findings highlight the need to balance microbiological safety with the preservation of freshness markers and bioactive compounds when selecting initial processing methods.

6.2 Heat treatment and changes in enzyme activity

Heat treatment is widely used to reduce viscosity, dissolve crystals, and lower moisture content, but it directly drives HMF formation and enzyme inactivation. Kinetic studies demonstrate that temperatures above about 60 °C cause a sharp increase in HMF and a concomitant decline in diastase, with both effects accelerating as time and temperature rise. Heating at 40 °C for long periods had little effect on HMF or diastase, whereas exposure at 60 °C-100 °C caused regular HMF increases and diastase decreases, implying relatively narrow thermal windows for safe processing. Similar patterns were observed in Apis florea honey, where treatments at 55 °C-65 °C for several hours raised HMF by up to ~45% and reduced diastase and invertase activities by ~60%-72%, clearly demonstrating enzyme deactivation at higher temperatures and longer durations (Wu et al., 2022).

Thermal processing also affects antioxidant-related compounds and activities. Experiments at 63 °C for up to 30 min on different floral honeys showed increases in HMF and reductions in total phenolic content, accompanied by declines in DPPH radical-scavenging and FRAP values in some honeys (Jaya et al., 2022). A broader review concludes that thermal treatment significantly influences honey color, moisture, HMF, diastase, microbial load, and antioxidant parameters, and that time-temperature combinations must be carefully managed to moderate these negative effects (Bhure et al., 2025). Overall, evidence indicates that maintaining temperatures at or below about 40 °C-45 °C during routine handling, and limiting exposure at 60 °C-65 °C to very short times, is critical to preserve enzyme activity and bioactive components while achieving technological goals (Al-Rubaie, 2022).

6.3 Impact of storage environment (temperature, light, duration) on quality

Storage temperature and duration are major drivers of long-term changes in freshness markers and sensory/nutritional quality. Multiple studies show that room-temperature or warm storage increases HMF and reduces diastase, whereas cool storage markedly slows these reactions (Ramly et al., 2021). For example, sunflower honey stored 18 months at ~22 °C in the dark exhibited a 17-fold increase in HMF and a two-fold decrease in diastase, though moisture and free acidity remained relatively stable. Two-year storage of varietal honeys at room temperature caused about a 79% rise in HMF and ~67% reduction in diastase, whereas storage at 4 °C or below limited HMF increases to ~25%-33% and produced smaller enzyme losses, also reducing color changes (Kędzierska-Matysek et al., 2025).

A systematic review of 43 studies confirms that prolonged storage can deteriorate sensory, nutritional, and antioxidant properties and promote fermentation, granulation, and quality indicators such as increased HMF and decreased diastase and invertase (Manickavasagam et al., 2024). Work on stingless bee and other honeys further indicates that storage at 40 °C accelerates HMF formation and loss of phenolics and antioxidants, while storage at 4 °C-5 °C preserves bioactive compounds and antimicrobial activity much better (Rababah et al., 2024).

7 Case Study: Comparative Analysis of Honey Quality Under Different Regional Environmental Conditions

7.1 Selection of study areas and sample collection methods

Comparative evaluation of regional environmental effects on honey quality requires sampling areas that differ clearly in climate, land use, pollution history, and topography. Recent work has contrasted honeys from multiple Romanian regions with distinct geological and anthropogenic backgrounds, using 61 samples from eight areas to capture gradients in soil composition, industrial activity, and atmospheric inputs (Shakoori et al., 2023). Other studies designed regional comparisons by selecting contrasting agroecological zones or climatic regions (e.g., cold vs. hot climates, or temperate vs. tropical zones), ensuring that differences in humidity, temperature, vegetation and land use were adequately represented for subsequent chemometric analysis (Rosiak et al., 2021).

Sampling strategies typically combine spatial replication with careful control of production variables to isolate environmental effects. Multifloral honeys are often collected directly from beekeepers or apiaries to avoid market adulteration and to link samples reliably to specific landscapes and pollution sources (Rosiak et al., 2021).