with sensitive detection methods to analyse small molecules over a broad dynamic range (6).

This approach was generally used for the characterization of rice aging and traceability to provide a potential pathway and determine the changes in biological phenotypes.

Metabolite composition of rice seeds

Rice seeds contained a wide range of metabolites which were broadly classified into primary and secondary metabolites. Understanding these metabolites was crucial for enhancing rice cultivation, nutritional value and storage properties.

1. Primary metabolites of rice seeds

Primary metabolites in rice seeds were essential compounds involved in the growth, development and metabolic processes necessary for the seed's viability and successful germination. The major primary metabolites in rice included carbohydrates, proteins, lipids.

Carbohydrates were the most abundant primary metabolites in rice seeds, serving as the primary energy source during germination and early seedling growth. Seeds contained a variety of carbohydrates, including oligosaccharides and polysaccharides. Starch being the chief component comprised of 72 % to 82 % of the dry weight in brown rice grain (7) and roughly 90 % in milled rice grain (8). In the endosperm of "albuminous" members of Poaceae family, carbohydrates, particularly starch, dominated, comprising about 80 % of the seed's total composition. The main components of starch, amylose and amylopectin possessed unique characteristics. Amylopectin, which made up 65 % to 85 % of starch granules, was extensively branched due to α-(1,6) bonds, with waxy mutants possibly containing entirely amylopectin (9). In contrast, amylose consisted of linear α-1,4 linked glucose units, occasionally featuring α-1,6 branch points (10). Wild rice starch yielded less but contained higher amylose levels than long grain brown rice starch (11). Factors such as grain size and sucrose levels affected the rate at which starch accumulated in rice grains (12).

While starch predominated, rice seeds also contained other carbohydrates, but in smaller quantities which include monosaccharides like glucose, fructose and oligosaccharides like raffinose and sucrose. In the early stages of seed development, monosaccharides like glucose and to a lesser extent, fructose were relatively abundant, but their levels decreased as the seed matured. In mature seeds, these monosaccharides comprised only a very small portion of the total sugar content, representing less than 0.2 % of the dry weight in cereals (13). In rice seeds, disaccharides were present in small quantities, with sucrose being the most notable. The sucrose concentration was significantly higher in the embryo compared to the endosperm of cereals (14). Sucrose was the predominant one, but the cereals also contained notable amounts of raffinose, stachyose and occasionally verbascose.

Lipid represented another important class of primary metabolites. The lipid content was higher in the embryo, followed by the aleurone layer of the seed where they were arranged in the lipid droplets and in sphaerosomes (15). In the endosperm, lipids were more concentrated in the outer layer, leading to a progressive decline towards the centre of kernel (16-18). Lipids in crops like rice were classified as starch lipids and non-starch lipids based on their cellular distribution and association; they were also categorized as neutral lipids, glycolipids and phospholipids (19).

Rice seed storage proteins (SSPs) were exclusively synthesized in the endosperm and accumulated in large quantities. These proteins were classified into albumins, globulins, prolamins and glutelins based on their solubility, using the Classical Osborne Fractionation method developed in 1924 (20). Within the ripe grain (caryopsis) of rice, there are twenty primary amino acids were present, including eight essential amino acids crucial for human and animal health. The concentration of free amino acids in a mature grain was very low, typically ranging from 0.35 % to 0.55 % of the total amino acid content.

2. Secondary metabolites of rice seeds

Secondary metabolites performed a variety of physiological roles, including regulating rice growth and development, enhancing disease resistance, providing anti-insect properties and exhibiting allelopathic effects. Additionally, they possessed a range of biological activities, such as antimicrobial, antioxidant properties (21). Some important secondary metabolites in rice seeds included phenolic compounds, flavonoids and terpenoids.

Ferulic acid and p-coumaric acid were the predominant phenolic acids in rice, with higher concentrations found in brown rice compared to milled rice (22). Earlier studies revealed that rice bran had the highest total phenolic and flavonoid contents (23).

Flavonoids were typically classified into several categories including flavones, flavonols, flavanols (also known as flavan-3-ols), flavanonols, isoflavones and flavanones. As per previous report Tricin, among the seven typically identified flavonoids in rice, emerged as the predominant one in the bran, constituting 77 % of the total amount of these seven flavonoids (24).

The other major secondary metabolites were the terpenoids, which were further divided into monoterpenoids, diterpenoids and triterpenoids.

The major secondary metabolites of rice seeds and their biological roles were illustrated in Table 1.

Factors affecting metabolite composition during storage

The three important external factors - temperature, relative humidity and oxygen exposure determined the storage potential of rice seeds (25). Besides these, the genetic factors and the storage duration also predominantly influenced the metabolite composition (Fig. 1).

1. Temperature

The influence of temperature on the metabolite composition of rice seeds was a complex phenomenon

Table 1. Major secondary metabolites in rice seeds and their functions

Class of Metabolite	Examples	Biological Role	References
Phenolic Compounds	Ferulic acid, p-Coumaric acid	Antioxidant, antimicrobial	(21, 22, 23)
Flavonoids	Tricin, Anthocyanins, Flavones, Flavonols, Flavanols, Isoflavones, Flavanones	Antioxidant, pigmentation	(24)
Terpenoids	Gibberellins, Momilactone B, Lupeol, Cycloartenol *etc.*	Aroma, phytohormones, defence, allelopathy	(21)

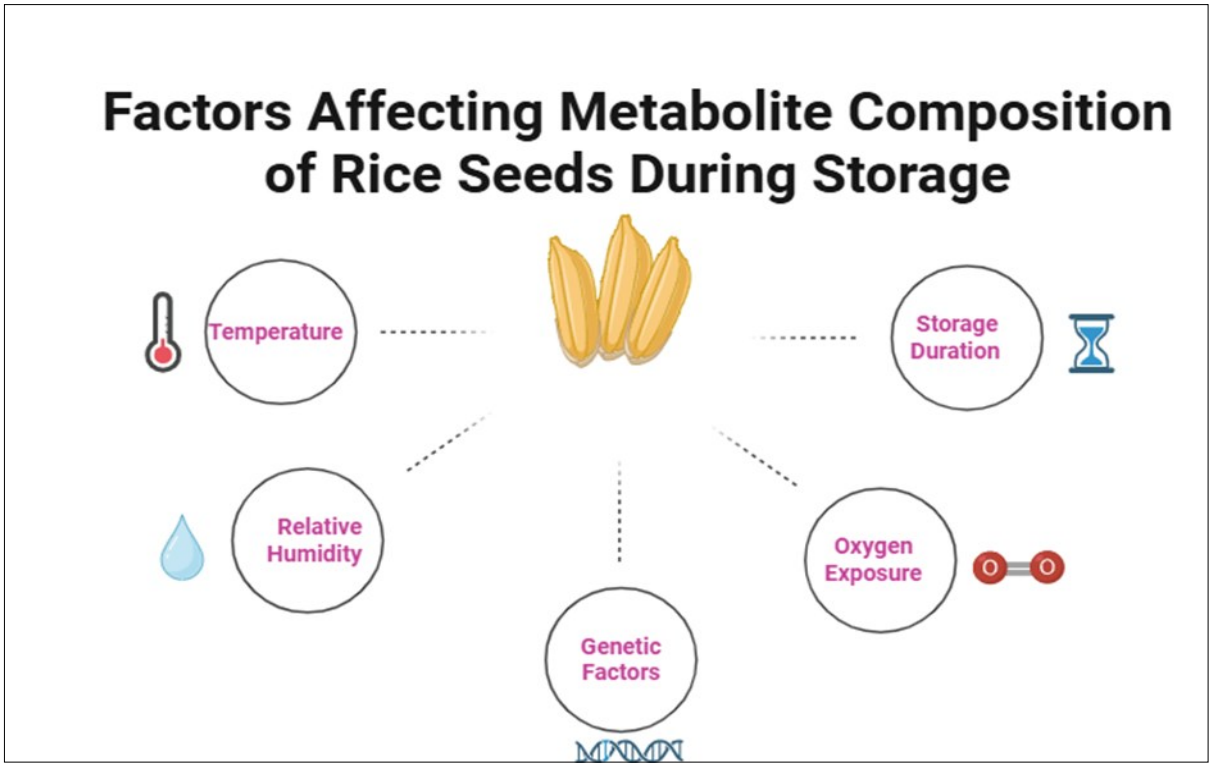

Fig.1. Factors affecting metabolite composition of rice seeds during storage.

As per report the storage at 25 °C caused a decrease in sucrose and glucose levels in most Basmati rice varieties. Basmati 86 showed a reduction in sucrose from 3.10 to 2.20 mg/ kg and in glucose from 37.50 to 1.70 mg/ kg, while Basmati 515 showed a reduction in sucrose from 5.80 to 2.40 mg/ kg and in glucose from 95.60 to 28.70 mg/ kg. At 5 °C, fructose increased in Basmati 86 from 9.70 to 15.70 mg/ kg, Basmati Super from 9.00 to 11.80 mg/ kg and Basmati Kainat from 7.60 to 15.00 mg/ kg, while sucrose and glucose decreased in most varieties. Basmati S. fine showed an increase in glucose from 64.90 to 97.00 mg/ kg at 5 °C. After 3 months of storage at 25 °C, Basmati 86 showed a significant decrease in palmitic acid (17.49 to 8.07 g/100 g), oleic acid (38.01 to 9.89 g/100 g) and linoleic acid (33.45 to 8.06 g/100 g). After 6 months of storage at 5 °C, palmitoleic acid increased to 17.80 g/ 100 g and stearic acid to 3.69 g/ 100 g. In Basmati Super, after 3 months at 25 °C, oleic acid decreased from 46.21 to 21.88 g/ 100 g, linoleic acid from 28.64 to 10.30 g / 100 g and palmitic acid from 17.3 to 10.72 g/ 100 g. After 6 months at 5 °C, oleic acid decreased to 10.93 g / 100 g, linoleic acid to 16.73 g / 100 g and palmitic acid to 8.26 g / 100 g (26).

The effect of storage temperature on the fatty acid composition of Kusabue and Katakutara rice showed notable differences, at 30°C resulted in Kusabue had higher levels of 16:0 (palmitic acid-53.33 %) and 18:1 (oleic acid-(42.06 %) compared to Katakutara. At 60 °C, Kusabue exhibited a decrease in 16:0 (39.32 %) and an increase in 18:2 (linoleic acid) (36.83 %), while Katakutara showed a slight increase in 16:0 (29.98 %) and a decrease in 18:2 (29.81 %). Both cultivars had increased 18:1 and 18:2 in neutral and polar lipids at 60 °C (27). Together these studies indicated that the temperature had a great influence on the metabolite composition of rice seeds.

2. Relative humidity

The influence of humidity on the metabolite composition of rice seeds was multifaceted. In a previous report on rice seeds stored under various conditions, it was noted that at 20 °C and 40 % humidity, the initial fatty acid content measured 3.27 mg/ 100 g and began to increase over time, with aging effects becoming evident from the 35th day onward (28). At 60 % humidity, the content also increased, peaking by the 40th day. In a high-humidity environment (80 %), fatty acid content rose steadily before decreasing after reaching the peak, with a rapid increase starting on the 35th day. At 30 °C and 35 °C, fatty acid levels increased significantly, with 80 % humidity accelerating the rise. Temperature and humidity together influenced the increase in fatty acids, with 30 °C and 35 °C showing the most rapid increase in fatty acid content, especially at 80 % humidity. The total starch content (%) in rice decreased under all relative humidity (RH) conditions, with higher RH (80 %) causing faster degradation than lower RH (40 %). At 20 °C, starch content dropped from 65 % to 52 % (40 % RH), 50 % (60 % RH) and 48 % (80 % RH) by day 10, stabilizing around 47- 45 % by day 50. At 30 °C, it declined from 58 % to 50 % (40 % RH), 48 % (60 % RH) and 46 % (80 % RH) by day 6, stabilizing at 48-45 % by day 24. At 35 °C, it fells from 62 % to 50 % (40 % RH), 48 % (60 % RH) and 46 % (80 % RH) by day 4, stabilizing at 48-46 %

by day 12. Lower RH slowed degradation, while higher RH accelerated it.

Primed seeds stored under LT-V (low temperature-vacuum), RT-V (Room temperature-vacuum) and RT-A-LH (Room temperature-aerobic-low RH) maintained stable α-amylase activity and sugar content for 60 days. In contrast, RT-A-HH (Room temperature-aerobic-high RH) storage caused sharp declines, with α-amylase activity decreasing by 55.0 %, 59.6 %, 75.3 % and 70.6 % after 15, 30, 45 and 60 days, respectively and sugar content dropping by 51.3 %, 49.3 % and 49.4 % compared to LT-V, RT-V and RT-A-LH, highlighting high humidity's adverse effects (29).

Together, these studies indicated that humidity can indeed influence the metabolite composition of seeds.

3. Oxygen exposure

The impact of oxygen exposure on the metabolite composition of cereals during storage was a complex phenomenon. The use of oxygen absorbers has been shown to inhibit lipid degradation in unpolished grains like brown rice and whole grain wheat by reducing the accumulation of free fatty acids (35). Rice seeds stored under elevated partial pressure of oxygen (EPPO) conditions exhibited significant changes in lipid and volatile compound composition. Oxidized lipids, which were negatively correlated with seed viability ($r < -0.98$), increased during storage, while triacylglyceride (TAG) 52:3 + O, initially present, declined to non-detectable levels, transforming into other oxidized TAGs that negatively affected germination. Lipids like coenzyme Q9 and tri-linoleoyl-glycerol, positively correlated with viability ($r > 0.85$), decreased with storage. Volatile profiling identified 183 compounds, with EPPO-stored samples showing marked increases in volatiles such as 3,5-octadien-2-one, 2-methyl-2-propanol, hexanal, 2-heptanone, acetic acid and heptanal, all of which were correlated with reduced seed germination and extended storage (36). Conversely, under anaerobic conditions, only rice seeds were capable of degrading non-boiled, soluble starch, indicating the presence of a complete set of starch-degrading enzymes (37).

4. Genetic factors

The metabolite composition of rice was notably affected by genetic factors, as evidenced by studies conducted (30, 31). Investigation into rice grains demonstrated that various metabolites were under the control of distinct genetic factors. Research further supported this notion, indicating that the metabolic makeup of rice kernels correlated with genetic diversity and can serve as a predictor of quality traits. Furthermore, a previous study emphasized the influence of crossing parentage and environmental conditions on metabolite profiles, particularly in wild-type cultivars, which had a significant impact on the metabolite composition of low phytic acid rice offspring.

In the study, among the IIYou 998 (IIY) and BoYou 998 (BY) seeds, the IIY seeds showed significant changes in 19 metabolites during storage whereas BoYou 998 (BY) seeds exhibited significant changes only in 8 metabolites (33). The raffinose levels were also lower in IIY seeds before and after storage compared to BY seeds indicating the lower storage potential of IIY seeds. As per previous report that after 20 days of storage at 10.9 % moisture content and 45 °C, "IR65483" (long-lived) seeds showed increased levels of kaempferide, quercetin-3-arabinoside, S-sulfocysteine and D-glucose, while "WAS170" (short-lived) seeds did not, instead they had higher levels of thiamine monophosphate and harmaline, indicating seed deterioration which are due to key metabolic and genetic factors in seed longevity (34).

5. Storage duration

The impact of storage duration on the metabolite composition of cereals varied depending on the specific metabolite and cereal type. The report observed changes in the fatty components of cereals during prolonged storage (38). The metabolite composition of rice varieties changed significantly during storage. Total starch content decreased after storage, while amylose content increased by 9.63-11.65 % in japonica, 2.99-4.67 % in indica and 8.07-8.97 % in indica-japonica hybrids. Fat content decreased sharply by 60.00-65.00 %, 37.21 -46.51 % and 41.67-42.42 %, respectively. Abscisic acid (ABA) content gradually decreased throughout the year, while raffinose content initially increased by 19.35-45.45 %, 7.02-10.77 % and 16.13-28.13 % after 4 months but later declined to the lowest levels after one year. Additionally, antioxidant enzyme activity decreased, leading to increased fatty acid values and malondialdehyde (MDA) levels. These chemical changes over one year contributed to deteriorated cooking quality, evidenced by reduced viscosity, increased gelatinization temperature and harder cooked rice (39). Over the 120-day storage period, rice seed metabolite composition showed significant changes where the starch content decreased from 77 % at 60 days to 75 % at 120 days (40). As per previous report, the rice was stored at 25 °C for 0 to 7 months (41). The total protein content of fresh harvest rice (0 months) decreased by 19.81 % over the 7-month storage period.

Metabolomics analysis techniques

Metabolomics was a technology for analysing metabolites in organisms including plant metabolites which were produced for growth, development and defence against natural predators in plants. In addition, metabolomics helped in understanding plant metabolic states, functions of unknown genes and breeding crops as valuable traits like taste and yield were closely related to metabolic conditions (4).

Metabolomics studies on seed storage revealed significant changes in metabolite profiles during storage, affecting seed longevity and quality deterioration. The steps in metabolomics analysis of rice seed metabolites were briefed in Fig. 2. Comparative analyses of rice cultivars with different storability showed changes in amino acids and sugars, with raffinose potentially playing a role in seed storability (33). These insights enhanced our knowledge of seed storage processes and could have helped devise strategies to preserve seed quality.

Improving the rice cultivars with valuable traits had been a constant challenge as rice was the most important staple crop in the world (42). It was believed that adopting metabolomic techniques in rice was useful in enhancing its quality, taste and nutritive value along with the understanding of useful traits like those of yield and defence response (33). Metabolomics utilized a few analytical platforms such as nuclear magnetic resonance (NMR) spectroscopy, mass

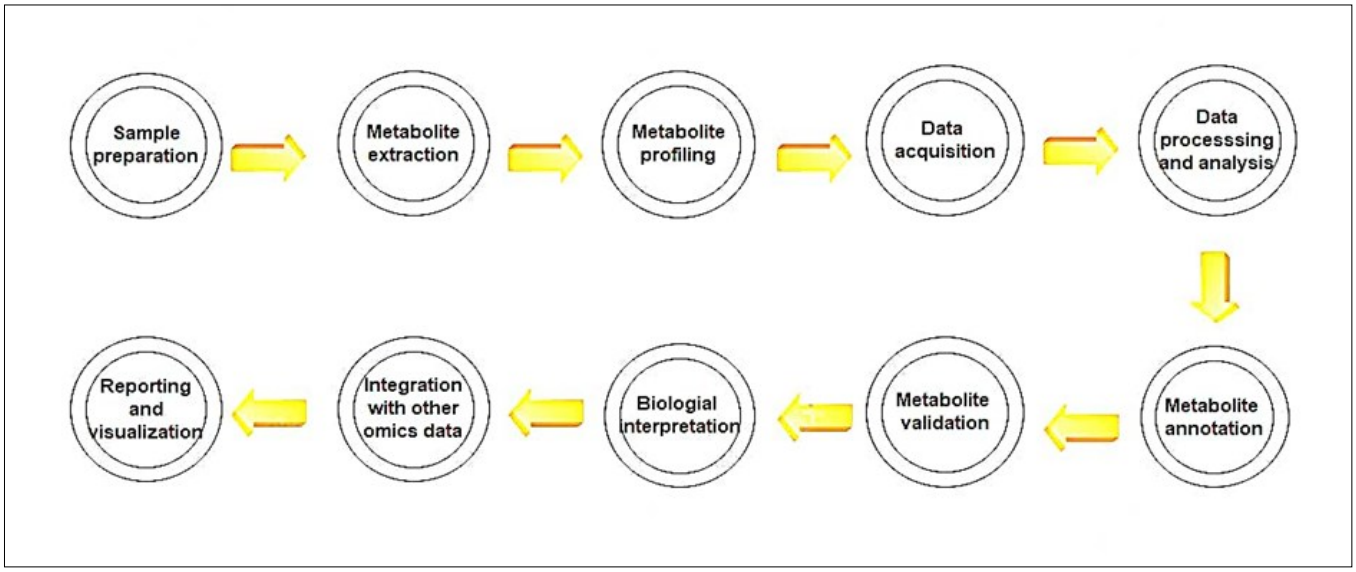

Fig.2. Steps in metabolomics analysis.

Note: The steps involved in metabolomics of rice seed metabolites such as sample preparation in which sample homogenization or extraction methods are employed to extract metabolites from the seeds. Following sample preparation, metabolite profiling is carried out in which metabolites are extracted from the rice seed samples using appropriate solvents or extraction methods. A range of analytical platforms are accessible for data acquisition, based on mass spectrometry (MS) or nuclear magnetic resonance (NMR) techniques (44). Additionally, Fourier transform infrared spectroscopy (FTIR) is gaining recognition in metabolomics for its capability for rapid analysis and characterizing intricate molecular structures simultaneously (100). These techniques separate, detect and quantify the metabolites present in the samples. Subsequently, the analytical devices produce raw data which includes retention times, mass spectra and peak intensities of identified metabolites and the data is processed and analysed using software. This includes statistical analysis to identify significant differences in metabolite abundance between samples.

Subsequently, the analytical devices produce raw data which includes retention times, mass spectra and peak intensities of identified metabolites and the data is processed and analysed using software. This includes statistical analysis to identify significant differences in metabolite abundance between samples. Data annotation is done by making comparisons between their mass spectra and retention times with entries in reference databases, or via supplementary experiments like tandem mass spectrometry (MS/MS). Then the identified and validated metabolites are further analyzed to understand their biological significance and metabolic pathways they are involved in. This step helps in elucidating the metabolic processes occurring in rice seeds and their roles in various physiological and biochemical functions. Metabolomics data is integrated with other omics data such as genomics, transcriptomics and proteomics. The results of the metabolomics analysis are reported and visualization tools are used to present the data effectively.

spectrometry (MS) and separation methods based on chromatography and electrophoresis.

1. Nuclear Magnetic Resonance (NMR) Spectroscopy

Nuclear Magnetic Resonance (NMR) spectroscopy was an essential tool in metabolomics, known for its high reproducibility, quantitative capabilities and non-invasive nature. It was able to identify unknown metabolites in complex mixtures and trace downstream products of isotope-labelled substrates. Although NMR was less sensitive than mass spectrometry, it could monitor content differences among thousands of metabolites and observe dynamic biochemical profile characteristics (43). The simplicity of sample preparation, the ability to quantify metabolite levels and its non-destructive nature made NMR particularly suitable for extensive or long-term clinical metabolomic studies. However, its lower sensitivity compared to other analytical techniques remains a drawback (44). Advancements in two-dimensional (2D) and multidimensional (nD) NMR greatly improved its sensitivity and resolution despite those limitations(44,45). Despite longer run times, nD-NMR provided valuable structural and functional information on biomolecules. Ultra-fast (UF) 2D NMR was developed to reduce analysis time by acquiring various spectra in a single scan (44-46). Comprehensive multiphase (CMP) NMR, a recent innovation, was able to simultaneously analyze all three states (solid, gel and liquid forms) with minimal run time increase and was successfully used for structural elucidation in seeds and during plant growth (45, 47, 48).

2. Mass Spectrometry (MS)

Mass spectrometry (MS), in contrast, was frequently paired with various chromatography systems in one or two dimensions (49). Two-dimensional liquid chromatography (LC) and gas chromatography (GC), along with multidimensional LC/GC technologies, gained attention as analytical techniques. These methods combined two or more columns with different stationary phase selectivities, enhancing resolution and peak capacities (50). Ion mobility MS became increasingly popular due to its ability to quickly analyze samples, eliminate interferences and separate isomers and isobars, as well as its capability to identify compounds based on both ion size-to-charge and ion mass-to-charge (m/z) ratios (51). Liquid chromatography-mass spectrometry (LC-MS) and capillary electrophoresis-mass spectrometry (CE-MS) have become essential tools. Advances in column technology, miniaturized systems and interfacing techniques have enhanced their reproducibility and sensitivity (52).

3. Gas Chromatography Coupled to Mass Spectrometry (GC-MS)

GC-MS was the most widely used analytical technique in metabolite profiling which combines high separation efficiency and resolution with mass-selective detection. It could analyse a wide range of volatile and/or derivatized non-volatile

metabolites with high analytical reproducibility and lower cost compared to other techniques such as LC-MS or LC-NMR.GC time-of-flight MS (TOF-MS) was popular due to its higher mass accuracy and resolution. One of the important requirements for GC-MS analysis was analyte volatility and thermal stability. Hence the metabolites had to be made volatile through chemical derivatization, which added time and complexity to sample preparation (53-57). Through chemical derivatization, GC-MS was also able to detect hydrophilic metabolites such as organic acids, sugars and amino acids. It also involved the electron-impact ionisation analysis, which identified fragment peaks, provided structure information for known metabolites (4).

4. Liquid Chromatography Coupled to Mass Spectrometry (LC-MS)

LC-MS was an effective method for profiling hydrophobic secondary metabolites including alkaloids, flavonoids and phenylpropanoids as it offered high chromatographic performance for separation of these substances (4). LC-MS operated at lower temperatures, allowing for the analysis of heat-labile metabolites. LC was a versatile separation technique that could be used for targeted or non-targeted analysis of metabolites as it allowed for analyte recovery through fraction collection or concentration, which was more challenging than using GC separations. ESI was the most commonly used ionization technique for LC-MS, as it reduced ionization competition and increased the number of detectable analytes.LC-MS operated at lower temperatures, which enabled for the analysis of heat labile metabolites (56-58).

5. Capillary Electrophoresis Mass Spectrometry (CE-MS)

CE-MS was able to detect ionic metabolites such as amino acids, organic acids, nucleotides and sugar phosphates without utilizing chemical derivatization experiments, primarily those belonging to central metabolic pathways like glycolytic and tricarboxylic acid cycles (4). CE-MS was considered a powerful separation technique for charged metabolites, with superior separation efficiencies compared to LC. Capillary zone electrophoresis (CZE) was the major CE mode used for metabolite analysis due to its simplicity and lack of additives. Other CE modes, such as micellar electrokinetic chromatography (MEKC) or capillary electrochromatography (CEC), were also employed to achieve simultaneous separation of charged and neutral metabolites (57-60).

6. Fourier Transform Infrared (FTIR) Spectroscopy

Fourier transform mass spectrometry (FT/MS) instruments, such as FT-ICR and FTICR-MS, proven effective in metabolomics for comprehensive metabolite profiling, precise quantification and structural elucidation (61-64). These instruments offered precise mass measurements with exceptional resolving power, enabling efficient high-throughput metabolomics analyses (63, 64). Notably, FTICR-MS was highlighted for its suitability in high-throughput metabolomic investigations, being capable of profiling over 400 metabolites within 24 hours (64).

7. Near Infrared (NIR) Spectroscopy

Near-infrared (NIR) spectroscopy stood as a valuable method for metabolite analysis, offering benefits such as non-destructive sample examination and the capacity to analyze intact tissue samples (44). Its application extended to understanding, preventing, diagnosing and managing human diseases .When paired with other analytical methods such as mass spectrometry and chromatography, NIR spectroscopy facilitated thorough qualitative and quantitative metabolite analysis within intricate mixtures (65). Despite its lower sensitivity compared to mass spectrometry, NIR spectroscopy's notable reproducibility and quantitative capability made it an invaluable asset in the realm of metabolomics.

Metabolite changes during seed ageing and deterioration

Metabolites served as a potential marker as their diagnostic procedures for their detection could be developed with ease (66). The metabolites with biomarker potential played an important role in seed ageing detection by having a significant function in seeds (Table 2).

1. Fatty acid metabolism

Alterations in the fatty acid content of rice during storage were evident, characterized by a decline in total fat content (67) and rise in fatty acid values (68). As per report the changes in lipid profile, with an increase in malondialdehyde content along with a decrease in antioxidant enzyme activity all contribute to the loss of rice quality during storage (67). A significant element in the decline of grain quality was the lipase activity (69). The hydrolysis of glycerol phospholipids and glycerides increased the amount of free fatty acids, which in turn caused lipid oxidation, a significant spoiling event that occurred during rice storage. Stored rice samples showed higher linoleic acid levels, disrupting the linoleic acid metabolism pathway, resulting in various oxidation products (70). The degradation of phosphatidylcholine (PC) was considered a trigger for rice aging due to which rice developed rancid flavour (71).

As reported previously, assessed eight lipid subclasses in Ezhong and Liaoxing rice varieties over 540 days of storage (70). In Ezhong, Phosphatidylcholine (PC), Phosphatidylethanolamine (PE) and Phosphatidylglycerol (PG) decreased by 7.99 %, 11.4 % and 6.52 %, respectively, while in Liaoxing, these lipids dropped by 36.07 %, 39.32 % and 22.10 %.

Table 2. Metabolites with biomarker potential for seed aging detection in rice

Metabolite	Trend in Ageing	Function in Seed	Reference
Malondialdehyde (MDA)	Increases progressively with aging	Marker of lipid peroxidation, indicator of oxidative damage	(67)
Raffinose	Shows an initial increase, then declines.	Helps maintain membrane stability and storability	(33, 73)
γ-Aminobutyric acid (GABA)	Varies by stress level and variety	Acts as antioxidant and stress signal molecule	(33, 74, 75)
Linoleic acid	Initially accumulates, later oxidizes forming rancid compounds	Component of membrane lipids	(70, 72)
Glucose (Reducing Sugar)	Gradual increase due to starch breakdown	Byproduct of starch degradation	(33, 39, 69)

Saturated fatty acids (SFA) Monounsaturated fatty acids (MUFA) and increased in Ezhong by 15.55 %, 30.88 % and in Liaoxing by 9.30 %, 6.01 %. Polyunsaturated fatty acid (PUFA) levels remained stable in Ezhong but rose by 11.56 % in Liaoxing reflecting difference in storage impact between the varieties.

In the study, Brown rice had higher levels of petroleum ether extractable lipids (PEE-L) (22.5–28.2 mg/g) compared to milled rice (3.0-4.5 mg/g), whereas milled rice contained greater amounts of aqueous propan-1-ol extractable fatty acids (PWE-FA) (72). Storage at 37 °C led to reductions in oleic and linoleic acids in brown rice PWE-FA and linoleic acid in milled rice PEE-L, suggesting that PWE-L were more stable. Total lipid content showed minimal variation, ranging from 30.5-39.2 mg / g in brown rice and 11.4-12.1 mg / g in milled rice. (In a previous report, a reduction in fatty acids in brown rice was noted following 12 months of storage, accompanied by notable shifts in lipid metabolism (69). It was further emphasized the increase in fatty acid levels during prolonged storage, coupled with a decline in rice quality (39). These investigations collectively underscore the dynamic nature of fatty acid composition in rice seeds during storage.

2. Carbohydrates metabolism

The carbohydrates were involved in metabolic pathways like Pentose Phosphate Pathway (PPP), glycolysis, TCA cycle, starch & sucrose metabolism etc. Reducing sugars like glucose and fructose were observed to increase during storage. During storage, the enzymatic degradation eventually led to the increased contents of reducing sugars in the storage sensitive seeds. Among the seeds of BY and IIY during natural storage, the sugars like glucose, sucrose, cellobiose, glucopyranose, gentiobiose, kestose, erythritol, sorbitol, mannitol, gluconic acid, glycerol and glycerol-3-phophate were higher in the relative storage sensitive IIY seeds, while only the raffinose level were lower in IIY than in BY seeds after the 24 month natural storage period (33). Certain cultivars with lower levels of raffinose exhibited poorer storability. Raffinose was shown to improve sucrose's ability in retaining the dry condition of the membranes in liquid crystalline condition and inhibit its natural tendency to crystallize and lose its protective properties (73).

Changes in carbohydrate composition occurred in rice seeds during storage, including a reduction in total starch content, increased amylose content and decreased fat content (39). The storage of brown rice resulted in diminished level of carbohydrates, amino acids and fatty acids, along with increased levels of sugar alcohols, amines and aldehydes (69).

Two japonica, two indica and two indica-japonica hybrid varieties were assessed and the results shown that the amylose content increased by 9.63-11.65 % in japonica rice, 2.99-4.67 % in indica rice and 8.07-8.97 % in indica-japonica hybrids (39). Incontrast, fat content decreased by 60.00-65.00 %, 37.21-46.51 % and 41.67-42.42 %, respectively. After one year of storage, the raffinose levels decreased, although initially rose by 19.35-45.45 %, 7.02-10.77 % and 16.13-28.13 % after four months, before dropping to the lowest levels after a year.

3. Amino acids metabolism

Amino acids which have showed significant changes during storage include glycine, phenylalanine, proline, serine, tyrosine, GABA, glutamic acid etc. The altered amino acids were mainly involved in pathways like in glyoxylate and dicarboxylate metabolism, glycine, serine and threonine metabolism, butanoate metabolism, C5- branched dibasic acid metabolism (74). A comparative study between the rice varieties of IIY and BY revealed that,15 out of 18 amino acids in the storage sensitive IIY seeds which included valine, leucine, glutamine, tryptophan, lysine, phenylalanine, isoleucine, alanine, asparagine, tyrosine, glycine, GABA, serine, aspartic acid and glutamic acid- significantly decreased during the course of 24 month storage period, whereas methionine, glutamine and proline however, remained constant (33). GABA was associated with the antioxidant properties and reactive oxygen species (ROS) scavenging. The amount of free amino acids was higher at elevated temperatures compared to lower temperatures during storage (75).

In a study, it was reported that initially, the total free amino acid content was 219.8 mg /100 g dry weight (3). After 1 year, this decreased to 146.4 mg /100 g at 30 °C and 169.2 mg/100 g at 4 °C. After 3 years, the content dropped further to 53.1 mg /100 g in paddy rice stored at 4 °C and 58.1 mg /100 g in brown rice stored at the same temperature. Significant reductions were observed for individual amino acids like lysine, glutamic acid and serine, with greater losses at higher temperatures and prolonged storage. These changes highlighted the impact of storage conditions on amino acid stability in rice grains. Significant variations in amino acid levels, especially those involved in glutathione metabolism was observed between the two rice varieties (76). Glutathione (GSH) was identified as vital for reducing oxidative damage and activating metabolism-related enzymes. L-cysteine levels were upregulated in indica rice (JZ) and downregulated in japonica rice (NJ), suggesting that indica rice possessed a stronger defense mechanism against abiotic stress. Another report state that the primary aroma-active compounds and taste components of Jasmine rice, including free amino acids, altered during storage, potentially affecting the rice's flavor profile (75). Collectively, these studies highlighted the dynamic nature of amino acid composition in rice during storage.

Role of antioxidants during storage

Hydrogen peroxide (H_2O_2) was a persistent reactive oxygen species (ROS) which is known as a significant signalling molecule has the ability in causing oxidative damage linked to damage to cellular components (77). ROS served dual functions they acted as a crucial messengers that started cellular defences against biotic and abiotic stresses, while at higher concentrations, they also caused oxidative damage, leading to cell death (78). Prolonged seed storage resulted in gradual build-up of ROS thereby elevating the risk of oxidative damage. Increased ROS concentrations led to damage to proteins, DNA and phospholipids consequently resulting in reduction of seed viability and associated physiological changes. The regulation of ROS by enzymatic and non-enzymatic (antioxidant activities) mechanisms and DNA repair mechanisms in the embryo needed to be optimally maintained to preserve seed viability (79).

An antioxidant was a compound that, even when present in a smaller amount compared to an oxidizable

substance, could slow down or stop the oxidation of that substance (80, 81). Antioxidants could be enzymatic (superoxide dismutase, catalase, glutathione peroxidase) and non-enzymatic (glutathione, proteins like ferritin, transferrin, vitamin C, vitamin E, EDTA and low molecular weight scavengers, like uric acid, co-enzyme Q & lipoic acid) (82). They were also classified as water-soluble antioxidants like flavonoids, ascorbic acid, uric acid and glutathione, as well as lipid-soluble antioxidants such as carotenoids, tocopherols and ascorbylpalmitate/stearate.

The seed coat (Testa) which was brown in colour in most seeds provide protection to the developing embryos from oxidative damage as it contains phenolic compounds that acted as antioxidants. Major antioxidant enzymes included superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase and glutathione peroxidase (83).

Sugars helped in preserving the structural integrity of the proteins and membranes in dry conditions by forming a glassy state that inhibited deteriorative reactions (84-86). Changes in sugar levels during storage could trigger degenerative changes in seeds. Raffinose family oligosaccharides (RFOs) which were the most common oligosaccharides in higher plants contained D -glucose. The addition of activated galactose moieties provided by galactinol enables sucrose to be converted into raffinose (87). Raffinose helped protect sucrose by inhibiting lipid crystallisation & other deteriorative effects, thereby preserving membrane integrity (88, 89). One of the key enzymes of the raffinose family oligosaccharide (RFO) pathway was the Galactinol Synthase (66).

Vitamin E, a naturally occurring essential nutrient comprised of 8 tocochromanols which are divided into α, β, γ & δ tocopherols and α, β, γ & δ tocotrienols. Tocopherols are the micronutrients with the properties of antioxidants (90). Tocopherols and Tocotrienols were collectively referred to as tocochromanols -lipophilic antioxidants that accumulated mainly in seeds. Tocopherols were found in most dicot seeds and in the embryos of monocots whereas the tocotrienols were mostly restricted to the endosperm of monocot seeds and in certain dicots. In rice, tocotrienols accumulated in higher levels in the pericarp and endosperm. Structurally tocotrienols differ from tocopherols due to the presence of three trans-double bonds in their hydrocarbon tail (91, 92). The rice germ fraction, which made up 4.6 % of the seed, contained the highest concentration of tocopherols (480 Kg ha^{-1}) and tocotrienols (90 Kg ha^{-1}), comparable to that of the pericarp whereas 16.9 % of the seed contained lower tocopherols levels (38.1 Kg ha^{-1}) but high concentration of tocotrienols (90.3 Kg ha^{-1}) (83). Tocopherols and tocotrienols helped protect PUFAs from oxidation and subsequently contributing to the seed longevity, as lipid oxidation was considered a major factor that influencing it (93). A study reported that the tocopherols function in protecting the embryo from ROS attack during ageing (including accelerated ageing) and also during stress conditions by ensuring optimum germination while tocotrienols in the pericarp may assist in lowering the seed's metabolic activity during accelerated ageing (83). Tocopherols possessed antioxidative and free radical scavenging properties (94, 95)and aided in maintaining the structure and integrity of membranes (96).

Future Prospects

- Metabolomics involved the analysis of a wide range of metabolites including amino acids, lipids, nucleic acids, carbohydrates, amines, vitamins and secondary metabolites such as flavonoids, polyphenols, terpenoids, steroids and alkaloids (46).
- Metabolomics was applied in numerous areas including agriculture, medical science and useful in metabolite

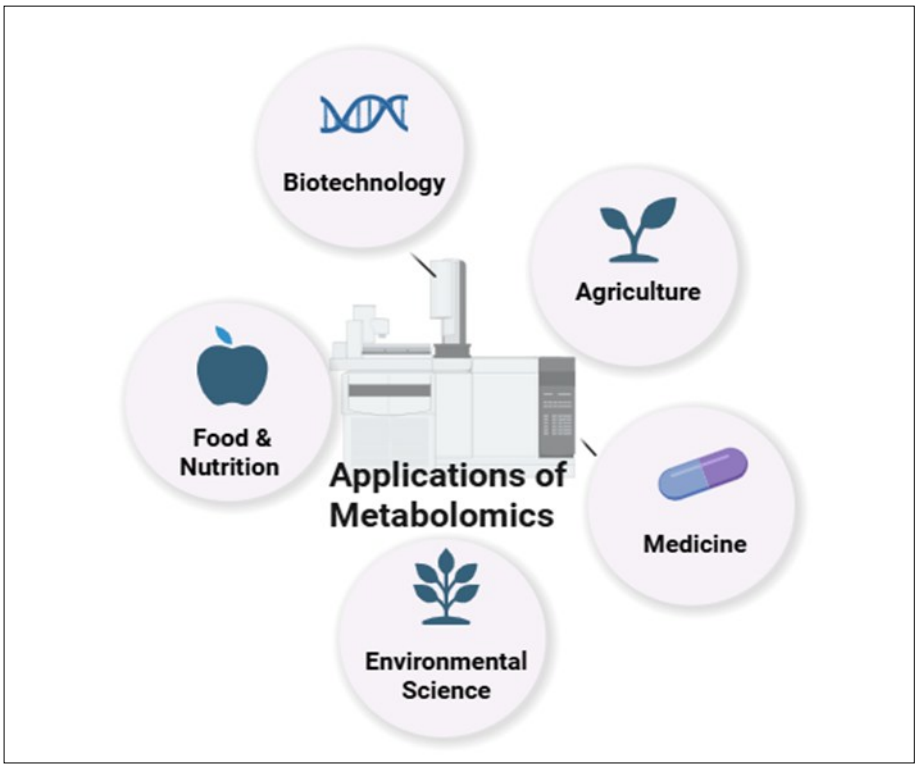

Fig. 3. Applications of metabolomics.

profiling of microbial and plant metabolites. It served as a valuable tool, as the metabolite profiling of plant metabolites helped in understanding the metabolic pathways present and how these were influenced by genetic and environmental factors. The applications of metabolomics in various areas were illustrated in Fig. 3.

◊ Plant metabolomics was an advancing field of research, characterized by large, chemically diverse metabolomes that are often under-represented in public database (97).

◊ In food and agriculture metabolites served as a powerful tool for crop quality improvement, analysis of metabolite composition and changes at various stages of plant growth and understanding metabolic responses to various environmental stresses. The metabolomics was a crucial tool for assessing the metabolite changes in agriculture and it also found applications in medicine, food industry (4).

◊ Annotating detected metabolites, however, remained a major challenge, with ongoing efforts to isolate and determine their structures.

◊ In spite of the challenges, integrating metabolomics with other methods such as phytochemical genomics showed potential to enhance rice grain quality and progress our knowledge of rice metabolism (98).

◊ Food shortages were a significant global issue exacerbated by population growth, which drove the need for innovative solutions in agriculture.

◊ Metabolomics, a promising advancement in biological research, offered potential to address these challenges by enhancing agricultural research.

◊ Utilizing high-throughput technologies, metabolomics enables the discovery and analysis of new bio products by examining microorganisms and their genetic, protein, RNA and metabolic components.

◊ Despite current limitations, such as inadequate processing tools, analytical skills and reference databases, metabolomics remains a valuable and evolving research field.

◊ Addressing these limitations could significantly improve its effectiveness in tackling critical issues such as climate change, crop stress responses, breeding and nutritional improvements in crops (99).

Conclusion

The study of rice seed metabolomics during storage was essential for understanding the biochemical changes that influences seed viability, vigor and overall crop performance. Storage conditions such as temperature, humidity and duration can significantly alter the seed's metabolic profile, including changes in amino acids, sugars, organic acids and lipids. These shifts impaired germination and led to quality deterioration, often indicated by increased lipid peroxidation. Metabolomics enables comprehensive profiling of these compounds, providing insights into the mechanisms of seed ageing and deterioration. It also supports the identification of biomarkers linked to seed viability, aiding in the selection of resilient rice varieties and the development of optimized storage protocols. Ultimately, metabolomics plays a vital role in preserving seed quality, improving storage practices and contributing to sustained rice production and food security.

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Authors' contributions

PS and RV performed the conceptualization. The original draft of the manuscript was written by PS and RV. Review and editing were carried out by VM, PB and RR. All authors have read and approved the final version of the manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

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