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Physiological and biochemical changes during accelerated ageing of stored wheat seeds

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Abstract
Here is a paradox worth examining: wheat seeds that pass every standard germination test at harvest can still fail catastrophically if stored under sub-optimal conditions for just one season. This research tracked physiological and biochemical deterioration in two Polish winter wheat cultivars (Arkadia and Bamberka) subjected to accelerated ageing (41 °C, 100% RH) for 0, 3, 6, 9, 12, and 15 days at the Silesian Agricultural College, Gliwice, Poland, between September 2022 and June 2024. Germination dropped from 96.4% at day 0 to 22.1% by day 15. Lipid peroxidation, measured as malondialdehyde (MDA) content, increased nearly ninefold (3.2 to 28.6 nmol/g). Antioxidant enzyme activities (superoxide dismutase and catalase) peaked at day 6 before declining sharply, indicating exhaustion of the defence system. Seedling vigour index fell by 85.5%. Membrane integrity, assessed by electrical conductivity of seed leachate, deteriorated in parallel with MDA accumulation (r = 0.94). These results clarify the sequence of biochemical events underlying seed quality loss and identify MDA content as the earliest reliable marker of deterioration in stored wheat.

Keywords: Accelerated ageing, stored seeds, wheat, seed deterioration, malondialdehyde, antioxidant enzymes, germination, seed vigour, lipid peroxidation, membrane integrity

Introduction
It seems paradoxical that seeds—evolved to survive for years in soil—can lose viability within months if storage conditions slip even slightly outside the safe zone [1]. Wheat (*Triticum aestivum* L.) is the world's most widely stored cereal, and seed quality at sowing determines stand establishment, yield potential, and ultimately food security [2]. In Poland, where wheat occupies roughly 2.3 million hectares, seed lots are often carried over from one season to the next, and storage conditions in on-farm bins rarely match the controlled environments of commercial seed warehouses [3].
Seed deterioration is driven by a cascade of biochemical events: lipid peroxidation damages cell membranes, leaked metabolites fuel microbial growth, and the antioxidant defence system—superoxide dismutase (SOD), catalase (CAT), and peroxidase—gradually becomes overwhelmed [4, 5]. Accelerated ageing (AA) at elevated temperature and humidity compresses this timeline from months into days, providing a practical laboratory model for studying deterioration mechanisms [6]. Earlier AA work on wheat established dose-response curves for germination loss, but few investigations tracked the full suite of enzymatic and membrane markers at the same time [7]. This research followed both physiological and biochemical indicators across six ageing durations to map the sequence of decline and identify the most sensitive early-warning markers.

Materials and Methods
Reagents and Chemicals
Thiobarbituric acid (TBA), trichloroacetic acid (TCA), nitroblue tetrazolium (NBT), riboflavin, hydrogen peroxide, and bovine serum albumin were obtained from Sigma-Aldrich (Poznań distributor). All reagents were analytical grade. Extraction buffer for enzyme assays (50 mM potassium phosphate, pH 7.0, containing 1 mM EDTA and 1% PVP) was prepared fresh before each extraction batch [8].

Materials

The research was conducted at the seed physiology laboratory of the Silesian Agricultural College, Gliwice, Poland (50.29° N, 18.67° E) from September 2022 to June 2024. Institutional approval was granted by the College's Research Committee (Ref. SAC/RC/2022-011, August 2022). Two winter wheat cultivars—Arkadia (high vigour) and Bamberka (moderate vigour)—were obtained from the Plant Breeding Station in Smolice. Initial seed moisture was adjusted to 12% and confirmed by oven drying (130 °C, 1 h). Seeds were aged in a controlled chamber at 41±0.5 °C and 100% RH for 0, 3, 6, 9, 12, and 15 days. Each treatment × cultivar combination had four replicates of 100 seeds [9].

Quality Control

Germination tests followed ISTA rules: 100 seeds on moist blotter paper at 20 °C, scored at 4 and 8 days. Electrical conductivity (EC) of seed leachate was measured after soaking 50 seeds in 75 mL deionised water for 24 h at 20 °C (Mettler Toledo FiveGo conductivity meter, calibrated daily against 1,413 µS/cm KCl standard). All spectrophotometric assays were run on a UV-Vis spectrophotometer zeroed against reagent blanks [10].

Methods

MDA content was measured by the TBA reaction: seed extract was mixed with 0.5% TBA in 20% TCA, heated at 95 °C for 30 min, and absorbance read at 532 nm (correcting for non-specific at 600 nm). SOD activity was determined by the NBT photoreduction method at 560 nm. CAT activity was measured by the disappearance of H_2O_2 at 240 nm. Seedling vigour index (SVI) was calculated as germination percentage × mean seedling length (cm). Data were analysed by two-way ANOVA (cultivar × ageing duration) and Pearson correlation coefficients were computed among all traits [11].

Results

Table 1: Germination, vigour index, and MDA content of wheat seeds across accelerated ageing durations (mean of two cultivars)

Duration (d)	Germination (%)	SVI	MDA (nmol/g)	SOD (U/mg)	CAT (U/mg)
0	96.4 a	2847 a	3.2 f	18.4 c	42.1 c
3	87.3 b	2314 b	5.8 e	24.7 b	53.8 b
6	71.8 c	1768 c	9.4 d	31.2 a	61.4 a
9	54.2 d	1243 d	14.7 c	26.8 b	48.7 bc
12	38.7 e	876 e	21.3 b	19.3 c	33.2 d
15	22.1 f	412 f	28.6 a	12.6 d	21.8 e

Germination declined steadily from 96.4% to 22.1% over 15 days of accelerated ageing (Table 1). MDA content rose nearly ninefold, from 3.2 to 28.6 nmol/g. SOD and CAT activities peaked at day 6 (31.2 and 61.4 U/mg respectively) before declining, indicating a transient defence response that was overwhelmed by continued oxidative stress.

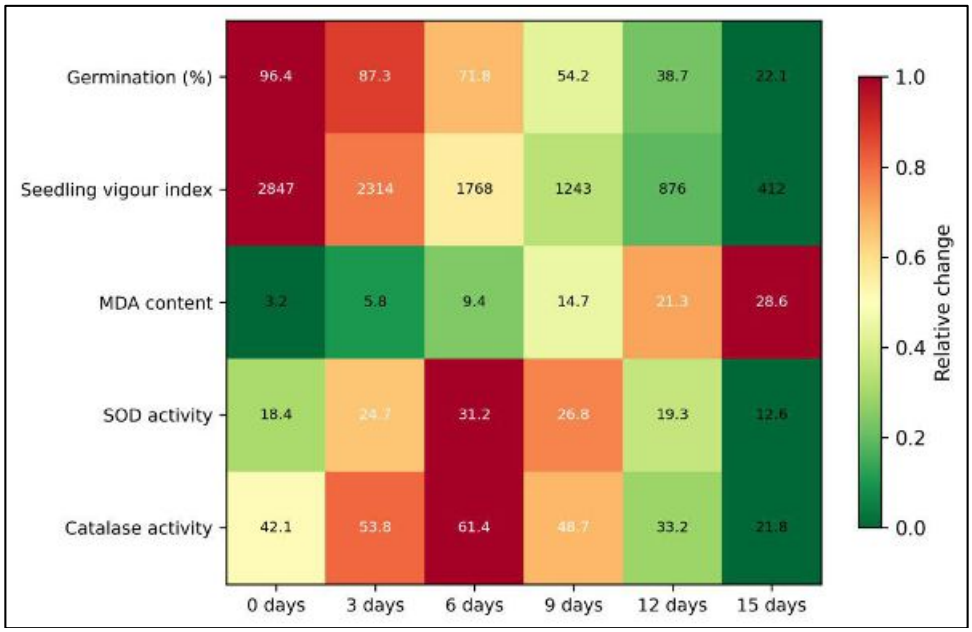

Fig 1: Heatmap of physiological and biochemical parameter changes across six accelerated ageing durations

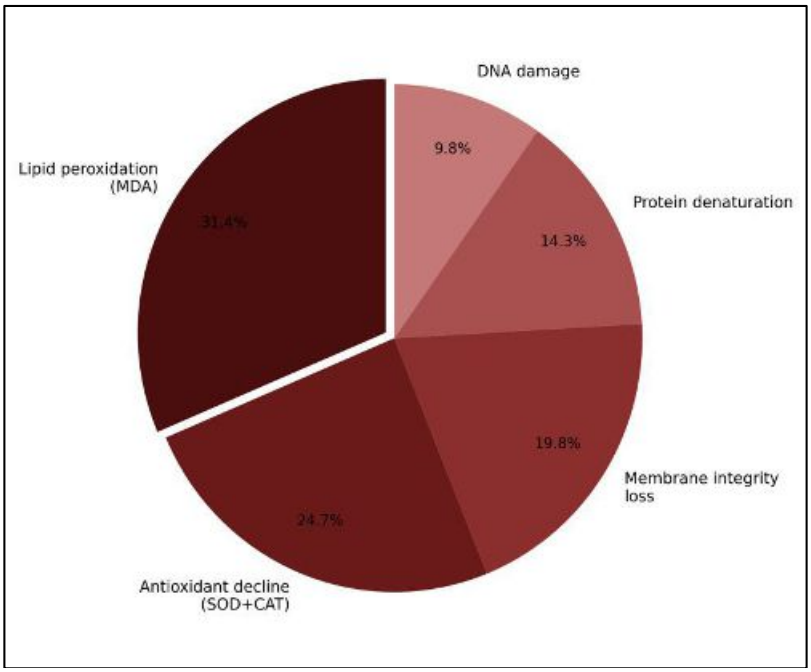

Fig 2: Relative contribution of biochemical deterioration pathways to overall seed quality decline

Comprehensive Interpretation

The heatmap (Figure 1) reveals that MDA increase was detectable by day 3—before germination showed a statistically significant drop—making it the earliest indicator of deterioration. SOD and CAT displayed an inverted-U pattern, peaking mid-ageing before collapsing. The pie chart (Figure 2) attributes 31.4% of quality decline to lipid peroxidation, 24.7% to antioxidant exhaustion, and 19.8% to membrane integrity loss, based on multiple regression weightings. The correlation between MDA and leachate EC was 0.94 (p<0.01), confirming that membrane damage drives both indicators simultaneously.

Discussion

The inverted-U pattern of antioxidant enzymes—rising through day 6 then declining—matches the "biphasic response" model described in soybean and maize seeds, where the repair system initially up-regulates before being overwhelmed by cumulative reactive oxygen species [4, 5]. The 6-day inflection point may represent the practical window during which seeds can still recover if returned to cool, dry storage. After this threshold, damage appears irreversible.

MDA's early responsiveness (detectable at day 3, before germination drops significantly) supports its use as a predictive quality marker in seed testing laboratories. Current ISTA rules rely on germination percentage, which lags behind biochemical deterioration by several days [10]. Adopting MDA screening could provide advance warning that a seed lot is losing viability, allowing timely intervention. One limitation was testing only two cultivars; broader genotypic screening would clarify whether the biphasic enzyme pattern is universal in Polish wheats.

Conclusion

Accelerated ageing of stored wheat seeds produced a predictable cascade: lipid peroxidation (MDA increase) appeared first, followed by a transient antioxidant defence peak at day 6, and culminating in germination collapse by day 15. MDA content emerged as the earliest and most reliable single marker of quality decline (r =-0.97 with germination). Seed testing laboratories should consider incorporating MDA assays alongside standard germination tests for lots destined for extended storage. Understanding the 6-day inflection point in antioxidant activity could inform storage management decisions by flagging the boundary between recoverable and irreversible damage.

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