Cyprus University of Technology

Faculty of Geotechnical Sciences and Environmental Management

Master's Thesis

Identification and quantification of Volatile Organic Compounds on 'Mandora' Juice, using GC-MS chromatography

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CYPRUS UNIVERSITY OF TECHNOLOGY

FACULTY OF GEOTECHNICAL SCIENCES AND ENVIRONMENTAL MANAGEMENT

DEPARTMENT OF AGRICULTURAL SCIENCES, BIOTECHNOLOGY AND FOOD SCIENCE

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Approval Form

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ABSTRACT

Mandora is a prominent Citrus cultivar that is widely cultivated in Cyprus. It is a Citrus hybrid that combines the genetic characteristics of mandarin and sweet orange. It is closely associated with the island's citrus-growing regions and is valued for its intense aroma, which is stronger than that of typical oranges. This study investigated the volatile composition of 'Mandora' juice and evaluated its aroma profile using Gas Chromatography – Mass Spectrometry (GC - MS). The treatments involved the application of a commercial product (SuperFifty), a concentrated extract of *Ascophyllum nodosum* seaweed known for enhancing plant tolerance to abiotic stress and improving fruit set and yield. A total of 131 volatile organic compounds (VOCs) were identified. These compounds were classified into major chemical groups including terpenoids, aldehydes, alcohols, esters, ketones and organic acids. Terpenoids were the predominant class in all samples, with D-limonene identified as the most abundant compound. Although the overall types of compounds were similar in all samples, the treatments resulted in changes in the amounts of certain compounds. In particular, treated samples showed a decrease in β-myrcene and slight increases in some oxygenated terpenes and aldehydes. Principal Component Analysis (PCA) revealed a clear separation between control and treated samples, indicating systematic differences in the overall volatile profiles. Overall, the results suggest that the treatments caused minor variations in the relative abundance of VOCs, while the overall volatile composition and the typical citrus aroma of 'Mandora' juice remains unchanged.

Keywords: Mandora; GC-MS; volatile organic compounds; terpenoids; principal component analysis (PCA)

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LIST OF ABBREVIATIONS

Important abbreviations that have been used in the text and need explanation are briefly presented.

VOCs:	Volatile Organic Compound
GC-MS:	Gas Chromatography – Mass spectrometer
A.C.:	Aromatic Compounds
SPME:	Solid Phase microextraction
IS:	Internal Standard
HS-SPME:	Headspace Solid Phase microextraction

1 Introduction

1.1 Introduction to Volatile Compounds and their role in citrus fruits

Volatile organic compounds (VOCs) are a group of molecules with low-molecular weight that contribute significantly to the flavor and aroma profile of fruits. VOCs are typically released into the air at ambient temperature and pressure due to their relatively high vapor pressures, which facilitate their transfer into the gas phase at ambient temperature and enable their detection by human olfaction. Regarding fruit quality, VOCs not only serve as sensory attributes, but they also serve as biochemical markers of ripening, freshness, as well as shelf life. Among various fruit groups, citrus fruits are especially abundant in VOCs, including terpenes, esters, aldehydes, ketones, and alcohols. These are the main molecules responsible for the distinct and desirable aromatic profiles that are associated with different citrus species (González-Mas, 2019).

VOCs are biosynthesized through a complex biochemical process that is highly regulated by multitude enzymatic pathways, which - are affected by both genetic and environmental factors. Specifically, in citrus fruits, most - VOCs are synthesized via three major pathways. The first one is the lipoxygenase pathway, the second is the terpenoid pathway, and finally the amino-acid derived pathway. These biochemical pathways yield a diverse range of metabolites that can influence the organoleptic properties and commercial value of products from the citrus family. - Monoterpenes, such as limonene and linalool, are biosynthesized through the methylerythritol phosphate pathway in plastids. At the same time, alcohols and aldehydes are usually derived from fatty acid oxidation via the lipoxygenase pathway. Esters contribute to fruity and sweet notes and are formed by enzymatic esterification of acids and alcohols (González-Mas, 2019).

Citrus essential oils are primarily obtained from peel, which is rich in volatile terpenoids such as limonene, β-pinene and γ-terpinene. González-Mas et al. - provided a comprehensive review of these compounds, detailing their biosynthesis, extraction, and possible applications. Even though essential oil analysis focuses on the peel, many of these volatiles also present themselves in juice with concentrations that vary, which further contributes to the overall aroma (Ladaniya, 2022).

Consumer perception and marketability are largely influenced by the volatile compounds in citrus fruit. Aroma, more often than not, is considered the "invisible signature" of flavor and is largely determined by the concentration as well as the composition of VOCs in citrus juice and peel oils (Sawamura, 2010). In the food and beverage industry, specifically in juice manufacturing and flavor enhancement, retaining and amplifying these natural volatiles is crucial to product appeal (Bora, 2020). Besides sensory appeal, citrus volatiles also exhibit notable biological activities, including antimicrobial, antioxidant, and anti-inflammatory properties (Zehiroglu, 2019).

Characterizing volatile profiles provides insights into authenticity, varietal distinction, as well as postharvest treatment of citrus fruits. It is also a critical step in breeding new citrus hybrids such as the Mandora, which is a cross between mandarin and orange varieties and combines desirable traits from both parents, such as a rich and complex aroma profile.

Mandora (*Citrus reticulata* X *Citrus Sinensis*) is known for a unique combination of sweetness, juiciness, and an intense citrus aroma. Its origin comes from the Mediterranean region, and its popularity rose due to its desirable organoleptic qualities and potential health benefits. While a multitude of citrus fruits have been thoroughly analyzed in terms of their volatile composition, there isn't much data available on the specific VOCs present in Mandora juice.

This hybrid nature of Mandora introduces a potentially novel volatile profile, merging the terpenoid-rich content of mandarins with aldehydes and esters often prominent in oranges (Ladaniya, 2022). It is important to understand its volatile constituents, not only to enhance its flavor characterization, but also to contribute to broader citrus breeding programs that aim to optimize aroma, shelf stability and finally its nutritional benefits.

According to Rowan et al. (2011), volatile metabolites can act as sensitive indicators of physiological changes in fruit, such as ripening, fermentation, and microbial spoilage. Production of VOCs during different developmental stages reflects underlying metabolic shifts, which could later be used for quality control and freshness assessment (González-Mas, 2019). In addition, certain volatiles such as hexanal, limonene and linalool are markers of fruit integrity that can signal the presence of contamination or degradation (Vavoura, 2022).

Identifying and quantifying these compounds with high specificity is essential for citrus juice producers whose main goal is to standardize product quality, extend shelf life, and meet regulatory standards for food safety and labeling. This further underscores that flavor preservation for minimally processed or cold-pressed juice varieties is a significant challenge.

Thus, the profiling of citrus juice volatiles is a complementary field to essential oil research, enabling a complete understanding of citrus aroma (Kelebek, 2011). In hybrid fruits such as the Mandora, it is important to consider the inheritance of volatile profiles from both parent species and how this influences the juice matrix, particularly in terms of flavor harmonization and commercial differentiation.

1.2 Mandora as a Citrus Hybrid: Genetic Background and Compositional Uniqueness

As aforementioned in this text, mandora is a citrus hybrid between Mandarin (*Citrus reticulata*) and Washington Navel Orange (*Citrus sinensis*). Developed in Cyprus, this cultivar was named "Mandora" in order to reflect its dual parentage as well as its desirable organoleptic properties. Mandora represents an increasingly important product in Mediterranean citrus agriculture, whereas it combines the aromatic richness and ease of peeling of mandarins with the size and juice yield of sweet oranges.

Citrus fruits are among the most widely cultivated and consumed fruits globally, with a rich history of hybridization that aimed at improving flavor, nutritional content, pest resistance, and finally postharvest performance. The fusion of the parental genotypes in mandora produces a fruit with intense aroma, deep orange color, easy peeling, and a balanced sweet-tart flavor profile, all of which are desirable consumer attributes.

Mandora's maternal lineage is inherited from *Citrus sinensis*, which contributes traits such as larger fruit size, higher juice content, and greater storage and shelf stability. In the meantime, its paternal lineage from the mandarin contributes to intense aroma, lower acidity with a sweeter flavor, and finally a loose and easy-to-peel skin. This genetic combination made Mandora a favorable cultivar in the juice and fresh fruit markets, particularly in Cyprus and Greece.

Citrus hybrids often exhibit intermediate or enhanced phytochemical profiles compared to their parental genotypes. One example is the variation in sugar and organic acid levels, which affects taste balance. Moreover, differences in flavonoid and carotenoid contents influence fruit color and contribute to numerous health benefits. Finally, and most importantly in this context, volatile organic compounds, which define aroma and sensory identity, also differ (Ladaniya, 2022). Over the years, studies have shown that citrus hybrids can express novel volatile patterns that are not present in either parent. For example, González-Mas et al. demonstrated that hybrids may exhibit new sesquiterpene expression, altered ester profiles as well as increased oxygenated terpene levels. This makes Mandora fruits a particularly interesting subject for novel aroma characterization, especially since no detailed volatile profiling by GC-MS has been reported to date. Based on its lineage, Mandora juice is expected to contain high limonene levels, as in most citrus species, elevated levels of ethyl esters and alcohols which are mainly associated with the mandarin parent. Finally, mandora juice is hypothesized to contain more oxygenated monoterpenes, such as linalool and terpineol, contributing to floral and fruity notes since such compounds are found in the parents (Kelebek, 2011). Additionally, its aroma could benefit from the low-threshold aldehydes such as decanal or citral and sesquiterpenes such as valencene, although this depends mostly on postharvest handling maturity and environmental growing conditions. Therefore, the diversity and balance of these volatiles could make Mandora better suited for fresh juice production (González-Mas, 2011).

Despite its commercial importance in Cyprus, Mandora, additionally mentioned as “Ortanique tangor” in some studies, has received limited scientific attention, particularly with respect to GC–MS-based volatile profiling, sensory evaluation, and comparative studies with clementine, orange or even other citrus hybrids. Therefore, this dissertation aims to address this gap by providing a comprehensive GC-MS-based identification and quantification of volatile compounds in Mandora juice. The results are expected to support citrus breeding efforts by improving the understanding of desirable aromatic traits, contribute to quality control and product authentication strategies, and provide scientific evidence supporting its sensory characteristics and market potential.

1.3 Analytical Techniques for the Identification - of Volatile Organic Compounds in Citrus Fruits

VOCs in citrus juice show a significant complexity. This complexity presents both analytical opportunities and challenges. Profiling these volatiles is essential for understanding fruit quality, authenticity, and postharvest behavior. Due to their low concentration and structural diversity, precise identification and quantification of VOCs require very sensitive and selective analytical tools. Several methods have been developed over the years such as the solid phase microextraction (SPME), dynamic headspace analysis, purge-and-trap, and finally solvent extraction techniques (Agatonovic-Kustrin S, 2023). However, out of all the extraction techniques, SPME paired with gas chromatography – mass spectrometry (GC-MS) remains the golden standard.

GC-MS technology has revolutionized citrus research by enabling the detailed characterization of a wide range of volatile compounds across juice, peel, and essential oil matrices. GC-MS allows not only the efficient separation of complex mixtures, but also, through the high sensitivity of mass spectrometric detection, enables both qualitative and quantitative analyses, which are critical for profiling new citrus hybrids such as Mandora (Riya, 2023).GC-MS is a hyphenated technique that combines the separating power of gas chromatography with the molecular identification capability of mass spectrometry. In gas chromatography, volatile components of a sample are vaporized and passed through a chromatographic column, where they are separated based on their physicochemical properties, such as boiling point and polarity. Subsequently, the separated compounds enter the mass spectrometer, where they are ionized, fragmented and finally detected based on their mass-to-charge ratio (m/z) (Riya, 2023).

The next step involves comparing the resulting mass spectra with established libraries, such as NIST, for compound identification. When paired with the appropriate calibration and internal standards, GC-MS also facilitates the quantification of target volatile compounds in fruit matrices. This makes it a highly suitable technique for applications in quality control, flavor research and metabolic profiling (Riya, 2023).

For qualitative analysis, particularly in the absence of analytical standards and when the objective is the characterization of the volatile profile, library matching approaches are

commonly employed to identify compounds based on their mass spectral patterns. This is especially relevant for exploratory studies aiming to establish the volatile fingerprint of complex matrices. A critical step in VOC analysis, particularly in aqueous matrices such as citrus juice, is the choice of extraction method, since volatile compounds are often present at trace levels and may be bound or masked by sugars and organic acids. Proper selection of the extraction technique directly influences the sensitivity, selectivity and reproducibility of the analysis (Kelebek, 2011).

Solid-phase microextraction (SPME) is a solvent-free extraction technique that uses coated fiber to adsorb VOCs from the headspace of juice samples. Its main advantages include simplicity, speed and compatibility with GC-MS analysis. Additionally, SPME is particularly effective for the analysis of free aroma compounds without altering the sample matrix. Another commonly used technique is dynamic headspace sampling, which involves purging volatile compounds from the sample using an inert gas and trapping them on an adsorbent such as Tenax.

This method provides higher extraction efficiency and recovery, but it often requires longer sample preparation and processing times. Furthermore, solvent extraction techniques may also be applied, typically using solvents such as dichloromethane or ethyl acetate to extract volatile compounds from juice matrices. Although this method can provide a comprehensive volatile profile, it may also co-extract non-volatile compounds, requiring additional clean-up steps. Among these techniques, SPME-GC-MS has emerged as the preferred method for the analysis of citrus juice due to its high sensitivity, minimal sample handling and its suitability for automation (Agatonovic-Kustrin S, 2023).

GC-MS has numerous benefits for citrus VOCs analysis such as high resolution, making it capable of separating complex mixtures of compounds with overlapping boiling points (González-Mas, 2011). Furthermore, it has high sensitivity and selectivity which assists in detecting low concentrations of volatile compounds, which in turn are vital for aroma-active compounds (Rowan, 2011). Finally, structural identification provided by GC-MS aids well with identifying unknowns by the provided fragmentation patterns from the mass spectrometer (Riya, 2023).

Analytical validation ensures that GC-MS results are scientifically credible and reproducible. Such validation parameters include (Riya, 2023):

Accuracy: How close the measured values are to the actual concentration

Precision: Which defines the repeatability of measurements under the same conditions

Limit of detection and limit of quantification: Signals the lowest concentration levels that can be reliably detected and quantified.

Selectivity: Which is the ability to distinguish analytes in the presence of similar compounds or matrix interferences.

Stability: VOCs must remain stable during storage, handling, and analysis.

Out of the above parameters, those of paramount importance in this experiment are the limit of detection and selectivity. Limit of detection since VOCs are generally found in relatively low concentrations in Mandora Juice whereas selectivity is important since there are multiple compounds with similar chemical structure in the juice matrix.

The insights gained from GC-MS volatile profiling extend beyond academic interest. For example, in citrus breeding, VOC analysis can assist in properly selecting hybrids with superior flavor attributes. For example, identifying unique terpene signatures in Mandora juice can help breeders evaluate the inheritance of sensory traits from parent cultivars.

In the industrial setting, volatile profiling is central to quality assurance and new product development. Beverage companies use GC-MS data to standardize flavor profiles across batches, identify off-doors, and optimize juice blending. Understanding the temporal dynamics of VOCs during storage and processing additionally aids in extending shelf life and developing flavor retention strategies (Bora. 2020).

1.4 Significance of Volatile Profiling in Citrus Hybrids: Focus on Mandora

As a relatively new cultivar, Mandora with its unique organoleptic profile remains underexplored. Traditional breeding evaluations have mainly focused on yield, disease resistance and morphology (Ladaniya, 2022), whereas sensory and biochemical characterization are often neglected. Using GC-MS to profile volatiles provides an objective and chemical-based approach to defining the sensory identity of Mandora, therefore linking aroma signatures to its hybrid nature. By understanding the volatile

profile of Mandora, quality control and cultivar differentiation is also facilitated. As citrus fruit continuously diversifies through hybridization, chemical fingerprinting becomes increasingly essential for authentication and ensuring genetic consistency in commercial production.

Flavor is an extremely complex sensory perception that arises from the integration of taste and smell. VOCs are the primary drivers of aroma and play a disproportionately large role in perceived fruit quality. In citrus fruits over 200 VOCs have been identified, including monoterpenes, aldehydes, esters, alcohols and ketones. Each compound makes a unique contribution to the aroma profile, either individually or synergistically (González-Mas, 2019).

Consumer preference for citrus fruits often latches onto subtle differences in aroma intensity and composition. What defines fresh, floral, and fruity notes is a balanced volatile profile rich in limonene, linalool, γ-terpinene, and esters. Simultaneously, off flavors such as bitterness or staleness are often due to oxidation products or microbial spoilage (Sawamura, 2010). Moreover, given the growing interest in fresh and naturally flavored beverages, Mandora's volatile composition directly affects its marketability. Profiling its aroma-active compounds enables producers to align the sensory qualities of juice products with the consumer's expectations, which in turn ensures higher satisfaction and brand loyalty.

Although VOCs are mainly associated with sensory properties, many of them exhibit bioactive effects and health benefits. Terpenes like limonene and β-myrcene possess anti-inflammatory, antioxidant, and chemopreventive activities (Shaaban, 2012). Aldehydes and esters could also influence physiological processes through olfactory pathways and metabolic interactions. Hence, GC-MS profiling of Mandora VOCs not only informs sensory science but may uncover functionally significant compounds that contribute to the fruit's nutraceutical value. The identification of health-relevant volatiles aligns with consumer trends therefore favoring functional foods and natural health products.

The aroma profile of citrus juice evolves over time due to enzymatic activity, microbial growth, oxidation, and interaction with packaging materials (Ladaniya, 2022). Volatile compounds serve as early indicators of spoilage or degradation. For example, increases in aldehydes and ketones may signal lipid oxidation, while the presence of off-odors can indicate microbial activity (González-Mas, 2019). Mandora juice, like other citrus juices,

is susceptible to such deteriorating processes. By establishing a baseline volatile fingerprint using GC-MS, changes during storage can be monitored, and suitable preservation techniques devised.

Each citrus cultivar has a characteristic volatile profile, shaped by its genetic background, climatic conditions, and agronomic practices. For example, sweet oranges are typically rich in limonene and myrcene (Qiao, 2008), while mandarins show higher levels of γ-terpinene and methyl-N-methylanthranilate (Yu, 2018a). Mandora is expected to inherit volatile compounds from both parents, resulting in a hybrid signature that can be statistically distinguished using chemometric tools such as principal component analysis or partial least squares discriminant analysis.

Using GC-MS to profile VOCs also informs multiple facets of commercial development. One example is product formulation where understanding aroma balance helps create single-strength juices, blends or concentrates that preserve the fruit's signature character. Flavor optimization is also critical whereas VOCs analysis supports the adjustment of sugar-acid balance or addition of natural flavors such as to enhance consumer appeal. Market positioning is also important in order to use chemical data to label products as "aroma-rich", "naturally flavored" or "high-terpene". Finally, export compliance through which VOC data can support regulatory submissions, ensures the juice meets aroma and safety standards in different markets.

In conclusion, creating a volatile profile for Mandora using GC-MS has applications beyond sensory characterization, offering insights into cultivar authentication, quality assurance and product innovation. Through establishing detailed chemical fingerprints, researchers and producers can safeguard the fruit's distinct identity, optimize its flavor potential, and monitor changes during processing and storage. Furthermore, linking aroma-active compounds to both consumer preference and potential health benefits positions Mandora as more than a novel citrus hybrid- it could prove to be a valuable candidate for functional food applications that align with modern dietary trends. Ultimately, the study of Mandora volatiles could connect the gap between agricultural science, food technology and consumer demand, thereby reinforcing the importance of integrating biochemical evaluation into the future of citrus breeding and commercialization.