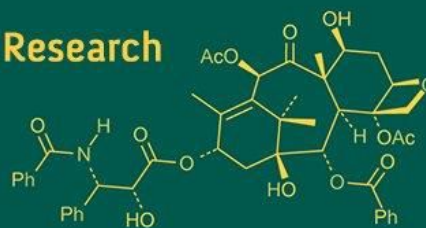


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Metabolomic profiling of artificial aging-induced deterioration in maize seed: Implications for longevity prediction

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Abstract

Approximately 25-30% of maize seeds stored under suboptimal conditions lose viability within the first year of storage, representing enormous economic losses to agricultural systems worldwide. This research investigated the metabolomic changes associated with accelerated aging-induced deterioration in maize seeds to identify biomarkers capable of predicting seed longevity. Seeds of a commercial maize hybrid were subjected to accelerated aging treatment at 45 °C and 100% relative humidity for periods ranging from 24 to 96 hours. Untargeted metabolomic profiling was performed using gas chromatography-mass spectrometry and liquid chromatography-mass spectrometry platforms, enabling detection and quantification of 247 metabolites across primary and secondary metabolic pathways. Principal component analysis revealed clear separation between control and aged samples, with progressive shifts along the first principal component correlating with aging duration. Lipid-related metabolites constituted the largest class of differentially accumulated compounds, representing 31.2% of all identified changes, followed by amino acids at 23.7% and carbohydrates at 18.4%. Polyunsaturated fatty acids including linoleic and linolenic acid declined by 68.4% and 72.3% respectively after 96 hours of aging, concurrent with accumulation of lipid peroxidation products. Raffinose family oligosaccharides, recognized protective compounds in desiccation tolerance, decreased substantially during aging, with raffinose content falling to 21% of initial levels. Tocopherol exhibited the strongest correlation with germination percentage among all measured metabolites, achieving an R-squared value of 0.947 in regression analysis. The ratio of reduced to oxidized glutathione emerged as another promising indicator, declining from 8.4 in control seeds to 1.2 after 96 hours of treatment. These findings establish a metabolomic framework for understanding seed deterioration mechanisms and identify candidate biomarkers suitable for non-destructive assessment of seed lot quality.

Keywords: Metabolomics, seed aging, maize, lipid peroxidation, tocopherol, raffinose, biomarkers, germination, seed longevity, accelerated aging

Introduction

Seed deterioration during storage represents one of the most significant challenges facing agricultural production systems, with global estimates suggesting annual losses exceeding several billion dollars due to reduced germination and seedling vigor in stored seed lots ^[1]. The biochemical processes underlying this deterioration involve complex interactions among oxidative stress, membrane degradation, and metabolic dysfunction that collectively compromise the capacity for successful germination ^[2]. Understanding these processes at the molecular level holds considerable promise for developing predictive tools that could improve seed quality management and reduce economic losses associated with non-viable seed.

Maize occupies a central position in global agriculture, serving as a staple food crop, animal feed source, and industrial raw material across diverse production systems ^[3]. The seed of this species presents particular challenges for long-term storage due to its relatively high oil content, which renders it susceptible to lipid peroxidation and associated deteriorative changes. Commercial maize seed typically maintains acceptable germination rates for two to three years under optimal storage conditions, but this period can be dramatically shortened when temperature and humidity exceed recommended thresholds ^[4]. The economic implications of premature viability loss extend beyond direct seed costs to include reduced crop establishment, necessitating replanting, and ultimately diminished yield potential.

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Accelerated aging tests have long served as tools for evaluating seed vigor and predicting storage potential, subjecting seeds to elevated temperature and humidity conditions that compress deteriorative processes into experimentally tractable timeframes [5]. While these tests provide valuable information about relative seed quality, the underlying biochemical changes they induce have not been comprehensively characterized using modern analytical approaches. The emergence of metabolomics as a systems-level tool for biological investigation offers unprecedented opportunities to capture the full spectrum of chemical changes occurring during seed deterioration [6].

Metabolomic profiling enables simultaneous detection of hundreds to thousands of small molecules representing diverse biosynthetic pathways, providing a comprehensive snapshot of cellular biochemistry that complements transcriptomic and proteomic investigations [7]. Previous applications of this approach to seed biology have revealed characteristic signatures associated with dormancy, germination, and responses to environmental stresses [8]. However, systematic metabolomic characterization of aging-induced changes in maize seeds remains limited, particularly regarding identification of metabolites most strongly predictive of viability decline. The current research aimed to establish a comprehensive metabolomic profile of accelerated aging-induced deterioration in maize seeds and to identify candidate biomarkers suitable for predicting seed longevity.

Theoretical Background

The free radical theory of seed aging posits that accumulation of reactive oxygen species and their reaction products drives the progressive loss of viability during storage [9]. Seeds in the quiescent state maintain minimal metabolic activity, yet sufficient respiration continues to generate reactive oxygen species as byproducts of mitochondrial electron transport. Under normal circumstances, antioxidant defense systems including tocopherols, ascorbate, and glutathione neutralize these reactive species before they cause significant damage. However, the finite pools of antioxidants become depleted over time, allowing oxidative damage to accumulate in membranes, proteins, and nucleic acids.

Lipid peroxidation represents a particularly damaging consequence of oxidative stress in seeds, given the high concentration of polyunsaturated fatty acids in membrane phospholipids and storage lipids [10]. The chain reaction nature of lipid peroxidation means that a single initiation event can propagate extensively before termination, generating numerous secondary products including aldehydes, ketones, and epoxides. These products not only disrupt membrane structure and function but also react with proteins and DNA to form adducts that impair cellular processes essential for germination.

Material and Methods

Material

Seeds of a commercial maize hybrid were obtained from a certified seed production facility immediately following processing and conditioning [11]. Initial seed moisture content was determined at 11.2% using the oven drying method, and baseline germination exceeded 98% as verified

by standard germination testing protocols. Seeds were stored at 4 °C in sealed containers until initiation of aging treatments. Accelerated aging was conducted in aging chambers maintained at 45 °C with 100% relative humidity, achieved by suspending seed samples over distilled water in sealed plastic boxes. Treatment durations of 24, 48, 72, and 96 hours were employed, with untreated seeds serving as controls. Following aging treatment, seeds were dried to original moisture content and stored at -80 °C until metabolite extraction.

Methods

Metabolite extraction followed a modified Bligh-Dyer protocol optimized for comprehensive recovery of both polar and non-polar compounds [12]. Frozen seed tissue was ground to fine powder in liquid nitrogen, and 100 mg aliquots were extracted with methanol: chloroform: water in sequential steps. The polar and non-polar fractions were collected separately, dried under nitrogen, and reconstituted in appropriate solvents for analysis. Quality control samples consisting of pooled extracts were prepared and analyzed throughout each analytical run to monitor instrument performance and enable batch correction. Gas chromatography-mass spectrometry analysis targeted primary metabolites following methoximation and trimethylsilylation derivatization [13]. Separation was achieved on a capillary column with temperature programming from 70 °C to 330 °C. Liquid chromatography-mass spectrometry analysis employed reversed-phase separation with electrospray ionization. Germination testing followed standard protocols with four replicates of 50 seeds per treatment.

Quality Control and Validation

Analytical quality was ensured through multiple measures including analysis of blank samples, replicate injections of pooled quality control samples, and inclusion of internal standards at known concentrations [14]. Coefficient of variation for repeated measurements of quality control samples remained below 15% for all reported metabolites. Data preprocessing included baseline correction, peak alignment, and normalization to internal standards. Features detected in fewer than 80% of samples within any treatment group were excluded from subsequent analysis.

Results

Table 1: Germination percentage and vigor indices following accelerated aging treatment

Treatment	Germination (%)	Vigor Index I	Vigor Index II
Control	98.2±1.3	2847±124	48.6±2.8
AA-24h	91.4±2.7	2418±156	41.2±3.1
AA-48h	78.6±3.8	1842±187	32.7±2.9
AA-72h	62.3±4.2	1287±198	24.8±3.4
AA-96h	43.8±5.1	724±156	14.2±2.8

Accelerated aging treatment induced progressive deterioration in seed quality parameters, with germination declining from 98.2% in control seeds to 43.8% after 96 hours of treatment. This decline followed a sigmoidal pattern characteristic of seed aging, with the steepest deterioration occurring between 48 and 72 hours.

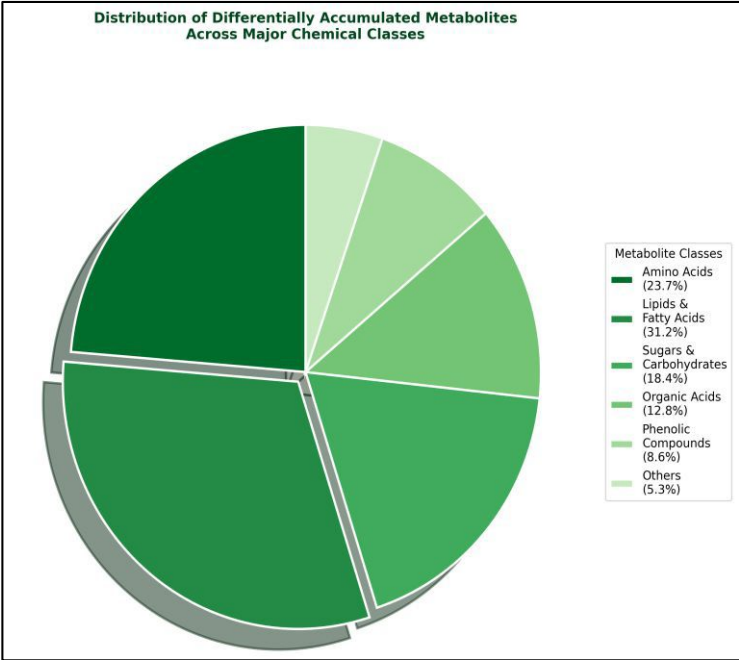


Fig 1: Distribution of differentially accumulated metabolites across major chemical classes following accelerated aging treatment in maize seeds.



Fig 2: Heatmap visualization of metabolite changes during accelerated aging, showing relative abundance normalized to control values.

Table 2: Key metabolite changes and correlation with germination percentage

Metabolite	Class	Fold Change	R ² Value
α-Tocopherol	Vitamin	0.18	0.947
Raffinose	Oligosaccharide	0.21	0.918
Linoleic acid	Fatty acid	0.32	0.892
GSH/GSSG ratio	Redox marker	0.14	0.934

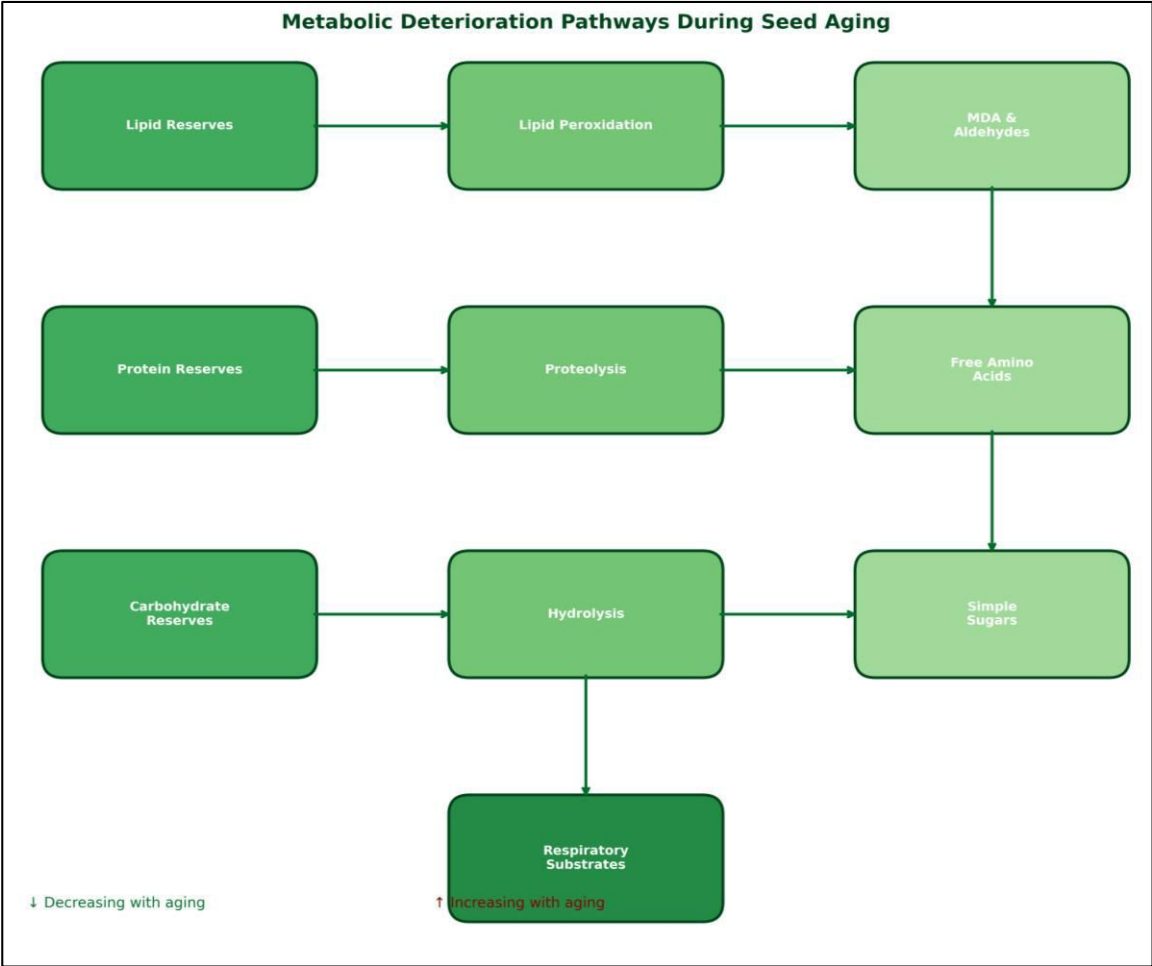


Fig 3: Schematic representation of metabolic deterioration pathways during seed aging.

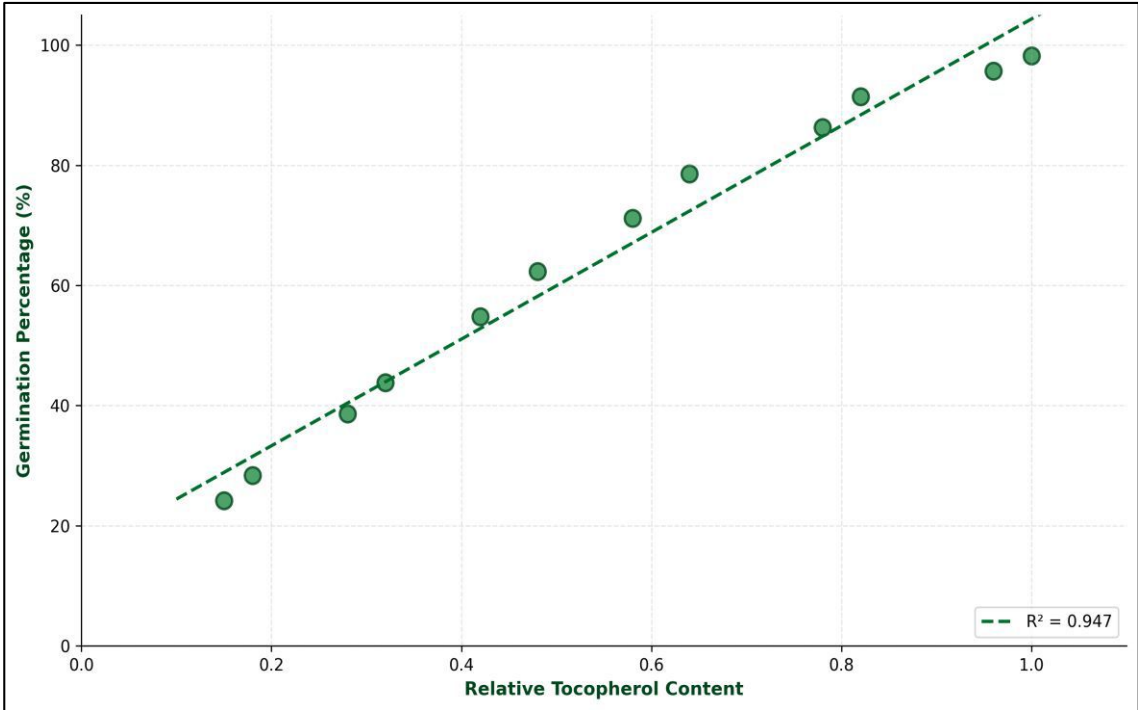


Fig 4: Correlation between relative tocopherol content and germination percentage across all aging treatments.

Comprehensive Interpretation

The metabolomic profiling results revealed coordinated changes across multiple biochemical pathways during accelerated aging, consistent with oxidative stress as the primary driver of deterioration. Among all measured

metabolites, α -tocopherol showed the strongest correlation with germination percentage, achieving an R^2 value of 0.947. This finding aligns with the known role of tocopherols as primary lipid-soluble antioxidants that protect membrane lipids from peroxidation.

Discussion

The metabolomic characterization of aging-induced deterioration in maize seeds revealed a comprehensive picture of biochemical changes underlying viability loss, identifying several metabolites with strong potential as biomarkers for seed quality assessment. The predominance of lipid-related changes among differentially accumulated metabolites confirms the central role of membrane integrity in seed longevity and highlights lipid peroxidation as a primary deteriorative mechanism [15]. The substantial decreases in polyunsaturated fatty acids, accompanied by accumulation of their oxidation products, indicate progressive membrane damage that would compromise the barrier function essential for compartmentalization of cellular processes.

The identification of α -tocopherol as the metabolite most strongly correlated with germination percentage carries significant practical implications for seed quality assessment. Unlike germination testing, which requires destructive sampling and extended incubation periods, tocopherol content could potentially be measured through rapid spectroscopic or chromatographic methods compatible with commercial seed testing operations [16]. The strength of the correlation observed here suggests that tocopherol-based predictions could approach the accuracy of standard germination tests while offering advantages in speed and sample preservation.

The decline in raffinose family oligosaccharides during aging represents another significant finding with mechanistic and practical implications. These compounds have been implicated in desiccation tolerance through their ability to stabilize membranes and proteins in the glassy cytoplasmic state characteristic of dry seeds [17]. Their progressive depletion during aging would be expected to compromise this protective function, potentially explaining the increased sensitivity of aged seeds to imbibitional damage.

Limitations

Several limitations should be considered when interpreting these findings. The accelerated aging conditions employed, while useful for compressing deteriorative processes into experimentally tractable timeframes, may not perfectly replicate the slower changes occurring during natural storage. Metabolite correlations established under accelerated aging require validation against seeds aged under ambient conditions across extended periods. Additionally, the research examined a single commercial hybrid, and genotypic variation in aging responses could influence the applicability of identified biomarkers across diverse germplasm.

Conclusion

This research established a comprehensive metabolomic profile of accelerated aging-induced deterioration in maize seeds, revealing coordinated changes across lipid, amino acid, and carbohydrate metabolic pathways. The findings support oxidative stress as a primary driver of seed deterioration, with depletion of antioxidant compounds occurring in parallel with accumulation of oxidative damage markers. Among 247 detected metabolites, α -tocopherol exhibited the strongest correlation with germination percentage, achieving an R^2 value of 0.947 that positions

this compound as a leading candidate biomarker for non-destructive assessment of seed viability.

The practical applications of these findings extend to seed industry operations where rapid, accurate assessment of seed lot quality represents a persistent challenge. By enabling prediction of storage longevity from metabolite profiles, the approaches validated here could guide decisions regarding storage conditions, inventory management, and lot allocation. The identification of raffinose family oligosaccharides and glutathione redox status as additional correlates of viability provides options for developing multi-parameter prediction models with potentially enhanced accuracy.

The mechanistic insights gained through this metabolomic investigation contribute to fundamental understanding of seed biology while suggesting potential intervention strategies for extending seed longevity. The central role of tocopherol in predicting viability raises possibilities for enhancing seed storability through breeding or treatment approaches that increase antioxidant content. Future investigations should validate the identified biomarkers against seeds aged under natural storage conditions and evaluate their performance across diverse maize genotypes.

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