

The Differences in Mitochondrial Activity at the Cellular and Molecular Levels 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 identify differences in TTC staining between the sampled organic and non-organic apples; however, the agricultural or biochemical mechanisms underlying these differences were not directly measured. 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 questions and experimental biology, this research demonstrates an accessible approach for investigating measurable cellular differences in consumer-grade produce and motivates further controlled study of their potential causes.

Keywords: mitochondrial activity, 2,3,5-Triphenyltetrazolium chloride (TTC) assay, organic produce, redox biology, formazan, antioxidants, cellular respiration, apples, plant biology, molecular & cell biology, biology, environmental science, food science

1. Background

1.1. 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.

1.2. 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 are 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.

1.3. 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), which 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 in organic versus non-organic samples.

1.4. 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.



1.5. 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.

1.6. 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?

2. Materials and Methods

An initial pilot experiment used three organic and three non-organic Granny Smith apples. Following this preliminary trial, a second experiment was conducted using six new organic and six new non-organic Granny Smith apples. Only the second experiment ($n = 6$ apples per group) was used for the quantitative HSV analysis and reported statistical tests. Each apple contributed one approximately 1-cm-thick central slice. 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. For the second experiment, the jars were labeled Organic #1 through Organic #6 and Non-organic #1 through Non-organic #6 to prevent sample mix-ups during subsequent analysis. 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.

2.1. 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 twelve 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. For the second experiment, the jars were labeled Organic #1 through Organic #6 and Non-organic #1 through Non-organic #6 to prevent sample mix-ups during subsequent 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, and 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. The three sampled locations within each slice were treated as technical subsamples rather than independent biological replicates. Their values were averaged before statistical analysis so that each apple slice contributed one independent observation for each HSV component. Thus, the second experiment contained six independent observations per group ($n = 6$ organic and $n = 6$ non-organic), rather than 18 observations per group. 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.

2.2. 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 (initial pilot labeled Organic #1, #2, #3 in Fig. 2) exhibited red staining.
- Non-Organic Samples (initial pilot 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.

3. Statistical Analysis of Colorimetric Data

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, and a Mann-Whitney U-test was used as a non-parametric confirmation in case the



small sample size violated normality assumptions.

Statistical comparisons were performed using the six slice-level observations from each group in the second experiment ($n = 6$ organic; $n = 6$ non-organic). Welch's independent two-sample t-test was used to compare group means without assuming equal population variances. A two-sided Mann-Whitney U-test was additionally performed as a non-parametric comparison. The three within-slice color measurements were averaged before testing and were not treated as independent observations.

Results:

- **Hue:**
 - Welch's independent two-sample t-test: $t(7.27) = -49.79$, $p = 1.78 \times 10^{-10}$, $n = 6$ per group.
 - Two-sided Mann-Whitney U-test: $U = 0$, $p = 0.00216$, $n = 6$ per group.
- **Saturation:**
 - Welch's independent two-sample t-test: $t(6.68) = 56.19$, $p = 3.48 \times 10^{-10}$, $n = 6$ per group.
 - Two-sided Mann-Whitney U-test: $U = 36$, $p = 0.00216$, $n = 6$ per group.
- **Value:**
 - Welch's independent two-sample t-test: $t(10.00) = -56.48$, $p = 7.35 \times 10^{-14}$, $n = 6$ per group.
 - Two-sided Mann-Whitney U-test: $U = 0$, $p = 0.00216$, $n = 6$ per group.

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

Across all three color components—Hue, Saturation, and Value—both the parametric (t-test) and non-parametric (Mann-Whitney U) statistical analyses confirm large, 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.

4. Analysis and Broader Implications

4.1. 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 characterize variability within the second experiment, 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 slices. For the organic group ($n = 6$), mean Hue was 12.98° (SD = 0.29, SEM = 0.12), mean Saturation was 66.23% (SD = 0.45, SEM = 0.18), and mean Value was 54.95% (SD = 0.19, SEM = 0.08). For the non-organic group ($n = 6$), mean Hue was 26.52° (SD = 0.60, SEM = 0.24), mean Saturation was 55.05% (SD = 0.19, SEM = 0.08), and mean Value was 61.05% (SD = 0.19, SEM = 0.08). These descriptive statistics correspond to the second-experiment data reported in Figure 4.

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 the sampled apples, including the initial pilot observations and the expanded second experiment. The staining pattern suggests that mitochondrial activity, as indicated by TTC reduction, was consistently more robust in organic samples. Although experimental conditions and procedural steps were standardized, unmeasured factors such as harvest timing, storage history, ripeness, and farm-specific growing conditions could also have contributed to the observed differences between the sampled groups. 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 possible explanation 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.

Differences in agricultural practices represent another hypothesis that could be investigated in future controlled studies. 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. 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. Second, although the final quantitative experiment expanded the sample size to six organic and six non-organic apples, the sample size remains relatively small and represents only one apple cultivar and one purchasing source. Additional trials with larger and more diverse samples would improve the generalizability of the findings. 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 observed TTC staining differences demonstrate a measurable distinction between the sampled groups, while further controlled research is required to determine whether this pattern is reproducible and attributable to agricultural practices. 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.”

4.2. 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 sampled organic apple slices motivates further controlled investigation of whether agricultural practices influence post-harvest metabolic activity.

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 may 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 et al. (2024) mention, 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. These possibilities warrant controlled investigation into whether cultivation conditions contribute to measurable differences in post-harvest cellular metabolism (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.

4.3. 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 a measurable approach for comparing cellular characteristics among produce samples grown under different agricultural classifications. 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.

5. 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 generate testable questions. 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.



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Author Biography

Shambhavi Sinha is a high school student at L.D. Bell High School with interests in biomedical research, cellular biology, and medicine. Her research experience includes investigating mitochondrial activity through TTC staining, CRISPR research using *C. elegans*, and cellular metabolism and circadian biology research at the University of Pittsburgh Medical Center and the University of Pittsburgh. She has also contributed to research at the Perot Museum of Nature and Science in collaboration with UTA, studying elemental concentrations in avian specimens. Beyond research, Shambhavi serves as Texas DECA District 12 President, representing more than 3,000 students across 11 counties, and leads community initiatives centered on public health, environmental awareness, and service. She hopes to pursue medicine while continuing to explore research at the intersection of cellular biology and human health.

Mentor Contribution Statement

Ms. Kathleen Fisher served as an academic mentor by providing the initial opportunity and framework through which Shambhavi Sinha developed this research project. As part of an English research paper assignment, Ms. Fisher encouraged students to pursue topics aligned with their individual academic interests. This opportunity allowed Shambhavi to combine her interest in cellular biology and mitochondrial function with the research and writing skills developed in the course.

Mrs. Ricki Tucker supported the experimental component of the project by providing Shambhavi Sinha access to laboratory space at Lawrence Dale Bell High School. She permitted Shambhavi to use the laboratory during morning hours and provided access to available laboratory materials and equipment necessary to conduct the experiment safely and effectively. Her contribution primarily involved facilitating access to an appropriate experimental workspace and resources.



7. Appendix



Figure A1. 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.

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 A2. In-Depth Color Analysis of Both Initial Pilot Apple Samples with a table containing colorimetric values.

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

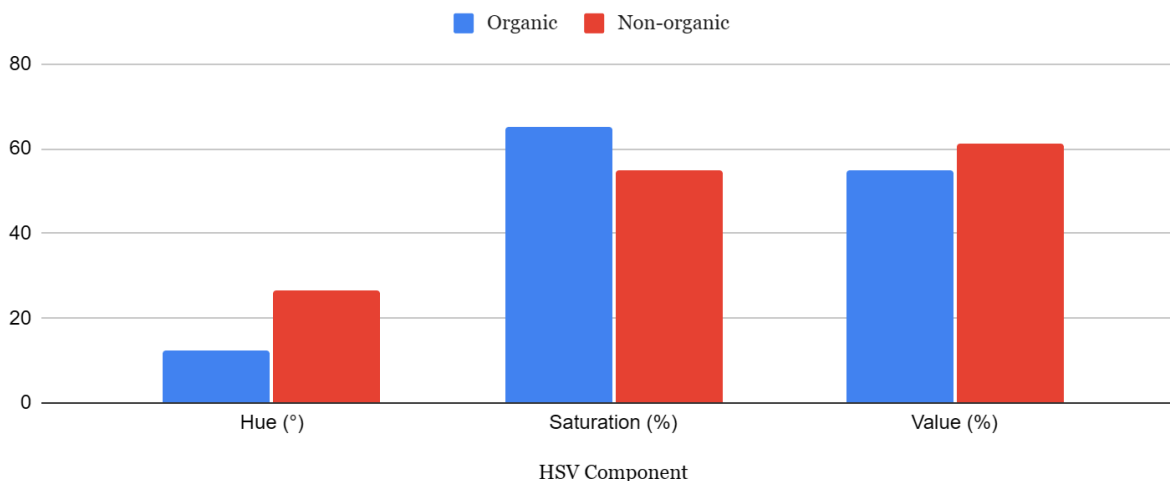


Figure A3. Quantitative HSV Comparison Between Organic and Non-Organic Apple Slices. Graph showing the average Hue, Saturation, and Value (HSV) percentages across organic (blue) and non-organic (red) groups after TTC staining.

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 A4. Summary of HEX and HSV Color Values for Organic and Non-Organic Apple Slices Following Second TTC Incubation (n = 6).



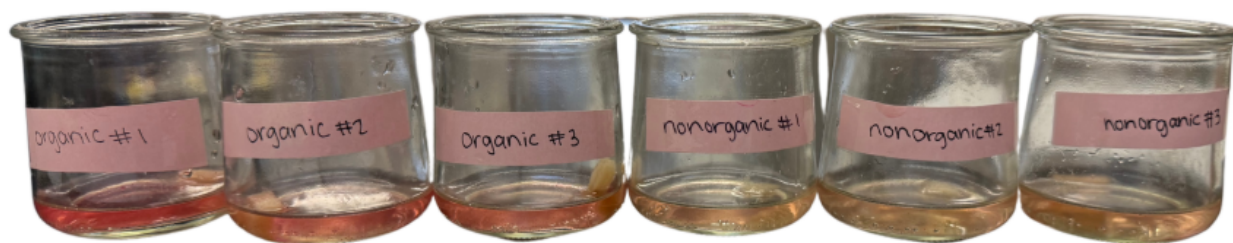


Figure A5. Visual Comparison of Post-TTC Incubation Staining Across Organic and Non-Organic Samples.