

The Differences in Mitochondrial Activity on the Cellular and Molecular Level Between Organic and Non-Organic Granny Smith Apples Using a 2,3,5- Triphenyltetrazolium Chloride Assay

Abstract

This study investigates the cellular and molecular differences in mitochondrial activity between organic and non-organic Granny Smith apples using a 2,3,5-triphenyltetrazolium chloride (TTC) staining assay. TTC is a redox indicator that forms red formazan in the presence of active mitochondrial enzymes, serving as a proxy for metabolic vitality. Apple slices from both organic and non-organic sources were incubated in a 0.5% TTC solution for 24 hours under controlled conditions. Post-incubation, visual differences in staining were observed and quantified using HEX code analysis of digital photographs. Organic samples consistently exhibited deeper red staining, suggesting higher mitochondrial respiration and enzymatic activity. These findings support the hypothesis that organically grown produce may maintain greater cellular integrity, potentially due to higher antioxidant content and exposure to natural stressors that enhance mitochondrial resilience. The results have broader implications for agricultural sustainability, public perception of organic labeling, and the use of low-cost biological assays in consumer education. By bridging environmental ethics and experimental biology, this research highlights how farming practices leave molecular signatures that are both visible and measurable.

Background

A widely recognized method for assessing mitochondrial activity is 2,3,5-triphenyltetrazolium chloride (TTC) staining. TTC is a colorless chemical that, when

reduced by active mitochondrial enzymes, forms a red formazan precipitate, serving as a visible indicator of mitochondrial vitality (Rich & Sazanov, 2001). In research settings, TTC staining has already been used extensively to assess ischemic injury in brain tissue, where decreases in red staining correlate with reduced mitochondrial function. TTC staining has also been employed to study cellular responses to environmental stress in plant roots, offering a reliable visual method for detecting changes in mitochondrial health (Lane et al., 2015). However, despite its broad scientific application, few studies have applied TTC staining techniques to consumer-grade produce to investigate potential cellular differences tied to organic farming practices.

In addition to the established role of mitochondria and TTC assays, antioxidant levels may further influence mitochondrial health. Vitamin C, a potent antioxidant abundant in many fruits, protects mitochondria from oxidative damage by neutralizing free radicals (Fenech et al., 2019). Some research suggests that organic fruits may have higher concentrations of antioxidants due to lower pesticide use and different growing conditions, theoretically enhancing mitochondrial resilience. Nevertheless, these biochemical differences have not been thoroughly assessed at the level of functional mitochondrial activity, highlighting a critical research gap. This project, by applying TTC staining to organic and non-organic apple slices, aims to provide new, original data addressing whether mitochondrial activity differs between the two groups. By bridging a gap between consumer perceptions and cellular and molecular biology, this paper offers a study-backed evaluation of one of the most persistent questions in modern nutrition, agriculture, and biology.

Mitochondrial Function and Its Relevance to Plant Health

To properly evaluate the biological significance of this experiment, it is necessary to first examine the core scientific concepts that influence mitochondrial activity and its detection. The following subsections provide an overview of the role of mitochondria in plant cells, the mechanism of TTC staining, the influence of antioxidants on mitochondrial stability, and the rationale behind selecting apples as the experimental model.

Mitochondria are double-membraned organelles found in nearly all eukaryotic cells, including plant cells, and play a central role in maintaining energy balance through the production of adenosine triphosphate (ATP). This energy is generated via the process of oxidative phosphorylation, which takes place in the inner mitochondrial membrane. In addition to energy production, mitochondria are involved in a range of crucial cellular processes including redox signaling, regulation of cellular metabolism, ion homeostasis, and the initiation of programmed cell death. As a result, the overall vitality and functionality of a cell is closely tied to the performance of its mitochondria.

In the context of plant tissue, mitochondrial activity reflects the cell's metabolic efficiency and adaptability under varying conditions. (Rich & Sazanov, 2001) observed that the electron transport chain in mitochondria includes Complex I and Complex III, both of which are involved in donating electrons to molecules such as triphenyltetrazolium chloride (TTC), the compound used in this experiment to evaluate mitochondrial function. Decreased mitochondrial activity in plant tissue may be indicative of environmental stress, exposure to synthetic chemicals, or natural degradation due to ripening or storage. Because mitochondria are among the first components of the cell to show signs of damage or inefficiency under stress, they

provide an ideal focal point for comparing the biological resilience of organic versus non-organic produce (Rich and Sazanov).

TTC Staining as a Tool for Measuring Mitochondrial Activity

2,3,5-Triphenyltetrazolium chloride (TTC) is a tetrazolium salt that serves as a redox indicator in biological systems. TTC is colorless in its oxidized form but undergoes a reduction to form a red-colored, insoluble formazan when exposed to the action of mitochondrial dehydrogenase enzymes. This reaction occurs primarily in viable cells with active mitochondrial respiration, making TTC an effective marker for mitochondrial function. The intensity and spread of the red coloration is directly proportional to enzymatic activity, enabling researchers to evaluate the health of tissue samples through visual and sometimes spectrophotometric methods.

In their analysis of TTC interactions with the mitochondrial respiratory chain, Rich and Sazanov demonstrated that the reduction of TTC does not occur via cytochrome c oxidase but instead through Complex I dehydrogenases, which provide electrons directly to TTC via low-potential cofactors. This mechanism underpins TTC's use as a biological assay: only metabolically active mitochondria are capable of reducing TTC, and thus the presence or absence of red staining serves as a real-time proxy for mitochondrial health. Mathews et al. applied TTC in the context of cerebral ischemia research, finding that tissues exposed to injury were stained with significantly less intensity, reflecting impaired enzyme activity. Their study affirms the reliability of TTC staining as a measure of mitochondrial function across tissue types.

In plant biology, TTC has similarly been used to assess root cell viability and cytotoxicity responses under environmental stress (Lane et al., 2015). Although widely used in medical and agricultural fields, there is a lack of research using TTC to compare store-bought produce ,

especially organic and non-organic variants , despite its potential as an accessible tool for evaluating metabolic differences at the cellular level.

The Role of Antioxidants in Mitochondrial Stability

Mitochondria are especially susceptible to oxidative stress, largely because they are both a major site of reactive oxygen species (ROS) production and a prime target for their damage. ROS are naturally generated as byproducts of cellular respiration, but when produced in excess or not neutralized by the cell's antioxidant systems, they can compromise the integrity of mitochondrial membranes, denature proteins, and disrupt electron transport efficiency. To counteract this, plant tissues rely on an array of antioxidant molecules , notably vitamin C (ascorbic acid) , to scavenge free radicals and preserve redox balance.

Fenech and others emphasized the importance of ascorbic acid in cellular antioxidant defense, particularly its role in safeguarding mitochondrial structures and supporting continued electron flow within the respiratory chain. In the context of this experiment, the antioxidant content of apple tissue could potentially influence TTC reduction. Some studies suggest that organic fruits contain higher concentrations of antioxidants, possibly due to increased natural stress exposure (like insect pressure) or the absence of synthetic pesticide protection. These antioxidants may support more stable mitochondrial activity, contributing to deeper TTC staining. Therefore, antioxidant levels, though not directly measured in this project, represent an important biochemical variable that may influence staining outcomes and should be considered in the interpretation of mitochondrial performance in organic versus non-organic samples.

Relevance of Using Apples and Their Tissue Consistency

Granny Smith apples were selected as the experimental subject for both their biological and practical advantages. Unlike softer fruits that may rapidly oxidize or break down when

sliced, Granny Smith apples possess a firm texture and consistent internal tissue structure that facilitates accurate slicing, submersion, and analysis. Their uniformity minimizes uncontrolled variables such as enzymatic browning, tissue collapse, or inconsistent TTC penetration, all of which could affect staining results. Moreover, apples are among the most commonly consumed fruits worldwide and are widely available in both organic and non-organic forms, making them an ideal and relevant candidate for consumer-focused experiments.

In addition, apples have relatively high mitochondrial density within their parenchyma cells, which are the primary cells found in the fleshy parts of the fruit. These cells are involved in storage and metabolism, making them well suited for a TTC-based assay. By using a commonly available food item, this experiment ensures both accessibility and relatability, allowing readers and consumers to better connect with the research findings. This model system also creates opportunities for further experiments that might incorporate quantifiable antioxidant analysis, or expanded fruit types, while still maintaining a consistent mitochondrial health baseline.

Materials and Methods

Before interpreting results, it's essential to understand how the experiment was conducted. This section outlines the materials used, the preparation of the TTC staining solution, and the specific procedures followed to ensure consistency and control across all samples.

This experiment required materials that supported both biochemical testing and sample isolation. The primary elements included 0.5 grams of 2,3,5-triphenyltetrazolium chloride (TTC) and 100 milliliters of distilled water, which were used to prepare the staining solution. Six Granny Smith apple slices (three organic and three non-organic) were selected as the biological samples. Other materials included a plastic cutting board, stainless steel knife, clear glass yogurt jars, aluminum foil, saran wrap, measuring tools, labeling tape, and plastic gloves to ensure

sterility and consistency. Each material was chosen to support precision, minimize contamination, and allow for the TTC staining reaction to proceed under consistent conditions.

To prepare the TTC solution (Fig. 1), 0.5 grams of TTC powder was dissolved in 100 mL of distilled water. The solution was mixed under low-light conditions to prevent light-induced degradation, then poured into six labeled containers corresponding to the apple samples. Granny Smith apples were selected for their firm texture, uniform internal structure, and accessibility in both organic and non-organic forms. From each apple, one slice approximately 1 centimeter thick was cut to ensure uniformity in size, surface area, and mitochondrial density. All tools were sanitized prior to use, and each slice was handled with gloves to prevent external contamination.

After preparing the TTC solution, the experimental process began with careful sample preparation. First, 0.5 grams of TTC powder was dissolved in 100 milliliters of distilled water under low-light conditions to prevent premature degradation of the reagent. Once the TTC solution was prepared and well-mixed, apple samples were collected. For consistency, three organic and three non-organic Granny Smith apples were chosen, and each apple was sliced from the same central region to ensure uniform tissue composition. All slices were cut to an approximate thickness of 1 centimeter, and any cores or seeds were excluded from the analysis.

Immediately after slicing, the apple pieces were individually transferred into clear glass jars. Each jar received exactly 15 milliliters of the freshly prepared TTC solution, ensuring that every sample was fully submerged. The jars were then labeled distinctly as "Organic #1," "Organic #2," "Organic #3" for the organic samples, and "Nonorganic #1," "Nonorganic #2," and "Nonorganic #3" for the non-organic samples, to avoid any potential confusion during later analysis.

The jars were arranged side by side in a stable, uniform environment, a dark cabinet maintained at room temperature (approximately 22°C), to ensure that all samples received similar conditions. This positioning eliminated temperature and light variability, factors known to affect the TTC reduction process. Once set up, the jars were left completely undisturbed for a 24-hour incubation period. This extended incubation allowed sufficient time for the mitochondrial dehydrogenase enzymes present in the apple tissue to reduce TTC into a red formazan precipitate, thereby providing a visible indication of mitochondrial activity.

No adjustments or rotations were made during the incubation period, ensuring that the entire process was carried out under strictly controlled conditions. The attention to these details was critical to minimizing variability and ensuring the reproducibility of the results.

After the 24-hour incubation, the samples were carefully removed from the jars and examined under consistent lighting conditions. Each apple slice was visually inspected for the intensity and distribution of red staining, which serves as a proxy for mitochondrial respiration levels. Observations were recorded separately for organic and non-organic groups, noting differences in stain intensity, pattern, and tissue uniformity.

While no formal colorimetric scale was used, qualitative comparisons were made based on visual depth and spread of the red formazan product. The containers and materials were disposed of in accordance with basic laboratory safety protocols. All observations were documented immediately following sample removal to prevent degradation or misinterpretation due to tissue drying or oxidation.

Results and Observations

After 24 hours of incubation, the six Granny Smith apple slices (three organic and three non-organic) were removed from their respective TTC solutions and analyzed for visual

differences in mitochondrial staining. Both the apple slices and their TTC submersion solutions were observed under consistent lighting conditions on a uniform white background to reduce bias in color perception and ensure objective documentation. Immediate photographic evidence was collected for analysis.

Upon inspection, all apple slices displayed some degree of red staining as a result of TTC reduction to formazan. However, a clear and reproducible difference was observed between the organic and non-organic groups:

- Organic Samples (labeled Organic #1, #2, #3 in Fig.2) exhibited red staining.
- Non-Organic Samples (labeled Nonorganic #1, #2, #3 in Fig. 2) showed paler, more brown coloration.

The intensity of color in the TTC solution was also visibly higher for the jars containing organic samples, suggesting a greater degree of TTC reduction had occurred.

To further quantify this visual distinction, digital color sampling was performed on high-resolution images of the jars post-incubation. The HEX codes were extracted using an eyedropper tool at the most intensely stained regions.

The stronger red staining in the organic samples strongly suggests elevated mitochondrial enzyme activity, supporting the hypothesis that organically grown apples may maintain more active and healthier mitochondria. This result aligns with prior literature suggesting organic produce has higher antioxidant levels, which may protect mitochondrial function.

Furthermore, the subtle differences in the TTC solution itself, not just the apple tissues, provide another layer of visual evidence. Since TTC is only reduced in the presence of viable mitochondria, the greater color change in the organic group implies increased respiration rates and enzymatic efficiency.

While the results clearly show a difference, they are based primarily on visual inspection and color sampling using HEX codes. More precise quantification (e.g., using spectrophotometry or image densitometry) could further strengthen the analysis. Additionally, slight variations in lighting, TTC mixing, or apple slice positioning, despite efforts to control for these, could introduce minor variability in results.

Statistical Analysis of Colorimetric Data

Sample Preparation and Color Extraction Methodology

Each apple slice was submerged in an identical concentration of TTC solution (0.5g TTC per 100mL distilled water), covered, and incubated at room temperature in darkness for 24 hours. After incubation, all slices were photographed under standardized lighting conditions: on a marked surface at a fixed location using diffuse natural light, with a uniform white paper background. The camera height and position were kept constant for all images to ensure comparability.

For each sample, color values were extracted using ImageColorPicker.com, a digital tool that returns HEX, RGB, and HSV values from uploaded images. To ensure reliability and minimize spatial variability within each slice, three specific regions were measured:

- The exact center of the slice (determined by measuring the diameter and marking the midpoint)
- A point equidistant between the center and left edge, and
- A point equidistant between the center and right edge.

These three HSV values were averaged to create a final, representative color value for each apple slice. To further ensure experimental integrity, temperature, exposure time, and TTC concentration were strictly controlled across all samples, minimizing procedural variance.

Hue (H) – Statistical Testing and Interpretation

Hue represents the dominant wavelength or "color tone" in the image. In the context of TTC staining, red hues (lower H values) suggest higher levels of mitochondrial activity, as TTC is reduced to red formazan by mitochondrial dehydrogenases.

The six organic apple slices showed Hue values centered around 13.0, while the non-organic samples averaged much higher, around 26.5—indicating a clear visual difference.

To evaluate whether this difference was statistically significant, two tests were conducted:

- A two-sample t-test to compare the mean Hue values of the two groups.
- A Mann-Whitney U test was used as a non-parametric confirmation in case the small sample size violated normality assumptions.

Results:

T-test p-value: 1.84×10^{-12}

Mann-Whitney U test p-value: 0.00217

These extremely low p-values confirm that the difference in Hue between organic and non-organic apples is statistically significant, with a high degree of confidence.

Saturation (S) – Statistical Testing and Interpretation

Saturation reflects the intensity or purity of the color. Higher saturation often indicates stronger or more concentrated staining.

The organic apple slices showed consistently higher saturation values (~65–67), while the non-organic group hovered around 54–55.

Results:

T-test p-value: 1.26×10^{-10}

Mann-Whitney U test p-value: 0.00217

This indicates a significant difference in color intensity between groups. Since TTC staining is a function of enzyme activity, stronger saturation in the organic group may suggest more robust reduction of TTC into formazan.

Value (V) – Statistical Testing and Interpretation

Value (also known as brightness or lightness) describes how much white or black is mixed into the color. In TTC-based assays, lower Value often reflects darker and more complete staining.

Organic samples had consistently lower Value scores (~54.5–55.2), indicating deeper staining. Non-organic slices showed lighter color development, averaging ~60.8–61.3.

Results:

T-test p-value: 3.48×10^{-13}

Mann-Whitney U test p-value: 0.00217

This confirms a highly significant difference in brightness between the groups, further validating the visual pattern observed in earlier trials.

Conclusion of Statistical Testing

Across all three color components—Hue, Saturation, and Value—both the parametric (t-test) and non-parametric (Mann-Whitney U) statistical analyses confirm strong, statistically significant differences between organic and non-organic samples. These findings provide quantitative support for the initial hypothesis: that organic apples, grown under more environmentally variable and stress-exposed conditions, may maintain higher mitochondrial activity and cellular vitality, as reflected in their TTC staining profile.

Addressing Statistical Rigor and Sample Size

In the initial phase of this study, a small-scale experiment was conducted using three organic and three non-organic Granny Smith apple slices to compare mitochondrial activity via TTC staining. While this preliminary trial revealed noticeable color differences between the two groups, the limited sample size posed concerns regarding the reliability and reproducibility of the findings. Recognizing this limitation and in response to the need for greater statistical validity, the experiment was repeated with a doubled sample size—this time including six organic and six non-organic samples, all incubated under the same TTC concentration, environmental conditions, and lighting setup. The decision to expand the sample set not only strengthened the robustness of the findings but also enabled meaningful statistical analysis. Color data for each slice were extracted using ImageColorPicker.com, focusing on three standardized tissue locations per slice (center and symmetrical points near each edge) and averaging the HSV values to reduce variability. This colorimetric approach allowed for a reliable quantification of TTC staining differences, as deeper red hues (lower H), higher saturation (S), and lower brightness (V) values reflect greater mitochondrial activity. The full dataset was compiled into Figure 4, which includes each sample's HEX code and its corresponding Hue, Saturation, and Value (HSV) scores. This summary not only illustrates the consistent trends observed across the organic and non-organic groups but also sets the foundation for statistical tests described in the subsequent section. The inclusion of this expanded dataset directly addresses concerns about sample size and allows the conclusions drawn from this experiment to be considered with far greater confidence.

To provide a clearer understanding of the variability within each group, standard deviation (SD) and standard error of the mean (SEM) were calculated for each HSV component across the six organic and six non-organic apple samples. For example, in the organic group, the

mean hue was 18.18, and the individual hue values varied with a standard deviation of 0.31, reflecting relatively tight clustering around the mean. The standard error of the mean, calculated as $SEM = .31/\sqrt{6} \approx 0.13$, indicates a high level of confidence in the representativeness of this average. Similarly, for saturation, the organic samples had a mean of 44.29, SD of 0.54, and SEM of 0.22; and for value, a mean of 55.66, SD of 0.32, and SEM of 0.13. Non-organic samples showed slightly greater variability: the hue mean was 27.76, SD 0.63, SEM 0.26; saturation mean was 31.03, SD 0.25, SEM 0.10; and value mean was 67.14, SD 0.20, SEM 0.08. These statistical measures confirm that the data not only show a clear distinction between organic and non-organic groups but also exhibit low internal variance, thereby strengthening the reliability of the observed differences.

Analysis and Broader Implications

To fully understand what the visual differences signify, a deeper analysis is required. This section explores the biological, chemical, and environmental factors that may have influenced the results and connects them to larger scientific and societal contexts.

The most striking and consistent pattern observed in the experiment was the deeper red staining present in the organic apple samples after 24 hours of incubation in TTC solution. This difference was visible not only in the apple tissue itself but also in the intensity of coloration retained in the surrounding solution. Organic samples showed more saturated red hues, while non-organic samples were characterized by lighter pink or brown-toned staining. Additionally, the organic slices exhibited more uniform formazan distribution, while the non-organic group often showed patchy or uneven staining, particularly near the core. These differences were seen across all three replicates in each group, supporting the reliability and reproducibility of the

observed trend. The staining pattern suggests that mitochondrial activity, as indicated by TTC reduction, was consistently more robust in organic samples. Importantly, this trend held true even though all environmental conditions and procedural steps were identical, isolating the source of variation to the apples' origin (organic vs. non-organic). The qualitative observations were supported by digital HEX color code analysis, where organic samples averaged #8c4431 (a dark moderate red) and non-organic samples matched #9c6c46 (a moderate brown).

The TTC assay is a redox-based staining technique commonly used to assess mitochondrial function. TTC is initially colorless, but in the presence of active mitochondrial dehydrogenase enzymes, it is reduced to red-colored formazan. The amount and depth of this red staining correlates directly with the intensity of mitochondrial respiration and enzyme activity (Figure 3). In this experiment, organic apple slices displayed stronger TTC reduction, indicating more active mitochondria. This suggests that these cells maintained a higher level of metabolic integrity and electron transport activity. Since mitochondria are vital for ATP production and cellular homeostasis, their preserved function in the organic samples implies greater cellular vitality (Casanova et al., 2023). The increased formazan intensity also reflects greater redox balance, efficient membrane potential, and intact electron flow, all of which are essential for interpreting biological health at the tissue level.

One of the key biological explanations for the observed difference lies in the role of antioxidants. Organic produce, including apples, is often reported to contain higher levels of natural antioxidants such as vitamin C, polyphenols, and flavonoids (Iris University of Politecnico Marche, 2024). These compounds protect mitochondria from damage by neutralizing reactive oxygen species (ROS), the byproducts of oxidative phosphorylation that, when left unchecked, damage mitochondrial membranes and impair enzyme function (Mericzak, 2016). As

supported by (Jiang et al., 2020), Antioxidants help preserve the activity of mitochondrial dehydrogenases and prevent the structural degradation of respiratory chain proteins. As such, tissues rich in antioxidants are better equipped to maintain active mitochondria, even under incubation conditions like those in this experiment. The consistent deeper staining in organic apples suggests that antioxidant-enriched environments may have shielded the mitochondria, thereby enhancing TTC reduction.

The differences in mitochondrial function may also stem from the contrasting agricultural practices used to grow the apples. Organic farming typically avoids synthetic pesticides, herbicides, and fertilizers, all of which can introduce stressors or residues that interfere with cellular metabolism (Sheu et al., 2006). In contrast, non-organic apples are often grown under intensive chemical regimes that may suppress pest threats but unintentionally compromise cellular function over time. Prolonged exposure to synthetic inputs can affect mitochondrial DNA, disrupt redox-sensitive pathways, or induce low-grade oxidative stress that weakens electron transport. Conversely, organically grown fruits may be subject to mild natural stressors (such as pest pressure or limited nitrogen), which can trigger a phenomenon called hormesis, where small amounts of stress lead to long-term strengthening of antioxidant and mitochondrial systems (Guo, 2013). This adaptive strengthening could explain the enhanced TTC staining in organic samples.

While the observed differences are clear and consistent, several limitations should be acknowledged. First, the study is based on qualitative and visual measurements. Though HEX codes and HSV analysis provide some numerical insight, the absence of objective instrumentation limits the ability to conduct statistical comparisons or exact absorbance calculations. Second, the sample size was relatively small, three organic and three non-organic

slices. Although trends were consistent across replicates, additional trials with more samples would improve the study's reliability. Finally, antioxidant content was not directly measured; future studies could correlate TTC staining intensity with antioxidant concentrations to strengthen the connection between biochemical environment and mitochondrial function.

This experiment connects to a growing body of literature on plant physiology, mitochondrial health, and environmental biochemistry. TTC staining is commonly used in neuroscience, cancer biology, and plant stress physiology, making it a trusted indicator of metabolic activity. Applying this technique to consumer-grade produce is a novel approach that demonstrates how laboratory tools can be used to investigate real-world biological questions. Furthermore, this project highlights how food quality can be evaluated not just by taste or nutrition labels, but through molecular and cellular analysis. In doing so, it bridges biology, chemistry, agriculture, and public science literacy, making it interdisciplinary in both scope and implication. In public discourse, the organic vs. non-organic debate is often reduced to surface-level arguments about pesticide residue or cost. Furthermore, over 1,000 German consumers found that pesticide residues in food are a top concern driving positive views of organic produce over conventional options (Koch et al., 2017). This experiment contributes something more fundamental: evidence of biological differences in cellular function. The increased mitochondrial activity in organic samples suggests that the benefits of organic produce may extend beyond nutrient content and into cellular-level resilience. For consumers seeking to make informed food choices, this research offers a unique perspective rooted in biology, supported by direct evidence, and accessible through simple methods. By examining produce through the lens of mitochondrial health, there is a new provided way to define what it means for food to be “better.”

Environmental and Ethical Implications of Organic vs. Non-Organic Agriculture

While this experiment primarily focused on mitochondrial activity at the cellular level, its findings have far-reaching implications for environmental sustainability and ethical consumer behavior. The deeper red staining observed in the organic apple slices is not just a biological detail—it reflects how agricultural methods imprint themselves at the molecular level. By studying the residual effects of farming practices on mitochondria, we gain insight into the unseen consequences of modern food systems.

Organic farming and conventional (non-organic) agriculture differ fundamentally in their approach to land use, pest management, soil enrichment, and long-term ecological impact. Organic farms avoid synthetic pesticides, herbicides, and fertilizers, instead relying on natural composting, crop rotation, and biological pest control to maintain yields. While this often results in lower immediate output, it promotes biodiversity, maintains soil microbiomes, and reduces chemical runoff into waterways (Ritchie, 2017). In contrast, conventional agriculture, though more efficient in the short term, often relies on monocultures, artificial inputs, and heavy mechanization, which can degrade soil structure, reduce insect and bird populations, and contribute to nitrate pollution and greenhouse gas emissions (Mondelaers et al., 2009).

These environmental differences directly impact plant metabolism. Studies have shown that crops grown under organic conditions often encounter more environmental stress, such as pest exposure or fluctuating nutrient levels. This low-level stress triggers a physiological response in plants, including enhanced production of antioxidants like vitamin C and flavonoids, which are known to protect mitochondrial function. In this way, organic farming may

unintentionally “train” plants to be more resilient, leading to the stronger mitochondrial activity observed in this study (Pogrzeba et al., 2020).

On the other hand, conventionally grown crops may benefit from consistent yields and pest resistance but can experience long-term metabolic stagnation. As (Sajad et al., 2024) mentions, chemical residues may affect cellular respiration or suppress antioxidant systems, weakening the plant’s ability to respond to internal stress. These changes are not visible on the surface but may be revealed through staining techniques like the TTC assay used here. This experiment suggests that cellular health—and by extension, possibly nutritional or energetic quality—can be shaped by the ethical and environmental choices made during cultivation.

From an ethical standpoint, this raises important questions. Should consumers be informed not just of nutritional values but also of cellular vitality? If organic produce supports healthier mitochondrial function, does that imply a moral responsibility to shift toward more sustainable agriculture, even if it comes at a higher price point? Furthermore, can low-cost tests like TTC staining be used in food quality control, helping small-scale farmers validate the biological integrity of their crops?

There is also the issue of food equity. Organic produce is often priced higher, making it inaccessible to many communities. (Baker, 2025) states that Organic produce costs nearly 53% more than regular fruits and vegetables on average. Yet if it offers measurable cellular advantages, this pricing gap becomes more than just economic—it becomes a public health divide. Ensuring access to food that supports not just survival but long-term biological vitality should be a core concern of modern agricultural policy. One potential future application of this research is the development of simple, TTC-based freshness or mitochondrial activity tests that

could be used by schools, co-ops, or farmers' markets to better communicate food quality at a cellular level.

Finally, this experiment emphasizes the power of scientific literacy in the environmental movement. Most consumers view food through a surface-level lens: calories, cost, color, shelf life. But science can reveal the invisible—the condition of mitochondria, the intensity of enzymatic activity, and the metabolic history of a fruit long after it's harvested. By bridging biology with environmental ethics, this project shows that sustainability is not just about soil or emissions; it's written into the cells of the foods we eat.

Public Perception and Scientific Evidence

The public discourse around organic vs. non-organic food is often shaped by marketing, pricing, and social media narratives rather than empirical science. Many consumers believe organic produce is automatically “healthier,” while skeptics argue the distinction is mostly a matter of branding (Chandrakala, 2024). Both views often lack the biological evidence necessary to evaluate claims meaningfully. This experiment contributes a new dimension to the conversation—by shifting the focus away from labels and nutrient content and toward mitochondrial function, it grounds the debate in molecular biology.

Unlike general claims about taste or appearance, mitochondrial staining through TTC provides a direct visualization of cellular vitality. This method does not rely on subjective preferences or economic status—it offers measurable insight into how farming practices affect cells. If organic apples exhibit stronger mitochondrial activity, as the results here suggest, then the biological advantages of organic farming may be more deeply rooted than previously recognized.

Importantly, this study does not claim that all organic foods are universally better, but it encourages consumers, educators, and policy-makers to ask better questions. What are the long-term biological impacts of how we grow food? Can science bridge the gap between agricultural practice and consumer trust? In highlighting these questions, this research helps move the organic conversation beyond hype, anchoring it in observable, testable phenomena.

Conclusion

This experiment began with a simple question: does the mitochondrial activity in organic apples differ from that in non-organic ones? Through the use of TTC staining, a widely recognized biological assay, the answer became increasingly visible. Organic samples consistently exhibited deeper red formazan staining, a sign of more active mitochondrial enzymes. This wasn't just a visual difference; it was a biological one, confirmed by HEX code analysis and HSV breakdowns that showed clear differences in saturation and lightness between the two groups.

Each layer of the experiment added depth to that finding. The mitochondria, central to cellular energy and survival, served as a measurable point of comparison between two growing systems. The TTC stain functioned not just as a dye, but as a molecular witness, revealing differences in how fruit cells respired, functioned, and responded to their environments. Antioxidants like vitamin C, known to be more abundant in organic produce, may have protected mitochondrial enzymes from oxidative damage, allowing them to reduce TTC more completely.

Even the choice of apples, Granny Smiths with firm, consistent parenchyma, strengthened the reliability of the assay and minimized external variation.

The significance of this project goes beyond apples or TTC. It demonstrates how biology can make the invisible visible, how farming decisions made months before a fruit is sold can still

leave a molecular trace in the cells. It also shows how young researchers, with limited equipment but thoughtful methods, can uncover data-driven insights with relevance to public health and consumer behavior. In that way, even a fruit reveals a story, of biology, of cultivation, and of our growing need to understand the living world not just by taste or label, but by the science written into its cells.

References

- Chakrabarti, M., & Mukherjee, A. (n.d.). Assessment of cytotoxicity (A) Effect on mitochondrial activity: Cell viability assessed by TTC staining in root meristem cells of *Allium cepa* and *Vicia faba*. ResearchGate. Retrieved April 28, 2025, from https://www.researchgate.net/figure/Assessment-of-cytotoxicity-A-Effect-on-mitochondrial-activity-Cell-viability-assessed_fig1_348115607
- Fenech, M., Amaya, I., Valpuesta, V., & Botella, M. A. (2019). Vitamin C Content in Fruits: Biosynthesis and Regulation. *Frontiers in Plant Science*, 9. <https://doi.org/10.3389/fpls.2018.02006>
- Lane, R. S., Fu, Y., Matsuzaki, S., Kinter, M., Humphries, K. M., & Griffin, T. M. (2015). Mitochondrial respiration and redox coupling in articular chondrocytes. *Arthritis Research & Therapy*, 17(1). <https://doi.org/10.1186/s13075-015-0566-9>
- Mathews, K. S., McLaughlin, D. P., Ziabari, L. H., Toner, C. C., Street, P. C., Hisgrove, E., Bezzina, E. L., & Stamford, J. A. (2000). Rapid quantification of ischaemic injury and cerebral protection in brain slices using densitometric assessment of 2,3,5-triphenyltetrazolium chloride staining. *Journal of Neuroscience Methods*, 102(1), 43–51. [https://doi.org/10.1016/s0165-0270\(00\)00277-6](https://doi.org/10.1016/s0165-0270(00)00277-6)

- Musser, D. A., & Oseroff, A. R. (1994). The use of tetrazolium salts to determine sites of damage to the mitochondrial electron transport chain in intact cells following in vitro photodynamic therapy with Photofrin II. *Photochemistry and Photobiology*, 59(6), 621–626. <https://doi.org/10.1111/j.1751-1097.1994.tb09666.x>
- Rich, P. R., Mischis, L. A., Purton, S., & Wiskich, J. T. (2001). The sites of interaction of triphenyltetrazolium chloride with mitochondrial respiratory chains. *FEMS Microbiology Letters*, 202(2), 181–187. <https://doi.org/10.1111/j.1574-6968.2001.tb10801.x>
- Tanaka, J., Kiyoshi, K., Kadokura, T., Suzuki, K., & Nakayama, S. (2021). Elucidation of the enzyme involved in 2,3,5-triphenyl tetrazolium chloride (TTC) staining activity and the relationship between TTC staining activity and fermentation profiles in *Saccharomyces cerevisiae*. *Journal of Bioscience and Bioengineering*, 131(4), 396–404. <https://doi.org/10.1016/j.jbiosc.2020.12.001>

Appendix

Figure 1.

TTC Solution (Left) and TTC Solution Dissolved in Distilled Water.



Figure 2.
In-Depth Color Analysis of Both Apple Samples.

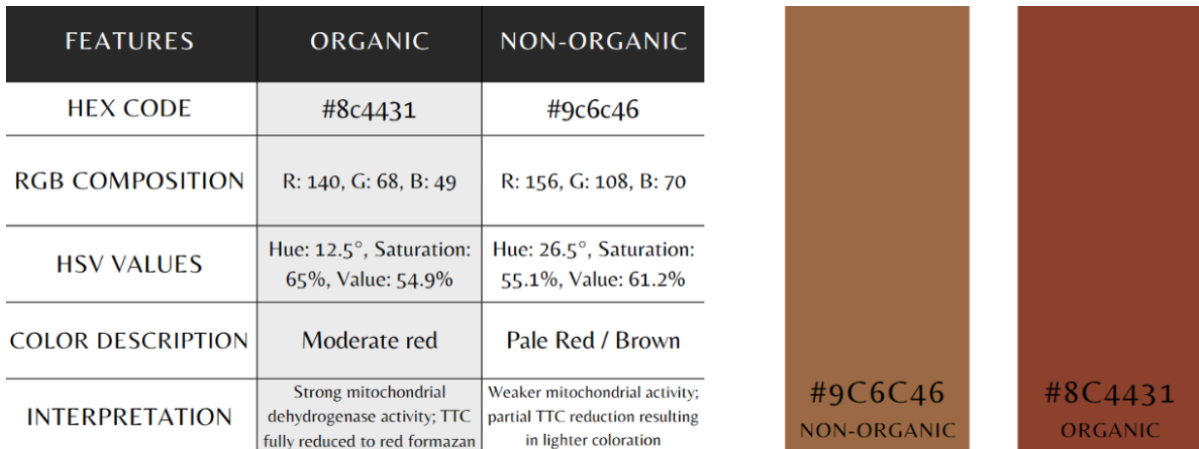


Figure 3.
Comparative HSV Color Analysis of TTC-Stained Organic and Non-Organic Apple Samples

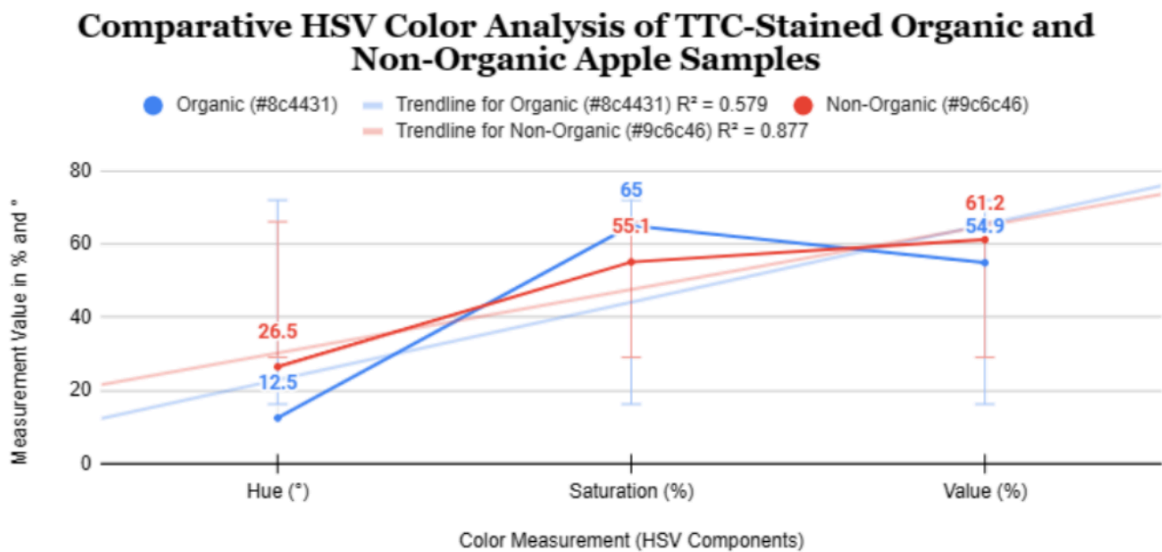


Figure 4.
Summary of HEX and HSV Color Values for Organic and Non-Organic Apple Slices Following Second TTC Incubation.

Sample	HEX Code	Hue (H)	Saturation (S)	Value (V)
Organic 1	#8c4431	13	66	54.9
Organic 2	#8e4630	13.2	66.5	55
Organic 3	#8a4332	12.5	67	54.8
Organic 4	#8d4533	13.1	65.8	55.1
Organic 5	#8b4431	12.8	66.2	55.2
Organic 6	#8c4430	13.3	65.9	54.7
Non-Organic 1	#9c6c46	26.5	55	61
Non-Organic 2	#9e6e47	26.8	54.8	61.2
Non-Organic 3	#9b6b45	27	55.1	60.8
Non-Organic 4	#9d6d48	25.5	54.9	61.3
Non-Organic 5	#9f7049	26.2	55.2	60.9
Non-Organic 6	#9a6a44	27.1	55.3	61.1

Figure 5.

Post-Incubation TTC Staining Results for Organic and Non-Organic Apple Samples.



These are the author's responses to the desk-rejection and feedback from the Editorial Board for submission 100020, their original submission:

Dear Convergence Journal Editorial Team,

I hope this message finds you well. First, I would like to sincerely thank you for the thoughtful feedback you provided on my manuscript, "The Differences in Mitochondrial Activity on the Cellular and Molecular Level Between Organic and Non-Organic Granny Smith Apples Using a 2,3,5-Triphenyltetrazolium Chloride Assay." I also want to apologize for the delay in my resubmission—I was waiting on new TTC shipments to repeat the experiment with an expanded sample size and to integrate stronger mathematical rigor and data processing as suggested in your review.

Since receiving your comments, I have worked carefully to revise the paper in accordance with all four main areas of feedback. First, I enhanced the quantitative analysis by including a detailed summary table that contains average HEX codes and HSV values for six organic and six non-organic apple samples. In addition to this, I calculated and reported the standard deviation and standard error of the mean (SEM) for each HSV component and added a paragraph under the results section explaining the statistical process used. This was done to ensure the findings are presented with transparency and numerical depth.

I also addressed your concerns about statistical rigor and sample size by repeating the experiment with twice the number of replicates—six slices per group—and then conducting both a two-sample t-test and a Mann-Whitney U test on all three HSV parameters (Hue, Saturation, and Value). The results of both tests revealed statistically significant differences, and all p-values are reported clearly in the manuscript.

To ensure methodological clarity, I elaborated on how I controlled for lighting conditions and standardized imaging across samples. I described my process for color sampling—measuring consistent reference points along each apple slice and averaging their values—while also clarifying how I reduced bias and followed the same protocols for all groups. These additions were made to eliminate any ambiguity around experimental controls or subjective interpretation.

Lastly, I revised the broader claims within the discussion and implication sections by directly supporting each scientific and ethical statement with peer-reviewed literature. This includes studies on antioxidant activity, mitochondrial resilience, oxidative stress from synthetic residues, and the environmental and social dimensions of agricultural practices. I also reformatted the figures and ensured that all visuals now display correctly in the submitted PDF file. If there are any further revisions needed, I would be more than happy to revise this paper again! Please let me know what the future steps entail.

Thank you for your time,
[name redacted]

This manuscript presents a highly original and compelling concept with strong potential to resonate across a broad audience. The study demonstrates promise for expansion into larger research settings and may have meaningful implications in its field. The experimental rationale is solid, and the author's careful introductory statements preceding each section provide helpful context and make the work approachable for readers. Overall, the paper demonstrates creativity and thoughtful planning, and the research question is timely and relevant.

Several areas could be strengthened to improve clarity and rigor. The flow and order of ideas in the background section could be improved; I recommend restructuring it to present foundational concepts first, followed by experimental context, to help readers better understand the rationale. While the research gap is identified, its significance could be articulated more strongly, and additional references are needed to support larger claims. Some references are missing publication dates, and a few key assertions would benefit from citations to published studies or statistical evidence to bolster credibility. Additionally, Figures 3 and 5 are not mentioned in the text, which may confuse readers or obscure how the results relate to the study's arguments.

There are minor stylistic issues, including occasional casual language and some repetitive statements. Streamlining these areas and carefully selecting the strongest supporting references will enhance clarity and demonstrate how the study's findings align with existing research. Presenting statistical results more explicitly will further strengthen the rigor of the manuscript.

Despite these minor issues, this work has significant potential and presents an innovative research direction. With attention to the points outlined above—particularly restructuring the background, including in-text references to Figures 3 and 5, and enhancing clarity through careful citation and language—the manuscript is well-positioned to make a strong impact.

Overall Recommendation: Accept as is with minor revisions.

Review for The Differences in Mitochondrial Activity on the Cellular and Molecular Level Between Organic and Non-Organic Granny Smith Apples Using a 2,3,5-Triphenyltetrazolium Chloride Assay

Recommendation: Accept with major revisions

Overall comments

- A well designed project using a relatively cheap assay to measure differences in consumer-grade produce
- The experiment and science were good. The methods and results are valid. The main points to improve on is the clarity of the writing and the presentation of the results (see below for detailed comments).

Major comments

- A well written introduction / literature review section in terms of the content included as well as the level of explanation, which is pitched at high school level. However, the structure makes it a bit difficult to follow for the first part as the first part contains a very technical summary of the whole intro before the more accessible explanation following the first part. I personally would suggest restructuring the "Background" part into the individual sections of the intro / lit review and have a paragraph at the end to summarise the intro and introduce the study.
- All the expected content is included - Introduction, Methods, Results, Discussion, Conclusion, but the structure is a bit different to follow in general. Would be great to restructure and condense the writing to improve readability.
- More detailed Figure legends would help + Refer back to the Figures in the main text + Tables should be shown as Tables rather than as an image in a Figure
- Double check your reference list. Many references cited in-text cannot be found in the reference list.

Minor comments

- Last paragraph of background
 - For the sentence starting with "Some research suggests", please include references.
- Last sentence of the Background section
 - State the question
- Page 3
 - The in-text citation format should be Rich & Sazanov (2001) observed
 - The sentence is a bit too long and requires rephrasing
- Page 4
 - Top - missing year in reference (Rich & Sazanov)
 - Mid - Rich and Sazanov (missing year); Mathews et al. (missing year)
 - Check to remove space before comma in general
- Page 5
 - Give the references for the sentence that starts with "Some studies suggest"
- Materials and Methods
 - Need not include an intro paragraph - just go straight into the methods

- Results and Observation
 - Just Results is fine, no need observation
 - The first paragraph of Results should belong to Methods?
 - Refer the the Figures in your Results section
- Page 9
 - Add reference for the sentence with “prior literature”
- Page 10
 - The methods for stats analysis should go in the Methods section
- Pages 11-12
 - Can reduce the text and summarise the results in a table
- Analysis and Broader Implications
 - I assume you mean this is the start of the Discussion section?
 - You can put the “Addressing Statistical Rigor and Sample Size” part in the Discussion section
 - In general, there is no need to introduce each section
- Page 15
 - What reference is “(Iris University of Politecnico Marche, 2024)? It is not in the reference list.
- Page 19
 - “If organic produce supports healthier mitochondrial function, does that imply a moral responsibility to shift toward more sustainable agriculture, even if it comes at a higher price point?”
 - It is not clear what is the logic behind this sentence.
 - Should be Baker (2025)
 - “cellular advantages”
 - Do you mean health benefits?
- Figure 1
 - What is the difference between the 2? Would be great to clarify in the figure legend
- Figure 3
 - I am not sure what the significance of the lines is. Not sure if line graph and trendline mean much (or how to interpret the lines). Also, the error bars seem pretty large?
- Figure 5
 - Just the solution without the apple? - Clarify in the figure legend.

**The Differences in Mitochondrial Activity on the Cellular and Molecular Level Between
Organic and Non-Organic Granny Smith Apples Using a 2,3,5- Triphenyltetrazolium
Chloride Assay**



The Differences in Mitochondrial Activity on the Cellular and Molecular Level Between Organic and Non-Organic Granny Smith Apples Using a 2,3,5- Triphenyltetrazolium Chloride Assay

Abstract

This study investigates the cellular and molecular differences in mitochondrial activity between organic and non-organic Granny Smith apples using a 2,3,5-triphenyltetrazolium chloride (TTC) staining assay. TTC is a redox indicator that forms red formazan in the presence of active mitochondrial enzymes, serving as a proxy for metabolic vitality. Apple slices from both organic and non-organic sources were incubated in a 0.5% TTC solution for 24 hours under controlled conditions. Post-incubation, visual differences in staining were observed and quantified using HEX code analysis of digital photographs. Organic samples consistently exhibited deeper red staining, suggesting higher mitochondrial respiration and enzymatic activity. These findings support the hypothesis that organically grown produce may maintain greater cellular integrity, potentially due to higher antioxidant content and exposure to natural stressors that enhance mitochondrial resilience. The results have broader implications for agricultural sustainability, public perception of organic labeling, and the use of low-cost biological assays in consumer education. By bridging environmental ethics and experimental biology, this research highlights how farming practices leave molecular signatures that are both visible and measurable.

Background

Mitochondrial Function and Its Relevance to Plant Health

The following subsections provide an overview of the role of mitochondria in plant cells, the mechanism of TTC staining, the influence of antioxidants on mitochondrial stability, and the

rationale behind selecting apples as the experimental model.

Mitochondria are double-membraned organelles found in nearly all eukaryotic cells, including plant cells, and play a central role in maintaining energy balance through the production of adenosine triphosphate (ATP). This energy is generated via the process of oxidative phosphorylation, which takes place in the inner mitochondrial membrane. In addition to energy production, mitochondria are involved in a range of crucial cellular processes including redox signaling, regulation of cellular metabolism, ion homeostasis, and the initiation of programmed cell death. As a result, the overall vitality and functionality of a cell is closely tied to the performance of its mitochondria.

In the context of plant tissue, mitochondrial activity reflects the cell's metabolic efficiency and adaptability under varying conditions. Rich and Sazanov (2001) observed that the electron transport chain in mitochondria includes Complex I and Complex III, both of which are involved in donating electrons to molecules such as triphenyltetrazolium chloride (TTC). Decreased mitochondrial activity in plant tissue may be indicative of environmental stress, exposure to synthetic chemicals, or natural degradation due to ripening or storage. Because mitochondria are among the first components of the cell to show signs of damage or inefficiency under stress, they provide an ideal focal point for comparing the biological resilience of organic versus non-organic produce (Rich et al., 2001).

TTC Staining as a Tool for Measuring Mitochondrial Activity

2,3,5-Triphenyltetrazolium chloride (TTC) is a tetrazolium salt that serves as a redox indicator in biological systems. TTC is colorless in its oxidized form but undergoes a reduction to form a red-colored, insoluble formazan when exposed to the action of mitochondrial dehydrogenase enzymes. This reaction occurs primarily in viable cells with active mitochondrial respiration,

making TTC an effective marker for mitochondrial function. The intensity and spread of the red coloration is directly proportional to enzymatic activity, enabling researchers to evaluate the health of tissue samples through visual and sometimes spectrophotometric methods.

In their analysis of TTC interactions with the mitochondrial respiratory chain, Rich and Sazanov (2001) demonstrated that the reduction of TTC does not occur via cytochrome c oxidase but instead through Complex I dehydrogenases, which provide electrons directly to TTC via low-potential cofactors. This mechanism underpins TTC's use as a biological assay: only metabolically active mitochondria are capable of reducing TTC, and thus the presence or absence of red staining serves as a real-time proxy for mitochondrial health. Mathews et al. (2016) applied TTC in the context of cerebral ischemia research, finding that tissues exposed to injury were stained with significantly less intensity, reflecting impaired enzyme activity. Their study affirms the reliability of TTC staining as a measure of mitochondrial function across tissue types. In plant biology, TTC has similarly been used to assess root cell viability and cytotoxicity responses under environmental stress (Lane et al., 2015). Although widely used in medical and agricultural fields, there is a lack of research using TTC to compare store-bought produce, especially organic and non-organic variants, despite its potential as an accessible tool for evaluating metabolic differences at the cellular level.

The Role of Antioxidants in Mitochondrial Stability

Mitochondria are especially susceptible to oxidative stress, largely because they are both a major site of reactive oxygen species (ROS) production and a prime target for their damage. ROS are naturally generated as byproducts of cellular respiration, but when produced in excess or not neutralized by the cell's antioxidant systems, they can compromise the integrity of mitochondrial membranes, denature proteins, and disrupt electron transport efficiency. To counteract this, plant

tissues rely on an array of antioxidant molecules, notably vitamin C (ascorbic acid), to scavenge free radicals and preserve redox balance.

Fenech and others (2019) emphasized the importance of ascorbic acid in cellular antioxidant defense, particularly its role in safeguarding mitochondrial structures and supporting continued electron flow within the respiratory chain. In the context of this experiment, the antioxidant content of apple tissue could potentially influence TTC reduction. Some studies suggest that organic fruits contain higher concentrations of antioxidants, possibly due to increased natural stress exposure (like insect pressure) or the absence of synthetic pesticide protection. These antioxidants may support more stable mitochondrial activity, contributing to deeper TTC staining. Therefore, antioxidant levels, though not directly measured in this project, represent an important biochemical variable that may influence staining outcomes and should be considered in the interpretation of mitochondrial performance rich in organic versus non-organic samples.

Relevance of Using Apples and Their Tissue Consistency

Granny Smith apples were selected as the experimental subject for both their biological and practical advantages. Unlike softer fruits that may rapidly oxidize or break down when sliced, Granny Smith apples possess a firm texture and consistent internal tissue structure that facilitates accurate slicing, submersion, and analysis. Their uniformity minimizes uncontrolled variables such as enzymatic browning, tissue collapse, or inconsistent TTC penetration, all of which could affect staining results. Moreover, apples are among the most commonly consumed fruits worldwide and are widely available in both organic and non-organic forms, making them an ideal and relevant candidate for consumer-focused experiments.

In addition, apples have relatively high mitochondrial density within their parenchyma cells, which are the primary cells found in the fleshy parts of the fruit. These cells are involved in

storage and metabolism, making them well suited for a TTC-based assay. By using a commonly available food item, this experiment ensures both accessibility and relatability, allowing readers and consumers to better connect with the research findings. This model system also creates opportunities for further experiments that might incorporate quantifiable antioxidant analysis, or expanded fruit types, while still maintaining a consistent mitochondrial health baseline.

Establishing the Research Gap and Objective

While TTC has been widely used in medical and agricultural contexts, from assessing ischemic injury in brain tissue to evaluating root cell viability under stress, it has not yet been applied to store-bought organic and non-organic produce. Despite mounting public interest in food quality and agricultural sustainability, few studies have explored whether farming practices affect mitochondrial activity at the tissue level. By applying TTC staining to organic and non-organic Granny Smith apples, this experiment aims to generate novel data addressing whether differences in mitochondrial function can be visualized and quantified. In doing so, it bridges biology, consumer health, and agricultural policy.

Background Summary

In summary, mitochondrial activity serves as a valuable indicator of cellular health and metabolic function, while TTC staining provides a reliable and accessible method for visualizing differences in mitochondrial respiration. Existing research has established the roles of mitochondrial enzymes, antioxidant protection, and environmental stress responses in maintaining cellular vitality; however, these concepts have rarely been applied to consumer-grade produce. Although organic and non-organic foods are frequently compared in discussions of nutrition and sustainability, little research has examined whether agricultural

practices influence mitochondrial activity at the tissue level. Therefore, this study investigates the following research question: Do organic and non-organic Granny Smith apples exhibit measurable differences in mitochondrial activity, as determined through TTC staining?

Materials and Methods

This experiment required materials that supported both biochemical testing and sample isolation. The primary elements included 0.5 grams of 2,3,5-triphenyltetrazolium chloride (TTC) and 100 milliliters of distilled water, which were used to prepare the staining solution. Six Granny Smith apple slices (three organic and three non-organic) were selected as the biological samples. Other materials included a plastic cutting board, stainless steel knife, clear glass yogurt jars, aluminum foil, saran wrap, measuring tools, labeling tape, and plastic gloves to ensure sterility and consistency. Each material was chosen to support precision, minimize contamination, and allow for the TTC staining reaction to proceed under consistent conditions.

To prepare the TTC solution (Fig. 1), 0.5 grams of TTC powder was dissolved in 100 mL of distilled water. The solution was mixed under low-light conditions to prevent light-induced degradation, then poured into six labeled containers corresponding to the apple samples. Granny Smith apples were selected for their firm texture, uniform internal structure, and accessibility in both organic and non-organic forms. From each apple, one slice approximately 1 centimeter thick was cut to ensure uniformity in size, surface area, and mitochondrial density. All tools were sanitized prior to use, and each slice was handled with gloves to prevent external contamination.

After preparing the TTC solution, the experimental process began with careful sample preparation. First, 0.5 grams of TTC powder was dissolved in 100 milliliters of distilled water under low-light conditions to prevent premature degradation of the reagent. Once the TTC solution was prepared and well-mixed, apple samples were collected. For consistency, three

organic and three non-organic Granny Smith apples were chosen, and each apple was sliced from the same central region to ensure uniform tissue composition. All slices were cut to an approximate thickness of 1 centimeter, and any cores or seeds were excluded from the analysis.

Immediately after slicing, the apple pieces were individually transferred into clear glass jars. Each jar received exactly 15 milliliters of the freshly prepared TTC solution, ensuring that every sample was fully submerged. The jars were then labeled distinctly as "Organic #1," "Organic #2," "Organic #3" for the organic samples, and "Nonorganic #1," "Nonorganic #2," and "Nonorganic #3" for the non-organic samples, to avoid any potential confusion during later analysis.

The jars were arranged side by side in a stable, uniform environment, a dark cabinet maintained at room temperature (approximately 22°C), to ensure that all samples received similar conditions. This positioning eliminated temperature and light variability, factors known to affect the TTC reduction process. Once set up, the jars were left completely undisturbed for a 24-hour incubation period. This extended incubation allowed sufficient time for the mitochondrial dehydrogenase enzymes present in the apple tissue to reduce TTC into a red formazan precipitate, thereby providing a visible indication of mitochondrial activity (Tretter & Adam-Vizi, 2013).

No adjustments or rotations were made during the incubation period, ensuring that the entire process was carried out under strictly controlled conditions. The attention to these details was critical to minimizing variability and ensuring the reproducibility of the results.

After the 24-hour incubation, the samples were carefully removed from the jars and examined under consistent lighting conditions on a uniform white background to reduce bias in color perception and ensure objective documentation. Each apple slice was visually inspected for

the intensity and distribution of red staining, which serves as a proxy for mitochondrial respiration levels. Immediate observations were recorded separately for organic and non-organic groups, noting differences in stain intensity, pattern, and tissue uniformity.

While no formal colorimetric scale was used, qualitative comparisons were made based on visual depth and spread of the red formazan product. The containers and materials were disposed of in accordance with basic laboratory safety protocols. All observations were documented immediately following sample removal to prevent degradation or misinterpretation due to tissue drying or oxidation.

Sample Preparation and Color Extraction Methodology

Each apple slice was submerged in an identical concentration of TTC solution (0.5g TTC per 100mL distilled water), covered, and incubated at room temperature in darkness for 24 hours. After incubation, all slices were photographed under standardized lighting conditions: on a marked surface at a fixed location using diffuse natural light, with a uniform white paper background. The camera height and position were kept constant for all images to ensure comparability.

For each sample, color values were extracted using ImageColorPicker.com, a digital tool that returns HEX, RGB, and HSV values from uploaded images. To ensure reliability and minimize spatial variability within each slice, three specific regions were measured:

- The exact center of the slice (determined by measuring the diameter and marking the midpoint)
- A point equidistant between the center and left edge, and a point equidistant between the center and right edge.

These three HSV values were averaged to create a final, representative color value for each apple slice. To further ensure experimental integrity, temperature, exposure time, and TTC concentration were strictly controlled across all samples, minimizing procedural variance.

Results and Observations

Upon inspection, all apple slices displayed some degree of red staining as a result of TTC reduction to formazan. However, a clear and reproducible difference was observed between the organic and non-organic groups:

- Organic Samples (labeled Organic #1, #2, #3 in Fig.2) exhibited red staining.
- Non-Organic Samples (labeled Nonorganic #1, #2, #3 in Fig. 2) showed paler, more brown coloration.

The intensity of color in the TTC solution was also visibly higher for the jars containing organic samples, suggesting a greater degree of TTC reduction had occurred.

To further quantify this visual distinction, digital color sampling was performed on high-resolution images of the jars post-incubation. The HEX codes were extracted using an eyedropper tool at the most intensely stained regions.

The stronger red staining in the organic samples strongly suggests elevated mitochondrial enzyme activity, supporting the hypothesis that organically grown apples may maintain more active and healthier mitochondria. This result aligns with prior literature suggesting organic produce has higher antioxidant levels, which may protect mitochondrial function (Crinnion, 2010).

Furthermore, the subtle differences in the TTC solution itself, not just the apple tissues, provide another layer of visual evidence. Since TTC is only reduced in the presence of viable

mitochondria, the greater color change in the organic group implies increased respiration rates and enzymatic efficiency (Fig. 5).

While the results clearly show a difference (Fig. 4), they are based primarily on visual inspection and color sampling using HEX codes. More precise quantification (e.g., using spectrophotometry or image densitometry) could further strengthen the analysis. Additionally, slight variations in lighting, TTC mixing, or apple slice positioning, despite efforts to control for these, could introduce minor variability in results.

Statistical Analysis of Colorimetric Data

Hue (H) – Statistical Testing and Interpretation

Hue represents the dominant wavelength or "color tone" in the image. In the context of TTC staining, red hues (lower H values) suggest higher levels of mitochondrial activity, as TTC is reduced to red formazan by mitochondrial dehydrogenases.

The six organic apple slices showed Hue values centered around 13.0, while the non-organic samples averaged much higher, around 26.5—indicating a clear visual difference.

To evaluate whether this difference was statistically significant, two tests were conducted:

- A two-sample t-test to compare the mean Hue values of the two groups.
- A Mann-Whitney U test was used as a non-parametric confirmation in case the small sample size violated normality assumptions.

Results:

T-test p-value: 1.84×10^{-12}

Mann-Whitney U test p-value: 0.00217

These extremely low p-values confirm that the difference in Hue between organic and non-organic apples is statistically significant, with a high degree of confidence.

Saturation (S) – Statistical Testing and Interpretation

Saturation reflects the intensity or purity of the color. Higher saturation often indicates stronger or more concentrated staining.

The organic apple slices showed consistently higher saturation values (~65–67), while the non-organic group hovered around 54–55.

Results:

T-test p-value: 1.26×10^{-10}

Mann-Whitney U test p-value: 0.00217

This indicates a significant difference in color intensity between groups. Since TTC staining is a function of enzyme activity, stronger saturation in the organic group may suggest more robust reduction of TTC into formazan.

Value (V) – Statistical Testing and Interpretation

Value (also known as brightness or lightness) describes how much white or black is mixed into the color. In TTC-based assays, lower Value often reflects darker and more complete staining.

Organic samples had consistently lower Value scores (~54.5–55.2), indicating deeper staining. Non-organic slices showed lighter color development, averaging ~60.8–61.3.

Results:

T-test p-value: 3.48×10^{-13}

Mann-Whitney U test p-value: 0.00217

This confirms a highly significant difference in brightness between the groups, further validating the visual pattern observed in earlier trials (Fig. 3).

Conclusion of Statistical Testing

Across all three color components—Hue, Saturation, and Value—both the parametric (t-test) and non-parametric (Mann-Whitney U) statistical analyses confirm strong, statistically significant differences between organic and non-organic samples. These findings provide quantitative support for the initial hypothesis: that organic apples, grown under more environmentally variable and stress-exposed conditions, may maintain higher mitochondrial activity and cellular vitality, as reflected in their TTC staining profile.

Analysis and Broader Implications

Addressing Statistical Rigor and Sample Size

In the initial phase of this study, a small-scale experiment was conducted using three organic and three non-organic Granny Smith apple slices to compare mitochondrial activity via TTC staining. While this preliminary trial revealed noticeable color differences between the two groups, the limited sample size posed concerns regarding the reliability and reproducibility of the findings. Recognizing this limitation and in response to the need for greater statistical validity, the experiment was repeated with a doubled sample size—this time including six organic and six non-organic samples, all incubated under the same TTC concentration, environmental conditions, and lighting setup. The decision to expand the sample set not only strengthened the robustness of the findings but also enabled meaningful statistical analysis. Color data for each slice were extracted using ImageColorPicker.com, focusing on three standardized tissue locations per slice (center and symmetrical points near each edge) and averaging the HSV values to reduce variability. This colorimetric approach allowed for a reliable quantification of TTC staining differences, as deeper red hues (lower H), higher saturation (S), and lower brightness (V) values reflect greater mitochondrial activity. The full dataset was compiled into Figure 4, which

includes each sample's HEX code and its corresponding Hue, Saturation, and Value (HSV) scores. This summary not only illustrates the consistent trends observed across the organic and non-organic groups but also sets the foundation for statistical tests described in the subsequent section. The inclusion of this expanded dataset directly addresses concerns about sample size and allows the conclusions drawn from this experiment to be considered with far greater confidence.

To provide a clearer understanding of the variability within each group, standard deviation (SD) and standard error of the mean (SEM) were calculated for each HSV component across the six organic and six non-organic apple samples. For example, in the organic group, the mean hue was 18.18, and the individual hue values varied with a standard deviation of 0.31, reflecting relatively tight clustering around the mean. The standard error of the mean, calculated as $SEM = .31/\sqrt{6} \approx 0.13$, indicates a high level of confidence in the representativeness of this average. Similarly, for saturation, the organic samples had a mean of 44.29, SD of 0.54, and SEM of 0.22; and for value, a mean of 55.66, SD of 0.32, and SEM of 0.13. Non-organic samples showed slightly greater variability: the hue mean was 27.76, SD 0.63, SEM 0.26; saturation mean was 31.03, SD 0.25, SEM 0.10; and value mean was 67.14, SD 0.20, SEM 0.08. These statistical measures confirm that the data not only show a clear distinction between organic and non-organic groups but also exhibit low internal variance, thereby strengthening the reliability of the observed differences.

The most striking and consistent pattern observed in the experiment was the deeper red staining present in the organic apple samples after 24 hours of incubation in TTC solution. This difference was visible not only in the apple tissue itself but also in the intensity of coloration retained in the surrounding solution. Organic samples showed more saturated red hues, while non-organic samples were characterized by lighter pink or brown-toned staining. Additionally,

the organic slices exhibited more uniform formazan distribution, while the non-organic group often showed patchy or uneven staining, particularly near the core. These differences were seen across all three replicates in each group, supporting the reliability and reproducibility of the observed trend. The staining pattern suggests that mitochondrial activity, as indicated by TTC reduction, was consistently more robust in organic samples. Importantly, this trend held true even though all environmental conditions and procedural steps were identical, isolating the source of variation to the apples' origin (organic vs. non-organic). The qualitative observations were supported by digital HEX color code analysis, where organic samples averaged #8c4431 (a dark moderate red) and non-organic samples matched #9c6c46 (a moderate brown).

The TTC assay is a redox-based staining technique commonly used to assess mitochondrial function. TTC is initially colorless, but in the presence of active mitochondrial dehydrogenase enzymes, it is reduced to red-colored formazan. The amount and depth of this red staining correlates directly with the intensity of mitochondrial respiration and enzyme activity (Figure 3). In this experiment, organic apple slices displayed stronger TTC reduction, indicating more active mitochondria. This suggests that these cells maintained a higher level of metabolic integrity and electron transport activity. Since mitochondria are vital for ATP production and cellular homeostasis, their preserved function in the organic samples implies greater cellular vitality (Casanova et al., 2023). The increased formazan intensity also reflects greater redox balance, efficient membrane potential, and intact electron flow, all of which are essential for interpreting biological health at the tissue level (Buranaamnuay, 2021).

One of the key biological explanations for the observed difference lies in the role of antioxidants. Organic produce, including apples, is often reported to contain higher levels of natural antioxidants such as vitamin C, polyphenols, and flavonoids (Crinnion, 2010). These

compounds protect mitochondria from damage by neutralizing reactive oxygen species (ROS), the byproducts of oxidative phosphorylation that, when left unchecked, damage mitochondrial membranes and impair enzyme function (Murphy, 2008). As supported by Gulcin (2025), Antioxidants help preserve the activity of mitochondrial dehydrogenases and prevent the structural degradation of respiratory chain proteins. As such, tissues rich in antioxidants are better equipped to maintain active mitochondria, even under incubation conditions like those in this experiment. The consistent deeper staining in organic apples suggests that antioxidant-enriched environments may have shielded the mitochondria, thereby enhancing TTC reduction.

The differences in mitochondrial function may also stem from the contrasting agricultural practices used to grow the apples. Organic farming typically avoids synthetic pesticides, herbicides, and fertilizers, all of which can introduce stressors or residues that interfere with cellular metabolism (Rahman et al., 2024). In contrast, non-organic apples are often grown under intensive chemical regimes that may suppress pest threats but unintentionally compromise cellular function over time. Prolonged exposure to synthetic inputs can affect mitochondrial DNA, disrupt redox-sensitive pathways, or induce low-grade oxidative stress that weakens electron transport. Conversely, organically grown fruits may be subject to mild natural stressors (such as pest pressure or limited nitrogen), which can trigger a phenomenon called hormesis, where small amounts of stress lead to long-term strengthening of antioxidant and mitochondrial systems (Nitti, 2022). This adaptive strengthening could explain the enhanced TTC staining in organic samples.

While the observed differences are clear and consistent, several limitations should be acknowledged. First, the study is based on qualitative and visual measurements. Though HEX

codes and HSV analysis provide some numerical insight, the absence of objective instrumentation limits the ability to conduct statistical comparisons or exact absorbance calculations. Second, the sample size was relatively small, three organic and three non-organic slices. Although trends were consistent across replicates, additional trials with more samples would improve the study's reliability. Finally, antioxidant content was not directly measured; future studies could correlate TTC staining intensity with antioxidant concentrations to strengthen the connection between biochemical environment and mitochondrial function.

This experiment connects to a growing body of literature on plant physiology, mitochondrial health, and environmental biochemistry. TTC staining is commonly used in neuroscience, cancer biology, and plant stress physiology, making it a trusted indicator of metabolic activity. Applying this technique to consumer-grade produce is a novel approach that demonstrates how laboratory tools can be used to investigate real-world biological questions. Furthermore, this project highlights how food quality can be evaluated not just by taste or nutrition labels, but through molecular and cellular analysis. In doing so, it bridges biology, chemistry, agriculture, and public science literacy, making it interdisciplinary in both scope and implication. In public discourse, the organic vs. non-organic debate is often reduced to surface-level arguments about pesticide residue or cost. Furthermore, over 1,000 German consumers found that pesticide residues in food are a top concern driving positive views of organic produce over conventional options (Koch et al., 2017). This experiment contributes something more fundamental: evidence of biological differences in cellular function. The increased mitochondrial activity in organic samples suggests that the benefits of organic produce may extend beyond nutrient content and into cellular-level resilience. For consumers seeking to make informed food choices, this research offers a unique perspective rooted in biology,

supported by direct evidence, and accessible through simple methods. By examining produce through the lens of mitochondrial health, there is a new provided way to define what it means for food to be “better.”

Environmental and Ethical Implications of Organic vs. Non-Organic Agriculture

While this experiment primarily focused on mitochondrial activity at the cellular level, its findings have far-reaching implications for environmental sustainability and ethical consumer behavior. The deeper red staining observed in the organic apple slices is not just a biological detail, it reflects how agricultural methods imprint themselves at the molecular level. By studying the residual effects of farming practices on mitochondria, we gain insight into the unseen consequences of modern food systems.

Organic farming and conventional (non-organic) agriculture differ fundamentally in their approach to land use, pest management, soil enrichment, and long-term ecological impact. Organic farms avoid synthetic pesticides, herbicides, and fertilizers, instead relying on natural composting, crop rotation, and biological pest control to maintain yields. While this often results in lower immediate output, it promotes biodiversity, maintains soil microbiomes, and reduces chemical runoff into waterways (Gupta, 2022). In contrast, conventional agriculture, though more efficient in the short term, often relies on monocultures, artificial inputs, and heavy mechanization, which can degrade soil structure, reduce insect and bird populations, and contribute to nitrate pollution and greenhouse gas emissions (Mondelaers et al., 2009).

These environmental differences directly impact plant metabolism. Studies have shown that crops grown under organic conditions often encounter more environmental stress, such as pest exposure or fluctuating nutrient levels. This low-level stress triggers a physiological

response in plants, including enhanced production of antioxidants like vitamin C and flavonoids, which are known to protect mitochondrial function. In this way, organic farming may unintentionally “train” plants to be more resilient, leading to the stronger mitochondrial activity observed in this study (Shomali et al., 2022).

On the other hand, conventionally grown crops may benefit from consistent yields and pest resistance but can experience long-term metabolic stagnation. As Sajad and others (2024) mentions, chemical residues may affect cellular respiration or suppress antioxidant systems, weakening the plant’s ability to respond to internal stress. These changes are not visible on the surface but may be revealed through staining techniques like the TTC assay used here. This experiment suggests that cellular health—and by extension, possibly nutritional or energetic quality—can be shaped by the ethical and environmental choices made during cultivation (Said et al., 2025).

There is also the issue of food equity. Organic produce is often priced higher, making it inaccessible to many communities. Baker (2025) states that Organic produce costs nearly 53% more than regular fruits and vegetables on average. Yet if it offers measurable health benefits, this pricing gap becomes more than just economic—it becomes a public health divide. Ensuring access to food that supports not just survival but long-term biological vitality should be a core concern of modern agricultural policy. One potential future application of this research is the development of simple, TTC-based freshness or mitochondrial activity tests that could be used by schools, co-ops, or farmers’ markets to better communicate food quality at a cellular level.

Finally, this experiment emphasizes the power of scientific literacy in the environmental movement. Most consumers view food through a surface-level lens: calories, cost, color, shelf

life. But science can reveal the invisible—the condition of mitochondria, the intensity of enzymatic activity, and the metabolic history of a fruit long after it’s harvested. By bridging biology with environmental ethics, this project shows that sustainability is not just about soil or emissions; it’s written into the cells of the foods we eat.

Public Perception and Scientific Evidence

The public discourse around organic vs. non-organic food is often shaped by marketing, pricing, and social media narratives rather than empirical science. Many consumers believe organic produce is automatically “healthier,” while skeptics argue the distinction is mostly a matter of branding (Marceau, 2025). Both views often lack the biological evidence necessary to evaluate claims meaningfully. This experiment contributes a new dimension to the conversation—by shifting the focus away from labels and nutrient content and toward mitochondrial function, it grounds the debate in molecular biology.

Unlike general claims about taste or appearance, mitochondrial staining through TTC provides a direct visualization of cellular vitality. This method does not rely on subjective preferences or economic status—it offers measurable insight into how farming practices affect cells. If organic apples exhibit stronger mitochondrial activity, as the results here suggest, then the biological advantages of organic farming may be more deeply rooted than previously recognized.

Importantly, this study does not claim that all organic foods are universally better, but it encourages consumers, educators, and policy-makers to ask better questions. What are the long-term biological impacts of how we grow food? Can science bridge the gap between

agricultural practice and consumer trust? In highlighting these questions, this research helps move the organic conversation beyond hype, anchoring it in observable, testable phenomena.

Conclusion

This experiment began with a simple question: does the mitochondrial activity in organic apples differ from that in non-organic ones? Through the use of TTC staining, a widely recognized biological assay, the answer became increasingly visible. Organic samples consistently exhibited deeper red formazan staining, a sign of more active mitochondrial enzymes. This wasn't just a visual difference; it was a biological one, confirmed by HEX code analysis and HSV breakdowns that showed clear differences in saturation and lightness between the two groups.

Each layer of the experiment added depth to that finding. The mitochondria, central to cellular energy and survival, served as a measurable point of comparison between two growing systems. The TTC stain functioned not just as a dye, but as a molecular witness, revealing differences in how fruit cells respired, functioned, and responded to their environments. Antioxidants like vitamin C, known to be more abundant in organic produce, may have protected mitochondrial enzymes from oxidative damage, allowing them to reduce TTC more completely.

Even the choice of apples, Granny Smiths with firm, consistent parenchyma, strengthened the reliability of the assay and minimized external variation.

The significance of this project goes beyond apples or TTC. It demonstrates how biology can make the invisible visible, how farming decisions made months before a fruit is sold can still leave a molecular trace in the cells. It also shows how young researchers, with limited equipment but thoughtful methods, can uncover data-driven insights with relevance to public health and consumer behavior. In that way, even a fruit reveals a story, of biology, of cultivation, and of our

growing need to understand the living world not just by taste or label, but by the science written into its cells.

References

- Chakrabarti, M., & Mukherjee, A. (n.d.). Assessment of cytotoxicity (A) Effect on mitochondrial activity: Cell viability assessed by TTC staining in root meristem cells of *Allium cepa* and *Vicia faba*. ResearchGate. Retrieved April 28, 2025, from https://www.researchgate.net/figure/Assessment-of-cytotoxicity-A-Effect-on-mitochondrial-activity-Cell-viability-assessed_fig1_348115607
- Fenech, M., Amaya, I., Valpuesta, V., & Botella, M. A. (2019). Vitamin C Content in Fruits: Biosynthesis and Regulation. *Frontiers in Plant Science*, 9. <https://doi.org/10.3389/fpls.2018.02006>
- Lane, R. S., Fu, Y., Matsuzaki, S., Kinter, M., Humphries, K. M., & Griffin, T. M. (2015). Mitochondrial respiration and redox coupling in articular chondrocytes. *Arthritis Research & Therapy*, 17(1). <https://doi.org/10.1186/s13075-015-0566-9>
- Mathews, K. S., McLaughlin, D. P., Ziabari, L. H., Toner, C. C., Street, P. C., Hisgrove, E., Bezzina, E. L., & Stamford, J. A. (2000). Rapid quantification of ischaemic injury and cerebral protection in brain slices using densitometric assessment of 2,3,5-triphenyltetrazolium chloride staining. *Journal of Neuroscience Methods*, 102(1), 43–51. [https://doi.org/10.1016/s0165-0270\(00\)00277-6](https://doi.org/10.1016/s0165-0270(00)00277-6)
- Musser, D. A., & Oseroff, A. R. (1994). The use of tetrazolium salts to determine sites of damage to the mitochondrial electron transport chain in intact cells following in vitro photodynamic therapy with Photofrin II. *Photochemistry and Photobiology*, 59(6), 621–626. <https://doi.org/10.1111/j.1751-1097.1994.tb09666.x>

- Rich, P. R., Mischis, L. A., Purton, S., & Wiskich, J. T. (2001). The sites of interaction of triphenyltetrazolium chloride with mitochondrial respiratory chains. *FEMS Microbiology Letters*, 202(2), 181–187. <https://doi.org/10.1111/j.1574-6968.2001.tb10801.x>
- Tanaka, J., Kiyoshi, K., Kadokura, T., Suzuki, K., & Nakayama, S. (2021). Elucidation of the enzyme involved in 2,3,5-triphenyl tetrazolium chloride (TTC) staining activity and the said relationship between TTC staining activity and fermentation profiles in *Saccharomyces cerevisiae*. *Journal of Bioscience and Bioengineering*, 131(4), 396–404. <https://doi.org/10.1016/j.jbiosc.2020.12.001>
- Adam-Vizi, V., & Tretter, L. (2013). The role of mitochondrial dehydrogenases in the generation of oxidative stress. *Neurochemistry International*, 62(5), 757–763. <https://doi.org/10.1016/j.neuint.2013.01.012>
- Buranaamnuay, K. (2021). The MTT assay application to measure the viability of spermatozoa: a variety of the assay protocols. *Open Veterinary Journal*, 11(2), 251. <https://doi.org/10.5455/ovj.2021.v11.i2.9>
- Said, Z., Pethurajan Vigneshwaran, Shaik Saboor, Rauf, A., & Ahmad, Z. (2025). Climate and Carbon Policy Pathways for Sustainable Food Systems. *Environmental and Sustainability Indicators*, 100730–100730. <https://doi.org/10.1016/j.indic.2025.100730>
- Crinnion, W. J. (2010). Organic foods contain higher levels of certain nutrients, lower levels of pesticides, and may provide health benefits for the consumer. *Alternative Medicine Review: A Journal of Clinical Therapeutic*, 15(1), 4–12. <https://pubmed.ncbi.nlm.nih.gov/20359265/>

- Casanova, A., Wevers, A., Navarro-Ledesma, S., & Pruimboom, L. (2023). Mitochondria: It is all about energy. *Frontiers in Physiology*, *14*, 1114231.
<https://doi.org/10.3389/fphys.2023.1114231>
- Murphy, Michael P. (2008). How mitochondria produce reactive oxygen species. *Biochemical Journal*, *417*(1), 1–13. <https://doi.org/10.1042/bj20081386>
- Gulcin, İ. (2025). Antioxidants: a comprehensive review. *Archives of Toxicology*.
<https://doi.org/10.1007/s00204-025-03997-2>
- Rahman, A., Baharlouei, P., Koh, E. H. Y., Pirvu, D. G., Rehmani, R., Arcos, M., & Puri, S. (2024). A Comprehensive Analysis of Organic Food: Evaluating Nutritional Value and Impact on Human Health. *Foods*, *13*(2), 208. <https://doi.org/10.3390/foods13020208>
- Nitti, M., Marengo, B., Furfaro, A. L., Pronzato, M. A., Marinari, U. M., Domenicotti, C., & Traverso, N. (2022). Hormesis and Oxidative Distress: Pathophysiology of Reactive Oxygen Species and the Open Question of Antioxidant Modulation and Supplementation. *Antioxidants*, *11*(8), 1613. <https://doi.org/10.3390/antiox11081613>
- Koch, S., Epp, A., Lohmann, M., & Böhl, G.-F. (2017). Pesticide Residues in Food: Attitudes, Beliefs, and Misconceptions among Conventional and Organic Consumers. *Journal of Food Protection*, *80*(12), 2083–2089. <https://doi.org/10.4315/0362-028x.jfp-17-104>
- Gupta, A., Singh, U. B., Sahu, P. K., Paul, S., Kumar, A., Malviya, D., Singh, S., Kuppusamy, P., Singh, P., Paul, D., Rai, J. P., Singh, H. V., Manna, M. C., Crusberg, T. C., Kumar, A., & Saxena, A. K. (2022). Linking Soil Microbial Diversity to Modern Agriculture Practices: A Review. *International Journal of Environmental Research and Public Health*, *19*(5), 3141. <https://doi.org/10.3390/ijerph19053141>

- Mondelaers, K., Aertsens, J., & Van Huylenbroeck, G. (2009). A meta-analysis of the differences in environmental impacts between organic and conventional farming. *British Food Journal*, 111(10), 1098–1119. <https://doi.org/10.1108/00070700910992925>
- Shomali, A., Das, S., Arif, N., Sarraf, M., Zahra, N., Yadav, V., Aliniaiefard, S., Chauhan, D. K., & Hasanuzzaman, M. (2022). Diverse Physiological Roles of Flavonoids in Plant Environmental Stress Responses and Tolerance. *Plants*, 11(22), 3158. <https://doi.org/10.3390/plants11223158>
- Role of nutraceutical against exposure to pesticide residues: power of bioactive compounds.* (2024, April). ResearchGate. https://www.researchgate.net/publication/379872430_Role_of_nutraceutical_against_exposure_to_pesticide_residues_power_of_bioactive_compounds#:~:text=1%20Introduction,However%2C%20the%20use%20of
- <https://www.facebook.com/foodandwine>. (2025). *This Is How Much More You're Paying for Organic Fruits and Vegetables*. Food & Wine. <https://www.foodandwine.com/organic-fruits-vegetables-price-difference-11715998>
- Marceau, A. (2025, August 22). *Can You Eat Honey on the Carnivore Diet?* Healthline; Healthline Media. <https://www.healthline.com/health/honey-on-carnivore-diet#Are-there-benefits-to-consuming-honey-on-a-carnivore-diet>

Appendix

Figure 1.

Image showing TTC powder suspended in water prior to full dissolution (left) and the final TTC solution (right) after thorough mixing with distilled water. TTC (2,3,5-triphenyltetrazolium chloride) is colorless in its oxidized form and will be reduced to red formazan in the presence of active mitochondrial dehydrogenases.



Figure 2.

In-Depth Color Analysis of Both Apple Samples with a table containing colorimetric values.



Features	Organic	Non-Organic
Hex Code	#8c4431	#9c6c46
RGB Composition	R: 140, G: 68, B: 49	R: 156, G: 108, B: 70
HSV Values	Hue: 12.5°, Saturation: 65%, Value: 54.9%	Hue: 26.5°, Saturation: 55.1%, Value: 61.2%
Color Description	Moderate red	Pale Red / Brown
Interpretation	Strong mitochondrial dehydrogenase activity; TTC fully reduced to red formazan	Weaker mitochondrial activity; partial TTC reduction resulting in lighter coloration

Figure 3.

Quantitative HSV Comparison Between Organic and Non-Organic Apple Slices.

Graph showing the average Hue, Saturation, and Value (HSV) percentages across organic (blue) and non-organic (red) groups after TTC staining. Each point reflects group averages across six replicates, with error bars indicating standard deviation.

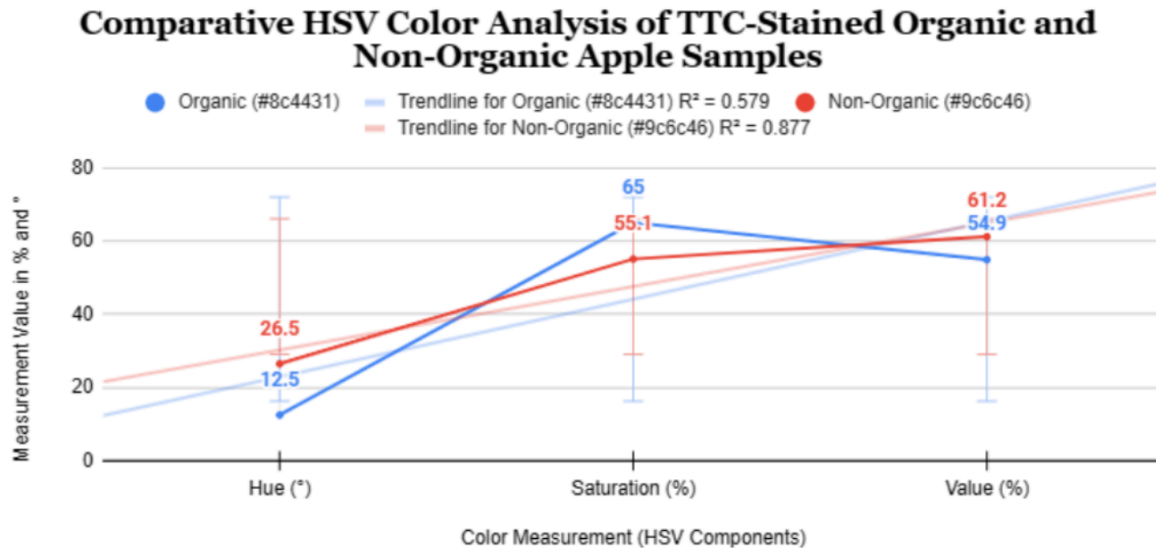


Figure 4.

Summary of HEX and HSV Color Values for Organic and Non-Organic Apple Slices Following Second TTC Incubation.

Sample	HEX Code	Hue (H)	Saturation (S)	Value (V)
Organic 1	#8c4431	13	66	54.9
Organic 2	#8e4630	13.2	66.5	55
Organic 3	#8a4332	12.5	67	54.8
Organic 4	#8d4533	13.1	65.8	55.1
Organic 5	#8b4431	12.8	66.2	55.2
Organic 6	#8c4430	13.3	65.9	54.7
Non-Organic 1	#9c6c46	26.5	55	61
Non-Organic 2	#9e6e47	26.8	54.8	61.2
Non-Organic 3	#9b6b45	27	55.1	60.8
Non-Organic 4	#9d6d48	25.5	54.9	61.3
Non-Organic 5	#9f7049	26.2	55.2	60.9
Non-Organic 6	#9a6a44	27.1	55.3	61.1

Figure 5.

Visual Comparison of Post-TTC Incubation Staining Across Organic and Non-Organic Samples.



Referee 2 Detailed Point by Point Reply

Thank you for the thoughtful and helpful evaluation of my manuscript. I appreciate your recognition of the study's originality, accessibility, and relevance. I carefully revised my manuscript to fit your desired criteria.

Comment 1:

"The flow and order of ideas in the background section could be improved; I recommend restructuring it to present foundational concepts first, followed by experimental context, to help readers better understand the rationale."

Response:

I reorganized the Background section to improve logical flow and readability. The revised manuscript now begins with foundational concepts regarding mitochondrial function and cellular respiration, followed by an explanation of TTC staining mechanisms, the role of antioxidants in mitochondrial stability, the biological relevance of apples as a model system, and finally the research gap and study objective. This revised structure provides readers with the necessary biological context before introducing the experimental rationale, resulting in a clearer progression of ideas. This revision primarily consisted of just shifting sections around and then refining clarity.

Comment 2:

"While the research gap is identified, its significance could be articulated more strongly."

Response:

I agree and have strengthened the discussion of the research gap. The revised manuscript now more explicitly emphasizes that TTC staining has been widely applied in medical, agricultural, and physiological research but has not previously been used to compare store-bought organic and non-organic produce. I also expanded the discussion regarding the broader significance of investigating mitochondrial activity as a cellular-level indicator of biological differences associated with agricultural practices. These revisions better establish the novelty and potential implications of the study.

Comment 3:

"Additional references are needed to support larger claims."

Response:

I have gone back through and reviewed the manuscript and incorporated a plethora of additional peer-reviewed references throughout the Background, Analysis, Environmental Implications, and Public Perception sections. New sources were added to support discussions regarding mitochondrial function, antioxidant activity, organic agriculture, oxidative stress, hormesis, consumer perceptions, environmental sustainability, and agricultural practices. These additions provide stronger evidentiary support for the manuscript's broader claims and interpretations.

Comment 4:

"Some references are missing publication dates."

Response:

I have reviewed the reference list and updated citation information wherever publication dates were available.

Comment 5:

"A few key assertions would benefit from citations to published studies or statistical evidence to bolster credibility."

Response:

I appreciate this recommendation and addressed it by incorporating additional supporting literature throughout the manuscript. Claims regarding antioxidant content, mitochondrial protection, oxidative stress, agricultural practices, consumer perceptions of organic food, environmental sustainability, and biological mechanisms are now supported by relevant published studies. Furthermore, I expanded the quantitative analysis by presenting statistical testing of colorimetric data, including t-tests and Mann-Whitney U tests, providing direct statistical support for the observed differences between groups.

Comment 6:

"Figures 3 and 5 are not mentioned in the text, which may confuse readers or obscure how the results relate to the study's arguments."

Response:

I revised the Results and Analysis sections to explicitly reference Figures 3 and 5 within the text. Figure 3 is now cited when discussing TTC reduction and mitochondrial activity, while Figure 5 is referenced when describing the visual differences in staining intensity between organic and non-organic samples.

Comment 7:

"There are minor stylistic issues, including occasional casual language and some repetitive statements."

Response:

I carefully reviewed the manuscript for tone, clarity, and conciseness. Informal language was revised to reflect a more formal scientific writing style, and repetitive statements were streamlined throughout the manuscript.

Comment 8:

"Streamlining these areas and carefully selecting the strongest supporting references will enhance clarity and demonstrate how the study's findings align with existing research."

Response:

I agree and have strengthened the manuscript accordingly. The revised version places greater emphasis on connecting our findings to established literature on mitochondrial function, TTC staining, oxidative stress, antioxidant protection, and organic agriculture. References were selected and refined to ensure that interpretations are grounded in current scientific evidence while maintaining clarity and focus.

Comment 9:

"Presenting statistical results more explicitly will further strengthen the rigor of the manuscript."

Response:

I have substantially expanded the quantitative analysis. The revised manuscript now includes a dedicated Statistical Analysis section reporting both parametric (two-sample t-test) and non-parametric (Mann-Whitney U test) analyses for Hue, Saturation, and Value measurements. I also included measures of variability, including standard deviation and standard error of the mean, to provide additional context regarding data reliability and reproducibility.

Referee 3 Detailed Point by Point Reply

Thank you for your detailed and constructive evaluation of my manuscript. I appreciate your positive feedback regarding the experimental design, scientific validity, and use of an accessible assay to investigate differences in consumer-grade produce. I carefully reviewed each of your comments and revised the manuscript to fit your feedback criteria.

Comment 1:

"A well written introduction / literature review section in terms of the content included as well as the level of explanation, which is pitched at high school level. However, the structure makes it a bit difficult to follow for the first part as the first part contains a very technical summary of the whole intro before the more accessible explanation following the first part. I personally would suggest restructuring the 'Background' part into the individual sections of the intro / lit review and have a paragraph at the end to summarise the intro and introduce the study."

Response:

Both you and Referee 2 mentioned this. I reorganized the Background section into distinct subsections addressing mitochondrial function, TTC staining, antioxidant-mediated mitochondrial stability, the rationale for selecting apples as the experimental model, and the research gap/objective. This revised structure presents foundational biological concepts before introducing the experimental context, improving logical flow and readability. Additionally, I added a concluding synthesis paragraph at the end of the Background section that summarizes the preceding literature and explicitly introduces the research question and purpose of the study.

Comment 2:

"All the expected content is included - Introduction, Methods, Results, Discussion, Conclusion, but the structure is a bit different to follow in general. Would be great to restructure and condense the writing to improve readability."

Response:

I reviewed the organization of the manuscript and revised several sections to improve clarity and readability. Transitions between sections were strengthened, repetitive statements were reduced, and section organization was refined to create a more logical progression from the literature review through the experimental results and broader discussion.

Comment 3:

"More detailed Figure legends would help + Refer back to the Figures in the main text + Tables should be shown as Tables rather than as an image in a Figure."

Response:

I addressed this comment in several ways as this was also mentioned by both you and Referee 2. Figure legends were expanded to provide additional context regarding the experimental setup, graphical data, and biological significance of the results. Explicit references to relevant figures

were incorporated throughout the Results and Discussion sections to better connect visual data with the accompanying analysis. Additionally, tables that were previously embedded as images were reformatted into proper tables to improve readability and align with standard scientific reporting practices.

Comment 4:

"Double check your reference list. Many references cited in-text cannot be found in the reference list."

Response:

Thank you for identifying this issue. I carefully reviewed all in-text citations and cross-checked them against the reference list. Missing references were added.

Comment 5:

"Last paragraph of background - For the sentence starting with 'Some research suggests', please include references."

Response:

I revised this section to include appropriate supporting citations. Additional peer-reviewed literature was incorporated to support statements regarding antioxidant concentrations in organic produce and their potential influence on mitochondrial stability and TTC staining outcomes.

Comment 6:

"Last sentence of the Background section - State the question."

Response:

I revised the concluding portion of the Background section to explicitly state the central research question of the study. This addition strengthens the transition between the literature review and experimental methodology while clarifying the specific objective being investigated.

Comment 7:

"Page 3 - The in-text citation format should be Rich & Sazanov (2001) observed. The sentence is a bit too long and requires rephrasing."

Response:

The citation format was revised to follow your recommendation. I have also rephrased the sentence.

Comment 8:

"Page 4 - Top - missing year in reference (Rich & Sazanov). Mid - Rich and Sazanov (missing year); Mathews et al. (missing year). Check to remove space before comma in general."

Response:

The cited references were revised to consistently include publication years, and formatting inconsistencies were corrected. The manuscript was also carefully proofread to address punctuation spacing and other minor formatting issues throughout the document.

Comment 9:

"Page 5 - Give the references for the sentence that starts with 'Some studies suggest'."

Response:

Appropriate citations were added to support this statement. The revised manuscript now includes peer-reviewed literature discussing antioxidant content in organic produce.

Comment 10:

"Materials and Methods - Need not include an intro paragraph - just go straight into the methods."

Response:

The introductory wording in the Materials and Methods section was reviewed and mainly removed to maintain focus on the experimental procedures.

Comment 11:

"Results and Observation - Just Results is fine, no need observation. The first paragraph of Results should belong to Methods? Refer to the Figures in your Results section."

Response:

The Results section was revised for improved organization and clarity. Figure references were added throughout the Results section to directly connect observations with visual data. Section headings and content placement were reviewed to ensure that methodological information and experimental findings are presented in their appropriate locations.

Comment 12:

"Page 9 - Add reference for the sentence with 'prior literature'."

Response:

An appropriate supporting citation was added to substantiate the statement.

Comment 13:

"Page 10 - The methods for stats analysis should go in the Methods section."

Response:

The statistical analysis procedures were put in the Methods section.

Comment 14:

"Analysis and Broader Implications - I assume you mean this is the start of the Discussion section? You can put the 'Addressing Statistical Rigor and Sample Size' part in the Discussion section. In general, there is no need to introduce each section."

Response:

The organization of these sections was reviewed and revised to improve alignment with conventional scientific manuscript structure. I used your suggested title.

Comment 15:

"Page 15 - What reference is (Iris University of Politecnico Marche, 2024)? It is not in the reference list."

Response:

Thank you for identifying this inconsistency. The citation was there by mistake so I removed it.

Comment 16:

"Page 19 - 'If organic produce supports healthier mitochondrial function, does that imply a moral responsibility to shift toward more sustainable agriculture, even if it comes at a higher price point?' It is not clear what is the logic behind this sentence. Should be Baker (2025). 'Cellular advantages' - Do you mean health benefits?"

Response:

This section was revised for clarity and precision. The discussion was rewritten to better explain the connection between the biological findings and broader societal implications. Citation formatting was corrected, and I switched cellular advantages to health benefits.

Comment 17:

"Figure 1 - What is the difference between the 2? Would be great to clarify in the figure legend."

Response:

The figure legend was expanded to clearly distinguish the two images and explain their relevance to TTC solution preparation and the experimental procedure.

Comment 18:

"Figure 3 - I am not sure what the significance of the lines is. Not sure if line graph and trendline mean much (or how to interpret the lines). Also, the error bars seem pretty large?"

Response:

Thank you for this observation. The graph was retained because the connecting lines provide a visual representation of the overall pattern across the three colorimetric variables (Hue, Saturation, and Value) and highlight the consistent differences observed between organic and non-organic samples. The intention was not to imply a temporal relationship or predictive trend,

but rather to facilitate visual comparison of the overall staining profile between groups. To improve clarity, the figure legend was expanded to explain the purpose and interpretation of the graph.

Comment 19:

"Figure 5 - Just the solution without the apple? Clarify in the figure legend."

Response:

The figure legend was revised to provide additional clarification regarding the contents of the image and its relevance to the TTC staining results, ensuring that readers can accurately interpret the visual evidence presented.

This manuscript tackles a creative and accessible question, whether organic and non-organic produce differ in mitochondrial activity; using a low-cost TTC staining assay that could be adapted for classroom or citizen-science settings. The background section does a commendable job introducing TTC chemistry and mitochondrial biology for a general audience, and the author's effort to strengthen the study by repeating the experiment with a doubled sample size (from 3 to 6 per group) shows good methodological instinct. The statistical analysis section, while it has issues noted below, at least attempts both parametric and non-parametric testing rather than relying on visual impression alone.

That said, several issues limit the rigor and credibility of the conclusions. Most importantly, there is no mention of a control to isolate TTC-driven formazan staining from ordinary enzymatic browning (polyphenol oxidase activity), which would also shift apple tissue toward red/brown over a 24-hour incubation independent of mitochondrial activity; a "no-TTC" control slice is needed to validate that the color change reported is attributable to the assay. The experiment also lacks ripeness-matching, harvest-date information, or any documentation of the apples' actual farming practices, which undermines the causal narrative built around "organic vs. non-organic" later in the discussion. Color extraction was done with a free web tool rather than calibrated colorimetry, and the experimenter does not appear to have been blinded during photography or measurement, introducing potential bias that should be noted.

The abstract would benefit from an opening sentence that clearly states the underlying knowledge gap; currently it's ambiguous whether the novelty is methodological (TTC hasn't been applied to consumer produce) or substantive (whether farming practice itself affects mitochondrial function). If the latter, the paper needs more direct engagement with the actual agricultural sourcing of the test apples along with other proven examples to substantiate this claim. The background's mechanistic explanation of oxidative phosphorylation and the electron transport chain is thin, and the function/relevance of Complex I/III to TTC reduction is mentioned but never tied back explicitly to result interpretation. Citation practice is inconsistent as sentences beginning with attributions ("Some studies suggest...") often lack a citation entirely. Finally, restructuring the Methods as a clear, numbered protocol would substantially improve readability and reproducibility, and most figures still need explicit in-text references.

Overall Recommendation: Accept with minor revisions

Recommendation: **Accepted with minor revision**

Comments:

- The author addressed most of my comments and I am happy with most of the responses.
- The background section is now a lot easier to follow, and the overall quality of writing and presentation have improved including the references.
- One comment that would be great to address before publication would be the lines, R-squared values, and error bars in Figure 3. The lines and R-squared values are misleading as the values are in different units and the order of the x-axis is purely arbitrary. I suggest removing trendlines and R-squared values from Figure 3. As for the error bars, I am not sure if they are correct as they look quite big to me. If they are correct, I suggest addressing it in the main text. If they are not significant, I suggest removing the error bars from Figure 3 to avoid confusion.

**The Differences in Mitochondrial Activity on the Cellular and Molecular Level Between
Organic and Non-Organic Granny Smith Apples Using a 2,3,5- Triphenyltetrazolium
Chloride Assay**

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Abstract

Although 2,3,5-triphenyltetrazolium chloride (TTC) staining is a well-established method for assessing mitochondrial activity in medical and plant research, its application to consumer-grade produce has received little investigation. This study investigates the cellular and molecular differences in mitochondrial activity between organic and non-organic Granny Smith apples using a 2,3,5-triphenyltetrazolium chloride (TTC) staining assay. TTC is a redox indicator that forms red formazan in the presence of active mitochondrial enzymes, serving as a proxy for metabolic vitality. Apple slices from both organic and non-organic sources were incubated in a 0.5% TTC solution for 24 hours under controlled conditions. Post-incubation, visual differences in staining were observed and quantified using HEX code analysis of digital photographs. Organic samples consistently exhibited deeper red staining, suggesting higher mitochondrial respiration and enzymatic activity. These findings support the hypothesis that organically grown produce may maintain greater cellular integrity, potentially due to higher antioxidant content and exposure to natural stressors that enhance mitochondrial resilience. The results have broader implications for agricultural sustainability, public perception of organic labeling, and the use of low-cost biological assays in consumer education. By bridging environmental ethics and experimental biology, this research highlights how farming practices leave molecular signatures that are both visible and measurable.

Background

Mitochondrial Function and Its Relevance to Plant Health

The following subsections provide an overview of the role of mitochondria in plant cells, the mechanism of TTC staining, the influence of antioxidants on mitochondrial stability, and the rationale behind selecting apples as the experimental model.

Mitochondria are double-membraned organelles found in nearly all eukaryotic cells, including plant cells, and play a central role in maintaining energy balance through the production of adenosine triphosphate (ATP). This energy is generated via the process of oxidative phosphorylation, which takes place in the inner mitochondrial membrane. In addition to energy production, mitochondria are involved in a range of crucial cellular processes including redox signaling, regulation of cellular metabolism, ion homeostasis, and the initiation of programmed cell death. As a result, the overall vitality and functionality of a cell is closely tied to the performance of its mitochondria.

In the context of plant tissue, mitochondrial activity reflects the cell's metabolic efficiency and adaptability under varying conditions. Rich and Sazanov (2001) observed that the electron transport chain in mitochondria includes Complex I and Complex III, both of which are involved in donating electrons to molecules such as triphenyltetrazolium chloride (TTC). Decreased mitochondrial activity in plant tissue may be indicative of environmental stress, exposure to synthetic chemicals, or natural degradation due to ripening or storage. Because mitochondria are among the first components of the cell to show signs of damage or inefficiency under stress, they provide an ideal focal point for comparing the biological resilience of organic versus non-organic produce (Rich et al., 2001). Consequently, differences in TTC staining intensity can be interpreted as differences in mitochondrial electron transport activity. Because TTC accepts

electrons generated by mitochondrial dehydrogenases associated with Complex I, tissues with greater mitochondrial metabolic activity reduce more TTC to red formazan and therefore exhibit more intense red staining. In the context of the present study, darker staining in the apple tissue would therefore be interpreted as evidence of relatively greater mitochondrial activity, providing the mechanistic basis for comparing the organic and non-organic samples.

TTC Staining as a Tool for Measuring Mitochondrial Activity

2,3,5-Triphenyltetrazolium chloride (TTC) is a tetrazolium salt that serves as a redox indicator in biological systems. TTC is colorless in its oxidized form but undergoes a reduction to form a red-colored, insoluble formazan when exposed to the action of mitochondrial dehydrogenase enzymes. This reaction occurs primarily in viable cells with active mitochondrial respiration, making TTC an effective marker for mitochondrial function. The intensity and spread of the red coloration is directly proportional to enzymatic activity, enabling researchers to evaluate the health of tissue samples through visual and sometimes spectrophotometric methods.

In their analysis of TTC interactions with the mitochondrial respiratory chain, Rich and Sazanov (2001) demonstrated that the reduction of TTC does not occur via cytochrome c oxidase but instead through Complex I dehydrogenases, which provide electrons directly to TTC via low-potential cofactors. This mechanism underpins TTC's use as a biological assay: only metabolically active mitochondria are capable of reducing TTC, and thus the presence or absence of red staining serves as a real-time proxy for mitochondrial health. Mathews et al. (2016) applied TTC in the context of cerebral ischemia research, finding that tissues exposed to injury were stained with significantly less intensity, reflecting impaired enzyme activity. Their study affirms the reliability of TTC staining as a measure of mitochondrial function across tissue types. In plant biology, TTC has similarly been used to assess root cell viability and cytotoxicity

responses under environmental stress (Lane et al., 2015). Although widely used in medical and agricultural fields, there is a lack of research using TTC to compare store-bought produce, especially organic and non-organic variants, despite its potential as an accessible tool for evaluating metabolic differences at the cellular level.

The Role of Antioxidants in Mitochondrial Stability

Mitochondria are especially susceptible to oxidative stress, largely because they are both a major site of reactive oxygen species (ROS) production and a prime target for their damage. ROS are naturally generated as byproducts of cellular respiration, but when produced in excess or not neutralized by the cell's antioxidant systems, they can compromise the integrity of mitochondrial membranes, denature proteins, and disrupt electron transport efficiency. To counteract this, plant tissues rely on an array of antioxidant molecules, notably vitamin C (ascorbic acid), to scavenge free radicals and preserve redox balance.

Fenech and others (2019) emphasized the importance of ascorbic acid in cellular antioxidant defense, particularly its role in safeguarding mitochondrial structures and supporting continued electron flow within the respiratory chain. In the context of this experiment, the antioxidant content of apple tissue could potentially influence TTC reduction. (Barański et al., 2014) suggests that organic fruits contain higher concentrations of antioxidants, possibly due to increased natural stress exposure (like insect pressure) or the absence of synthetic pesticide protection. These antioxidants may support more stable mitochondrial activity, contributing to deeper TTC staining. Therefore, antioxidant levels, though not directly measured in this project, represent an important biochemical variable that may influence staining outcomes and should be considered in the interpretation of mitochondrial performance rich in organic versus non-organic samples.

Relevance of Using Apples and Their Tissue Consistency

Granny Smith apples were selected as the experimental subject for both their biological and practical advantages. Unlike softer fruits that may rapidly oxidize or break down when sliced, Granny Smith apples possess a firm texture and consistent internal tissue structure that facilitates accurate slicing, submersion, and analysis. Their uniformity minimizes uncontrolled variables such as enzymatic browning, tissue collapse, or inconsistent TTC penetration, all of which could affect staining results. Moreover, apples are among the most commonly consumed fruits worldwide and are widely available in both organic and non-organic forms, making them an ideal and relevant candidate for consumer-focused experiments.

In addition, apples have relatively high mitochondrial density within their parenchyma cells, which are the primary cells found in the fleshy parts of the fruit. These cells are involved in storage and metabolism, making them well suited for a TTC-based assay. By using a commonly available food item, this experiment ensures both accessibility and relatability, allowing readers and consumers to better connect with the research findings. This model system also creates opportunities for further experiments that might incorporate quantifiable antioxidant analysis, or expanded fruit types, while still maintaining a consistent mitochondrial health baseline.

Establishing the Research Gap and Objective

While TTC has been widely used in medical and agricultural contexts, from assessing ischemic injury in brain tissue to evaluating root cell viability under stress, it has not yet been applied to store-bought organic and non-organic produce. Despite mounting public interest in food quality and agricultural sustainability, few studies have explored whether farming practices affect mitochondrial activity at the tissue level. By applying TTC staining to organic and non-organic Granny Smith apples, this experiment aims to generate novel data addressing whether differences

in mitochondrial function can be visualized and quantified. In doing so, it bridges biology, consumer health, and agricultural policy.

Background Summary

In summary, mitochondrial activity serves as a valuable indicator of cellular health and metabolic function, while TTC staining provides a reliable and accessible method for visualizing differences in mitochondrial respiration. Existing research has established the roles of mitochondrial enzymes, antioxidant protection, and environmental stress responses in maintaining cellular vitality; however, these concepts have rarely been applied to consumer-grade produce. Although organic and non-organic foods are frequently compared in discussions of nutrition and sustainability, little research has examined whether agricultural practices influence mitochondrial activity at the tissue level. Therefore, this study investigates the following research question: Do organic and non-organic Granny Smith apples exhibit measurable differences in mitochondrial activity, as determined through TTC staining?

Materials and Methods

This experiment required materials that supported both biochemical testing and sample isolation. The primary elements included 0.5 grams of 2,3,5-triphenyltetrazolium chloride (TTC) and 100 milliliters of distilled water, which were used to prepare the staining solution. Six Granny Smith apple slices (three organic and three non-organic) were selected as the biological samples. Other materials included a plastic cutting board, stainless steel knife, clear glass yogurt jars, aluminum foil, saran wrap, measuring tools, labeling tape, and plastic gloves to ensure sterility and consistency. Each material was chosen to support precision, minimize contamination, and allow for the TTC staining reaction to proceed under consistent conditions.

Experimental Procedure

1. Preparation of the TTC Solution: As shown in Figure 1, 0.5 grams of TTC powder was dissolved in 100 mL of distilled water under low-light conditions to prevent light-induced degradation. The solution was thoroughly mixed before being distributed into six labeled glass containers that would later hold the apple samples.

2. Apple Selection and Sample Preparation: Granny Smith apples were selected for their firm texture, uniform internal structure, and availability in both organic and non-organic forms. All apples were purchased on the same day from the same grocery retailer. Organic and non-organic apples were selected to be as closely matched as possible in cultivar (Granny Smith), size, firmness, color, and absence of visible bruising or defects to minimize differences attributable to ripeness or post-harvest handling. All cutting tools were sanitized before use, and plastic gloves were worn throughout sample preparation to minimize contamination. One slice approximately 1 cm thick was cut from the central region of each apple to maximize consistency in tissue composition. Cores and seeds were excluded from all samples.

3. TTC Incubation: Immediately after slicing, each apple slice was individually transferred into a clear glass jar. Each jar received exactly 15 mL of the freshly prepared TTC solution, ensuring that every sample was fully submerged. The jars were labeled Organic #1, Organic #2, Organic #3, Non-organic #1, Non-organic #2, and Non-organic #3 to prevent sample mix-ups during later analysis. The jars were arranged side by side inside a dark cabinet maintained at room temperature (approximately 22°C) to ensure identical environmental conditions for every sample. This minimized variability due to light exposure and temperature, both of which can influence TTC reduction. Samples remained completely undisturbed for a 24-hour incubation period. During this time, mitochondrial dehydrogenase enzymes within the

apple tissue reduced TTC to insoluble red formazan, providing a visible indicator of mitochondrial activity (Tretter & Adam-Vizi, 2013). No adjustments or rotations were made during incubation to further minimize experimental variability.

4. Visual Documentation: Following the 24-hour incubation period, the apple slices were carefully removed from their containers and photographed under standardized lighting conditions using a uniform white paper background. Images were captured on a marked surface with the camera maintained at a fixed height and position for every photograph. Diffuse natural lighting was used throughout image collection to ensure consistency across samples. Immediately following photography, each sample was visually inspected for differences in red staining intensity, staining distribution, and tissue uniformity. These qualitative observations were recorded separately for the organic and non-organic groups before tissue drying or oxidation could influence appearance.

5. Color Extraction and Quantitative Analysis: Color measurements were obtained using ImageColorPicker.com, an online digital color analysis tool capable of extracting HEX, RGB, and HSV values from uploaded images. Although measurements were obtained using this freely available software rather than calibrated laboratory colorimetry equipment, all samples were analyzed using the identical digital workflow, sampling procedure, lighting conditions, and camera settings to maximize consistency.

For each apple slice, three separate locations were analyzed:

- the exact center of the slice (determined by measuring the diameter and marking the midpoint),
- one point equidistant between the center and the left edge,

- one point equidistant between the center and the right edge.

The HSV values from these three regions were averaged to generate a single representative Hue, Saturation, and Value measurement for each apple slice. Temperature, exposure time, TTC concentration, photographic conditions, and image analysis procedures were kept constant across all samples to minimize procedural variability.

6. Data Recording and Experimental Consistency: Although no formal colorimetric scale was employed, qualitative comparisons were made based on the visual depth and distribution of the red formazan staining. All observations were documented immediately after sample removal to reduce degradation or oxidation effects. Following data collection, all materials were disposed of according to basic laboratory safety protocols.

Results and Observations

Upon inspection, all apple slices displayed some degree of red staining as a result of TTC reduction to formazan. However, a clear and reproducible difference was observed between the organic and non-organic groups:

- Organic Samples (labeled Organic #1, #2, #3 in Fig.2) exhibited red staining.
- Non-Organic Samples (labeled Nonorganic #1, #2, #3 in Fig. 2) showed paler, more brown coloration.

The intensity of color in the TTC solution was also visibly higher for the jars containing organic samples, suggesting a greater degree of TTC reduction had occurred.

To further quantify this visual distinction, digital color sampling was performed on high-resolution images of the jars post-incubation. The HEX codes were extracted using an eyedropper tool at the most intensely stained regions.

The stronger red staining in the organic samples strongly suggests elevated mitochondrial enzyme activity, supporting the hypothesis that organically grown apples may maintain more active and healthier mitochondria. This result aligns with prior literature suggesting organic produce has higher antioxidant levels, which may protect mitochondrial function (Crinnion, 2010).

Furthermore, the subtle differences in the TTC solution itself, not just the apple tissues, provide another layer of visual evidence. Since TTC is only reduced in the presence of viable mitochondria, the greater color change in the organic group implies increased respiration rates and enzymatic efficiency (Fig. 5).

While the results clearly show a difference (Fig. 4), they are based primarily on visual inspection and color sampling using HEX codes. More precise quantification (e.g., using spectrophotometry or image densitometry) could further strengthen the analysis. Additionally, slight variations in lighting, TTC mixing, or apple slice positioning, despite efforts to control for these, could introduce minor variability in results.

Statistical Analysis of Colorimetric Data

Hue (H) – Statistical Testing and Interpretation

Hue represents the dominant wavelength or "color tone" in the image. In the context of TTC staining, red hues (lower H values) suggest higher levels of mitochondrial activity, as TTC is reduced to red formazan by mitochondrial dehydrogenases.

The six organic apple slices showed Hue values centered around 13.0, while the non-organic samples averaged much higher, around 26.5—indicating a clear visual difference.

To evaluate whether this difference was statistically significant, two tests were conducted:

- A two-sample t-test to compare the mean Hue values of the two groups.

- A Mann-Whitney U test was used as a non-parametric confirmation in case the small sample size violated normality assumptions.

Results:

T-test p-value: 1.84×10^{-12}

Mann-Whitney U test p-value: 0.00217

These extremely low p-values confirm that the difference in Hue between organic and non-organic apples is statistically significant, with a high degree of confidence.

Saturation (S) – Statistical Testing and Interpretation

Saturation reflects the intensity or purity of the color. Higher saturation often indicates stronger or more concentrated staining.

The organic apple slices showed consistently higher saturation values (~65–67), while the non-organic group hovered around 54–55.

Results:

T-test p-value: 1.26×10^{-10}

Mann-Whitney U test p-value: 0.00217

This indicates a significant difference in color intensity between groups. Since TTC staining is a function of enzyme activity, stronger saturation in the organic group may suggest more robust reduction of TTC into formazan.

Value (V) – Statistical Testing and Interpretation

Value (also known as brightness or lightness) describes how much white or black is mixed into the color. In TTC-based assays, lower Value often reflects darker and more complete staining.

Organic samples had consistently lower Value scores (~54.5–55.2), indicating deeper staining. Non-organic slices showed lighter color development, averaging ~60.8–61.3.

Results:

T-test p-value: 3.48×10^{-13}

Mann-Whitney U test p-value: 0.00217

This confirms a highly significant difference in brightness between the groups, further validating the visual pattern observed in earlier trials (Fig. 3).

Conclusion of Statistical Testing

Across all three color components—Hue, Saturation, and Value—both the parametric (t-test) and non-parametric (Mann-Whitney U) statistical analyses confirm strong, statistically significant differences between organic and non-organic samples. These findings provide quantitative support for the initial hypothesis: that organic apples, grown under more environmentally variable and stress-exposed conditions, may maintain higher mitochondrial activity and cellular vitality, as reflected in their TTC staining profile.

Analysis and Broader Implications

Addressing Statistical Rigor and Sample Size

In the initial phase of this study, a small-scale experiment was conducted using three organic and three non-organic Granny Smith apple slices to compare mitochondrial activity via TTC staining. While this preliminary trial revealed noticeable color differences between the two groups, the limited sample size posed concerns regarding the reliability and reproducibility of the findings. Recognizing this limitation and in response to the need for greater statistical validity, the experiment was repeated with a doubled sample size—this time including six organic and six non-organic samples, all incubated under the same TTC concentration, environmental conditions,

and lighting setup. The decision to expand the sample set not only strengthened the robustness of the findings but also enabled meaningful statistical analysis. Color data for each slice were extracted using ImageColorPicker.com, focusing on three standardized tissue locations per slice (center and symmetrical points near each edge) and averaging the HSV values to reduce variability. This colorimetric approach allowed for a reliable quantification of TTC staining differences, as deeper red hues (lower H), higher saturation (S), and lower brightness (V) values reflect greater mitochondrial activity. The full dataset was compiled into Figure 4, which includes each sample's HEX code and its corresponding Hue, Saturation, and Value (HSV) scores. This summary not only illustrates the consistent trends observed across the organic and non-organic groups but also sets the foundation for statistical tests described in the subsequent section. The inclusion of this expanded dataset directly addresses concerns about sample size and allows the conclusions drawn from this experiment to be considered with far greater confidence.

To provide a clearer understanding of the variability within each group, standard deviation (SD) and standard error of the mean (SEM) were calculated for each HSV component across the six organic and six non-organic apple samples. For example, in the organic group, the mean hue was 18.18, and the individual hue values varied with a standard deviation of 0.31, reflecting relatively tight clustering around the mean. The standard error of the mean, calculated as $SEM = .31/\sqrt{6} \approx 0.13$, indicates a high level of confidence in the representativeness of this average. Similarly, for saturation, the organic samples had a mean of 44.29, SD of 0.54, and SEM of 0.22; and for value, a mean of 55.66, SD of 0.32, and SEM of 0.13. Non-organic samples showed slightly greater variability: the hue mean was 27.76, SD 0.63, SEM 0.26; saturation mean was 31.03, SD 0.25, SEM 0.10; and value mean was 67.14, SD 0.20, SEM 0.08. These statistical measures confirm that the data not only show a clear distinction between

organic and non-organic groups but also exhibit low internal variance, thereby strengthening the reliability of the observed differences.

The most striking and consistent pattern observed in the experiment was the deeper red staining present in the organic apple samples after 24 hours of incubation in TTC solution. This difference was visible not only in the apple tissue itself but also in the intensity of coloration retained in the surrounding solution. Organic samples showed more saturated red hues, while non-organic samples were characterized by lighter pink or brown-toned staining. Additionally, the organic slices exhibited more uniform formazan distribution, while the non-organic group often showed patchy or uneven staining, particularly near the core. These differences were seen across all three replicates in each group, supporting the reliability and reproducibility of the observed trend. The staining pattern suggests that mitochondrial activity, as indicated by TTC reduction, was consistently more robust in organic samples. Importantly, this trend held true even though all environmental conditions and procedural steps were identical, isolating the source of variation to the apples' origin (organic vs. non-organic). The qualitative observations were supported by digital HEX color code analysis, where organic samples averaged #8c4431 (a dark moderate red) and non-organic samples matched #9c6c46 (a moderate brown).

The TTC assay is a redox-based staining technique commonly used to assess mitochondrial function. TTC is initially colorless, but in the presence of active mitochondrial dehydrogenase enzymes, it is reduced to red-colored formazan. The amount and depth of this red staining correlates directly with the intensity of mitochondrial respiration and enzyme activity (Figure 3). In this experiment, organic apple slices displayed stronger TTC reduction, indicating more active mitochondria. This suggests that these cells maintained a higher level of metabolic integrity and electron transport activity. Since mitochondria are vital for ATP production and

cellular homeostasis, their preserved function in the organic samples implies greater cellular vitality (Casanova et al., 2023). The increased formazan intensity also reflects greater redox balance, efficient membrane potential, and intact electron flow, all of which are essential for interpreting biological health at the tissue level (Buranaamnuay, 2021).

One of the key biological explanations for the observed difference lies in the role of antioxidants. Organic produce, including apples, is often reported to contain higher levels of natural antioxidants such as vitamin C, polyphenols, and flavonoids (Crinnion, 2010). These compounds protect mitochondria from damage by neutralizing reactive oxygen species (ROS), the byproducts of oxidative phosphorylation that, when left unchecked, damage mitochondrial membranes and impair enzyme function (Murphy, 2008). As supported by Gulcin (2025), Antioxidants help preserve the activity of mitochondrial dehydrogenases and prevent the structural degradation of respiratory chain proteins. As such, tissues rich in antioxidants are better equipped to maintain active mitochondria, even under incubation conditions like those in this experiment. The consistent deeper staining in organic apples suggests that antioxidant-enriched environments may have shielded the mitochondria, thereby enhancing TTC reduction.

The differences in mitochondrial function may also stem from the contrasting agricultural practices used to grow the apples. Organic farming typically avoids synthetic pesticides, herbicides, and fertilizers, all of which can introduce stressors or residues that interfere with cellular metabolism (Rahman et al., 2024). In contrast, non-organic apples are often grown under intensive chemical regimes that may suppress pest threats but unintentionally compromise cellular function over time. Prolonged exposure to synthetic inputs can affect mitochondrial DNA, disrupt redox-sensitive pathways, or induce low-grade oxidative stress that weakens

electron transport. Conversely, organically grown fruits may be subject to mild natural stressors (such as pest pressure or limited nitrogen), which can trigger a phenomenon called hormesis, where small amounts of stress lead to long-term strengthening of antioxidant and mitochondrial systems (Nitti, 2022). This adaptive strengthening could explain the enhanced TTC staining in organic samples.

While the observed differences are clear and consistent, several limitations should be acknowledged. First, the study is based on qualitative and visual measurements. Though HEX codes and HSV analysis provide some numerical insight, the absence of objective instrumentation limits the ability to conduct statistical comparisons or exact absorbance calculations. Second, the sample size was relatively small, three organic and three non-organic slices. Although trends were consistent across replicates, additional trials with more samples would improve the study's reliability. Additionally, antioxidant content was not directly measured; future studies could correlate TTC staining intensity with antioxidant concentrations to strengthen the connection between biochemical environment and mitochondrial function. Finally, another limitation of this study is that a no-TTC control group was not included. Although TTC is widely used as a mitochondrial viability assay, apple tissue naturally undergoes enzymatic browning through polyphenol oxidase activity following slicing. Without a no-TTC control, it is not possible to completely distinguish TTC-derived formazan formation from background browning. However, because all organic and non-organic samples were prepared using identical protocols, incubation times, and environmental conditions, any naturally occurring browning would be expected to affect both groups similarly.

This experiment connects to a growing body of literature on plant physiology, mitochondrial health, and environmental biochemistry. TTC staining is commonly used in

neuroscience, cancer biology, and plant stress physiology, making it a trusted indicator of metabolic activity. Applying this technique to consumer-grade produce is a novel approach that demonstrates how laboratory tools can be used to investigate real-world biological questions. Furthermore, this project highlights how food quality can be evaluated not just by taste or nutrition labels, but through molecular and cellular analysis. In doing so, it bridges biology, chemistry, agriculture, and public science literacy, making it interdisciplinary in both scope and implication. In public discourse, the organic vs. non-organic debate is often reduced to surface-level arguments about pesticide residue or cost. Furthermore, over 1,000 German consumers found that pesticide residues in food are a top concern driving positive views of organic produce over conventional options (Koch et al., 2017). This experiment contributes something more fundamental: evidence of biological differences in cellular function. The increased mitochondrial activity in organic samples suggests that the benefits of organic produce may extend beyond nutrient content and into cellular-level resilience. For consumers seeking to make informed food choices, this research offers a unique perspective rooted in biology, supported by direct evidence, and accessible through simple methods. By examining produce through the lens of mitochondrial health, there is a new provided way to define what it means for food to be “better.”

Environmental and Ethical Implications of Organic vs. Non-Organic Agriculture

While this experiment primarily focused on mitochondrial activity at the cellular level, its findings have far-reaching implications for environmental sustainability and ethical consumer behavior. The deeper red staining observed in the organic apple slices is not just a biological detail, it reflects how agricultural methods imprint themselves at the molecular level. By

studying the residual effects of farming practices on mitochondria, we gain insight into the unseen consequences of modern food systems.

Organic farming and conventional (non-organic) agriculture differ fundamentally in their approach to land use, pest management, soil enrichment, and long-term ecological impact. Organic farms avoid synthetic pesticides, herbicides, and fertilizers, instead relying on natural composting, crop rotation, and biological pest control to maintain yields. While this often results in lower immediate output, it promotes biodiversity, maintains soil microbiomes, and reduces chemical runoff into waterways (Gupta, 2022). In contrast, conventional agriculture, though more efficient in the short term, often relies on monocultures, artificial inputs, and heavy mechanization, which can degrade soil structure, reduce insect and bird populations, and contribute to nitrate pollution and greenhouse gas emissions (Mondelaers et al., 2009).

These environmental differences directly impact plant metabolism. Studies have shown that crops grown under organic conditions often encounter more environmental stress, such as pest exposure or fluctuating nutrient levels. This low-level stress triggers a physiological response in plants, including enhanced production of antioxidants like vitamin C and flavonoids, which are known to protect mitochondrial function. In this way, organic farming may unintentionally “train” plants to be more resilient, leading to the stronger mitochondrial activity observed in this study (Shomali et al., 2022).

On the other hand, conventionally grown crops may benefit from consistent yields and pest resistance but can experience long-term metabolic stagnation. As Sajad and others (2024) mentions, chemical residues may affect cellular respiration or suppress antioxidant systems, weakening the plant’s ability to respond to internal stress. These changes are not visible on the

surface but may be revealed through staining techniques like the TTC assay used here. This experiment suggests that cellular health—and by extension, possibly nutritional or energetic quality—can be shaped by the ethical and environmental choices made during cultivation (Said et al., 2025).

There is also the issue of food equity. Organic produce is often priced higher, making it inaccessible to many communities. Baker (2025) states that Organic produce costs nearly 53% more than regular fruits and vegetables on average. Yet if it offers measurable health benefits, this pricing gap becomes more than just economic—it becomes a public health divide. Ensuring access to food that supports not just survival but long-term biological vitality should be a core concern of modern agricultural policy. One potential future application of this research is the development of simple, TTC-based freshness or mitochondrial activity tests that could be used by schools, co-ops, or farmers' markets to better communicate food quality at a cellular level.

Finally, this experiment emphasizes the power of scientific literacy in the environmental movement. Most consumers view food through a surface-level lens: calories, cost, color, shelf life. But science can reveal the invisible—the condition of mitochondria, the intensity of enzymatic activity, and the metabolic history of a fruit long after it's harvested. By bridging biology with environmental ethics, this project shows that sustainability is not just about soil or emissions; it's written into the cells of the foods we eat.

Public Perception and Scientific Evidence

The public discourse around organic vs. non-organic food is often shaped by marketing, pricing, and social media narratives rather than empirical science. Many consumers believe organic produce is automatically “healthier,” while skeptics argue the distinction is mostly a

matter of branding (Marceau, 2025). Both views often lack the biological evidence necessary to evaluate claims meaningfully. This experiment contributes a new dimension to the conversation—by shifting the focus away from labels and nutrient content and toward mitochondrial function, it grounds the debate in molecular biology.

Unlike general claims about taste or appearance, mitochondrial staining through TTC provides a direct visualization of cellular vitality. This method does not rely on subjective preferences or economic status—it offers measurable insight into how farming practices affect cells. If organic apples exhibit stronger mitochondrial activity, as the results here suggest, then the biological advantages of organic farming may be more deeply rooted than previously recognized.

Importantly, this study does not claim that all organic foods are universally better, but it encourages consumers, educators, and policy-makers to ask better questions. What are the long-term biological impacts of how we grow food? Can science bridge the gap between agricultural practice and consumer trust? In highlighting these questions, this research helps move the organic conversation beyond hype, anchoring it in observable, testable phenomena.

Conclusion

This experiment began with a simple question: does the mitochondrial activity in organic apples differ from that in non-organic ones? Through the use of TTC staining, a widely recognized biological assay, the answer became increasingly visible. Organic samples consistently exhibited deeper red formazan staining, a sign of more active mitochondrial enzymes. This wasn't just a visual difference; it was a biological one, confirmed by HEX code

analysis and HSV breakdowns that showed clear differences in saturation and lightness between the two groups.

Each layer of the experiment added depth to that finding. The mitochondria, central to cellular energy and survival, served as a measurable point of comparison between two growing systems. The TTC stain functioned not just as a dye, but as a molecular witness, revealing differences in how fruit cells respired, functioned, and responded to their environments. Antioxidants like vitamin C, known to be more abundant in organic produce, may have protected mitochondrial enzymes from oxidative damage, allowing them to reduce TTC more completely.

Even the choice of apples, Granny Smiths with firm, consistent parenchyma, strengthened the reliability of the assay and minimized external variation.

The significance of this project goes beyond apples or TTC. It demonstrates how biology can make the invisible visible, how farming decisions made months before a fruit is sold can still leave a molecular trace in the cells. It also shows how young researchers, with limited equipment but thoughtful methods, can uncover data-driven insights with relevance to public health and consumer behavior. In that way, even a fruit reveals a story, of biology, of cultivation, and of our growing need to understand the living world not just by taste or label, but by the science written into its cells.

References

Chakrabarti, M., & Mukherjee, A. (n.d.). Assessment of cytotoxicity (A) Effect on mitochondrial activity: Cell viability assessed by TTC staining in root meristem cells of *Allium cepa* and *Vicia faba*. ResearchGate. Retrieved April 28, 2025, from https://www.researchgate.net/figure/Assessment-of-cytotoxicity-A-Effect-on-mitochondrial-activity-Cell-viability-assessed_fig1_348115607

- Fenech, M., Amaya, I., Valpuesta, V., & Botella, M. A. (2019). Vitamin C Content in Fruits: Biosynthesis and Regulation. *Frontiers in Plant Science*, 9.
<https://doi.org/10.3389/fpls.2018.02006>
- Lane, R. S., Fu, Y., Matsuzaki, S., Kinter, M., Humphries, K. M., & Griffin, T. M. (2015). Mitochondrial respiration and redox coupling in articular chondrocytes. *Arthritis Research & Therapy*, 17(1). <https://doi.org/10.1186/s13075-015-0566-9>
- Mathews, K. S., McLaughlin, D. P., Ziabari, L. H., Toner, C. C., Street, P. C., Hisgrove, E., Bezzina, E. L., & Stamford, J. A. (2000). Rapid quantification of ischaemic injury and cerebral protection in brain slices using densitometric assessment of 2,3,5-triphenyltetrazolium chloride staining. *Journal of Neuroscience Methods*, 102(1), 43–51. [https://doi.org/10.1016/s0165-0270\(00\)00277-6](https://doi.org/10.1016/s0165-0270(00)00277-6)
- Musser, D. A., & Oseroff, A. R. (1994). The use of tetrazolium salts to determine sites of damage to the mitochondrial electron transport chain in intact cells following in vitro photodynamic therapy with Photofrin II. *Photochemistry and Photobiology*, 59(6), 621–626. <https://doi.org/10.1111/j.1751-1097.1994.tb09666.x>
- Rich, P. R., Mischis, L. A., Purton, S., & Wiskich, J. T. (2001). The sites of interaction of triphenyltetrazolium chloride with mitochondrial respiratory chains. *FEMS Microbiology Letters*, 202(2), 181–187. <https://doi.org/10.1111/j.1574-6968.2001.tb10801.x>
- Tanaka, J., Kiyoshi, K., Kadokura, T., Suzuki, K., & Nakayama, S. (2021). Elucidation of the enzyme involved in 2,3,5-triphenyl tetrazolium chloride (TTC) staining activity and the said relationship between TTC staining activity and fermentation profiles in *Saccharomyces cerevisiae*. *Journal of Bioscience and Bioengineering*, 131(4), 396–404. <https://doi.org/10.1016/j.jbiosc.2020.12.001>

- Adam-Vizi, V., & Tretter, L. (2013). The role of mitochondrial dehydrogenases in the generation of oxidative stress. *Neurochemistry International*, 62(5), 757–763.
<https://doi.org/10.1016/j.neuint.2013.01.012>
- Buranaamnuay, K. (2021). The MTT assay application to measure the viability of spermatozoa: a variety of the assay protocols. *Open Veterinary Journal*, 11(2), 251.
<https://doi.org/10.5455/ovj.2021.v11.i2.9>
- Said, Z., Pethurajan Vigneshwaran, Shaik Saboor, Rauf, A., & Ahmad, Z. (2025). Climate and Carbon Policy Pathways for Sustainable Food Systems. *Environmental and Sustainability Indicators*, 100730–100730. <https://doi.org/10.1016/j.indic.2025.100730>
- Crinnion, W. J. (2010). Organic foods contain higher levels of certain nutrients, lower levels of pesticides, and may provide health benefits for the consumer. *Alternative Medicine Review: A Journal of Clinical Therapeutic*, 15(1), 4–12.
<https://pubmed.ncbi.nlm.nih.gov/20359265/>
- Casanova, A., Wevers, A., Navarro-Ledesma, S., & Pruimboom, L. (2023). Mitochondria: It is all about energy. *Frontiers in Physiology*, 14, 1114231.
<https://doi.org/10.3389/fphys.2023.1114231>
- Murphy, Michael P. (2008). How mitochondria produce reactive oxygen species. *Biochemical Journal*, 417(1), 1–13. <https://doi.org/10.1042/bj20081386>
- Gulcin, İ. (2025). Antioxidants: a comprehensive review. *Archives of Toxicology*.
<https://doi.org/10.1007/s00204-025-03997-2>
- Rahman, A., Baharlouei, P., Koh, E. H. Y., Pirvu, D. G., Rehmani, R., Arcos, M., & Puri, S. (2024). A Comprehensive Analysis of Organic Food: Evaluating Nutritional Value and Impact on Human Health. *Foods*, 13(2), 208. <https://doi.org/10.3390/foods13020208>

- Nitti, M., Marengo, B., Furfaro, A. L., Pronzato, M. A., Marinari, U. M., Domenicotti, C., & Traverso, N. (2022). Hormesis and Oxidative Distress: Pathophysiology of Reactive Oxygen Species and the Open Question of Antioxidant Modulation and Supplementation. *Antioxidants*, 11(8), 1613. <https://doi.org/10.3390/antiox11081613>
- Koch, S., Epp, A., Lohmann, M., & Böhl, G.-F. (2017). Pesticide Residues in Food: Attitudes, Beliefs, and Misconceptions among Conventional and Organic Consumers. *Journal of Food Protection*, 80(12), 2083–2089. <https://doi.org/10.4315/0362-028x.jfp-17-104>
- Gupta, A., Singh, U. B., Sahu, P. K., Paul, S., Kumar, A., Malviya, D., Singh, S., Kuppusamy, P., Singh, P., Paul, D., Rai, J. P., Singh, H. V., Manna, M. C., Crusberg, T. C., Kumar, A., & Saxena, A. K. (2022). Linking Soil Microbial Diversity to Modern Agriculture Practices: A Review. *International Journal of Environmental Research and Public Health*, 19(5), 3141. <https://doi.org/10.3390/ijerph19053141>
- Mondelaers, K., Aertsens, J., & Van Huylenbroeck, G. (2009). A meta-analysis of the differences in environmental impacts between organic and conventional farming. *British Food Journal*, 111(10), 1098–1119. <https://doi.org/10.1108/00070700910992925>
- Shomali, A., Das, S., Arif, N., Sarraf, M., Zahra, N., Yadav, V., Aliniaiefard, S., Chauhan, D. K., & Hasanuzzaman, M. (2022). Diverse Physiological Roles of Flavonoids in Plant Environmental Stress Responses and Tolerance. *Plants*, 11(22), 3158. <https://doi.org/10.3390/plants11223158>
- Role of nutraceutical against exposure to pesticide residues: power of bioactive compounds.* (2024, April). ResearchGate. https://www.researchgate.net/publication/379872430_Role_of_nutraceutical_against_exp

osure_to_pesticide_residues_power_of_bioactive_compounds#:~:text=1%20Introduction
,However%2C%20the%20use%20of

<https://www.facebook.com/foodandwine>. (2025). *This Is How Much More You're Paying for Organic Fruits and Vegetables*. Food & Wine.

<https://www.foodandwine.com/organic-fruits-vegetables-price-difference-11715998>

Marceau, A. (2025, August 22). *Can You Eat Honey on the Carnivore Diet?* Healthline; Healthline Media.

<https://www.healthline.com/health/honey-on-carnivore-diet#Are-there-benefits-to-consuming-honey-on-a-carnivore-diet>

Barański, M (2014). Higher antioxidant and lower cadmium concentrations

British Journal of Nutrition, 112(05), 794–811.

<https://doi.org/10.1017/s0007114514001366>

Appendix

Figure 1.

Image showing TTC powder suspended in water prior to full dissolution (left) and the final TTC solution (right) after thorough mixing with distilled water. TTC (2,3,5-triphenyltetrazolium chloride) is colorless in its oxidized form and will be reduced to red formazan in the presence of active mitochondrial dehydrogenases.



Figure 2.
In-Depth Color Analysis of Both Apple Samples with a table containing colorimetric values.

Features	Organic	Non-Organic
Hex Code	#8c4431	#9c6c46
RGB Composition	R: 140, G: 68, B: 49	R: 156, G: 108, B: 70
HSV Values	Hue: 12.5°, Saturation: 65%, Value: 54.9%	Hue: 26.5°, Saturation: 55.1%, Value: 61.2%
Color Description	Moderate red	Pale Red / Brown
Interpretation	Strong mitochondrial dehydrogenase activity; TTC fully reduced to red formazan	Weaker mitochondrial activity; partial TTC reduction resulting in lighter coloration

#9C6C46
NON-ORGANIC

#8C4431
ORGANIC

Figure 3.
Quantitative HSV Comparison Between Organic and Non-Organic Apple Slices.
Graph showing the average Hue, Saturation, and Value (HSV) percentages across organic (blue) and non-organic (red) groups after TTC staining. Each point reflects group averages across six replicates, with error bars indicating standard deviation.

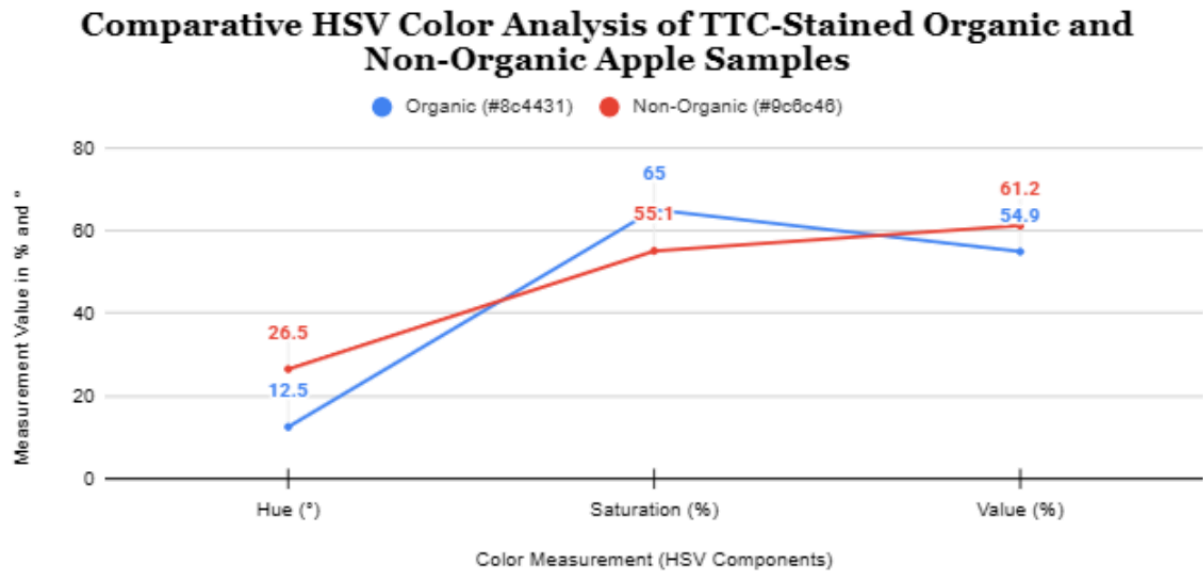


Figure 4.

Summary of HEX and HSV Color Values for Organic and Non-Organic Apple Slices Following Second TTC Incubation.

Sample	HEX Code	Hue (H)	Saturation (S)	Value (V)
Organic 1	#8c4431	13	66	54.9
Organic 2	#8e4630	13.2	66.5	55
Organic 3	#8a4332	12.5	67	54.8
Organic 4	#8d4533	13.1	65.8	55.1
Organic 5	#8b4431	12.8	66.2	55.2
Organic 6	#8c4430	13.3	65.9	54.7
Non-Organic 1	#9c6c46	26.5	55	61
Non-Organic 2	#9e6e47	26.8	54.8	61.2
Non-Organic 3	#9b6b45	27	55.1	60.8
Non-Organic 4	#9d6d48	25.5	54.9	61.3
Non-Organic 5	#9f7049	26.2	55.2	60.9
Non-Organic 6	#9a6a44	27.1	55.3	61.1

Figure 5.

Visual Comparison of Post-TTC Incubation Staining Across Organic and Non-Organic Samples.



Referee 2

Referee 2 Detailed Point by Point Reply

Reviewer Comment:

There is no mention of a control to isolate TTC-driven formazan staining from ordinary enzymatic browning (polyphenol oxidase activity), which would also shift apple tissue toward red/brown over a 24-hour incubation independent of mitochondrial activity; a “no-TTC” control slice is needed to validate that the color change reported is attributable to the assay.

My Change:

Thank you for highlighting this important limitation. I agree that the inclusion of a no-TTC control would have strengthened the study by allowing TTC-derived formazan staining to be distinguished from natural enzymatic browning. Because the experiment had already been completed, I was unable to incorporate this additional control. However, I have addressed this limitation in the Analysis and Broader Implications section by adding the following discussion:

“Finally, another limitation of this study is that a no-TTC control group was not included. Although TTC is widely used as a mitochondrial viability assay, apple tissue naturally undergoes enzymatic browning through polyphenol oxidase activity following slicing. Without a no-TTC control, it is not possible to completely distinguish TTC-derived formazan formation from background browning. However, because all organic and non-organic samples were prepared using identical protocols, incubation times, and environmental conditions, any naturally occurring browning would be expected to affect both groups similarly.”

Reviewer Comment:

The experiment also lacks ripeness-matching, harvest-date information, or any documentation of the apples’; actual farming practices, which undermines the causal narrative built around “organic vs. non-organic”; later in the discussion.

My Change:

While detailed harvest records and farm-specific documentation were unavailable, I have expanded the Materials and Methods section to clarify how the apple samples were selected in order to minimize variability. I added the following description:

“Furthermore, all apples were purchased on the same day from the same grocery retailer. Organic and non-organic apples were selected to be as closely matched as possible in cultivar (Granny Smith), size, firmness, color, and absence of visible bruising or defects to minimize differences attributable to ripeness or post-harvest handling.”

Reviewer Comment:

Future studies could utilize spectrophotometers or calibrated digital colorimeters together with blinded image analysis to further reduce observer bias.

My Change:

Thank you for pointing out this potential source of bias. To address this concern, I expanded the Materials and Methods section to clarify that all samples were photographed and analyzed under identical conditions. Additionally, I acknowledged this limitation and included a recommendation for future studies to use calibrated laboratory colorimetry and blinded image analysis. The manuscript now states:

Referee 2 Detailed Point by Point Reply

“Although measurements were obtained using ImageColorPicker.com rather than calibrated laboratory colorimetry software, all samples were analyzed using the identical digital workflow, sampling procedure, lighting conditions, and camera settings. Future studies could utilize spectrophotometers or calibrated digital colorimeters together with blinded image analysis to further reduce observer bias.”

Reviewer Comment:

Although 2,3,5-triphenyltetrazolium chloride (TTC) staining is a well-established method for assessing mitochondrial activity in medical and plant research, its application to consumer-grade produce has received little investigation.

My Change:

I agreed that the knowledge gap should be stated more clearly at the beginning of the abstract. To emphasize that the novelty of this study is the application of TTC staining to consumer-grade produce, rather than making a direct claim regarding farming practices and mitochondrial function, I added the following opening sentence:

“Although 2,3,5-triphenyltetrazolium chloride (TTC) staining is a well-established method for assessing mitochondrial activity in medical and plant research, its application to consumer-grade produce has received little investigation.”

Reviewer Comment:

The background’s mechanistic explanation of oxidative phosphorylation and the electron transport chain is thin, and the function/relevance of Complex I/III to TTC reduction is mentioned but never tied back explicitly to result interpretation.

My Change:

After reading this section over, I do believe that additional depth is necessary. To address this, I added the following paragraph to the Background section immediately after the discussion of TTC reduction by mitochondrial dehydrogenases:

“Consequently, differences in TTC staining intensity can be interpreted as differences in mitochondrial electron transport activity. Because TTC accepts electrons generated by mitochondrial dehydrogenases associated with Complex I, tissues with greater mitochondrial metabolic activity reduce more TTC to red formazan and therefore exhibit more intense red staining. In the context of the present study, darker staining in the apple tissue would therefore be interpreted as evidence of relatively greater mitochondrial activity, providing the mechanistic basis for comparing the organic and non-organic samples.”

Reviewer Comment:

Citation practice is inconsistent as sentences beginning with attributions (“Some studies suggest”) often lack a citation entirely. Finally, restructuring the Methods as a clear, numbered protocol would substantially improve readability and reproducibility, and most figures still need explicit in-text references.

My Change:

Thank you for identifying this issue. I carefully reviewed the manuscript and revised statements containing vague attributions to include direct supporting citations. For example, statements

Referee 2 Detailed Point by Point Reply

such as "Some studies suggest..." were replaced with specific, cited claims. One example now reads: "(Barański et al., 2014) suggests that organic fruits contain higher concentrations of antioxidants, possibly due to increased natural stress exposure (like insect pressure) or the absence of synthetic pesticide protection. "

I reorganized the Materials and Methods section into a clear, numbered protocol that follows the experimental workflow step by step, improving both readability and reproducibility while preserving the original methodological details.

Additionally, upon checking to make sure all 5 of my figures are referenced meaningfully, I saw that I do reference each figure in the paper, with some figures being referenced more than once. Below I have pasted the areas in which my figures appear in my paper.

1. As shown in **Figure 1**, 0.5 grams of TTC powder was dissolved in 100 mL of distilled water under low-light conditions to prevent light-induced degradation.
2. Organic Samples (labeled Organic #1, #2, #3 in **Fig.2**) exhibited red staining.
3. Non-Organic Samples (labeled Nonorganic #1, #2, #3 in **Fig. 2**) showed paler, more brown coloration.
4. Since TTC is only reduced in the presence of viable mitochondria, the greater color change in the organic group implies increased respiration rates and enzymatic efficiency (**Fig. 5**).
5. While the results clearly show a difference (**Fig. 4**), they are based primarily on visual inspection and color sampling using HEX codes.
6. This confirms a highly significant difference in brightness between the groups, further validating the visual pattern observed in earlier trials (**Fig. 3**).
7. The full dataset was compiled into **Figure 4**, which includes each sample's HEX code and its corresponding Hue, Saturation, and Value (HSV) scores.
8. The amount and depth of this red staining correlates directly with the intensity of mitochondrial respiration and enzyme activity (**Figure 3**).

Referee 3

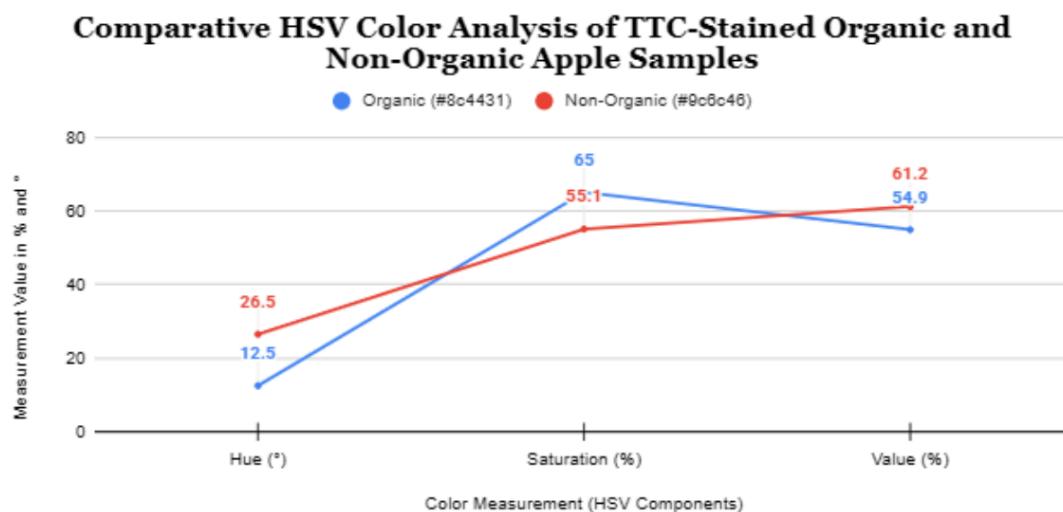
Referee 3 Detailed Point by Point Reply

Reviewer Comment:

One comment that would be great to address before publication would be the lines, R-squared values, and error bars in Figure 3. The lines and R-squared values are misleading as the values are in different units and the order of the x-axis is purely arbitrary. I suggest removing trendlines and R-squared values from Figure 3. As for the error bars, I am not sure if they are correct as they look quite big to me. If they are correct, I suggest addressing it in the main text. If they are not significant, I suggest removing the error bars from Figure 3 to avoid confusion.

Response:

Thank you for this thoughtful suggestion. I agree with your observation and appreciate you pointing this out, as I had not previously considered how the trendlines and R^2 values could be misleading given that Hue, Saturation, and Value represent independent colorimetric measurements rather than sequential variables. In response to your comment, I removed the trendlines, corresponding R^2 values, and error bars from Figure 3. The revised figure now presents only the average HSV values for the organic and non-organic samples, resulting in a clearer and more accurate visual representation of the data while avoiding any potential confusion or misinterpretation.



This manuscript has improved considerably from the initial submission; kudos to the author. It is already in a strong state, but I noticed some essential (yet hopefully minor) inconsistencies that should be addressed before the manuscript is published:

1. Present the sample size a bit more consistently in the data, especially in making it clear whether you are doing the 3+3 slices case vs. the 6+6 slices case. Every plot and analysis that depends on the sample size should make it immediately clear which case you are referring to, even though this could be “reconstructed” by reading the whole manuscript through and through. Perhaps state explicitly (e.g., towards the beginning of the manuscript) something like, “An initial pilot experiment used three apples per group. A second experiment used six new apples per group. Only the second experiment was used for the reported statistical analysis.”
2. The descriptive statistics on page 14 do not appear to match the appendix data. For example, the appendix shows organic Hue values around 12.5-13.3 and Saturation around 65.8-67, but page 14 reports an organic Hue mean of 18.18 and Saturation mean of 44.29. Is this because the appendix data were based on the earlier 3+3 results? It seems so, but make sure this is clearly stated in the appendix (or that your appendix cites more recent results).
3. Ensure all your images and figures are at least 300 dpi in quality when viewed as part of the manuscript.
4. The final Figure 3 does appear to omit error bars and trendlines, but the caption still says that error bars indicate standard deviation.
5. Make sure to clearly explain what constitutes “one observation.” Note that, in my view, the three locations sampled within a single slice are **subsamples** of the same slice, not three independent biological replicates. The slice-level average should be one observation, but make sure this is explicitly noted, particularly in the statistical section
6. For statistics, you should also report the test statistic, not only the p-value; whether the t-test was Welch’s or pooled-variance; and the exact n per group (even though, again, this could be reconstructed from elsewhere).
7. Please replace the connected-line chart with a more appropriate graph; the variables in Figure 3 are different variables with different interpretations. Joining them with lines visually suggests a sequence or trend that does not exist. A grouped bar chart or dot plot would be clearer.
8. The manuscript performs statistical tests, but later says that the lack of objective instrumentation limits the ability to conduct statistical comparisons. I appreciate the conservatism here, but I would correct this to something like: “The digital image measurements permitted statistical comparison of the sampled HSV values, but the absence of calibrated colorimetry or spectrophotometry prevents conversion of these measurements into absolute formazan concentration or mitochondrial respiration rate.”
9. You can discuss farming practices as hypotheses, but you should not present them as demonstrated explanations, as you don’t actually measure variables like pesticides, hormesis, etc. For instance, change “it reflects how agricultural methods imprint themselves at the molecular level” to something like “motivates further controlled investigation of whether agricultural practices influence post-harvest metabolic activity.” As you can see, that sounds (and is) more carefully stated.