

**FORENSIC CHEMICAL CHARACTERISATION OF TOMATOES AND
TOMATO SAUCES BY HIGH PERFORMANCE LIQUID
CHROMATOGRAPHY**

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CERTIFICATE

This is to certify that the dissertation entitled “Forensic Chemical Characterisation of Tomatoes and Tomato Sauces by High Performance Liquid Chromatography” is the bona fide record of research work done by Ms Lesanya a/p Ramalingam 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 standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation to be submitted in partial fulfillment for the degree of Bachelor of Science in Forensic Science (Honours).

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DECLARATION

I hereby declare that this dissertation is the result of my own investigations, except where otherwise stated and duly acknowledged. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees 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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21 January 2026

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LIST OF ABBREVIATIONS

HPLC	High Performance-Liquid Chromatography
GC	Gas Chromatography
UHPLC	Ultra-High Performance Liquid Chromatography
ICP	Inductively Coupled Plasma
PCA	Principal Component Analysis
PDA	Photodiode array
MD	Mahalanobis Distance
PLSR	Partial Least Square Regression
LC-MS	Liquid Chromatography-Mass Spectrometry

CIRIAN KIMIA FORENSIK TOMATO DAN SOS TOMATO MELALUI KROMATOGRAFI CECAIR BERPRESTASI TINGGI

ABSTRAK

Produk yang dihasilkan daripada tomato digunakan secara meluas, dan kandungan kimianya berbeza-beza bergantung kepada kaedah pemprosesan dan formulasi. Oleh itu, kaedah analisis diperlukan untuk membandingkan ciri-ciri produk tomato segar dan diproses. Kajian ini bertujuan untuk mencirikan secara kimia tomato segar dan tiga jenama sos tomato komersial menggunakan kromatografi cecair berprestasi tinggi (HPLC). Dalam kajian ini, sampel tomato dan sos tomato menjalani proses pengekstrakan pelarut sebelum dianalisis menggunakan HPLC. Karotenoid, yang terdapat dengan banyak dalam tomato, berjaya diekstrak daripada sampel tomato dan sos tomato menggunakan aseton dan dianalisis menggunakan HPLC fasa songsang yang dilengkapi dengan pengesanan tatasusunan fotodiod (PDA). Selain itu, kehadiran likopena turut dikenal pasti melalui profil kromatografi. Melalui analisis komponen utama (PCA), data telah diuraikan di mana dua komponen utama pertama menerangkan sebanyak 94.1% daripada jumlah varians. Skor PCA kemudiannya dianalisis menggunakan analisis diskriminan linear (LDA), yang mencapai ketepatan pengelasan keseluruhan sehingga 80%. Dapatan kajian menunjukkan bahawa penggunaan HPLC yang digabungkan dengan PCA dan LDA membolehkan pencirian kimia serta pembezaan produk berasaskan tomato. Hasil kajian ini mencadangkan potensi aplikasi kaedah tersebut dalam pengesanan forensik makanan.

ABTRACT

Products derived from tomatoes are widely consumed, and their chemical composition vary depending on how they are processed and formulated. Therefore, analytical methods are necessary to compare the characteristics of fresh and processed tomato products. This study was aimed to chemically characterise the tomato and three commercial tomato sauce brands utilising high-performance liquid chromatography (HPLC). In this study, tomato and tomato samples underwent solvent extraction before being subjected to HPLC. Carotenoids, which are found abundantly in tomatoes, were successfully extracted from the tomato and tomato sauce samples using acetone and analysed using reverse-phase HPLC equipped with photodiode array detector. Apart from that, the presence of lycopene was also revealed through the chromatographic profiles. Through principal component analysis (PCA), the data was decomposed in which the first two principal components explained 94.1% of the total variance. The PCA scores were then subjected to linear discriminant analysis (LDA), which achieved an overall classification accuracy of up to 80%. The findings demonstrated that the use of HPLC coupled with PCA and LDA allowed for chemical characterization and discrimination of tomato-based products. The outcome suggested the potential of the application for food forensic authentication.

CHAPTER 1: INTRODUCTION

1.1 Background of study

Tomatoes (*Solanum lycopersicum*) rank among the most significant vegetables grown around the globe. It belongs to the nightshade family, known as Solanaceae, which comprises over 3000 species (Padmanabhan et al., 2015a). Tomatoes and their derived products are the primary dietary source of lycopene and provide notable levels of antioxidants, including phenolics, flavonoids and ascorbic acid (Toor et al., 2005). However, certain tomato-based products, such as sauces, paste and puree, undergo industrial processing, and their chemical profile may not be similar to that of a fresh tomato. The antioxidant contents of the tomato and tomato sauces may also differ according to brands and how they were produced. While tomatoes are transformed into paste or sauces, a significant amount of carotenoids and polyphenols may be lost along with the peels and seeds, since these compounds are chemically attached to proteins and dietary fibres (Tagliamonte et al., 2023).

Despite the growing research on food products, real-world problems are still not addressed using chemical characterisation. From the forensic aspect, a blood stain found at the crime scene might be mistakenly determined as tomato sauce, which would lead to the misdirection of the investigating procedure at the very beginning stage. From the food perspective, certain manufacturers may adulterate a tomato product by adding colourants or substitutes to mimic the colour and flavour of fresh tomatoes. Despite current regulations, the frequency of food fraud is also on the rise, frequently motivated by the intention to reduce production expenses or improve product attractiveness for higher profits. Detecting food fraud proves challenging since it is not readily apparent to

consumers, and perpetrators often employ advanced techniques to avoid being caught (Habib et al., 2025).

Previous studies have shown that the chemical composition of tomato products can be greatly affected by industrial processing, and this can be identified using chromatographic techniques like HPLC. For instance, Kamiloglu et al. (2014) reported that paste processing and drying could significantly increase the bio accessible total lycopene content, total phenolic content, total flavonoid content, and total antioxidant capacity compared to fresh tomatoes. HPLC analysis showed a notable reduced level of rutin in both puree and juice. Similarly, Višnjevec et al. (2021), identified that 40 different phenolic compounds were detected in various tomato extracts, representing several classes of phenolic compounds, such as simple phenolic acid derivatives, hydroxycinnamoylquinic acid, flavones, flavonones, flavanol, and dihydrochalcone. The crude protein obtained from tomatoes could have retained most of these compounds, demonstrating that valuable phenolic compounds remained intact during the food processing of these co-product streams.

High-performance liquid chromatography, a modern analytical method, is frequently employed during compositional profiling of food products, such as separating lycopene, beta-carotene and other antioxidants in tomatoes (Daood et al., 2014). However, their application in forensic-related cases is not well studied especially in distinguishing the chemical profiles of fresh tomatoes and tomato-based products. By implementing the use of HPLC, chemical compounds such as lycopene, flavonoids and phenolics can be used to determine if a tomato-based product was subjected to adulteration by analysing the chromatographic profiles upon the analysis.

1.2 Problem statement

Tomato-based products are among the most widely used condiments worldwide. However, many of the products claiming to be tomato-based may not actually contain any tomatoes at all. Such falsification presented to customers raises serious issues regarding the authenticity of tomato-based products being sold. Supporting the concern, Londoño-Giraldo (2021) and colleagues reported that wild cherry tomatoes contain significantly higher and more diverse carotenoid profiles than commercial cultivars. While the carotenoid composition in wild tomatoes has been extensively studied, its forensic application for verifying the authenticity of commercial tomatoes is still not thoroughly investigated, especially through the use of HPLC.

1.3 Research objectives

1.3.1 General objectives

To characterise the chemical profiles of fresh tomatoes and tomato sauces using HPLC.

1.3.2 Specific objectives

1. To extract chemical compounds from three varieties of fresh tomatoes and three different commercial tomato sauces.
2. To determine the chemical compounds in tomato and tomato sauces based on HPLC chromatographic profiles.
3. To differentiate the HPLC profiles of tomato and tomato sauces through PCA and LDA.

1.4 Significance of study

Many commercial tomato sauces might include additives or alternatives that change their natural chemical composition. Therefore, High-Performance Liquid Chromatography analysis provides a forensic method to chemically differentiate authentic tomato content from processed tomato products. By separating and quantifying important chemical markers such as carotenoids like lycopene or beta-carotene, HPLC produces a distinct chromatographic profile that serves as a reference standard for comparing commercial products. The findings can enhance forensic approaches in verifying authenticity and tracing the origin of tomato-based products.

CHAPTER 2: LITERATURE REVIEW

2.1 Tomatoes

Tomatoes are the sole domesticated species of the section *Lycopersicon* of the genus *Solanum*, which includes 13 species or subspecies. It originated from the western coastal region of South America. Its domestication began in Mexico, and the fruit was brought to Europe by Spanish explorers in the mid-1500s (Padmanabhan et al., 2015b). For many years, tomatoes were considered toxic and unsafe for consumption, as they belong to the nightshade family. Today, tomato ranks as the second most widely consumed vegetable, followed by potato. The global average production of tomatoes is estimated to be around 159 million tonnes (Padmanabhan et al., 2015b).

Tomatoes are not just a tasty ingredient but also a source of significant nutrition. Tomatoes also play a crucial role in various sectors, including food processing, agriculture, biotechnology, beverages, and pharmaceuticals (Sattar et al., 2024). Tomato breeding initiatives are concentrated on creating new varieties that offer increased yields and resistance to stress, along with improved uniformity in fruit size, enhanced colour, and extended shelf life. However, these approaches often overlook the organoleptic qualities and nutritional benefits, negatively impacting commercial tomatoes. In recent years, there has been a rising consumer demand for flavourful and healthy products (Paolo et al., n.d.).

2.1.1 Chemical composition of tomatoes

The chemical composition of tomatoes determines their nutritional value, sensory characteristics, and health benefits. Tomatoes are known to contain several important nutrients, including vitamins, minerals, fibre, protein, essential amino acids, carotenoids, and phytosterols (Ali et al., 2021). Tomatoes are an excellent source of bioactive

compounds, commonly known as secondary metabolites. In addition to water, the primary components of tomatoes include carbohydrates, which contribute a slight sweetness, along with various organic acids that provide their characteristic flavour. Citric acid and malic acid are the most prevalent, although other acids like acetic, formic, lactic, and fumaric acids have also been identified (Raga Amrutha et al., 2019). They are rich in natural antioxidants, which include carotenoids (β -carotene and lycopene), ascorbic acid (vitamin C), tocopherol (Vitamin E), and important phenolic compounds (like quercetin, kaempferol, naringenin, and lutein). The primary carotenoid found in tomatoes is lycopene, giving them their characteristic red hue (Ali et al., 2021).

2.1.2 Factors affecting chemical composition

Factors influencing the quality of tomatoes can be categorised into two main groups, which are external factors (such as colour, shape and the absence of defects) and internal characteristics, including texture, sweetness, acidity, aroma, flavour, shelf life and nutritional content (Amr & Raie, 2022). The quality of tomato fruits and their acceptance by consumers, as evaluated through sensory analysis, can be strongly correlated with their chemical composition. The type of growth media, fertilizers and the sources of salinity could have influenced the composition of the fruit and its desirability (Amr & Raie, 2022).

There is a vast array of tomato cultivars exhibiting a wide variety of morphological and sensory traits that determine their suitability for various applications. The specific qualities and nutritional attributes of tomatoes are predominantly influenced by cultivar type, harvest timing, and various agronomic practices. The overall quality of tomatoes is determined by factors such as their appearance, firmness and chemical composition (Amr & Raie, 2022).

2.2 Major bioactive compounds

A bioactive compound is a material that exhibits biological activity, influencing a living organism directly (Mahfoudhi et al., 2016). Tomatoes are recognised as a vegetable with several health benefits due to their high concentration of bioactive compounds. The levels of biologically active compounds and their antioxidant properties can vary depending on factors such as ripeness and environmental conditions (Dūma et al., 2018).

2.2.1 Lycopene

Lycopene is a vibrant red pigment that is primarily present in red fruits and vegetables, including tomatoes, papayas, pink grapefruits, pink guavas, and watermelons. The pigment was initially identified as tomato by Millardet in 1876. Subsequently, it was named lycopene by Schunck (Kong et al., 2010a). Lycopene is also a hydrocarbon carotenoid, sometimes referred to as carotene. This significant characteristic of these compounds is their extensive conjugated double bond system, which is crucial for the vibrant colours of carotenoids, as it creates the light-absorbing chromophore (Rodriguez-Amaya & Kimura, n.d.).

Furthermore, lycopene is a linear, unsaturated carotenoid featuring 11 conjugated double bonds and two non-conjugated double bonds as shown in Figure 2.1. It is known to be the most effective singlet oxygen quencher among carotenoids, with its quenching capability primarily influenced by the quantity of conjugated double bonds, while the presence of cyclic or acyclic end groups has a lesser effect (Kong et al., 2010b) .

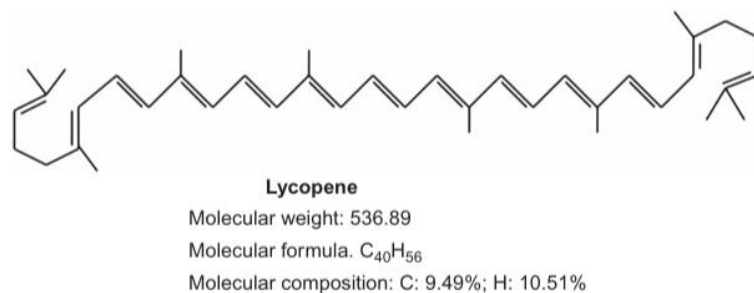


Figure 2.1: Chemical structure of lycopene

The acyclic open chain structure has 13 double bonds, which can undergo isomerisation, resulting in different cis isomers as observed in plants and blood plasma. Naturally, lycopene is present in all of its trans-isomers; however, the cis-isomers are the predominant forms found in tissues and plasma (Imran et al., 2020). During its metabolic process, lycopene oxidation initially yields lycopene 1,2- epoxide, and subsequently leads to the formation of lycopene 5,6-epoxide as the primary metabolites as shown in Figure 2.2. Lycopene does not dissolve in water, has limited solubility in methanol and ethanol, and can dissolve in organic solvents like carbon disulfide, ethyl ether, petroleum ether, chloroform and benzene (Cámara et al., 2013).

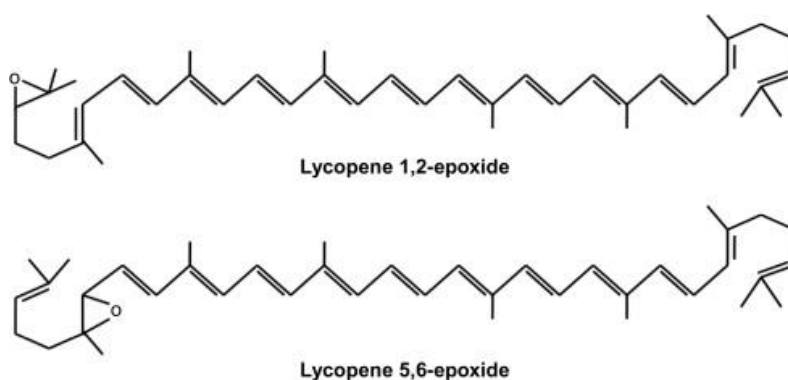


Figure 2.2: Chemical structure of common lycopene metabolites

2.2.2 β -carotene

Carotenoids are derived from various green components of plants, including leaves, seeds, flowers, tubers, fruits, and roots. β -carotene is synthesised within the thylakoid membranes of chloroplasts in plant cells as a byproduct of photosynthesis (Singh & Sambyal, 2022). β -carotene has a molecular weight of 536.888 g/mol and a chemical formula of $C_{40}H_{56}$ (Rudzińska et al., 2023). Its polycarbonate structure contributes significant hydrophobicity, making it a lipophilic substance. With a melting point of 183°C , it is able to withstand the heat of cooking, although it is not suitable for frying. It is first produced by plants, particularly carrots, and can be extracted through physiochemical methods (Meléndez-Martínez et al., 2010). Figure 2.3 shows the chemical structure of β -carotene consisting of a long symmetrical chain of 40 carbon atoms with 9 alternating double bonds.

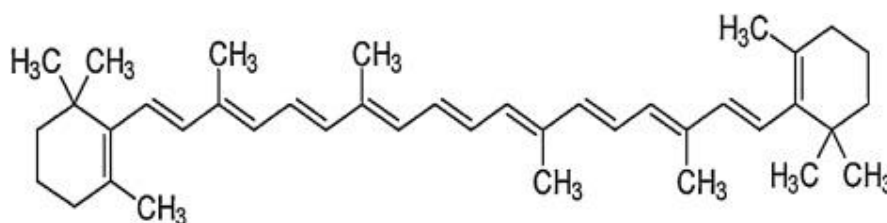


Figure 2.3: Chemical structure of β -carotene

High temperatures facilitate the isomerisation of these double bonds, enhancing the brightness of the resulting colour utilised in industries as an orange-red food colouring pigment (Meléndez-Martínez et al., 2010). The bioactivity of β -carotene not only contributes to its value as a food additive, but its industrial significance also stems from its coloring characteristics (Bogacz-Radomska & Harasym, 2018a). On an industrial level, molds such as *Blakeslea trispora* and the alga *Dunaliella salina* are utilised to produce β -

carotene. The cultivation process is carried out in three primary phases: increasing the inoculum, synthesising biomass and extracting carotenoids. Based on the desired final product of β -carotene, the biomass undergoes suitable technological processes (Bogacz-Radomska & Harasym, 2018b).

2.3 Tomato-based products and processing effects

Fresh tomatoes are often processed into a variety of products that hold a longer shelf life. The processing effects to enable these products to last longer might affect the original tomato content to some extent. Tomatoes that undergo processing might have a change in the nutritional content and bioactive compounds of tomatoes.

2.3.1 Common tomato-based products

Tomatoes are consumed in a variety of forms. The fresh fruits can be included in salads, sandwiches, and salsa, while the processed versions are available dried or as pastes, preserves, sauces, soups, and juices (Beckles, 2012). These products are utilised to improve the flavour of various forms of food items. Tomato puree serves as an alternative to fresh tomatoes in a recipe. Ketchup is a sweeter and more diluted form of puree (pulp). Tomato sauces have a sweet and tangy flavour. Both sauce and ketchup are enjoyed with meals and snacks.

2.3.2 Processing effects

Typical food-processing techniques, namely freezing, canning and drying are known to affect the nutrients and bioactive compounds found in fruits and vegetables. While processed foods are viewed as less desirable compared to unprocessed foods, the term “processing” is not inherently negative, and not all processed foods lack nutrients or are

unhealthy. Food processing can provide advantages like enhanced digestibility and better nutrient absorption, enhancing food safety (Wu et al., 2022). Certain processing methods, on the other hand, can help in reducing or eliminating harmful chemicals in food to some degree (Singh et al., 2023). Fresh tomatoes and processed tomato products contain different levels of moisture as well as total and soluble solids. For instance, most fresh processing tomatoes typically have around 5-6% soluble solids, while tomato paste can be manufactured to achieve soluble solid content ranging from 20% to 37%, depending on the requirements of the production process (Wu et al., 2022b).

Stahl et al. (1992) have proposed that the absorption of lycopene was higher from heat-processed juice compared to unprocessed tomato juice, while a more recent investigation by Agarwal and Are (2000) indicated that lycopene from both tomato juice and tomato sauce was easily absorbed. Wang et al. (2023) found that heat-processed tomato juice exhibited significantly greater antioxidant activity than fresh tomatoes; however, the reason for the increase in antioxidant activity due to processing was not explored.

2.4 Analytical techniques for chemical characterisation

The chemical composition of food is essential in defining its traits and properties. Modern analytical techniques have enabled the identification and quantification of the diverse chemical constituents found in food. These methods allow accurate assessment of nutrients, minerals and additional compounds within a specific food sample (De Santis, 2024).

2.4.1 Overview of analytical methods

Analytical chemistry techniques are used to detect, identify, characterise, and quantify chemical compounds (Shivhare & Chincholikar, n.d.). It is also used to determine a compound or element's concentration (Sakinala, 2021). The development of analytical methods for food matrices is challenging due to their diverse physiochemical features, including physical state, lipid content, and pH, which might impact analyte structure and extraction efficiency (Delahaut & Marega, 2022). Mass spectrometry (MS), often coupled with liquid chromatography or gas chromatography (GC), capillary electrophoresis, infrared spectroscopy and nuclear magnetic resonance spectroscopy, represents the most commonly used analytical methods for evaluating food quality (Kaloo et al., 2023). Other than that, the composition of food is usually analysed through various specialised traditional analytical methods such as HPLC, GC, ultra-high-performance liquid chromatography (UHPLC), and inductively coupled plasma (ICP) in conjunction with mass spectrometry (Kaloo et al., 2023).

Several analytical approaches have been created to identify antioxidants in food matrices. Typically, these methods fall into three categories which are electrochemical, spectroscopic, and chromatographic techniques. Electrochemical methods offer quick and sensitive detection, spectroscopic techniques facilitate extensive compositional analysis, whereas chromatographic methods, including HPLC, provide precise separation and quantification of specific antioxidant compounds (Patil et al., 2025).

2.4.2 Principle of high-performance liquid chromatography

Chromatography is an analytical technique used in labs to separate substances. The mixture is dispersed in a liquid known as the mobile phase, which moves it through an apparatus containing another substance known as the stationary phase (B, 2024). HPLC

is a type of chromatography used to separate, identify, and measure dissolved chemicals in solution (Ahmed, 2024). HPLC is a more advanced form of liquid chromatography that operates on the same chemical separation principles and is used as a powerful tool in chemical and biological research (Ahmed, 2024b). This form of chromatography separates substances based on their unique polarity. The interaction of these compounds with the stationary phase of the column is also considered. HPLC requires a pump that used to move the mobile phase and analyte through the column, a stationary phase, as well as a detector (Bachhav et al., 2023).

The stationary phase is generally a packed column filled with porous particles, whereas the mobile phase is a liquid solvent or solvent mixture pumped through the column at high pressure. Components in the sample mixture are divided between the stationary and mobile phases based on their affinity for each phase. Compounds having greater interactions with the stationary phase elute longer, whereas those with weaker interactions elute sooner (Walters, 2024). Retention time is a significant chromatographic and the fundamental parameter used to separate, identify, and quantify molecules of interest from complex mixtures (Etxebarria et al., 2009). The size, polarity and interactions of compounds with the stationary phase are the variables that could affect retention duration (Walters, 2024). HPLC is also based on the Van Deemter equation, which links the efficacy of the chromatographic column to particle size, molecular diffusion, and stationary phase thickness (Ingale et al., 2023). HPLC is known for its great resolution, precision, and efficiency, making it useful in industries such as pharmaceuticals, biotechnology, environmental analysis and forensic research. The technique's adaptation to different detectors, such as UV-visible, fluorescence and MS, increases its versatility and precision in assessing a wide range of chemicals (Ahmed, 2024a). Figure 2.4 illustrates the setup of HPLC system adapted from (Ahmed, 2024c).

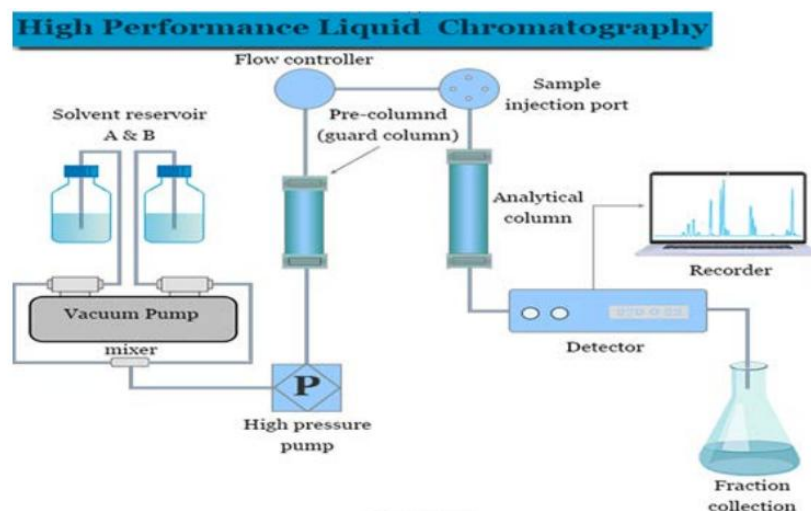


Figure 2.4: Setup of HPLC system

2.4.3 Application of HPLC in food and forensic analysis

To evaluate the quality of food, HPLC has been widely utilised for quantitative analysis of many different substances, such as vitamins, carbohydrates and additives, and has also proved useful with food flavourings, additives and contaminants (Esteki et al., 2019). HPLC, which has been proven to be optimal for identifying or measuring the great majority of food analytes, is becoming the foundation of food analysis techniques (Yiasmin & Waleed, 2021). HPLC has also emerged as a crucial method for enhancing food traceability and understanding the bioactivity of food and its components within the food sector (Nie & Nie, 2019).

In many cases, HPLC procedures have replaced complicated analyses, and chromatographic methods are generally more specific and exact, resulting in considerable reductions in the analysis times (Yiasmin & Waleed, 2021). In the context of food analysis and quality assessment, HPLC primarily concentrates on assessing nutrients (such as

amino acids, proteins, carbohydrates), bioactive substances (including phenolic compounds, isoflavones, ellagitannins, flavonoids), and harmful substances (like mycotoxins, herbicides, fungicides, and certain food additives) (Nie & Nie, 2019). HPLC has also been known to have low sensitivity to matrix interferences by components such as fatty acids, cholesterol and carbohydrates (Pragst, n.d.).

HPLC is utilised in forensic science to measure the concentration of drugs in biological samples, identify steroids in blood and urine, analyse textile dyes in forensic cases, and determine the presence of cocaine and other substances of abuse in biological matrices (Abdu Hussen, 2022). HPLC is also one of the most common separation methods used in forensic toxicology apart from GC, as it offers distinct benefits for non-volatile, thermally sensitive, and high molecular weight compounds under mild conditions without derivatisation (Pragst, n.d.). Soil is of particular interest in forensic investigation because it provides valuable associative evidence linking suspects, victims or crime scene and HPLC has proven to be an effective method for forensic soil analysis, particularly in distinguishing soils from nearby locations (Lee et al., 2022).

2.4.4 Application of HPLC in analysis of tomato and tomato-based products

Previous studies have successfully applied HPLC to separate and quantify major carotenoid compounds such as lycopene and beta-carotene. In a study conducted by Sérino et al. (2009), employed reverse-phase HPLC with photodiode array detection to separate and evaluate lycopene, beta-carotene, lutein and phytoene. The carotenoids in the cited study were extracted by agitation and centrifugation before being analysed. The study demonstrated that a rapid extraction method coupled with HPLC enabled the analysis of major tomato carotenoids. Apart from fresh tomato analysis, HPLC was also

used for processed tomato products. Monteiro et al. (2009), also quantified lycopene using HPLC in various tomato sauces with different concentrations of mushrooms or their extracts. The extraction process in this study explored various solvents from dichloromethane, hexane and ethanol, discovering that ethanol was the most effective solvent due to the quality of chromatographic peaks obtained. The study revealed that when tomato sauce and raw materials underwent heat treatment, the type of treatment did not affect the lycopene bioavailability.

Another study by Vallverdú-Queralt et al. (2011), tomato-based products were analysed upon extraction using high-performance liquid chromatography/electrospray ionization-time of flight-mass spectrometry (HPLC-ESI-QTOF) with negative ion detection using information-dependent acquisition (IDA) to determine their phenolic composition. The results found that phenolic content varied across ketchups, gazpachos and tomato liquids, with gazpacho having the greatest concentration. Similarly, Alharthi et al. (2025), conducted a study on the nutritional quality of tomato fruits harvested from hybridized bio-agents assisted microbial remediation using HPLC. The findings implied that the remediation program significantly impacted vitamin B, vitamin C and total carotenoid concentration.

CHAPTER 3: METHODOLOGY

3.1 Instrumentation

The general instruments used in this study are listed in Table 3.1.

Table 3.1: General instruments and apparatus used in this study

Name (model)	Manufacturer
Freeze Dryer	Buchi (Germany)
High-Performance Liquid Chromatography	Shimadzu (Japan)
Analytical balance	Sartorius (Germany)
Pipette	Eppendorf (Germany)

3.2 Reagents

The chemical reagents used in this study are listed in Table 3.2.

Table 3.2: List of chemical reagents used in this study

Name	Manufacturer
AR-grade acetone	Bendosen (Malaysia)
AR-grade petroleum ether	Hmbg (Germany)
HPLC-grade acetone	Fischer (Germany)
AR-grade methanol	Bendosen (Malaysia)
Anhydrous Sodium Sulphate	Hmbg (Germany)

3.3 Tomato sauce samples

Three different brands of commercial tomato sauces were selected to be used in this study, namely Eonsave brand tomato sauce (Malaysia), Longson brand tomato sauce (Malaysia) and McDonald's (MCD) tomato sauce (Malaysia). All three tomato sauce samples for each brand were purchased from the local market to represent various market segments, comprising a supermarket private label (Eonsave), a widely used retail sauce brand (Longson), and a product designed for food service (MCD). To compare the profile of fresh tomato with the commercial product, a fresh tomato was also bought from the local market.

3.4 Sample preparation and analysis

This section explains the procedures for sample preparation and analysis before HPLC determination. The procedures involved include preparation of samples for repeatability and reproducibility testing, sample freeze-drying, solvent extraction, liquid-liquid partitioning, and extraction protocols. Pre-analytical treatment was also performed before samples were subjected to HPLC analysis.

3.4.1 Repeatability and reproducibility

To assess the repeatability and reproduction of the sample preparation and HPLC analysis, the sample from a single Econsave tomato sauce bottle was selected as the representative sample and analysed in duplicate. The variation between the three bottles or three packets of tomato sauce of the same brand was investigated for consistency among the samples. The fresh tomato was analysed on its known for the purpose of comparing the chromatographic profile.

3.4.2 Freeze-drying procedure

The tomato sauces from each brand and a fresh sliced tomato were transferred to a urine bottle for the freeze-drying procedure. A total of 10 urine bottles containing sample were sent for freeze-drying for a period of three days (72 hours). Upon freeze-drying, any form of moisture from the sample was removed, leaving a dry sample. The freeze-dried samples obtained were kept in a desiccator at room temperature to avoid moisture absorption until they undergo further analysis.

3.4.3 Chemical extraction

The extraction of carotenoids from samples was performed using previously described protocols for carotenoid analysis in vegetables, with slight adjustments to fit the current study (de Castro et al., 2021). This step was crucial to ensure a concentrated analyte is obtained for the HPLC analysis.

Extraction was performed on the freeze-dried sample by using acetone-based solvent extraction method. A spatula was used to transfer the freeze-dried sample to be analysed and was placed in a weighing boat. A weight of 1.0 g sample was accurately measured by using an analytical balance. The weighed sample was then placed in a mortar, and 100 mL of acetone was utilized for the extraction process. The acetone was gradually added in five 20 mL portions measured using a measuring cylinder. For every addition, the sample was carefully ground with a pestle for several minutes to guarantee thorough homogenization and dissolution of the compounds. The first homogenised mixture was then passed through a Buchner funnel containing filter paper into a 250 mL conical flask for vacuum filtration. The procedure was carried out repeatedly until the total volume of acetone had been utilised to the point where the filtrate was clear, indicating a complete extraction.

3.4.4 Liquid-liquid partitioning

The acetone filtrate underwent a liquid-liquid partitioning to transfer desired compounds into a more non-polar solvent. Firstly, into a 250 mL separating funnel, 20 mL of petroleum ether was measured and added. Next, the acetone filtrate was slowly transferred into the separating funnel containing the petroleum ether. Following that, 500 mL of distilled water was added by using five 50 mL portions. Upon adding each 50 mL

portion, the lower layer was then carefully discarded into a separate beaker after each wash, while retaining the upper desired layer.

3.4.5 Pre-analytical treatment

The upper collected layer was then discarded through a glass funnel containing a layer of glass wool containing anhydrous sodium sulphate to remove any remaining water or moisture. The purified extract was collected in a 25 mL conical flask. The funnel was then washed with a small amount of petroleum ether that was added to the flask until the meniscus reached the 25 mL calibration mark.

3.5 High-performance liquid chromatography analysis

After chemical extraction, the analyte was then subjected to HPLC for separation and determination of compounds.

3.5.1 Sample preparation for HPLC

The purified extracts in petroleum ether were subjected to HPLC analysis. The sample was transferred into the 2 mL vial. The vials were then sealed with a Teflon-lined cap to prevent solvent loss through evaporation and labelled accordingly. They were respectively placed at the sample tray of the HPLC system prior to injection. To ensure proper documented analysis, the vials were positioned in the autosampler tray in a specific sequence that corresponds to the samples list in the HPLC instrument software. The labelling was done to avoid confusion and mixing of samples.

3.5.2 HPLC instrumental conditions

Chromatography analysis was then performed using HPLC system. The reversed-phase HPLC settings and detection wavelength for carotenoid measurements were determined using previously published techniques for vegetable matrices (Will@ & Ranggab, 1996). The settings used throughout the analysis of the tomato sauce samples are demonstrated in Table 3.3.

As for the gradient elution, a starting mobile phase of 0% acetone was used and was then gradually increased to 70% over a span of 15 minutes to elute moderately polar to non-polar compounds. Finally, the concentration was increased to 100% acetone by 25 minutes to remove any strongly retained and highly non-polar compounds. The wavelength for detection was set to 450nm as it was the maximum absorption for major carotenoid pigments like lycopene and β -carotene which could be highly present in tomatoes.

Table 3.3: HPLC instrumental conditions for analysis

Parameters	Specifications
HPLC Type	Reverse-Phase
Column	C18 Column Brand: Shimazdu (Japan) Dimensions: 4.6x250 mm
Mobile Phase	Binary Gradient of Acetone (HPLC grade) and Distilled Water Solvent A: Distilled water Solvent B: Acetone

Table 3.3: Continued

Gradient Programme	<ul style="list-style-type: none"> • Start: 0% solvent B (Less polar)/ 100% Solvent A (Water) • At 15 minutes: 70% Solvent B/ 30% Solvent A • At 25 minutes: 100% Solvent B
Flow Rate	1.0 mL/min
Injection Volume	10 μ L
Column Temperature	25°C
Detection Wavelength	450 nm
Total Run Time	25 minutes

3.5.3 Data acquisition and analysis

LabSolutions (version 5.87 SP 1, Shimadzu, Japan) was used as software in operating and handling the HPLC system, in which chromatogram, retention times, areas and spectral information were obtained. As for repeatability, the tomato sauce sample from Econsave was evaluated three times by injecting. Reproducibility was evaluated based on the deviation of three individual measurements for three samples. The chromatogram generated was further compared for analysis and compared among the samples of various sources. The possible identity of the chromatographic peaks was determined based on tentative identification using their retention time and spectral characteristics. The peak assignments were cross-referenced with previous literature on the carotenoid profiles in tomatoes. Next, the chromatograms from fresh tomatoes and commercial tomato sauces were evaluated for observable similarities and differences.

3.5.4 Statistical analysis

The resulting data obtained in this study were analysed using Minitab® Statistical Software 22 (Minitab, LLC, United States). Principal component analysis (PCA) was performed on the data to observe the patterns and similarities between samples. PCA is a widely used multivariate approach that reduces data to a few characteristic dimensions for visualisation by creating a new set of variables known as principal component (PC) (McKenzie et al., 2011). The use of PCA enabled data obtained from commercial tomato sauces to be identified objectively, offering a clear overview of similarities and differences between samples. Prior to analysis, the chromatographic data of major peaks from the PDA detector was processed using Microsoft® Excel (Microsoft Corporation, United States).

In the Excel® spreadsheet document, values were compiled in a way where samples represent rows and peak areas as variables. To minimise variation and ensure each variable contributes equally, the data was normalized before being imported to Minitab software. Upon PCA, linear discriminant analysis (LDA) was performed as a supervised classification technique to differentiate sample groups and to develop discriminant functions for the purpose of classifying sample (Gardner-Lubbe, 2021). In this study, PCA score-LDA model was used to assess the ability of the chromatographic peak to distinguish commercial tomato sauce samples based on their sources and origins.

CHAPTER 4: RESULTS

4.1 Comparison between fresh tomato and tomato sauce profile

The visual and qualitative differences were examined and compared between the fresh tomato and commercial tomato sauce samples based on their respective HPLC chromatograms. Their peak retention times and intensities between the samples, in which the chromatograms are demonstrated in Figure 4.1.

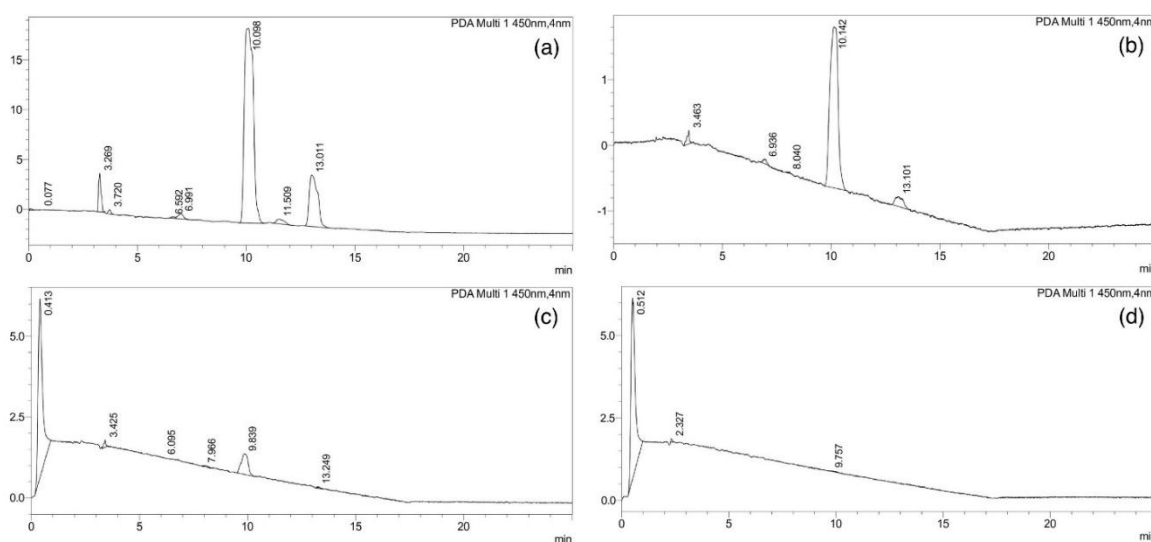


Figure 4.2: chromatograms of fresh tomato and commercial tomato sauces, where (a) Fresh tomato sample, (b) Econsave tomato sauce sample, (c) McD tomato sauce sample, (d) Longson tomato sauce sample

Figure 4.1 (a) shows the HPLC chromatogram of a fresh tomato fruit that had been extracted using the procedure described in Section 3.4.2. Through visual observation, several distinct peaks were evident in the profile, suggesting a tomato-like profile. Most peaks were observed in the early to mid-retention time region. From the PDA (Photodiode Array) detector, the major peaks appeared at approximately 3.0 to 3.5 minutes, 6.0 to 7.0