

Behavioural Feeding Patterns of Storage Insect Pests and Their Biochemical Impact on Stored Cereals and Pulses

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Abstract

This study investigated the behavioural responses of four major stored-product insect pests—saw-toothed grain beetle (*Oryzaephilus surinamensis*), lesser grain borer (*Rhyzopertha dominica*), granary weevil (*Sitophilus granarius*) and red flour beetle (*Tribolium castaneum*)—to different grain substrates and evaluated the physicochemical and molecular changes induced by their infestation. Behavioural observations were conducted using buckwheat, wheat flour, lentil and rice as substrates at different times of the day. In addition, changes in moisture, ash, titratable acidity, starch, protein and lipid oxidation (TBARS) were analyzed, and multivariate statistical analysis and FTIR spectroscopy were applied to determine relationships between insect activity and grain quality. The results showed that insect activity varied significantly depending on grain type and time of day. Wheat flour and buckwheat exhibited the highest levels of insect activity, particularly for the granary weevil, whereas lentil was the least preferred substrate. Insect infestation caused significant physicochemical alterations in grains, including increased moisture content, ash levels and titratable acidity, as well as decreased starch content. Lipid oxidation levels increased in all infested samples, indicating enhanced oxidative deterioration. FTIR analysis revealed molecular changes in protein and carbohydrate structures, particularly in the amide I and starch vibration regions. Hierarchical clustering analysis indicated that samples were primarily grouped according to grain type rather than insect species. Overall, the findings demonstrate that insect feeding behaviour and infestation severity are strongly influenced by grain structure and biochemical composition, and that insect activity can significantly accelerate biochemical deterioration in stored grains. These results contribute to a better understanding of insect–grain interactions and highlight the importance of effective postharvest management strategies to reduce storage losses and maintain grain quality.

1. Introduction

Cereals, pulses and pseudo-cereals constitute the structural foundation of global food systems, supplying essential macronutrients and micronutrients to populations worldwide. These commodities play a central role in human nutrition due to their high content of carbohydrates, proteins, dietary fiber, vitamins and minerals, while also serving as key raw materials for a wide range of food products, including bread, pasta and cereal-based formulations (Serna-Saldivar & Perez-Carrillo, 2019; FAO, 2023). In recent years, pseudo-cereals such as buckwheat and quinoa have gained increasing attention due to their gluten-free nature, favorable amino acid composition and high levels of bioactive compounds with antioxidant capacity (Alvarez-Jubete et al., 2010; Repo-Carrasco-Valencia et al., 2010). This growing interest is further driven by global population expansion, urbanization and dietary diversification. According to the Food and Agriculture Organization, global production of cereals and pulses is projected to exceed 3.2 billion tons by 2025, accompanied by a steady increase in per capita consumption (FAO, 2023). Among these commodities, wheat, rice, lentil and buckwheat are of particular importance due to their nutritional value, functional properties and widespread consumption.

Despite their critical contribution to global food security, significant postharvest losses continue to affect these commodities. It is estimated that 10–30% of total production is lost after harvest, with considerably higher losses occurring in developing regions due to inadequate storage infrastructure, suboptimal environmental conditions and limited access to modern preservation technologies (Gustavsson et al., 2011; Affognon et al., 2015; Nath et al., 2024). These losses encompass both quantitative reductions and qualitative deterioration, including degradation of proteins, starch and lipids, as well as the development of off-flavors, discoloration and mycotoxin contamination (Kiaya, 2014; Tadesse, 2020). In developing countries, where smallholder farming systems dominate, storage losses can reach up to 50%, posing serious threats to food availability and economic sustainability (Affognon et al., 2015). On a global scale, hundreds of millions of tons of grain are lost annually during storage, highlighting the urgent need for improved postharvest management strategies (Mesterházy et al., 2020). Among the various factors contributing to postharvest losses, insect pests represent one of the most destructive biological agents affecting stored grains. Key species such as the confused flour beetle (*Tribolium castaneum*), the lesser grain borer (*Rhyzopertha dominica*), the sawtoothed grain beetle (*Oryzaephilus surinamensis*) and the granary weevil (*Sitophilus granarius*) are widely distributed and capable of causing extensive damage during storage (Hagstrum et al., 2013; Phillips & Throne, 2010). These pests induce both direct losses through feeding and indirect losses by facilitating microbial growth and accelerating biochemical degradation processes. Infestation by these species has been associated with significant reductions in protein and carbohydrate content, increased lipid oxidation and alterations in moisture dynamics, ultimately compromising both nutritional quality and safety (Hassan et al., 2021; Stathas et al., 2023). The confused flour beetle and lesser grain borer, in particular, are known for their ability to rapidly colonize stored products and intensify deterioration under favorable environmental conditions.

The susceptibility of grains to insect infestation is strongly influenced by their physicochemical properties. Factors such as kernel hardness, moisture content, phenolic composition and nutrient profile play critical roles in determining pest development, feeding behavior and reproductive success (Beckett et al., 2007; Tefera et al., 2011). In addition to these intrinsic properties, plant-derived volatile organic compounds (VOCs) have been identified as key mediators of host selection and insect orientation behavior. These compounds enable pests to detect and discriminate among potential food sources, thereby shaping infestation dynamics (Bruce et al., 2005; D'Isita et al., 2024). Variations in VOC composition, combined with differences in grain structure and biochemical content, significantly influence pest preference and performance. For example, previous studies have demonstrated that grain hardness, tannin content and seed coat integrity can act as physical and chemical barriers, reducing susceptibility to infestation and limiting pest development (Tefera et al., 2011; Mwangangi & Mutisya, 2013). The granary weevil, for instance, exhibits strong sensitivity to such grain characteristics, which directly affect its oviposition behavior and developmental cycle. Despite considerable progress in understanding insect–grain interactions, existing research remains fragmented and predominantly focused on isolated parameters. Most studies have evaluated infestation outcomes using macroscopic indicators such as weight loss, damage percentage and population growth (Hagstrum et al., 2013). In

contrast, comprehensive investigations into the molecular and biochemical alterations induced by insect activity remain limited. Moreover, the relationship between insect behavioral preferences and subsequent biochemical deterioration has not been systematically explored. This represents a critical knowledge gap, as early-stage behavioral decisions by pests may directly influence the extent and nature of grain degradation over time. The saw-toothed grain beetle and confused flour beetle, for example, exhibit distinct feeding strategies that may differentially affect grain composition, yet such interactions are rarely examined within an integrated framework.

The present study addresses this gap by adopting a comprehensive and interdisciplinary approach that integrates behavioral analysis with biochemical and spectroscopic characterization. Specifically, the behavioral preferences of the confused flour beetle, lesser grain borer, saw-toothed grain beetle and granary weevil were evaluated using a custom-designed multi-arm olfactometer system, allowing simultaneous assessment of insect orientation toward different grain substrates. Temporal variations in insect activity were also considered by conducting observations at multiple time points, thereby incorporating a dynamic dimension into behavioral analysis. In parallel, the long-term effects of insect infestation on grain composition were investigated using Fourier Transform Infrared (FTIR) spectroscopy alongside conventional biochemical analyses, including moisture content, ash content, titratable acidity, starch, protein and lipid oxidation (TBARS). The novelty of this study lies in its holistic integration of behavioral ecology with biochemical and spectroscopic analyses, providing a mechanistic understanding of pest-induced deterioration processes. By directly linking insect preference patterns with subsequent compositional changes in grains, this research advances beyond conventional descriptive approaches and offers new insights into the progression of postharvest losses. Furthermore, the application of FTIR spectroscopy as a rapid and non-destructive analytical tool introduces methodological innovation, enabling the detection of subtle molecular alterations associated with insect activity. From a broader perspective, the findings of this study are expected to contribute to the development of predictive frameworks for assessing grain susceptibility and deterioration risk based on insect behavior and grain characteristics. Such insights have important implications for improving storage practices, optimizing pest management strategies and enhancing the sustainability of postharvest systems. Ultimately, this work supports global efforts to reduce food losses and strengthen food security by advancing the scientific understanding of insect–grain interactions.

2. Materials and Methods

2.1. Materials

2.1.1. Insect Species

This study utilized four stored-product insect species: *T. castaneum* (red flour beetle), *S. granarius* (granary weevil), *R. dominica* (lesser grain borer) and *O. surinamensis* (saw-toothed grain beetle). These species were selected due to their ecological and behavioral diversity in grain infestation. All insects were provided by the Department of Plant Protection at Kahramanmaraş Sütçü İmam University and

reared under controlled conditions of $28 \pm 2^\circ\text{C}$ and $65 \pm 5\%$ relative humidity in a dedicated insect growth chamber. These conditions mimicked typical grain storage environments. Each species was maintained in isolated colonies and only healthy, adult individuals of uniform age were selected for experimentation. These insects were used both for behavioral assays and to evaluate their long-term biochemical impacts on different cereal-based substrates.

2.1.2. Grain Samples

The plant-derived food materials used in the experiments were lentils (*Lens culinaris*), buckwheat (*Fagopyrum esculentum*), rice (*Oryza sativa*) and wheat flour (*Triticum aestivum*). All samples were obtained from a local retail store in Gaziantep, Turkey, using the same supplier and brand to minimize variability. Upon acquisition, the grains were stored in sterile, dry and aerated containers at room temperature until use. Prior to experimental procedures, each sample was visually inspected to ensure the absence of pre-existing insect infestation, mold, or physical damage. These samples served both as substrates in the behavioral tests and as experimental material for post-infestation chemical analyses.

2.2. Experimental Design and Setup

Insect preference among the four grain types was assessed using a custom-designed olfactometer (Figure 1). The apparatus consisted of a central release chamber connected to eight transparent arms extending radially. Each grain type was presented in duplicate arms to reduce positional bias, allowing simultaneous access to all options. Behavioral observations were conducted during three specific time intervals: morning, midday and evening, in order to detect potential diurnal variation. Environmental parameters such as temperature ($28 \pm 2^\circ\text{C}$), relative humidity ($65 \pm 5\%$) and light intensity were held constant throughout all trials. In each test, approximately 10 g of a given grain type was placed at the end of each respective arm. Insects were released in the central zone and their movement into the arms was recorded after 2 hours. Each species was tested in separate, sanitized olfactometer units to eliminate cross-contamination or chemical trace effects. Individuals remaining in the central chamber were excluded from the preference analysis.

2.3. Long-Term Exposure and Post-Exposure Sample Processing

Following behavioral trials, insects were exposed to 100 g of each grain type in separate containers for six weeks under the same controlled environmental conditions. This setup simulated extended storage conditions and allowed for interaction between insects and grain over time. Each grain–insect combination was replicated three times using adult beetles only. At the end of the exposure period, samples were sieved to remove insects, larvae, eggs, excreta and other debris. The cleaned grains were finely ground to pass through a 0.5 mm sieve. The homogenized powders were stored in airtight containers at 4°C until biochemical analyses were performed.

2.4. Analytical Methods

2.4.1. Moisture Content

Moisture content was determined following AOAC Method 925.10 using a gravimetric technique. Approximately 2 g of ground sample was placed in aluminum crucibles and dried in a hot-air oven at $130\pm 5^{\circ}\text{C}$ until a constant weight was achieved. After cooling in a desiccator, the weight loss was used to calculate the moisture percentage.

2.4.2. Ash Content

Total mineral content (ash) was determined using AOAC Method 923.03. Between 5 g of each powdered sample was incinerated in porcelain crucibles at 550°C for 6–8 hours in a muffle furnace until a white, carbon-free ash was obtained. After cooling in a desiccator, crucibles were reweighed and ash content was expressed as a percentage of the initial weight.

2.4.3. Titratable Acidity

Titratable acidity was measured by extracting 10 g of ground sample with 50 mL of 67% ethanol under constant shaking for 15 minutes. The filtrate (Whatman No.1) was titrated using 0.1 N NaOH and phenolphthalein as an indicator. The appearance of a persistent pink color indicated the endpoint. The acidity level was calculated as equation below (1):

$$\text{TA, \% HC} = \frac{(\text{Normality of NaOH (C, N)} \times \text{Volume of NaOH (V, mL)})}{\text{Weight of Sample (m, g)}} \quad (1)$$

where C is the normality of NaOH (N), V is the volume of NaOH used (mL) and m is the mass of the sample (g).

2.4.4. Starch Content

Starch content was analyzed via acid hydrolysis and polarimetry. A 1.25 g sample was hydrolyzed with 12.5 mL of 1% HCl at 100°C for 15 minutes, cooled and diluted with 7.5 mL distilled water. Then, 2.5 mL of 4% phosphotungstic acid was added and the final volume was adjusted to 25 mL. After filtration, optical rotation was measured using a polarimeter. Starch concentration was calculated using the formula (2):

$$\% \text{Starch} = \left(\frac{V_{ts} \times \alpha_{20}}{(\alpha_{20}^d \times L_p \times W_s)} \right) \times 100 \quad (2)$$

where V_{ts} = volume of test solution, α_{20} = observed rotation, α_{20}^d = specific rotation constant (182.7), L_p = tube length in dm, W_s = sample weight.

2.4.5. Protein Content

Protein concentration was determined by the Kjeldahl method according to AOAC Method 920.87. One gram of sample was digested with 25 mL concentrated H_2SO_4 and 10 g of catalyst (potassium sulfate and copper sulfate) at 420°C. The resulting ammonia was distilled and titrated with 0.2 N HCl. The percentage of nitrogen was multiplied by a conversion factor of 5.7 to estimate total protein content (Equation 3):

$$\text{Protein Content}(\%, \text{wb}) = \frac{(V_2 - V_1) \times \text{Normality} \times 14 \times \text{Factor}}{\text{Sample Weight}} \quad (3)$$

Where: V_1 was consumption of 0.2 N HCl for blank (mL), V_2 was consumption of 0.2 N HCl for the sample (mL), normality was true normality of the HCl solution and the factor value were 5.70 for grain and grain products (AOAC International, 1995).

2.4.6. TBARS (Thiobarbituric Acid Reactive Substances)

Lipid oxidation was assessed using the TBARS assay. A 2 g sample was extracted three times with 10 mL of 0.4% perchloric acid. The supernatant (centrifuged at 1790×g for 5 minutes) was mixed with 5 mL

of TBA reagent and heated in a boiling water bath for 35 minutes. After cooling, absorbance was measured at 538 nm using a UV-vis spectrophotometer. Malondialdehyde (MDA) equivalents were calculated from a standard curve and reported as mg MDA/kg of sample (Bozkurt, 2009).

2.4.7. FTIR Spectroscopy

Fourier Transform Infrared (FTIR) spectroscopy was performed to detect structural changes in macromolecules. About 2–3 mg of each ground sample was mixed with dry KBr and pressed into thin transparent pellets. Spectra were recorded in the 4000–400 cm^{-1} range at 4 cm^{-1} resolution. Specific absorption bands corresponding to lipids (C–H stretching), proteins (amide I and II) and carbohydrates (C–O stretching) were analyzed. This technique enabled the identification of biochemical alterations at the functional group level.

2.5. Behavioral Observations and Insect Counting

Insect entry into each arm of the olfactometer was recorded visually during each time point (morning, afternoon, evening). No automated or invasive methods were used to avoid disturbing natural movement. Only individuals that fully entered the test arms were counted; those remaining in the center were excluded from the statistical analysis. The time-of-day effect on insect preference was evaluated using consistent observation protocols.

2.6. Statistical Analysis

A multivariate analysis of variance (MANOVA) was employed to evaluate the impact of insect species and grain type on behavioral choices and biochemical parameters. Significance was set at $p \leq 0.05$. Post-hoc comparisons were made using Tukey's HSD test. Additionally, multiple regression analyses were used to determine how grain type influenced each biochemical attribute, statistically analyzed using IBM SPSS Statistics 25.0 (IBM Corp., USA). Correspondence Analysis (CA) was performed to explore the associations among insect species, periods of the day, and grain types using R statistical software (R version 4.3.2, R Core Team, Austria). Furthermore, hierarchical clustering analysis was conducted to classify insect-infested grain samples based on their physicochemical properties, and dendrograms were generated.

3. Results and discussion

This study investigated the activity patterns of four major stored-product insect species—saw-toothed grain beetle, lesser grain borer, granary weevil and red flour beetle—on different grain substrates and at different times of the day. In addition to behavioral observations, the effects of insect infestation on the physicochemical properties of buckwheat, wheat flour, lentil and rice were evaluated by analyzing moisture, ash, titratable acidity, starch, protein and TBARS values. Multivariate statistical analyses and FTIR spectroscopy were also applied to determine the relationships between insect activity, grain substrates and infestation-induced molecular changes.

The activity of stored-product insects varied significantly depending on both the food substrate and the time of day (Table 1). In general, higher insect responses were observed on buckwheat and wheat flour, whereas lentil was the least preferred substrate for most species. The saw-toothed grain beetle exhibited moderate activity across most substrates, with the highest response recorded on buckwheat at midday (6.50 ± 0.71) and the lowest on lentil in the evening (0.50 ± 0.71). Activity on rice remained relatively stable throughout the observation periods, suggesting that this insect species is capable of exploiting rice substrates but without strong temporal fluctuations. Similarly, the lesser grain borer demonstrated its highest activity on buckwheat during midday (6.00 ± 0.00) and evening (5.00 ± 0.00), whereas almost no activity was observed on lentil in the morning. Among the tested insect species, the granary weevil showed the highest overall activity, particularly on wheat flour where values ranged from 11.50 ± 0.71 to 14.50 ± 0.71 throughout the day. This clearly indicates a strong preference of this species for finely milled substrates. In contrast, the red flour beetle exhibited comparatively lower activity levels across most substrates, although its response increased on flour during the evening period (6.50 ± 0.71), suggesting that environmental conditions or feeding opportunities may influence its activity rhythm. In the correspondence analysis (Fig. 2), revealed clear associations between insect species, time of day and grain types. Rice was positioned close to OS-M and OS-N, indicating that the saw-toothed beetle was more strongly associated with rice during the morning and midday periods. Flour appeared near SG-M, SG-N and SG-E, suggesting that the Granary weevil showed a consistent association with flour throughout the day. Buckwheat was located closer to RD-M and RD-N, indicating that the Lesser grain beetle was relatively more related to buckwheat, particularly during the morning and midday. In contrast, lentil was positioned separately in the upper part of the plot, suggesting a weaker or less specific association with the insect–time combinations. Points related to TC (TC-M, TC-N and TC-E) were more dispersed in the ordination space, indicating that *Tribolium castaneum* did not show a strong preference for a single grain type and its association varied depending on the time of day. These results indicated that substrate composition and accessibility strongly influence insect feeding and movement behavior. Finely milled substrates such as flour are generally easier for insects to penetrate and exploit compared with intact grains, which may explain the strong association between granary weevil and wheat flour observed in this study. Previous studies have also reported that insect feeding preferences are closely related to grain hardness, particle size and nutrient availability (Hagstrum and Subramanyam, 2009). In addition, temporal differences in insect activity observed in the present study may reflect circadian behavioral rhythms commonly reported in stored-product insects. Campbell et al. (2016) reported that environmental conditions such as temperature and humidity can significantly influence locomotor activity and feeding behavior in storage pests. According to the results presented in Fig. 2, the saw-toothed grain beetle showed moderate activity on buckwheat throughout the day. In contrast, its activity on wheat flour and lentil remained lower. Rice exhibited moderate levels of activity during morning and evening periods. The lesser grain borer showed moderate activity on buckwheat but relatively low activity on wheat flour and lentil. The granary weevil displayed the highest activity on wheat flour across all observation periods, whereas the red flour beetle exhibited relatively low to moderate activity across most substrates.

Table 1
Effect of food type and time of day on the activity/response of selected stored-product insects.

Species of Stored-Grain Insects	Observation Period	Tested Food Substrates			
		Buckwheat	Flour	Lentil	Rice
Saw-toothed grain beetle	Morning	4.50 ± 0.71 ^{Bc}	1.50 ± 0.71 ^{Bd}	1.00 ± 0.00 ^{Ba}	4.00 ± 0.00 ^{Bb}
	Midday	6.50 ± 0.71 ^{Bc}	2.50 ± 0.71 ^{Bd}	1.50 ± 0.71 ^{Ba}	4.50 ± 0.71 ^{Bb}
	Evening	5.00 ± 1.41 ^{Bc}	3.50 ± 0.71 ^{Bd}	0.50 ± 0.71 ^{Ba}	5.00 ± 0.00 ^{Bb}
Lesser grain borer	Morning	3.50 ± 0.71 ^{ABc}	1.50 ± 0.71 ^{ABd}	0.00 ± 0.00 ^{Aba}	2.00 ± 1.41 ^{ABb}
	Midday	6.00 ± 0.00 ^{ABc}	2.00 ± 0.00 ^{ABd}	0.50 ± 0.71 ^{Aba}	3.00 ± 1.41 ^{ABb}
	Evening	5.00 ± 0.00 ^{ABc}	3.50 ± 0.71 ^{ABd}	1.50 ± 0.71 ^{Aba}	4.50 ± 0.71 ^{ABb}
Granary weevil	Morning	5.50 ± 0.71 ^{Cc}	12.50 ± 0.71 ^{Cd}	2.50 ± 0.71 ^{Ca}	3.00 ± 1.41 ^{Cb}
	Midday	5.00 ± 1.41 ^{Cc}	11.50 ± 0.71 ^{Cd}	2.50 ± 0.71 ^{Ca}	3.00 ± 0.00 ^{Cb}
	Evening	4.00 ± 1.41 ^{Cc}	14.50 ± 0.71 ^{Cd}	2.50 ± 0.71 ^{Ca}	2.50 ± 2.12 ^{Cb}
Red flour beetle	Morning	2.00 ± 0.00 ^{Ac}	3.50 ± 0.71 ^{Ad}	0.00 ± 0.00 ^{Aa}	0.50 ± 0.71 ^{Ab}
	Midday	3.00 ± 0.00 ^{Ac}	2.50 ± 0.71 ^{Ad}	0.50 ± 0.71 ^{Aa}	2.00 ± 0.00 ^{Ab}
	Evening	4.50 ± 0.71 ^{Ac}	6.50 ± 0.71 ^{Ad}	0.50 ± 0.71 ^{Aa}	1.50 ± 0.71 ^{Ab}
Values are presented as mean ± standard deviation (SD). Means followed by different uppercase letters within the same column indicate significant differences among insect species, while different lowercase letters within the same row indicate significant differences among food materials according to Tukey's HSD test (P < 0.05).					

Infestation by these insects caused substantial changes in the physicochemical properties of the grains (Table 2; Fig. 3). Moisture content increased significantly in grains exposed to insects compared with the control group. For instance, wheat flour moisture increased from 8.45 ± 0.28 g/100 g in the control to

12.38 ± 0.08 g/100 g following infestation by the red flour beetle. Similarly, buckwheat moisture content increased from 8.36 ± 0.57 g/100 g to 9.83 ± 0.66 g/100 g after infestation by the saw-toothed grain beetle. This increase in moisture is most likely associated with the metabolic activity of insects, as respiration processes release water vapor that accumulates within the grain environment. Additionally, insect movement and feeding can disrupt grain structures, facilitating moisture absorption and creating conditions favorable for microbial growth and biochemical deterioration. Ash content, which represents the mineral fraction of the grains, remained highest in lentil samples, reaching up to 3.32 ± 0.13 g/100 g. Slight but consistent increases were also observed in buckwheat and lentil samples following insect infestation. For example, ash content in buckwheat increased to 3.26 ± 0.10 g/100 g in samples infested by the red flour beetle. These increases may be associated with the accumulation of insect fragments, excreta (frass) and metabolic residues within the grain matrix. Such contamination can alter the mineral profile of stored grains and contribute to quality deterioration. Titratable acidity increased in most infested samples, suggesting intensified biochemical reactions within the grain substrates. Lentil samples showed an increase from 0.17 ± 0.03 mL HCl/100 mL in the control group to 0.26 ± 0.03 mL HCl/100 mL after infestation by the saw-toothed grain beetle and lesser grain borer. This increase in acidity may indicate enzymatic degradation of carbohydrates and proteins as well as microbial fermentation processes promoted by insect damage. Insect feeding often breaks the protective structures of grains, exposing internal nutrients to microbial colonization and accelerating spoilage reactions. Starch content declined significantly in all grain types following insect infestation. Buckwheat starch decreased from 57.76 ± 1.90 g/100 g to 51.11 ± 0.88 g/100 g under infestation by the saw-toothed grain beetle, while wheat flour starch decreased from 71.98 ± 0.89 g/100 g to 62.16 ± 1.27 g/100 g in samples exposed to the red flour beetle. These reductions are most likely caused by direct consumption by insects as well as enzymatic hydrolysis of starch molecules into simpler sugars that can be metabolized more easily by insect digestive systems. Such degradation not only reduces the nutritional quality of grains but can also influence their functional properties during food processing. Protein content showed more complex changes depending on the grain type and insect species. In buckwheat and wheat flour samples, protein levels decreased considerably after infestation. For example, buckwheat protein decreased from 15.26 ± 0.69 g/100 g to 7.79 ± 0.12 g/100 g following infestation by the lesser grain borer. In contrast, lentil samples showed relatively stable or slightly increased protein levels, reaching 26.77 ± 0.75 g/100 g in samples infested by the red flour beetle. This apparent increase may be explained by a concentration effect resulting from starch degradation or selective feeding behavior targeting carbohydrate-rich components of the grain matrix. Lipid oxidation, evaluated through TBARS values, increased consistently in infested grain samples. For example, TBARS values in buckwheat increased from 0.05 ± 0.00 mg MDA/kg in the control to 0.08 ± 0.00 mg MDA/kg following infestation by the red flour beetle. Increased TBARS values indicate enhanced lipid peroxidation processes, which can negatively affect both the nutritional value and storage stability of grains. Lipid oxidation products may also contribute to undesirable flavors and odors, further reducing the quality of stored grain products. Infestation by these insects also caused pronounced changes in the physicochemical properties of the grains. Moisture content increased markedly in grains exposed to insects compared with the control group. For example, wheat flour moisture rose from 8.45 g/100 g in

the control to 12.38 g/100 g after infestation by the red flour beetle, while buckwheat moisture content increased following infestation by the saw-toothed grain beetle. This increase can be attributed to insect respiration and metabolic activity, which release water vapor within the grain mass. Similar increases in grain moisture during insect infestation have been reported in previous studies and are known to promote microbial growth and accelerate grain deterioration (Phillips & Throne, 2010; Fleurat-Lessard, 2017). Ash content remained highest in lentil samples and showed slight increases in some infested samples. These increases may result from the accumulation of insect fragments, frass and metabolic residues within the grain matrix. Hubert et al. (2018) reported that contamination by stored-product insects can significantly alter the mineral composition and hygienic quality of stored grains due to the presence of insect debris. Titratable acidity also increased in most infested samples, indicating intensified biochemical reactions within the grains. Lentil samples showed an increase in acidity following infestation by both the saw-toothed grain beetle and the lesser grain borer. Such increases may reflect enzymatic degradation processes and microbial fermentation promoted by insect damage (Fleurat-Lessard, 2017). Starch content decreased significantly across all grains following infestation. Buckwheat starch declined from 57.76 to 51.11 g/100 g following infestation by the saw-toothed grain beetle, while wheat flour starch decreased considerably in samples exposed to the red flour beetle. This reduction likely results from direct consumption by insects as well as enzymatic hydrolysis of starch molecules into simpler sugars. Digestive enzymes such as amylases enable insects to efficiently utilize carbohydrate reserves as metabolic energy sources (Campbell et al., 2016). Protein content showed more complex responses. In buckwheat and wheat flour samples, protein levels decreased after infestation, whereas lentil protein content remained relatively stable or slightly increased. This apparent increase may reflect a concentration effect caused by starch degradation or selective insect feeding on carbohydrate-rich fractions. Lipid oxidation, evaluated through TBARS values, increased consistently in infested grains. Elevated TBARS values indicate intensified lipid peroxidation processes that negatively affect both nutritional quality and storage stability. Oxidative deterioration of lipids during insect infestation has been previously reported and is often associated with insect metabolic activity and increased oxygen exposure in damaged grain tissues (Hubert et al., 2018).

Table 2

Impact of infestation by insect species (saw-toothed grain beetle, lesser grain borer, granary weevil and red flour beetle) on chemical composition (moisture content, ash, titratable acidity, starch, protein and lipid oxidation measured by TBARS) of various grains (buckwheat, wheat flour, lentil and rice), compared control group

Groups	Grain	Moisture Content (wb, g/100 g sample)	Ash Content (wb, g/100 g sample)	Titratable Acidity (mL HCl/100 mL)	Starch Content (wb, g/100 g sample)	Protein Content (wb, g/100 g sample)	TBARS (mg MDA/kg)
Control Group	Buckwheat	8.36 ± 0.57 ^{Ab}	2.44 ± 0.10 ^{A^{Bb}}	0.16 ± 0.02 ^{A^{ab}}	57.76 ± 1.90 ^{Ac}	15.26 ± 0.69 ^{Ac}	0.05 ± 0.00 ^{Ab}
	Flour	8.45 ± 0.28 ^{Ab}	1.01 ± 0.27 ^{A^{Ba}}	0.12 ± 0.01 ^{A^a}	71.98 ± 0.89 ^{A^a}	9.30 ± 1.06 ^{A^a}	0.01 ± 0.00 ^{A^a}
	Lentil	7.63 ± 1.52 ^{Ab}	3.32 ± 0.13 ^{A^{Bb}}	0.17 ± 0.03 ^{A^{bc}}	40.37 ± 1.88 ^{Ab}	27.41 ± 1.70 ^{Ad}	0.05 ± 0.00 ^{Ac}
	Rice	4.63 ± 0.34 ^{A^a}	0.95 ± 0.04 ^{A^{Ba}}	0.15 ± 0.04 ^{Ac}	81.66 ± 3.13 ^{A^a}	10.77 ± 1.17 ^{Ab}	0.02 ± 0.00 ^{A^a}
Saw-toothed grain beetle	Buckwheat	9.83 ± 0.66 ^{B^b}	2.89 ± 0.42 ^{B^b}	0.20 ± 0.03 ^{B^{ab}}	51.11 ± 0.88 ^{B^c}	9.37 ± 0.62 ^{B^{Cc}}	0.08 ± 0.00 ^{B^{Cb}}
	Flour	8.84 ± 2.54 ^{B^b}	0.80 ± 0.28 ^{B^a}	0.15 ± 0.04 ^{B^a}	68.58 ± 0.81 ^{B^a}	7.18 ± 0.25 ^{B^{Ca}}	0.06 ± 0.00 ^{B^{Ca}}
	Lentil	8.62 ± 0.41 ^{B^b}	3.07 ± 0.07 ^{B^b}	0.26 ± 0.03 ^{B^{bc}}	38.83 ± 0.65 ^{B^b}	25.46 ± 1.10 ^{B^{Cd}}	0.05 ± 0.00 ^{B^{Cc}}
	Rice	8.77 ± 0.33 ^{B^a}	0.99 ± 0.02 ^{B^a}	0.32 ± 0.05 ^{B^c}	66.89 ± 0.09 ^{B^a}	9.65 ± 0.78 ^{B^{Cb}}	0.03 ± 0.00 ^{B^{Ca}}
Lesser grain borer	Buckwheat	9.12 ± 0.42 ^{B^b}	2.90 ± 0.08 ^{A^{Bb}}	0.15 ± 0.03 ^{A^{Bab}}	52.83 ± 2.67 ^{B^{Cc}}	7.79 ± 0.12 ^{C^c}	0.08 ± 0.00 ^{C^b}
	Flour	10.42 ± 0.59 ^{B^b}	0.90 ± 0.14 ^{A^{Ba}}	0.16 ± 0.04 ^{A^{Ba}}	64.42 ± 2.76 ^{B^{Ca}}	7.26 ± 1.12 ^{C^a}	0.02 ± 0.01 ^{C^a}
	Lentil	9.47 ± 0.35 ^{B^b}	2.57 ± 0.01 ^{A^{Bb}}	0.26 ± 0.03 ^{A^{Bbc}}	39.01 ± 0.08 ^{B^{Cb}}	24.38 ± 0.69 ^{C^d}	0.05 ± 0.01 ^{C^c}
	Rice	7.24 ± 1.20 ^{B^a}	0.76 ± 0.01 ^{A^{Ba}}	0.21 ± 0.04 ^{A^{Bc}}	62.68 ± 3.81 ^{B^{Ca}}	8.39 ± 0.99 ^{C^b}	0.03 ± 0.00 ^{C^a}

Different capital letters (A–C) in the same row indicate statistically significant differences ($p < 0.05$) occurring depending on insect species within each cereal type; different lowercase letters (a–d) in the same column indicate significant differences ($p < 0.05$) observed between different grains.

Groups	Grain	Moisture Content (wb, g/100 g sample)	Ash Content (wb, g/100 g sample)	Titrateable Acidity (mL HCl/100 mL)	Starch Content (wb, g/100 g sample)	Protein Content (wb, g/100 g sample)	TBARS (mg MDA/kg)
Granary weevil	Buckwheat	5.69 ± 0.31 ^{Ab}	2.2 ± 0.01 ^{Ab}	0.16 ± 0.04 ^{ABab}	54.82 ± 0.35 ^{Bc}	13.65 ± 1.24 ^{Bc}	0.08 ± 0.01 ^{Bb}
	Flour	5.94 ± 0.04 ^{Ab}	0.99 ± 0.01 ^{Aa}	0.18 ± 0.04 ^{ABa}	68.66 ± 2.76 ^{Ba}	8.93 ± 0.01 ^{Ba}	0.02 ± 0.00 ^{Ba}
	Lentil	8.65 ± 1.77 ^{Ab}	2.69 ± 0.14 ^{Ab}	0.17 ± 0.02 ^{ABbc}	38.91 ± 1.83 ^{Bb}	25.90 ± 0.49 ^{Bd}	0.05 ± 0.01 ^{Bc}
	Rice	6.19 ± 0.35 ^{Aa}	0.93 ± 0.10 ^{Aa}	0.25 ± 0.04 ^{ABc}	64.88 ± 3.63 ^{Ba}	8.58 ± 1.48 ^{Bb}	0.03 ± 0.01 ^{Ba}
Red flour beetle	Buckwheat	9.23 ± 0.38 ^{Bb}	3.26 ± 0.10 ^{Bb}	0.23 ± 0.03 ^{Bab}	52.12 ± 0.74 ^{Cc}	13.48 ± 0.74 ^{Bc}	0.08 ± 0.00 ^{Bb}
	Flour	12.38 ± 0.08 ^{Bb}	0.90 ± 0.13 ^{Ba}	0.22 ± 0.04 ^{Ba}	62.16 ± 1.27 ^{Ca}	6.30 ± 0.25 ^{Ba}	0.05 ± 0.01 ^{Ba}
	Lentil	9.33 ± 0.15 ^{Bb}	2.88 ± 0.16 ^{Bb}	0.24 ± 0.04 ^{Bbc}	37.93 ± 0.88 ^{Cb}	26.77 ± 0.75 ^{Bd}	0.05 ± 0.01 ^{Bc}
	Rice	7.28 ± 1.02 ^{Ba}	0.84 ± 0.07 ^{Ba}	0.22 ± 0.04 ^{Bc}	55.82 ± 1.75 ^{Ca}	9.48 ± 0.53 ^{Bb}	0.03 ± 0.00 ^{Ba}
Different capital letters (A–C) in the same row indicate statistically significant differences ($p < 0.05$) occurring depending on insect species within each cereal type; different lowercase letters (a–d) in the same column indicate significant differences ($p < 0.05$) observed between different grains.							

Hierarchical clustering analysis (Fig. 4) based on physicochemical parameters indicated that samples were grouped primarily according to grain type rather than insect species. Lentil samples formed a compact cluster regardless of infestation status, suggesting that their physicochemical properties remained relatively stable across treatments. Buckwheat samples formed another cluster with moderate variation among treatments, whereas flour and rice samples showed greater dispersion, indicating higher susceptibility to insect-induced biochemical alterations. Previous research has demonstrated that variations in grain morphology and biochemical composition can significantly influence insect host preference and reproductive success (Nietupski et al., 2017). Also, FTIR spectroscopy further revealed molecular-level changes in infested grain samples (Fig. 5). Increased absorbance bands associated with moisture and protein structural changes were observed in insect-infested samples, particularly in the amide I region around 1645 cm⁻¹. Rice samples infested with the red flour beetle exhibited a 42% increase in absorbance in this region, suggesting disruption of protein secondary structures due to proteolytic enzyme activity. Changes in carbohydrate fractions were also detected in the 1000–1150 cm⁻¹ region corresponding to starch vibrations. Wheat flour infested by the granary weevil showed a 65%

decrease in peak intensity at 1042 cm^{-1} , indicating extensive starch hydrolysis into simpler sugars. These molecular-level changes demonstrate that insect infestation can significantly alter the structural integrity of grain macromolecules.

The results demonstrated that stored-product insect infestations are governed by complex interactions between insect biology and grain physicochemical properties. Grain structure, nutrient composition and biochemical defenses collectively determine the extent of insect feeding activity and the magnitude of quality deterioration during storage.

4. Conclusions

This study provides a comprehensive evaluation of the interactions between stored-product insect pests and different grain substrates by integrating behavioral observations, physicochemical analyses and spectroscopic characterization. The results demonstrate that insect feeding activity and host preference are strongly influenced by the structural and biochemical properties of grain substrates. Among the tested commodities, wheat flour exhibited the highest susceptibility to infestation, particularly by the granary weevil, likely due to its finely milled structure and greater accessibility of nutrients. In contrast, substrates such as lentil showed comparatively lower levels of insect activity, suggesting that grain hardness, composition and structural barriers play an important role in limiting pest colonization. The behavioral assays conducted with the multi-arm olfactometer revealed distinct host-selection patterns among the four insect species studied. These patterns indicate that insects rely on both physical accessibility and chemical cues when locating suitable feeding substrates. Temporal observations further suggested that insect activity may vary throughout the day, reflecting circadian behavioral rhythms commonly reported in stored-product insects. Such behavioral variability highlights the importance of considering both environmental conditions and grain characteristics when evaluating infestation risk in storage systems.

In addition to behavioral responses, prolonged insect exposure resulted in significant physicochemical alterations in the examined grains. Moisture content increased consistently in infested samples, likely due to metabolic processes and respiration associated with insect activity. Elevated moisture levels are particularly concerning because they promote microbial growth and accelerate deterioration during storage. Similarly, titratable acidity increased in several samples, suggesting intensified biochemical reactions and potential microbial fermentation triggered by insect damage. Changes in ash content also indicated contamination from insect residues, including fragments, excreta and metabolic by-products. The degradation of major macromolecules was another key finding of this study. Starch content declined significantly in most infested samples, demonstrating that carbohydrate reserves represent a primary nutritional source for stored-product insects. Protein content exhibited more variable responses depending on grain type, with reductions observed in some substrates and apparent increases in others, possibly due to concentration effects following starch degradation. Lipid oxidation, measured through TBARS values, increased across infested samples, indicating enhanced oxidative stress and

deterioration of grain lipids. Such oxidative processes negatively affect both nutritional quality and storage stability.

The results highlight that postharvest grain deterioration is driven by complex interactions between insect biology and grain physicochemical properties. Grain structure, nutrient composition and accessibility collectively determine insect feeding behavior and the extent of biochemical degradation during storage. By integrating behavioral ecology with biochemical and spectroscopic analyses, this study advances current understanding of insect–grain interactions and provides a more holistic framework for evaluating storage-related losses. From a broader perspective, the results underscore the importance of improving postharvest management strategies to mitigate insect-induced losses in grain storage systems. Understanding how insect preferences relate to grain characteristics may help inform the development of targeted pest management approaches and predictive models for infestation risk. Such knowledge is particularly relevant for regions where postharvest losses remain high and storage infrastructure is limited. Ultimately, reducing insect-related grain deterioration contributes not only to improved food quality and economic sustainability but also to global efforts aimed at minimizing food loss and enhancing food security within agricultural supply chains.

Declarations

CRediT authorship contribution statement

Ali Yilmaz: Conceptualization, Methodology, Formal analysis, Acquisition of data, Visualization, Writing-original draft, Writing-review & editing.

Mustafa Bayram: Investigation, Conceptualization, Project administration, Supervision, Writing-review & editing.

Declaration of Competing Interest

The researchers acquaint that they have no competing financial or individual connections that may influence the work reported in this manuscript.

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References

1. Al-Hayali, T. S. A., & Qader, F. A. (2024, July). The effect of five wheat varieties on khapra beetle, *Trogoderma granarium* evarts (Coleoptera: Dermestidae) during four storage months. In *IOP Conference Series: Earth and Environmental Science* (Vol. 1371, No. 3, p. 032023). IOP Publishing.

2. AOAC International. (1995). *Official methods of analysis of AOAC International* (Method 923.03).
3. AOAC International. (1997). *Official methods of analysis of AOAC International* (Method 925.10).
4. Astuti, L. P., Mario, M. B., & Widjayanti, T. (2018). Preference, growth and development of *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae) on red, white and black rice in whole grain and flour form. *Journal of Entomological Research*, 42(4), 461-468.
5. Astuti, L. P., Rizali, A., Firnanda, R., & Widjayanti, T. (2020). Physical and chemical properties of flour products affect the development of *Tribolium castaneum*. *Journal of Stored Products Research*, 86, 101555.
6. Bozkurt, H. (2007). Comparison of the effects of sesame and *Thymbra spicata* oil during the manufacturing of Turkish dry-fermented sausage. *Food Control*, 18(2), 149-156.
7. Campbell, J. F. (2006). Stored-product insect behavior. *Insect Management for Food Storage and Processing, 2nd Edition*. American Association of Cereal Chemists Press, St. Paul, Minn, 39-54.
8. D'Isita, I. (2024). Suscettibilità dei grani antichi ad insetti in postraccolta: interazioni allelochimiche e caratteristiche tecnologiche.
9. Fleurat-Lessard, F. (2017). Integrated management of the risks of stored grain spoilage by seedborne fungi and contamination by storage mould mycotoxins—An update. *Journal of Stored Products Research*, 71, 22-40.
10. Food and Agriculture Organization of the United Nations (FAO). (2023). *Crops and livestock products*. FAOSTAT. <https://www.fao.org/faostat/en/#data/QCL/visualize>
11. Hassan, M. W., Sarwar, G., Farooqi, M. A., & Jamil, M. (2021). Extent and pattern of damage in wheat caused by three different species of storage insect pests. *International Journal of Tropical Insect Science*, 41, 593-599.
12. Hagstrum, D., & Subramanyam, B. (2017). *Stored-product insect resource*. Elsevier.
13. Hubert, J., Stejskal, V., Athanassiou, C. G., & Throne, J. E. (2018). Health hazards associated with arthropod infestation of stored products. *Annual review of entomology*, 63, 553-573.
14. Keskin, Ş., Özkaya, H. (2012). Buğdayların *Sitophilus granarius* L. ile bulaştırılmasının unların reolojik özelliklerine etkisi. *Akademik Gıda*, 10(3), 40-46.
15. Kiaya, V. (2014). Post-harvest losses and strategies to reduce them. *Technical Paper on Postharvest Losses, Action Contre la Faim (ACF)*, 25(3), 1-25.
16. Mario, M. B., Taufik, M., Melina, M., Joshi, R. C., Anshar, I., Paridah, P., ... & Fernando, I. (2025). Resistance of several Indonesian cereal grain cultivars to the maize weevil, *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae). *Crop Protection*, 190, 107105.
17. Mesterházy, Á., Oláh, J., & Popp, J. (2020). Losses in the grain supply chain: Causes and solutions. *Sustainability*, 12(6), 2342.
18. Naseri, B., & Majd-Marani, S. (2020). Assessment of eight rice cultivars flour for feeding resistance to *Tribolium castaneum* (Herbst)(Coleoptera: Tenebrionidae). *Journal of Stored Products Research*, 88, 101650.

19. Nath, B., Chen, G., O'Sullivan, C. M., & Zare, D. (2024). Research and technologies to reduce grain postharvest losses: a review. *Foods*, 13(12), 1875.
20. Nietupski, M., Kwiatkowski, J., & Kosewska, A. (2017). Physicochemical properties of achenes of buckwheat cultivars affecting the development of grain weevil (*Sitophilus granarius* L.) and lesser grain borer (*Rhyzopertha dominica* F.).
21. Özkaya, H., Özkaya, B., & Colakoglu, A. S. (2009). Technological properties of a variety of soft and hard bread wheat infested by *Rhyzopertha dominica* (F.) and *Tribolium confusum* du Val.
22. Phillips, T. W., & Throne, J. E. (2010). Biorational approaches to managing stored-product insects. *Annual review of entomology*, 55(1), 375-397.
23. Rajnincová, D., Spaleková, A., Gálová, Z., & Romanavá, K. (2019). The protein profile of cereals, pseudocereals and legumes. *J. Food Sci. Technol*, 7, 49-53.
24. Serna Saldívar, S. O., & Hernández, D. S. (2020). Dietary fiber in cereals, legumes, pseudocereals and other seeds. *Science and technology of fibers in food systems*, 87-122.
25. Stathas, I. G., Sakellaridis, A. C., Papadelli, M., Kapolos, J., Papadimitriou, K., & Stathas, G. J. (2023). The effects of insect infestation on stored agricultural products and the quality of food. *Foods*, 12(10), 2046.
26. Tadesse, M. (2020). Post-harvest loss of stored grain, its causes and reduction strategies. *Food Science and Quality Management*, 96, 26-35.
27. Wanniarachchi, P. C., Pavalakumar, D., & Jayasinghe, M. A. (2023). Pseudocereals: Nutrition, Health Benefits and Potential Applications in Gluten-free Food Product Developments. *Current Nutrition & Food Science*, 19(4), 377-385.

Figures

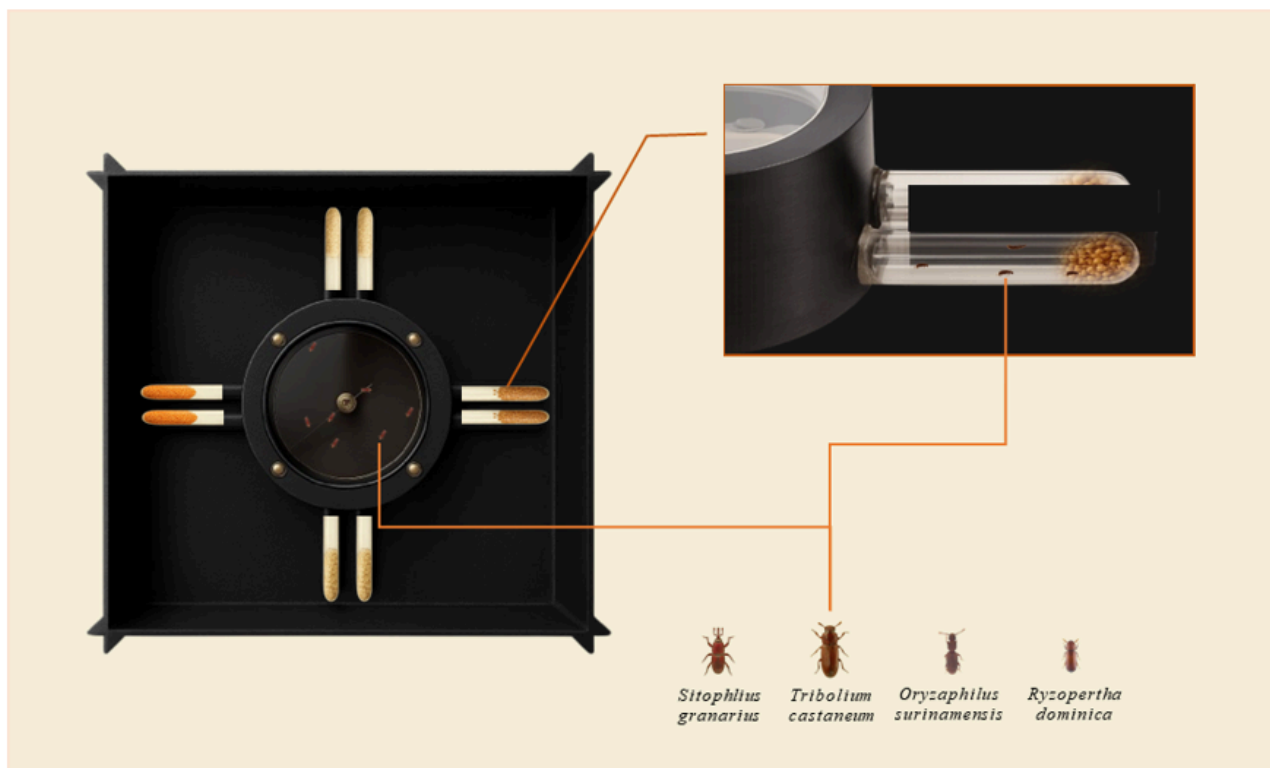


Figure 1

Schematic illustration of the multi-choice experimental arena used to assess grain preference of stored-product insects.

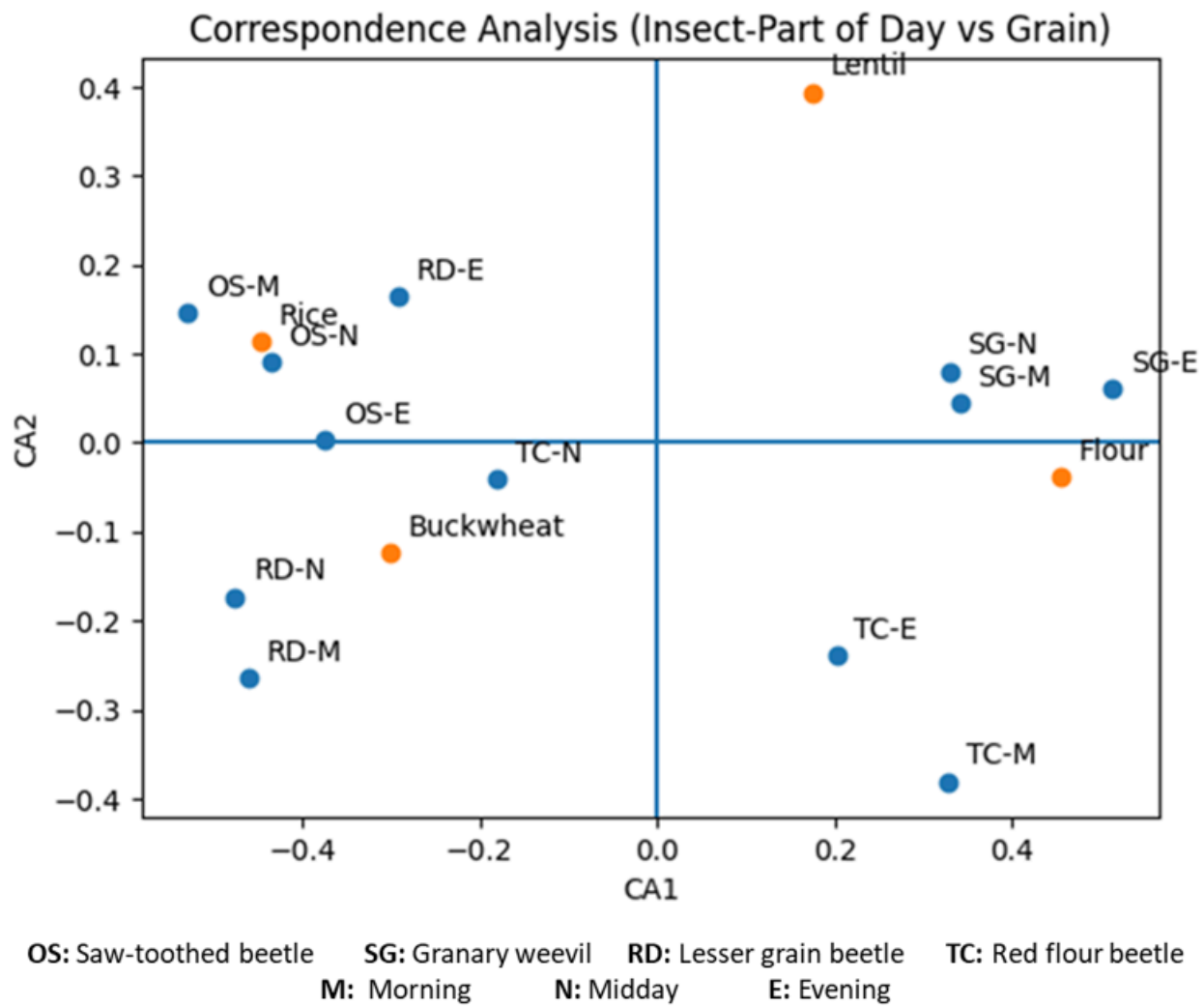


Figure 2

Correspondence analysis (CA) showing the relationship between insect species, parts of the day, and grain types.

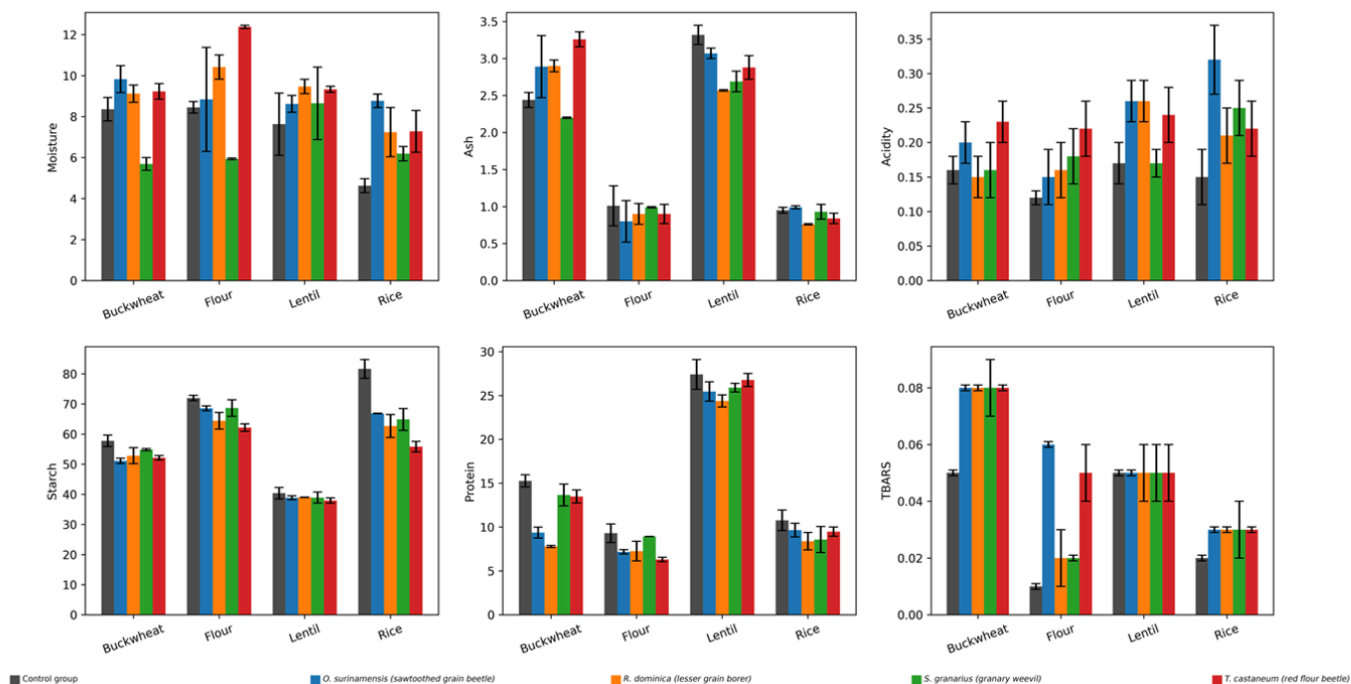


Figure 3

Effects of stored-product insect infestation on the physicochemical properties of different grains during storage conditions, including changes in moisture content, ash content, titratable acidity, starch content, protein content and TBARS values of buckwheat, flour, lentil and rice samples, where colors represent the control group and insect species: *Oryzaephilus surinamensis* (sawtoothed grain beetle), *Rhyzopertha dominica* (lesser grain borer), *Sitophilus granarius* (granary weevil) and *Tribolium castaneum* (red flour beetle).

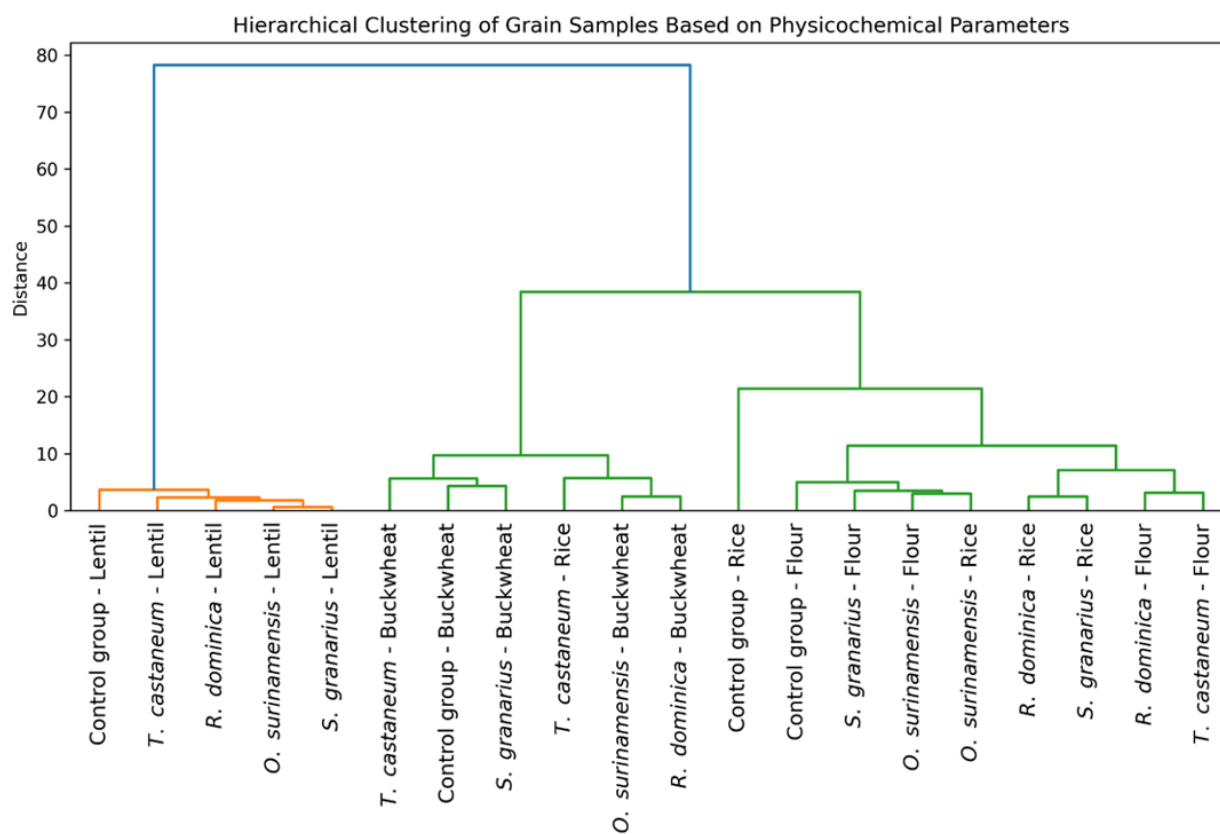


Figure 4

Hierarchical clustering dendrogram showing the similarity among insect-infested grain samples based on physicochemical properties.

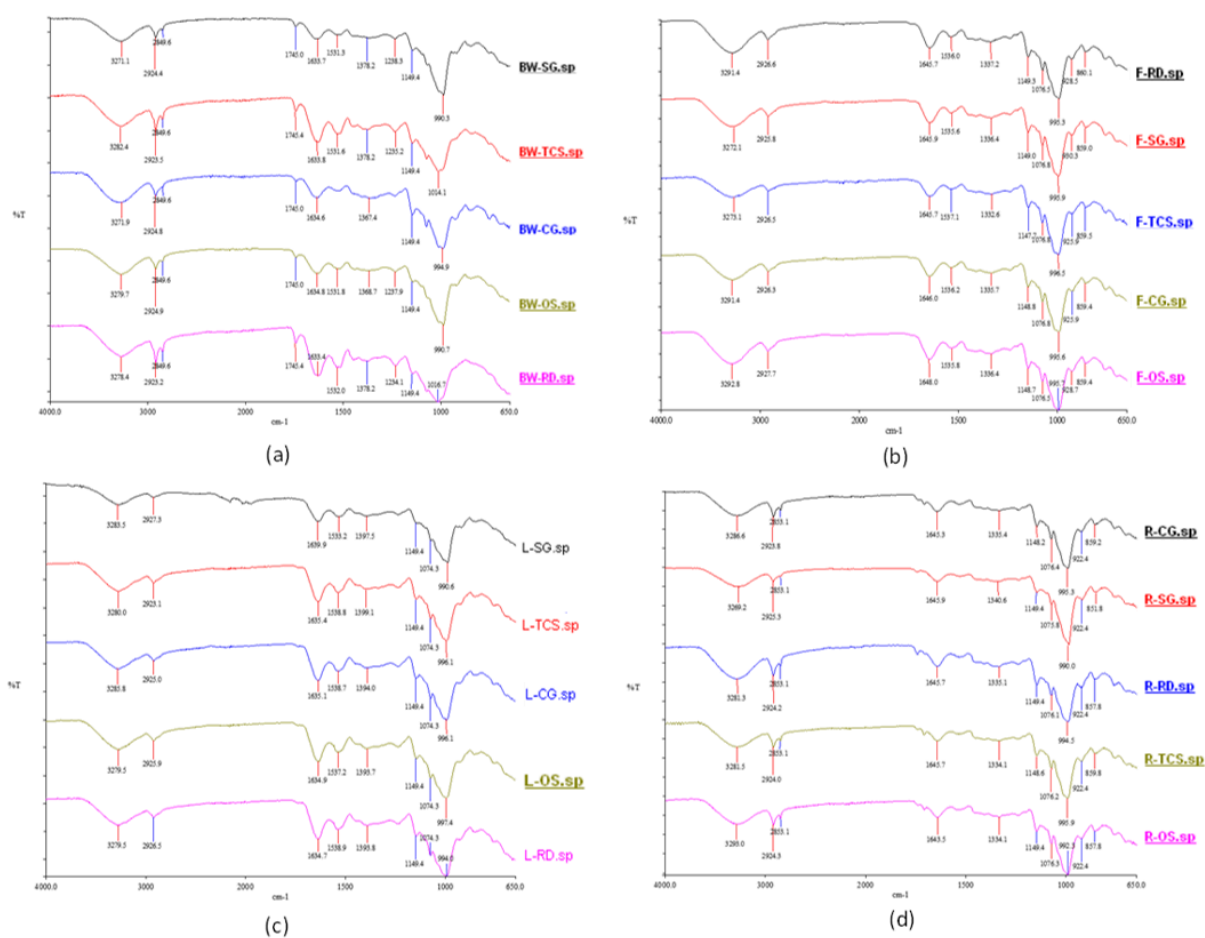


Figure 5

FTIR spectra of grains from the control group (CG) and samples infested by *Tribolium castaneum* (red flour beetle, TCS), *Sitophilus granarius* (granary weevil, SG), *Rhyzopertha dominica* (lesser grain borer, RD) and *Oryzaephilus surinamensis* (sawtoothed grain beetle, OS): (a) buckwheat (BW), (b) flour (F), (c) lentil (L) and (d) rice (R). Spectra are presented as percentage transmittance (%T).