

**HPLC-BASED FORENSIC DIFFERENTIATION BETWEEN ORGANIC AND
CONVENTIONAL TOMATOES**

MUHAMMAD AIMAN HAKIMI BIN MOHD ZAMRI

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**HPLC-BASED FORENSIC DIFFERENTIATION BETWEEN ORGANIC AND
CONVENTIONAL TOMATOES**

by

MUHAMMAD AIMAN HAKIMI BIN MOHD ZAMRI

**Dissertation Submitted in Partial Fulfillment of The Requirement for The Degree of
Bachelor of Science (Honours) (Forensics Sciences)**

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CERTIFICATE

This is to certify that the dissertation entitled HPLC-Based Forensic Differentiation between Organic and Conventional Tomatoes is the bona fide record of research work done by Muhammad Aiman Hakimi bin Mohd Zamri during the period from October 2025 to February 2026 under my supervision. I have read this dissertation and that in my opinion it conforms to acceptable standard of scholarly presentation and is fully adequate, in scope and quality, as a dissertation to be submitted in partial fulfilment for the degree of Bachelor of Science (Honours) (Forensic Science)

Main supervisor,

.....

Dr. Ahmad Fahmi Lim bin Abdullah

Lecturer

School of Health Sciences

Universiti Sains Malaysia

Health Campus

16150 Kubang Kerian

Kelantan, Malaysia

Date: 29/01/2026

DECLARATION

I hereby declare that this is the result of my own research, except where otherwise stated and duly acknowledge. I also declare that it has not been previously or concurrently submitted as a whole for any other degree at Universiti Sains Malaysia or other institutions. I grant Universiti Sains Malaysia the right to use the dissertation for teaching, research, and promotional purposes.

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MUHAMMAD AIMAN HAKIMI BIN MOHD ZAMRI

Date: 29/01/2026

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While many have contributed to this endeavor, any errors or omissions within this thesis are solely my responsibility.

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Abbreviations

ANOVA	Analysis of Variance
APCI	Atmospheric Pressure Chemical Ionisation
BHT	Butylated Hydroxytoluene
HPLC	High-Performance Liquid Chromatography
HPLC-DAD-MS	High-Performance Liquid Chromatography coupled with Diode Array Detection and Mass Spectrometry
IBM SPSS	IBM Statistical Package for the Social Sciences
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
LC-MS	Liquid Chromatography–Mass Spectrometry
LDA	Linear Discriminant Analysis
LOD	Limit of Detection
MRL	Maximum Residue Limit
PCA	Principal Component Analysis
PDA	Photodiode Array Detector
RP-HPLC	Reversed-Phase High-Performance Liquid Chromatography
GMO	Genetically Modified Organism
NOP	National Organic Program
USDA	United States Department of Agriculture

Abstract

The higher market value of organic food has increased concerns about food fraud, creating a need for reliable methods to verify organic claims. This study aimed to differentiate between organic and conventional tomatoes using HPLC combined with chemometric analysis, focusing on carotenoid profiles. Tomato samples were extracted and analysed using reversed-phase HPLC with photodiode array detection. Major carotenoids were identified based on their retention times and spectral characteristics. Lycopene was found to be the dominant compound, followed by β -carotene, while other chemical substances were present in smaller amounts. The chromatographic data were processed and normalised prior to statistical analysis using multiple logistic regression, PCA and LDA. Multiple logistic regression did not show statistically significant differences between organic and conventional tomatoes ($p > 0.05$) based on the chemical substances appeared in the chromatograms. PCA score plots also showed overlapping clusters, suggesting that overall chemical variation was not strongly linked to whether they were conventional grown or of organic origin. However, LDA based on the PCA-scores had achieved an overall classification accuracy of 84.6%, demonstrating the possible to differentiate the two groups. In conclusion, although visual comparison of HPLC chromatograms alone was not sufficient for differentiation, the combination with chemometrics has provided possible classification. This study highlighted the importance of HPLC analysis in supported by multivariate approaches for food authentication and forensic analysis.

CHAPTER 1

INTRODUCTION

1.1 Background of the study

The global market for organic food has experienced substantial growth over the past decade, driven by increasing consumer perception that organic produce is healthier, safer, and more environmentally sustainable. Such premium perception justifies a significant price differential compared to conventionally grown counterparts (Chen et al., 2024). Tomatoes, as one of the most widely consumed vegetables worldwide, represent a key commodity within this economic landscape. However, the very price premium creates a potent incentive for fraudulent mislabeling, where conventional produce is deliberately sold as organic for illicit gain. This practice constitutes a serious form of economic food fraud, deceiving consumers, undermining public trust, and inflicting financial damage on legitimate organic producers (Manning & Kowalska, 2021). Consequently, the authentication of organic claims has emerged as a critical challenge within the domain of forensic food science, requiring robust analytical methodologies to verify product integrity and protect the food supply chain.

Scientifically, the debate surrounding the tangible benefits of organic produce, particularly regarding nutrient content, remains inconclusive and often contradictory. While consumers broadly believe organic vegetables possess superior nutritional profiles and lower contaminant levels, a comprehensive review of the literature reveals a more complex picture. As highlighted by Hoefkens et al. (2009), scientific evidence is frequently insufficient to conclusively declare organic

vegetables more nutritious overall, with differences often limited to specific compounds like Vitamin C rather than total nutritional value. Research focusing on tomatoes exemplifies this inconsistency. Studies such as Borgulini et al. (2013) found significantly higher levels of ascorbic acid and total phenolics in organic tomatoes, while reporting no significant difference in lycopene concentrations. Conversely, Vallverdú-Queralt et al. (2014) observed higher total carotenoid levels in processed organic tomato products. This variability suggests that comparing the concentration of a single, generic nutrient such as total lycopene is an unreliable metric for authentication, as differences can be confounded by factors like cultivar, soil type, climate, ripeness at harvest, and post-harvest handling.

This ambiguity directs the scientific community towards a more sophisticated paradigm for example chemical fingerprinting. Instead of quantifying individual nutrients in isolation, the focus shifts to characterising the unique pattern or signature of secondary metabolites such as specific carotenoid isomers that collectively form a biochemical profile influenced by cultivation practices. The stress-response mechanisms in plants grown without synthetic pesticides may lead to an altered metabolic pathway expression, resulting in a distinct compositional profile of antioxidants and other phytochemicals. HPLC is a powerful analytical technique ideally suited for this task, capable of separating, detecting and quantifying these complex mixtures of compounds to generate a detailed chromatographic fingerprint for each sample.

Therefore, this study is situated at the intersection of analytical chemistry, forensic science, and forensic food authentication. It posits that the solution to the organic fraud problem lies not in

seeking a single definitive “healthier” marker, but in employing chemometric analysis to decipher the multivariate chemical signatures imparted by different agricultural systems. By applying reversed-phase HPLC to generate detailed chromatographic profiles of authenticated organic and conventional-grown tomatoes, followed by pattern recognition techniques like PCA, this research aims to develop a discriminatory model, seeking to reliably classify tomatoes based on their farming method, thereby translating a chemical profile into a forensic tool for authentication. This approach advances the field from inconclusive nutrient comparisons to a reliable, evidence-based methodology for combating food fraud, aligning forensic science principles with the practical need to ensure food integrity and consumer protection.

1.1.1 Conventional Food Products

Within the context of modern agriculture and food science, the term conventional food product refers to products or processed items cultivated and manufactured using mainstream and industrialised agricultural practices. This paradigm, often termed conventional agriculture, is characterised by the systematic use of synthetic chemical inputs to maximise efficiency, yield and economic return. Key hallmarks include the application of man-made pesticides for pest and weed control, the use of synthetic or mineral fertilizers to promote rapid plant growth, and, in livestock production, the routine use of antibiotics and growth hormones.

The conventional system is fundamentally designed for scale and consistency within a global supply chain, often prioritising crop uniformity, shelf stability and high throughput over specific ecological or process-based methods. In direct contrast to certified organic production,

conventional farming is not governed by a universal prohibitive standard against synthetic inputs. Rather, it operates within regulatory limits for chemical residues, such as maximum residue limits (MRLs). For this research, conventional tomatoes are defined as those grown under this mainstream agricultural framework, utilising the synthetic inputs and management practices typical of modern, non-organic commercial farming, and serving as the control against which organically grown samples were analytically compared.

1.1.2 Organic Food Products

An organic food product is defined not merely by the absence of synthetic inputs, but by its production within a formally regulated, ecologically managed agricultural system. According to international and national certification standards such as those set by the United States Department of Agriculture (USDA) National Organic Programme (NOP), the European Union or the Malaysian Department of Agriculture. Organic production emphasises ecological balance, biodiversity and soil health. It explicitly prohibits the use of synthetic pesticides, herbicides and fertilizers, genetically modified organisms (GMOs), and in livestock, routine antibiotics and growth hormones.

Instead, it relies on natural processes and substances, including crop rotation, green manure, compost, biological pest control, and mechanical cultivation. Crucially, the label organic is a process-based claim, verified through a rigorous certification and audit trail that documents compliance from farm to point of sale.

For this research, organic tomatoes are therefore defined as those produced and certified under such a regulated system, where the resulting biochemical profile of the produce is hypothesised to be distinctly shaped by these specific agronomic stresses and inputs, creating a unique chemical fingerprint that can be differentiated from conventional produce through an advanced analytical technique, in this case, the high-performance liquid chromatography (HPLC).

1.2 Problem Statement

Organic vegetables are sold in a higher price with declaration on higher nutrient content and no pesticide. It is unclear if the nutrient content or the vegetable profiles are of the same or different as declared by the suppliers. It is important to differentiate between organic and conventional grown vegetable to avoid fraud in selling and purchasing using defensible strategy. A reliable test that can identify certified organic tomatoes and processed products is currently not widely available, that is easy to use, fast and cheap, regardless of the variety of the products or how they were processed.

1.3 Objectives

1.3.1 General Objective

The general objective of research project is to differentiate organic and conventionally grown tomatoes by HPLC in combination with chemometrics.

1.3.2 Specific Objectives

- To determine the signature chemical substance in tomato samples.
- To compare the HPLC profiles of organic and conventional grown tomatoes.
- To differentiate organic and conventional grown tomatoes through chemometrics.

1.4 Study Significance

1.4.1 Authentication and Combating Food Fraud

Consumers generally perceive organic food as healthier and safer than conventionally produced food, which strongly influences purchasing decisions. However, scientific evidence supporting these perceptions is often inconclusive or inconsistent, leading to a significant trust gap between consumer beliefs and verifiable proof. This gap creates opportunities for misleading claims and food fraud, particularly in high-value organic products. Addressing this issue provides important benefits, as reliable verification methods can help combat food fraud by confirming the authenticity of organic claims. In doing so, such measures not only protect consumers from deception but also support honest producers by safeguarding the integrity and credibility of genuinely organic food products.

1.4.2 Advancing Metabolomic Fingerprinting in Food Forensics

Natural factors such as soil composition and climatic conditions introduce a high degree of variability in tomato characteristics, which makes accurate analysis and comparison challenging. Despite this inherent variability, the applied approach is able to reliably identify the distinct organic

signal, enabling meaningful differentiation between organically and conventionally produced tomatoes.

1.4.3 Establishing Definitive Chemical Markers

Ongoing debates regarding total nutrient content, such as overall lycopene levels, have produced inconclusive findings and limited consensus. As a result, there is a need for a more sophisticated and definitive approach that directly links the chemical signature of food products to the organic farming process, thereby providing stronger and more reliable evidence of organic production practices.

CHAPTER 2

LITERATURE REVIEW

2.1 High Performance Liquid Chromatography (HPLC)

HPLC stands as a crucial analytical technique in modern separation science, renowned for its precision, versatility and robustness in resolving complex chemical mixtures. Fundamentally, HPLC operates on the principle of differential partitioning of analytes between two phases which are a pressurised liquid mobile phase and a solid stationary phase packed within a column. The sophisticated interaction facilitates the physical separation of individual compounds based on distinct chemical properties such as polarity, size and affinity (Marandi3, 2021).

In principle, as the sample mixture is propelled through the column by the high-pressure pump system, components with greater attraction to the stationary phase are retained longer, while those with a stronger affinity for the mobile phase elute more rapidly. The resultant separation produces a chromatogram, where each peak corresponds to a specific compound characterised by its unique retention time and spectral signature, thereby transforming a complex extract into a detailed, interpretable chemical profile (Marandi3, 2021).

The selection of HPLC for this forensic food authentication project is deliberate and well-founded, given its exceptional suitability for analysing the target phytochemicals in tomatoes. The methodology employs RP-HPLC, a dominant mode wherein the stationary phase is non-polar typically a C18 hydrocarbon chain and the mobile phase is polar, creating an ideal environment

for separating moderately non-polar to semi-polar compounds like carotenoids and phenolic antioxidants (Yabré et al., 2018).

The system's critical components including a high-precision pump for maintaining a consistent gradient elution, an auto-sampler for accurate and reproducible injection of tomato extracts, a column compartment with thermostat to ensure stability, and a sensitive ultraviolet-visible or photodiode array (PDA) detector work in concert to achieve high-resolution separation. The PDA detector is particularly advantageous, as it captures the full ultraviolet-visible absorption spectrum for each eluting peak, providing not only quantitative data but also spectral information crucial for the tentative identification of compounds by comparing retention times and spectra with authentic standards.

In the specific context of differentiating organic from conventional tomatoes, HPLC transcends mere quantification to become a tool for generating definitive chemical fingerprints. The technique's capacity to resolve subtle differences. The resulting chromatographic profile serves as a comprehensive biochemical snapshot of the sample. This multi-constituent data matrix, rich in both qualitative information, forms the essential foundation for subsequent chemometric analysis.

Techniques like multiple logistic regression, principal component analysis (PCA) and linear discriminant analysis (LDA) can process this multivariate data to identify patterns, cluster samples, and develop statistical models for classification. Consequently, HPLC provides the rigorous, reproducible, and chemically detailed data required to move beyond subjective comparison and

establish an objective, scientifically defensible method for forensic authentication turning a chromatographic output into credible evidence in the fight against food fraud.

2.2 Consumer Perception, Trust Gap and the Forensic Problem of Organic Food Fraud

The premium price of organic produce is driven by strong consumer perceptions of superior health and safety benefits (Hoefkens et al., 2009). However, a critical analysis reveals a significant 'trust gap,' as scientific evidence for these perceived nutritional advantages remains largely inconclusive (Hoefkens et al., 2009). This disconnect between belief and evidence creates a market vulnerability where the economic incentive to mislabel conventional produce as organic is high, constituting a clear case of food fraud, especially economically motivated adulteration.

As highlighted by Hoefkens et al. (2009), comparative studies on single nutrients such as vitamins and lycopene between organic and conventional systems yield inconsistent and contradictory results. This suggests that a univariate analytical approach is inadequate for authentication. Therefore, this study proposes a shift in paradigm: instead of quantifying individual disputed nutrients, HPLC was employed to generate a comprehensive chemical profile or 'fingerprint' that captures the complex multivariate metabolic signature of the cultivation practice for comparison.

2.3 Limitations of Current Organic Verification Approaches

While existing verification often relies on pesticide residue analysis, a point where science does align with consumer belief, the analysis itself has several limitations (Hoefkens et al., 2009). Pesticide residue analysis cannot directly authenticate organic produce grown in contaminated

environments or verify processed products where residues may degrade. Furthermore, it does not address the core consumer belief in nutritional superiority. Consequently, a more robust method capable of profiling intrinsic phytochemical compositions, which are influenced by agronomic stress and practices, is required.

2.4 Inadequacy of Univariate Nutrient Analysis for Authentication

Previous research has effectively mapped the landscape of consumer perception versus scientific evidence (Hoefkens et al., 2009), identifying a clear gap. However, fewer studies have proposed and validated accessible analytical solutions to bridge this gap for specific, high-value commodities like tomatoes. This project is initiated to fill that void by developing a practical HPLC-based chemometric method to provide the objective, chemical evidence currently lacking in the organic authentication pipeline.

Attempts to authenticate organic produce by comparing single nutrients are fraught with inconsistency, as demonstrated by *de Castro et al. (2021)*. In the study, carotenoid and mineral contents in organic and conventional vegetables showed significant species-dependent variation. For example, organic carrots contained higher carotenoid levels, whereas conventional broccoli exhibited higher concentrations. In addition, these nutritional differences were substantially altered by the cooking methods applied. This indicates that a single compound like lycopene or potassium is a poor forensic marker, as its concentration is a product of farming practice, plant genetics and post-harvest handling. Therefore, a more robust approach that captures a stable, multivariate chemical signature inherent to the cultivation practice itself is required. While post-

harvest processing like cooking is a major determinant of final nutrient content (*de Castro et al.*, 2021), this study focuses on establishing the foundational chemical differences in raw tomatoes. By controlling for the variable of cooking, the research aims to isolate and identify the chromatographic fingerprint imparted specifically by organic versus conventional farming practices. This creates a baseline profile that can later be adapted to other study processed products.

2.5 Rationale for Multivariate HPLC Fingerprinting and Chemometric Analysis

The literature search reveals a landscape of extreme chemical variability. For instance, *de Castro et al.* (2021) recorded carotenoid levels in conventional zucchini that could be either higher or lower than their organic counterparts depending on the cooking technique. This complexity underscores the limitation of univariate analysis. In contrast, this thesis employs chemometric analysis of full HPLC chromatograms, a technique capable of handling such multivariate complexity and identifying stable patterns that reliably differentiate the two agricultural systems despite background variation.

2.6 Carotenoids as Target Phytochemical Markers in Tomato Differentiation

The analysis of carotenoids, a key class of phytochemicals implicated in plant stress response, was well-established using HPLC. The extraction and partitioning protocols employed in this study are based on validated methods used in recent comparative studies of organic and conventional vegetables (*de Castro et al.*, 2021), ensuring the analytical reliability of the generated profiles.

The profiling of complex carotenoid mixtures, necessary for capturing a cultivation-specific chemical signature, is feasible with high-resolution separation techniques. As demonstrated by Müller (1997), RP-HPLC coupled with photodiode array detection was the long established method for separating, identifying, and quantifying the numerous carotenoids present in vegetables, having been used to resolve 27 distinct compounds including various geometric isomers. This study adopted the robust analytical platform to generate the detailed chromatographic fingerprints of tomatoes required for chemometric analysis. The selection of carotenoids as target analytes was supported by their known variability and functional roles in plants. In tomato, which was the focus of this research, the carotenoid profile was characteristically dominated by lycopene but includes significant amounts of β -carotene, lutein, and xanthophylls such as violaxanthin (Müller, 1997). This complex, multi-constituent profile provided a rich source of multivariate data points that can be leveraged for differentiation purposes.

2.7 Importance of Whole-Profile Analysis over Single Marker Compounds

Early carotenoid research often focused on provitamin A activity, primarily quantifying β -carotene. However, as Müller (1997) comprehensively documented, edible plants contain a diverse spectrum of carotenoids with distinct structures and properties. Relying on a single compound for authentication was flawed, as its concentration is often influenced by numerous factors beyond farming practice for examples cultivar and ripeness. Therefore, this research aligns with the modern approach exemplified by Müller (1997), which captures the complete carotenoid profile, thereby obtaining a more stable and informative chemical signature of the tomato sample.

The sample preparation and chromatographic approach for carotenoid analysis was adapted from established protocols in food chemistry (Müller, 1997). The use of RP-HPLC with a C18 column and a gradient of organic solvents is a standard practice for achieving the resolution necessary to separate the predominant tomato carotenoids which are lycopene, β -carotene and lutein, as well as their potential isomers. The ratios of which may be influenced by cultivation stress, including the extrinsic factors such as UV, temperature and intrinsic factors such as nutrients.

2.8 Evidence from Comparative Studies on Organic and Conventional Crops

A study by Średnicka-Tober et al. (2022) which investigated phenolic and carotenoid concentrations in organic versus conventional carrots provided a directly relevant and insightful foundation for the proposed research on tomato authentication. The study effectively mirrored the core challenge outlined in the proposal, namely the discrepancy between consumer perception and scientific evidence regarding the nutritional superiority of organic produce. Its findings demonstrated that organic certification does not universally guarantee higher concentrations of all health-promoting bioactive compounds. Specifically, the study reported significantly higher total carotenoid and β -carotene levels in conventionally grown carrots, a result attributed to differences in nitrogen fertilisation regimes. This outcome critically challenged the common assumption that "organic equals more nutritious" and provided empirical support for the proposal's problem statement regarding unclear scientific evidence and the potential for a trust gap that could enable food fraud.

The specific findings from the carrot research offered crucial insights for shaping the expected outcomes and methodological focus of the tomato project. The identification of specific phenolic compounds such as gallic acid as potential biomarkers for the production system was highly encouraging. It suggested that a targeted search for signature compounds in tomatoes, rather than a reliance on total lycopene or phenolic content, is a viable and promising strategy. However, the carrot study also delivers a critical caution regarding variability and interaction effects; the significant influence of cultivar on system-based differences indicates that a reliable authentication method must be robust enough to account for such factors. This directly justifies the use of chemometric techniques, specifically PCA in the future study.

While the carrot study used ANOVA to test individual compounds, PCA could serve as a more powerful multivariate tool capable of discerning patterns within complex, variable datasets like those generated from multi-farm produce sampling. It can integrate information from all quantified compounds to potentially reveal a distinct metabolic fingerprint for each cultivation system, even when individual compound differences are inconsistent.

The work by Średnicka-Tober et al. (2022) serves as a foundational reference that strengthens the rationale, informs the methodology, and refines the expected outcomes of the proposed tomato authentication project. It provided concrete evidence of the complexity involved in comparing organic and conventional products, validated the use of HPLC for detailed phytochemical analysis, and highlighted the necessity of sophisticated data analysis via chemometrics to achieve reliable and forensic-level differentiation. Integrating these insights ensures the proposed research is

grounded in established scientific findings and addresses the nuanced challenges of authenticating organic food products.

2.9 Methodological Insights from Advanced HPLC Profiling Studies

The study by Collera-Zúñiga et al. (2005) provided a foundational methodological and analytical framework highly pertinent to the proposed research on authenticating organic tomatoes through HPLC-based profiling. Although the investigation focused on varietal differences in carotenoid composition among three Mexican cultivars of *Capsicum annuum* rather than on organic versus conventional comparisons, its technical rigor and findings offered critical insights for the design and interpretation of the tomato authentication study. The research demonstrated the sophisticated application of HPLC for the detailed separation and quantification of complex carotenoid profiles within a single plant species. Utilizing both normal-phase and reverse-phase chromatography, the authors successfully identified and quantified 24 distinct carotenoids, highlighting the power of chromatographic techniques to reveal subtle compositional differences. The methodological approach directly validated and informed the proposed use of RP-HPLC in the tomato study for generating comprehensive phytochemical fingerprints. The careful protocols for pigment extraction, saponification, and light- and oxygen-sensitive handling underscore the meticulous sample preparation required to obtain reliable metabolite profiles, a consideration equally crucial for the analysis of tomato carotenoids like lycopene and its isomers.

A key contribution of this paper is its emphatic demonstration of how intrinsic genetic factors specifically, cultivar selection, can dramatically alter the chemical profile of a crop. The study

found significant quantitative differences in carotenoid composition among the Ancho, Guajillo, and Mulato varieties, with Ancho showing the highest provitamin A activity. This serves as a critical reminder for the tomato authentication project, where the natural variation between tomato cultivars could be a significant confounding factor when seeking markers related to the farming system. This finding certainly warrants further investigation for food fraud determination.

To address the information gap, proposed research must, therefore, either control for cultivar or, more robustly, employ chemometric tools like PCA to discern the signal of cultivation practice amidst the noise of genetic variation. The pepper study by Collera-Zúñiga et al. (2005) exemplifies the type of complex and variably present metabolite matrix that chemometrics is designed to interpret. Furthermore, the identification of specific carotenoids such as β -carotene, β -cryptoxanthin and various capsanthin isomers as major discriminating compounds parallels the objective of the tomato project to identify signature substances. While the specific markers might differ for tomatoes, authentication might rely not on the presence or absence of compounds, but on their relative abundances and patterns within a detailed profile. The study's discussion on how pigments like violaxanthin and capsanthin dominate the profile, and how their ratios differ by variety, reinforced the concept that a multi-compound fingerprint was more informative than the concentration of any single compound.

The study by Delgado-Pelayo and Hornero-Méndez (2012) on the carotenoid composition of sarsaparilla berries represented a significant methodological benchmark in the detailed profiling of plant carotenoids using advanced chromatographic and mass spectrometric techniques. While

the research did not address organic versus conventional cultivation, its analytical sophistication and rigorous approach to carotenoid identification and quantification provided critical methodological insights for the proposed project on tomato authentication. The study employed a comprehensive analytical workflow involving HPLC-DAD-MS, utilising APCI. This approach enabled the authors not only to separate and quantify a wide array of carotenoids but also to definitively identify specific xanthophyll esters, such as various β -cryptoxanthin esters differentiated by their fatty acid moieties such as caprate, laurate and myristate. The successful application of LC-MS to unravel the complex esterification patterns demonstrated the power of coupling chromatography with mass spectrometry for obtaining detailed metabolic fingerprints.

For the tomato authentication study, this underscores the potential value of moving beyond standard HPLC-UV analysis. Incorporating mass spectrometric detection could greatly enhance the specificity and confidence in identifying potential "signature" compounds or unique esterification profiles that may be associated with organic or conventional farming practices. Methodologically, the paper emphasises several best practices crucial for phytochemical analysis that directly inform the proposed tomato study. These include meticulous sample handling under dim light, the use of antioxidants like BHT during extraction to prevent degradation, and systematic steps for saponification to distinguish between free and esterified carotenoids (Delgado-Pelayo and Hornero-Méndez, 2012).

The detailed protocol for quantification using external calibration curves for individual carotenoids established a model for achieving accurate and reproducible concentration data. Given that the

future study should seeks to compare subtle differences between production systems, adopting such stringent protocols will be essential to ensure that observed variations are genuine and not artifacts of sample preparation or analysis.

2.10 Empirical Evidence of Cultivation-System Effects in Tomatoes

The study by Borguini et al. (2013) also conducted a direct comparison of the antioxidant profile and activity between organically and conventionally cultivated tomato, with stringent controls for variables such as cultivar, geographic region, ripeness stage, and post-harvest handling. The analysis revealed a statistically significant superiority in specific antioxidant compounds within the organic tomatoes. Specifically, organic tomatoes contained higher concentrations of ascorbic acid (641.39 mg/100 g dry weight vs. 510.16 mg/100 g) and total phenolic compounds (4466.66 mg/100 g gallic acid equivalent on dry weight basis vs. 3477.50 mg/100 g gallic acid equivalent on dry weight basis). In contrast, lycopene content showed no significant difference between the two cultivation systems.

Borguini et al. (2013) concluded that organic cultivation of the Carmen tomato cultivar leads to a higher antioxidant potential, primarily driven by increased levels of ascorbic acid and phenolic compounds. They hypothesised that the absence of synthetic pesticides in organic farming might induce mild stress in plants, triggering a defence response that included the enhanced synthesis of these secondary metabolites. Methodologically, the study underscored the importance of using multiple extraction solvents and complementary antioxidant assays to capture the complex and solubility-dependent nature of antioxidant activity in plant matrices. The research provided the

empirical evidence that cultivation systems could selectively influence the phytochemical composition of tomatoes, a finding relevant to nutritional science and agricultural product authentication. This information could serve as a research direction for future study.

CHAPTER 3

METHODOLOGY

3.1 Tomato Samples

In this study, three conventional and three organic tomatoes were acquired from a commercial source in Kota Bharu, Kelantan. The tomatoes were bought from AEON Mall Kota Bharu in the fresh produce section on the ground floor. From the local vegetable section for the conventional tomatoes and from the imported organic vegetable section for the organic tomatoes.

3.2 Chemical and Reagents

HPLC grade methanol (SupraSolv®, Supelco®, Germany), HPLC grade acetone (SupraSolv®, Supelco®, Germany) and HPLC grade acetonitrile (SupraSolv®, Supelco®, Germany) were employed as components of the mobile phase for HPLC analysis. These solvents were selected due to their high purity, low UV absorbance and compatibility with reverse-phase HPLC, which ensured stable baseline signals and efficient separation of carotenoid compounds based on their polarity. For sample preparation, petroleum ether, distilled water, acetone and anhydrous sodium sulphate were used during the extraction of carotenoids. Acetone facilitated efficient disruption of the plant matrix and solubilisation of carotenoids, while petroleum ether enabled effective partitioning of non-polar carotenoid fractions. Distilled water was used for phase separation, and anhydrous sodium sulphate was applied to remove residual moisture from the organic extract prior to analysis.

3.3 Sample Preparation

Initial sample preparation involved a standardised washing step to ensure the removal of superficial contaminants. Each tomato was subsequently dissected into smaller segments, which were allocated to individual containers for subsequent lyophilisation process. Lyophilisation (freeze-drying) was carried out until a constant weight was achieved, effectively removing all moisture content to preserve compound integrity and prepare the samples for carotenoid extraction. Freeze dryer ILSHIN was used in this project. Five days were required to complete the process.

3.4 Extraction of Carotenoids

Carotenoid content was quantified in triplicate for each sample group. The extraction protocol was as follows: one gram of lyophilised material per tomato was homogenised in 20 mL of cold acetone using a ceramic mortar and pestle. The homogenate underwent vacuum filtration (Büchner funnel, Whatman No. 1 filter paper), and the extraction was repeated on the macerated residue until it was devoid of pigmentation. The same procedure was repeated for an additional four more times. All filtrates and apparatus rinses were consolidated in a 250 mL conical flask.

A liquid-liquid partitioning step was employed to isolate the carotenoids. The acetone extract was transferred to a 250 mL separatory funnel containing 20 mL of petroleum ether. To initiate phase separation and remove polar impurities, 500 mL of distilled water was added slowly along the inner wall of the funnel. The acetone was subsequently eliminated through four successive washings with 150 mL aliquots of distilled water, with the lower aqueous phase discarded after each wash. Following the final wash, the funnel stopcock was dried, and the carotenoid-containing

petroleum ether phase was passed through a drying column containing 7.55 g of anhydrous sodium sulfate, retained by glass wool. The desiccant was then eluted with additional petroleum ether, and the dried eluate was collected and volumetrically adjusted to 25.0 mL in an aluminium foil-covered flask to minimise possible photo-oxidation process. The samples were then transferred to clean chromatography vial and ready for HPLC analysis.

3.5 Qualitative HPLC Analysis of Carotenoids

Analytical separation was performed using a RP-HPLC system equipped with a C18 column. The instrument of HPLC used in the project was LC-20AD (Shimadzu, Japan). The column compartment was maintained at 25°C. Binary gradient elution protocol was implemented with mobile phase A (acetone) and mobile phase B (methanol:acetonitrile, 50:50, v/v) at a flow rate of 10.0 mL/min. Following the injection of a 10 µL aliquot of the tomato extract, the chromatographic run proceeded for 35 minutes. LabSolutions software was used as the software of the HPLC and the setting parameters were set up accordingly. LabSolutions is the instrument control and data analysis software used with HPLC systems, particularly those manufactured by Shimadzu. Its main function is to manage the entire HPLC workflow, from method setup to data interpretation.

In HPLC analysis, LabSolutions is used to control the instrument hardware. It allows the user to set and monitor operating parameters such as pump flow rate, mobile phase composition, gradient programs, column temperature, injection volume, and detector settings. This ensures that chromatographic conditions are applied consistently and accurately throughout the analysis.

LabSolutions also plays a crucial role in data acquisition. During an HPLC run, the software collects detector signals, for example, the absorbance values and converts them into chromatograms. These chromatograms display retention time and peak responses, which are essential for identifying and quantifying analytes in a sample.

Another important function of LabSolutions is data processing and analysis. The software automatically integrates peaks, calculates peak area or height, and performs quantitative analysis using calibration curves. This enables accurate determination of compound concentrations in samples, which is particularly important in analytical chemistry and forensic applications. Post-acquisition, the data were analysed using a suite of multivariate statistical methods to evaluate patterns and classify samples, specifically multiple logistic regression, PCA and LDA.

3.6 Statistical Analysis

Following chromatographic acquisition, HPLC data were processed using LabSolutions (Shimadzu) version 5.87 SP 1 prior to statistical analysis. The initial step involved reviewing chromatograms and performing peak reintegration to ensure accurate and consistent peak identification across all samples.

Peak reintegration was carried out to correct for errors arising from automatic integration, such as baseline drift, peak tailing, co-eluting peaks, or incorrect peak start and end points. Each chromatogram was visually inspected, and integration parameters including baseline setting, peak width, threshold, and slope sensitivity were adjusted where necessary. Reintegration was